Characterization of OQM for Quantitative Measures of Embryo Viability

A Dissertation Presented

by

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to

The Department of Electrical and Computer Engineering

in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy

in

Electrical Engineering

in the Field of

Electromagnetics

Northeastern University
Boston, Massachusetts

January, 2009
Abstract

Since 1978 in vitro fertilization (IVF) procedures have resulted in the birth of over three million babies. Yet, despite the plethora of qualitative viability markers utilized in various embryo scoring methods, IVF procedures in the United States had a live birth rate of only 34% in 2005, with 32% of these successful pregnancies resulting in multiple births. These multiple pregnancies were directly attributed to the transfer of multiple embryos to increase the probability that a single, healthy embryo was included, because the current qualitative measures are inadequate to measure embryo viability reliably. The use of quantitative, three dimensional viability measures could create a definitive method of embryo scoring that will produce a successful pregnancy from the transfer of a single embryo.

Optical quadrature microscopy (OQM) was invented at Northeastern University to measure the amplitude and phase of an optically transparent sample. Since its conception, OQM has been built into the Keck 3D Fusion Microscope that combines brightfield, differential interference contrast (DIC), epi-fluorescence, OQM, confocal fluorescence, confocal reflectance, and two-photon on a single microscope stage, and has been used in conjunction with DIC to create the phase-subtraction cell-counting method that has counted the number of cells in live mouse embryos accurately beyond the developmental stage at which DIC can be used alone.

In this dissertation, we derive a thorough signal-to-noise ratio analysis that provides the minimum phase error in the current system, and present the ability of OQM to measure accurate phase for spherical objects that are much larger than the depth of field. We also provide an overview of multimodal imaging of mouse embryos and describe how the rate of development, cell size, symmetry, and fragmentation, and oocyte mitochondrial distribution measurements could be quantified with the extension of the phase-subtraction cell count and the measurement of relative dry mass. If verified with human embryos, these methodologies could provide the means to determine which viability measures are truly indicative of embryo health.

Supplementary File 1 shows a brightfield time-lapse of embryos developing from the 2-cell to the morula stage and Supplementary File 2 provides a visualization of the phase-subtraction cell-counting method.
Acknowledgements

First, I want to acknowledge all of the great colleagues that I have had the privilege to work with from Northeastern University and from various affiliations around the world. I especially want to thank Judy Newmark and Carol Warner for their patience and insight, as this project would have never materialized without their help. I would also like to thank all of my friends from the OSL that made my long hours in the lab enjoyable.

I want to make a special acknowledgement for my advisor, Chuck DiMarzio. Chuck has been my graduate advisor, mentor, and friend for the past five and a half years, and I have never encountered an educator that was more caring and compassionate about the education of their students. Chuck always puts the students before himself and will do everything in his power to provide every opportunity for them to excel. He has touched the lives of so many of us throughout his career, and we have all flourished and become who we are because of him. Unfortunately, Chuck never receives the just recognition that his efforts deserve, but I’m sure he would shy away from it if he did. I would have never had the opportunity to try and show that I could be a slightly successful graduate student without Chuck taking a chance on me, and I am forever indebted to him for the success that I am able to achieve professionally.

I also want to thank Maya Silvis for her love and support during this whole ordeal. She believed in me from the beginning, and has helped me better myself to become the person that I am today. She has always been the one constant that I could count on no matter how grim things appeared at the time. I would not have been able to reach this level of success without her, and I will never be able to make up for the sacrifices and hardships that she has had to endure. I appreciate all that you have done for me and I am thankful for all the years that we have been able to spend together.
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1. Introduction

Since 1978 in vitro fertilization (IVF) procedures have resulted in the birth of over three million babies [Steptoe et al. 1980]. Yet, despite the plethora of qualitative viability markers utilized in various embryo scoring methods amongst the clinics, IVF procedures in the United States had a live birth rate of only 34% in 2005, with 32% of these successful pregnancies resulting in multiple births [Centers for Disease Control and Prevention 2007]. These multiple pregnancies were directly attributed to the transfer of multiple embryos to increase the probability that a single, healthy embryo was included, because the current qualitative measures are inadequate to measure embryo viability reliably. The use of quantitative, three dimensional viability measures could create a definitive method of embryo scoring that will produce a successful pregnancy from the transfer of a single embryo.

Optical quadrature microscopy (OQM) was invented by Charles DiMarzio at Northeastern University in 1997 to measure the amplitude and phase of an optically transparent object without having to acquire multiple views of the object or introduce multiple light sources [Hogenboom et al. 1998; DiMarzio 1999, 2000]. Since its conception, OQM has been built into the Keck 3D Fusion Microscope [Warger et al. 2007a], which combines brightfield, differential interference contrast (DIC), epi-fluorescence, confocal fluorescence, confocal reflectance, two-photon, and OQM on a single microscope stage, and has been used in conjunction with DIC to count the number of cells in live mouse embryos beyond the developmental stage at which DIC can be used alone [Warger et al. 2008]. With the progressive use of OQM for embryo viability measures and the recent popularity of full-field quantitative phase imaging, many questions needed to be answered in regard to the accuracy and ability of OQM to measure the phase induced by thick objects.

1.1 Dissertation Overview

This dissertation contains three major parts: (1) imaging of live mouse embryos, (2) characterizing the phase measurements of thick samples with OQM, and (3) quantifying current qualitative embryo viability markers, which are described in Chapters 2, 3, and 4, respectively. Chapter 2 provides an overview of embryo development and in vitro fertilization (IVF), followed by a discussion on the fundamentals behind the imaging modalities used on the Keck 3D Fusion Microscope to image live mouse embryos. Chapter 3 provides an overview of the principles and techniques behind quantitative phase imaging, followed by descriptions of a thorough signal-to-noise ratio (SNR) analysis that provides the minimum phase error in the current system, and the ability of OQM to measure accurate phase for spherical objects that are much larger than
the depth of field of the instrument. Chapter 4 provides a general description of the phase-subtraction cell-counting method that we have created from the combination of OQM and DIC, and additional viability measurements that could be quantified with the extension of the phase-subtraction cell count and the measurement of relative dry mass. The three main chapters are followed by a chapter on the future work that contains preliminary designs and analyses to: (1) improve the OQM instrumentation, which includes repeatable, more accurate phase measurements with near-real-time acquisition, (2) improve the models used in the quantitative embryo viability measures to reduce the error of the techniques and gain a better understanding of embryo structures within the images, and (3) incorporate image processing techniques to move toward automation of the quantitative viability measures.

1.2 Dissemination of Results

To date the work that has been described in this dissertation has provided the foundation for: one masters project on a potential cell-counting technique using the OQM image alone [Chang 2004], and one masters thesis [Braganza 2008b] and one ongoing Masters project on speeding up the phase reconstruction and unwrapping algorithms with FPGA and GPU devices [Mistry in preparation 2009] at Northeastern University, two Masters theses on automatic image registration between OQM and DIC images at National Chung Cheng University in Taiwan [Chen 2007; Liang 2008], and two funded proposals on automatic image registration and cell boundary detection from the National Science Council in Taiwan. In addition, the results described in this dissertation have been acknowledged with one best techniques paper award, one best student proceedings paper finalist, one best poster award, and an alternate position for the NSF East Asia and Pacific Summer Internship (EAPSI) program to work in Singapore with Colin Sheppard. Overall this work has resulted in five journal publications:


   – 2008 Best Techniques paper within Microscopy and Microanalysis

seven conference proceedings:


   – Best Student Paper Award Finalist


nine posters, presentations, and invited talks:


and one patent application:

2. Background

2.1 In Vitro Fertilization

In the United States, one in six couples suffers from problems related to infertility [Chandra et al. 2005]. Assisted reproductive technologies (ART), such as in vitro fertilization (IVF), have given these couples a second option after natural attempts at reproduction have proven unsuccessful. However, after more than a quarter century of administered IVF procedures that have resulted in over three million babies, only 24% of transfers completed world-wide resulted in a successful delivery in 2000 [Adamson et al. 2006], and U.S. clinics were still only able to provide a live birth rate of 34% with fresh nondonor eggs or embryos in 2005 [Centers for Disease Control and Prevention 2007]. A major cause for the low success rate is the inability to determine which embryos are viable and will lead to a successful pregnancy. As a result, clinicians transfer multiple embryos to increase the chances of including one viable embryo that will produce a successful pregnancy. In 2000, an average of 2.6 embryos were transferred per procedure world-wide, with some countries averaging as many as 3.6 embryos per transfer, which led to twin pregnancies in 26.5% and triplet pregnancies in 2.9% of successful transfers [Adamson et al. 2006]. In 2005, 32% of successful IVF procedures resulted in multiple pregnancies because three or more embryos were transferred in 47% of procedures and four or more embryos were transferred in 18% [Centers for Disease Control and Prevention 2007]. The practice of transferring multiple embryos introduces complications for both mother and child. Multiple pregnancy leads to the increased risk of pregnancy complications, including preterm delivery, prematurity, low birth weight, congenital malformations, and infant death [Centers for Disease Control and Prevention 2007; Martin and Park 1999; ESHRE Campus Course Report 2001; Ozturk et al. 2001; De Neubourg et al. 2002; Gerris et al. 2002; Sutcliffe 2002; Nowak et al. 2003; Kissin et al. 2005]. Multiple-birth infants from IVF also face an increased risk of neurological problems, especially cerebral palsy [Strömberg et al. 2002; Centers for Disease Control and Prevention 2007]. For all of these reasons, there is a worldwide effort toward single embryo transfer [Gurgan and Demirol 2004; Schieve 2006].

Human embryos that are cultured in IVF clinics are given a grade based on two major criteria: (1) the number of cells at specific time points during development and (2) overall morphology [Cummins et al. 1986; Puissant et al. 1987; Ziebe et al. 1997; Gardner and Schoolcraft 1999; Tesarik and Greco 1999; Racowsky et al. 2000; Scott et al. 2000; Warner and Brenner 2001; Baczkowski et al. 2004; Warner et al. 2004; Shen et al. 2005; Shen et al. 2006]. Some of the morphological parameters that have been considered are symmetry, size, fragmentation, vacuoles, perivitelline space, position of pronuclei, orientation of nucleoli, thickness and structure of the zona pellucida, and spindle integrity, but none of these parameters has proven to be good enough to
determine embryo viability reliably. There is agreement that the first criterion, embryo cell number, shows that faster developing embryos are more likely to give rise to a successful pregnancy than slower developing embryos [Warner and Brenner 2001; Baczkowski et al. 2004; Warner et al. 2004]. However, current non-toxic microscopy techniques are unable to count the number of cells accurately past the 8-cell stage. Thus, the number of cells can be used for day 3 transfers where embryos containing less than 10 cells are returned to the uterine environment, but cannot be used for blastocyst stage transfers that occur on day 5 of development. Blastocyst transfers are advantageous because the embryo does not reach the uterus until day 5 in a natural pregnancy [Gardner and Lane 1997], and the ability to develop to the blastocyst stage is also a viability marker [Balaban et al. 2006]. As a result, the decision on embryo quality in blastocyst transfers is based on morphological markers such as the expansion of the blastocoel cavity and the characteristics of the inner cell mass and the trophectoderm [Balaban et al. 2006]. Therefore, the creation of hardware and software that could quantify the current qualitative viability measures could help to determine what markers are indicative of embryo health and move toward a definitive method of embryo scoring that will produce a successful pregnancy from the transfer of a single embryo.

2.2 Embryo Development

To date, ethical, religious, and political concerns do not allow human embryos to be used for research in the United States. As a result, U.S. embryology research generally begins with mice and then continues with bovine and primate embryos. Mice are an excellent animal model for human research and have been classified as a model organism by the National Institutes of Health. The mouse model also provides many advantages over human embryos for research, including genetically identical inbred mouse strains, embryo availability, and low cost [Quinn and Horstman 1998].
The diameter of a mouse embryo is approximately 100 μm, including the zona pellucida, and the diameter of a human embryo is approximately 130 μm [Warner and Brenner 2001]. A DIC image of a typical 8-cell live mouse embryo is shown in Figure 2.1(a). Each cell, or blastomere, cleaves into two cells with a combined volume approximately equal to the original cell before division. Therefore, the total volume of the embryo is relatively constant during preimplantation development. The first polar body extrudes during meiosis and degenerates shortly after fertilization of the oocyte. The second polar body extrudes on fertilization and is maintained at least through the 8-cell stage of development, after which it begins to degenerate. The second polar body can be distinguished from the cells easily because it is considerably smaller in size. The zona pellucida is a spherical encasement with a thickness of approximately 7 μm that surrounds the cells and polar body and keeps them contained. The space between the blastomeres and the zona pellucida is the perivitelline space. During imaging, we have assumed that the perivitelline space is filled with culture medium because the zona pellucida is a loose matrix that is porous to macromolecules, including proteins. A DIC image of a typical embryo at the blastocyst stage is shown in Figure 2.1(b). Some of the cells shown in the 8-cell embryo will form a spherical shell inside of the zona pellucida called the trophectoderm. Within the trophectoderm, the remaining cells group together on one side to form the inner cell mass and the remaining volume is called the blastocoel cavity. The blastocoelic fluid within the blastocoel cavity acts as a culture medium for the inner cell mass and is filled with water and proteins [Dardik and Schultz 1991; Dardik et al. 1993].

Early mouse embryo development is very similar to human embryo development. The embryo begins as an unfertilized egg, called an oocyte in Figure 2.2(a). After fertilization, the egg becomes a zygote and the chromosomes of the sperm combine with the chromosomes of the oocyte to form a diploid nucleus [pp. 1154-1155, Alberts et al. 2002]. A DIC image of a zygote appears very similar to an oocyte because the majority of the change is internal. As the embryo continues to develop, the nucleus of the zygote divides (cleaves) into two nuclei, and the large single cell divides into two cells in Figure 2.2(b). The cells continue to divide to the 8-cell stage in Figure 2.2(c), and into the morula stage in Figure 2.2(d) where the mouse embryo contains between 9 and 30 cells and begins to undergo compaction. During compaction, the cells begin to form a relatively smooth mass with indistinguishable cell outlines [pp. 61-63, Veeck and Zaninović 2003]. The development after the zygote and before compaction is also referred to as the cleavage stage. After compaction, the embryo undergoes cavitation, where the cells differentiate into the trophectoderm and inner cell mass that form the blastocyst stage in Figure 2.2(e) [p. 63, Veeck and Zaninović 2003]. During implantation, the blastocyst hatches out of the zona pellucida, and the trophectoderm becomes the protective layer that attaches to the wall of the uterus and later become the placenta. The primary difference between the development of human and mouse embryos is the speed
at which the embryos develop. A human embryo will be fertilized on day 1, and then
become a 2-cell embryo on day 2, an 8-cell embryo on day 3, a morula on day 4, and a
blastocyst on day 5. A mouse embryo develops slightly faster and becomes a 2-cell
embryo on day 2, an 8-cell embryo during the beginning of day 3, a morula toward the
end of day 3, and a blastocyst on day 4 [p. 1224, Alberts et al. 2002]. Supplementary File
1 contains an avi file that shows embryos developing from the 2-cell to the morula stage
using brightfield time-lapse images acquired every 30 minutes for 72 hours.

![DIC images of live mouse embryos at different stages](image)

Figure 2.2: DIC images of live mouse embryos at the (a) oocyte, (b) 2-cell, (c) 8-cell,
(d) morula, and (e) blastocyst stages.

2.3 Optical Imaging of Mouse Embryos

Until recent years, most new microscopy techniques were developed into separate
instruments such that multimodal microscopy was limited to working with separate
microscopes and then combining images through digital processing techniques [Glasbey
and Martin 1996]. This method is obviously problematic for ensuring spatial registration
of the images while maintaining physiologically correct structure and function. To
address this problem, we built the Keck 3D Fusion Microscope that combines differential
interference contrast (DIC), epi-fluorescence, optical quadrature microscopy (OQM),
confocal reflectance, confocal fluorescence, and two-photon fluorescence on a single
Nikon TE2000 microscope base [Warger et al. 2007a]. This microscope allows the user
to place a sample on the microscope stage and acquire any combination of images
without having to disturb the sample. An example of the images acquired of a live 3-cell
mouse embryo stained with Hoechst and MitoTracker Green dye is shown in Figure 2.3, followed by a general discussion of resolution and the individual imaging techniques.

Figure 2.3: Multimodal images of a live 3-cell mouse embryo collected on the Keck 3D Fusion Microscope in (a) brightfield, (b) DIC, (c) OQM, (d) epi-fluorescence with Hoechst dye, (e) epi-fluorescence with MitoTracker Green dye, (f) two-photon with Hoechst dye, (g) confocal fluorescence with MitoTracker Green dye, and (h) confocal reflectance. The Hoechst dye binds to the DNA within the nuclei and the MitoTracker Green dye binds to the mitochondria. The light sources and filters used to acquire these images are provided in the discussions of the individual modes.

2.3.1 Full-field Imaging Techniques

In a conventional transmission microscope, a light source illuminates the entire field of view for full-field imaging. A schematic for such a system using a non-infinity corrected objective lens is shown in Figure 1. A point source located at the object plane of the objective (within the sample) will image to a diffraction-limited spot in the image plane of the objective (-), where a two-dimensional detector is mounted. Identical point
sources positioned along the optical axis and in out-of-focus transverse planes before or after the object plane will image to planes after or before the image plane (\(\cdots\)).

Figure 2.4: Optical layout of a full-field imaging system. The light source illuminates a 2D area with a condenser lens, and the objective images each point within a 2D field of view onto a 2D detector array. Point sources from out-of-focus planes spread over the detector area and degrade the quality of the in-focus image.

Assuming a diffraction-limited system and a uniform, non-absorbing sample, the irradiance from each of the point sources will be the same at the image plane, but the out-of-focus light will spread across a larger area. The spreading of the light intensity in transverse planes through the focal region of a lens is shown in Figure 2.5.

Figure 2.5: Intensity distribution of the light within transverse planes through the focus of a lens [Cagnet 1962].

A point source from the object plane of the objective will appear in-focus, but the spreading of the out-of-focus planes will contribute to out-of-focus blur. The thickness of the sample that appears in-focus is referred to as the depth of field of the imaging system:

\[ d_z = \frac{n \lambda}{N A^2} + \frac{n}{N A} d_{\text{pixel}} \]  

(1)
where $n$ is the refractive index between the lens and the focused spot, $\lambda$ is the center wavelength for the illumination light in vacuum, $NA$ is the numerical aperture of the objective, and $d_{\text{pixel}}$ is the width of a pixel in the image found by dividing the physical pixel size by the total magnification of the system [Inoué and Spring 1997]. The desired depth of field of an imaging system is dependent on the application. A shallow depth of field provides fine details within a small section of the sample, while a large depth of field provides information about the entire sample, but in less detail.

### 2.3.1.1 Resolution in Conventional Full-field Imaging Techniques

The performance of any imaging instrument is generally described in terms of the system’s point spread function (PSF). The PSF of a lens describes the ability of the lens to focus light to a diffraction-limited spot, in comparison to the geometric focal point described by ray tracing. But the PSF is also a measure of the ability to image a point object, thereby providing a measure of the instrument’s ability to resolve point objects within a sample.

Mathematically, the PSF of a lens provides the amplitude distribution of the light in the planes near the geometric focal point:

$$h(u, v) = U(u, v) = -i \frac{2\pi NA^2}{\lambda n} A e^{\frac{i n^2 u^2}{\lambda}} \int_0^1 J_0(v \rho) e^{-i \frac{\pi}{2} u \rho^2} \rho d\rho,$$

assuming the lens has uniform, plane-wave illumination in the pupil plane and follows the paraxial approximation [p. 487, Born and Wolf 1999]. Eq. (2) is also considered the coherent point spread function because it includes the magnitude and phase information. From a linear systems perspective [Gaskill 1978], the PSF is often considered the impulse response of the imaging system for a point object, which is generally described by the variable $h$, so the variables $U(u, v)$ and $h(u, v)$ have been used interchangeably in the literature depending on whether the author is describing the PSF of an imaging system $h(u,v)$ or the electromagnetic field within the focal region of a lens $U(u,v)$. In Eq. (2), $J_0$ is the zeroth-order Bessel function, $i$ is $\sqrt{-1}$, and $A$ and $\rho$ are the amplitude of the light and the radial coordinate centered on the optical axis in the pupil plane, respectively. The optical coordinates $u$ and $v$ in the focal plane relate to the transverse (lateral) direction from the optical axis ($r$) and the axial direction parallel to the optical axis ($z$) by:

$$u = \frac{2\pi NA^2}{\lambda} z, \quad v = \frac{2\pi}{\lambda} NA r.$$  

The optical coordinates in Eq. (3) are provided in terms of NA to stay consistent with the resolution equations that are generally provided in terms of NA. However, the optical
units are often times expressed in terms of the half angle of illumination $\theta$ or the radius of the pupil $a$ and focal length of the lens $f$ from the relation \( NA = n \sin \theta \approx a/f \) that results from the paraxial approximation. The paraxial approximation does not hold once \( NA > 1/\sqrt{2} \sim 0.7 \), but the resolution expressions derived using the paraxial approximation can be extended to \( NA \leq 0.87n \) [Sheppard and Matthews 1987] by substituting the axial optical coordinate:

\[
u = \frac{8\pi}{\lambda} n z \sin^2 \frac{\theta}{2}, \tag{4}\]

where $n$ is the refractive index of the immersion medium, and ensuring the refractive index of the sample is greater than the refractive index of the immersion medium [Wilson and Juskaitis 1995]. For this reason, many authors define the axial optical coordinate by Eq. (4) and specify that resolution equations must be derived from a more in-depth mathematical treatment when $NA > 0.87n$ [Sheppard and Matthews 1987; Wilson and Juskaitis 1995; Gu 1996; Török and Wilson 1997]. It is important to emphasize that any physical distance can be converted into optical units by substitution of the distance into Eq. (3) or Eq. (4).

Because the amplitude in Eq. (2) cannot be seen or measured directly, the PSF is often described by the intensity distribution:

\[
l(u, v) = |h(u, v)|^2 = I_0 \left| \int_0^1 J_0(v \rho) e^{-i \frac{z}{2} \rho^2} \rho d\rho \right|^2, \tag{5}\]

where:

\[
I_0 = \left( \frac{2\pi NA^2}{\lambda n} \right)^2 \tag{6}\]

is the intensity at the geometric focal point $u = v = 0$. Eq. (5) is also considered the incoherent point spread function because it does not contain the phase information. The solution to the integral in Eqs. (2) and (5) [pp. 484-492, Born and Wolf 1999] can be calculated by separating the solution to the integral into the real and imaginary parts and solving for the points within the geometric focus (beam according to ray tracing):

\[
l(u, v) = \left( \frac{\lambda}{2 \pi} \right)^2 [U_1^2(u, v) + U_2^2(u, v)]I_0 \tag{7}\]

and within the geometric shadow:

\[
l(u, v) = \left( \frac{\lambda}{2 \pi} \right)^2 \left[ 1 + V_0^2(u, v) + V_1^2(u, v) - 2V_0(u, v) \cos \left[ \frac{1}{2} (u + v^2) \right] \right. - \\
\left. 2V_1(u, v) \sin \left[ \frac{1}{2} (u + v^2) \right] \right] I_0, \tag{8}\]
where:

\[ U_n(u, v) = \sum_{s=0}^{\infty} (-1)^s \left( \frac{u}{v} \right)^{n+2s} J_{n+2s}(v) \]
\[ V_n(u, v) = \sum_{s=0}^{\infty} (-1)^s \left( \frac{v}{u} \right)^{n+2s} J_{n+2s}(v) \]  \hspace{1cm} (9)

are the Lommel functions, and the boundary between the geometric focus and the geometric shadow corresponds to the points \(|u| = |v|\). To complete the solution, approximations must be used for points along the focal plane \((u = 0)\):

\[ I(0, v) = I_0 \left( \frac{2J_1(v)}{v} \right)^2, \]  \hspace{1cm} (10)

the geometric focal point \((u = v = 0)\):

\[ I(0,0) = I_0, \]  \hspace{1cm} (11)

and the points along the boundary between the geometric focus and the geometric shadow:

\[ I(|u|, |u|) = I_0 \left( \frac{1 - 2J_0(u) \cos u + J_0^2(u)}{u^2} \right)^2. \]  \hspace{1cm} (12)

It is important to note that the first 50 terms provides an accurate approximation to the infinite series in Eq. (9), and the points within the geometric focus provide a satisfactory approximation for the points along the boundary between the geometric focus and the geometric shadow for a field of view limited by at least \(|u| = |v| = 40\), which were the limits in Figure 2.14.

Since the condenser lens illuminates the entire field of view, the resolution in a conventional full-field imaging system is primarily based on the objective’s ability to resolve point objects. The optics within the system are generally assumed to be diffraction limited with the objective lens as the limiting resolving element, so the PSF of the microscope is simply the PSF of the objective lens in Eqs. (2) or (5). Assuming uniform plane-wave illumination in the pupil plane, the intensity distribution of the focused spot along the lateral and axial axes normalized by \(I_0\) reduces to:

\[ I_{\text{conv}}(v) = \left[ \frac{2J_1(v)}{v} \right]^2, \]  \hspace{1cm} (13)
\[ I_{\text{conv}}(u) = \left[ \frac{\sin(u/4)}{u/4} \right]^2, \]  \hspace{1cm} (14)
respectively, where $I_{conv}(v)$ is a one-dimensional Airy pattern. Figure 2.6(a) shows a plot of Eqs. (13) and (14) to show the width of the focused spot along the lateral and axial directions. The Rayleigh criterion for resolution states that two point objects of equal intensity are resolvable if the points are separated by the radius of the Airy disk, where the diameter of the Airy disk is defined as the distance between the first zeros of the Airy function. According to the Rayleigh criterion, the lateral resolution of a conventional microscope is the width between the origin and $v$ such that $J_1(v) = 0$, or $v = \frac{2\pi}{\lambda} NA r = 3.83$, which reduces to the well known:

$$\Delta x_{conv} = 0.61 \frac{\lambda}{NA}$$

for an evenly-illuminated circular aperture. Following the same procedure in the axial direction with the axial coordinate in Eq. (3) provides the axial resolution:

$$\Delta z_{conv} = 2 \frac{n\lambda}{NA^2}.$$  

The resolution may also be interpreted in regard to the saddle-to-peak intensity ratio of the sum of two normalized Airy functions, where the ratio is the dip in intensity between the two maximum points [p. 371, Born and Wolf 1999]. Figure 2.6(b) shows the plot for two Airy functions from Eq. (13) separated by the Rayleigh criterion, and that the two points will be resolved if the saddle-to-peak intensity ratio is 0.735 or the depth of the saddle is 26.5%.

\[Figure 2.6: \text{ (a) Intensity distribution along the lateral (--) and axial (-) axes. (b) Sum of two Airy patterns (-) representing the image of two point sources (--) displaced by the minimum resolvable distance (v = 3.83) using a uniformly illuminated circular aperture in a conventional microscope.}\]
2.3.1.2 Brightfield

Brightfield images are the typical white light images that the general public associates with microscopes. A white light source, such as a Halogen or Xenon lamp, illuminates the entire field of view, and a tube lens creates an image from the light that transmits through the sample and is within the NA of the infinity-corrected objective lens at an intermediate image plane. If the objective is not infinity corrected, an intermediate image plane exists between 160 mm and 200 mm from the pupil plane of the objective depending on the manufacturer’s design. The image plane after the tube lens for both infinity- and non-infinity-corrected objective lenses is either located at a camera port of the microscope, where a 2D detector array acquires an image, or before the oculars. The combination of the ocular lens and the lens within a human’s eye relays the image to the retina of the operator.

The contrast in a brightfield image is related to the absorption, scattering, refraction, and diffraction of the illumination light. The contrast of a transparent or semi-transparent object is very low, which makes the embryo appear flat in Figure 2.3(a). A clinician may use brightfield imaging to locate or move an embryo quickly within a dish or make a quick judgment on overall viability, but will not use it to assess any of the scoring techniques.

2.3.1.3 DIC

DIC microscopy produces images in which contrast is related to one component of the gradient of the object’s phase [pp. 153-167, Murphy 2001]. The DIC image can be interpreted as the sum of a constant bias and the derivative of the object’s phase in one direction. The addition of the bias provides the mean grayscale background of the image in Figure 2.3(b) with dark or light contrast corresponding to an increase or decrease in the phase of the object along the direction of the derivative. The shading that results along the edges of the sample in DIC images provides a three-dimensional appearance of a semi-transparent object, compared to the flat appearance in brightfield images. However, it is important to emphasize that the shading is dependent on the derivative of the phase along a single direction, called the shear axis. This property makes it extremely difficult to apply conventional image processing techniques and obtain quantitative information about the object.

DIC uses the same optical path as brightfield, but includes two polarizers and two Wollaston prisms. The first polarizer ensures the illumination light is at 45 degrees to provide equal amounts of S and P polarized light with respect to the Wollaston prism. The 45-degree polarized light is split spatially by a birefringent Wollaston prism in the back-focal (pupil) plane of the condenser lens. The condenser lens collimates the two
paths separated by a distance (shear) that is less than the resolution of the microscope. The direction of the shear (shear axis) defines the direction of the derivative. The two paths travel through the sample separated by the shear, and are collected by the objective lens. A second Wollaston prism positioned near the pupil plane of the objective recombines the two paths, and conceptually, the second polarizer (analyzer) oriented orthogonally to the first polarizer blocks the light that contains no difference in phase between the two paths. Tilting the second Wollaston prism provides a phase shift between the two paths, which effectively shifts the zeroth-order interference fringe out of the field of view, resulting in the addition of the bias [p. 161, Murphy 2001]. Strictly speaking, the analyzer creates the interference between the two orthogonal polarization states as described in §3.2.1.

![DIC images focused to 5 planes through the thickness of a live mouse embryo at the (a)-(e) 8-cell stage, (f)-(j) morula stage containing 26 cells, and (k)-(o) blastocyst stage, corresponding to days 3, 4, and 5 in human embryo development. After the 8-cell stage the out-of-focus cells on top of the embryo hinder the ability to resolve cells on the bottom, making accurate cell counts unattainable.](image)

**Figure 2.7:** DIC images focused to 5 planes through the thickness of a live mouse embryo at the (a)-(e) 8-cell stage, (f)-(j) morula stage containing 26 cells, and (k)-(o) blastocyst stage, corresponding to days 3, 4, and 5 in human embryo development. After the 8-cell stage the out-of-focus cells on top of the embryo hinder the ability to resolve cells on the bottom, making accurate cell counts unattainable.

Clinicians currently use DIC, or the similar Hoffman optics [pp. 169-172, Murphy 2001] to analyze the morphological characteristics of embryos and to make their best determination of the number of cells in the embryo noninvasively. A DIC image of an
embryo provides distinct cell boundaries for cells within the depth of field of the microscope because the cell boundaries provide the greatest difference in optical path between the two waves. As the embryo develops, the cells begin to overlap and the cells on the top layer begin to obstruct the edges of the bottom layer of cells. Cell edges are visible under a single layer of overlapped cells, providing the ability to count accurately up to two layers of four cells in an 8-cell embryo in Figure 2.7(a)-2.7(e). Once the embryo forms a third layer, the cell edges of the bottom layer cannot be resolved, thereby making accurate cell counts unattainable by using DIC microscopy alone in Figure 2.7(f)-2.7(j). Figure 2.7(k)-2.7(o) also shows a sequence of focal planes through a blastocyst for comparison.

2.3.1.4 Epi-Fluorescence

Fluorescence microscopy images the distribution of individual molecules tagged to specific organelles by collecting only the fluorescent wavelengths that are emitted after excitation with a particular band of wavelengths [pp. 177-199, Murphy 2001]. An epi-fluorescence system uses an epitaxial imaging configuration where the objective illuminates the sample with the excitation wavelengths and collects the fluorescent wavelengths from the excited fluorophores within the sample. An “epi cube” positioned after the light source, which is generally a mercury lamp, contains an excitation filter, dichroic mirror, and emission filter. The excitation filter is a bandpass filter that passes the desired excitation wavelengths from the broadband light source. The dichroic mirror is typically a long pass filter that reflects the excitation wavelengths toward the sample and passes the Stokes-shifted fluorescent wavelengths toward the detector. The emission filter is also a bandpass filter that passes the fluorescent wavelengths and blocks any reflections of the excitation light.

Figure 2.3(d) shows an epi-fluorescence image of Hoechst 33342 dye (Invitrogen Corporation, Carlsbad, California) that binds the fluorophores to the DNA within the nucleus of each cell. The epi cube used to acquire this image contained a 360 nm ± 20 nm excitation filter, 400 nm long pass dichroic, and a 460 nm ± 25 nm emission filter that were optimized for the fluorescence spectra shown in Figure 2.8(a). Figure 2.3(e) shows an epi-fluorescence image of MitoTracker Green dye (Invitrogen Corporation, Carlsbad, California) that binds the fluorophores to the mitochondria throughout the cells. The epi cube used to acquire this image contained a 480 ± 15 nm excitation filter, a 505 nm long pass dichroic, and a 535 nm ± 20 nm emission filter that were optimized for the fluorescence spectra shown in Figure 2.8(b). All of the brightfield, DIC, and epi-fluorescence images were acquired with an air-cooled CCD camera (Diagnostic Instruments, Inc., Sterling Heights, MI).
We have used a piezoelectric z-stage (Piezosystems Jena, Jena, Germany) to step the embryo through the focus of the objective to provide a z-stack of images through the sample. Counting the stained nuclei within the z-stack provides the ground truth for the number of nuclei within an embryo and was used to determine the total number of cells [Ebert et al. 1985]. This method of cell counting is easy to use and offers a relatively fast measurement for the cell number, but the Hoechst stain permanently binds to the DNA of the nuclei and is a known mutagen. Its use is therefore considered an invasive and potentially mutagenic procedure that is not permitted in a clinical setting.

![Fluorescence spectra](image)

**Figure 2.8:** Fluorescence spectra for (a) Hoechst [Invitrogen Corporation, Hoechst] and (b) MitoTracker Green [Invitrogen Corporation, MitoTracker Green] dyes.

2.3.1.5 **Optical Quadrature Microscopy**

Optical quadrature microscopy (OQM) is an interferometric imaging technique that reconstructs the change in magnitude and phase of the coherent light that travels through an optically transparent object [Hogenboom et al. 1998]. Assuming a non-scattering sample with a single uniform refractive index, the change in phase ($\alpha$) is related to the thickness ($h$) and refractive index ($n_s$) of the object by:

$$\alpha = \frac{2\pi(n_s-n_0)}{\lambda}h$$  \hspace{1cm} (17)

where $\lambda$ is the wavelength of the laser and $n_0$ is the refractive index of the immersion medium. The phase image in Figure 2.3(c) can then be generalized as values proportional to the thickness of the embryo, where the dark red regions (large phase values) correspond to the overlap of the cells.

OQM uses a Mach-Zehnder interferometer, where the signal path travels along the optical axis of the microscope through the camera port and mixes with the reference
path outside of the microscope housing. The signal path is polarized at 45 degrees to provide equal amounts of the two orthogonal polarization states, and the reference path is circularly polarized to provide a 90-degree phase shift between the two orthogonal polarization states. The reference and signal paths recombine at a non-polarizing 50/50 beamsplitter and a polarizing beamsplitter after each output of the recombining beamsplitter separates the two orthogonal polarization states that are acquired with 4 synchronized CCD cameras. A reconstruction algorithm uses images from the 4 CCD cameras to reconstruct the magnitude and phase changes in the signal path induced by the sample. A detailed description of OQM is provided in Chapter 3.

OQM has been shown to be non-toxic to embryo development, with 2-cell embryos developing to the blastocyst stage after extended imaging exposure [Newmark et al. 2007]. OQM images have also been combined with DIC images to create the phase-subtraction cell-counting technique that has extended the ability to count the number of cells in live mouse embryos accurately beyond the 8-cell stage [Warger et al. 2008]. A description of the phase-subtraction cell-counting method is provided in §4.2.

### 2.3.2 Point-Scanning Imaging Techniques

Point-scanning systems create an image of a single plane within a translucent material by removing most of the out-of-focus light that degrades the quality of the image in a conventional microscope. Achieving this provides the ability to image individual sections (optically section) without having to cut the sample into thin slices. It is important to note that some full-field imaging techniques, such as structured illumination [Neil et al. 1997; Gustafsson 2000] and deconvolution [McNally et al. 1999; Wallace et al. 2001], provide optical sectioning capabilities, but these techniques are more complicated than conventional full-field microscopes.

![Figure 2.9: Optical layout for a point-scanning system with a non-infinity corrected objective lens. The condenser lens illuminates a focused spot within the sample with a point source of light. The objective lens focuses the light from a point-source at the object plane to a spot in the image plane.](image)
In a point-scanning imaging system, the condenser lens focuses the light source to a diffraction-limited spot within the sample, and an objective lens images the light from the illumination spot to a spot in the image plane. The optical layout for a transmission configuration for a single pixel is shown in Figure 2.9. The type of point-scanning system defines the method in which the out-of-focus light is removed from each individual point within the image. The optical layout for a reflection (epitaxial) confocal point-scanning system with a non-infinity corrected objective lens is shown in Figure 2.10. The objective lens focuses illumination light to a focused spot within the sample, and collects the resultant backscattered or fluorescent light. A dichroic beamsplitter separates illumination wavelengths from detection wavelengths in a confocal fluorescence system and uses an emission filter to block unwanted reflections of the light source. A polarizing beamsplitter and quarter-wave plate separate illumination and backscattered light in a confocal reflectance system.

**Figure 2.10:** Optical layout for a reflection (epitaxial) confocal point-scanning system with a non-infinity corrected objective lens. The objective lens focuses illumination light to a focused spot within the sample, and collects the resultant backscattered or fluorescent light. A dichroic beamsplitter separates illumination wavelengths from detection wavelengths in a confocal fluorescence system and uses an emission filter to block unwanted reflections of the light source. A polarizing beamsplitter and quarter-wave plate separate illumination and backscattered light in a confocal reflectance system.

Point-scanning systems generally utilize a reflection or epitaxial imaging configuration shown in Figure 2.10 where the objective lens illuminates and collects the backscattered or fluorescent signal within the first 100 μm – 2 mm of the sample surface depending on the illumination wavelength, objective NA, and turbidity of the tissue [White et al. 1999; Chapter 1, Bouma and Tearney 2001; Wang and Wu 2007; González et al. 2008]. A fluorescence point-scanning system uses a dichroic beamsplitter to separate the excitation wavelengths that illuminate the sample (illumination path) and the fluorescent wavelengths that emit from the sample (detection path). An emission filter positioned before the pinhole ensures the detected signal is from the fluorescent wavelengths desired. A reflectance point-scanning system typically uses polarization to separate the illumination and detection paths and increase the throughput of the system because the same wavelengths exist in the illumination and detection paths. In a reflectance system, the illumination light transmits through a polarizing beamsplitter with \( P \)-polarization and becomes circularly polarized after transmitting through a quarter-wave plate with axes oriented at 45 degrees with respect to the laser polarization. The circularly
polarized light illuminates a spot within the sample and the backscattered light from the object plane passes back through the objective and quarter-wave plate. On the return path, the light has passed through the quarter-wave plate twice converting the incident $P$-polarization into $S$-polarization that will reflect from the polarizing beamsplitter, provided the returning light retains its state of polarization as it interacts with the sample. This will be the case for single-scattered (ballistic) light that retains circular polarization and a small fraction of the multiply-scattered light that comes back randomly polarized.

In practice, infinity-corrected objective lenses have become the norm for microscope design because they provide an infinity space between the light source and the objective in an epitaxial configuration such that a scanning system can be incorporated. The light collected at the front focal-plane of an infinity-corrected objective returns collimated, and the back-focal plane of a tube or detector lens becomes the image plane. A collimated beam is required for a diffraction-limited imaging system because aberrations will be induced by a converging or diverging beam traveling through a thick piece of glass, such as the beamsplitter, quarter-wave plate, or emission filter.

The benefit of point-scanning systems over conventional full-field imaging techniques is the dramatic reduction of the out-of-focus signal that degrades the quality of an image. However, point scanning is inherently slower than full-field imaging because each pixel is generally acquired separately, in comparison to capturing all of the pixels simultaneously in a full-field imaging system. The time required to capture one point-scanning image (frame rate) is dependent on the speed at which the illumination point scans across each pixel within the field of view.

Early point-scanning microscopes used a fixed optical path and scanned the sample incrementally. This technique provides diffraction-limited imaging throughout the field of view within a very stable, compact system, but it cannot be used to image large objects that cannot be translated on a microscope stage. In addition, potential vibrations induced by sudden starts and stops of the stage limit the scanning speed, because the sample may move in between the collection of individual pixels. This reduces the ability to scan the sample quickly and produces long frame rates on the order of seconds for a field of view comparable to a conventional full-field imaging microscope.

To increase the versatility of point-scanning systems, microscope developers use beam steering techniques to keep the sample stationary while the illumination light points to each pixel location within the sample. The fundamental concept behind most scanning techniques is the relation of tilt and translation between the back-focal (pupil) and front-focal planes of a lens.
Figure 2.11: Concept of optical scanning. A collimated beam parallel to the optical axis focuses to a spot on the optical axis at the focal length $f$ of the lens. A collimated beam tilted at an angle $\beta$ and centered on the optical axis in the pupil plane of the lens focuses to a spot $L/2$ from the optical axis at the focal length of the lens.

A collimated beam traveling along the optical axis will focus to a spot at the intersection of the optical axis and the focal plane of the lens in Figure 2.11. Applying a tilt of angle $\beta$ to the collimated beam, such that the beam is still centered on the optical axis at the pupil plane of the lens, results in a translation $L/2$ of the focused spot from the optical axis in the focal plane, where $L = 2f \tan \beta / M$ is the total distance scanned in one dimension of the field of view for a system with magnification $M$. Thus, each pixel within the field of view can be illuminated by adjusting the angle of the collimated beam in the pupil plane of the lens.

For many years, the pupil plane was always positioned at the shoulder of the objective housing where the threads meet the barrel. Unfortunately, this standard has begun to follow the standard 160 mm tube length, and many objective manufacturers are designing the pupil further into the housing, making it even less assessable. Unless an on-axis beam deviation technique is incorporated inside the objective housing, the pupil must be relayed to a conjugate plane outside of the microscope housing in order to pivot the beam in the pupil while taking advantage of the full pupil diameter. Reducing the beam diameter less than the pupil diameter reduces the effective NA of the objective.

Most point-scanning microscopes have incorporated a raster scan that samples a rectangular field of view. One technique to create a raster scan uses two single-axis pivoting (galvanometer) mirrors, where one mirror scans the horizontal direction (fast scan) and the second mirror scans the vertical direction (slow scan). However, this technique requires two relay telescopes for both mirrors to pivot exactly in a pupil plane. The optical layout of a confocal reflectance microscope that utilizes two galvanometric scanners is shown in Figure 2.12. The collimated illumination light exits the laser and passes through the polarizing beamsplitter (PBS). The first scanner reflects the beam through a deviation angle $\pm \beta_1$ to sample points along a one-dimensional scan line. Each
point within the scan line travels through the first relay telescope between the two scanners to ensure the pivot plane of the first scanner (P₁) and the pivot plane of the second scanner (P₂) are optically conjugate. The second scanner then reflects each point along the scan line through a second deviation angle ±β₂ in the orthogonal direction in order to sample points throughout a two-dimensional scan area. Each point within the scan area travels through the second relay telescope between the second scanner and the quarter-wave plate (λ/4) that ensures the pivot plane of the second scanner (P₂) and the pupil plane of the objective (P₃) are optically conjugate. This results in planes P₁, P₂, and P₃ being in optically conjugate focal planes, and ensures the beam will always be centered on the optical axis at the pupil plane of the objective and have a deviation angle dependent on the combined contributions of both scanners. Each point then fills the back pupil plane of the objective lens to use the full NA and illuminates a single point within the sample at the image plane I₁. The backscattered light collected at the front-focal plane and within the NA of the infinity-corrected objective lens returns collimated and passes back through the quarter-wave plate and relay telescope. After the backscattered light reflects from Scanner 2, the light is descanned from a two-dimensional scan area to a one-dimensional scan line. In turn, the scan line is descanned by Scanner 1 to a single stationary point. The descanned light reflects from the polarizing beamsplitter and the tube lens focuses the light through a stationary pinhole to a point detector.

Figure 2.12: Optical layout for a confocal reflectance microscope that uses two galvanometric scanners to scan the focused spot in the image plane I₁. Two relay telescopes produce two optically conjugate pupil planes P₁ and P₂, so the two one-dimensional scanners can deviate the beam within the inaccessible pupil plane of the objective.
The scanners and relay telescopes add size and weight to the overall instrument and generally result in a large, bulky device. Many additional techniques have been developed to reduce the size and increase the speed of point-scanning systems [Chapters 3 and 6, Pawley 2006; Warger and DiMarzio 2007b], but they are all limited by the fact that each pixel must remove the out-of-focus light separately.

### 2.3.2.1 Resolution in Point-Scanning Techniques

In a point-scanning microscope, the condenser lens focuses the illumination light to a diffraction limited spot according to Eqs. (2) or (5), and the objective lens images the resulting scattered or fluorescent light from the illumination spot to a diffraction limited spot at the image plane. The PSF is then the PSF of the condenser lens multiplied by the PSF of the objective lens. In an epitaxial imaging system the objective lens acts as both the condenser and imaging lenses and the normalized intensity distribution of the focused spot along the lateral and axial axes reduces to:

$$ I_{ptscan}(v) = \left[ \frac{2J_1(v_{ex})}{v_{ex}} \right]^{2p} \left[ \frac{2J_1(v_{em})}{v_{em}} \right]^2 = \left[ \frac{2J_1(v)}{v} \right]^4 $$

$$ I_{ptscan}(u) = \left[ \frac{\sin(u_{ex}/4)}{u_{ex}/4} \right]^{2p} \left[ \frac{\sin(u_{em}/4)}{u_{em}/4} \right]^2 = \left[ \frac{\sin(u/4)}{u/4} \right]^4 $$

where the subscripts $ex$ and $em$ correspond to the optical units for the excitation and emission wavelengths in a fluorescence system respectively, $p$ is the number of photons in the excitation ($p = 1$: one-photon fluorescence and $p = 2$: two-photon fluorescence), the optical units without the subscript correspond to a reflectance system where the same wavelengths are used in illumination and detection, and a delta function pinhole was assumed to be positioned in the image plane in both the fluorescence and reflectance configurations. Strictly following the Rayleigh criterion, the resolution of a point-scanning microscope would be considered the same as a conventional microscope because the first minima of Eqs. (13) and (14) are the same as the first minima of Eqs. (18) and (19). However, the plot in Figure 2.13(a) shows two point sources separated by the Rayleigh criterion in a point-scanning reflectance microscope have a much larger saddle-to-peak intensity ratio and will be much easier to distinguish than two points in a conventional full-field microscope. Figure 2.13(b) shows that by following the Rayleigh criterion of a 26.5% dip in intensity for a circular pupil, the two points will be resolved in a point-scanning reflectance system if they are separated by $v = \frac{2\pi}{\lambda} NA r = 2.77$, which results in a lateral resolution:
\[ \Delta x_{ptscan,ref} = 0.44 \frac{\lambda}{NA}, \]  

assuming uniform plane-wave illumination in the pupil of the objective and a delta function for the pinhole. Following the same procedure in the axial direction with optical units in Eq. (3) provides the axial resolution:

\[ \Delta z_{ptscan,ref} = 1.52 \frac{n \lambda}{NA^2}. \]  

It is important to emphasize that the axial resolution should not be confused with optical sectioning. The ability to optically section is dependent on the intensity across each transverse plane through the focused spot [Sheppard and Wilson 1978], and is a measure of the sample thickness that contributes to the image. As will be discussed in §2.3.2.2, a conventional microscope does not have the ability to optically section because every transverse plane contributes to the final image. To distinguish axial resolution from optical sectioning, it may be helpful to think of the axial resolution in Eq. (21) as the smallest distance two point sources can be separated and still be resolved along the axial direction, but there may be many point sources within a single optical section that are separated by the axial resolution.

The diffraction-limited lateral resolution multiplicative factor 0.44 in Eq. (20) assumes the pupil of the objective has uniform, plane-wave illumination and a pinhole size equivalent to a delta function. However, the light sources used in most point-scanning microscopes contain a Gaussian distribution and a pinhole radius \( \geq 3 \) optical units. Sheppard and Gu have derived the solution for Eq. (2) with a Gaussian pupil function, and have shown that the effective change on the PSF is small assuming the

![Figure 2.13](image)

Figure 2.13: (a) Sum of two Airy patterns (-) for two point sources (--) within a confocal reflectance microscope separated by the minimum resolvable distance in a conventional microscope. (b) Sum of two Airy patterns (-) for the same point sources (--) separated by the minimum resolvable distance (\( v = 2.77 \)) in a confocal reflectance microscope.
beam fills the pupil of the objective, and the pinhole is approximately equal to the size of the focused spot [Sheppard and Gu 1994]. Wilson and Carlini have shown that the lateral resolution within a confocal microscope that contains a pinhole radius \( \geq 3 \) optical units approaches the lateral resolution of a conventional microscope [Wilson and Carlini 1987].

Fourier optics theory states that the expression for diffraction of the light from the pupil plane to the front focal plane of a lens reduces to a Fourier transform [Goodman 2004]. We can then use this relation to quantify the change in the lateral resolution by Gaussian illumination in the pupil. A circ function, corresponding to a plane wave apodized by the diameter of the pupil, Fourier transforms to an Airy function that matches the ideal result of Eq. (13). The multiplication of the pixel size and the number of pixels corresponding to the radius of the Airy disk \( r_{\text{plane}} \) is equivalent to \( 0.61\lambda/\text{NA} \) in Eq. (15). We can then assume a Gaussian distribution in the pupil of the objective:

\[
I(\rho) = I_0 e^{-2\rho^2/(\hbar\sigma)^2},
\]  

(22)

where \( I_0 \) is the intensity of the beam along the optical axis, \( \hbar \) is the fill factor found by dividing the diameter of the beam by the diameter of the pupil, and \( \sigma \) is the radius of the collimated beam assuming the measured beam diameter corresponds to the \( 1/e^2 \) points of the Gaussian distribution. Apodizing Eq. (22) by the same circ function and taking the Fourier transform provides the Gaussian illumination function at focus with the number of pixels of the disk radius \( r_{\text{gauss}} \). The new multiplier \( m \) for the lateral resolution can be found by equating the ratios \( r_{\text{gauss}}/m = r_{\text{plane}}/0.61 \) resulting in the diffraction-limited lateral resolution:

\[
\Delta x_{\text{conf.ref}} = \frac{0.61 r_{\text{gauss}} \lambda}{r_{\text{plane}} \text{NA}},
\]  

(23)

for a point-scanning reflectance system with Gaussian illumination in the pupil and a pinhole radius \( \geq 3 \) optical units. The multiplier for the lateral resolution can then be converted to a measure of the full width at half of the maximum (FWHM) or the 90/10 points of an edge spread function by measuring the number of pixels between the FWHM of the Gaussian illumination function \( (r_{\text{FWHM}}) \) or the 90/10 points of the cumulative sum of the Gaussian illumination function \( (r_{90/10}) \) and multiplying \( 0.61 r_{\text{gauss}}/r_{\text{plane}} \) by \( r_{\text{FWHM}}/r_{\text{gauss}} \) or \( r_{90/10}/r_{\text{gauss}} \), respectively.

This shows that the lateral resolution is dependent on the fill factor of the objective pupil. When the beam overfills the pupil \( (\hbar > 1) \) the beam approaches a uniform plane wave and the resolution approaches the ideal case, but the laser power that does not pass through the pupil will be lost. When the beam underfills the objective \( (\hbar < 1) \), less of the NA will be used thereby losing focusing power of the lens and broadening the PSF (worse resolution and optical sectioning). Matching the beam diameter to the diameter of
2.3.2.2 Optical Sectioning

Optical sectioning describes the ability of a microscope to provide fine details within a small axial thickness of the sample without degradation by out-of-focus light. Another interpretation of optical sectioning is a measure of the sample thickness in the axial direction that contributes to the resultant image. Mathematically [Sheppard and Wilson 1978], optical sectioning is dependent on the sum of the intensity distribution of the focused spot over each lateral plane along the axial direction expressed by:

$$ I_{int}(u) = \int_0^\infty I(u,v) \, v \, dv. $$

(24)

The optical section thickness can be measured by plotting Eq. (24) versus the axial coordinates through the focused spot. A contour map of the intensity distribution through the focused spot of a conventional full-field microscope described by Eq. (5) is shown in Figure 2.14(a), where the dashed lines correspond to the geometric focus of the light described by ray tracing $|u| = |v|$. A plot of the normalized sum of each plane over the field of view is shown in Figure 2.14(c). Theoretically, the plot in Figure 2.14(c) should be a straight line according to conservation of energy and Parseval’s theorem, but the limit on the integral was bounded by the field of view in this example and not infinity as described in Eq. (24). In practice, the integral will be bounded by the size of the two-dimensional detector used to image the point source. The straight line result of Eq. (24) means that all planes will contribute equally to the image, and for this reason a conventional microscope does not have optical sectioning capabilities.

A contour map of the intensity distribution through the focused spot of a confocal reflectance microscope with a pinhole diameter equal to a delta function, described by the square of Eq. (5), is shown in Figure 2.14(b). A confocal fluorescence system will have a slightly broader result than the confocal reflectance system and the two-photon system without a pinhole will be broader still, because a change of wavelength is required in the optical units between the excitation and emission wavelengths for the respective imaging modalities [Chapter 5, Diaspro 2002]. The contour plot in Figure 2.14(b) shows that the light that contributes to the image is from a much smaller area compared to a conventional microscope. The dramatic drop off in the plot of the normalized sum of each plane over the field of view, shown in Figure 2.14(d), confirms that a finite thickness of the sample will contribute to the image. Measuring the FWHM of the plot provides a diffraction-limited optical-section thickness:
\[ \Delta z_{FWHM} = 1.36 \frac{n \lambda}{NA^2} \]  

(25)

for a confocal reflectance system using uniform, plane-wave illumination in the pupil of the objective and a delta function for the pinhole. Using a larger pinhole will allow more out-of-focus light to reach the detector, which broadens the PSF [Wilson and Carlini 1987].

Figure 2.14: Intensity distribution of a focused spot in (a) a conventional microscope and (b) a confocal reflectance microscope. The dashed lines correspond to the geometric focus found by ray tracing, and the bold contour line highlights the visible \(1/e^2\) cross section. Optical sectioning plot from Eq. (24) showing that (c) a conventional microscope has no optical sectioning capabilities, while the (d) confocal microscope can optically section. Contour lines are plotted for intensities: 0.9, 0.7, 0.5, 0.3, 0.2, 0.135, 0.05, 0.03, 0.02, 0.015, 0.01, 0.005, 0.002, 0.001, 0.0005, 0.0002, 0.0001, 0.00005, and 0.00002.
2.3.2.3 Confocal Reflectance and Fluorescence

Confocal microscopy [Minsky 1955, 1988] uses a pinhole in the image plane to block most of the out-of-focus light that spreads across the image plane according to Figure 2.5. Figure 2.15 shows the optical layout for a transmission confocal configuration for a single point, where the condenser lens focuses the light source to a diffraction-limited spot within the sample and a pinhole is mounted in the image plane. The light source is then optically conjugate to the focused illumination spot within the sample via the condenser lens, and the illumination spot is optically conjugate to the pinhole via the objective lens. Light from the point sources along the optical axis pass through the objective as in the case for full-field imaging, but the pinhole blocks most of the irradiance from the out-of-focus points, and allows the irradiance from the point source at the object plane to pass to a point detector. In theory, the size of the detector aperture is matched to the size of the illumination spot, thereby blocking most of the light that does not originate from the object plane.

![Figure 2.15: Optical layout for a confocal imaging system.](image)

Figure 2.15: Optical layout for a confocal imaging system. The condenser lens illuminates a focused spot within the sample with a point source of light. The objective lens focuses the light from a point at the object plane to a spot at the image plane, and focuses the out-of-focus points to planes before and after the image plane. The pinhole blocks most of the out-of-focus light that is spread across the image plane and passes the light from the object plane to a point detector.

In a confocal fluorescence system, the signal is proportional to the excitation of the same fluorophores imaged in an epi-fluorescence system, but only the fluorophores within an optical section contribute to the image. This can be seen by comparing Figures 2.3(e) and 2.3(g) where the MitoTracker dye from the third out of focus cell degrades the quality of the epi-fluorescence image but does not contribute to the confocal fluorescence image. The image in Figure 2.3(g) was acquired with a line-tunable Argon laser at 488 nm, 505 longpass dichroic, 535 nm ± 15 nm emission filter, 300 μm pinhole, and a photomultiplier tube (PMT). In a confocal reflectance system, the signal is proportional to the backscatter of the illumination light provided by native variations in the refractive indices of organelles and microstructures within the sample [Dunn et al. 1996]. The
image in Figure 2.3(h) visualizes all of the refractive index discontinuities within an optical section of the embryo that correspond to the MitoTracker seen in the confocal fluorescence image in addition to other organelles that do not bind to the dye. The image in Figure 2.3(h) was acquired with a titanium-sapphire laser in continuous-wave mode at 920 nm, 150 μm pinhole, and an avalanche photodiode (APD).

The optical sectioning capabilities of confocal microscopy would make it a prime candidate for use with transparent mouse embryos, but it has been shown that confocal imaging with illumination wavelengths of 514 nm, 532 nm, and 568 nm hindered the development of 2-cell embryos from reaching the blastocyst stage in both reflection and fluorescence configurations [Squirrell et al. 1999]. As a result, all confocal imaging has been considered toxic to embryo development, and a researcher would have to repeat the toxicity study at another wavelength before confocal imaging would be reconsidered for use within the clinic.

2.3.2.4 Two-Photon

Two-photon microscopy [Göppert 1929; Sheppard and Kompfner 1978; Denk et al. 1990] images the same fluorescence signal as epi-fluorescence and confocal fluorescence, but the illumination light uses two photons at twice the wavelength (half the frequency) to excite the fluorophore. One photon contains the energy $E = hν = hc/λ$, where $h$ is Planck’s constant, $ν$ is the frequency of the light, $c$ is the speed of light, and $λ$ is the wavelength of the photon. In epi- and confocal fluorescence, a single photon excites an electron to an energy state $hν$ in Figure 2.16(a). In two-photon, an electron is excited to the same energy state when two photons at half the frequency ($2hν/2$) or twice the wavelength are absorbed simultaneously in Figure 2.16(b).

![Jablonski energy diagrams for (a) one-photon (epi- and confocal fluorescence) and (b) two-photon fluorescence.](image)

Figure 2.16: Jablonski energy diagrams for (a) one-photon (epi- and confocal fluorescence) and (b) two-photon fluorescence.
The probability of two photons being absorbed simultaneously is proportional to the square of the light intensity [Göppert 1929, 1931, English translations pp. 42-59 Masters and So 2008]. This leads to a normalized two-photon fluorescence intensity distribution from the illumination path equivalent to the confocal reflectance intensity distribution in Figure 2.14(b) from both illumination and detection, but with optical units equivalent to the two-photon excitation wavelength. Since the fluorescent light that results from two-photon illumination originates from the focal spot alone, all of the fluorescent light can be collected without descanning to a pinhole. Descanning to a stationary pinhole can provide a slight theoretical improvement in resolution [Chapter 11, Masters and So 2008], but it is not common practice. In addition to the optical sectioning capabilities, the advantage of two-photon imaging is the use of red to near-infrared wavelengths to excite the UV and blue excitation dyes because UV and blue light is highly absorbed in tissue compared to red and infrared light.

The drawback to two-photon microscopy is the need for an expensive, pulsed laser source. The time-averaged two-photon fluorescence intensity per molecule for a continuous-wave (CW) laser has been defined:

$$\langle I_{f,cw}(t) \rangle = \delta_{2p} P_{cw}^2 \left( \frac{NA^2}{2\hbar\lambda} \right)^2,$$  \hspace{1cm} (26)

where $\delta_{2p}$ is the two-photon cross section, $\hbar$ is Planck’s constant divided by $2\pi$, and $P_{cw}$ and $\lambda$ are the average power and wavelength of the laser respectively [Chapter 3, Diaspro 2002]. If the laser is pulsed with a pulse width $\tau_p$ and repetition rate $f_p$, the time-averaged two-photon fluorescence intensity per molecule becomes:

$$\langle I_{f,p}(t) \rangle = \frac{\delta_{2p} P_{ave}^2}{\tau_p f_p} \left( \frac{NA^2}{2\hbar\lambda} \right)^2,$$  \hspace{1cm} (27)

where $P_{ave}$ is the product of the peak power out of the laser and $\tau_p f_p$. This leads to the probability $n_a$ of two-photons being absorbed simultaneously during a single pulse within the paraxial approximation [Denk et al. 1990]:

$$n_a = \frac{\delta_{2p} P_{ave}^2}{\tau_p f_p^2} \left( \frac{NA^2}{2\hbar\lambda} \right)^2.$$  \hspace{1cm} (28)

Thus, a two-photon system with a continuous-wave laser must illuminate the sample at $1/\sqrt{\tau_p f_p}$ times more power than a two-photon system using a pulsed-laser. As an example, a 3.5 W CW laser would be needed to produce the same two-photon fluorescence as a 10 mW pulsed-laser with a pulse width of 100 fsec and a repetition rate of 80 MHz.
Two-photon fluorescence imaging requires exogenous fluorescent dyes so it cannot be used within the clinic to determine embryo viability. However, Figure 2.3(f) shows that two-photon images of Hoechst stained nuclei provide much clearer images of the individual nuclei, and may provide a simpler, more accurate cell count for large cell numbers compared to epi-fluorescence imaging. In addition, a z-stack of images may be able to provide insight to the distribution of the nuclear DNA to create a more accurate model for nuclei instead of the spherical ball that is now assumed [Aiken 2004; Pogorelov 2008]. The image in Figure 2.3(f) was acquired with a titanium-sapphire laser mode-locked at 920 nm ± 10 nm and a 675 nm long-pass hot mirror for excitation, and a 505 nm long-pass dichroic and 535 nm ± 20 nm emission filter for detection with a PMT.

2.3.3 Fusion of Image Modalities

The combination of brightfield, DIC, epi-fluorescence, OQM, confocal reflectance, confocal fluorescence, and two-photon on one microscope stage is very convenient to observe the various contrasts available from each mode, but the real power of the Keck 3D Fusion Microscope is the pixel-to-pixel registration among the image modalities. Image fusion allows the contrast and measures provided by the separate images to be combined to form new measures that would not have been possible from using any of the image techniques individually.

The general procedure to create one common image space between the different image modalities is to acquire a set of images of a target (1 from each mode), choose corresponding landmarks in each image, and apply a registration transform to the landmarks. The procedure can be very monotonous and prone to human error if completed manually, so we developed a method that automatically finds the landmarks within each image of a target and outputs the registration transform for each mode [Tsai et al. 2008]. It is important to note that an automatic alignment algorithm was proposed [Sandoz et al. 2007] that makes use of a pattern of aluminum dots etched on the top of the cover slip used to image the sample. The pattern enables images of a sample from various imaging modalities to be transformed to the same coordinate system, but the spatial transformation provides only rotation and translation. To register images from different imaging modalities, the technique requires the user to change the focus of the microscope to the top of the cover slip after acquiring an image in one modality, refocusing to the same plane within the sample to acquire an image in another modality, refocusing to the top of the cover slip to acquire a second image of the pattern, and repeating for the desired number of imaging modalities. However, the aluminum pattern may not be compatible with all imaging modalities, such as OQM, since the pattern acts as a diffraction grating with coherent illumination, and the aluminum blocks at least 20% of the detected signal that may be essential for samples with low light levels. It is also
important to note that a book on image fusion has been published recently with other registration techniques [Stathaki 2008].

The technique that we have developed images a chrome-on-glass target with all desired imaging modalities of a multimodal microscope and calibrates the imaging units before imaging the sample to ensure the multimodal images acquired during the session are aligned accurately. The technique has been developed specifically for the Keck 3D Fusion Microscope, but the methodology was designed for any multimodal microscope that combines a number of imaging modalities on a common platform. It is important to note that this method registers images of stationary samples, where the spatial relationship of multimodal images can be determined through calibration of the imaging units, and provides the starting point for image registration of dynamic samples using more computationally expensive algorithms to eliminate the remaining alignment error caused by sample movement [Tsai et al. 2005].

![Image](image.jpg)

**Figure 2.17:** Brightfield image of the custom chrome-on-glass target we designed and had manufactured for the automatic registration algorithm.

Our algorithm was based on a procedure shown to register blood vessels in the retina [Stewart et al. 2003]. Accordingly, we designed and had manufactured a custom chrome-on-glass target (Applied Image, Inc., Rochester, NY) that contains 10 μm thick chrome lines intersecting at random angles in Figure 2.17. This ensures that an intersection of two lines in one image, which serves as a landmark, can be matched to the same intersection point in another image automatically by using the crossing angles as the signature for matching. The algorithm inputs an image from each modality and uses exploratory tracing to determine the width of the lines and the pixel location for the intersection points between the chrome lines [Can et al. 1999]. Each landmark was defined as the pixel location where at least three traces come together. The intersection of three traces corresponds to two chrome lines forming a T, and the intersection of four traces corresponds to two chrome lines forming a cross. Every landmark stores the pixel coordinate \((x, y)\) of the intersection and the average tangential directions and widths of
the traces in the local neighborhood of the landmark [Tsai et al. 2004]. The algorithm then iterates through the possible solutions to align the landmarks [Tsai et al. 2008], and calculates the affine transform. An affine transform, which incorporates rotation, translation, and shear, was chosen to compensate for aberrations that may be in one imaging modality but not in another and the variation in pixel sizes between the detectors. A similarity transform that only incorporates a rotation and translation would suffice for image modes that contain the same pixels sizes if the imaging system was perfected and it was known that no aberrations existed within any of the modes.

One difficulty with using a chrome-on-glass target is the acquisition of an image with confocal and two-photon fluorescence. The chrome lines block the white light to acquire an image with the DIC and OQM CCD cameras, and reflect the laser light for confocal reflectance, but the target does not provide a change in wavelength that will pass through the dichroics and filters in the fluorescence paths. To overcome this difficulty, we administered a 4 \(\mu\)L drop of Fluorescein on top of the chrome lines and placed a coverslip on top. When the slide is oriented up, such that the objective images the chrome lines through the full thickness of the glass slide, the fluorescence images show dark lines where the chrome blocks the excitation of the Fluorescein in Figure 2.18. When the slide is oriented down, such that the objective images the lines through the coverslip, the fluorescence images show bright lines because the surface tension between the slide and the coverslip produces a larger concentration of Fluorescein on the chrome in Figure 2.19. It is important to note that the dye placed on the registration target should require approximately the same excitation wavelengths as the dye that will be used within the experiment to account for chromatic misalignment of the light coming out of the laser and within the system.

Although the fluorescence images of the chrome lines provide much more contrast when the chrome lines are oriented up, away from the objective, there was a concern that the aberrations caused by imaging through the full thickness of glass would distort the registration transforms. To address this concern, transforms were created for each set of images in Figures 2.18 and 2.19 separately. Subtracting the created transforms showed that they were identical with a maximum difference of 0.002 within the rotation and shear variables and 0.9 pixels within the total translation of the affine transform. Therefore, the transforms can be created by imaging the chrome lines through the coverslip or through the full thickness of glass with sub-pixel accuracy. This result makes sense because imaging through the full thickness of glass produces aberrations in all of the images and the registration algorithm is registering individual pixels in the detector planes regardless of the image. It is important to note that this concept also applies to the use of various objective lenses with different magnifications and numerical apertures because a 10x objective was required to determine the pixel locations of the landmarks accurately. Ideally, an additional target would be created for use with higher
magnification objectives, but we are currently limited to the 10 μm line width by the manufacturing process.

![Images](image1.png)

**Figure 2.18:** Images of the registration target through the full thickness of the glass slide within the original field of view (before registration) of the (a) DIC/epi-fluorescence, (b) OQM, (c) confocal reflectance, (d) confocal fluorescence, and (e) two-photon detectors.

![Images](image2.png)

**Figure 2.19:** Images of the registration target through the coverslip within the original field of view (before registration) of the (a) DIC/epi-fluorescence, (b) OQM, (c) confocal reflectance, (d) confocal fluorescence, and (e) two-photon detectors.

We have validated our algorithm with target images from the detectors of five modalities: OQM, DIC, confocal reflectance, confocal fluorescence, and two-photon using a 10x, 0.45 NA objective. The DIC and epi-fluorescence images are acquired with the same imaging optics and CCD camera, so the transformation developed for the DIC image is also applicable for the epi-fluorescence images. To validate the robustness of the algorithm using the target, we acquired images of ten different fields of view to ensure sufficient coverage of the 1 mm x 1 mm target. Ten additional sets of images from the four OQM CCD cameras were obtained for validation of intra-modal registration. For registration of each set of images acquired for OQM, three pairwise registrations were performed to align the image of camera 0 (reference space) with the images from the three other cameras. All 30 pairs were registered successfully, with an average alignment error of 0.24 pixel ± 0.16 pixel. A registration was successful if the alignment was below 1.5 pixels. For inter-modal registration, a white light image acquired with the OQM camera 0 was used as the reference image because it has the smallest field of view of all the imaging modes. We define the success rate as the percentage of successfully registered pairs out of all the image pairs from the same two modalities. The average success rate was 95%, with an average alignment error of 0.43 pixel and a maximum error of 1 pixel. Failures only occurred for registration between two sets of images.
acquired with the two-photon and OQM detectors due to low contrast caused by a low signal-to-noise ratio (SNR) in the two-photon images. The low SNR was caused by photobleaching of the Fluorescein during the acquisition of the entire data set of ten images with each of the detectors. In practice, the problem with low SNR seldom arises because only a single data set will be required, thus imaging of the target is often completed before photobleaching can take effect.

Figure 2.20 shows images from the Molecular Probes FluoCells® prepared slide no. 3 (F24630, Invitrogen, Carlsbad, CA) that contains a mouse kidney section stained with Alexa Fluor 488 WGA, Alexa Fluor 568 phalloidin and DAPI, that were acquired with DIC, OQM, epi-fluorescence, confocal fluorescence and two-photon using a 20x, 0.75 NA objective. A confocal reflectance image was not acquired because the monolayer of cells does not provide much contrast in confocal reflectance. Figure 2.20(g) shows an overlay of OQM in blue and the absolute value of the difference between the DIC image and its mean grey scale value, which provides contrast for the edges of the sample alone. Figure 2.20(h) shows an overlay of OQM in blue, two-photon in green, and epi-fluorescence of the DAPI in red to visualize the result of registering the image modalities to the same field of view qualitatively.

Figure 2.20: Images acquired of the Molecular Probes FluoCells® prepared slide no. 3 within the original field of view (before registration) of (a) DIC, (b) OQM, (c) epi-fluorescence with UV excitation and blue emission, (d) epi-fluorescence with blue excitation and green emission, (e) confocal fluorescence, and (f) two-photon. Overlays of (g) OQM in blue and a modified DIC image that highlights the boundaries in green (described in text) and (h) OQM in blue, two-photon in green, and epi-fluorescence with UV excitation and blue emission in red to show the result of the registration qualitatively. Scale bars are 50 μm.
3. Optical Quadrature Microscopy

3.1 Introduction

Optical quadrature microscopy (OQM) was invented in 1997 under the name optical quadrature interferometry to measure the amplitude and phase of an optically transparent object without having to acquire multiple views of the object or introduce multiple light sources [Hogenboom et al. 1998; DiMarzio 1999, 2000]. The instrument was based on techniques originally developed for radio frequency Doppler radar, described in [Bringi and Chandrasekar 2001], that were later modified for laser radar [Hogenboom and DiMarzio 1998]. The next generation instrument was implemented within a microscope that was named the quadrature tomographic microscope (QTM) because it could be used to acquire tomographic phase information and potentially provide viability measurements of live mouse embryos [Glina et al. 1999; Stott et al. 2001; Townsend et al. 2003; Warner et al. 2004]. Since work had not been completed to rotate the sample [Barty et al. 2000; Charrière et al. 2006] or rotate the incident angle of the light source [Choi et al. 2007], the microscopy technique was named optical quadrature microscopy (OQM) to differentiate between the technique used to acquire a single full-field image of quantitative phase, and an instrument that creates a 3D tomographic reconstruction from the acquisition of multiple views.

3.2 Full-field Quantitative Phase Imaging

Full-field quantitative phase imaging has made a resurgence in recent years since the work in the 1950s and 1960s to produce a commercial instrument to measure the dry mass of biological samples [Ross 1967] due to the sensitivity of the measurement and a reduction in the complexity of interferometry from various technological developments. In a transmission geometry, light with a field magnitude \( E \) and phase \( \phi \) illuminates a sample that induces a change in magnitude \( A \) and phase \( \alpha \) in Figure 3.1. Assuming a non-scattering sample with a single uniform refractive index, \( (1-A) \) is proportional to the amount light absorbed within the sample, and \( \alpha \) is related to the thickness and refractive index:

\[
\alpha = \frac{2\pi}{\lambda}(n_s - n_0)h,
\]

where \( \lambda \) is the wavelength of the light, \( n_0 \) is the refractive index of the immersion medium, and \( n_s \) and \( h \) are the refractive index and thickness of the sample respectively. To provide a general idea of the sensitivity of the technique, a refractive index mismatch \( n_s - n_0 = 0.02 \) and a phase measurement accurate to 0.08 radians can provide an accurate
measurement of sample thickness down to 0.64\(\lambda\), or 405 nm using a wavelength of 633 nm.

![Figure 3.1](image.png)

**Figure 3.1:** In quantitative phase imaging light illuminates a sample with refractive index \(n_s\) immersed in a medium \(n_0\) that induces a magnitude \(A\) and phase \(\alpha\). The phase induced by the sample is dependent on the refractive index mismatch \(n_s - n_0\) and thickness of the bead \(h\).

### 3.2.1 Interference

The difficulties associated with phase imaging derive from the fact that phase cannot be measured directly. Optical detectors measure the irradiance of the incident light, resulting in a detector current proportional to the amplitude of the electric field squared:

\[
I_s \propto |E_s e^{j\varphi_s}|^2 = E_s^2, \tag{2}
\]

without any phase information. To visualize the phase, most quantitative phase imaging techniques use interferometry [Hariharan 2003]. In an interferometer, a light source is split into two paths, one path propagating through the sample (signal path) and one path traveling the same distance without a sample (reference path or local oscillator). The two paths recombine and their constructive and destructive interference produces fringes that are associated with the difference in phase between the two paths:

\[
I = |E_s e^{j\varphi_s} + E_R e^{j\varphi_R}|^2 = E_s^2 + E_R^2 + 2E_sE_R \cos(\varphi_s - \varphi_R), \tag{3}
\]

assuming the amplitudes of the signal and reference fields are real. The contrast (visibility) between the fringes can be defined by a multiplicative factor:

\[
V = \frac{l_{\text{max}} - l_{\text{min}}}{l_{\text{max}} + l_{\text{min}}} |\gamma||\cos \psi| = \frac{2E_sE_R}{E_s^2 + E_R^2} |\gamma||\cos \psi|, \tag{4}
\]
contingent on the irradiance from the two paths, degree of coherence $|\gamma|$ that includes both the spatial and temporal coherence, and polarization angle between the fields in the two paths $\psi$. In Eq. (4), $I_{\text{max}}$ is the maximum irradiance from constructive interference ($\cos(\phi_S - \phi_R) = 1$) and $I_{\text{min}}$ is the minimum irradiance from destructive interference ($\cos(\phi_S - \phi_R) = -1$). Accounting for the contrast, the irradiance of the fringe pattern becomes:

$$ I = E_S^2 + E_R^2 + 2E_SE_RV\cos(\phi_S - \phi_R), $$

such that a contrast of zero corresponds to the incoherent addition of the signal and reference paths.

The contrast of the interference between two linearly polarized beams follows a $|\cos \psi|$ dependence such that two beams with the same polarization provide the greatest contrast, while two beams with orthogonal polarization do not interfere. However, it is important to note that parts of two beams with orthogonal polarization states can interfere with the addition of a polarizer in the path. Light polarized at 0 degrees can be described by equal amounts of the two orthogonal polarization states at $\pm 45$ degrees, and light polarized at 90 degrees can be described by equal amounts of the orthogonal polarization states at $+45$ and $+135$ degrees. The combination of the two beams alone will not produce interference, but if a polarizer oriented at $+45$ degrees is positioned after the combination of the two beams, the $+45$ degree component from both beams will transmit through the polarizer and interfere. The same is true for a polarizer oriented at $+135$ or $-45$ degrees because they share the same axis. This is the underlying concept behind the interference produced in a DIC system where the birefringent Wollaston prisms separate and combine the orthogonal polarization states into the two paths that travel through the sample.

The irradiance (brightness) of the light can be interpreted physically as the number of photons per unit area. When the irradiance of both the signal and reference beams are equal ($E_S^2 = E_R^2 = I$), the fringe pattern will have an average irradiance $2I$ with an oscillation $\pm 2I$. Destructive interference will then provide a minimum irradiance of zero and constructive interference will provide a maximum irradiance of $4I$. As the difference between the irradiance of the two beams increases, the minimum irradiance from destructive interference will increase thereby decreasing the contrast.

Spatial coherence is a measure of the correlation of the light within the two-dimensional plane transverse to the direction of propagation. The concept of spatial coherence has generally been described by Young’s double slit experiment where light travels through a screen with a circular aperture of radius $\rho$, and illuminates a second screen at a distance $R$ from the first screen that contains two slits separated by a distance $d$. The beam illuminates both slits and the light that passes through the slits acts as two point sources that illuminate a third screen. Interference will be seen on the third screen where the light from the two slits overlap if the distance between the slits is within the
spatial coherence of the beam: $d < \lambda R/\rho$ [Milonni and Eberly 1988]. In general, a light source of finite size, such as a tungsten lamp, can be described by a collection of independent point sources that emit light at random phase, so the white light source is said to provide no spatial coherence. However, the Cittert-Zernike theorem [Milonni and Eberly 1988] states that the light from an incoherent light source that evenly illuminates a circular aperture of radius $\rho$ has a degree of coherence:

$$|\gamma| = \left| \frac{2J_1(x)}{x} \right|,$$

where $x = 2\pi pd/(\lambda R)$, assuming perfect temporal coherence. As an example, $|\gamma| = 0.88$ when $x = 1$, and the spatial coherence of the beam is $\geq 88\%$ when $2\pi pd/(\lambda R) \leq 1$, or the beam is spatially coherent over an area $\pi(0.08\lambda R/\rho)^2$. A laser that can emit single-mode light would have perfect spatial coherence, but in general, lasers output multiple transverse modes. The spatial coherence of a laser is still considered high, but focusing the light through a single-mode optical fiber or a pinhole will spatially filter the beam and provide spatial coherence according to Eq. (6). It is important to note that the general expression for the Cittert-Zernike theorem is the diffraction pattern of the illuminated aperture. However, we have provided the result for a circular aperture in Eq. (6) because non-circular apertures are not often used in practice.

Temporal coherence is a measure of the correlation of the light within the direction of propagation and is based on the ability to interfere when a time delay has been introduced between the signal and reference arms of the interferometer. Temporal coherence is generally described in terms of a coherence length that can be thought of physically as the largest difference in optical distance between the signal and reference arms ($\Delta l$) such that the two beams will still produce interference. The coherence time $\tau_c$ is the longest time delay that can be introduced and still produce interference, and is dependent on the frequency bandwidth (linewidth) $dv$ of the light: $\tau_c = 1/dv$ through the Wiener-Khinchin theorem [pp. 48-49, Hariharan 2003]. The product of the coherence time and the speed of light is the coherence length: $z_c = \tau_c c$. As an example, a Helium-Neon laser that outputs two longitudinal modes separated in frequency by $c/(2L)$, where $L$ is the length of the laser cavity, has a coherence time $\tau_{coh} = L/(\pi c)$ and a coherence length $L/\pi$. The difference in optical path between the signal and reference arms will then be sufficient for interference as long as $\Delta l < z_c$. However, the bandwidth of a light source is generally thought of in terms of the spectral bandwidth $d\lambda$ because the light source is typically described in terms of wavelength, and the spectral bandwidth can be measured easily on a spectrometer. The relation between frequency bandwidth and spectral bandwidth: $|dv/\nu| = |d\lambda/\lambda|$ can be derived by taking the derivative of the equation: $\nu \lambda = c$ with respect to $\nu$, which leads to the coherence length: $z_c = \lambda^2/d\lambda$, where $\lambda$ is the mean wavelength of the spectral bandwidth. Thus, a pure monochromatic light source will have infinite temporal coherence, and a light source with a broad spectral bandwidth
will have a short coherence length. This is the reason optical coherence tomography (OCT) systems use a broadband light source, since the minimum resolvable axial feature is dependent on the coherence length of the system [Bouma and Tearney 2001]. From the Wiener-Khinchin theorem [p. 48, Hariharan 2003] the difference in optical path between the signal and reference arms $\Delta l$ provides a sinc-dependence on the degree of coherence:

$$|\gamma| = \left| \text{sinc} \left( \frac{\Delta l}{c \Delta v} \right) \right|$$

(7)

where the first zero corresponds to the difference in optical path equal to the coherence length ($\Delta l = z_c$). Thus, maximum contrast will be provided when the optical path lengths are identical, and the contrast will decrease according to the sinc function in Eq. (7) when $\Delta l > 0$.

In summary, the fringe pattern within an interferometer will have the greatest contrast when the light source originates from a point source such as an optical fiber or pinhole, and the signal and reference arms contain the same linear polarization angle, irradiances, and optical path lengths. However, interference will still be visible as long as the light in the two arms of the interferometer retain spatial and temporal coherence and contain similar irradiances and polarization angles.

### 3.2.2 DC Components

Once an interference pattern can be obtained with the interferometer, the phase information from the sample must be separated from the rest of the signal. Optical detectors measure the irradiance of the light incident upon them according to Eq. (3), which results in each pixel containing the sum of the signal path irradiance $E_s^2$, the reference path irradiance $E_r^2$, and the interference term $2E_sE_r \cos(\varphi_s - \varphi_r)$. $E_s^2$ and $E_r^2$ are called the DC terms because they correspond to the original light source in each path that is constant, assuming there are no fluctuations in the light source, and they contain no phase dependence. The primary difference among the quantitative phase imaging techniques is the way they remove the DC components. A general description of some of the techniques that have been applied to microscopy is provided in §3.3.

### 3.2.3 Interpreting Phase Images

Once the phase images are reconstructed, the data must be interpreted to provide meaningful information. Phase values cycle between $-\pi$ and $\pi$, such that an image of a sample with phase differences greater than $2\pi$ will contain discontinuities in the phase at every multiple of $2\pi$. Figure 3.2 shows an example of a PolyMethylMethAcrylate
(PMMA) bead immersed in oil that provides a maximum change in phase greater than $7\pi$ radians. The original image of wrapped phase values is shown in Figure 3.2(a), and a plot through the center of the bead (--) shows the phase discontinuities at every multiple of $2\pi$ in Figure 3.2(b). The wrapped phase values must be unwrapped to provide quantitative information. A 1D signal can be unwrapped by adding or subtracting $2\pi$ at every discontinuity (-) in Figure 3.2(b), and will provide a correct answer as long as the signal has been sampled appropriately and the noise in the signal is low. Appropriate sampling, defined by Nyquist, requires two pixels of wrapped phase for the quickest $2\pi$ phase change produced by the sample. Unwrapping a 2D image is not trivial and requires a computationally intensive algorithm to determine the appropriate boundaries between the discontinuities [Ghiglia and Pritt 1998]. Figure 3.2(c) shows an image of the unwrapped phase for the PMMA bead using the $L^p$-norm algorithm, which we have found to work the best for circular objects. Unfortunately, this algorithm requires time on the order of minutes to unwrap a single 640 x 480 image of quantitative phase using a computer with a 1.8 GHz processor and 1.5 GB of RAM. Additional work is underway to speed up this process, and is discussed in §5.1.4, but the difficulties associated with phase unwrapping have kept most quantitative phase imaging research to small samples that produce phase changes on the order of $2\pi$ radians or less.

![Figure 3.2: (a) Original image of wrapped phase. (b) Plot through the center of the bead with wrapped (--) and unwrapped phase (-). (c) Unwrapped image of phase from the $L^p$-norm algorithm.](image)

Referring back to Figure 3.1, the change in phase $\alpha$ is related to a physical distance by the wavenumber $k = \frac{2\pi}{\lambda}$ and the optical path length $OPL = \int n \, ds$:

$$\alpha = \frac{2\pi}{\lambda} OPL,$$

where $n$ is the refractive index of the medium along a path $s$ and $\lambda$ is the wavelength of light. The optical path length reduces to $OPL = nh$ when the distance is a straight line.
through an object with height \( h \). Strictly speaking, the interferometer measures the phase difference \( \phi_S - \phi_R \) between the reference and signal arms in Eq. (3), which relates to the optical path difference \( OPD = OPL_S - OPL_R \):

\[
\phi_S - \phi_R = \frac{2\pi}{\lambda} OPD,
\]

where the subscripts \( S \) and \( R \) correspond to the signal and reference paths respectively. Since phase values greater than \( 2\pi \) wrap, the phase within a blank image (no sample) can have any arbitrary value between \( -\pi \) and \( \pi \), assuming \( OPL_S - OPL_R \) is less than the coherence length of the light source. A section of the phase image must then contain a zero phase point to compare the remaining phase of the image. For this reason, most phase images are converted to \( OPD \) and the image is said to provide a relative mapping of \( OPD \) between the various structures within the sample.

It is important to note that ambiguities also exist between the refractive index and thickness of the sample. From the definition of \( OPL \), an object with a certain refractive index and thickness will appear the same as another object with half of the thickness and twice the refractive index.

### 3.3 Quantitative Phase Imaging Techniques

Here we provide a brief description of some of the techniques that have been applied to microscopy to reconstruct an image of full-field quantitative phase. This list is only meant to provide a general overview of the more popular techniques and is far from a complete overview of the field.

#### 3.3.1 Jamin-Lebedeff Interferometer

Jamin devised the first polarization interferometer in 1868, which was later applied to microscopy by Lebedeff in 1930 [Lebedeff 1930]. A birefringent beamsplitter separates the monochromatic light source into the ordinary and extraordinary beams that become the signal and reference paths, respectively. The distance between the beams is significantly greater than the shear created by Wollaston prisms for DIC imaging, discussed in §2.3.1.3, and is a sizeable fraction of the field of view. Both beams pass through a half-wave plate to rotate the polarization of each beam by 90º, thereby making the ordinary beam the extraordinary beam and vice versa. The signal beam passes through the sample while the reference beam passes through a different region of the slide that supports the sample. The two paths then combine within an identical
birefringent beamsplitter and a detector acquires an image of the interference [Francon and Mallick 1971; Spencer 1982; Slayter & Slayter 1992].

### 3.3.2 Phase-Shifting Interferometry

Phase-shifting interferometry is the basis for many of the quantitative phase imaging techniques and has been around since 1966 [Carré 1966, Creath 1988]. The signal acquired from the interference of the signal and reference paths in Eq. (5) can be broken down into three unknowns: the DC irradiance \( I_0 = E_s^2 + E_r^2 \), the modulation of the interference fringes \( \xi = 2E_sE_r\nu \), and the wavefront phase \( \phi = \varphi_s - \varphi_r \):

\[
I = I_0(1 + \xi \cos \phi). \tag{10}
\]

A minimum of three images with a constant phase value added to one arm of the interferometer is then required to calculate the phase. It is important to note that the constant phase shift must be between 0 and \( \pi \) to satisfy Nyquist sampling. While there are many ways to induce extra phase, the most common technique is to use a piezo to translate a mirror and add extra OPL. Images acquired with a \( \pi/2 \) phase shift between each image, with the first image at \( \pi/4 \):

\[
\begin{align*}
I_1(x,y) &= I_0(x,y)[1 + \xi \cos \left(\phi(x,y) + \frac{\pi}{2}\right)] \\
I_2(x,y) &= I_0(x,y)[1 + \xi \cos \left(\phi(x,y) + \frac{3\pi}{4}\right)] \\
I_3(x,y) &= I_0(x,y)[1 + \xi \cos \left(\phi(x,y) + \frac{5\pi}{4}\right)]
\end{align*}
\]

(11)

can then be used to reconstruct the phase:

\[
\phi(x,y) = \tan^{-1}\left(\frac{I_3(x,y) - I_2(x,y)}{I_1(x,y) - I_2(x,y)}\right). \tag{12}
\]

Because acquiring the images sequentially is prone to error from sample movement, many techniques acquire the phase-shifted images simultaneously. The same reconstruction in Eq. (12) could be used with 3 images, but there is no efficient way to acquire the three images without wasting some of the light. As a result, many of the techniques acquire four sequential images with a \( \pi/2 \) phase shift between each image:
\[ I_1(x, y) = I_0(x, y)[1 + \xi \cos (\phi(x, y))] \]
\[ I_2(x, y) = I_0(x, y)[1 + \xi \cos \left( \phi(x, y) + \frac{\pi}{2} \right)] = I_0(x, y)[1 - \xi \sin (\phi(x, y))] \]
\[ I_3(x, y) = I_0(x, y)[1 + \xi \cos (\phi(x, y) + \pi)] = I_0(x, y)[1 - \xi \cos (\phi(x, y))] \]
\[ I_4(x, y) = I_0(x, y)[1 + \xi \cos \left( \phi(x, y) + \frac{3\pi}{2} \right)] = I_0(x, y)[1 - \xi \cos (\phi(x, y))] \] (13)

that can be used to reconstruct the phase:

\[ \phi(x, y) = \tan^{-1}\left(\frac{I_3(x, y) - I_2(x, y)}{I_1(x, y) - I_4(x, y)}\right). \] (14)

It is important to note that three images could be acquired simultaneously with a \(2\pi/3\) phase shift between each image, but it is difficult to create a system with off the shelf components that will split the light into three equal paths with a \(2\pi/3\) shift between each path.

### 3.3.3 Digital Holography

Digital holography microscopy acquires a hologram from the interference of the reference and signal beams in a pupil plane and then mathematically reconstructs the amplitude and phase in the image plane. The primary difference between many of the digital holography techniques is the method used to remove the DC components and the twin image (negative frequency terms). The most commonly used technique is the use of a tilted reference beam that displaces the DC terms, and the real and twin images spatially within the field of view of the detector array [Cuche et al. 1999, 2000]. The advantage of digital holography is the flexibility of the mathematical reconstruction. Because the image is acquired in a pupil plane, the user can compute the image for various depths from a single hologram acquisition. The user can also incorporate models for many of the noise terms to remove the aberrations in the image. Other digital holography methods include phase reconstruction from amplitude images at different distances [Maleki and Devaney 1993] and phase-shifting interferometry [Guo and Devaney 2004].

### 3.3.4 Fourier Phase Microscopy

Fourier phase microscopy combines a phase-shifting reconstruction algorithm with a common path interferometer [Popescu et al. 2004]. A low coherence source illuminates the sample and forms an image at the camera port of the microscope. A pair of lenses, with one lens positioned at the image plane, produces the Fourier transform of the image on a programmable phase modulator (PPM). The PPM keeps a region of pixels
at the center of the Fourier transform (DC terms) stationary and modulates the remaining pixels in increments of $\pi/2$, thereby providing constant DC terms for the reference path and modulating the higher frequencies by multiples of $\pi/2$. The constant DC terms and modulated frequency terms reflect from the PPM, and interfere as they propagate back through the second lens, reflect from a beamsplitter, and form an image on a detector array. The quantitative phase image is then reconstructed with a phase-shifting algorithm.

3.3.5 Hilbert Phase Microscopy

Hilbert phase microscopy uses a tilted reference beam and acquires an image in an image plane to reconstruct the quantitative phase from a single camera acquisition [Ikeda et al. 2005]. The light source is split into two arms of a Mach-Zehnder interferometer, where the light in the signal arm illuminates the sample with a plane wave and is imaged with an objective and tube lens. A beamsplitter is positioned between the objective and the tube lens to recombine the plane-wave reference path that is tilted at 45 degrees with respect to the signal path to create fringes at a 45 degree angle on the detector array with respect to the $x$ and $y$ axes. The quantitative phase image is reconstructed by applying a high-pass spatial filter and Hilbert transforming the acquired image.

3.3.6 Diffraction Phase Microscopy

Diffraction phase microscopy uses a common-path interferometer to reconstruct an image of quantitative phase from a single camera acquisition [Popescu et al. 2006]. Coherent light illuminates a sample on a microscope and produces an image at the camera port. A relay lens positioned after the camera port collimates the beam and creates an intermediate image plane, where a grating is positioned. The grating diffracts the light into the zeroth and first orders, with each order containing all of the spatial information from the image. A two-lens relay is positioned between the grating and the detector array such that the two diffracted orders recombine at the detector. A spatial filter positioned at the focal length between the two lenses passes most of the first order with a large pinhole and low-pass filters the zeroth order with a small pinhole. Thus, the zeroth order approaches a uniform plane wave and is incident at a 45 degree angle upon the detector array, while the first order illuminates the detector array at normal incidence. The quantitative phase image is then reconstructed with the Hilbert phase microscopy reconstruction.
3.3.7 Quantitative Phase Microscopy

Quantitative phase imaging (QPI) uses partially coherent light and a non-interferometric configuration to reconstruct the quantitative phase from the axial irradiance gradient [Paganin and Nugent 1998; Barty et al. 1998]. The system illuminates the sample with partially coherent light and acquires two images simultaneously, one in-focus at an image plane and one slightly defocused. An algorithm analyzes the images and reconstructs the wavefront of the light that travels through the sample.

3.3.8 Reconstruction from DIC Images

DIC images provide information related to the derivative of the sample phase along the direction of the shear, as described in §2.3.1.3. Assuming an ideal system, the integral of the DIC image along the shear axis would provide an image of quantitative phase for the sample. However, much more complicated techniques are required to retrieve accurate quantitative phase information [Preza 2000; Arnison et al. 2000, 2004; Heise 2005; Ishiwata et al. 2006; Shribak and Inoue 2006].

3.3.9 Quadrature Detection

A wave is in quadrature with another wave if they are matched in frequency and amplitude, but differ in phase by 90 degrees. Quadrature detection is a technique that has been used in Doppler radar [Bringi and Chandrasekar 2001] as well as in communications systems [Couch 1993] to measure the amplitude and phase of an unknown sinusoidal signal by mixing with two reference, or local oscillator, signals that are in quadrature in Figure 3.3. The unknown and reference signals are split into two separate but equal parts, where one part of the reference has a 90-degree phase shift with respect to the second. Two multiplying mixers combine the two parts of the unknown signal with the reference signals that are in quadrature to produce two mixed signals. Low-pass filters block the double frequency signal inherent in the mixers and any leakage of the carrier frequencies. The mixed signal with the original reference is the in-phase channel (I channel), and the mixed signal with the 90-degree phase-shifted reference is the quadrature channel (Q channel). The I and Q channels can be interpreted mathematically as the real and imaginary values of a complex number to calculate the amplitude and phase of the unknown signal.

Optical quadrature microscopy utilizes a similar detection technique where the two orthogonal horizontal and vertical polarization states of the laser beam act as the two unknown signal paths in Figure 3.3. Polarizing the reference and signal paths at 45 degrees provides equal amounts of horizontal and vertical polarization. The reference
path then transmits through a quarter-wave plate to create circular polarization, where one polarization state has a 90-degree phase shift with respect to the other. The 45-degree polarized signal and circularly polarized reference beams mix by transmission through a non-polarized recombining beamsplitter, and a polarizing beamsplitter separates the two polarization states of the mixed signal into the I and Q channels that are acquired with two CCD cameras. In practice, both outputs of the recombining beamsplitter are separated into two separate I and Q channels in a balanced detection configuration to remove the DC components that result from the additive combination of the fields in the beamsplitters and the square-law detection required for optical frequencies.

It is important to note that a similar optical quadrature detection technique with polarizing beamsplitters was reported in 1997 for the use with DVDs [Marx and Psaltis 1997]. More recently, another technique to acquire quadrature images was developed with the use of two diffraction gratings [Yaqoob et al. 2006].

![Figure 3.3: Simplified block diagram for quadrature detection in a Doppler radar receiver [Bringi and Chandrasekar 2001].](image)

### 3.4 Optical Layout for OQM

The current optical layout for OQM is shown in Figure 3.4. A linearly polarized Helium-Neon laser (31-2082-000, Coherent, Santa Clara, California) is fiber coupled with a single-mode optical fiber to a non-polarizing 50/50 beamsplitter that splits the light into the reference and signal paths of a Mach-Zehnder interferometer. A lens at the fiber output of the signal path collimates the beam that travels through a polarizer oriented at 45 degrees relative to the \( \vec{x} \) and \( \vec{y} \) basis vectors of the system. The 45-degree linearly polarized light enters the optical path of the Nikon Eclipse TE2000U microscope by reflecting from a narrow bandstop dichroic splitter centered at 633 nm with a 30-nm
bandstop defined by the FWHM. The dichroic splitter reflects the 633 nm light from the laser and transmits the white light from the halogen light source of the microscope for brightfield and differential interference contrast (DIC) microscopy [Warger et al. 2007a]. The condenser lens focuses the light that travels through a sample where a change in phase is induced. An infinity-corrected objective lens collects the light that transmits through the sample and is within the numerical aperture, and a tube lens images the sample at the camera port of the microscope. Because the recombination of the reference and signal paths occurs outside of the microscope body, a lens relays the image of the sample at the camera port to the image plane of the CCD cameras. A linear polarizer oriented at 45 degrees relative to the $\vec{x}$ and $\vec{y}$ basis vectors of the system can be positioned before the recombining beamsplitter to ensure the signal path is polarized at 45 degrees before mixing with the reference path.

**Figure 3.4:** Optical layout for OQM. The $\vec{x}$ and $\vec{y}$ basis vectors are labeled along the optical path within the individual arms of the interferometer and after recombination. The unlabeled lenses are single element lenses.

A lens at the fiber output of the reference path collimates the beam that travels through a linear polarizer oriented at 45 degrees relative to the $\vec{x}$ and $\vec{y}$ basis vectors of the system, and a quarter-wave plate oriented at 45 degrees to the axis of the polarizer to produce circularly polarized light. A lens matches the wavefront of the beam in the reference path to the wavefront of the beam in the signal path, and the 45-degree linearly polarized signal path mixes with the circularly polarized reference path at the 50/50 non-polarizing recombining beamsplitter. A balanced detection configuration has been implemented such that both outputs of the recombining beamsplitter are acquired instead of just one.
The two mixed beams that are output from the recombining beamsplitter travel through polarizing beamsplitters to separate the quadrature components, and four synchronized 8-bit CCD cameras (XC-75, Sony Electronics Inc., Park Ridge, New Jersey) acquire images of the sample from each output of the two polarizing beamsplitters with a framegrabber (Matrox Genesis LC, Dorval, Canada) that has the ability to buffer four simultaneous video channels.

3.5 OQM Phase Reconstructions

While many of the previous OQM publications have provided a brief mathematical description of the reconstruction techniques used to compute the change in phase induced by a sample, a thorough signal-to-noise ratio (SNR) analysis had not been completed. Here we provide an overview of the reconstruction techniques followed by an in-depth analysis that includes noise terms caused by laser fluctuations, imperfections within the optics, and noise within the CCD cameras. In addition, we present a model of the reconstructions using images from the experimental setup to quantify the minimum amount of noise that is inherent in the resultant phase images.

3.5.1 Ideal System

To describe the general principles behind the phase reconstructions, we provide a mathematical description of OQM that assumes perfect optical elements free from optical aberrations. Laser light travels through a single-mode fiber to a non-polarizing beamsplitter that separates the light into the reference \( (E_{\text{ref}}) \) and signal \( (E_{\text{sig}}) \) paths. The 50/50 non-polarizing beamsplitter splits the irradiance of the incident laser light, which is proportional to the intensity or the square of the electric field, thereby providing \( 1/\sqrt{2} \) of the incident field in each path. Each path passes through a linear polarizer oriented at 45 degrees to the basis set to ensure 45-degree polarized light in each path. Each path passes through a linear polarizer oriented at 45 degrees to the basis set to ensure 45-degree polarized light in each path:

\[
E_{\text{ref}} = \frac{1}{\sqrt{2}} E_r e^{j(\omega t + \phi)} (\vec{x} + \vec{y})
\]

\[
E_{\text{sig}} = \frac{1}{\sqrt{2}} E_s e^{j(\omega t + \phi)} (\vec{x} + \vec{y}),
\]

where \( E_r/\sqrt{2} \) and \( E_s/\sqrt{2} \) are the amplitudes of the fields from the laser that pass through the linear polarizers, \( \phi \) is the phase of the laser output, \( j \) is \( \sqrt{-1} \), \( \omega \) is the angular frequency, \( t \) is time, and \( \vec{x} \) and \( \vec{y} \) are the basis vectors of the system, which are projections of the field in the \( S \) and \( P \) directions with respect to the polarizing beamsplitters. The reference path travels through a quarter-wave plate with axes oriented
at 45 degrees relative to the laser’s polarization axis thereby producing circular polarization:

$$\tilde{E}_{\text{ref}} = \frac{1}{\sqrt{2}} E_r e^{j(\phi_0 + \delta)} (\tilde{x} + j\tilde{y}).$$

(17)

The signal path travels through the sample inducing a change in magnitude (A) and phase ($\alpha$):

$$\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} A E_s e^{j(\alpha + \phi_0 + \alpha)} (\tilde{x} + j\tilde{y}).$$

(18)

The two paths recombine within a second 50/50 non-polarizing beamsplitter. The sum of the input intensity from the square of Eqs. (17) and (18) must be equal to the sum of the output intensity by conservation of energy. Assuming the beamsplitter is lossless the total output is:

$$\tilde{E}_{\text{sig}}^2 + \tilde{E}_{\text{ref}}^2 = \frac{1}{2} |(\tilde{E}_{\text{sig}} + \tilde{E}_{\text{ref}})|^2 + \frac{1}{2} |(\tilde{E}_{\text{sig}} - \tilde{E}_{\text{ref}})|^2,$$

(19)

where the two terms right of the equals sign correspond to the two outputs of the beamsplitter. Polarizing beamsplitters separate the quadrature components of each output by reflecting the $\tilde{x}$ component and transmitting the $\tilde{y}$ component, where the $\tilde{x}$ and $\tilde{y}$ components are defined as the $S$ and $P$ polarization states with respect to the beamsplitter. Four CCD cameras, numbered 0 to 3 and spatially registered with an affine transform [Tsai et al. 2008], capture the interferograms from each output of the polarizing beamsplitters. The two quadrature signals corresponding to the first output of the recombinign beamsplitter in Eq. (19) are:

$$M_0 = \left| \frac{1}{\sqrt{2}} \left( \tilde{E}_{\text{sig}} + \tilde{E}_{\text{ref}} \right) \cdot \tilde{x} \right|^2$$

$$M_1 = \left| \frac{1}{\sqrt{2}} \left( \tilde{E}_{\text{sig}} + \tilde{E}_{\text{ref}} \right) \cdot \tilde{y} \right|^2$$

(20)

and the two quadrature signals corresponding to the second output of the recombinign beamsplitter in Eq. (19) are:

$$M_2 = \left| \frac{1}{\sqrt{2}} \left( \tilde{E}_{\text{sig}} - \tilde{E}_{\text{ref}} \right) \cdot \tilde{x} \right|^2$$

$$M_3 = \left| \frac{1}{\sqrt{2}} \left( \tilde{E}_{\text{sig}} - \tilde{E}_{\text{ref}} \right) \cdot \tilde{y} \right|^2$$

(21)

where $\cdot$ denotes the dot product and:
Assuming $E_S$ and $E_R$ are real, the complex conjugates of the magnitudes can be removed and Eq. (22) reduces to:

$$
\begin{align*}
M_0 &= \frac{1}{4}(A^2 E_S^* + E_R^* E_S^* + \alpha E_S^* E_R^* e^{i\alpha} + \alpha E_R^* E_S^* e^{-i\alpha}) \\
M_1 &= \frac{1}{4}(A^2 E_S^* + E_R^* E_S^* - j\alpha E_S^* E_R^* e^{i\alpha} + j\alpha E_R^* E_S^* e^{-i\alpha}) \\
M_2 &= \frac{1}{4}(A^2 E_S^* + E_R^* E_S^* - \alpha E_S^* E_R^* e^{i\alpha} - \alpha E_R^* E_S^* e^{-i\alpha}) \\
M_3 &= \frac{1}{4}(A^2 E_S^* + E_R^* E_S^* + j\alpha E_S^* E_R^* e^{i\alpha} - j\alpha E_R^* E_S^* e^{-i\alpha}) .
\end{align*}
$$

(22)

where the squared terms are the DC components from the irradiance of the individual signal and reference paths, and the third terms are the mixing or interference terms that result from the interference between the two paths.

Ideally, balanced mixing via the summation:

$$
E_{BM} = \sum_{k=0}^{3} j^k M_k = \alpha e^{i\alpha} E_S E_R ,
$$

(24)

could remove the DC terms, but in practice variations in irradiance exist along the individual paths from imperfections within the optics and detectors, as shown in §3.5.2. In Eq. (24) the subscript $k$ defines the signal captured at a specific CCD camera. The subtraction of the pure signal ($S_k$), reference ($R_k$), and dark detector voltage ($D_k$) from the individual signals in Eq. (22) removes the aberrations in each path and leads to the reconstruction:

$$
E_{BM,DC} = \sum_{k=0}^{3} j^k (M_k - R_k - S_k + D_k) = \alpha e^{i\alpha} E_S E_R ,
$$

(25)

where the addition of $D_k$ simplifies the notation for the subtraction of the dark detector current from $M_k$, $R_k$, and $S_k$. The images for $D_k$ are subtracted from each image because a value proportional to $D_k$ exists in every image acquired with a CCD camera. Images are acquired for $S_k$, $R_k$, and $D_k$ by blocking the signal and reference arms individually and simultaneously. Additional noise results from imperfections in the beamsplitters, such as a 50/50 beamsplitter not splitting the intensity of the light into equal halves. Thus, the
division by the square root of the pure reference normalizes the signals from each of the
Cameras and leads to the current reconstruction used for OQM:

\[ E_{BM,DC,\text{Norm}} = \sum_{k=0}^{3} e^{j^k} \frac{M_k - R_k - S_k + D_k}{\sqrt{R_k - D_k}} = \frac{\alpha e^{j\alpha} E_S E_R}{|E_R|}. \] (26)

Moving the sample out of the field of view and reconstructing a second image with Eq.
(26) provides the blank image:

\[ E_{BM,DC,\text{Norm,blank}} = \frac{E_SE_R}{|E_R|}. \] (27)

Dividing the result of Eq. (26) by the blank in Eq. (27) provides the complex magnitude
and phase induced by the sample:

\[ \frac{E_{BM,DC,\text{Norm}}}{E_{BM,DC,\text{Norm,blank}}} = \alpha e^{j\alpha}. \] (28)

The primary difference between the two reconstructions in Eqs. (24) and (26) is
the method used to remove the DC components. With balanced mixing in Eq. (24), the
DC components are removed mathematically by the summation of the four images. With
the current OQM reconstruction in Eq. (26), separate images are acquired for the two DC
components and subtracted individually. Thus, the four outputs from the two polarizing
beamsplitters are required for balanced mixing, but only two are required for the current
OQM reconstruction. However, all four CCD cameras are still used with the current
OQM reconstruction to increase the SNR of the final image. The noise that is inherent in
each reconstruction is shown in the following section.

3.5.2 SNR Model

The description in the previous section assumes ideal components to explain the
concepts behind the phase reconstructions, but additional noise terms exist from
temperature fluctuations in the laser and aberrations caused by imperfection in the optics
and CCD cameras. In this section we describe a more complete mathematical analysis
[Warger and DiMarzio 2009] that includes additional noise terms throughout the system
for balanced mixing in Eq. (24), balanced mixing and subtraction of the DC terms in Eq.
(25), and balanced mixing, subtraction of the DC terms, and division by the square root of the reference image (camera normalization) in Eq. (26). The complete mathematical derivation is shown in Appendix A. §3.5.2.4 shows the result of each model side-by-side and §3.5.2.5 compares the noise that is inherent in each reconstruction.

3.5.2.1 Balanced Mixing

The Helium Neon laser outputs an electric field with temperature fluctuations that produce an additive complex noise term. The signal and reference beams are polarized at 45 degrees relative to the $\hat{x}$ and $\hat{y}$ basis vectors of the system, and the reference path is circularly polarized by the quarter-wave plate oriented at 45 degrees to the laser polarization:

$$\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (E_S e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) \times (\tilde{x} + \tilde{y}) \tag{29}$$

$$\tilde{E}_{\text{ref}} = \frac{1}{\sqrt{2}} (E_R e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) \times (\tilde{x} + j\tilde{y}) \tag{30}$$

where $E_N$ and $\zeta$ are the amplitude and phase of the laser fluctuations that pass through the linear polarizers, respectively, and $\times$ denotes a multiplication. The signal path transmits through the sample that induces a change in magnitude ($A$) and phase ($\alpha$):

$$\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (AE_S e^{j(\omega t + \phi + \alpha)} + AE_N e^{j(\omega t + \zeta + \alpha)}) \times (\tilde{x} + \tilde{y}) \quad \tag{31}$$

Imperfections within the optics aberrate the field in each path before reaching the recombining beamsplitter:

$$\tilde{E}_{\text{ref}} = \frac{1}{\sqrt{2}} (E_R e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) X_r e^{j\chi_r} E^R_{C_k} \times (\tilde{x} + j\tilde{y}) \tag{32}$$

$$\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (AE_S e^{j(\omega t + \phi + \alpha)} + AE_N e^{j(\omega t + \zeta + \alpha)}) X_s e^{j\chi_s} E^S_{C_k} \times (\tilde{x} + \tilde{y}) \tag{33}$$

where $X_r$ and $X_s$ are the amplitudes and $\chi_r$ and $\chi_s$ are the phases of the aberrations in the reference and signal paths, respectively. The two paths mix at the recombining beamsplitter and each output travels through a polarizing beamsplitter that separates the quadrature components. Imperfections within the beamsplitters and coherent noise in the CCD cameras provide fixed pattern noise in each path with a magnitude $E^R_{C_k}$ and $E^S_{C_k}$ in

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Eqs. (32) and (33), where the superscript $R$ and $S$ designate the reference and signal paths, respectively. Four CCD cameras convert a general irradiance $I_k$ into a current:

$$i_k = \frac{\eta_k q}{\hbar \nu} A_{\text{pixel}} I_k + i_{D,k},$$  \hspace{1cm} (34)

where $\frac{\eta_k q}{\hbar \nu}$ is the responsivity of the camera (A/W), $\eta_k$ is the quantum efficiency of the silicon chip (electrons/photon), $q$ is the charge of an electron (1.6 x 10^{-19} c), $\hbar$ is Planck's constant (6.6 x 10^{-34} Wsec^2), $\nu$ is the frequency of the laser (sec^{-1}), $A_{\text{pixel}}$ is the area of a pixel (m^2), $I_k$ is the irradiance of the beam (Wm^{-2}), and $i_{D,k}$ is the dark current noise. It is important to note that the dark current noise fluctuates randomly from image to image so we have included a different dark current noise term for every image acquired during the reconstruction. The images captured for the mix of the signal and reference paths ($M_k$) and containing all of the various noise terms can then be expressed:

$$M_k = \frac{1}{4} \frac{\eta_k q}{\hbar \nu} A_{\text{pixel}} \left[ (A^2 E_S E_R + A^2 E_S E_N e^{i(\phi_{-})} - E_S^E C_{i1} X_i) + (-j)^k (AE_S E_R e^{i\alpha} + AE_S E_N e^{i(\phi_{-} - \alpha)}) E_S^E C_{i1} X_i e^{i(2\chi - \zeta)} + (A^2 E_N E_N e^{i(\phi_{-})} + A^2 E_N E_N e^{i(\phi_{-} - \alpha)}) E_N^E C_{i1} X_i + (-j)^k (AE_N E_R e^{i(\phi_{-} - \alpha)} + AE_N E_N e^{i\alpha}) E_N^E C_{i1} X_i e^{i(2\chi - \zeta)} + j^k (AE_N E_S e^{-i\alpha} + AE_N E_N e^{i(\phi_{-} - \alpha)}) E_N^R E_R^E C_{i1} X_i X_j e^{i(2\chi - \zeta)} + (E_R E_R + E_N^R e^{i(\phi_{-})} + E_N e^{i(\phi_{-})} + E_N e^{i(\phi_{-})} + E_N e^{i(\phi_{-})} + E_N) E_N^R E_R^E C_{i1} X_i e^{i(2\chi - \zeta)} \right] + i_{M,k} \hspace{1cm} (35)$$

where $i_{M,k}$ is the dark current in the mix images and we have assumed the magnitude of each term is real. Balanced mixing of Eq. (35) via the summation in Eq. (24) provides the reconstructed current of the mixed signal:

$$E_{BM} = \frac{1}{4} \frac{A_{\text{pixel}} q}{\hbar \nu} \left[ A e^{i\alpha} (E_S E_R + E_S E_N e^{i(\phi_{-})} + E_N e^{i(\phi_{-})} + E_N e^{i(\phi_{-})} + E_N) X_i X_j \sum_{k=0}^{3} \eta_k E_S^E C_{i1} + A e^{-i\alpha} (E_R E_S + E_R e^{i(\phi_{-})} + E_N e^{i(\phi_{-})} + E_N e^{i(\phi_{-})} + E_N) X_i X_j \sum_{k=0}^{3} (-1)^k \eta_k E_N^E C_{i1} + A^2 (E_S^2 + E_S E_N (e^{i(\phi_{-})} + e^{-i(\phi_{-})}) + E_N^2) X_i X_j \sum_{k=0}^{3} j^k \eta_k (E_S^E)^2 + (E_R^2 + E_R E_N (e^{i(\phi_{-})} + e^{-i(\phi_{-})}) + E_N^2) X_i X_j \sum_{k=0}^{3} j^k \eta_k (E_N^E)^2 \right] + \sum_{k=0}^{3} j^k i_{M,k} \hspace{1cm} (36)$$

The reconstructed current in Eq. (36) reduces to:

$$E_{BM} = \frac{A_{\text{pixel}} q}{\hbar \nu} A e^{i\alpha} E_S E_R \hspace{1cm} (37)$$
which is proportional to the result in Eq. (24), assuming the detectors are ideal:

\[ i_{M, k} = 0 \]  \hspace{1cm} (38)

\[ \eta_k = 1 , \]  \hspace{1cm} (39)

there is no fixed pattern noise within the beamsplitters and CCD cameras:

\[ E_{C_k}^R = E_{C_k}^S = 1 , \]  \hspace{1cm} (40)

there are no aberrations in the system:

\[ X_s e^{i\chi_s} = X_r e^{i\chi_r} = 1 , \]  \hspace{1cm} (41)

and there are no noise fluctuations in the laser:

\[ E_N e^{i(\alpha_s + \zeta)} = 0 . \]  \hspace{1cm} (42)

Substituting a summation to remove redundant terms, and separating the signal (S) and noise (\(N_S\)) terms provides:

\[
S = \frac{1}{4} \frac{\lambda_{\text{eff}}}{\hbar v} \left[ A e^{i\alpha} e^{(\phi - \zeta)} + E_N e^{i(\phi - \zeta)} + E_N E_N e^{i(\phi - \zeta)} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R + \right.
\]
\[
\left. A^2 e^{i\alpha} e^{(\phi - \zeta)} + E_N e^{i(\phi - \zeta)} + E_N E_N e^{i(\phi - \zeta)} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R + \right.
\]
\[
\left. \sum_{k=0}^{3} \sum_{l=0}^{3} \eta_{kl} E_{C_k}^S E_{C_l}^R \right] + \sum_{k=0}^{3} \sum_{l=0}^{3} \sum_{m=0}^{3} \eta_{klm} E_{C_k}^S E_{C_l}^R \right] + \sum_{k=0}^{3} \sum_{l=0}^{3} \sum_{m=0}^{3} \eta_{klm} E_{C_k}^S E_{C_l}^R \right]
\]
\[
N_S = \frac{1}{4} \frac{\lambda_{\text{eff}}}{\hbar v} \left[ A e^{i\alpha} e^{(\phi - \zeta)} + E_N e^{i(\phi - \zeta)} + E_N E_N e^{i(\phi - \zeta)} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R + \right.
\]
\[
\left. A e^{i\alpha} e^{(\phi - \zeta)} + E_N e^{i(\phi - \zeta)} + E_N E_N e^{i(\phi - \zeta)} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R + \right.
\]
\[
\left. A^2 e^{i\alpha} e^{(\phi - \zeta)} + E_N e^{i(\phi - \zeta)} + E_N E_N e^{i(\phi - \zeta)} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R + \right.
\]
\[
\left. \sum_{k=0}^{3} \sum_{l=0}^{3} \sum_{m=0}^{3} \eta_{klm} E_{C_k}^S E_{C_l}^R \right] , \]  \hspace{1cm} (43)

where the total image is the sum of Eqs. (43) and (44).
Following the same procedure with laser noise $E_N e^{j(\alpha + \phi)}$ and dark current in the CCD cameras $i_{BM,k}$, the reconstruction in Eq. (24) for the blank image with the sample moved out of the field of view provides the signal $(B)$ and noise $(N_B)$ terms:

$$B = \frac{A_{max}}{N_D} \left[ E_S E_B X_k X_x e^{j(x_s - x_r)} \sum_{k=0}^{3} j^k \eta_i E_i^S E_i^R + E_B E_S X_k X_x e^{-j(x_s - x_r)} \sum_{k=0}^{3} (-1)^k \eta_i E_i^S E_i^R + \right. \left. E_S E_B X_k X_x, \sum_{k=0}^{3} j^k \eta_i E_i^S E_i^R + E_B E_S X_k X_x, \sum_{k=0}^{3} j^k \eta_i E_i^S E_i^R + \sum_{k=0}^{3} j^k i_{BM,k} \right]$$

$$N_B = \frac{A_{max}}{N_D} \left[ (E_S E_B e^{j(\phi - \beta)} - E_B E_s e^{-j(\phi - \beta)} + E_B E_B) X_k X_x e^{j(x_s - x_r)} \sum_{k=0}^{3} \eta_i E_i^S E_i^R + \right. \left. (E_S E_B e^{j(\phi - \beta)} + E_B E_s e^{-j(\phi - \beta)} + E_B E_B) X_k X_x e^{-j(x_s - x_r)} \sum_{k=0}^{3} \eta_i E_i^S E_i^R + \right. \left. (E_S E_B e^{j(\phi - \beta)} + E_B E_s e^{-j(\phi - \beta)} + E_B E_B) X_k X_x, \sum_{k=0}^{3} \eta_i E_i^S E_i^R + \right. \left. (E_S E_B e^{j(\phi - \beta)} + E_B E_s e^{-j(\phi - \beta)} + E_B E_B) X_k X_x, \sum_{k=0}^{3} \eta_i E_i^S E_i^R + \right. \left. \sum_{k=0}^{3} \eta_i E_i^S E_i^R \right].$$

Dividing the sum of the signal and noise terms of the sample image by the sum of the signal and noise terms of the blank image, and using a Taylor series expansion for the denominator provides:

$$\frac{S + N_S}{B + N_B} \approx \frac{S}{B} + \frac{N_S}{B} - \frac{N_B S}{B^2} \approx \frac{S}{B}$$

assuming any noise term squared or divided by a signal term is approximately equal to zero. Substituting Eqs. (43)-(46) into Eq. (47) and using Taylor series expansions to remove the denominators provides the reconstructed magnitude and phase induced by the sample:

$$E_{BM} = \Lambda e^{j \alpha} \left[ 1 - \Phi e^{-j2(x_s - x_r)} - (\Gamma_S + \Gamma_R) e^{-j(x_s - x_r) - I_{BM} e^{-j(x_s - x_r)}} \right] + \Lambda e^{-j \alpha} \left[ \Phi e^{-j2(x_s - x_r)} - \Phi(\Gamma_S + \Gamma_R) e^{-j3(x_s - x_r)} - \Phi^2 e^{-j4(x_s - x_r)} \right] + (A^2 \Gamma_S + \Gamma_R) \left[ e^{-j(x_s - x_r)} - (\Gamma_S + \Gamma_R) e^{-j2(x_s - x_r)} - \Phi e^{-j3(x_s - x_r)} \right] + I_{BM} e^{-j(x_s - x_r)}$$

where:
\[
\Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{BS_k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \tag{49}
\]

\[
\Gamma_R = \frac{\sum_{k=0}^{3} j^k R_k}{\sum_{k=0}^{3} \sqrt{BS_k} \sqrt{R_k}} \tag{50}
\]

\[
\Gamma_S = \frac{\sum_{k=0}^{3} j^k BS_k}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \tag{51}
\]

\[
I_{S,M} = \frac{\sum_{k=0}^{3} j^k i_{M,k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \tag{52}
\]

\[
I_{B,M} = \frac{\sum_{k=0}^{3} j^k i_{BM,k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \tag{53}
\]

assuming we can ignore the dark current terms to approximate the image of the pure signal path with the sample moved out of the field of view and the image of the pure reference path by:

\[
BS_k \approx \frac{A_{\text{phot}}}{h_0} \eta_k |E_{C_i}^S X_r E_S|^2 \tag{54}
\]

\[
R_k \approx \frac{A_{\text{phot}}}{h_0} \eta_k |E_{C_i}^R X_r E_R|^2 . \tag{55}
\]

Following the same procedure, a phase image can also be reconstructed without dividing by the blank image, resulting in the reconstructed magnitude and phase:

\[
E_{BM,NB} = \left[ A e^{i\alpha} + A e^{-i\alpha} \Phi e^{-i(2x_r-x_c)} + (\Gamma_S + \Gamma_R) e^{-i(x_r-x_c)} \right] e^{i(x_r-x_c)} \sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k} + I_M \tag{56}
\]

where:
\[ I_M = \sum_{k=0}^{3} f^k i_{M,k}. \]  

(57)

However, it is important to emphasize that the resultant phase of Eq. (56) includes the wavefront mismatch between the reference and signal paths in addition to the phase induced by the sample, as will be shown in the reconstructed images in §3.5.2.5. This reconstruction is then only useful when a constant wavefront mismatch exists across the field of view.

The expressions in Eqs. (48) and (56) for the images reconstructed with balanced mixing remove many of the noise terms in Eq. (35), but noise terms still exist from aberrations and fixed pattern noise in the individual arms of the interferometer (\( \Gamma_S \) and \( \Gamma_R \)). In the following subsection, we subtract images of the individual DC terms to reduce the noise within the resultant image.

### 3.5.2.2 Balanced Mixing and DC Term Subtraction

In addition to the mixed signals in Eq. (30), images can be acquired of the pure signal by blocking the reference path:

\[
S_k = \frac{i}{4} \eta_i \frac{A}{A_{\text{pixel}}} \left( A^2 E_S^2 + A^2 E_S E_N (e^{j(\phi - \zeta)} + e^{-j(\phi - \zeta)}) + A^2 E_N^2 \right) (E_{C_S}^S X_s)^2 + i_{S,k},
\]  

(58)

the pure reference by blocking the signal path:

\[
R_k = \frac{i}{4} \eta_i \frac{A}{A_{\text{pixel}}} \left( E_R^2 + E_R E_N (e^{j(\phi - \zeta)} + e^{-j(\phi - \zeta)}) + E_N^2 \right) (E_{C_R}^R X_r)^2 + i_{R,k},
\]  

(59)

and the dark detector current \( i_{D,k} \) by blocking both paths, where \( i_{S,k} \) and \( i_{R,k} \) are the dark current terms for the images of the signal and reference images, respectively. Subtracting \( i_{D,k} \) from Eqs. (35), (58), and (59) and substituting into the reconstruction in Eq. (25) provides:

\[
E_{BM,DC} = \frac{i}{4} \frac{A_{\text{pixel}}}{A} \left[ Ae^{i\alpha} (E_R E_S + E_S E_N e^{j(\phi - \zeta)} + E_N E_R e^{-j(\phi - \zeta)} + E_N^2 X_s X_e e^{j(\chi - \chi_e)} \sum_{k=0}^{3} \eta_k E_{C_S}^S E_{C_R}^R \right] + \n
\]

\[
Ae^{-i\alpha} (E_R E_S + E_R E_N e^{j(\phi - \zeta)} + E_N E_S e^{-j(\phi - \zeta)} + E_N^2 X_s X_e e^{-j(\chi - \chi_e)} \sum_{k=0}^{3} (-1)^k \eta_k E_{C_S}^R E_{C_S}^S \] + \n
\[
\sum_{k=0}^{3} f^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}),
\]  

(60)
which can be separated into the signal \((S)\) and noise \((N_S)\) terms:

\[
S = \frac{1}{4} \frac{A_{\text{pole}}}{h v} \left[ A e^{j\alpha} E S X S X S e^{j(x - X S)} \sum_{k=0}^{3} \eta_k E^S C_k E^R C_k + \right. \\
\left. A e^{-j\alpha} E R S X S X S e^{-j(x - X S)} \sum_{k=0}^{3} (-1)^k \eta_k E^R C_k E^S C_k \right] + \sum_{k=0}^{3} j^k \left( i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k} \right) \tag{61}
\]

\[
N_S = \frac{1}{4} \frac{A_{\text{pole}}}{h v} \left[ A e^{j\alpha} (E S N e^{j(\phi - \zeta)} + E N R e^{-j(\phi - \zeta)} + E^2 N) X S X S e^{j(x - X S)} \sum_{k=0}^{3} \eta_k E^S C_k E^R C_k + \right. \\
\left. A e^{-j\alpha} (E R N e^{j(\phi - \zeta)} + E N S e^{-j(\phi - \zeta)} + E^2 N) X S X S e^{-j(x - X S)} \sum_{k=0}^{3} (-1)^k \eta_k E^R C_k E^S C_k \right] \tag{62}
\]

The reconstructed blank image with the sample moved out of the field of view can also be separated into the signal \((B)\) and noise \((N_B)\) terms:

\[
B = \frac{1}{4} \frac{A_{\text{pole}}}{h v} \left[ E S B X S X S e^{j(x - X S)} \sum_{k=0}^{3} \eta_k E^S C_k E^B C_k + E R B X S X S e^{-j(x - X S)} \sum_{k=0}^{3} (-1)^k \eta_k E^B C_k E^S C_k \right] + \\
\sum_{k=0}^{3} j^k \left( i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k} \right) \tag{63}
\]

\[
N_B = \frac{1}{4} \frac{A_{\text{pole}}}{h v} \left[ (E S B e^{j(\phi - \beta)} + E R B e^{-j(\phi - \beta)} + E^2 B) X S X S e^{j(x - X S)} \sum_{k=0}^{3} \eta_k E^S C_k E^R C_k + \right. \\
\left. (E R B e^{j(\phi - \beta)} + E B S e^{-j(\phi - \beta)} + E^2 B) X S X S e^{-j(x - X S)} \sum_{k=0}^{3} (-1)^k \eta_k E^B C_k E^S C_k \right] \tag{64}
\]

where \(i_{BM,k}\) and \(i_{BS,k}\) are the dark current terms for the signal and reference images with the sample moved out of the field of view, respectively. Following the same procedure described in the previous subsection provides the reconstructed magnitude and phase induced by the sample:

\[
E_{BM,DC} = A e^{j\alpha} \left[ 1 - \Phi e^{-j2(x - X S)} - I_S e^{-j2(x - X S)} \right] + \\
A e^{-j\alpha} \left[ \Phi e^{-j2(x - X S)} - \Phi^2 e^{-j4(x - X S)} \right] + I_S e^{-j2(x - X S)} \tag{65}
\]

where:

60
\[
\Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{BS_k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}}
\]

(66)

\[
I_S = \frac{\sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}}
\]

(67)

\[
I_B = \frac{\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}}
\]

(68)

A phase image can also be reconstructed without dividing by the blank image resulting in the reconstructed magnitude and phase:

\[
E_{BM,DC,NB} = \left[ Ae^{j\alpha} + A e^{-j\alpha} \Phi e^{-j2(\varphi - \chi)} \right] e^{i(\varphi - \chi)} \sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k} + I_{BM}
\]

(69)

where:

\[
I_{BM} = \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}).
\]

(70)

The expressions in Eqs. (65) and (69) for the images reconstructed with balanced mixing and DC term subtraction removes the fixed pattern noise from the individual arms of the interferometer, and the primary source of noise now derives from the fixed pattern noise in the combination of the signal and reference paths (\(\Phi\)). If the beamsplitters and cameras had been perfect and the same irradiance was incident on each camera, \(\Phi\) would go to zero and the reconstruction would be detector noise limited. However, this is not possible with the commercial optics currently available, so we normalize the camera signals by dividing by the square root of the reference images in the following subsection.
3.5.2.3 Balanced Mixing, DC Term Subtraction, and Normalization

The square root of the difference of the pure reference path in Eq. (59) and the dark current image can be approximated:

\[
\sqrt{R_k - I_{D,k}} \approx \frac{1}{2} \sqrt{\eta_\text{nl} E_{\text{pixel}}} A_{\text{pix} C_x} E_R^R X_r |E_R + E_N|,
\]

(71)

assuming the difference of two dark current terms is much less than the signal terms of the reference image. Substituting Eq. (71) and the subtraction of \(i_{D,k}\) from Eqs. (35), (58), and (59) into the reconstruction in Eq. (26) provides:

\[
E_{\text{BM, Norm}} = \frac{1}{4|E_x + E_s|} \left[ A e^{j\alpha} (E_x E_R + E_x E_N e^{j(\phi - \zeta)} + E_N E_R e^{-j(\phi - \zeta)} + E_N^2) X_x X_R e^{j(x_x - x_r)} \sum_{k=0}^{3} \sqrt{\eta_\text{nl} E^R_{C_k}} E^R_{C_k} X_r +
\right.

\[
A e^{-j\alpha} (E_x E_R + E_x E_N e^{j(\phi - \zeta)} + E_N E_R e^{-j(\phi - \zeta)} + E_N^2) X_x X_R e^{j(x_x - x_r)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_\text{nl} E^R_{C_k}} E^R_{C_k} X_r
\]

\[
\left. + \sum_{k=0}^{3} j^k \frac{i_{M,k} - i_{g,k} - i_{s,k} + i_{D,k}}{2} E_{\text{pixel}} E_R E^R_{C_k} X_r + \frac{E_N}{E_R} E^R_{C_k} X_r \right],
\]

(72)

which can be separated into the signal (S) and noise (NS) terms:

\[
S = \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{\eta_\text{nl}}} \frac{1}{|E_x + E_s|} \left[ A e^{j\alpha} E_x E_R X_s X_x e^{j(x_x - x_r)} \sum_{k=0}^{3} \sqrt{\eta_\text{nl} E^R_{C_k}} E^R_{C_k} X_r +
\right.

\[
A e^{-j\alpha} E_x E_R X_s X_x e^{j(x_x - x_r)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_\text{nl} E^R_{C_k}} E^R_{C_k} X_r + \sum_{k=0}^{3} j^k \frac{i_{M,k} - i_{g,k} - i_{s,k} + i_{D,k}}{\sqrt{\eta_\text{nl} E^R_{C_k}} X_r |E_x + E_s|} \right]
\]

(73)

\[
N_S = \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{\eta_\text{nl}}} \frac{1}{|E_x + E_s|} \left[ A e^{j\alpha} (E_x E_N e^{j(\phi - \zeta)} + E_R e^{-j(\phi - \zeta)} + E_N^2) X_x X_R e^{j(x_x - x_r)} \sum_{k=0}^{3} \sqrt{\eta_\text{nl} E^R_{C_k}} E^R_{C_k} X_r +
\right.

\[
A e^{-j\alpha} (E_x E_N e^{j(\phi - \zeta)} + E_R e^{-j(\phi - \zeta)} + E_N^2) X_x X_R e^{j(x_x - x_r)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_\text{nl} E^R_{C_k}} E^R_{C_k} X_r
\]

(74)

The blank image with the sample moved out of the field of view can also be separated into the signal (B) and noise (NB) terms:
Following the same procedure described in the previous subsection provides the reconstructed magnitude and phase induced by the sample:

\[
E_{BM, DC, Norm} = A e^{j\alpha} \left[ 1 - \Psi e^{-j2(\chi_s - \chi_r)} - I_{B, Norm} e^{-j(\chi_s - \chi_r)} \right] +
\]

\[
A e^{-j\alpha} \left[ \Psi e^{-j2(\chi_s - \chi_r)} - \Psi^2 e^{-j4(\chi_s - \chi_r)} \right] + I_{S, Norm} e^{-j(\chi_s - \chi_r)}
\]

where:

\[
\Psi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{BS_k}}{\sum_{k=0}^{3} \sqrt{BS_k}}
\]

\[
I_{S, Norm} = \frac{\sum_{k=0}^{3} j^k \frac{i_{BM,k} e^{-i_{BS,k} - i_{BS,k} + i_{D,k}}}{\sqrt{R_k}}}{\sum_{k=0}^{3} \sqrt{BS_k}}
\]

\[
I_{B, Norm} = \frac{\sum_{k=0}^{3} j^k \frac{i_{BM,k} e^{-i_{BS,k} - i_{BS,k} + i_{D,k}}}{\sqrt{R_k}}}{\sum_{k=0}^{3} \sqrt{BS_k}}
\]

A phase image can also be reconstructed without dividing by the blank image resulting in the reconstructed magnitude and phase:
\[ E_{BM,DC,Norm,NB} = \left[ Ae^{i\alpha} + Ae^{-i\alpha}\Psi e^{-i2(x,-x_r)} \right] e^{i(x,-x_r)} \sum_{k=0}^{3} \sqrt{BS_k} + I_{BM,Norm} \]  

(81)

where:

\[ I_{BM,Norm} = \sum_{k=0}^{3} i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k} \frac{1}{\sqrt{R_k}}. \]  

(82)

The expressions in Eqs. (77) and (81) for the images reconstructed with balanced mixing, DC term subtraction, and camera normalization removes the noise from the individual arms of the interferometer and reduces the noise from the fixed pattern noise in the reference path. The sources of noise in these images are dependent on the fixed pattern noise in the signal path (\(\Psi\)) and the dark current noise in the detectors. The following section describes the images that were created for the noise terms in the SNR analysis to create a model for the resultant images that are acquired with the microscope.

3.5.2.4 Modeling of the Phase Reconstructions

Images from a data collection of a PolyMethylMethAcrylate (PMMA) bead immersed in oil were used to model the variables within the resultant expressions for each phase reconstruction described in Section 5. The difference of the phase aberration terms \(\chi - \chi_r\) corresponds to the wavefront mismatch between the reference and signal paths when the sample is not within the field of view. Using the same approximations described in §3.5.2.3, the reconstruction in Eq. (26) for the image of the blank provides the expression:

\[ E_{BM,DC,Norm,blank} = \left[ 1 + \Psi e^{-i2(x,-x_r)} \right] e^{i(x,-x_r)} \sum_{k=0}^{3} \sqrt{BS_k} + I_{BM,Norm}. \]  

(83)

Assuming the unwrapped phase of an experimental reconstruction using Eq. (26) with the bead moved out of the field of view is approximately equal to the wavefront mismatch between the reference and signal paths, the difference of the phase terms can be modeled by fitting the Zernike polynomials for bias, tilt, and focus [pp. 523-525, Born and Wolf 1999] in Figure 3.5.
Figure 3.5: (a) Experimental image of the wavefront mismatch between reference and signal paths. (b) Model of wavefront mismatch created from a Zernike polynomial fit for bias, tilt, and focus.

Figure 3.6: Images for the noise terms that are associated with the phase reconstructions. The real (Re) and imaginary (Im) parts of the complex noise terms are shown in separate images. All of the dark current noise terms have similar distributions to those shown in (g) and (h).
The images acquired with the reference path blocked and the sample moved out of the field of view provided the images for $B_{S,k}$ in Eq. (54), and the images acquired with the signal path blocked provided the images for $R_k$ in Eq. (55). Seven sets of images with the same field of view and containing random numbers between 0 and 2 were created for each camera to model the dark current noise terms $i_{M,k}$, $i_{BM,k}$, $i_{S,k}$, $i_{BS,k}$, $i_{R,k}$, $i_{BR,k}$, and $i_{D,k}$. The images for $B_{S,k}$, $R_k$, and the dark current noise were then substituted into Eqs. (49)-(52), (57), (66)-(68), (70), (78)-(80), and (82) to create the images shown in Figure 3.6 that model $\Psi$, $\Phi$, $\Gamma_S$, $\Gamma_R$, $I_{S,M}$, $I_{M,S}$, $I_M$, $I_S$, $I_B$, $I_{BM}$, $I_{S,Norm}$, $I_{B,Norm}$, and $I_{BM,Norm}$.

![Images of phase through unwrapped bead](image)

**Figure 3.7:** Comparison of the SNR model to experimental images of wrapped phase from balanced mixing in Eq. (24), balanced mixing and DC term subtraction in Eq. (25), and balanced mixing, DC term subtraction, and camera normalization in Eq. (26) when the sample images were divided by a blank image created from the same reconstruction. The plot for each phase reconstruction shows the unwrapped phase values through the center of both the model and experimental beads.
An image of an ideal PMMA bead immersed in oil was created to validate the model. A brightfield image of the PMMA bead was used to determine an accurate measure of the bead diameter. The maximum ideal phase of the bead was calculated to be -23.0 radians using Eq. (1), where the maximum thickness of the bead \(h\), the refractive index of the bead \(n_s\), and the refractive index of the immersion oil \(n_0\) were set to 99.12 \(\mu m\), 1.489, and 1.5124, respectively. An image of the ideal phase for the bead \(\alpha\) was created with the bead centered on the same pixel in the field of view as the experimental image. Figure 3.7(a), 3.7(d), and 3.7(g) show the resultant wrapped phase from the reconstructions in Eqs. (48), (65), and (77), and Figure 3.7(b), 3.7(e), and 3.7(h) show the wrapped experimental results from the reconstructions in Eqs. (24), (25), and (26) that were also divided by a blank image created from the same reconstructions with the sample moved out of the field of view, respectively. Figure 3.7(c), 3.7(f), and 3.7(i) show plots of the unwrapped phase values through the center of both the model and experimental phase reconstructions to show the accuracy of the phase measurement of a 99 \(\mu m\) PMMA bead immersed in oil, and the correlation of the noise inherent in each phase reconstruction. It is important to note that some of the fluctuations that exist in the experimental phase image and do not exist in the model could be imperfections in the bead, irregularities in the oil, or a change in wavefront mismatch between the image of the sample and the image of the blank.

To visualize the effect of dividing the sample image by the blank image, we also created images for the reconstructions in Eqs. (56), (69), and (81) that do not divide the image of the sample by the blank image. Figure 3.8(a), 3.8(d), and 3.8(g) show the resultant wrapped phase from reconstructions in Eqs. (56), (69), and (81), and Figure 3.8(b), 3.8(e), and 3.8(h) show the wrapped experimental results from the reconstructions in Eqs. (24), (25), and (26), respectively. Figure 3.8(c), 3.8(f), and 3.8(i) show plots of the unwrapped phase values through the center of both the model and experimental phase reconstructions to show the correlation of the noise inherent in each phase reconstruction. The wavefront mismatch is clearly visible in these images and must be accounted for to determine the phase induced by the sample alone. Thus, a constant wavefront mismatch must be incorporated into the system in order to remove the need to divide by the blank image. It is important to emphasize that images could be created from these reconstructions that are similar to the images in Figure 3.7 if the system was optimized to provide a constant wavefront mismatch across the field of view, as discussed in §5.1.1.
Figure 3.8: Comparison of the SNR model to experimental images of wrapped phase from balanced mixing in Eq. (24), balanced mixing and DC term subtraction in Eq. (25), and balanced mixing, DC term subtraction, and camera normalization in Eq. (26) when the sample images were not divided by a blank image. The wavefront mismatch between the reference and signal paths is clearly visible in the resultant images. The plot for each phase reconstruction shows the unwrapped phase values through the center of both the model and experimental beads.

3.5.2.5 Minimum Noise Inherent in Phase Reconstructions

The noise in the phase reconstructions was approximated by substituting a model of constant phase across the field of view for the phase induced by the sample, and the models of the noise terms discussed in the previous subsection into Eqs. (48), (65), and (77) for the sample images divided by the blank images, and into Eqs. (56), (69), and (81) for the reconstructions that include the wavefront mismatch between the reference and signal paths. It is important to note that a constant wavefront mismatch was assumed for Eqs. (56), (69), and (81) to provide a constant ideal phase across the field of view.
Incorporating the wavefront mismatch shown in Figure 3.5 would produce a range of phase values for the ideal phase image, and would not provide an accurate representation of the reconstruction error for the reconstructions that do not divide by a blank. The wavefront mismatch was included for the reconstructions that do divide by a blank.

**Figure 3.9:** Phase reconstruction error for balanced mixing from (a) Eq. (48) and (b) Eq. (56), balanced mixing and DC term subtraction from (c) Eq. (65) and (d) Eq. (69), and balanced mixing, DC term subtraction, and camera normalization from (e) Eq. (77) and (f) Eq. (81). The plots in the left column show the result of dividing the sample images by a blank image using the same reconstruction, while the plots in the right column do not divide the sample image by a blank. The plots in the right column also assume a constant wavefront mismatch across the field of view to calculate the error with a constant ideal phase.
The plots in Figure 3.9 show the mean phase error of each reconstruction, calculated by subtracting the expected phase from the result of each reconstruction, versus the constant phase values between $-4\pi$ and $4\pi$. The error bars show the standard deviation of the phase error across the field of view. The maximum RMS error across the $8\pi$ measurements was 0.31 radians, 0.14 radians, and 0.08 radians for the reconstructions in Eqs. (48), (65), and (77) that divide the sample image by the blank image, respectively, and 0.20 radians, 0.10 radians, and 0.05 radians for the reconstructions in Eqs. (56), (69), and (81) that assume a constant wavefront mismatch across the field of view and do not divide by a blank image, respectively. The periodicity of the error can be explained by the strong contribution of multiplicative noise in the reconstructions from the fixed pattern noise. DC term subtraction and camera normalization reduces the amplitude of the periodicity, but the fixed pattern noise must be reduced for the noise to approach a constant value over the range $-4\pi$ to $4\pi$. It is important to note that the amplitude of the periodic error is 0.06 radians for the current OQM reconstruction, which is less than 0.3% of the maximum phase that we are measuring from 100 $\mu$m diameter mouse embryos [Warger et al. 2008].

The balanced mixing reconstructions in Eqs. (48) and (56) are the same mathematically as a phase-shifting system that acquires four sequential images with a 90-degree phase shift in between each image [Creath 1988]. However, the imperfections within the beamsplitters and CCD cameras contribute to a maximum RMS error of 0.31 radians in the phase reconstruction when the sample image is divided by a blank image to remove the wavefront mismatch between the signal and reference paths. The maximum RMS error can be reduced from 0.31 radians to 0.14 radians by subtracting separate images of the reference and signal paths, and to 0.08 radians by also dividing by the square root of the reference image to normalize the camera signals. However, the fixed pattern noise within the system produces a periodic error over the range of phase values that is negligible when imaging large phase objects such as 100 $\mu$m PMMA beads or live mouse embryos that induce a change in phase on the order of $8\pi$ radians, but must be reduced to image samples that induce a change in phase on the order of 1 cycle.

A telecentric imaging system would provide a constant wavefront mismatch across the field of view and remove the need to divide by the blank. Such a system would allow the use of the balanced mixing phase reconstruction without dividing by a blank image and provide a maximum RMS error of 0.20 radians. The maximum RMS error can be further reduced to 0.10 radians by subtracting separate images of the reference and signal paths, and to 0.05 radians by also normalizing the camera signals, assuming the noise terms are comparable to the current experimental setup. Images must be collected with a telecentric system to recreate the models of the noise terms and calculate the noise in such a setup accurately. Phase unwrapping further complicates real-time imaging of samples that produce a change in phase greater than $2\pi$. Techniques to work around these obstacles and more toward real-time OQM imaging are discussed in §5.1.
The SNR model incorporates noise terms from the experimental setup, but it does not include sample dependent effects, such as refraction and diffraction. We have ignored these effects and assumed a projection model because the change in refractive index between the sample and the immersion medium is approximately 0.02 [Warger et al. 2008]. However, these effects will increase as the refractive index mismatch increases and as the location of the sample changes with respect to the focal plane of the microscope, as discussed in §3.6.

It is important to note that the phase reconstruction errors describe the noise inherent in the current system and are not the limiting noise of the techniques. Further optimization of the components and the optical layout will reduce the error in the resultant phase images. Until this optimization is complete, the RMS error of the OQM phase images can be recorded as 0.08 radians.

3.6 Depth of Field

The depth of field for a conventional microscope provides a measure of sample thickness that appears in focus in the image. The light from the remaining thickness of the sample spreads across the image plane and contributes to out-of-focus blur, as described in §2.3.1. One may then ignore the out-of-focus planes and interpret the depth of field as the only portion of the sample that provides useful information. From this perspective, it is reasonable to question how the depth of field affects quantitative phase imaging. Does the resultant phase measurement provide an accurate representation of the phase change induced by the entire sample, or by only the thickness of the sample within the depth of field? Since the depth of field of our imaging system is 1.6 μm, which is much smaller than the 100 μm diameter mouse embryos that we are imaging with a 20x, 0.75 NA air objective and a detector with 7.2 μm pixels, we had to determine what affect the depth of field has on OQM images.

To measure the accuracy of phase images from thick samples (larger than the depth of field) we purchased dry PolyMethylMethAcrylate (PMMA) beads that have a refractive index of 1.489 at 589 nm according to the manufacturer’s specifications (BB05N, Bangs Laboratories, Inc., Fishers, IN). Unfortunately, the manufacturer does not provide the refractive index of the PMMA beads at 633 nm, but others have shown that 1.489 is also a good approximation for the refractive index of PMMA at 633 nm [Nikolov and Ivanov 2000]. We immersed the beads in Nikon Type A nd = 1.515 (23° C) Immersion Oil that has a refractive index of 1.5124 at 633 nm according to the manufacturer’s specifications (Cargille Labs, Cedar Grove, NJ). The PMMA bead immersed in oil produces a refractive index mismatch approximately equal to 0.02, which is equivalent to the refractive index change between a mouse embryo and the culture medium. Images of the PMMA beads immersed in oil were acquired with brightfield and
OQM and registered with the technique described in §2.3.3. Determining the diameter of the bead \((h)\) from the boundary in the brightfield image, and combining it with the refractive index of the bead \((n_s)\) and immersion oil \((n_0)\) in Eq. (1) provides the maximum change in phase induced by the bead, assuming the bead is a perfect sphere. Substituting the maximum change in phase and the diameter of the bead into the equation of a sphere provides a model for the ideal phase measurement.

3.6.1 Ray Tracing Model

While we can approximate the refractive index of the PMMA beads to 1.489, the diameters varied between 70 \(\mu\)m and 110 \(\mu\)m. A circular least-squares fit was applied to points selected along the boundary of the bead to determine an accurate pixel location for the center point, but there was a question in regard to the exact pixel locations of the bead boundary. Figure 3.10 shows a brightfield image of a PMMA bead greater than 100 \(\mu\)m in diameter and a plot of the grayscale values for the pixels along the line drawn through the center of the bead.

![Figure 3.10: (a) Brightfield image and (b) plot of the grayscale values through the center of a PMMA bead immersed in oil.](image)

The bright boundary that surrounds the bead, called a Becke line, derives from the refractive index mismatch between the bead and the immersion oil and has been used for refractometry [Nesse 2004; Gustafsson and Sebesta 2004]. The simplest way to describe the formation of the Becke line is from a geometric optics perspective where we assume the light travels in straight rays. If the sample has the same refractive index as the immersion medium in Figure 3.11(a), the rays travel straight through the sample without refraction (projection theorem). If the refractive index of the sample is different from the immersion oil, the rays refract at every change in refractive index according to Snell’s law: \(n_1 \sin \theta_1 = n_2 \sin \theta_2\) in Figure 3.11(b).
Figure 3.11: (a) Rays do not refract when the refractive index of the sample ($n_{\text{sample}}$) equals the refractive index of the immersion oil ($n_{\text{oil}}$). (b) Rays refract at every change in refractive index.

Two phenomena are responsible for the Becke line, the lens effect in Figure 3.12(a)-3.12(b) and total internal reflection in Figure 3.12(c)-3.12(d). In the lens effect, the edges of the sample are thinner than at the center such that the sample focuses ($n_{\text{sample}} > n_{\text{oil}}$) or defocuses ($n_{\text{sample}} < n_{\text{oil}}$) the light like a lens. Total internal reflection reflects all of the rays incident at an angle greater than or equal to the critical angle ($\theta_1 = \sin^{-1} \frac{n_2}{n_1}$) when $n_1 > n_2$.

Figure 3.12: The Becke line is formed by the (a)-(b) lens effect and by (c)-(d) total internal reflection.

When the sample is in exact focus, the Becke line is said to be coincident with the edge of the sample. As the sample translates through the focal plane, the Becke line moves from the inside to the outside or from the outside to the inside of the sample depending on the sign of the refractive index mismatch between the sample and the immersion medium. When the sample is below the focal plane of the objective (closer to the objective), the Becke line is inside the medium with the lower refractive index. As the sample raises along the optical axis, the Becke line moves toward the edge of the sample and into the medium with the higher refractive index when the bead is above the focal plane. Thus, the relation between the refractive index of the sample and the immersion medium can be determined by translating the sample up and down through the focal plane.
plane and watching the movement of the Becke line, which is one of the methods used to
determine the approximate refractive index of minerals [Nesse 2004].

The Becke line is often accompanied by a dark line, as seen in Figure 3.10(a).
From a geometric optics perspective, the dark line can be explained as the absence of rays
that were refracted to form the Becke line. This is verified in the image in Figure 3.10(a)
because the refractive index of the PMMA bead is less than the refractive index of the
immersion oil, which will produce a defocusing effect. The rays that intersect the edge of
the bead refract away from the bead to produce the dark line between the Becke line and
the rays that travel within the boundary of the bead.

We developed a ray-tracing model that traces rays through an \(xz\)-cross section
through the center of the bead to verify the location of the bead boundary with respect to
the Becke line, where \(x\) and \(z\) correspond to the lateral and axial directions of the
microscope, respectively. The input for the model is the field of view in the \(xz\)-plane,
immersion refractive index, illumination angle for the rays to be traced, and the \((x,z)\)
pixel location, diameter, and refractive index of the bead. The model iterates the
refraction of each ray through the field of view with a unit vector representation of
Snell’s Law, while recording the corresponding average of the Fresnel coefficients
(amplitude) and phase values at every discontinuity of refractive index. Once the rays
reach the end of the field of view, the model back propagates the rays to a focal plane
with the final propagation vector to provide the effective location of the rays in the image
plane. The unit vector representation of Snell’s Law [Wolfe and Zissis 1978; Amirault
and DiMarzio 1985] calculates the propagation unit vector \(\vec{V}\) of the rays at each
discontinuity \(i\):

\[
\vec{V}_{i+1} = \frac{n_i}{n_{i+1}} \vec{V}_j + \left[ 1 - \left( \frac{n_i}{n_{i+1}} \right)^2 \left( 1 - (\vec{V}_i \cdot \vec{N}_i)^2 \right) - \frac{n_i}{n_{i+1}} \vec{V}_i \cdot \vec{N}_i \right] \vec{N}_i
\]

where \(\vec{V}_i\) is a unit vector parallel to the incident beam, \(\vec{V}_{i+1}\) is parallel to the refracted
beam, \(\vec{N}_i\) is the normal to the surface, and \(n_i\) is the refractive index of medium \(i\).

To validate the model, we input a 100 \(\mu m\) diameter PMMA sphere in air with
plane wave illumination at normal incidence in Figure 3.13. The rays that pass through
the bead within the 0.75 NA objective lens forms a focal point 76 \(\mu m\) from the center of
the bead, which correlates to the effective focal length of a ball lens:

\[
f = \frac{nD}{4(n-1)}, \tag{85}
\]

where \(n\) and \(D\) are the refractive index and diameter of the lens, respectively. It is
important to note that some rays toward the outer diameter of the sphere focus to points
less than 76 \(\mu m\) from the center of the bead. These rays produce the aberrations that result
from using the full diameter of a lens, and are the reason optical designers try to limit the beam diameter to the inner third of the lens diameter.

![Refractive Index](image)

**Figure 3.13:** Tracing the rays from plane wave illumination through a 100 μm diameter PMMA sphere in air produces a focal plane 76 μm from the center of the bead.

### 3.6.2 Incoherent Ray Tracing

After the ray tracing model was validated, we modeled brightfield illumination with the center of the bead positioned at every 5 μm within ±45 μm from the focal plane of the objective. The focal plane of the model was defined as the center of the field of view, and the center of the bead was translated along the optical axis to mimic the z-stage translating the bead through the fixed focus of the microscope. Because brightfield is an incoherent imaging technique, a complete ray trace was calculated for a plane wave incident at one degree increments through the full NA of the condenser lens (0.52), and the sum of the squared amplitudes for each ray backpropagated to a single pixel at the focal plane provided the effective irradiance in the image for that particular illumination angle. The contribution from each angular ray trace was weighted to a Gaussian profile, with illumination at the full NA equal to the $1/e^2$ points, and summed to provide the final result. Comparing the resultant ray traces with experimental images showed that modeling the illumination angles through 80% of the condenser NA provided the best fit to the experimental data in Figure 3.14.

We then computed ray traces for a bead positioned at the focal plane (center focus) with diameters between 10 μm and 100 μm in increments of 10 μm. Figure 3.15 shows three of the ray trace results with vertical lines corresponding to the bead boundary. From the plots, we determined that the pixel location of the bead boundary corresponds to the minimum point of the grayscale values plotted across the center of a bead at center focus, resulting in a bead diameter of 106 μm for the bead in Figure 3.10.
Figure 3.14: Comparison of incoherent ray tracing results to experimental images show the microscope used approximately 80% of the full NA to acquire the brightfield image of the PMMA bead immersed in oil.

Figure 3.15: Incoherent ray tracing results from PMMA beads immersed in oil with various diameters and positioned at center focus show that the boundary of the bead corresponds to the minimum grayscale values for a plot of the values across the center of the bead.

Because center focus must be determined by eye experimentally, there was a concern regarding determination of the bead diameter when the bead was not positioned at center focus. To address this concern, we computed ray traces for a 106 μm diameter PMMA bead immersed in oil at every 10 μm from 50 μm above to 50 μm below the focal plane in addition to the planes 2 μm and 5 μm above and below the focal plane. The plots in Figure 3.16 show that the minimum point moves inside the bead diameter as the bead translates more than 5 μm above focus. Once the bead is less than or equal to 5 μm above the focal plane, the Becke line reaches a minimum width outside of the bead boundary and the pixels with the minimum grayscale values through the center of the bead correspond to the bead diameter. Thus, the bead diameter can be determined accurately by measuring the distance between the minimum points of the brightfield grayscale values through the center of the bead if the operator ensures the thinnest Becke line width outside of the bead boundary.
Figure 3.16: Incoherent ray trace results for a 106 μm diameter PMMA bead immersed in oil translated through the focal plane. The vertical lines represent the boundary of the bead.

3.6.3 OQM Images at Center Focus

With knowledge of the pixel location for the PMMA bead boundary, we can use the registered brightfield image to create a model of the ideal phase, assuming the bead is a perfect sphere. A circular least-squares fit was computed for points selected along the boundary of the bead to determine the pixel location of the center point. The grayscale values through the center of the bead in the brightfield image were plotted and the pixel distance between the minimum points in the plot were recorded as the diameter of the bead. The bead diameter and refractive indices of the bead and the oil were input into Eq. (1) to calculate the maximum change in phase induced by the bead. The maximum phase, bead diameter, and center pixel location were put into the equation for a sphere to create the model of ideal phase. A plot through the center of the model bead and through the center of the corresponding OQM bead were overlaid to determine the accuracy of the OQM image qualitatively. Figures 3.17 – 3.20 show that accurate phase measurements were obtained for the change in phase induced by the full PMMA bead with diameters of 77 μm, 91 μm, 99 μm, and 106 μm. It is important to note that the bead may move between collection of the brightfield and OQM images because the images are not
acquired simultaneously, and additional registration may be necessary to create the bead model at the same pixel location as the OQM image.

**Figure 3.17**: Experimental (a) OQM and (b) brightfield images of a 77 μm diameter PMMA bead immersed in oil. (c) Plots through the center of the experimental and model phase images show good agreement.

**Figure 3.18**: Experimental (a) OQM and (b) brightfield images of a 91 μm diameter PMMA bead immersed in oil. (c) Plots through the center of the experimental and model phase images show good agreement.

**Figure 3.19**: Experimental (a) OQM and (b) brightfield images of a 99 μm diameter PMMA bead immersed in oil. (c) Plots through the center of the experimental and model phase images show good agreement.
Figure 3.20: Experimental (a) OQM and (b) brightfield images of a 106 μm diameter PMMA bead immersed in oil. (c) Plots through the center of the experimental and model phase images show good agreement.

### 3.6.4 Coherent Ray Tracing

The ray tracing model can also be used for coherent illumination to model the signal and reference paths in OQM by recording the optical path in addition to the refraction unit vector and Fresnel coefficients at each refractive index discontinuity. In the coherent case, a single ray trace is calculated with an illumination wavefront equivalent to the output of the condenser lens, and the sum is performed with the complex magnitude and phase from every ray backpropagated to the focal plane. This results in complex values across the focal plane that correspond to the magnitude and phase of the rays through the $xz$-section, and can be used to calculate a synthetic OQM image.

To model the wavefront that illuminates the bead, we had to determine the focal point of the illumination light through the condenser lens with respect to the focal plane of the objective. We placed a business card on the piezo stage with the writing facing the objective, illuminated the card with the halogen lamp, and adjusted the position of the objective until the writing on the card came into focus. We then aligned the condenser lens for Köhler illumination, removed the objective from the path, placed the business card on the objective turret, turned off the halogen lamp, and turned on the Helium-Neon laser. A piece of glass with one side ground was placed within the optical path with the ground side facing the condenser lens, and was translated along the optical path until the largest speckle pattern was observed on the business card. Measuring the distance from the top of the piezo stage, where the business card was originally positioned, to the position of the ground glass surface that provided the largest speckles showed that the condenser lens focus was 4.7 mm beyond the focal plane. Assuming a spherical wavefront with the same focal point as the condenser lens provided an accurate model of the illumination rays in the coherent ray tracing model.
Figure 3.21: Coherent illumination and coherent ray tracing result for a 106 μm PMMA bead immersed in oil with the center of the bead positioned at (a) 45 μm above the focal plane, (b) center focus, and (c) 45 μm below the focal plane.

To validate the coherent model, we calculated a coherent ray trace for the 106 μm diameter PMMA bead in oil with the center of the bead positioned at every 5 μm within ±45 μm from the focal plane, and compared the results with images of the bead in the pure signal path (without interference with the reference path). Figure 3.21 shows three of the bead images at ±45 μm and at the focal plane, and the corresponding coherent ray trace. When the bead is at center focus, the resultant ray trace is similar to the incoherent ray trace, but when the center of the bead is above or below the focal plane, the coherent light creates constructive and destructive interference fringes within the region corresponding to the Becke line. It is important to note that the striations in the background of the coherent images correspond to the coherent noise caused by the acquisition of coherent light with a CCD camera and imperfections within the optics.

3.6.5 OQM Z-Stacks

The coherent ray trace can also be used to create a model of the ideal phase measured by OQM. A coherent ray trace of the 106 μm diameter PMMA bead in immersion oil provides a model of the field in the signal path $E_{\text{sig}}$ and a coherent ray trace
of the immersion oil without a PMMA bead provides a model of the field in the reference path $E_{ref}$. Substituting $E_{sig}$ and $E_{ref}$ into:

\[
M_0 = \frac{1}{2}(E_{sig} + E_{ref})(E_{sig}^* + E_{ref}^*)
\]
\[
M_1 = \frac{1}{2}(E_{sig} + jE_{ref})(E_{sig}^* + (jE_{ref})^*)
\]
\[
M_2 = \frac{1}{2}(E_{sig} - E_{ref})(E_{sig}^* - E_{ref}^*)
\]
\[
M_3 = \frac{1}{2}(E_{sig} - jE_{ref})(E_{sig}^* - (jE_{ref})^*)
\]

provides the ideal signals captured by each of the four CCD cameras. Balanced mixing of Eq. (86) with Eq. (24) provides the ideal magnitude and phase of the sample that would be acquired with OQM assuming no noise or aberrations within the system, same optical path in the signal and reference arms, perfect alignment of the wavefront between the signal and reference paths (single interference fringe larger than the field of view), and no changes in polarization.

Computing coherent ray traces for the 106 $\mu$m PMMA bead immersed in oil with the center of the bead positioned every 5 $\mu$m between 45 $\mu$m above the focal plane (-45 $\mu$m) and 45 $\mu$m below the focal plane (+45 $\mu$m) provides the wrapped phase $xz$-section through the center of the bead in Figure 3.22(a), and compares to the experimental $z$-stack in Figure 3.22(b). From the coherent ray tracing model, it is apparent that the projection theorem is valid for the inner 80% of the bead diameter, but there is a slight focusing effect induced by the edges of the bead. This result is interesting because the refractive index of the bead is greater than the refractive index of the immersion oil, and it may be intuitive to assume a defocusing effect. However, this result makes sense when considering the location of the focal plane with respect to the sphere. If a sphere with an index greater than the background was positioned above the focal plane, the illumination rays could focus to a point at the focal plane. As the sphere moves along the optical path toward the objective, the focal point induced by the sphere will traverse past the focal plane resulting in spreading of the light in the focal plane. Thus the $xz$-slice of a bead with a refractive index greater than the immersion will defocus as the bead translates from above the focal plane and a bead with a refractive index less than the immersion will focus as seen in Figure 3.22(a) and 3.22(b). It is important to note that the fluctuation in the background of the experimental $z$-stack was caused by the wavefront mismatch of the sample images changing with respect to the wavefront mismatch of the blank image. Further optimization of the system must be completed to provide a constant fringe pattern during imaging, as discussed in §5.1.
Figure 3.22: (a) Coherent ray tracing and (b) experimental $xz$-section of wrapped phase for a 106 $\mu$m PMMA bead immersed in oil. Unwrapped $xz$-section of (c) the model using a 1D phase unwrapping algorithm, and (d) the experimental images using the 2D phase unwrapping algorithm $L^p$-norm for each slice through the stack.

Figure 3.22(c) shows the unwrapped phase using the Matlab 1D unwrap function for each ray trace in the $z$-stack, and compares to the unwrapped $z$-stack from the experimental images in Figure 3.22(d). The experimental $z$-stack was unwrapped by applying the 2D phase unwrapping algorithm $L^p$-norm to each 2D image of the bead and recording a single row through the center of the bead to create the $xz$-section. It is important to note that the incorrect phase at 25 $\mu$m in the lateral direction and -20 $\mu$m from the focal plane in Figure 3.22(d) corresponds to an unwrap failure, where the 2D phase unwrapping algorithm chose the boundary of the sample incorrectly. The phase unwraps correctly in the model image when the center of the bead is at the focus, within 20 $\mu$m below focus, and at 40 $\mu$m below focus. The reason for the incorrect unwraps in the remaining slices derives from the constructive and destructive interference of the light that is within the Becke line.
Figure 3.23: (a) Wrapped and unwrapped phase from a coherent ray trace of a 106 μm diameter PMMA bead immersed in oil with the bead at center focus. (b) Exploded view of the plot to show the phase unwraps correctly.

Figure 3.24: (a) Wrapped and unwrapped phase from a coherent ray trace of a 106 μm diameter PMMA bead immersed in oil positioned 5 μm above the focal plane. (b) Exploded view of the plot to show the phase wrap that the unwrapping algorithm did not unwrap correctly.

Figure 3.23 shows the wrapped (+) and unwrapped (-) coherent ray tracing results for the bead positioned at the focal plane. When the 1D signal reached the boundary of the bead, the unwrapping algorithm correctly subtracted $2\pi$ at the first phase discontinuity resulting in the correct phase for the overall bead. Figure 3.24 shows the wrapped and unwrapped coherent ray tracing results for the bead positioned 5 μm above focus. When the 1D signal reached the boundary of the bead at this slice, the unwrapping algorithm incorrectly assumed the phase discontinuity was an increase of $0.9\pi$ instead of a decrease of $1.1\pi$ resulting in $2.2\pi$ radians less phase than the actual maximum phase of the bead. However, the 2D phase unwrapping algorithm was able to unwrap this slice correctly, because the 2D phase unwrap uses the phase discontinuities throughout the entire field of view.
view to determine the unwrap direction or because the noise in the system resulted in a smaller change in phase in the correct direction. Thus, it appears that it is possible to unwrap the phase of a spherical object located ±20 μm from the focal plane correctly, but the phase may not unwrap correctly for slices above the focal plane with the current pixel size.

The 2D phase unwrapping algorithm was unable to unwrap the slices greater than 20 μm from the focal plane in both directions due to the displacement of the rays that form the Becke line. When the bead was positioned 30 μm above the focal plane in Figure 3.25, the rays that traveled through the outer diameter of the bead form the Becke line outside of the bead diameter in the image plane resulting in constructive and destructive interference between the rays that do not pass through the bead and the rays that refract from inside the bead diameter to the outside. This can be seen in Figure 3.25(c) where the phase was unwrapped by hand starting from the center of the bead, and resulting in $2\pi$ less phase at the center of the bead compared to the unwrapped phase of the bead at the focal plane.

When the bead was positioned 30 μm below the focal plane, the rays that traveled through the outer diameter of the bead form the Becke line positioned inside the bead diameter in the image plane. Figure 3.26(b) shows quick $2\pi$ phase changes on the inside edge of the dark ring seen in Figure 3.21(c). These $2\pi$ phase changes were not unwrapped correctly by the 1D technique resulting in less phase at the center of the bead. Unwrapping the phase from the center of the bead by hand in Figure 3.26(c) shows that less phase will result within the dark ring in the 2D image in Figure 3.21(c) and the correct phase can be unwrapped within the inner boundary of the dark ring compared to

**Figure 3.25:** (a) Wrapped and unwrapped phase from a coherent ray trace of a 106 μm diameter PMMA bead immersed in oil positioned 30 μm above the focal plane. (b) Exploded view of the plot to show the constructive and destructive interference produced by the Becke line outside of the bead boundary. (c) Unwrapping the phase by hand from the center of the bead shows that the total phase of the bead is incorrect because the phase from the rays that form the Becke line do not contribute to the phase within the boundary of the phase.
the phase at center focus if we assume a $2\pi$ phase change in the desired direction at every phase discontinuity. Thus, it appears that it is possible to unwrap the phase within the center of the bead when the bead is greater than 20 $\mu$m below focus, but the phase values at the edge of the bead may be incorrect. However, it is possible that the correct phase was unwrapped because the pixel size was too large to sample the interference properly. Smaller pixels would provide increased sampling within the interference and may have led to the inability to unwrap the phase in the center of the bead.

Figure 3.26: (a) Wrapped and unwrapped phase from a coherent ray trace of a 106 $\mu$m diameter PMMA bead immersed in oil positioned 30 $\mu$m below the focal plane. (b) Exploded view of the plot to show the constructive and destructive interference produced by the Becke line outside of the bead boundary. (c) Unwrapping the phase by hand from the center of the bead shows that the total phase of the bead is incorrect because the phase from the rays that form the Becke line provide less phase near the boundary of the bead.

In summary, OQM can measure the correct phase for spherical objects much larger than the depth of field when the object is at center focus, and we can assume a projection model when the sphere is near center focus. The limitation on the distance from center focus is dependent on the diameter of the sample because the diameter determines the location of the Becke line. When the bead is above the upper limit from center focus and the refractive index of the bead is less than the immersion medium, the rays at the edge of the bead refract outside the bead boundary in the image plane, and the phase along those rays produces interference outside of the bead boundary. When the bead is below the lower limit from center focus and the refractive index of the bead is less than the immersion medium, the rays at the edge of the bead refract inside the bead boundary in the image plane, and the phase along those rays provides less phase near the bead boundary. The inverse is true for the effects above and below center focus for a bead with a refractive index greater than the immersion medium. Thus, according to the ray tracing model, the formation of the Becke line is the fundamental reason for the difficulty in acquiring OQM z-stacks, and refraction must be incorporated into the model to provide quantitative phase information in OQM images acquired away from center focus.
4. Quantitative 3D Mouse Embryo Viability Measurements

4.1 Introduction

Since 1978 in vitro fertilization (IVF) procedures have resulted in the birth of over three million babies [Steptoe et al. 1980]. Yet, despite the plethora of qualitative viability markers utilized in various embryo scoring methods amongst the clinics, IVF procedures in the United States had a live birth rate of only 34% in 2005, with 32% of these successful pregnancies resulting in multiple births [Centers for Disease Control and Prevention 2007]. These multiple pregnancies were directly attributed to the transfer of multiple embryos to increase the probability that a single, healthy embryo was included, because the current qualitative measures are inadequate to measure embryo viability reliably. The use of quantitative, three dimensional measures could create a definitive method of embryo scoring that will produce a successful pregnancy from the transfer of a single embryo.

Current viability markers used for IVF, such as the cell number, symmetry, size, and fragmentation, are analyzed qualitatively while focusing through the embryo with differential interference contrast (DIC) microscopy. However, this method is not ideal for quantitative measures beyond the 8-cell stage of development because the cells overlap and obstruct the view within and below the cluster of cells. Because embryo transfers at the blastocyst stage are increasingly believed to be more likely to succeed, we have developed the phase-subtraction cell-counting method that uses the combination of DIC and OQM to extend the measure of cell number beyond the 8-cell stage of development [Warger et al. 2008]. We have also created preliminary quantitative analyses to measure the mitochondrial distribution in oocytes and the cell symmetry, size, and fragmentation during the cleavage stages using the relative dry mass from the OQM image in conjunction with the phase-subtraction count. If verified with human embryos and adopted into the clinic, these methodologies could provide the means to determine which viability markers are truly indicative of embryo health and produce a more accurate method to score embryo viability that will help move toward successful single embryo transfer.

4.2 Phase-Subtraction Cell-Counting Method

The rate of development has been considered one of the key viability measures for embryos during the cleavage stage [Baczkowski et al. 2004], which includes the 2-cell to morula stages. However, the number of cells can only be measured accurately up to the 8-cell stage of development because the cells begin to overlap and obstruct the view of additional cells on the bottom of the cell cluster, as seen in Figure 2.7. Thus, the
rate of development after the 8-cell stage has been characterized by the formation of the 
blastocyst. Since human embryos form the 8-cell and blastocyst stages on days 3 and 5, 
respectively, the rate of development is not characterized during the morula stage on day 
4. Some studies have investigated qualitative viability measures and transfers of embryos 
during the morula stage and have shown pregnancy rates greater than and equal to 
pregnancy rates using day 5 viability measures and transfers [Tao et al. 2002; Feil et al. 
2008].

We have developed the phase-subtraction cell-counting method that uses the 
combination of the OPD information from the OQM image and the boundary information 
from the DIC image to provide a means for counting the number of cells past the 8-cell 
stage [Wager et al. 2008]. The technique begins by registering the pixel size and field of 
view between the two image modalities [Tsai et al. 2008], and then registering the 
embryo to remove sample movement and provide pixel-to-pixel registration between the 
features in each image. Registration of the embryo has been completed by selecting 
corresponding landmarks manually where the boundaries of the cells intersect along the 
perimeter of the cell cluster. Landmarks cannot be chosen within the interior of the cell 
cluster because out-of-focus cells may create or distort the appearance of additional 
landmarks. Techniques are underway to automate registration of the embryo that are 
discussed in §5.2.2.

The user analyzes the registered OPD and DIC images and selects a model cell 
that is along the perimeter of the cell cluster, approximately equal in size to the majority 
of cells, has a uniform distribution of OPD, and contains the least amount of cell overlap. 
A plot of the OPD values along a line in Figure 4.1(a) that traverses from the culture 
medium (outside the zona pellucida), through the center of the chosen cell, and into the 
region of overlapped cells is shown in Figure 4.1(b). At the bottom of the plot is a 
relatively flat line of constant OPD that represents the culture medium. As the line on the 
OPD image begins to go into the zona pellucida, the plot of OPD increases following a 
parabolic shape. Once the line reaches the cell, the plot increases again in a parabolic 
fashion, and continues until the line reaches the region of overlapped cells. Points are 
selected along the plot manually to produce a parabolic fit to the cell. The OPD between 
the maximum point of the parabolic fit and the sum of the culture medium and zona 
pellucida corresponds to the OPD diameter of the cell. The user then creates an elliptical 
boundary for the cell by selecting points along the boundary in the DIC image in Figure 
4.1(c). Combining the radii of the ellipse (a and b) with the OPD diameter of the model 
cell (c) creates an ellipsoidal model cell of OPD in Figure 4.1(d). The OPD diameter is 
used for c instead of the radius to account for the full optical thickness of the cell. The 
equation for a slightly flattened ellipsoid:

\[
\left(\frac{x^2}{a^2} + \frac{y^2}{b^2}\right)^2 + \frac{z}{c} = 1
\] (1)
was originally determined to be the best fit for cells in the OPD image, where \( x, y, \) and \( z \) are the Cartesian coordinates with \( z \) parallel to the optical axis of the microscope. All of the cells were assumed to contain the same OPD diameter in the \( z \)-direction so all model cells used the same value for \( c \) in Eq. (1). Subtracting the model cell from the OPD image in Figure 4.1(e) reveals either the background culture medium and zona pellucida or additional cells. The user creates elliptical boundaries for all the visible cells in the DIC image and subtracts the corresponding model cells from the OPD image. The user fits elliptical boundaries to elliptically shaped regions or clusters with the approximate size of the cells previously subtracted in the OPD image, and subtracts the model cells until no such regions remain in Figure 4.1(f). A complete visualization of the phase-subtraction cell-counting method has been provided on the website for the Keck microscope: http://www.keck3dfm.neu.edu/samplevideo, and in Supplementary File 2.

![Figure 4.1:](image)

**Figure 4.1:** The optical path differences along the line drawn on the OPD image in (a) is plotted in (b). A parabolic fit is used to determine the OPD of a single cell, which is combined with the ellipse drawn on the DIC image in (c) to create a model cell in (d). The model cell is subtracted from the OPD image to show the background culture media or additional cells above or below the subtracted cell in (e). Model cells are subtracted from the OPD image until no more cells remain in (f).

### 4.2.1 Image Collection and Cell-Counting Results

C57BL/6 female mice (Jackson Laboratory, Bar Harbor, ME) were superovulated with eCG and hCG (Sigma, St. Louis, MO) to increase their number of eggs [Nagy *et al.* 2003], and mated with single CBA/Ca male mice. Plug positive female mice were
sacrificed on days 3 and 4, post hCG injection, and 8-cell through morula-stage embryos were collected in M2 medium (Specialty Media, Phillipsburg, NJ). The embryos were stained for 30 minutes in 1 μg/ml Hoechst 33342 dye, which binds to the DNA within the nucleus of each cell. Morphologically normal appearing embryos (no visible fragmentation, intact/healthy appearing zona pellucida and cells) were placed in M2 microdrops, under equilibrated oil, in a Mat-Tek imaging dish with a coverslip-bottom (Mat-Tek, Ashland, MA). Single images were acquired at the center focus plane of the embryo in DIC and OQM with a 20x, 0.45 NA objective lens and a 0.52 NA condenser lens. Center focus was found when the outer boundary of the zona pellucida was in focus. A z-stack of images was also acquired of the Hoechst stained nuclei with epi-fluorescence, and analyzed using the count cells tool in Metamorph software (Molecular Devices, Downingtown, PA) to determine the number of cells.

<table>
<thead>
<tr>
<th>Fluorescence Count</th>
<th>Phase-Subtraction Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13 cells</td>
</tr>
<tr>
<td>2</td>
<td>14 cells</td>
</tr>
<tr>
<td>3</td>
<td>16 cells</td>
</tr>
<tr>
<td>4</td>
<td>17 cells</td>
</tr>
<tr>
<td>5</td>
<td>25 cells</td>
</tr>
</tbody>
</table>

**Table I:** Results of cell counts produced by epi-fluorescence imaging of Hoechst stained nuclei and the phase-subtraction cell-counting method for a training set of five live mouse embryos. The number of cells was known before the phase-subtraction count was completed.

<table>
<thead>
<tr>
<th>Fluorescence Count</th>
<th>Phase-Subtraction Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 cells</td>
</tr>
<tr>
<td>2</td>
<td>8 cells</td>
</tr>
<tr>
<td>3</td>
<td>8 cells</td>
</tr>
<tr>
<td>4</td>
<td>12 cells</td>
</tr>
<tr>
<td>5</td>
<td>12 cells</td>
</tr>
<tr>
<td>6</td>
<td>15 cells</td>
</tr>
<tr>
<td>7</td>
<td>16 cells</td>
</tr>
<tr>
<td>8</td>
<td>16 cells</td>
</tr>
<tr>
<td>9</td>
<td>21 cells</td>
</tr>
<tr>
<td>10</td>
<td>26 cells</td>
</tr>
</tbody>
</table>

**Table II:** Results of cell counts produced by epi-fluorescence imaging of Hoechst stained nuclei and the phase-subtraction cell-counting method for ten live mouse embryos. The number of cells was not known before the phase-subtraction count was completed.
Phase-subtraction cell counts were completed on 15 morphologically normal, live mouse embryos. The first 5 samples were used as a training set where the number of cells was known before the cell count was complete. This training set was used to determine the potential variation in cell sizes for embryos with different cell numbers because the cells divide asynchronously [Rafferty 1970]. As seen in Table I, accurate cell counts were obtained for the five samples once the correct cell boundaries were chosen. The second set of 10 samples was completed blind where the number of cells remained unknown until after the cell count was complete. The phase-subtraction cell-counting method had a maximum error of one cell for the 10 blind samples (samples 7 and 8) and accurately counted up to 26 cells as shown in Table II.

4.2.2 Model Equation to Fit Cells

To further analyze the ellipsoid equation for fitting the OPD of the cells in the phase-subtraction count, the cell counts were repeated with the equation for a perfect ellipsoid:

\[
\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1
\]

in place of the slightly flattened ellipsoid in Eq. (1), and the same results presented in Tables I and II were obtained. The cell count worked in both cases because the primary difference between the two equations is the distribution of the OPD and not the total amount of OPD, as seen in the plots in Figure 4.2.

![Figure 4.2](image)

**Figure 4.2:** (a) Plot of the cross section of optical path for model cells created by Eq. (1) with a dashed line (--) and Eq. (2) with a solid line with \( c \) equal to the OPD radius. (b) Plot of the cross section of positive OPD with \( c \) equal to the OPD diameter.

The perfect ellipsoid provides an even distribution of OPD and the flattened ellipsoid...
provides more OPD within the center of the cell and less along the edges. There is a minimal difference between the total volume between the two equations, so as long as the correct cell boundaries are chosen, the subtraction of either equation will not remove enough extra OPD to make a single cell appear as two, or subtract too little to provide the appearance of an extra cell.

$$\text{OPD (μm)}$$

50 μm

0 0.5 1 1.5 2

(a) (b) (c)

Figure 4.3: (a) OPD, (b) brightfield, and (c) MitoTracker epi-fluorescence images of an oocyte (egg). (d) Plot of OPD along line drawn on image and ellipsoidal equations for fit. (f) Plot of OPD and distribution of MitoTracker dye within the depth of field to show the effect of mitochondria in OPD image.

We then analyzed multimodal images of an oocyte (egg) and a zygote (1-cell embryo) to compare the two equations without the overlap of multiple cells. Figures 4.3(a)-4.3(c) show respectively the images from OQM, brightfield, and epi-fluorescence of MitoTracker dye of an oocyte with a zona pellucida. The plot in Figure 4.3(d) shows the OPD values for the line drawn through the center of the oocyte and the equations for perfect and slightly flattened ellipsoids. Both equations appear to fit the edge of the egg relatively well, but the flattened ellipsoid fits the center of the egg much better than the perfect ellipsoid. It is important to note that the small parabolic increases of OPD on either side of the egg correspond to the zona pellucida that is modeled as a spherical shell that surrounds the egg.
Figure 4.4: (a) OPD, (b) brightfield, (c) MitoTracker epi-fluorescence, and (d) Hoechst epi-fluorescence images of a zygote without a zona pellucida. (e) Plot of OPD along line drawn on image and ellipsoidal equations for fit. (f) Plot of OPD and distribution of fluorescent dyes within the depth of field to show effect of mitochondria and nucleus in OPD image.

Figure 4.4(a)-4.4(d) show respectively the images for OQM, brightfield, epi-fluorescence of MitoTracker dye, and epi-fluorescence of Hoechst dye of a zygote that happened to not have a zona pellucida. The plot in Figure 4.4(e) shows the OPD values for the line drawn through the center of the zygote and the equations for the perfect and slightly flattened ellipsoids respectively. In this plot, the flattened ellipsoid appears to fit along the right boundary of the cell, but contains too much OPD along the left boundary. The perfect ellipsoid appears to fit the general shape of the cell with some extra fluctuations near the center of the cell. These fluctuations that are seen in both plots in Figure 4.4(e) can be explained using the plots in Figures 4.4(e) and 4.4(f) that show the OPD values from the OQM image in comparison to the distribution of the dye from the epi-fluorescence images. The plot in Figure 4.4(e) shows the fluctuations from the distribution of mitochondria and the plot in Figure 4.4(f) shows the fluctuations from the nucleus and the mitochondria. These plots show how the fluctuations of the fluorescent dye from both the mitochondria and the nucleus correlate to the fluctuation in OPD from the model equations. It is important to note that the locations of the fluctuations are better correlated than the amplitudes are because the fluorescence signal is proportional to the total amount of dye that is within the depth of field. A confocal fluorescence or two-photon z-stack [Squirrell et al. 2003] would be required to correlate the amplitude of the...
fluorescence fluctuation with the fluctuation of OPD. From the comparison of these datasets, the perfect ellipsoid fits the general shape for the OPD from the cell with some random fluctuation corresponding to the mitochondria and the nucleus, and the flattened ellipsoid includes the contribution from the zona pellucida that surrounds the embryo. Thus, the perfect ellipsoid in Eq. (2) should be used to model the cells during the phase-subtraction count.

4.3 Dry Mass

Additional characteristics of the embryo can be derived from the dry mass. The dry mass of a dry object is a measure of density (g/cm³), and the dry mass of a biological object has been described as a measure of the dry protein concentration (g/ml). The refractive index of a solute in a solution ($n_0$) follows a linear relation:

$$n = n_0 + \chi C,$$

where $\chi$ is the specific refraction increment and $C$ is the concentration of the solute expressed in terms of mass per unit volume. Cells are primarily made of water, but they also contain inorganic salts, carbohydrates, lipids, nucleic acids, and proteins that change the refractive index of the sample from the refractive index of water. Proteins provide the greatest proportion of all of these components, so the refractive index of a cell has been summarized as the concentration of protein in the cell or dry mass [Barer 1957].

The dry mass ($M$) of protein [Barer 1952; Davies and Wilkins 1952] in a living cell has been defined:

$$M = \frac{OPD \times AREA}{\chi},$$

where $\times$ denotes a multiplication, $AREA$ is a projection of the two-dimensional cell area, and $\chi$ is the specific refraction increment:

$$\chi = \frac{n_p - n_0}{C},$$

where $n_p$ is the refractive index of the protein, $n_0$ is the refractive index of the immersion medium, and $C$ is the concentration of grams of dry protein per ml of solution. Eqs. (4) and (5) are derived by the definitions of OPD:
\[ \text{OPD} = (n_p - n_i)h, \]  
\[ \text{and of concentration:} \]
\[ C = \frac{M}{\text{AREA} \times h}, \]

where \( h \) is the thickness of the cell. It is important to note that many of the original papers defined the concentration in grams of dry protein per 100 ml of solution, thereby substituting \( \alpha \) into Eq. (4) for \( \chi \) and defining \( \chi = 100 \alpha \). However, we have converted the concentration to cgs units of grams per ml for clarity of the method.

4.3.1 Relative Dry Mass

The confocal reflectance image of the 3-cell embryo in Figure 2.3(h) visualized the variations in refractive index within the cells that are caused by organelles dispersed throughout the cytoplasm. Had the cells been homogeneous, scattering would not occur until a refractive index mismatch was created between the cytoplasm and the nucleus. The variations in refractive index lead to the assumption that a single specific refraction increment may not be appropriate for the entire cell, or for every stage of development [Brinster 1967; Biggers and Borland 1976]. To account for the heterogeneity of the cells, we have defined the relative dry mass of the cell cluster:

\[ M_R = \sum_{\text{AREA}} \text{OPD} \times dx \times dy = \chi \times M, \]  

where \( dx \) and \( dy \) are the pixel sizes in the image plane, such that a single specific refractive increment does not need to be assumed.

To measure the relative dry mass, we differentiate the cells from the background of the image by tracing the boundary of the cell cluster in the DIC image in Figure 4.5(a) to create a binary mask. Multiplying the mask by the OPD image and subtracting the minimum OPD of the reference cell removes the contribution from the culture medium and the zona pellucida. Summing the remaining OPD in Figure 4.5(b) provides the total OPD of the cell cluster, and multiplying by the pixel sizes in the image plane provides the relative dry mass. It is important to note that the inner or outer boundary of the zona pellucida could be used as the boundary of the cell cluster if the optical path of the zona pellucida can be modeled and subtracted accurately from the OPD image.
Figure 4.5: (a) OPD image of a live 8-cell mouse embryo. (b) Tracing the boundary of the cell cluster creates a mask to segment the OPD of the cells from the OPD of the background zona pellucida and culture medium. (c) Segmenting the cell cluster and subtracting the OPD of the zona pellucida and culture medium provides the OPD of the cells alone. Summing the OPD in (c) and multiplying by the 2D pixel size provides a measure of the relative dry mass for the total cell cluster.

The 15 morphologically normal, live mouse embryos counted with the phase-subtraction method were also analyzed for relative dry mass. The 15 samples, with an average cell cluster radius \( (r_{xy}) \) of 40 μm, had an average relative dry mass of 4831 μm³ ± 368 μm³, which corresponds to a radius \( (r_z) \) of 36 μm ± 2.75 μm in the z-direction by:

\[
M_R = \frac{4}{3} \pi \ (r_{xy})^2 \ r_z \ \Delta n ,
\]

assuming the difference in refractive index between the cells and the culture medium \( (\Delta n) \) is 0.02. Assuming a value of 0.18 g/ml for \( \chi \) in Eq. (4) [Davies et al. 1954; Barer 1957], and remembering 1 ml = 10¹² μm³, provides an average dry mass of 26.8 ng ± 2.0 ng, which is in the range of previous work that showed 20.6 ng of protein in the morula stage [Brinster 1967], 22.6 ng of dry weight at the morula/early blastocyst stage [Biggers and Borland 1976], and 32.59 ng of dry mass at the morula stage (using a value of 0.17 g/ml for \( \chi \)) [Turner et al. 1992].

4.4 Relative Dry Mass Cell Count

A relative dry mass cell count can be completed by dividing the relative dry mass of the total cell cluster by that of a single cell in Table III, where the relative dry mass of a single cell was calculated from the first cell subtracted in the phase-subtraction count. Counting the number of cells by the analysis of relative dry mass provided an RMS error of 2.3 cells from the epi-fluorescence count. However, we believe more error will result from an analysis of a larger dataset because this method is only appropriate for a sample where all of the cells are approximately the same size. The cells within an embryo are the same size when the number of cells is equal to a power of 2, more specifically 2, 4, 8, 16,
or 32 cells, which correspond to the five cleavage stages. All other cell numbers will have cells that are approximately half or twice the volume of the first chosen cell in the phase-subtraction count and will produce errors within the relative dry mass count. We can assume that all cells will divide to a single cleavage stage before any cell divides to the next cleavage stage by following the trend of development, i.e. 1 cell on day one, 2 cells on day two, 4 cells between days two and three, 8 cells on day three, 16 cells between days three and four, and 32 cells (blastocyst stage) on day four. Accordingly, the error can be approximated because each cell number is created by only one combination of cell sizes. As an example, an 8-cell embryo will have 8 cells at the third cleavage stage that are approximately the same size, and a 9-cell embryo will have 7 cells at the third cleavage stage and 2 cells that are approximately half the size at the fourth cleavage stage. Additional error will also exist because the boundary for the total cell cluster will include any fragmentation or polar bodies in the embryo that may contribute to more OPD than that of a single cell.

<table>
<thead>
<tr>
<th>Fluorescence Count (cells)</th>
<th>Total Relative Dry Mass (μm$^3$)</th>
<th>1st Cell Relative Dry Mass (μm$^3$)</th>
<th>Relative Dry Mass Count (cells)</th>
<th>Error (cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>4300</td>
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<td>7</td>
<td>-1</td>
</tr>
<tr>
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<td>7</td>
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Table III: Cell count results from the analysis of relative dry mass for the 15 samples analyzed with the phase-subtraction cell count.

It is important to note that there was minimal error in the phase-subtraction count with a constant value for the OPD diameter of each subtracted cell even though the samples were among various cleavage stages. Accurate cell counts were obtained because the boundary was changed to fit each cell in the 2D image plane. Subtracting the relative dry mass of a cell by twice or half of the relative dry mass for a cell at the previous or following cleavage stages respectively, will provide an error of a whole cell in the count.
However, the difference in OPD between a cell fit perfectly and a cell that has the proper radii in 2 dimensions and the OPD diameter of the previous or following cleavage stage will be $\sqrt{2}$ of a cell, which is not enough difference to appear as an additional cell or remove enough of a cell to make one cell appear as two during the phase-subtraction count. However, the OPD diameter of each cell will require an accurate model to extend the phase-subtraction count for use with the additional viability measures discussed in §4.5 and §4.6.

4.5 Cell Symmetry/Size Analysis

One of the key viability measures during the cleavage stage in addition to the rate of development is the relation between the sizes of the individual cells [Baczkowski et al. 2004]. However, the characterization of cell size is dependent on the 2D cross-sectional area provided by DIC images. Additional methods have produced 3D volumetric models of the cells, but they use stains with confocal and two-photon imaging that cannot be used in a clinical setting [Aiken 2004; Pogorelov 2008].

Assuming each cell can be modeled perfectly during the phase-subtraction count, as discussed in §5.2.1, the relative dry mass of each model cell would provide a measure proportional to the volume of the individual cells. Comparing the relative dry mass of each cell would then provide a quantitative measure of the cell symmetry and size. Embryo scoring techniques characterize cell size into equal and unequal, but analyzing the relative dry mass of each cell would provide a quantitative comparison of the individual cells.

Cytoplasm inclusions (texture) and cell expansion (cell touching the zona pellucida with minimal perivitelline space) have also been analyzed in addition to cell size. Cytoplasm inclusions such as pitting and a granular appearance could be characterized as the difference between the overall OPD of the cell and the model cell, but the operator may not be able to determine the difference between cytoplasm defects and fragmentation with the current technique. In addition, OPD fluctuations from mitochondria and the nucleus could appear similar to the defects. Cell expansion could be measured by creating a 3D model of the embryo with the OPD from all of the subtracted model cells and comparing that volume with an ellipsoid created with the inside edge of the zona pellucida. However, this technique would rely on assuming the zona is symmetric with the 2D area seen within the DIC image. It is also important to note that some studies have shown that the texture of the cells has no predictive value for embryo viability [Rienzi et al. 2005].
4.6 Fragmentation Analysis

The third key viability marker for cleavage stage embryos in addition to the rate of development and cell symmetry and size is the location and amount of fragmentation. Fragmentation is caused by individual cells breaking up into little spheres that disperse throughout the embryo volume, within the zona pellucida, as seen in Figure 4.6(a). Some scoring systems quantify the percent of fragmentation per volume in numbers, such as 0%, <30%, 30%-50%, and >50%, while other scoring systems generalize the amount and specify the location, such as minimal fragments, some small fragments localized in the perivitelline space, many small fragments throughout the embryo, many fragments with uneven cell sizes, and so much fragmentation that the cells cannot be distinguished [Baczkowski et al. 2004]. Both of these measurements are completed by focusing through the embryo with DIC imaging and approximating the volumetric percentages by eye. Some studies have emphasized the importance of fragmentation and have considered it almost as important as the rate of development when determining embryo viability [Alikani et al. 1999; della Ragione et al. 2007].

The relative dry mass analysis could be expanded to provide a quantitative three-dimensional fragmentation and polar body analysis. Tracing the boundary of the cell cluster, while making sure to include all of the fragments in Figure 4.6(c), segments the OPD of the cell cluster from the background culture medium and zona pellucida in Figure 4.6(d). Assuming the OPD of each cell was subtracted perfectly during the phase-subtraction count, the relative dry mass of the remaining OPD would correspond to the polar body and fragmentation throughout the entire volume in Figure 4.6(f), and compares to the fragmentation visible in a single DIC image in Figure 4.6(e). Dividing the relative dry mass after the phase-subtraction count by the relative dry mass of the total cell cluster would provide a quantitative percentage of the fragmentation and polar body per volume. Segmenting the OPD of the polar body from the fragmentation would provide a percent of fragmentation alone. This type of measurement may provide a more accurate measure of the fragmentation throughout the embryo and could be applied at any time during preimplantation development before the blastocyst stage. However, it is important to emphasize that precise modeling of each cell is crucial for the fragmentation measurement to be accurate.
Figure 4.6: (a) DIC and (b) OPD images of a live fragmented 3-cell mouse embryo. Tracing the boundary of the cell cluster on the DIC image in (c) creates a mask to segment the OPD in the cell cluster from the background zona pellucida and culture medium in (d). (e) Trace of fragmentation visible in a single DIC image compares to (f) OPD after phase-subtraction count corresponding to total fragmentation throughout the embryo.

4.7 Modeling the Various OPD Contributions within an Oocyte

Another approach to gain a better understanding of the OPD images from oocytes and embryos is to model each of the components individually. The zona pellucida is a spherical shell that surrounds the egg or developing cells, and can be modeled by tracing the inner and outer boundaries in the brightfield image in Figure 4.7(b) with a circular least-squares fit. Assuming the zona pellucida is symmetric, we created two spheres from the boundaries, subtracted the smaller sphere from the larger sphere, and normalized the values from the difference of the two spheres to create a model for the phase of the spherical shell. Multiplying the normalized phase values from the model by the maximum phase value for the zona pellucida in a plot through the center of the oocyte creates the model for the zona pellucida in Figure 4.7(c). A plot through the center of the original OQM image of the oocyte and through the center of the zona pellucida model is shown in Figure 4.7(d). The subtraction of the zona pellucida model from the original OQM image in Figure 4.7(e) shows that the model subtracts the zona pellucida relatively well where the circular boundary fits the zona pellucida boundary, as confirmed by the plot Figure 4.7(f). However, the boundary of the zona pellucida is often not perfectly
circular and should be fit with an ellipse. Additional work is then required to gain an understanding of the effective 3D shape of the zona pellucida when the cross section is elliptical, as discussed in §5.2.1. It is important to note that this technique has also shown an approximate refractive index for the zona pellucida of $0.0076 \pm 0.0015$ above the refractive index of the culture medium. However, this result was only based on two oocytes, and additional samples must be analyzed to determine a more accurate measure.

Figure 4.7: (a) OQM image of a live mouse oocyte. (b) Brightfield image of the oocyte with circular least-squares fits for the inner and outer boundaries of the zona pellucida and the egg. (c) Zona pellucida model and (d) plot of phase values through the center of the oocyte and the zona pellucida model. (e) Subtraction of zona pellucida model from the OQM image and (f) plot of phase values through the center of the oocyte before and after subtraction of the zona pellucida model.

After the zona pellucida has been removed computationally, we can create a model of the egg cytoplasm using the circular least-square fit boundary created in Figure 4.7(b) and assuming the refractive index mismatch between the cytoplasm and the culture medium is 0.02. We can also approximate a model for the mitochondria within the oocyte from the epi-fluorescence image of the MitoTracker dye assuming the difference between the phase of the oocyte and the combination of phase from the cytoplasm and zona pellucida models is equivalent to the phase of the MitoTracker distribution. The grayscale values in the epi-fluorescence image in Figure 4.8(b) were normalized and then multiplied by the difference between the maximum phase value through the center of the oocyte in the OQM image and the sum of the cytoplasm and zona pellucida models. Combining the models for the zona pellucida, cytoplasm, and mitochondria produces the synthetic OQM image in Figure 4.8(c), which compares to the experimental image in Figure 4.8(a).
Figure 4.8: (a) OQM and (b) MitoTracker dye epi-fluorescence images of a live mouse oocyte. (c) Phase model of the oocyte after modeling the zona pellucida (Zona), mitochondria (Mito), and cytoplasm (Cyto). (d) Plot of the phase values through the center of the oocyte for each component that makes up the oocyte model.

A plot through the center of each model and through the center of the experimental image shows the contributions from each model and how the final model compares to the experimental image in Figure 4.8(d). The difference between the model and experimental images derives from the fact that an epi-fluorescence image was used to model the mitochondria and there is no known way to model the inclusions (dark spots) throughout the egg in Figure 4.7(b). A z-stack of confocal or two-photon images would provide a more accurate representation of the mitochondria [Squirrell et al. 2003] as discussed in §5.2.1. It is important to note the large change in phase between the maximum of the cytoplasm and the maximum of the oocyte in Figure 4.8(d). The large contribution of phase from the mitochondria could explain the difficulty in determining the location of the nucleus from an OQM image since the refractive index of the nucleus is greater than the cytoplasm, but less than the mitochondria. However, an accurate measure of the refractive index mismatch between the cytoplasm and the culture medium must be determined to validate this hypothesis.
4.7.1 Mitochondrial Distribution

Oocyte viability measures have received more attention in recent years due to legislative and moral concerns that limit the number of embryos that are created. In addition, the use of the best quality oocytes leads to the highest probability of producing healthier embryos for transfer back into the mother. Previous work has shown that a homogeneous distribution of mitochondria correlates to high developmental potential, which compares to localized mitochondrial distributions (clumping) in unhealthy oocytes [Muggleton-Harris and Brown 1988; Nagai et al. 2006]. However, mitochondria have only been visible with the use of fluorescent dyes such as MitoTracker [Squirrell et al. 2003], or transgenic embryos that express green fluorescence protein (GFP) [Nagai et al. 2006], thereby limiting their analysis to research oriented applications. The expansion of the oocyte model may provide the foundation to measure the mitochondrial distribution within an oocyte and become the first non-toxic method that could be used in a clinical setting.

Figure 4.9(a) and 4.9(d) show DIC images for two live mouse oocytes. Modeling the zona pellucida and cytoplasm of the cell with circular least-squares fits and assuming a refractive index mismatch of 0.02 between the cytoplasm and the culture medium, and subtracting the models from the experimental OQM image provides the results in Figure 4.9(b) and 4.9(e). The orange/red regions within the OQM images with phase values $\geq 3$ radians seem to match relatively well with the distribution of MitoTracker dye in the corresponding epi-fluorescence images in Figure 4.9(c) and 4.9(f). It is important to note that the integration times may differ between the two epi-fluorescence images so they can only be interpreted in terms of the distribution and not the strength of the signal. The remaining phase in the subtracted OQM image also corresponds to mismatch between the models and the experimental images and the inclusions seen in the DIC image. Improvements to the models, such as ellipsoidal least-square fits for the zona pellucida and an accurate refractive index mismatch between the cytoplasm and the culture medium will improve the subtraction, but a proper model for the inclusions is not known currently.

It is important to note that the epi-fluorescence image is not a good measure for ground truth because the image only shows the distribution of MitoTracker dye within the depth of field. Out-of-focus dye will spread across the pixels of the camera, as discussed in §2.3.1, and either have the appearance of a larger distribution or not be visible within the image. A z-stack of confocal fluorescence or two-photon images would be more appropriate to test whether the remaining phase in the subtracted OQM image will provide a valid measurement of the mitochondrial distribution [Squirrell et al. 2003].
Figure 4.9: DIC images of a live mouse oocyte in (a) and (d). Remaining phase ≥ 3 radians in (b) and (e) after subtraction of the zona pellucida and cytoplasm models from the original OQM images corresponds to the mitochondrial distribution shown in the epi-fluorescence images of MitoTracker dye in (c) and (f).
5. Future Work

While this dissertation is the culmination of much work in regard to embryo imaging with OQM, the results that have been presented are just a small part of the overall project to determine embryo viability with the combination of OQM and DIC images. Additional work must be completed to build a stable, easy-to-use instrument that can be brought into the clinic and assess additional aspects of the theory behind imaging live embryos to create a complete embryo model. In this chapter, we discuss many of the improvements and future work that are necessary to continue this project and work toward the long-term goal of determining which single embryo will provide the largest probability of developing into a successful pregnancy.

5.1 OQM Improvements

The three primary obstacles in regard to developing a stable OQM instrument that is capable of real-time acquisition are: (1) the ability to match the wavefront of the reference path to the wavefront of the signal path to create a large fringe pattern, (2) the need to acquire multiple images in order to reconstruct a single wrapped phase image, and (3) the computation time required to unwrap a two-dimensional phase image. Here we describe suggestions and preliminary results for techniques that will help overcome these obstacles and develop the next generation bench-top instrument. Once a stable, repeatable, next-generation instrument has been constructed, the knowledge that has been obtained can be used to design a bolt-on instrument that can attach to the microscopes that are used in the clinic.

5.1.1 Telecentric Imaging System

In the current optical configuration, the condenser lens focuses the light in the signal path that illuminates the sample in Figure 5.1, resulting in converging light at the camera port of the microscope, and diverging light incident on the detector array of the CCD cameras. A lens must then be positioned in the reference path to focus the collimated light and match the wavefront of the reference beam to the wavefront of the signal beam to create the largest fringe pattern possible. A small fringe pattern with thin fringes results from quick $2\pi$ phase changes from the high spatial frequencies that are within the field of view. A large fringe pattern, with a large, circular, zeroth-order interference fringe and wide fringes, results from matching the wavefronts such that only low spatial frequencies are within the field of view. As the fringe pattern increases, the
zeroth-order interference fringe becomes larger than the field of view and the wavefront mismatch between the reference and signal paths approaches a constant.

![Diagram of optical layout](image)

**Figure 5.1:** Current optical layout for OQM that has divergent light incident on the CCD cameras. L.P. represents a linear polarizer.

The use of divergent light in the reference and signal paths introduces instability to the image acquisition because the reference path must be realigned for a small change in the sample path, such as a curvature of the immersion medium. If the embryo is positioned near the edge of the culture drop, the interference pattern may be centered for the acquisition of the signal image, but will move as the sample translates out of the field of view. The user must then realign the reference path to ensure the fringe pattern is centered during acquisition of the blank image, or the background wavefront mismatch between the signal and reference paths will not be subtracted from the sample image correctly. A telecentric imaging system shown in Figure 5.2 could be developed to remove this instability. In a telecentric system, an extra lens would be positioned in the signal arm of the interferometer to focus the collimated light at the back-focal (pupil) plane of the condenser lens and provide a collimated beam illuminating the sample. A collimated beam at the sample would result in a collimated beam at the camera port, and a two-lens relay would be needed to relay the image at the camera port to the image plane of the CCD cameras with a collimated beam. Since the reference path is already collimated, it is possible that the lens used to match the wavefronts can be removed, but a beam expander would be needed to match the beam diameter of both paths. The addition
of these three elements would stabilize the image acquisition, remove most of the complication in regard to acquiring an OQM image, and reduce the aberrations since the system would have collimated beams traveling through the thick beamsplitters and polarization elements.

**Figure 5.2:** Optical layout for a telecentric imaging system, where the elements labeled in blue are the primary optical elements needed to modify the current system.

A design concern with this system is keeping the telecentricity with the condenser and objective lenses. The lens added to the signal path must be aligned accurately with respect to the condenser lens such that the light focuses at the pupil plane of the condenser. However, the condenser is often repositioned to provide Köhler illumination, so the lens must be repositioned with the condenser lens to ensure a collimated beam still illuminates the sample. The same concern exists with respect to the objective lens and the tube lens, because the objective is generally repositioned to find the best focus of the sample and the tube lens is stationary within the microscope housing. The position of the objective lens with respect to the tube lens is not a concern for DIC imaging because the objective is infinity-corrected resulting in a collimated beam traveling to the tube lens. This allows the infinity space to be expanded on the TE2000 microscope, where we have positioned the hot and cold mirrors for the scanning modes of the Keck 3D Fusion Microscope. The optical design of the Keck 3D Fusion Microscope would need to incorporate all of these factors and determine whether additional optical elements would be needed between the objective and the tube lenses to produce a telecentric system with.
the expanded infinity space. The optical design with the Nikon TE200 microscope would not be as complicated since the infinity space cannot be expanded, so the user would only have to remember to keep the objective fixed and use the piezoelectric z-stage to focus the sample once the system is aligned. However, the alignment of the condenser lens is still a concern with the TE200 microscope.

Another design concern is the extra optical path required for the relay positioned between the camera port and the CCD cameras. The simplest relay configuration would use two lenses with identical focal lengths to keep the magnification equal to one between the image plane at the CCD cameras and the image plane at the camera port. However, the focal length of the second lens must be long enough to pass through a linear polarizer and two beamsplitters to reach the CCD cameras. This optical distance is at least 170 mm in the current system, incorporating the 2 one-inch beamsplitters and rounding up 4.5 mm to allow extra room for the mounts and the thickness of the linear polarizer, which would require 680 mm of optical path between the camera port of the microscope and the CCD cameras for a basic two lens relay. A more elegant relay should be designed to reduce the optical path required after the camera port and still provide a collimated beam incident on the CCD cameras.

It is important to note that the goal of this new design is to provide a constant wavefront mismatch across the field of view to remove the need to divide by the blank. Thus, a perfect telecentric system may not be required, but additional work is needed to determine the tolerance for the beam divergence that will provide the necessary wavefront mismatch.

5.1.2 Polarization Maintaining Fiber

Another instability in regard to the fringe pattern is the polarization of the light output from the fiber coupled to the laser. While the fiber in the current configuration is single-mode, stresses on the fiber from compression, bending, twisting, and kinking produce birefringence effects that change the polarization state of the light along the fiber [Rashleigh 1983]. The birefringent effects are also time-variant with environmental changes, such as temperature fluctuations, vibration, and external electric and magnetic fields, resulting in an unstable output polarization. Polarization maintaining (PM) fiber typically induces a high birefringence along the length of the fiber with a non-cylindrical core to output a single polarization state [Rashleigh 1983; Noda et al. 1986]. The use of PM fiber also removes the need for the linear polarizers after the fiber outputs to ensure the paths contain the proper polarization, but two polarizers could still be used to adjust the laser power for a larger range of samples. However, the advantages of using PM fiber may be negated by the fact that the interferometer arms are in open-air and prone to path length changes and misalignment from environmental effects, such as air currents and...
vibrations. The interferometer arms should then be enclosed as much as possible when PM fiber is introduced into the system to help stabilize the imaging system.

### 5.1.3 Real-Time Phase Reconstruction

The SNR analysis presented in §3.5.2 provides a mathematical expression for the result of the phase resolutions to understand the contribution of each noise term to the final phase image. While DC term subtraction and camera normalization in the current OQM reconstruction in §3.5.1 (26) removes the most noise terms and provides the most accurate measurement of quantitative phase, this technique complicates the acquisition of real-time images because multiple images are required to reconstruct a single phase image. Using the SNR analysis, it is possible to develop a new phase reconstruction technique that minimizes the number of images that must be acquired.

Balanced mixing is the ideal phase reconstruction because only the acquisition of a single set of images from the mix of the signal and reference paths is required. However, balanced mixing currently provides a phase error of 0.20 radians with a telecentric system that does not divide by a blank to remove the wavefront mismatch between the signal and reference paths in the final image. The SNR analysis provides the expression for the magnitude and phase of balanced mixing:

\[
E_{BM} = \Theta e^{j(x_s-x_r)}[Ae^{j\alpha} + Ae^{-j\alpha}\Phi e^{-j2(x_s-x_r)} + (A^2\Gamma_S + \Gamma_R)e^{-j(x_s-x_r)}] + I_M
\]

where:

\[
\Theta = \sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}
\]  

is the sum of the square root of the irradiance in the signal path with the sample moved out of the field of view (\(BS_k\)) and the square root of the irradiance of the reference path (\(R_k\)),

\[
\Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{BS_k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}}
\]

is the error from an imbalance of the irradiance between the cameras in the blank image, such that \(\Phi\) would go to zero if the irradiance was the same on every camera,

\[
\Gamma_R = \frac{\sum_{k=0}^{3} j^k R_k}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}}
\]
\[ \Gamma_S = \frac{\sum_{k=0}^{3} j^k B S_k}{\sum_{k=0}^{3} \sqrt{B S_k}} \]

are the errors from fixed pattern noise in the individual reference and signal arms, such that \( \Gamma_R \) and \( \Gamma_S \) would go to zero if the coherent noise was the same on every camera,

\[ I_M = \sum_{k=0}^{3} j^k I_{M,k} \]

is the error from dark current noise, such that \( I_M \) would go to zero if the dark current was the same on every camera, and \( \chi_s - \chi_r \) is the wavefront mismatch between the reference and signal paths. Using a telecentric system described in §5.1.1, the wavefront mismatch would go to zero, Eq. (1) would reduce to:

\[ E_{BM} = \Theta \left[ A e^{j\alpha} + A e^{-j\alpha} \Phi + (A^2 \Gamma_S + \Gamma_R) \right] + I_M, \]

and the division by a blank image would not be required. Images can then be collected of the signal path with the sample moved out of the field of view, reference path, and dark current terms to model \( \Theta, \Gamma_S, \Gamma_R, \) and \( I_D \) before acquisition of the sample. A single set of mix images \( (M_k) \) of the sample can then be acquired and substituted into the phase reconstruction:

\[ E_{realtime} = \frac{\sum_{k=0}^{3} j^k M_k}{\Theta} - (\Gamma_S + \Gamma_R) = A e^{j\alpha} + A e^{-j\alpha} \Phi + \Gamma_S (A^2 - 1) + \frac{I_{M} - I_D}{\Theta} \]

which is equivalent to:

\[ E_{realtime} = \frac{\sum_{k=0}^{3} j^k (M_k - B S_k - R_k - I_D)}{\sum_{k=0}^{3} \sqrt{B S_k / R_k}} \]

The reconstruction in Eq. (9) is also similar to the current OQM reconstruction, but we have replaced the images of the signal path for the sample \( (S_k) \) with images of the signal path for the blank \( (BS_k) \), and summed the square root of both the blank signal and reference images in the denominator. However, the SNR analysis must be completed with Eq. (9) to ensure the output is equivalent to Eq. (8). The error of the phase from the reconstruction in Eq. (8) is then dependent on the imbalance of the irradiance between the cameras \( (\Phi) \) and a sample dependent contribution of the fixed pattern noise in the signal.
path \( (I_S) \). It is important to note that the subtraction of the dark current term \( I_D \) may provide a minimal change in the accuracy of the phase image because the division by \( \Theta \) brings the dark current term \( I_M \) near zero.

The comparison of wrapped phase of a PMMA bead from the phase reconstruction in Eq. (8) to balanced mixing and the current OQM phase reconstruction are shown in Figure 5.3. The balanced mixing image in Figure 5.3(a) shows striations within the background of the image corresponding to the fixed pattern noise within the system, but a distinct difference is not visible between the current OQM phase reconstruction without dividing by the blank in Figure 5.3(b) and the new phase reconstruction in Figure 5.3(c).

![Figure 5.3](image.png)

**Figure 5.3:** Wrapped phase result from (a) balanced mixing, (b) the current OQM reconstruction in §3.5.1 (26), and (c) the real-time algorithm in Eq. (8). The telecentric imaging system would remove the visible wavefront mismatch in the background of the image.

To compare the new phase reconstruction with the current OQM phase reconstruction, we repeated the analysis in §3.5.2.5 for the phase reconstruction in Eq. (8). The plots in Figure 5.4 shows the mean phase error of both phase reconstructions across the field of view *versus* the constant phase value between \(-4\pi\) and \(4\pi\), where the error bars depict the standard deviation of the phase error across the field of view. These plots show that the phase reconstruction in Eq. (8) will provide a slightly higher RMS error of 0.11 radians, compared to 0.08 radians with the current OQM reconstruction that divides by a blank. However, the slight inaccuracy may be acceptable considering the phase reconstruction in Eq. (8) could acquire images in real-time. It is important to note that the periodic error still exists in the phase reconstruction in Eq. (8) because it is dependent on the multiplicative noise term \( \Phi \) from the imbalance of irradiance between
the cameras. Additional work is required to balance the irradiance and the fixed pattern noise between the cameras in the new optical design to remove the periodicity and further reduce the RMS errors. If the fixed pattern noise can also be reduced, or at least matched across the cameras, it is possible that the images of the reference and blank signal paths may not be required and accurate phase information would be provided from balanced mixing alone.

![Figure 5.4](image.png)

**Figure 5.4:** Error in the phase reconstruction for (a) the current OQM reconstruction in §3.5.1 (26) that also divides an image by a blank and (b) the reconstruction in Eq. (8) that could be acquired in real-time without dividing by a blank in a telecentric imaging system.

It is also important to note that the phase reconstruction in Eq. (8) assumes the laser power and noise terms do not change between the acquisition of the reference and signal images used to model the noise terms and the acquisition of the mix of the signal and reference images of the sample. The current OQM reconstruction uses the same assumption in regard to the reference and blank images, but an image of the signal path has been acquired directly after acquisition of the mix of the signal and reference paths. To test the consistency of the irradiance incident on the cameras with the current instrumentation, 100 images were acquired of the reference and signal paths separately after allowing the laser to warm-up for 1 hour, 2 hours, and 3 hours.
Figure 5.5: Histograms for the standard deviation of each pixel from the mean grayscale value over the collection of 100 straight images.

The plots in Figure 5.5 show the standard deviation of the counts from the mean count of each pixel over the 100 images of both paths using one camera. After 1 hour, the majority of the pixels fluctuated less than 1.5 counts or 5.9% of the 25.5 count mean over the entire field of view for the reference path, and 4 counts or 5.2% of the 76.8 count mean over the entire field of view for the signal path. After 2 hours, the majority of the pixels fluctuated less than 1.5 counts or 3.8% of the 39.2 count mean in the reference path, and 4 counts or 13.6% of the 29.5 count mean in the signal path. After 3 hours, the majority of the pixels fluctuated less than 1.25 counts or 3.3% of the 38.2 count mean in the reference path, and 1.75 counts or 5.3% of the 33.2 count mean in the signal path. The fluctuation was relatively consistent in the reference path, with a slight decrease after
warming up for 2 hours, but the fluctuation in the signal path dropped dramatically after allowing the laser to warm up 3 hours. It is possible that the laser required 3 hours to warm up properly, but it is more likely that an environmental effect, such as air currents or vibration, was present during the acquisitions after 1 hour and 2 hours. However, this dataset shows that a fluctuation of $\leq 5\%$ is possible with both interferometer arms exposed to open air, and it is realistic to assume this percentage will decrease with the use of PM fiber and enclosing of the interferometer arms.

5.1.3.1 FPGA/GPU Phase Unwrap

Phase unwrapping further complicates real-time imaging of samples that produce a change in phase greater than $2\pi$. We have used the $L^p$-norm algorithm to unwrap our phase images [Ghiglia and Pritt 1998] because it seems to provide the most repeatable and accurate results for circular objects, such as PMMA beads and mouse embryos. However, this algorithm requires time on the order of minutes to unwrap a single $640 \times 480$ image of quantitative phase on a computer using a 1.8 GHz processor and 1.5 GB of RAM. Preliminary results have shown that a 95 second phase unwrap of a glass bead on a desktop computer could be reduced 42.6% to 40.5 seconds (2.35x speed up) by implementing the phase unwrap on a field programmable gate array (FPGA), or 19.1% to 18.1 seconds (5.24x speed up) with the use of a graphics processing unit (GPU) [Braganza 2008b]. Additional work to incorporate the phase reconstruction algorithm in conjunction with the phase unwrap on a GPU device has also shown promising preliminary results [Mistry in preparation 2009]. Thus, a telecentric system with PM fiber that processes the phase reconstruction in Eq. (8) and phase unwrap on a GPU could provide unwrapped phase images in near-real-time, with real-time images available after the development of a faster GPU or similar processing device.

5.2 Embryo Viability Measurement Improvements

The quantitative embryo viability measures derived from the combination of DIC and OQM images have shown promising preliminary results, but much work is required to prove that the measures are accurate and useful in a clinical application. Large datasets must be analyzed blind by a trained biologist in order to prove that accurate measures can be determined by an unbiased third party. Assuming accurate measurements can be developed, additional studies must be conducted in which embryos that are characterized by these new measures are transferred back into a mouse and the successful pregnancy statistics are evaluated to determine whether the measures are truly indicative of embryo
health. But before these long-term studies can be considered, additional work is required to improve the models and automate the measures from the images acquired with the next-generation OQM system described above, because the current manual procedure is too time consuming to conduct an experiment with embryo numbers in the 10’s or 100’s.

5.2.1 Embryo Modeling

The cell models created within the phase-subtraction cell-counting technique were able to provide accurate cell counts because the cells are relatively large in size. However, the accuracy of the cell model must be improved if the relative dry mass will be analyzed for the cell symmetry, size, and fragmentation in embryos, and the mitochondrial distribution in oocytes. Here we discuss various aspects of multimodal imaging of embryos and theory behind quantitative phase imaging of spherical beads that must be developed in order to improve our models.

Most microscopy analyses are completed by observing individual slices of a three-dimensional object in either the transverse or lateral planes, depending on the imaging technique, and making assumptions about how these slices work together in the third dimension. With all of the work that has been conducted on visualizing 3D data, people are still accustomed to looking at 2D images. There has been increased popularity in volumetric imaging in recent years with the availability of more computing power, but an accepted technique to display 3D information on a 2D screen has not been developed. We believe that using 3D information to develop accurate viability measures with 2D images already collected within the clinic will increase the rate at which our quantitative viability measures will be accepted for use, assuming we can make them user friendly and prove their clinical significance.

Studies have shown that volumetric data can be collected for various types of embryos [Boppart et al. 1997; Aiken et al. 2004; Tassy et al. 2006; Pogorelov and Pogorelova 2008]. The easiest technique to implement on the Keck 3D Fusion Microscope would be to acquire confocal and two-photon z-stacks of Hoechst dye that stains the nuclei (775 nm two-photon excitation, 400 nm - 480 nm emission), calcium dye Calcein AM that stains the cytoplasm (775 nm two-photon excitation, 505 nm – 545 nm emission), styryl dye FM4-64 that stains the membrane (568 nm confocal excitation, 600 nm – 700 nm emission) [Aiken et al. 2004], and MitoTracker dye that stains the mitochondria (488 nm confocal excitation, 510 nm – 550 nm emission). Fusing the z-stacks together would provide a volumetric rendering of the complete embryo, including many of the internal structures, for us to analyze.

The volumetric data would then be used to try to determine a relation for the thickness of the egg, zona pellucida, and cells during all stages of preimplantation development from the radii in the xy-imaging plane. Such a relation would allow each
cell to be modeled accurately in the phase-subtraction cell count to provide an accurate measure of the relative dry mass, and provide the means to model the zona pellucida within the measure of the mitochondrial distribution in oocytes. The volumetric data would also help derive accurate models of the nuclei and mitochondria within the cells to develop the ground truth for the analysis of oocytes and zygotes.

We have shown in §3.6.3 that accurate phase images can be acquired with OQM for large spherical objects much larger than the depth of field when the object is near center-focus, but we have not characterized the accuracy of overlapping spheres. We have assumed the total cluster of cells acts like a large sphere when the embryo develops beyond the 8-cell stage, but the accuracy of this assumption is unknown. The volumetric data could also be used to create an $xz$-slice of multiple overlapping spheres with a similar arrangement to the cells in an embryo. This slice can then be analyzed with the coherent ray tracing model to determine the accuracy of the OQM images of mouse embryos at the various stages of preimplantation development. Ideally, the model would be expanded to include diffraction, but the refraction model would suffice as a first approximation.

5.2.2 Automation Techniques

We have combined DIC images with OQM images to develop quantitative viability measures because clinicians currently use DIC to assess embryo health. We believe it is easier for a technique to gain clinical acceptance if it is similar to the current state of the art and does not require extensive training for proper use. The ideal technique would be fully automated such that a clinician would acquire the necessary images and then be given the number of cells, the percentage of fragmentation, etc. It would also be advantageous to show the sequence of steps for the measurement techniques so the clinician can be assured the measurement was completed accurately.

Unfortunately, the contrast mechanism of DIC makes the use of conventional image processing techniques extremely difficult to register the DIC image to the OQM image and automate the selection of boundaries in the DIC image. Figure 5.6 shows DIC images of a PMMA bead and an 8-cell live mouse embryo. The arrow in the bottom left corner of each image depicts the direction of the shear axis, which corresponds to the direction of the derivative, as described in §2.3.1.3. The shading in a DIC image is proportional to the derivative in the direction of the shear, resulting in a cosine dependent shading for a circular object. Thus, the diameter of the PMMA bead parallel to the shear axis provides maximum bright and dark shading, and there is zero shading along the diameter orthogonal to the shear axis. The contrast in the orthogonal direction results from refraction and diffraction alone. In the image of the embryo, we can see that the contrast for the individual cell boundaries is more prominent in the direction of the shear.
axis and minimal in the orthogonal direction. As a result, the anti-symmetric (dark and bright) and cosine-dependent shading does not provide the desired result with the use of thresholds and derivatives within many of the conventional image processing techniques. In this section we describe many of the image processing techniques that we have applied to DIC images in an attempt to automate the viability measures. We have included the techniques that have not worked in addition to the techniques that have shown promise in hopes that the information will be helpful for anyone continuing this work in the future.

![Figure 5.6: DIC images for (a) a PMMA bead and (b) an 8-cell live mouse embryo to show the cosine dependent shading inherent in DIC images. The arrow in the bottom left corner of the images depicts the shear axis.](image)

### 5.2.2.1 Hilbert Transform

One technique that has been shown to provide symmetrical shading in DIC images involves the use of a Hilbert transform [Arnison et al 2000; Heise et al. 2005]. The Hilbert transform reverses the sign of components less than zero and conserves the sign of components greater than zero to produce symmetric shading. Mathematically, the one-dimensional Hilbert transform is defined by the convolution:

\[
H[f(x)] = f(x) \ast \frac{1}{\pi x} = \frac{1}{\pi} \int_{-\infty}^{\infty} \frac{f(x')}{x-x'} dx',
\]  

which can be replaced by a multiplication in the frequency domain:

\[
H[f(x)] = F^{-1}\{ j \text{sgn}(s) \tilde{F}(s) \},
\]

where \( \tilde{F}(s) = F(f(x)) \), the operator \(F\) is the Fourier transform, \(F^{-1}\) is the inverse Fourier transform, \(j\) is the \(\sqrt{-1}\), and \(\text{sgn}(s)\) is the signum function defined as:
\[ sgn(s) = \begin{cases} 
-1 & s < 0 \\
0 & s = 0. \\
1 & s > 0 
\end{cases} \] (12)

The method described by Arnison applies the two dimensional form of Eq. (10) and requires the signum function to be parallel to the shear axis [Arnison et al. 2000]. Thus, the DIC image in Figure 5.6(b) must be rotated approximately 45°, as shown in Figure 5.7(a). Applying the two dimensional Hilbert transform with a signum function that contains all columns left of center equal to -1, all columns right of center equal to 1, and the center column equal to zero, produces the image in Figure 5.7(b).

![Figure 5.7](image)

**Figure 5.7:** (a) Rotated DIC image and (b) Hilbert transform result of a method to remove the anti-symmetric shading of DIC [Arnison et al. 2000].

The Hilbert transformed image in Figure 5.7(b) is symmetric such that all the boundaries have a dark shadow, in comparison to the dark and bright shadows in the original DIC image in Figure 5.7(a). However, the intensity of the shadow in the Hilbert transformed image now contains a cosine squared dependence that prevents techniques using a single threshold from producing a complete boundary of the sample.

The method described by Heise is an iterative technique that continually applies a Hilbert transform to remove the cosine squared dependence in order to produce a complete boundary [Heise et al. 2005]. The technique takes the difference of a Hilbert transform in the forward direction and a differentiation in the backward direction to create a residual image that is used during the next iteration. The summation of the Hilbert transform for each residual image provides the final image. The recursive steps amplify structures within the object on each step but they also amplify the noise, so a low-pass filter is included in the Hilbert transform to amplify the structures with lower frequencies but not the high frequency noise. The modified Hilbert transform can be expressed mathematically as:

\[
H\{f(x,y)\} = F^{-1}\{ j \ g_{\alpha}(u-v) \ \frac{\alpha}{\alpha+n(|u-v|+|u+v|)} \tilde{F}(u,v) \},
\] (13)
where \( \tilde{F}(u, v) = F(f(x, y)) \), the image \( u - v \) contains increasing values along the diagonal of the matrix shown in Figure 5.8(a), the image \( u + v \) contains increasing values along the orthogonal diagonal, the image of \( (|u - v| + |u + v|) \) is a two dimensional inverted saw tooth with zero at the origin and the maximum value at the extents of the two diagonals, \( n \) is the iteration number, and \( \alpha \) is the parameter that determines the width of the low-pass filter. An image of the low-pass filter with \( n = 2 \) and \( \alpha = 75 \) is shown in Figure 5.8(b), and a plot of the cross section along the center row is shown in Figure 5.8(c).

Another difference between the methods described by Arnison and Heise is the axis of the signum function. In the method described by Arnison, the axis of the signum function is parallel to the shear axis, but the axis is perpendicular in the method described by Heise. The function \( u - v \) was then used for the DIC image in Figure 5.6(b), where the shear axis is along the diagonal from the top left corner to the bottom right corner of the image. The technique described by Heise is broken down into two parts: the initialization and the recursion. The initialization includes the differential operator:

\[
D = \begin{bmatrix} 1 & 0 \\ 0 & -1 \end{bmatrix},
\]

(14)

where the diagonal can be parallel to the shear to obtain more boundary information with less resolution in the overall image after more iteration, and less boundary information with more resolution if the diagonal is perpendicular to the shear, the modified Hilbert transformed images:
\( I(0) = \text{dic} \) \hspace{1cm} (15)

\( I(1) = D * H_{\alpha}^0[I(0)] \), \hspace{1cm} (16)

where \text{dic} is the input DIC image, \( * \) is the convolution, and \( H_{\alpha}^0 \) is the modified Hilbert transform with iteration 0 and low-pass filter parameter \( \alpha \), the residual images:

\[
\Delta I(0) = 0 \hspace{1cm} (17)
\]

\[
\Delta I(1) = I(0) - I(1) \hspace{1cm} (18)
\]

and the resultant images for the first two iterations:

\[
R(0) = H_{\alpha}^0[I(0)] \hspace{1cm} (19)
\]

\[
R(1) = R(0) + H_{\alpha}^1[\Delta I(1)] \hspace{1cm} (20)
\]

The recursion for iterations \( n > 2 \) are:

\[
\Delta I(n) = \Delta I(n - 1) - I(n - 2) \hspace{1cm} (21)
\]

\[
I(n) = D * H_{\alpha}^n[\Delta I(n - 1)] \hspace{1cm} (22)
\]

\[
R(n) = R(n - 1) + H_{\alpha}^n[\Delta I(n)] \hspace{1cm} (23)
\]

The result of applying the method described by Heise with the DIC image in Figure 5.6(b), \( \alpha = 75 \), \( n = 1, 2, 3, 4 \), and the differential operator perpendicular to the shear axis are shown in Figure 5.9, and the results with the same values for \( \alpha \) and \( n \) but with the differential operator parallel to the shear axis are shown in Figure 5.10.
Figure 5.9: Result of an iterative method to remove the anti-symmetric cosine dependent shading in a DIC image with $\alpha = 75$, the differential operator perpendicular to the shear axis, and the iteration number equal to (a) 1, (b) 2, (c) 3, and (d) 4 [Heise et al. 2005].

Figure 5.10: Result of an iterative method to remove the anti-symmetric cosine dependent shading in a DIC image with $\alpha = 75$, the differential operator parallel to the shear axis, and the iteration number equal to (a) 1, (b) 2, (c) 3, and (d) 4 [Heise et al. 2005].
The results in Figures 5.9 and 5.10 may provide less cosine squared dependence in the intensity along the boundaries, but a single threshold is still not able to differentiate the complete boundary. There is also a streaking artifact introduced parallel to the shear axis after multiple iterations. This artifact also exists in the method described by Arnison, but is less noticeable. In addition, the method described by Heise blurs the shadow along the shear direction, which may lead to inaccurate boundary locations. As a result, the Hilbert transform techniques may help, but are not the solution to automate the analysis of DIC images.

5.2.2.2 Iterative Radial Voting

The iterative radial voting technique has been shown to segment cells within an image that contains an evenly illuminated and detected field of view, such as brightfield and fluorescence microscopy [Parvin, et al. 2007], and is based on the assumption that the centroid of an object is along the gradient from the object’s boundary. The algorithm begins with an input image, such as the DIC image in Figure 5.11(a), and computes the gradient in the $\hat{x}$ and $\hat{y}$ directions, which correspond to the direction of increasing columns ($dx$) and rows ($dy$) respectively. The algorithm computes the radial derivative from the individual gradients:

$$R = \sqrt{(dx)^2 + (dy)^2}$$ (24)

to produce the image in Figure 5.11(b).

![Figure 5.11: (a) Original DIC image and (b) the resulting normalized radial derivative.](image)

A threshold value $\gamma$ is set to ignore small radial changes in intensity in the original image by substituting a value of zero for all pixels with a radial derivative less than $\gamma$ in Figure 5.12. Radial changes in intensity could correspond to irregularities in the light source,
optics, or detector in an imperfect imaging system, or small biological characteristics in the sample assuming the imaging technique used to create the image does not produce a radially dependent intensity along the sample. The algorithm records the pixel locations (P) for all values in the radial derivative image greater than $\gamma$, which should correspond to the points along the boundary of the objects, and then creates the initial voting direction image by normalizing the individual $\hat{x}$ and $\hat{y}$ gradient images to values between [-1 1].

![Figure 5.12: Radial derivative of the DIC image with pixel values below $\gamma$ equal to (a) 0.1, (b) 0.15, and (c) 0.2 set to zero.](image)

The heart of the technique is the use of a sector of half angle $\Delta$ in Figure 5.13 that emanates from each point P to find the center point of the objects in the field of view. The algorithm determines the radial direction $g(P)$ for each point $P$ from the voting direction image, and projects the sector of angle $2\Delta$ and radius $r$ in the radial direction. A number proportional to the iteration number is added to each pixel within the minimum ($r_{min}$) and maximum ($r_{max}$) radii in the sector to the final voting image $V$, where $V$ is the same size as the input image and initially filled with zeros. After the sector was applied to each boundary pixel $P$, the algorithm creates each sector on $V$ and records the pixel location $U$ that contains the largest value. The largest value corresponds to the overlap of sectors from multiple points along the boundary. Calculating the radial gradient between $P$ and the corresponding pixel $U$ recreates the voting direction images. The algorithm reiterates the use of the sector on the same boundary pixels $P$ by calculating new radial directions $g(P)$ from the updated voting direction images, and applies a sector with a smaller $\Delta$. The algorithm continually reiterates until the sector nears a straight lines with a $\Delta$ approximately equal to zero.
Figure 5.13: Sector of half angle $\Delta$ from point $P$ along radial derivative direction $g(P)$ used in the iterative process to highlight pixels within $r_{\text{min}}$ and $r_{\text{max}}$ [Parvin, et al. 2007].

Figure 5.14: Resultant images from the iterative process with $\gamma$ equal to (a) 0.1, (b) 0.15, and (c) 0.2.

Figure 5.15: Pixel locations found on the DIC image using the iterative voting technique with $\gamma = 0.15$ and thresholding the resultant images $V$ by (a) 0, (b) 0.25, and (c) 0.5.

After the iterative process is complete, the final image $V$ contains a background corresponding to the first application of sectors, and the pixel values increase toward the center of the objects. The algorithm normalizes $V$ such that the pixels closer to the calculated center points are weighted toward 1 in Figure 5.14. The algorithm applies a
threshold to the normalized image V to provide the desired size of the center points and
plots all pixels above the threshold on the original image in Figure 5.15.

The iterative radial voting technique is a useful method for finding the center
point of curved objects within an image assuming the pixel intensity across each object is
relatively uniform. However, a DIC image emphasizes the gradient of the phase along the
shear direction, and does not provide an even intensity across the sample. As a result, the
radial derivative provides a cosine dependent second derivative of the sample with the
maximum along the shear axis. This can be seen in the zona pellucida in the radial
derivative images in Figure 5.12 where portions of the zona pellucida boundary in the
normalized radial derivative are below the 0.1 threshold, while portions of the boundary
along the shear axis are above the 0.2 threshold. This characteristic of the DIC image
prevents the iterative radial voting technique from developing a continuous boundary or
an accurate center point for each cell.

The other drawback of using the iterative radial voting technique to find the
boundaries of the cells is that the method was optimized for calculating the center point
for the cells. Assuming the input image had an even pixel intensity across the objects in
the image, the radial derivative would provide more edge information than the use of the
entire technique. Using a small maximum radius for the sector could highlight potential
features near the boundary, but the final result would be proportional to the radial
derivative. However, this is an advantage of the second derivative resulting from the use
of the DIC image. The second derivative of an edge provides one positive and one
negative pixel on either side of the edge. Assuming the pixel intensities were above the
threshold value, two sectors could be produced that overlap across the edge. The use of
the second derivative and a short maximum radius of the sector could then provide a
radial edge detection technique, but the DIC image only provides the derivative in a
single direction so half of the information is not available.

5.2.2.3 Random Walk

Segmenting the cell cluster from the background could be an important first step
to the automation of the cell counting techniques because the outer boundary could be
used to register the DIC image to the OQM image, and possibly provide the means to
determine the boundaries of the cells along the perimeter of the cell cluster. One
technique that has been investigated by Prof. Chia-Ling (Charlene) Tsai at Iona College
is the use of Canny edge detection and a Random Walk algorithm [Tsai et al. submitted
2008].
Figure 5.16: (a) Original DIC image. (b) Canny edge detection of the DIC image. (c) Seed points derived from Canny edge detection where the foreground points are yellow and the background points along the perimeter of the cell cluster are black. (d) Binary mask for the cell cluster in the DIC image after applying the Random Walk algorithm.

Canny edge detection, non-maximum suppression, and hysteresis with two thresholds provides boundary points within the cell cluster and along portions of the zona pellucida in Figure 5.16(b), and clustering the boundary points with another threshold differentiates boundary points within the cell cluster (foreground) from points outside the cell cluster (background) in Figure 5.16(c). The algorithm then uses the foreground points as seed points in the Random Walk algorithm [Grady 2006] to develop the mask for the cell cluster in Figure 5.16(d). The same algorithm was applied to create a mask for the corresponding OQM image, and boundaries of both masks are registered with an affine transform. A dataset of 28 image pairs were registered with this technique and 24 (85.7%) were registered successfully with an average alignment accuracy of 0.46 pixel. However, 3 of the 4 failed image pairs would have been registered successfully had a similarity transform been utilized instead of an affine transform. A similarity transform is more appropriate because it limits the registration algorithm from trying to over-fit the masks if the exact cell cluster boundary was not determined. §2.3.3 describes a registration algorithm that already aligns the image space of DIC and OQM such that the
pixel sizes and fields of view are equivalent, so the only misregistration between the images should be from rotation and translation of the embryo in between acquisition of the two modes. However, the dataset used to analyze this technique was acquired before the registration algorithm was developed, so an affine model was needed. A new dataset collected with the Keck 3D Fusion Microscope should be acquired to retest the accuracy of this technique with a similarity transform.

5.2.2.4 Texture

An image processing technique that appears to show promise in segmenting the cell cluster from the background of the image is the use of texture analysis. Texture is a statistical measure based on the intensity histogram [p. 464, Gonzalez et al. 2004], that segments a region of the image that is smooth (small deviation in intensity values) from a region that contains texture (variation in intensity values). Looking at the DIC image in Figure 5.6(b), we can see that the background of the image is smooth because it contains a relatively constant grayscale value, and a region within the cells has texture because it contains much more variation in the grayscale values.

One technique to analyze the texture of a DIC image is to use a variance filter [Feineigle et al. 1996]. It is important to note that the use of a variance filter was suggested by Tom Morgan, a student working at the Broad Institute that analyzed the DIC images from the phase-subtraction cell-counting results, provided us the Matlab code for the variance filter in Figure 5.17, and initiated the posting of our mouse embryo DIC images on the Broad Institute website:

http://www.broad.mit.edu/bbbc/mouse_embryos_dic.html,

for other researchers to download and develop techniques to segment the cell boundaries automatically.

```matlab
% Tom Morgan
% August 29, 2008
% Broad Imaging Platform

function out = variancefilter(im, WINDOWSIZE, STDEV)

WIND = floor((WINDOWSIZE-1)/2);
im = double(im);

[x, y] = meshgrid(-WIND:WIND,-WIND:WIND);
c = exp(-(x.^2+y.^2)/(2*STDEV^2)) / (sqrt(2*pi)*STDEV);
out = sqrt(im.*(sum(c(:))*im - 2*imfilter(im,c,'symmetric')) ... 
    + imfilter(im.^2,c,'symmetric'));
```
Figure 5.17: Matlab code provided by Tom Morgan to apply the variance filter.

The variance filter generates high output values for regions that contain a large variability in the intensity values, so a region containing both boundary and background pixels would return a high value, and a region containing all boundary or all background pixels would return a low value. Varying the input STDEV adjusts the scale for the returned values, with a higher STDEV providing a smaller range. Varying the input WINDOWSIZE adjusts the region size so the high values returned for the boundary can be spread across a larger region. A large value for WINDOWSIZE with the original DIC image also provides a similar result to a median filtered DIC image with a small value for WINDOWSIZE. A threshold can then be applied to the result of the variance filter and the boundary can be filled to provide a mask for the cell cluster in Figure 5.18, assuming the complete boundary of the cell cluster returned values greater than the background.

Figure 5.18: (a) Original DIC image and (b) the mask for the cell cluster created from the result of the variance filter.

Another texture analysis technique is the measurement of entropy. Entropy is a measurement of the randomness of the intensity values within a region and is expressed mathematically:

\[
e = -\sum_{i=0}^{L-1} p(z_i)\log_2 p(z_i),
\]

where \( L \) is the number of possible intensity values and \( p(z) \) is the probability of occurrence of the intensity value \( z \) [p. 466, Gonzalez et al. 2004]. The result of the Matlab function entropyfilt is shown in Figure 5.19, where higher values are returned for the regions with large variations and low values are returned for the regions with small variations within the default 9 pixel region.
Figure 5.19: (a) Original DIC image and (b) the result of the Matlab function entropyfilt with the default parameters to calculate the entropy of the DIC image.

While none of these image processing techniques has shown to be the single solution to automate the quantitative embryo-viability measurements, it is possible some combination will provide the first steps. Creating image processing techniques for DIC images is a difficult problem, but a successful methodology would be used extensively considering the wide-spread use of DIC as a non-invasive optical microscopy technique.
6. Conclusion

The two overall goals of this work were to: (1) gain a better understanding of the imaging limitations of OQM and (2) combine multiple imaging modalities together on one microscope base to produce quantitative measures of embryo viability. This dissertation has described the results in regard to these two goals and suggests ways to improve the current methods, and the direction in which to continue the work. These findings and suggestions are summarized below.

A thorough SNR analyses has been completed for the current OQM reconstructions that include noise terms for laser fluctuations, aberrations, and fixed pattern noise within the individual signal and reference paths, and dark current noise in the detectors. Substituting images from the experimental system produces a model for the resultant OQM images, and has shown that the minimum phase error in the current reconstruction is 0.08 radians. This phase error can be reduced by removing the division by the blank image, but the optical layout must be modified to provide a constant wavefront mismatch between the reference and signal paths across the field of view, i.e. the center interference fringe larger than the field of view. A telecentric system with polarization-maintaining optical fiber will provide the desired wavefront mismatch, and will improve the stability of the imaging system. Increased processing speed with FPGA or GPU devices in addition to a stable, telecentric system will also help move toward real-time phase acquisition with OQM.

We have shown accurate phase measurements of spherical objects at center focus with a refractive index mismatch approximately equal to 0.02 and that are much larger than the depth of field, on the order of 77-106 μm in diameter. Previous work [Goldstein and Hartmann-Goldstein 1974] has shown that accurate OPD measurements can be provided by samples that produce less than 1 wavelength of OPD, thereby following the projection theorem. However, our results from a ray tracing model have shown that samples producing more than 1 wavelength of OPD, must be relatively close to center focus for the unwrapping algorithm to unwrap the phase and provide correct OPD information. The limit on the distance the object can be translated from center focus is dependent on the location of the Becke line, which forms from the refractive index mismatch between the object and the immersion medium. The broadening of the Becke line from defocus produces constructive and destructive interference that leads to errors in the phase unwrap and inaccurate OPD information. Thus, refraction must be incorporated into a model to analyze z-stacks of OQM images.

We have developed the Keck 3D Fusion Microscope that combines brightfield, DIC, epi-fluorescence, confocal fluorescence, confocal reflectance, two-photon, and OQM on a single microscope stage [Warger et al. 2007a]. Second-harmonic generation (SHG) imaging has also been added to the microscope for imaging collagen fibrils, but was not used for imaging mouse embryos. An automatic registration algorithm has also
been created that aligns the imaging units (pixel size and field of view) between the various detectors to provide pixel-to-pixel registration of static samples [Tsai et al. 2008]. The combination of the Keck 3D Fusion Microscope and the registration algorithm fuses the various imaging modes, and leads to new measures that would not be possible from the use of a single imaging modality alone.

One area where we have created new measures from the combination of imaging modalities is embryo viability. Clinicians currently use DIC imaging to measure most, if not all, of the embryo viability markers by eye. DIC provides contrast at the edges of the transparent embryos, but is not ideal for development after the 8-cell stage because the cells overlap and obstruct the view of cells that are under more than a single layer of cells. It is also difficult to extract quantitative information from DIC images because the contrast is related to the derivative of the phase in only one direction. Using the combination of OPD information from the OQM image and the boundary information from the DIC image, we have developed the phase-subtraction cell-counting method that has counted the number of cells accurately in live mouse embryos beyond the 8-cell stage limit and up to 26 cells [Warger et al. 2008]. The dry mass of a sample provides a measure of the dry protein content within the volume of a biological sample and derives from the OPD. Since the embryos are heterogeneous, we have used the relative dry mass that does not require a definitive value for the specific refractive increment. Recording the relative dry mass of the total cell cluster before and after the phase-subtraction count, and of each cell subtracted during the count provides a quantitative measure of cell symmetry, size, and fragmentation. We have also created models for the zona pellucida and the cytoplasm of oocytes using the boundaries in the brightfield image to remove their contributions from the OPD image and provide the distribution of mitochondria.

For clinical acceptance, the viability measures must be automated because the clinicians do not want a method that is time consuming and requires special training. Various image processing techniques have been applied to the DIC image to extract the boundary information from the cells, but none of them has been successful due to the cosine dependent shading around a circular object that is inherent in DIC. The use of a variance filter [Feineigle et al. 1996] has shown the ability to segment the cluster of cells from the background, which may be useful in automatic registration between the DIC and OQM images, but will not provide the cell boundary locations. The DIC images and results with the variance filter for the samples counted with the phase-subtraction method have been posted on the Broad Institute website:

http://www.broad.mit.edu/bbbc/mouse_embryos_dic.html,

so researchers can download the data and attempt other techniques to extract the boundary information automatically.
Until the boundary information can be provided automatically, it may be advantageous to explore the use of OQM to provide a measure of the mitochondrial distribution within oocytes. Research in determining oocyte viability has become more popular in recent years due to the legislative and moral restrictions on the number of embryos created with assisted reproductive technologies. The mitochondrial distribution is one viability marker that cannot be measured currently with a non-toxic imaging technique [Squirrell et al. 2003]. In addition, the modeling and boundary extraction of oocytes is more straightforward than embryos because the egg is much closer to a spherical shape and the only cellular overlap results from polar bodies. Many questions still exist in regard to imaging non-ideal embryos that contain a large percentage of fragmentation, and there are concerns whether the automation of the phase-subtraction count is possible with DIC images. However, a measure of mitochondrial distribution would be much quicker to implement considering that PMMA beads immersed in oil provide an excellent model for oocytes, and image processing techniques that did not work with the embryos are more probable to work with the simplified structure of an oocyte. Thus, it is possible that OQM could be used to provide the first non-toxic images of the mitochondrial distribution within oocytes and provide an immediate impact within the biological community.
Appendix A. OQM SNR Analyses

Here we provide the full SNR analyses for the current phase reconstructions used for OQM. The overall analysis begins with the electric field in each arm of the interferometer and incorporates multiplicative and additive noise terms for the imperfections and aberrations within the optics along each path and within the detectors. §A.1 provides the full expressions for an image of a sample that induces a magnitude $A$ and phase $\alpha$ and §A.2 provides the full expressions for an image of the blank, where we have assumed the slide, coverslip, and immersion medium contribute a field of 1. §A.3 describes the assumptions that remove noise terms approximately equal to zero to reduce the expressions. §A.4 derives the expression for the final image using the phase reconstruction in §3.5.1 (26) that includes balanced mixing, subtraction of the DC terms, camera normalization (division by the square root of the reference images), and division by the blank image, and assumes the wavefront mismatch between the reference and signal path (fringes) does not change between the images of the sample and the blank. §A.4.1 removes the assumption of constant fringes and provides the expression for a change in the fringe pattern. §A.4.2 derives the expression for the same image in §A.4, but without dividing by the blank image, to provide a model for a telecentric system that provides negligible wavefront mismatch across the field of view. §A.5 and §A.6 derive the same expressions as each of the subsections in §A.4 for the phase reconstructions that include balanced mixing and subtraction of the DC terms in §3.5.1 (25) and balanced mixing alone in §3.5.1 (24), respectively.

It is important to note that these analyses do not include sample dependent effects such as refraction and diffraction. However, these effects could be included by substituting the resultant 2D image of the sample from a refraction and/or diffraction model for the magnitude and phase of the sample. The effect of various imaging aberrations could also be included by taking the Fourier transform of the ideal sample image, applying the Zernike polynomials for the desired aberrations, taking the inverse-Fourier transform, and substituting the result for the magnitude and phase of the sample in these analyses.
A.1 Optical Quadrature Image of Sample

Fiber splitter splits the Electric Field into Signal and Reference Paths (let $j = \sqrt{-1}$):

$$
\tilde{E}_{\text{ref}} = \frac{1}{\sqrt{2}} (E_R e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)})
$$

$$
\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (E_S e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)})
$$

Signal Beam is Linearly Polarized:

$$
\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (E_S e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)}) \cdot (\tilde{x} + \tilde{y})
$$

Reference Beam is Circularly Polarized:

$$
\tilde{E}_{\text{ref}} = \frac{1}{\sqrt{2}} (E_R e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)}) \cdot (\tilde{x} + j\tilde{y})
$$

Amplitude and Phase of Sample induced into Signal Beam:

$$
\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (AE_S e^{j(\alpha + \phi + \alpha)} + AE_N e^{j(\alpha + \zeta + \alpha)}) \cdot (\tilde{x} + \tilde{y})
$$

Fields Entering the Recombining Beamsplitter (where $Xe^{jx}$ is the aberrations within the path):

$$
\tilde{E}_{\text{ref}} = \frac{1}{\sqrt{2}} (E_R e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)}) X_e^{jx} \cdot (\tilde{x} + j\tilde{y})
$$

$$
\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (AE_S e^{j(\alpha + \phi + \alpha)} + AE_N e^{j(\alpha + \zeta + \alpha)}) X_e^{jx} \cdot (\tilde{x} + \tilde{y})
$$

Mix of Reference and Signal Fields after Recombining Beamsplitter:

Path 1 (Camera 0 & Camera 1):

$$
\frac{1}{\sqrt{2}} (\tilde{E}_{\text{sig}} + \tilde{E}_{\text{ref}})
$$

$$
\frac{1}{\sqrt{2}} \left( \frac{1}{\sqrt{2}} \left( (AE_S e^{j(\alpha + \phi + \alpha)} + AE_N e^{j(\alpha + \zeta + \alpha)}) X_e^{jx} \cdot (\tilde{x} + \tilde{y}) + \frac{1}{\sqrt{2}} (E_R e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)}) X_e^{jx} \cdot (\tilde{x} + j\tilde{y}) \right) \right) =
$$

$$
\frac{1}{\sqrt{2}} \left( (AE_S e^{j(\alpha + \phi + \alpha)} + AE_N e^{j(\alpha + \zeta + \alpha)}) X_e^{jx} \cdot (\tilde{x} + \tilde{y}) + (E_R e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)}) X_e^{jx} \cdot (\tilde{x} + j\tilde{y}) \right)
$$

Path 2 (Camera 2 & Camera 3):

$$
\frac{1}{\sqrt{2}} (\tilde{E}_{\text{sig}} - \tilde{E}_{\text{ref}})
$$

$$
\frac{1}{\sqrt{2}} \left( (AE_S e^{j(\alpha + \phi + \alpha)} + AE_N e^{j(\alpha + \zeta + \alpha)}) X_e^{jx} \cdot (\tilde{x} + \tilde{y}) - \frac{1}{\sqrt{2}} (E_R e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)}) X_e^{jx} \cdot (\tilde{x} + j\tilde{y}) \right) =
$$

$$
\frac{1}{\sqrt{2}} \left( (AE_S e^{j(\alpha + \phi + \alpha)} + AE_N e^{j(\alpha + \zeta + \alpha)}) X_e^{jx} \cdot (\tilde{x} + \tilde{y}) - (E_R e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)}) X_e^{jx} \cdot (\tilde{x} + j\tilde{y}) \right)
$$
Pure Reference (Signal Blocked) after Recombining Beamsplitter:
Both Paths:
\[ \frac{1}{\sqrt{2}} \vec{E}_{\text{ref}} \]
\[ \frac{1}{\sqrt{2}} \left( \frac{1}{\sqrt{2}} \left( E_R e^{j(\alpha+\phi)} + E_N e^{j(\alpha+\phi')} \right) X_r e^{j\alpha} \cdot (\hat{x} + j\hat{y}) \right) \]

Pure Signal (Reference Blocked) after Recombining Beamsplitter:
Both Paths:
\[ \frac{1}{\sqrt{2}} \left( \frac{1}{\sqrt{2}} \left( A E_S e^{j(\alpha+\phi+\alpha)} + A E_N e^{j(\alpha+\phi+\alpha')} \right) X_r e^{j\alpha} \cdot (\hat{x} + \hat{y}) \right) \]

Detector Noise:
\[ \frac{\eta}{\hbar} A_{\text{pixel}} P + i_D \]
where:
- \( \eta \) – quantum efficiency (unitless)
- \( q \) – charge of an electron (1.6 x 10^{-19} \text{ C})
- \( h \) – Planck’s constant (6.6 x 10^{-34} \text{ Wsec}^2)
- \( v \) – frequency of the laser (sec^{-1})
- \( A_{\text{pixel}} \) – area of a pixel (m^2)
- \( P \) – irradiance (Wm^{-2}): equivalent to \( |E|^2 \)
- \( i_D \) – dark current

Mixed Signal and Reference after Polarizing Beamsplitters (where \( E_C \) is fixed pattern noise in Sig & Ref)

Camera 0 (\( \hat{x} \)):
\[ \frac{1}{2} \left( (A E_S e^{j(\alpha+\phi+\alpha)}) E_{c_0}^S X_s e^{j\alpha} + (E_R e^{j(\alpha+\phi)} + E_N e^{j(\alpha+\phi)}) E_{c_0}^R X_r e^{j\alpha} \right) \cdot (\hat{x}) \]

\[ \frac{\eta q \eta}{\hbar} A_{\text{pixel}} \left| \frac{1}{2} \left( (A E_S e^{j(\alpha+\phi+\alpha)}) E_{c_0}^S X_s e^{j\alpha} + (E_R e^{j(\alpha+\phi)} + E_N e^{j(\alpha+\phi)}) E_{c_0}^R X_r e^{j\alpha} \right) \right|^2 = i_M,0 = \]

\[ \frac{1}{2} \left( (A E_S e^{j(\alpha+\phi+\alpha)}) E_{c_0}^S X_s e^{j\alpha} + (E_R e^{j(\alpha+\phi)} + E_N e^{j(\alpha+\phi)}) E_{c_0}^R X_r e^{j\alpha} \right) \cdot (\hat{x}) \]
Camera 1 ($\tilde{y}$):
\[
\frac{1}{2}((AE_S e^{j(\omega t + \phi)}) + AE_N e^{j(\omega t + \zeta + \alpha)}) E_{C_1}^S X_s e^{j\omega t} + (jE_R e^{j(\omega t + \phi)} + jE_N e^{j(\omega t + \zeta)}) E_{C_1}^R X_s e^{j\omega t}) \cdot \tilde{y} =
\]
\[
\frac{\eta g}{h} A_{pixel} \left[ \frac{1}{2}((AE_S e^{j(\omega t + \phi)}) + AE_N e^{j(\omega t + \zeta + \alpha)}) E_{C_1}^S X_s e^{j\omega t} + (jE_R e^{j(\omega t + \phi)} + jE_N e^{j(\omega t + \zeta)}) E_{C_1}^R X_s e^{j\omega t}) \right]^2 + i_{M,1} =
\]
\[
\frac{1}{2} (AE_S e^{j(\omega t + \phi)}) + AE_N e^{j(\omega t + \zeta + \alpha)}) E_{C_1}^S X_s e^{j\omega t} + (jE_R e^{j(\omega t + \phi)} + jE_N e^{j(\omega t + \zeta)}) E_{C_1}^R X_s e^{j\omega t}) \cdot \tilde{x} =
\]
\[
\frac{\eta g}{h} A_{pixel} \left[ \frac{1}{2}((AE_S e^{j(\omega t + \phi)}) + AE_N e^{j(\omega t + \zeta + \alpha)}) E_{C_1}^S X_s e^{j\omega t} + (jE_R e^{j(\omega t + \phi)} - E_N e^{j(\omega t + \zeta)}) E_{C_1}^R X_s e^{j\omega t}) \right]^2 + i_{M,2} =
\]
\[
\frac{1}{2} (AE_S e^{j(\omega t + \phi)}) + AE_N e^{j(\omega t + \zeta + \alpha)}) E_{C_1}^S X_s e^{j\omega t} + (jE_R e^{j(\omega t + \phi)} - E_N e^{j(\omega t + \zeta)}) E_{C_1}^R X_s e^{j\omega t}) \cdot \tilde{y} =
\]
\[
\frac{\eta g}{h} A_{pixel} \left[ \frac{1}{2}((AE_S e^{j(\omega t + \phi)}) + AE_N e^{j(\omega t + \zeta + \alpha)}) E_{C_1}^S X_s e^{j\omega t} + (jE_R e^{j(\omega t + \phi)} - E_N e^{j(\omega t + \zeta)}) E_{C_1}^R X_s e^{j\omega t}) \right]^2 + i_{M,3} =
\]
\[
\frac{1}{2} (AE_S e^{j(\omega t + \phi)}) + AE_N e^{j(\omega t + \zeta + \alpha)}) E_{C_1}^S X_s e^{j\omega t} + (jE_R e^{j(\omega t + \phi)} - jE_N e^{j(\omega t + \zeta)}) E_{C_1}^R X_s e^{j\omega t}) \cdot \tilde{y} =
\]
\[
\frac{\eta g}{h} A_{pixel} \left[ \frac{1}{2}((AE_S e^{j(\omega t + \phi)}) + AE_N e^{j(\omega t + \zeta + \alpha)}) E_{C_1}^S X_s e^{j\omega t} + (jE_R e^{j(\omega t + \phi)} - jE_N e^{j(\omega t + \zeta)}) E_{C_1}^R X_s e^{j\omega t}) \right]^2 + i_{M,3} =
\[ E_{BM, sample} = \sum_{k=0}^{3} j^k M_k \]

\[ E_{BM, sample} = \frac{1}{\frac{\eta}{4} A_{pixel}} \left( (A^2 E_s E_s^* + A^2 E_n E_n^*) e^{j(\phi - \zeta)} E_{s C_1}^S S_{x C_1}^S X X^* + (j A E_s E_n e^{i \alpha} + j A E_n E_n^r e^{j(\phi + \alpha - \zeta)}) E_{s C_1}^S R^S X X^* e^{j(\zeta - \zeta)} + (A^2 E_s E_s^r e^{j(\phi - \zeta)} + A^2 E_n E_n^r) E_{s C_1}^S S_{x C_1}^S X X^* + (j A E_n E_n^r e^{j(\phi + \alpha - \zeta)} + j A E_n E_n^r) E_{s C_1}^S R^S X X^* e^{j(\zeta - \zeta)} + (-j A E_n E_n^* e^{j(\phi + \alpha - \zeta)} - j A E_n E_n^r e^{j(\phi - \zeta)}) E_{s C_1}^R E_{s C_1}^S X X^* e^{j(\zeta - \zeta)} + (E_s E_n^* e^{j(\phi - \zeta)} + E_n E_n^r) E_{s C_1}^R E_{s C_1}^S X X^* + (E_s E_n^* e^{j(\phi + \alpha - \zeta)} + E_n E_n^r) E_{s C_1}^R E_{s C_1}^S X X^* + i M_3 \right) \]  

(M3)

Balanced Mixing:

\[ \frac{1}{4} \frac{\eta}{A_{pixel}} ((A^2 E_s E_s^* + A^2 E_n E_n^*) e^{j(\phi - \zeta)} E_{s C_1}^S S_{x C_1}^S X X^* + (A E_s E_n e^{i \alpha} + A E_n E_n^r e^{j(\phi + \alpha - \zeta)}) E_{s C_1}^S R^S X X^* e^{j(\zeta - \zeta)} + (A^2 E_s E_s^r e^{j(\phi - \zeta)} + A^2 E_n E_n^r) E_{s C_1}^S S_{x C_1}^S X X^* + (A E_n E_n^r e^{j(\phi + \alpha - \zeta)} + A E_n E_n^r) E_{s C_1}^S R^S X X^* e^{j(\zeta - \zeta)} + (-A E_n E_n^* e^{j(\phi + \alpha - \zeta)} - A E_n E_n^r e^{j(\phi - \zeta)}) E_{s C_1}^R E_{s C_1}^S X X^* e^{j(\zeta - \zeta)} + (A E_n E_n^* e^{j(\phi + \alpha - \zeta)} + A E_n E_n^r) E_{s C_1}^R E_{s C_1}^S X X^* + (A E_n E_n^* e^{j(\phi + \alpha - \zeta)} + A E_n E_n^r) E_{s C_1}^R E_{s C_1}^S X X^* + j (i M_3) + \]

(M3)
If there were no aberrations in the system:

$$E_{BM, sample} = \frac{1}{4} \frac{A_{\text{pixel}}}{\hbar \omega} (A e^{i \alpha} (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* e^{i(x, \omega - x)} ) .$$

$$(\hat{\eta}_0 E_S^S C_0^S E_R^* + \hat{\eta}_1 E_S^S E_C^S C_1^* + \hat{\eta}_2 E_S^S E_C^C C_2^* + \hat{\eta}_3 E_S^S E_R^S C_3^* ) +$$
$$A e^{i \alpha} (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* e^{i(x, \omega - x)} .$$

$$A^2 (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* (\hat{\eta}_0 E_S^S C_0^S E_R^* + \hat{\eta}_1 E_S^S E_C^S C_1^* + \hat{\eta}_2 E_S^S E_C^C C_2^* - \hat{\eta}_3 E_S^S E_R^S C_3^* ) +$$
$$(i_{M,0} - i_{M,2} + j(i_{M,1} - i_{M,3}))$$

If the detectors were ideal:

$$i_{M,0} = i_{M,1} = i_{M,2} = i_{M,3} \quad \text{and} \quad \hat{\eta}_0 = \hat{\eta}_1 = \hat{\eta}_2 = \hat{\eta}_3 = 1$$

$$E_{BM, sample} = \frac{1}{4} \frac{A_{\text{pixel}}}{\hbar \omega} (A e^{i \alpha} (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* e^{i(x, \omega - x)} ) (E_S^S E_R^* + E_S^S E_C^S C_1^* + E_S^S E_C^C C_2^* + E_S^S E_R^S C_3^* ) +$$
$$A e^{i \alpha} (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* (E_S^S E_C^S C_1^* - E_S^S E_C^C C_2^* + E_S^S E_R^S C_3^* ) +$$
$$A^2 (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* (E_S^S E_C^S C_1^* + jE_S^S E_C^C C_2^* - E_S^S E_R^S C_3^* ) +$$
$$(E_R E_R^* + E_R E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* (E_S^S E_C^S C_1^* + jE_S^S E_C^C C_2^* - E_S^S E_R^S C_3^* ) +$$
$$(i_{M,0} - i_{M,2} + j(i_{M,1} - i_{M,3}))$$

If the fixed pattern noise was the same in the signal and reference paths:

$$E_S^C = E_C^C = E_C$$

$$E_{BM, sample} = \frac{1}{4} \frac{A_{\text{pixel}}}{\hbar \omega} (A e^{i \alpha} (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) (E_S^S E_R^* + E_S^S E_C^S C_1^* + E_S^S E_C^S C_2^* + E_S^S E_R^S C_3^* ) +$$
$$A e^{i \alpha} (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* (E_S^S E_C^S C_1^* - E_S^S E_C^C C_2^* + E_S^S E_R^S C_3^* ) +$$
$$A^2 (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* (E_S^S E_C^S C_1^* + jE_S^S E_C^C C_2^* - E_S^S E_R^S C_3^* ) +$$
$$(E_R E_R^* + E_R E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) X_s X_s^* (E_S^S E_C^S C_1^* + jE_S^S E_C^C C_2^* - E_S^S E_R^S C_3^* ) +$$
$$(i_{M,0} - i_{M,2} + j(i_{M,1} - i_{M,3}))$$

If there was no fixed pattern noise:

$$E_S^C = E_C^C = E_C = 1$$

$$E_{BM, sample} = A e^{i \alpha} (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) \frac{A_{\text{pixel}}}{\hbar \omega} X_s X_s^* e^{i(x, \omega - x)} .$$

If there were no aberrations in the system:

$$X_s e^{i2x} = X_s e^{i2x} = 1$$

$$E_{BM, sample} = A e^{i \alpha} (E_S E_R^* + E_S E_N^* e^{i(\theta - \zeta)} + E_N E_R^* e^{-j(\theta - \zeta)} + E_N E_N^*) \frac{A_{\text{pixel}}}{\hbar \omega} .$$

If there was no noise in the laser:

$$E_N e^{j(\theta + \zeta)} = 0$$

$$E_{BM, sample} = A e^{i \alpha} (E_S E_R^*) \frac{A_{\text{pixel}}}{\hbar \omega} .$$

Converting to Irradiance:

$$\hat{I}_{BM, sample} = \frac{E_{BM, sample} \hbar \omega}{A_{\text{pixel}}}$$

$$\hat{I}_{BM, sample} = A e^{i \alpha} (E_S E_R^*) .$$
Pure Dark After Polarizing Beamsplitters:

Camera 0: \( i_{D,0} \) (D0)

Camera 1: \( i_{D,1} \) (D1)

Camera 2: \( i_{D,2} \) (D2)

Camera 3: \( i_{D,3} \) (D3)

Pure Reference after the Polarizing Beamsplitters:

Camera 0 (\( \vec{x} \)): 
\[
\frac{1}{2} (E_R e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) X_x e^{jx} C_0 \cdot (\vec{x})
\]

\[
\frac{\eta x}{h \nu} A_{\text{pixel}} \left| \frac{1}{2} (E_R e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) X_x e^{jx} C_0 \right|^2 + i_{R,0} =
\]

\[
\frac{\eta y}{h \nu} A_{\text{pixel}} \left| \frac{1}{2} (E_R e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) X_y e^{jy} C_0 \right|^2 + i_{R,0} =
\]

\[
\frac{1}{2} E^R_{C_0} X_y |E_R + E_N| \cdot \sqrt{\frac{\eta y}{h \nu} A_{\text{pixel}}} \cdot \sqrt{1 + \frac{\eta y}{h \nu} A_{\text{pixel}} (E_R E^*_R + E_N E^*_N e^{-j(\omega t + \zeta) + E_N E^*_N} C_0) E^R_{C_0} X_x X^*_x + i_{R,1}}
\]

Camera 1 (\( \vec{y} \)): 
\[
\frac{1}{2} (E_R e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) X_x e^{jx} C_1 \cdot (\vec{y})
\]

\[
\frac{\eta x}{h \nu} A_{\text{pixel}} \left| \frac{1}{2} (E_R e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) X_x e^{jx} C_1 \right|^2 + i_{R,1} =
\]

\[
\frac{1}{2} E^R_{C_1} X_y |E_R + E_N| \cdot \sqrt{\frac{\eta y}{h \nu} A_{\text{pixel}}} \cdot \sqrt{1 + \frac{\eta y}{h \nu} A_{\text{pixel}} (E_R E^*_R + E_N E^*_N e^{-j(\omega t + \zeta) + E_N E^*_N} C_1) E^R_{C_1} X_x X^*_x + i_{R,2}}
\]

Camera 2 (\( \vec{x} \)): 
\[
\frac{1}{2} (E_R e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) X_x e^{jx} C_2 \cdot (\vec{x})
\]

\[
\frac{\eta y}{h \nu} A_{\text{pixel}} \left| \frac{1}{2} (E_R e^{j(\omega t + \phi)} + E_N e^{j(\omega t + \zeta)}) X_x e^{jx} C_2 \right|^2 + i_{R,2} =
\]

\[
\frac{1}{2} E^R_{C_2} X_y |E_R + E_N| \cdot \sqrt{\frac{\eta y}{h \nu} A_{\text{pixel}}} \cdot \sqrt{1 + \frac{\eta y}{h \nu} A_{\text{pixel}} (E_R E^*_R + E_N E^*_N e^{-j(\omega t + \zeta) + E_N E^*_N} C_2) E^R_{C_2} X_x X^*_x + i_{R,3}}
\]
Camera 3 ($\tilde{y}$):

$$j \frac{1}{2} (E_R e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)}) X_s e^{jx_s} E^{R}_{C_3} \cdot (\tilde{y})$$

$$\frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} \left| j \frac{1}{2} (E_R e^{j(\alpha + \phi)} + E_N e^{j(\alpha + \zeta)}) X_s e^{jx_s} E^{R}_{C_3} \right|^2 + i_{R,3} =$$

$$\frac{1}{4} \frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} (E_R E_R^{*} + E_N E_N^{*} e^{j(\phi - \zeta)} + E_N E_N^{*} e^{-j(\phi - \zeta)} + E_N E_N^{*}) E^{R}_{C_3} X_s X_s^{*} + i_{R,3}$$  \hspace{1cm} (R3)

Square Root:

$$\frac{1}{2} E^R_{C_3} X_s E_{R} + E_{N} \cdot \sqrt{\frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} \cdot \frac{i_{R,3}}{1 + \frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} (E_R E_R^{*} + E_N E_N^{*} e^{j(\phi - \zeta)} + E_N E_N^{*} e^{-j(\phi - \zeta)} + E_N E_N^{*}) E^{R}_{C_3} X_s X_s^{*}} \sqrt{R_3}$$

Balanced Mixing of Pure Reference:

$$E_{BM,ref} = \sum_{k=0}^{3} j^k R_k$$

$$= \frac{1}{4} \frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} (E_R E_R^{*} + E_R e^{j(\phi - \zeta)} + E_N E_N^{*} e^{-j(\phi - \zeta)} + E_N E_N^{*}) E^{R}_{C_0} E^{R}_{C_0} X_s X_s^{*} + i_{R,0} +$$

$$j \frac{1}{4} \frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} (E_R E_R^{*} + E_R E_N^{*} e^{j(\phi - \zeta)} + E_N E_N^{*}) E^{R}_{C_0} E^{R}_{C_0} X_s X_s^{*} + j(i_{R,1}) +$$

$$- \frac{1}{4} \frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} (E_R E_R^{*} + E_R E_N^{*} e^{j(\phi - \zeta)} + E_N E_N^{*} e^{-j(\phi - \zeta)} + E_N E_N^{*}) E^{R}_{C_0} E^{R}_{C_0} X_s X_s^{*} - i_{R,2} +$$

$$- j \frac{1}{4} \frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} (E_R E_R^{*} + E_R E_N^{*} e^{j(\phi - \zeta)} + E_N E_N^{*} e^{-j(\phi - \zeta)} + E_N E_N^{*}) E^{R}_{C_0} E^{R}_{C_0} X_s X_s^{*} - j(i_{R,3})$$

$$E_{BM,ref} = \frac{1}{4} \frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} (E_R E_R^{*} + E_R E_N^{*} e^{j(\phi - \zeta)} + E_N E_N^{*}) E^{R}_{C_0} X_s X_s^{*} \cdot$$

$$(\eta_0 E^{R}_{C_0} E^{R}_{C_0} + j \eta_1 E^{R}_{C_1} E^{R}_{C_1} - \eta_2 E^{R}_{C_2} E^{R}_{C_2} - j \eta_3 E^{R}_{C_3} E^{R}_{C_3}) + (i_{R,0} - i_{R,2} + j(i_{R,1} - i_{R,3}))$$

If no fixed pattern noise $E^R_C = 1$ and ideal detectors $i_{M,0} = i_{M,1} = i_{M,2} = i_{M,3}$ and $\eta = 1$:

$$E_{BM,ref} = 0$$

Pure Signal after the Polarizing Beamsplitters:

Camera 0 ($\tilde{x}$):

$$\frac{1}{2} (AE_S e^{j(\alpha + \phi + \alpha)} + AE_N e^{j(\alpha + \alpha + \alpha + \zeta)}) X_s e^{jx_s} E^{S}_{C_0} \cdot (\tilde{x})$$

$$\frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} \left| \frac{1}{2} (AE_S e^{j(\alpha + \phi + \alpha)} + AE_N e^{j(\alpha + \alpha + \alpha + \zeta)}) X_s e^{jx_s} E^{S}_{C_0} \right|^2 + i_{S,0} =$$

$$\frac{1}{4} \frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} ((AE_S e^{j(\alpha + \phi + \alpha)} + AE_N e^{j(\alpha + \alpha + \alpha + \zeta)}) X_s e^{jx_s} E^{S}_{C_0} + ((AE_S e^{j(\alpha + \alpha + \alpha + \zeta)}) X_s e^{jx_s}) E^{S}_{C_0}^{*} + i_{S,0} =$$

$$\frac{1}{4} \frac{n_{\text{eq}}}{h_0} A_{\text{pixel}} (A^2 E_S E_S^{*} + A^2 E_S E_N^{*} e^{j(\phi - \zeta)} + A^2 E_N E_S^{*} e^{-j(\phi - \zeta)} + A^2 E_N E_N^{*}) E^{S}_{C_0} E^{S}_{C_0}^{*} X_s X_s^{*} + i_{S,0}$$  \hspace{1cm} (S0)
Camera 1 ($\vec{y}$):

\[
\frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S \cdot (\vec{y})
\]

\[
\frac{\eta \alpha}{\hbar} A_{\text{pixel}} \left\{ \frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S \right\}^2 + i_{s,1} =
\]

\[
\frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S \cdot \frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S + i_{s,1} =
\]

\[
\frac{1}{2} \frac{\eta \alpha}{\hbar} A_{\text{pixel}} (A^2 E_s E_s^* + A^2 E_s E_s^* e^{j(\phi - \zeta)} + A^2 E_s E_s^* e^{-j(\phi - \zeta)} + A^2 E_s E_s^*) E_s^S E_s^S E_s^S X_s X_s + i_{s,1} \tag{S_1}
\]

Camera 2 ($\vec{x}$):

\[
\frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S \cdot (\vec{x})
\]

\[
\frac{\eta \alpha}{\hbar} A_{\text{pixel}} \left\{ \frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S \right\}^2 + i_{s,2} =
\]

\[
\frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S \cdot \frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S + i_{s,2} =
\]

\[
\frac{1}{2} \frac{\eta \alpha}{\hbar} A_{\text{pixel}} (A^2 E_s E_s^* + A^2 E_s E_s^* e^{j(\phi - \zeta)} + A^2 E_s E_s^* e^{-j(\phi - \zeta)} + A^2 E_s E_s^*) E_s^S E_s^S E_s^S X_s X_s + i_{s,2} \tag{S_2}
\]

Camera 3 ($\vec{y}$):

\[
\frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S \cdot (\vec{y})
\]

\[
\frac{\eta \alpha}{\hbar} A_{\text{pixel}} \left\{ \frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S \right\}^2 + i_{s,3} =
\]

\[
\frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S \cdot \frac{1}{2} (AE_S e^{j(\alpha+\phi+\alpha)} + AE_N e^{j(\alpha+\alpha+\zeta)}) X_s e^{jx_c} E_s^S + i_{s,3} =
\]

\[
\frac{1}{2} \frac{\eta \alpha}{\hbar} A_{\text{pixel}} (A^2 E_s E_s^* + A^2 E_s E_s^* e^{j(\phi - \zeta)} + A^2 E_s E_s^* e^{-j(\phi - \zeta)} + A^2 E_s E_s^*) E_s^S E_s^S E_s^S X_s X_s + i_{s,3} \tag{S_3}
\]

Balanced Mixing of Pure Signal:

\[
E_{BM,sig} = \sum_{k=0}^{j} i_k S_k
\]

\[
\frac{1}{2} \frac{\eta \alpha}{\hbar} A_{\text{pixel}} (A^2 E_s E_s^* + A^2 E_s E_s^* e^{j(\phi - \zeta)} + A^2 E_s E_s^* e^{-j(\phi - \zeta)} + A^2 E_s E_s^*) E_s^S E_s^S E_s^S X_s X_s + i_{s,0} +
\]

\[
\frac{j}{2} \frac{\eta \alpha}{\hbar} A_{\text{pixel}} (A^2 E_s E_s^* + A^2 E_s E_s^* e^{j(\phi - \zeta)} + A^2 E_s E_s^* e^{-j(\phi - \zeta)} + A^2 E_s E_s^*) E_s^S E_s^S E_s^S X_s X_s + j(i_{s,1}) +
\]

\[
\frac{-1}{2} \frac{\eta \alpha}{\hbar} A_{\text{pixel}} (A^2 E_s E_s^* + A^2 E_s E_s^* e^{j(\phi - \zeta)} + A^2 E_s E_s^* e^{-j(\phi - \zeta)} + A^2 E_s E_s^*) E_s^S E_s^S E_s^S X_s X_s - i_{s,2} +
\]

\[
\frac{-j}{2} \frac{\eta \alpha}{\hbar} A_{\text{pixel}} (A^2 E_s E_s^* + A^2 E_s E_s^* e^{j(\phi - \zeta)} + A^2 E_s E_s^* e^{-j(\phi - \zeta)} + A^2 E_s E_s^*) E_s^S E_s^S E_s^S X_s X_s - j(i_{s,3})
\]

\[
E_{BM,sig} = \frac{A_{\text{pixel}}}{\hbar} \left[ A^2 (E_s E_s^* + E_s E_s^* e^{j(\phi - \zeta)} + E_s E_s^* e^{-j(\phi - \zeta)} + E_s E_s^*) X_s X_s \cdot (\eta_0 E_s^S E_s^S + j \eta_1 E_s^S E_s^S - \eta_2 E_s^S E_s^S + j \eta_3 E_s^S E_s^S) \right] +
\]

\[
(i_{s,0} - i_{s,2} + j(i_{s,1} - i_{s,3}))
\]

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If no fixed pattern noise $E_C^2 = 1$ and ideal detectors $i_{S,0} = i_{S,1} = i_{S,2} = i_{S,3}$ and $\eta = 1$:

$$E_{BM,sig} = 0$$

Simplify $\sqrt{R_D}$ with a Taylor Series: $f(x) = \sum_{n=0}^{\infty} \frac{f^{(n)}(a)}{n!} (x-a)^n$, (Let $a = 0$, thus using Maclaurin series):

$$\sqrt{1 + x} \approx 1 + \frac{x}{2} - \frac{x^2}{8} + \ldots$$

where: $x = \frac{i_{g-\nu}}{\frac{1}{12_\nu} A_{pixel} (E_R E_R^* + E_N E_N^*) + (E_R e^{-j(\phi - \zeta)} + E_N e^{j(\phi - \zeta)} + E_N E_N^*) E_C^R e^{Z^*} X_r X_r^*}$

$$\sqrt{1 + \frac{i_{g-\nu}}{\frac{1}{12_\nu} A_{pixel} (E_R E_R^* + E_N E_N^*) + (E_R e^{-j(\phi - \zeta)} + E_N e^{j(\phi - \zeta)} + E_N E_N^*) E_C^R e^{Z^*} X_r X_r^*}} = 1 + \frac{i_{g-\nu}}{\frac{1}{12_\nu} A_{pixel} (E_R E_R^* + E_N E_N^*) + (E_R e^{-j(\phi - \zeta)} + E_N e^{j(\phi - \zeta)} + E_N E_N^*) E_C^R e^{Z^*} X_r X_r^*}$$

Since $x$ is already near zero, we assume $x$ raised to any power is zero:

$$\sqrt{R_D} \approx \sqrt{\frac{1}{2} E_C^R X_r |E_R + E_N| \cdot \sqrt{\eta q \cdot \frac{\hbar \nu}{A_{pixel}}} \cdot \left(1 + \frac{i_{g-\nu}}{\frac{1}{12_\nu} A_{pixel} (E_R E_R^* + E_N E_N^*) + (E_R e^{-j(\phi - \zeta)} + E_N e^{j(\phi - \zeta)} + E_N E_N^*) E_C^R e^{Z^*} X_r X_r^*} \right)}$$

Substitution of Dark (subscript D):

$$\frac{1}{4 \frac{\eta q}{\hbar \nu}} A_{pixel} \cdot \left( \left( A^2 E_S^* + A^2 E_N e^{j(\phi - \zeta)} \right) e^{X_r^*} X_r \cdot X_r^* + (A E_S e^{jx} + A E_N e^{j(\phi - \zeta)}) e^{X_r^*} X_r \cdot X_r^* \right)$$

$$= \left( A^2 E_S^* + A^2 E_N e^{j(\phi - \zeta)} \right) e^{X_r^*} X_r \cdot X_r^* + (A E_S e^{jx} + A E_N e^{j(\phi - \zeta)}) e^{X_r^*} X_r \cdot X_r^*$$

Substitution of Dark (subscript D):

$$\frac{1}{4 \frac{\eta q}{\hbar \nu}} A_{pixel} \cdot \left( \left( A^2 E_S^* + A^2 E_N e^{j(\phi - \zeta)} \right) e^{X_r^*} X_r \cdot X_r^* + (A E_S e^{jx} + A E_N e^{j(\phi - \zeta)}) e^{X_r^*} X_r \cdot X_r^* \right)$$

Substitution of Dark (subscript D):

$$\frac{1}{2} E_C^R X_r |E_R + E_N| \cdot \sqrt{\frac{\eta q}{\hbar \nu} \cdot \frac{1}{A_{pixel}}} \cdot \left(1 + \frac{i_{g-\nu}}{\frac{1}{12_\nu} A_{pixel} (E_R E_R^* + E_N E_N^*) + (E_R e^{-j(\phi - \zeta)} + E_N e^{j(\phi - \zeta)} + E_N E_N^*) E_C^R e^{Z^*} X_r X_r^*} \right)$$

$$\sqrt{R_D}$$
\[
\frac{1}{\eta D} A_{\text{pixel}} \left[ (A^2 E_S E^*_S + A^2 E_S E^*_N) E^S_{C_1} E^S_{C_1} X_s X_s^* + (-jA^2 E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (-jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (jA^2 E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* \right] + i_{M,1} - i_{D,1} \]  
(M_d,1)  

\[
\frac{1}{\eta D} A_{\text{pixel}} \left[ (A^2 E_S E^*_S + A^2 E_S E^*_N) E^S_{C_1} E^S_{C_1} X_s X_s^* + (jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (-jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* \right] + i_{S,1} - i_{D,1}  
(S_d,1)  

\[
\frac{1}{\eta D} A_{\text{pixel}} \left[ (E_S E^*_S + E_S E^*_N) E^S_{C_1} E^S_{C_1} X_s X_s^* + (jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (-jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* \right] + i_{S,1} - i_{D,1}  
(R_d,1)  

\[
\frac{1}{\eta D} A_{\text{pixel}} \left[ \frac{1}{\eta D} \right] \sqrt{R(D)}  
\]  

\[
\frac{1}{\eta D} A_{\text{pixel}} \left[ (A^2 E_S E^*_S + A^2 E_S E^*_N) E^S_{C_1} E^S_{C_1} X_s X_s^* + (-jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (jA^2 E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (-jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* \right] + i_{M,2} - i_{D,2}  
(M_d,2)  

\[
\frac{1}{\eta D} A_{\text{pixel}} \left[ (A^2 E_S E^*_S + A^2 E_S E^*_N) E^S_{C_1} E^S_{C_1} X_s X_s^* + (jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (-jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* \right] + i_{S,2} - i_{D,2}  
(S_d,2)  

\[
\frac{1}{\eta D} A_{\text{pixel}} \left[ (E_S E^*_S + E_S E^*_N) E^S_{C_1} E^S_{C_1} X_s X_s^* + (jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (-jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* \right] + i_{S,2} - i_{D,2}  
(R_d,2)  

\[
\frac{1}{\eta D} A_{\text{pixel}} \left[ \frac{1}{\eta D} \right] \sqrt{R(D)}  
\]  

\[
\frac{1}{\eta D} A_{\text{pixel}} \left[ (A^2 E_S E^*_S + A^2 E_S E^*_N) E^S_{C_1} E^S_{C_1} X_s X_s^* + (jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* + (-jA\eta E_S E^*_N e^{i(\phi - \zeta)}) E^S_{C_1} E^S_{C_1} X_s X_s^* \right] + i_{M,3} - i_{D,3}  
(M_d,3)  

\[
\frac{1}{4} A_{\text{pixel}} \left( A^2 E_S E_S^* + A^2 E_S E_N e^{j(\phi-\zeta)} + A^2 E_N E_S^* e^{-j(\phi-\zeta)} + A^2 E_N E_N^* \right) E_C^R E_C^S X_s X_s^* + i_{S,3} - i_{D,3}
\]

\[
\frac{1}{4} A_{\text{pixel}} \left( E_R E_S^* + E_R E_N^* e^{j(\phi-\zeta)} + E_N E_R^* e^{-j(\phi-\zeta)} + E_N E_N^* \right) E_C^R E_C^S X_s X_s^* + i_{R,3} - i_{D,3}
\]

\[
\frac{1}{2} E_C^R X_s \left| E_R + E_N \right| \frac{1}{\sqrt{R_{D,3}}} \left( 1 + \frac{t_{S,3-i_{D,3}}}{\sqrt{R_{D,3}}} \right)
\]

Reconstruction Steps:

\[\text{M}_{D,0} - \text{R}_{D,0} - \text{S}_{D,0}:\]

\[
\frac{1}{4} A_{\text{pixel}} \left[ A e^{j\alpha} \left( E_S^* + E_N e^{j(\phi-\zeta)} + E_N^* e^{-j(\phi-\zeta)} + E_S \right) E_C^R E_C^S X_s X_s^* e^{j(\chi-\xi)} + A e^{-j\alpha} \left( E_R^* + E_N e^{j(\phi-\zeta)} + E_N^* e^{-j(\phi-\zeta)} + E_R \right) E_C^R E_C^S X_s X_s^* e^{-j(\chi-\xi)} \right] + ((i_{M,0} - i_{D,0}) - (i_{R,0} - i_{D,0}) - (i_{S,0} - i_{D,0}))
\]

\[\text{M}_{D,1} - \text{R}_{D,1} - \text{S}_{D,1}:\]

\[
\frac{1}{4} A_{\text{pixel}} \left[ A e^{j\alpha} \left( -jE_S^* - jE_N e^{j(\phi-\zeta)} - jE_N^* e^{-j(\phi-\zeta)} - jE_S \right) E_C^R E_C^S X_s X_s^* e^{j(\chi-\xi)} + A e^{-j\alpha} \left( jE_R^* + jE_N e^{j(\phi-\zeta)} + jE_N^* e^{-j(\phi-\zeta)} + jE_R \right) E_C^R E_C^S X_s X_s^* e^{-j(\chi-\xi)} \right] + ((i_{M,1} - i_{D,1}) - (i_{R,1} - i_{D,1}) - (i_{S,1} - i_{D,1}))
\]

\[\text{M}_{D,2} - \text{R}_{D,2} - \text{S}_{D,2}:\]

\[
\frac{1}{4} A_{\text{pixel}} \left[ A e^{j\alpha} \left( -jE_S^* - jE_N e^{j(\phi-\zeta)} - jE_N^* e^{-j(\phi-\zeta)} - jE_S \right) E_C^R E_C^S X_s X_s^* e^{j(\chi-\xi)} + A e^{-j\alpha} \left( jE_R^* + jE_N e^{j(\phi-\zeta)} + jE_N^* e^{-j(\phi-\zeta)} + jE_R \right) E_C^R E_C^S X_s X_s^* e^{-j(\chi-\xi)} \right] + ((i_{M,2} - i_{D,2}) - (i_{R,2} - i_{D,2}) - (i_{S,2} - i_{D,2}))
\]

\[\text{M}_{D,3} - \text{R}_{D,3} - \text{S}_{D,3}:\]

\[
\frac{1}{4} A_{\text{pixel}} \left[ A e^{j\alpha} \left( jE_S^* + jE_N e^{j(\phi-\zeta)} + jE_N^* e^{-j(\phi-\zeta)} + jE_S \right) E_C^R E_C^S X_s X_s^* e^{j(\chi-\xi)} + A e^{-j\alpha} \left( jE_R^* - jE_N e^{j(\phi-\zeta)} - jE_N^* e^{-j(\phi-\zeta)} - jE_R \right) E_C^R E_C^S X_s X_s^* e^{-j(\chi-\xi)} \right] + ((i_{M,3} - i_{D,3}) - (i_{R,3} - i_{D,3}) - (i_{S,3} - i_{D,3}))
\]
Summation with Subtraction of Reference and Signal:

\[ E_{DC,\text{sample}} = \sum_{k=0}^{3} M_{D,k} \cdot R_{D,k} \cdot S_{D,k} \]

\[ E_{DC,\text{sample}} = \frac{A_{\text{pixel}}}{\eta^2} \left[ A e^{j\alpha} (E_J E_R^* + E_S E_N^* e^{j(\phi - \zeta)} + E_N E_S^* e^{-j(\phi - \zeta)}) + E_N E_N^* \right] \]

\[ \left( \eta_0 E_J^* E_J^* C_0 + j \eta_1 E_J^* E_J^* C_1 - \eta_2 E_J^* E_J^* C_2 + j \eta_3 E_J^* E_J^* C_3 \right) + \]

\[ A e^{-j\alpha} (E_J E_S^* + E_S E_N^* e^{j(\phi - \zeta)} + E_N E_N^* E_J^* C_0 E_J^* C_0 X_r X_r^* e^{j(x, -\chi, r)}) \]

\[ (i_{M,0} - i_{D,0}) \cdot \left( (i_{M,0} - i_{D,0}) - (i_{S,0} - i_{D,0}) \right) \]

\[ \frac{1}{2} \sqrt{\frac{\eta_0}{\eta^2} A_{\text{pixel}}} \left( \frac{1}{2} \sqrt{\frac{\eta_0}{\eta^2} A_{\text{pixel}}} \right) \left( \frac{1}{2} \sqrt{\frac{\eta_0}{\eta^2} A_{\text{pixel}}} \right) \]

\[ \frac{1}{2} \sqrt{\frac{\eta_0}{\eta^2} A_{\text{pixel}}} \cdot \left( \frac{1}{2} \sqrt{\frac{\eta_0}{\eta^2} A_{\text{pixel}}} \right) \left( \frac{1}{2} \sqrt{\frac{\eta_0}{\eta^2} A_{\text{pixel}}} \right) \]

Simply denominator with a Taylor Series: \( f(x) = \sum_{n=0}^{\infty} \frac{f^{(n)}(a)}{n!} (x - a)^n \), \( \text{Let } a = 0, \text{ Maclaurin series} \):

\[ \frac{1}{1+x} \approx 1 - x + x^2 - \ldots \]

where: \( x = \frac{i_{D,0}}{i_{M,0} - i_{D,0}} \)
Since \( x \) is already near zero, we will assume \( x \) raised to any power is zero:

\[
1 + \frac{i_{r,0} - i_{d,0}}{\left( E_x E_Z + E_Z E_x e^{i(\phi - \zeta)} + E_x E_Y e^{-i(\phi - \zeta)} + E_z E_Y e^{i(\phi - \zeta)} + E_y E_z e^{-i(\phi - \zeta)} + E_y E_x e^{i(\phi - \zeta)} \right) X, X'} \approx 1 - \frac{i_{r,0} - i_{d,0}}{\left( E_x E_Z + E_Z E_x e^{i(\phi - \zeta)} + E_x E_Y e^{-i(\phi - \zeta)} + E_z E_Y e^{i(\phi - \zeta)} + E_y E_z e^{-i(\phi - \zeta)} + E_y E_x e^{i(\phi - \zeta)} \right) X, X'}
\]

\[
\left[ \frac{1}{\sqrt{\eta D}} A \right] \cdot \left( A e^{i \alpha (E_x E_z + E_z E_x e^{i(\phi - \zeta)} + E_y E_x e^{-i(\phi - \zeta)} + E_z E_y e^{i(\phi - \zeta)} + E_y E_z e^{-i(\phi - \zeta)} + E_y E_x e^{i(\phi - \zeta)})} + A e^{-i \alpha} (E_x E_z + E_z E_x e^{i(\phi - \zeta)} + E_x E_y e^{-i(\phi - \zeta)} + E_z E_y e^{i(\phi - \zeta)} + E_y E_z e^{-i(\phi - \zeta)} + E_y E_x e^{i(\phi - \zeta)}) + \frac{i_{M,0} - i_{r,0} - i_{s,0} + i_{d,0}}{\sqrt{\eta D} A} \right]
\]

\[
\frac{j^1 \left( M_{D,1} - R_{D,1} - S_{D,1} \right)}{\sqrt{R_{D,1}}} = \frac{1}{\frac{r_s}{\sqrt{\eta D} A}} \cdot \left( \frac{i_{r,1} - i_{d,1}}{i_{r,1} - i_{d,1}} \right)
\]

\[
\frac{1}{\sqrt{\eta D}} A \cdot \left( A e^{i \alpha (E_x E_z + E_z E_x e^{i(\phi - \zeta)} + E_y E_x e^{-i(\phi - \zeta)} + E_z E_y e^{i(\phi - \zeta)} + E_y E_z e^{-i(\phi - \zeta)} + E_y E_x e^{i(\phi - \zeta)})} + A e^{-i \alpha} (E_x E_z + E_z E_x e^{i(\phi - \zeta)} + E_x E_y e^{-i(\phi - \zeta)} + E_z E_y e^{i(\phi - \zeta)} + E_y E_z e^{-i(\phi - \zeta)} + E_y E_x e^{i(\phi - \zeta)}) + \frac{i_{M,0} - i_{r,0} - i_{s,0} + i_{d,0}}{\sqrt{\eta D} A} \right)
\]

\[
\left[ \frac{1}{\frac{r_s}{\sqrt{\eta D} A}} \cdot \left( \frac{i_{r,1} - i_{d,1}}{i_{r,1} - i_{d,1}} \right) \right]
\]

\[
\frac{1}{\sqrt{\eta D}} A \cdot \left( A e^{i \alpha (E_x E_z + E_z E_x e^{i(\phi - \zeta)} + E_y E_x e^{-i(\phi - \zeta)} + E_z E_y e^{i(\phi - \zeta)} + E_y E_z e^{-i(\phi - \zeta)} + E_y E_x e^{i(\phi - \zeta)})} + A e^{-i \alpha} (E_x E_z + E_z E_x e^{i(\phi - \zeta)} + E_x E_y e^{-i(\phi - \zeta)} + E_z E_y e^{i(\phi - \zeta)} + E_y E_z e^{-i(\phi - \zeta)} + E_y E_x e^{i(\phi - \zeta)}) + \frac{i_{M,0} - i_{r,0} - i_{s,0} + i_{d,0}}{\sqrt{\eta D} A} \right)
\]
Substitute Taylor Series:

\[
\left. \left[ \frac{1}{2} \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}} \cdot \left( Ae^{ia} (E_S E_R^* + E_S E_N^* e^{i(\phi-\zeta)} + E_N E_R^* e^{-i(\phi-\zeta)} + E_N E_N^*) E_{C_1}^S E_{C_2}^R X_1 X_2 e^{i(z_r-z_2)} + \right. \right. \right. \\
\left. \left. \left. Ae^{-ja} (-E_S E_S^* - E_R E_N^* e^{i(\phi-\zeta)} - E_N E_S^* e^{-i(\phi-\zeta)} - E_S E_N^*) E_{C_1}^R E_{C_2}^S X_1 X_2 e^{-i(z_r-z_2)}) \right]+ \right] \frac{i M_2 - i S_2 + i D_2}{\frac{1}{2} \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}}} \right] \\
\frac{1}{E_S E_X^* X_1 + E_S E_Y^* X_2} \left( 1 - \frac{i_{R,1} - i_{D,1}}{\frac{1}{2} \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}}} \right)
\]

\[
j^2(M_{D,2} - R_{D,2} - S_{D,2}) / \sqrt{R_{D,2}}
\]

\[
\frac{1}{2} \left| E_R E_{C_2} X_r + E_N E_{C_2} X_r \right| \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}} \left[ \frac{Ae^{-ja} (E_R E_S^* + E_S E_N^* e^{i(\phi-\zeta)} + E_N E_S^* e^{-i(\phi-\zeta)} + E_S E_N^*) E_{C_1}^R E_{C_2}^S X_1 X_2 e^{i(z_r-z_2)} +}{E_R E_{C_1}^R X_r + E_N E_{C_1}^R X_r} \right] \\
\frac{1}{2} \left| E_R E_{C_2} X_r + E_N E_{C_1} X_r \right| \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}} \left[ \frac{Ae^{-ja} (E_R E_S^* + E_S E_N^* e^{i(\phi-\zeta)} + E_N E_S^* e^{-i(\phi-\zeta)} + E_S E_N^*) E_{C_1}^R E_{C_2}^S X_1 X_2 e^{i(z_r-z_2)} +}{E_R E_{C_1}^R X_r + E_N E_{C_2}^R X_r} \right] \\
\frac{1}{2} \left| E_R E_{C_1} X_r + E_N E_{C_2} X_r \right| \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}} \left[ \frac{Ae^{-ja} (E_R E_S^* + E_S E_N^* e^{i(\phi-\zeta)} + E_N E_S^* e^{-i(\phi-\zeta)} + E_S E_N^*) E_{C_2}^R E_{C_1}^S X_1 X_2 e^{i(z_r-z_2)} +}{E_R E_{C_1} X_r + E_N E_{C_2} X_r} \right] \\
\frac{1}{2} \left| E_R E_{C_1} X_r + E_N E_{C_2} X_r \right| \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}} \left[ \frac{Ae^{-ja} (E_R E_S^* + E_S E_N^* e^{i(\phi-\zeta)} + E_N E_S^* e^{-i(\phi-\zeta)} + E_S E_N^*) E_{C_2}^R E_{C_1}^S X_1 X_2 e^{i(z_r-z_2)} +}{E_R E_{C_1} X_r + E_N E_{C_2} X_r} \right] \\
\frac{1}{2} \left| E_R E_{C_1} X_r + E_N E_{C_2} X_r \right| \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}} \left[ \frac{Ae^{-ja} (E_R E_S^* + E_S E_N^* e^{i(\phi-\zeta)} + E_N E_S^* e^{-i(\phi-\zeta)} + E_S E_N^*) E_{C_2}^R E_{C_1}^S X_1 X_2 e^{i(z_r-z_2)} +}{E_R E_{C_1} X_r + E_N E_{C_2} X_r} \right]
\]

Substitute Taylor Series:

\[
\left. \left[ \frac{1}{2} \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}} \cdot \left( Ae^{ia} (E_S E_R^* + E_S E_N^* e^{i(\phi-\zeta)} + E_N E_R^* e^{-i(\phi-\zeta)} + E_N E_N^*) E_{C_1}^S E_{C_2}^R X_1 X_2 e^{i(z_r-z_2)} + \right. \right. \right. \\
\left. \left. \left. Ae^{-ja} (-E_S E_S^* - E_R E_N^* e^{i(\phi-\zeta)} - E_N E_S^* e^{-i(\phi-\zeta)} - E_S E_N^*) E_{C_1}^R E_{C_2}^S X_1 X_2 e^{-i(z_r-z_2)}) \right]+ \right] \frac{i M_2 - i S_2 + i D_2}{\frac{1}{2} \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}}} \right] \\
\frac{1}{E_S E_X^* X_1 + E_S E_Y^* X_2} \left( 1 - \frac{i_{R,2} - i_{D,2}}{\frac{1}{2} \sqrt{\frac{\eta_0}{h_0}} A_{\text{pixel}}} \right)
\]
\[ j^3 \left( \text{MD}_{3} - \text{RD}_{3} - \text{SN}_{3} \right) / \sqrt{R_{D,3}} : \]

\[
\frac{\eta_{q}}{\pi \delta} A_{\text{pixel}} \left( \begin{array}{c}
\text{AE}^{j3} (E_{s} E_{r}^{*} + E_{s} E_{n}^{*} e^{j(\theta - \chi)} + E_{n} E_{r}^{*} e^{-j(\theta - \chi)} + E_{n} E_{n}^{*} E_{C_{1}}^{S} E_{C_{1}}^{R} X_{r} X_{r}^{*} e^{j(x_{r} - x_{r})}) + \\
\text{AE}^{-j3} (E_{s} E_{r}^{*} - E_{s} E_{n}^{*} e^{j(\theta - \chi)} - E_{n} E_{r}^{*} e^{-j(\theta - \chi)} - E_{n} E_{n}^{*} E_{C_{1}}^{S} E_{C_{1}}^{R} X_{r} X_{r}^{*} e^{-j(x_{r} - x_{r})}) + \\
- j(i_{M,3} - i_{D,3}) - (i_{R,3} - i_{D,3}) - (i_{S,3} - i_{D,3})
\end{array} \right) /
\]

\[
\frac{1}{2} E_{C_{1}}^{R} X_{r} E_{r} + E_{N} E_{C_{1}}^{R} X_{r} \sqrt{\eta_{q} \pi \delta A_{\text{pixel}}} \left( \begin{array}{c}
\text{AE}^{j3} (E_{s} E_{r}^{*} + E_{s} E_{n}^{*} e^{j(\theta - \chi)} + E_{n} E_{r}^{*} e^{-j(\theta - \chi)} + E_{n} E_{n}^{*} E_{C_{1}}^{S} E_{C_{1}}^{R} X_{r} X_{r}^{*} e^{j(x_{r} - x_{r})}) + \\
\text{AE}^{-j3} (E_{s} E_{r}^{*} - E_{s} E_{n}^{*} e^{j(\theta - \chi)} - E_{n} E_{r}^{*} e^{-j(\theta - \chi)} - E_{n} E_{n}^{*} E_{C_{1}}^{S} E_{C_{1}}^{R} X_{r} X_{r}^{*} e^{-j(x_{r} - x_{r})}) + \\
- j(i_{M,3} - i_{R,3} - i_{S,3} + i_{D,3})
\end{array} \right)
\]

Substitute Taylor Series:
Balanced Mixing, DC Term Subtraction, and Camera Normalization

\[
E_{BM,DC,\text{Norm, sample}} = \sum_{k=0}^{3} \frac{M_{D,k} - R_{D,k} - S_{D,k}}{\sqrt{R_{D,k}}} =
\]

\[
E_{BM,DC,\text{Norm, sample}} = \left[ \frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \right] \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{\eta_d}{h_D}} A_{\text{pixel}} \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right) \left( \frac{1}{\frac{\eta_d}{h_D} A_{\text{pixel}}} \right)
\]
\[ E_{BM,DC,Norm,sample} = \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{h_0}} \left[ A e^{j\alpha} (E_S E_R^* + E_S E_N^* e^{j(\phi-\varphi)} + E_N E_R^* e^{-j(\phi-\varphi)} + E_N E_N^*) X, X^* e^{j(x-x')}. \right] \]

\[ \left\{ \sqrt{\eta_0 E_C^R E_C^R} \right\} \left( 1 - \frac{i_{D,0}}{2} A_{\text{pixel}} (E_S E_R^* + E_S E_N^* e^{j(\phi-\varphi)} + E_N E_R^* e^{-j(\phi-\varphi)} + E_N E_N^*) E_C^0 X, X^* \right) + \]

\[ \left\{ \sqrt{\eta_0 E_C^R E_C^R} \right\} \left( 1 - \frac{i_{D,1}}{2} A_{\text{pixel}} (E_S E_R^* + E_S E_N^* e^{j(\phi-\varphi)} + E_N E_R^* e^{-j(\phi-\varphi)} + E_N E_N^*) E_C^1 X, X^* \right) + \]

\[ \left\{ \sqrt{\eta_0 E_C^R E_C^R} \right\} \left( 1 - \frac{i_{D,2}}{2} A_{\text{pixel}} (E_S E_R^* + E_S E_N^* e^{j(\phi-\varphi)} + E_N E_R^* e^{-j(\phi-\varphi)} + E_N E_N^*) E_C^2 X, X^* \right) + \]

\[ \left\{ \sqrt{\eta_0 E_C^R E_C^R} \right\} \left( 1 - \frac{i_{D,3}}{2} A_{\text{pixel}} (E_S E_R^* + E_S E_N^* e^{j(\phi-\varphi)} + E_N E_R^* e^{-j(\phi-\varphi)} + E_N E_N^*) E_C^3 X, X^* \right) + \]

\[ \left\{ \sqrt{\eta_0 E_C^R E_C^R} \right\} \left( 1 - \frac{i_{D,0}}{2} A_{\text{pixel}} (E_S E_R^* + E_S E_N^* e^{j(\phi-\varphi)} + E_N E_R^* e^{-j(\phi-\varphi)} + E_N E_N^*) E_C^0 X, X^* \right) - \]

\[ \left\{ \sqrt{\eta_0 E_C^R E_C^R} \right\} \left( 1 - \frac{i_{D,1}}{2} A_{\text{pixel}} (E_S E_R^* + E_S E_N^* e^{j(\phi-\varphi)} + E_N E_R^* e^{-j(\phi-\varphi)} + E_N E_N^*) E_C^1 X, X^* \right) - \]

\[ \left\{ \sqrt{\eta_0 E_C^R E_C^R} \right\} \left( 1 - \frac{i_{D,2}}{2} A_{\text{pixel}} (E_S E_R^* + E_S E_N^* e^{j(\phi-\varphi)} + E_N E_R^* e^{-j(\phi-\varphi)} + E_N E_N^*) E_C^2 X, X^* \right) - \]

\[ \left\{ \sqrt{\eta_0 E_C^R E_C^R} \right\} \left( 1 - \frac{i_{D,3}}{2} A_{\text{pixel}} (E_S E_R^* + E_S E_N^* e^{j(\phi-\varphi)} + E_N E_R^* e^{-j(\phi-\varphi)} + E_N E_N^*) E_C^3 X, X^* \right) \]
Assuming: \( i_R - i_D < \frac{1}{2} \eta \frac{q}{h} A_{\text{pixel}} (E_R E_R^* + E_R E_N^* e^{i(\phi - \gamma)} + E_N E_R^* e^{-i(\phi - \gamma)} + E_N E_N^*) E_C^R E_C^R X, X^* \)

\[
E_{BM,DC,Norm, sample} = \frac{1}{2} \sqrt{\frac{\eta q}{h}} A_{\text{pixel}} \left[ \begin{array}{c}
\sqrt{\eta_0 E_C^R E_C^R} \\
\sqrt{\eta_1 E_C^R E_C^R} \\
\sqrt{\eta_2 E_C^R E_C^R} \\
\sqrt{\eta_3 E_C^R E_C^R}
\end{array} \right] \left[ \begin{array}{c}
E_R E_C^R X_r + E_N E_C^R X_r \\
E_R E_C^R X_r + E_N E_C^R X_r \\
E_R E_C^R X_r + E_N E_C^R X_r \\
E_R E_C^R X_r + E_N E_C^R X_r
\end{array} \right]
\]

\[
A e^{-i\alpha} (E_R E_S^* + E_R E_N^* e^{i(\phi - \gamma)} + E_N E_R^* e^{-i(\phi - \gamma)} + E_N E_N^*) X_r X^* e^{i(\chi_r - \chi_x)}.
\]

\[
\frac{i_{M,0} - i_{R,0} - i_{S,0} + i_{D,0}}{2} + j \frac{i_{M,1} - i_{R,1} - i_{S,1} + i_{D,1}}{2} \sqrt{\frac{\eta q}{h}} A_{\text{pixel}} E_R E_C^R X_r + E_N E_C^R X_r \]

\[
\frac{i_{M,2} - i_{R,2} - i_{S,2} + i_{D,2}}{2} - j \frac{i_{M,3} - i_{R,3} - i_{S,3} + i_{D,3}}{2} \sqrt{\frac{\eta q}{h}} A_{\text{pixel}} E_R E_C^R X_r + E_N E_C^R X_r
\]

If the detectors were ideal: \( i_M = i_R = i_S = i_D \) \text{ and } \( \eta_0 = \eta_1 = \eta_2 = \eta_3 = 1 \)

\[
E_{BM,DC,Norm, sample} = \frac{1}{2} \sqrt{\frac{\eta q}{h}} A_{\text{pixel}} \left[ \begin{array}{c}
E_S E_C^R E_C^R \\
E_C^R E_C^R \\
E_C^R E_C^R \\
E_C^R E_C^R
\end{array} \right] \left[ \begin{array}{c}
E_R E_C^R X_r + E_N E_C^R X_r \\
E_R E_C^R X_r + E_N E_C^R X_r \\
E_R E_C^R X_r + E_N E_C^R X_r \\
E_R E_C^R X_r + E_N E_C^R X_r
\end{array} \right]
\]

\[
A e^{-i\alpha} (E_R E_S^* + E_R E_N^* e^{i(\phi - \gamma)} + E_N E_R^* e^{-i(\phi - \gamma)} + E_N E_N^*) X_r X^* e^{i(\chi_r - \chi_x)}.
\]

\[
\frac{E_S E_C^R E_C^R}{E_R E_C^R X_r + E_N E_C^R X_r} - \frac{E_C^R E_C^R}{E_R E_C^R X_r + E_N E_C^R X_r} + \frac{E_C^R E_C^R}{E_R E_C^R X_r + E_N E_C^R X_r} - \frac{E_C^R E_C^R}{E_R E_C^R X_r + E_N E_C^R X_r}
\]

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If the fixed pattern noise was the same in the signal and reference paths: \[ E_C^S = E_C^R = E_C \]

\[
E_{BM,DC,Norm,sample} = \frac{1}{2} \sqrt{\frac{A_{noise}}{h \nu}} \cdot \left[ A e^{j\alpha} (E_S E_R^* + E_S^* E_N e^{j(\phi - \xi)} + E_N E_R^* e^{-j(\phi + \xi)} + E_N^* E_S e^{j(\phi + \xi)}), \frac{E_{C_0} + E_{C_1} + E_{C_2} + E_{C_3}}{|E_r X_r + E_N X_r|} \right]
\]

\[
A e^{-j\alpha} (E_R E_S^* + E_R^* E_N e^{j(\phi - \xi)} + E_N E_S^* e^{-j(\phi - \xi)} + E_N^* E_R e^{j(\phi - \xi)}), \frac{E_{C_0} - E_{C_1} + E_{C_2} - E_{C_3}}{|E_r X_r + E_N X_r|}
\]

If there was no fixed pattern noise: \[ E_{C_0} = E_{C_1} = E_{C_2} = E_{C_3} = 1 \]

\[
E_{BM,DC,Norm,sample} = \frac{2 \sqrt{A_{noise}}}{|E_r X_r + E_N X_r|} \cdot \left( A e^{j\alpha} (E_S E_R^* + E_S^* E_N e^{j(\phi - \xi)} + E_N E_R^* e^{-j(\phi - \xi)} + E_N^* E_S e^{j(\phi - \xi)}), (E_S E_R^* + E_S^* E_N e^{j(\phi - \xi)} + E_N E_R^* e^{-j(\phi - \xi)} + E_N^* E_S e^{j(\phi - \xi)}) \right)
\]

If there were no aberrations in the system: \[ X_s e^{j\xi} = X_s e^{j\xi} = 1 \]

\[
E_{BM,DC,Norm,sample} = \frac{2 \sqrt{A_{noise}}}{|E_r + E_N|} \cdot A e^{j\alpha} (E_S E_R^* + E_S^* E_N e^{j(\phi - \xi)} + E_N E_R^* e^{-j(\phi - \xi)} + E_N^* E_S e^{j(\phi - \xi)})
\]

If there was no noise in the laser: \[ E_N e^{j(\alpha + \xi)} = 0 \]

\[
E_{BM,DC,Norm,sample} = \frac{2 \sqrt{A_{noise}}}{|E_r|^2} E_S E_R^* \cdot A e^{j\alpha}, \text{ which is proportional to the electric field for the ideal system.}
\]
Fiber splitter splits Electric Field into Signal and Reference Paths (let $j = \sqrt{-1}$):

\[
\tilde{E}_{\text{ref}} = \frac{1}{\sqrt{2}} (E_R e^{j(x+\phi)} + E_S e^{j(\alpha + \beta)})
\]
\[
\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (E_S e^{j(\alpha + \phi)} + E_R e^{j(\alpha + \beta)})
\]

Signal Beam is Linearly Polarized:

\[
\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (E_S e^{j(\alpha + \phi)} + E_R e^{j(\alpha + \beta)}) \cdot (x + y)
\]

Reference Beam is Circularly Polarized:

\[
\tilde{E}_{\text{ref}} = \frac{1}{\sqrt{2}} (E_S e^{j(\alpha + \phi)} + E_R e^{j(\alpha + \beta)}) \cdot (x + jy)
\]

Amplitude and Phase of Sample induced into Signal Beam:

\[
\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (E_S e^{j(\alpha + \phi)} + E_R e^{j(\alpha + \beta)}) \cdot (x + y)
\]

Fields Entering the Recombining Beamsplitter (where $X e^{j\chi}$ is the aberrations within the path):

\[
\tilde{E}_{\text{ref}} = \frac{1}{\sqrt{2}} (E_R e^{j(\alpha + \phi)} + E_S e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + iy)
\]
\[
\tilde{E}_{\text{sig}} = \frac{1}{\sqrt{2}} (E_S e^{j(\alpha + \phi)} + E_R e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + iy)
\]

Mix of Reference and Signal Fields after Recombining Beamsplitter:

Path 1 (Camera 0 & Camera 1):

\[
\frac{1}{\sqrt{2}} (\tilde{E}_{\text{sig}} + \tilde{E}_{\text{ref}})
\]
\[
\frac{1}{\sqrt{2}} \left( \frac{1}{\sqrt{2}} (E_S e^{j(\alpha + \phi)} + E_R e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + y) + \frac{1}{\sqrt{2}} (E_R e^{j(\alpha + \phi)} + E_S e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + jy) \right) =
\]
\[
\frac{1}{2} ((E_S e^{j(\alpha + \phi)} + E_R e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + y) + (E_R e^{j(\alpha + \phi)} + E_S e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + jy))
\]

Path 2 (Camera 2 & Camera 3):

\[
\frac{1}{\sqrt{2}} (\tilde{E}_{\text{sig}} - \tilde{E}_{\text{ref}})
\]
\[
\frac{1}{\sqrt{2}} \left( \frac{1}{\sqrt{2}} (E_S e^{j(\alpha + \phi)} + E_R e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + y) - \frac{1}{\sqrt{2}} (E_R e^{j(\alpha + \phi)} + E_S e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + jy) \right) =
\]
\[
\frac{1}{2} ((E_S e^{j(\alpha + \phi)} + E_R e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + y) - (E_R e^{j(\alpha + \phi)} + E_S e^{j(\alpha + \beta)}) X_s e^{j\chi_s} \cdot (x + jy))
\]
Pure Reference (Signal Blocked) after Recombining Beamsplitter:
Both Paths:
$$\frac{1}{\sqrt{2}} E_{\text{ref}}$$

$$\frac{1}{\sqrt{2}} \left( \frac{1}{\sqrt{2}} (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) X_r e^{jx} \right) \cdot (\tilde{x} + j\tilde{y})$$

Pure Signal (Reference Blocked) after Recombining Beamsplitter:
Both Paths:
$$\frac{1}{\sqrt{2}} E_{\text{sig}}$$

$$\frac{1}{\sqrt{2}} \left( \frac{1}{\sqrt{2}} (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) X_r e^{jx} \right) \cdot (\tilde{x} + j\tilde{y})$$

Detector Noise:
$$\frac{n_l}{h\nu} A_{\text{pixel}} P + i_D$$

where:
$$\eta$$ – quantum efficiency (unitless)
$$q$$ – charge of an electron ($1.6 \times 10^{-19} \text{c}$)
$$h$$ – Planck’s constant ($6.6 \times 10^{-34} \text{Wsec}^2$)
$$\nu$$ – frequency of the laser (sec$^{-1}$)
$$A_{\text{pixel}}$$ – area of a pixel ($\text{m}^2$)
$$P$$ – irradiance ($\text{Wm}^{-2}$): equivalent to $|E|^2$
$$i_D$$ – dark current

Mixed Signal and Reference after Polarizing Beamsplitters (where $E_C$ is fixed pattern noise in Sig & Ref)

Camera 0 ($\tilde{x}$):
$$\frac{1}{2} ((E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) \cdot (\tilde{x})$$

$$\frac{n_l}{h\nu} A_{\text{pixel}} \left[ \frac{1}{2} ((E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) \right]^2 + i_{BM,0} =$$

$$\frac{n_l}{h\nu} A_{\text{pixel}} \left[ \frac{1}{2} ((E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) \right]^2 + i_{BM,0} =$$

$$\frac{n_l}{h\nu} A_{\text{pixel}} [(E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + i_{BM,0} =$$

$$\frac{n_l}{h\nu} A_{\text{pixel}} [(E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + (E_s e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^S X_S e^{jx} + (E_R e^{i(\omega t + \phi)} + E_B e^{i(\omega t + \beta)}) E_c^R X_r e^{jx}) + i_{BM,0} =$$

BM =
Camera 1 (\(\hat{\gamma}\)):

\[
\frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_1}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_1}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,1} =
\]

\[
\frac{1}{2} \sum_{B,b} A_{pixel} \left| \frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_1}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_1}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,1} =
\]

\[
\frac{1}{2} \sum_{B,b} A_{pixel} \left| \frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_1}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_1}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,1} =
\]

\[
\frac{1}{2} \sum_{B,b} A_{pixel} \left| \frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_1}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_1}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,1} =
\]

\[
BM_1
\]

Camera 2 (\(\hat{x}\)):

\[
\frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_2}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_2}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,2} =
\]

\[
\frac{1}{2} \sum_{B,b} A_{pixel} \left| \frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_2}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_2}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,2} =
\]

\[
\frac{1}{2} \sum_{B,b} A_{pixel} \left| \frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_2}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_2}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,2} =
\]

\[
BM_2
\]

Camera 3 (\(\hat{y}\)):

\[
\frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_3}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_3}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,3} =
\]

\[
\frac{1}{2} \sum_{B,b} A_{pixel} \left| \frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_3}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_3}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,3} =
\]

\[
\frac{1}{2} \sum_{B,b} A_{pixel} \left| \frac{1}{2} \left( |(E_S e^{j(\omega t+\phi)} + E_g e^{j(\omega t+\beta)}) E_{C_3}^S X_s e^{j\lambda t_r} + (j E_R e^{j(\omega t+\phi)} + j E_g e^{j(\omega t+\beta)}) E_{C_3}^R X_s e^{j\lambda t_r} \right)^2 + i_{BM,3} =
\]

\[
BM_3
\]
Balanced Mixing:

\[ E_{BM, blank} = \sum_{k=0}^{3} j^k BM_k \]
\[ E_{BM,\text{blank}} = \frac{1}{4} \frac{A_{\text{geom}}}{h_0} \left( (E_x E_y^* + E_y E_x^*) e^{j(\phi - \beta)} + E_x E_y e^{-j(\phi - \beta)} + E_y E_y^*) X_s X_s^* e^{j(\chi, - \chi_0)} \right) \]

\[ (\eta E_{C_0} E_{R_0}^* + \eta E_{C_1} E_{R_1}^* + \eta E_{C_2} E_{R_2}^* + \eta E_{C_4} E_{R_4}^*) + (\eta E_{C_0} E_{R_0}^* + \eta E_{C_1} E_{R_1}^* + \eta E_{C_2} E_{R_2}^* + \eta E_{C_4} E_{R_4}^*) \]

\[ (\eta_0 E_{C_0} E_{R_0}^* - \eta E_{C_1} E_{R_1}^* + \eta_2 E_{C_2} E_{R_2}^* - \eta_3 E_{C_3} E_{R_3}^*) + (\eta_0 E_{C_0} E_{R_0}^* + \eta E_{C_1} E_{R_1}^* + \eta_2 E_{C_2} E_{R_2}^* - \eta_3 E_{C_3} E_{R_3}^*) \]

\[ (E_y E_y^*) X_s X_s^* \cdot (\eta_1 E_{C_0} E_{R_0}^* + j \eta_2 E_{C_1} E_{R_1}^* - j \eta_3 E_{C_2} E_{R_2}^*) + (E_y E_y^*) X_s X_s^* \cdot (\eta_1 E_{C_0} E_{R_0}^* + j \eta_2 E_{C_1} E_{R_1}^* - j \eta_3 E_{C_2} E_{R_2}^*) \]

\[ (i_{BM,0} - i_{BM,2} + j(i_{BM,1} - i_{BM,3})) \]

If the detectors were ideal: \[ i_{BM,0} = i_{BM,1} = i_{BM,3} = i_{BM} \quad \text{and} \quad \eta_0 = \eta_1 = \eta_2 = \eta_3 = 1 \]

\[ E_{BM,\text{blank}} = \frac{1}{4} \frac{A_{\text{geom}}}{h_0} \left( (E_x E_y^* + E_y E_x^*) e^{j(\phi - \beta)} + E_x E_y e^{-j(\phi - \beta)} + E_y E_y^*) X_s X_s^* e^{j(\chi, - \chi_0)} \right) \]

\[ (E_y E_y^*) X_s X_s^* \cdot (E_{C_0} E_{R_0}^* + E_{C_1} E_{R_1}^* + E_{C_2} E_{R_2}^* + E_{C_4} E_{R_4}^*) + \]

\[ (E_y E_y^*) X_s X_s^* \cdot (E_{C_0} E_{R_0}^* - E_{C_1} E_{R_1}^* + E_{C_2} E_{R_2}^* - E_{C_4} E_{R_4}^*) \]

If the fixed pattern noise was the same in the signal and reference paths: \[ E_C = E_C^* \]

If there was no fixed pattern noise: \[ E_{C_0} = E_{C_1} = E_{C_2} = E_{C_3} = 1 \]

\[ E_{BM,\text{blank}} = (E_x E_y^* + E_y E_x^*) e^{j(\phi - \beta)} + E_x E_y e^{-j(\phi - \beta)} + E_y E_y^*) X_s X_s^* e^{j(\chi, - \chi_0)} \]

If there were no aberrations in the system: \[ X_s e^{j\chi} = X_s e^{j\chi} = 1 \]

\[ E_{BM,\text{blank}} = (E_x E_x^* + E_y E_y^*) e^{j(\phi - \beta)} + E_y E_y e^{-j(\phi - \beta)} + E_y E_y^*) \frac{A_{\text{geom}}}{h_0} X_s X_s^* e^{j(\chi, - \chi_0)} \]

If there was no noise in the laser: \[ E_{\beta} e^{j(\omega t + \beta)} = 0 \]

\[ E_{BM,\text{blank}} = (E_x E_x^*) \frac{A_{\text{geom}}}{h_0} \]

Converting to Irradiance: \[ \hat{I}_{BM,\text{blank}} = \frac{E_{BM,\text{blank}} h_0}{A_{\text{geom}}} \]

\[ \hat{I}_{BM,\text{blank}} = E_x E_x^* \]
Pure Dark After Polarizing Beamsplitters:

Camera 0: \( i_{D,0} \) \hspace{1cm} (D_0)

Camera 1: \( i_{D,1} \) \hspace{1cm} (D_1)

Camera 2: \( i_{D,2} \) \hspace{1cm} (D_2)

Camera 3: \( i_{D,3} \) \hspace{1cm} (D_3)

Pure Reference after the Polarizing Beamsplitters:

Camera 0 (\( \vec{x} \)):
\[
\frac{\eta \Upsilon}{h \nu} A_{pixel} \left( \frac{1}{2} (E_R e^{i(\alpha+\phi)} + E_B e^{i(\alpha+\beta)}) X_r e^{i\lambda R} E_C^R (\vec{x}) \right)^2 + i_{BR,0} = \]

\[
= \frac{1}{4} \eta \Upsilon A_{pixel} (E_R E_R^* + E_B E_B^*) e^{i(\phi-\beta)} + E_B E_B^* e^{-i(\phi-\beta)} + E_B E_B^*) E_C^R X_r X_r^* + i_{BR,0} \tag{BR_0}
\]

Square Root:
\[
\frac{1}{2} E_C^R X_r |E_R + E_B| \cdot \sqrt{\frac{\eta \Upsilon A_{pixel}}{4} \cdot \left( \frac{1}{2} (E_R e^{i(\alpha+\phi)} + E_B e^{i(\alpha+\beta)}) X_r e^{i\lambda R} E_C^R \right)^2 + i_{BR,0}} \sqrt{BR_0}
\]

Camera 1 (\( \vec{y} \)):
\[
\frac{\eta \Upsilon}{h \nu} A_{pixel} \left( j \frac{1}{2} (E_R e^{i(\alpha+\phi)} + E_B e^{i(\alpha+\beta)}) X_r e^{i\lambda R} E_C^R (\vec{y}) \right)^2 + i_{BR,1} = \]

\[
= \frac{1}{4} \eta \Upsilon A_{pixel} (E_R E_R^* + E_B E_B^*) e^{i(\phi-\beta)} + E_B E_B^* e^{-i(\phi-\beta)} + E_B E_B^*) E_C^R X_r X_r^* + i_{BR,1} \tag{BR_1}
\]

Square Root:
\[
\frac{1}{2} E_C^R X_r |E_R + E_B| \cdot \sqrt{\frac{\eta \Upsilon A_{pixel}}{4} \cdot \left( j \frac{1}{2} (E_R e^{i(\alpha+\phi)} + E_B e^{i(\alpha+\beta)}) X_r e^{i\lambda R} E_C^R \right)^2 + i_{BR,1}} \sqrt{BR_1}
\]

Camera 2 (\( \vec{z} \)):
\[
\frac{\eta \Upsilon}{h \nu} A_{pixel} \left( \frac{1}{2} (E_R e^{i(\alpha+\phi)} + E_B e^{i(\alpha+\beta)}) X_r e^{i\lambda R} E_C^R (\vec{z}) \right)^2 + i_{BR,2} = \]

\[
= \frac{1}{4} \eta \Upsilon A_{pixel} (E_R E_R^* + E_B E_B^*) e^{i(\phi-\beta)} + E_B E_B^* e^{-i(\phi-\beta)} + E_B E_B^*) E_C^R X_r X_r^* + i_{BR,2} \tag{BR_2}
\]

Square Root:
\[
\frac{1}{2} E_C^R X_r |E_R + E_B| \cdot \sqrt{\frac{\eta \Upsilon A_{pixel}}{4} \cdot \left( \frac{1}{2} (E_R e^{i(\alpha+\phi)} + E_B e^{i(\alpha+\beta)}) X_r e^{i\lambda R} E_C^R \right)^2 + i_{BR,2}} \sqrt{BR_2}
\]
Camera 3 ($\hat{y}$):

$$j \frac{1}{2}(E_R e^{j(\alpha + \phi)} + E_g e^{j(\alpha + \beta)}) \chi e^{i\chi} E^R_{C_3} \cdot (\hat{y})$$

$$\frac{\eta y}{\eta y} A_{\text{pixel}} \left| j \frac{1}{2}(E_R e^{j(\alpha + \phi)} + E_g e^{j(\alpha + \beta)}) \chi e^{i\chi} E^R_{C_3} \right|^2 + i_{BR,3} =$$

$$\frac{1}{4} \frac{\eta y}{\eta y} A_{\text{pixel}} \left( E_R E^*_R + E_R E^*_g e^{j(\phi - \beta)} + E_g E^*_g e^{-j(\phi - \beta)} + E_R E^*_g \right) E^R_{C_3} E^S_{C_3} x, x^* + i_{BR,3} \tag{BR_3}$$

Square Root:

$$\frac{1}{2} E^R_{C_3} x, E_R + E_B \left| \sqrt{\frac{\eta y}{\eta y} A_{\text{pixel}} \left( 1 + \frac{1}{4} \frac{\eta y}{\eta y} A_{\text{pixel}} \left( E_R E^*_R + E_R E^*_g e^{j(\phi - \beta)} + E_g E^*_g e^{-j(\phi - \beta)} + E_R E^*_g \right) E^R_{C_3} E^S_{C_3} x, x^* \right)^*} \right| \sqrt{BR}$$

Balanced Mixing of Pure Reference:

$$E_{BM,\text{blank ref}} = \sum_{k=0}^{3} j^k BR_k$$

$$= \frac{1}{4} \frac{\eta y}{\eta y} A_{\text{pixel}} \left( E_R E^*_R + E_R E^*_g e^{j(\phi - \beta)} + E_g E^*_g e^{-j(\phi - \beta)} + E_R E^*_g \right) E^R_{C_3} E^S_{C_3} x, x^* + i_{BR,0} +$$

$$j \frac{1}{4} \frac{\eta y}{\eta y} A_{\text{pixel}} \left( E_R E^*_R + E_R E^*_g e^{j(\phi - \beta)} + E_g E^*_g e^{-j(\phi - \beta)} + E_R E^*_g \right) E^R_{C_3} E^S_{C_3} x, x^* + j(i_{BR,1}) +$$

$$- \frac{1}{4} \frac{\eta y}{\eta y} A_{\text{pixel}} \left( E_R E^*_R + E_R E^*_g e^{j(\phi - \beta)} + E_g E^*_g e^{-j(\phi - \beta)} + E_R E^*_g \right) E^R_{C_3} E^S_{C_3} x, x^* - i_{BR,2} +$$

$$- j \frac{1}{4} \frac{\eta y}{\eta y} A_{\text{pixel}} \left( E_R E^*_R + E_R E^*_g e^{j(\phi - \beta)} + E_g E^*_g e^{-j(\phi - \beta)} + E_R E^*_g \right) E^R_{C_3} E^S_{C_3} x, x^* - j(i_{BR,3})$$

$$E_{BM,\text{blank ref}} = \frac{1}{4} \frac{\eta y}{\eta y} A_{\text{pixel}} \left( E_R E^*_R + E_R E^*_g e^{j(\phi - \beta)} + E_g E^*_g e^{-j(\phi - \beta)} + E_R E^*_g \right) X, x^* \cdot \left( \eta_0 E^R_{C_0} E^R_{C_0} + j \eta_0 E^R_{C_1} E^R_{C_1} - \eta_2 E^R_{C_2} E^R_{C_2} - j \eta_3 E^R_{C_3} E^R_{C_3} \right) + (i_{BR,0} - i_{BR,2} + j(i_{BR,1} - i_{BR,3}))$$

If no fixed pattern noise $E^R_C = 1$ and ideal detectors $i_{BM,0} = i_{BM,1} = i_{BM,2} = i_{BM,3} = i_{BM}$ and $\eta = 1$: $E_{BM,\text{blank ref}} = 0$

Pure Signal after the Polarizing Beamsplitters:

Camera 0 ($\hat{x}$):

$$\frac{1}{2}(E_x e^{j(\alpha + \phi)} + E_g e^{j(\alpha + \beta)}) X_x e^{i\chi} E^S_{C_0} \cdot (\hat{x})$$

$$\frac{\eta y}{\eta y} A_{\text{pixel}} \left| \frac{1}{2}(E_x e^{j(\alpha + \phi)} + E_g e^{j(\alpha + \beta)}) X_x e^{i\chi} E^S_{C_0} \right|^2 + i_{BS,0} =$$

$$\frac{1}{2} \frac{\eta y}{\eta y} A_{\text{pixel}} \left( E_x E^*_x + E_x E^*_g e^{j(\phi - \beta)} + E_g E^*_g e^{-j(\phi - \beta)} + E_x E^*_g \right) E^S_{C_0} E^S_{C_0} x, x^* + i_{BS,0} \tag{BS_0}$$

Camera 1 ($\hat{y}$):

$$\frac{1}{4} \frac{\eta y}{\eta y} A_{\text{pixel}} \left( E_x E^*_x + E_x E^*_g e^{j(\phi - \beta)} + E_g E^*_g e^{-j(\phi - \beta)} + E_x E^*_g \right) E^S_{C_0} E^S_{C_0} x, x^* + i_{BS,0} \tag{BS_0}$$

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\[
\frac{1}{2}(E_se^{j(\alpha t + \phi)} + E_be^{j(\alpha t + \beta)}) X_s e^{jx} E_{C_1}^S \quad (\bar{y})
\]

\[
\frac{\eta_g}{\hbar v} A_{pixel} \left[ \frac{1}{2}(E_se^{j(\alpha t + \phi)} + E_be^{j(\alpha t + \beta)}) X_s e^{jx} E_{C_1}^S \right]^2 + i_{BS,1} = \]

\[
\frac{1}{2} \frac{\eta_g}{\hbar v} A_{pixel} (E_se^{j(\alpha t + \phi)} + E_be^{j(\alpha t + \beta)}) X_s e^{jx} E_{C_1}^S \cdot \frac{1}{2}(E_s e^{-j(\alpha t + \phi)} + E_b e^{-j(\alpha t + \beta)}) X_s e^{jx} E_{C_1}^S + i_{BS,1} = \]

\[
\frac{1}{4} \frac{\eta_g}{\hbar v} A_{pixel} (E_s E_s^* + E_s E_b e^{j(\phi - \beta)} + E_b E_s e^{-j(\phi - \beta)} + E_b E_b^*) E_{C_1}^S X_s^* X_s^* + i_{BS,1} \quad (BS_1)
\]

**Camera 2 (\bar{x}):**

\[
\frac{1}{2}(E_se^{j(\alpha t + \phi)} + E_be^{j(\alpha t + \beta)}) X_s e^{jx} E_{C_2}^S \cdot (\bar{x})
\]

\[
\frac{\eta_g}{\hbar v} A_{pixel} \left[ \frac{1}{2}(E_se^{j(\alpha t + \phi)} + E_be^{j(\alpha t + \beta)}) X_s e^{jx} E_{C_2}^S \right]^2 + i_{BS,2} = \]

\[
\frac{1}{2} \frac{\eta_g}{\hbar v} A_{pixel} (E_se^{j(\alpha t + \phi)} + E_be^{j(\alpha t + \beta)}) X_s e^{jx} E_{C_2}^S \cdot \frac{1}{2}(E_s e^{-j(\alpha t + \phi)} + E_b e^{-j(\alpha t + \beta)}) X_s e^{jx} E_{C_2}^S + i_{BS,2} = \]

\[
\frac{1}{4} \frac{\eta_g}{\hbar v} A_{pixel} (E_s E_s^* + E_s E_b e^{j(\phi - \beta)} + E_b E_s e^{-j(\phi - \beta)} + E_b E_b^*) E_{C_2}^S X_s^* X_s^* + i_{BS,2} \quad (BS_2)
\]

**Camera 3 (\bar{y}):**

\[
\frac{1}{2}(E_se^{j(\alpha t + \phi)} + E_be^{j(\alpha t + \beta)}) X_s e^{jx} E_{C_3}^S \cdot (\bar{y})
\]

\[
\frac{\eta_g}{\hbar v} A_{pixel} \left[ \frac{1}{2}(E_se^{j(\alpha t + \phi)} + E_be^{j(\alpha t + \beta)}) X_s e^{jx} E_{C_3}^S \right]^2 + i_{BS,3} = \]

\[
\frac{1}{2} \frac{\eta_g}{\hbar v} A_{pixel} (E_se^{j(\alpha t + \phi)} + E_be^{j(\alpha t + \beta)}) X_s e^{jx} E_{C_3}^S \cdot \frac{1}{2}(E_s e^{-j(\alpha t + \phi)} + E_b e^{-j(\alpha t + \beta)}) X_s e^{jx} E_{C_3}^S + i_{BS,3} = \]

\[
\frac{1}{4} \frac{\eta_g}{\hbar v} A_{pixel} (E_s E_s^* + E_s E_b e^{j(\phi - \beta)} + E_b E_s e^{-j(\phi - \beta)} + E_b E_b^*) E_{C_3}^S X_s^* X_s^* + i_{BS,3} \quad (BS_3)
\]

**Balanced Mixing of Pure Signal:**

\[
E_{BM\text{ blanksg}} = \sum_{k=0}^{3} j^k BS_k
\]

\[
\frac{1}{4} \frac{\eta_g}{\hbar v} A_{pixel} (E_s E_s^* + E_s E_b e^{j(\phi - \beta)} + E_b E_s e^{-j(\phi - \beta)} + E_b E_b^*) E_{C_0}^S X_s X_s^* + i_{BS,0} +
\]

\[
+ \sum_{k=0}^{3} j^k BS_k
\]

\[
\frac{1}{4} \frac{\eta_g}{\hbar v} A_{pixel} (E_s E_s^* + E_s E_b e^{j(\phi - \beta)} + E_b E_s e^{-j(\phi - \beta)} + E_b E_b^*) E_{C_1}^S X_s X_s^* + j(i_{BS,1}) +
\]

\[
- \frac{1}{4} \frac{\eta_g}{\hbar v} A_{pixel} (E_s E_s^* + E_s E_b e^{j(\phi - \beta)} + E_b E_s e^{-j(\phi - \beta)} + E_b E_b^*) E_{C_1}^S X_s X_s^* - j(i_{BS,2}) +
\]

\[
- \frac{1}{4} \frac{\eta_g}{\hbar v} A_{pixel} (E_s E_s^* + E_s E_b e^{j(\phi - \beta)} + E_b E_s e^{-j(\phi - \beta)} + E_b E_b^*) E_{C_2}^S X_s X_s^* - j(i_{BS,3})
\]

\[
E_{BM\text{ blanksg}} = \frac{1}{4} \frac{\eta_g}{\hbar v} \left[ (E_s E_s^* + E_s E_b e^{j(\phi - \beta)} + E_b E_s e^{-j(\phi - \beta)} + E_b E_b^*) X_s X_s^* \cdot \left( \eta_s E_s E_s E_s^* - \eta_s E_s E_s E_s^* - j \eta_s E_s E_s E_s^* + j(i_{BS,0}) \right) \right] +
\]

\[
(i_{BS,0} - i_{BS,2} + j(i_{BS,1} - i_{BS,3}))
\]
If no fixed pattern noise \( E_C^S = 1 \) and ideal detectors \( i_{BS,0} = i_{BS,1} = i_{BS,2} = i_{BS,3} = i_{BS} \) and \( \eta = 1 \):

\[ E_{BM,blanking} = 0 \]

Simplify \( \sqrt{R_D} \) with a Taylor Series: \( f(x) = \sum_{n=0}^{\infty} \frac{f^{(n)}(a)}{n!} (x-a)^n \), (Let \( a = 0 \), thus using Maclaurin series):

\[ \sqrt{1 + x} \approx 1 + \frac{i_{BM} - i_D}{4 \eta x} + \ldots \quad \text{where:} \quad x = i_{BM} - i_D \]

\[ \frac{i_{BM} - i_D}{4 \eta x} = 1 + \frac{i_{BM} - i_D}{4 \eta x} - \frac{(i_{BM} - i_D)^2}{32 \eta^2 x^2} + \ldots \]

Since \( x \) is already near zero, we assume \( x \) raised to any power is zero:

\[ \sqrt{R_D} \approx \frac{1}{2} E_C^R X_r |E_R + E_B| \cdot \frac{4 \eta q}{h \nu \text{A}_{\text{pixel}}} \left( 1 + \frac{i_{BM} - i_D}{4 \eta x} \right) \]

Substitution of Dark:

\[ \frac{4 \eta q}{h \nu \text{A}_{\text{pixel}}} \left( (E_S E_C^S + E_S E_C^R e^{j(\phi - \beta)}) E_c^S X_c^S + (E_S E_R^* + E_S E_B^* e^{j(\phi - \beta)}) E_c^S X_c^S + (E_S E_R^* + E_S E_B^* e^{j(\phi - \beta)}) E_c^R X_c^R e^{j(\lambda - \chi)} + (E_S E_R^* + E_S E_B^* e^{j(\phi - \beta)}) E_c^R X_c^R e^{j(\lambda - \chi)} + (E_S E_R^* + E_S E_B^* e^{j(\phi - \beta)}) E_c^R X_c^R e^{j(\lambda - \chi)} \right) \]

\[ (E_R E_B^* + E_R E_B^* e^{j(\phi - \beta)}) E_c^R X_c^R e^{j(\lambda - \chi)} + (E_R E_B^* + E_R E_B^* e^{j(\phi - \beta)}) E_c^R X_c^R e^{j(\lambda - \chi)} + (E_R E_B^* + E_R E_B^* e^{j(\phi - \beta)}) E_c^R X_c^R e^{j(\lambda - \chi)} + (E_R E_B^* + E_R E_B^* e^{j(\phi - \beta)}) E_c^R X_c^R e^{j(\lambda - \chi)} + (E_R E_B^* + E_R E_B^* e^{j(\phi - \beta)}) E_c^R X_c^R e^{j(\lambda - \chi)} + i_{BM,0} - i_{D,0} \]  

\[ (BM_{D,0}) \]

\[ \frac{4 \eta q}{h \nu \text{A}_{\text{pixel}}} \left( E_S E_R + E_S E_B^* e^{j(\phi - \beta)} + E_B E_S^* e^{j(\phi - \beta)} + E_B E_B^* e^{j(\phi - \beta)} \right) E_c^S X_c^S + i_{BS,0} - i_{D,0} \]

\[ (BS_{D,0}) \]

\[ \frac{4 \eta q}{h \nu \text{A}_{\text{pixel}}} \left( E_R E_R + E_R E_B^* e^{j(\phi - \beta)} + E_B E_R^* e^{j(\phi - \beta)} + E_B E_B^* e^{j(\phi - \beta)} \right) E_c^R X_c^R + i_{BR,0} - i_{D,0} \]

\[ (BR_{D,0}) \]

\[ \frac{1}{2} E_C^R X_r |E_R + E_B| \cdot \sqrt{\frac{4 \eta q}{h \nu \text{A}_{\text{pixel}}} \left( 1 + \frac{i_{BR,0} - i_D}{4 \eta x} \right)} \]

\[ \sqrt{BR_{D,0}} \]

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\[ \frac{\eta_d}{4} A_{\text{pixel}} \left[ \left( E_S E_S' + E_S E_B' e^{i(\phi-\beta)} \right) E_C E_C^* X, X^* + \left( -jE_S E_S' - jE_S E_B' e^{i(\phi-\beta)} \right) E_C E_C^* X, X^* \right] + \]
\[ \left( E_B E_B' e^{-j(\phi-\beta)} + E_B E_B' \right) E_C E_C^* X, X^* + \left( -jE_B E_B' + jE_B E_B' e^{i(\phi-\beta)} \right) E_C E_C^* X, X^* \right] + \]
\[ \left( jE_B E_B' e^{-j(\phi-\beta)} + jE_B E_B' \right) E_C E_C^* X, X^* + \left( E_B E_B' + jE_B E_B' e^{i(\phi-\beta)} \right) E_C E_C^* X, X^* \right] + \]
\[ \left( jE_B E_B' e^{-j(\phi-\beta)} + jE_B E_B' \right) E_C E_C^* X, X^* + \left( jE_B E_B' e^{i(\phi-\beta)} + jE_B E_B' \right) E_C E_C^* X, X^* \right] \]
\[
\frac{1}{4} \eta_{4D} A_{\text{pixel}} \left( E_S E_S^* + E_S E_B^* e^{i(\theta - \beta)} + E_B E_S^* e^{-i(\theta - \beta)} + E_B E_B^* \right) E_C^S \left[ X, X_r^* \right] + i_{BS,3} - i_{D,3}
\]  
(\text{BD}_3)

\[
\frac{1}{4} \eta_{4D} A_{\text{pixel}} \left( E_R E_R^* + E_R E_B^* e^{i(\theta - \beta)} + E_B E_R^* e^{-i(\theta - \beta)} + E_B E_B^* \right) E_C^R \left[ X, X_r^* \right] + i_{BR,3} - i_{D,3}
\]  
(\text{BR}_3)

\[
\frac{1}{2} E_C^R \left[ X, X_r^* \right] E_R + \frac{\eta_{4D}}{\eta_{4D} A_{\text{pixel}}} \left[ \left( i_{BM,0} - i_{D,0} \right) - \left( i_{BR,0} - i_{D,0} \right) - \left( i_{BS,0} - i_{D,0} \right) \right]
\]  
(\sqrt{BR}_3)

**Reconstruction Steps:**

**BM\text{D,0} - BR\text{D,0} - BD\text{D,0}:**

\[
\frac{1}{4} \eta_{4D} A_{\text{pixel}} \left[ -jE_S E_R^* e^{i(\theta - \beta)} + jE_S E_B^* e^{i(\theta - \beta)} - jE_B E_R^* e^{-i(\theta - \beta)} + jE_B E_B^* \right] E_C^S \left[ X, X_r^* \right] + \\
\left( i_{BM,1} - i_{D,1} \right) - \left( i_{BR,1} - i_{D,1} \right) - \left( i_{BS,1} - i_{D,1} \right)
\]  
**BM\text{D,1} - BR\text{D,1} - BD\text{D,1}:**

\[
\frac{1}{4} \eta_{4D} A_{\text{pixel}} \left[ -jE_S E_B^* e^{i(\theta - \beta)} - jE_S E_R^* e^{i(\theta - \beta)} + jE_B E_R^* e^{-i(\theta - \beta)} - jE_B E_B^* \right] E_C^S \left[ X, X_r^* \right] + \\
\left( i_{BM,2} - i_{D,2} \right) - \left( i_{BR,2} - i_{D,2} \right) - \left( i_{BS,2} - i_{D,2} \right)
\]  
**BM\text{D,2} - BR\text{D,2} - BD\text{D,2}:**

\[
\frac{1}{4} \eta_{4D} A_{\text{pixel}} \left[ \left( jE_S E_R^* + jE_S E_B^* e^{i(\theta - \beta)} + jE_B E_R^* e^{-i(\theta - \beta)} + jE_B E_B^* \right) E_C^S \left[ X, X_r^* \right] + \\
\left( i_{BM,3} - i_{D,3} \right) - \left( i_{BR,3} - i_{D,3} \right) - \left( i_{BS,3} - i_{D,3} \right)
\]  
**BM\text{D,3} - BR\text{D,3} - BD\text{D,3}:**
Summation with Subtraction of Reference and Signal:

\[ E_{DC,blank} = \sum_{k=0}^{3} BM_{D,k} - BR_{D,k} - BS_{D,k} \]

\[ E_{DC,blank} = \frac{1}{4} \frac{\partial^2}{\partial t^2} \left| E_{D_{R}} \right| \left( E_{D_{R}} \right)^* + E_{D_{R}}^* e^{i(\phi - \beta)} + E_{D_{R}} e^{-i(\phi - \beta)} + E_{D_{R}}^* e^{i(\phi - \beta)} + E_{D_{R}} e^{-i(\phi - \beta)} \right) X_r X_r^* e^{i(x_r - x_r)} \cdot \]

\[ \left( \eta_0 E_{C_0}^* E_{C_0} - j \eta_1 E_{C_1} E_{C_1}^* - \eta_2 E_{C_2} E_{C_2}^* + j \eta_3 E_{C_3} E_{C_3}^* \right) + \]

\[ \left( E_{B} E_{B}^* + E_{B} E_{B} e^{i(\phi - \beta)} + E_{B} E_{B} e^{-i(\phi - \beta)} + E_{B} E_{B}^* e^{i(\phi - \beta)} + E_{B} E_{B}^* e^{-i(\phi - \beta)} \right) X_r X_r^* e^{i(x_r - x_r)} \cdot \]

\[ \left( \eta_0 E_{D_{R}} E_{D_{R}}^* + j \eta_1 E_{D_{R}} E_{D_{R}}^* - \eta_2 E_{D_{R}} E_{D_{R}}^* - j \eta_3 E_{D_{R}} E_{D_{R}}^* \right) + \]

\[ (i_{BM,0} + i_{BM,1} + i_{BM,2} + i_{BM,3} - (i_{BR,0} + i_{BR,1} + i_{BR,2} + i_{BR,3}) - (i_{BS,0} + i_{BS,1} + i_{BS,2} + i_{BS,3}) + i_{D,0} + i_{D,1} + i_{D,2} + i_{D,3}) \]

\[ j^0 \left( BM_{D,0} - BR_{D,0} - BS_{D,0} \right) / \sqrt{BR_{D,0}} : \]

\[ \frac{1}{2} \sqrt{n} A_{pixel} \cdot \left( E_{D_{R}} E_{D_{R}}^* + E_{D_{R}} E_{D_{R}} e^{i(\phi - \beta)} + E_{D_{R}} E_{D_{R}} e^{-i(\phi - \beta)} + E_{D_{R}} E_{D_{R}}^* e^{i(\phi - \beta)} + E_{D_{R}} E_{D_{R}}^* e^{-i(\phi - \beta)} \right) X_r X_r^* e^{i(x_r - x_r)} \cdot \]

\[ \left( E_{D_{R}} E_{D_{R}}^* + E_{D_{R}} E_{D_{R}} e^{i(\phi - \beta)} + E_{D_{R}} E_{D_{R}} e^{-i(\phi - \beta)} + E_{D_{R}} E_{D_{R}}^* e^{i(\phi - \beta)} + E_{D_{R}} E_{D_{R}}^* e^{-i(\phi - \beta)} \right) X_r X_r^* e^{i(x_r - x_r)} \cdot \]

\[ \left( E_{D_{R}} E_{D_{R}}^* + E_{D_{R}} E_{D_{R}} e^{i(\phi - \beta)} + E_{D_{R}} E_{D_{R}} e^{-i(\phi - \beta)} + E_{D_{R}} E_{D_{R}}^* e^{i(\phi - \beta)} + E_{D_{R}} E_{D_{R}}^* e^{-i(\phi - \beta)} \right) X_r X_r^* e^{i(x_r - x_r)} \cdot \]

Simply denominator with a Taylor Series: \( f(x) = \sum_{n=0}^{\infty} \frac{f^{(n)}(a)}{n!} (x - a)^n \), (Let a = 0, Maclaurin series):

\[ \frac{1}{1+x} \approx 1 - x + x^2 - \ldots \quad \text{where: } x = \frac{i_{BR,0} - i_{D,0}}{i_{BR,0} - i_{D,0}} \]
Since $x$ is already near zero, we will assume $x$ raised to any power is zero:

$$1 + \frac{i_{BR,0} - i_{D,0}}{1 + \frac{i_{BR,0} - i_{D,0}}{\frac{R_{BR,1} - R_{BS,1}}{2} A_{pixel}}} \approx 1 - \frac{i_{BR,0} - i_{D,0}}{\frac{R_{BR,1} - R_{BS,1}}{2} A_{pixel} E_N E^*_B e^{(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))}} X, X'$$

$$\frac{1}{2} \sqrt{\frac{\eta_\beta}{\eta_0}} A_{pixel} \left[ (E_S E_N + E^*_S e^{(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))}) E^*_E E^*_C X, X' e^{j(\chi - \zeta)} + (E^*_S + E^*_B e^{(j(\phi - \beta))} + E^*_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))}) E^*_E E^*_C X, X' e^{j(\chi - \zeta)} \right] + \frac{i_{BM,0} - i_{BR,0} - i_{BS,0} + i_{D,0}}{2}$$

$$j^1 (BM_{D,1} - BR_{D,1} - BS_{D,1}) / \sqrt{BR_{D,1}} :$$

$$\frac{1}{2} \frac{\eta_\beta}{\eta_0} A_{pixel} \left[ (E_S E_N + E^*_S e^{(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))}) E^*_E E^*_C X, X' e^{j(\chi - \zeta)} + (E^*_S + E^*_B e^{(j(\phi - \beta))} - E^*_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))}) E^*_E E^*_C X, X' e^{j(\chi - \zeta)} \right] + \frac{i_{BM,1} - i_{D,1}}{2}$$

$$\frac{1}{2} \sqrt{\frac{\eta_\beta}{\eta_0}} A_{pixel} \left[ (E_S E_N + E^*_S e^{(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))}) E^*_E E^*_C X, X' e^{j(\chi - \zeta)} + (E^*_S + E^*_B e^{(j(\phi - \beta))} - E^*_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))} + E^*_B E_B e^{-(j(\phi - \beta))}) E^*_E E^*_C X, X' e^{j(\chi - \zeta)} \right] + \frac{i_{BM,1} - i_{BR,1} - i_{BS,1} + i_{D,1}}{2}$$

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Substitute Taylor Series:

\[
\left[ \frac{1}{2} \sqrt{\frac{q_B}{h \nu}} A_{\text{pixel}} \right] \left( (E_s E^*_r + E_s E^*_e e^{j(\phi - \beta)} + E_b E^*_r e^{-j(\phi - \beta)} + E_b E^*_b) E^S_{C_1} E^R_{C_1} X_\ast X^\ast e^{j(x_\ast - x_\ast)} + \right. \\
\left. (E_s E^*_r - E_s E^*_e e^{j(\phi - \beta)} - E_b E^*_r e^{-j(\phi - \beta)} - E_b E^*_b) E^S_{C_1} E^R_{C_1} X_\ast X^\ast e^{-j(x_\ast - x_\ast)} \right) + j i_{BM,2} - i_{BR,2} + i_{D,2} \right].
\]

\[
\frac{1}{2} \sqrt{\frac{q_B}{h \nu}} A_{\text{pixel}} \left( 1 - \frac{i_{BM,2} - i_{BR,2}}{2} \right)
\]

\[
\frac{1}{2} \sqrt{\frac{q_B}{h \nu}} A_{\text{pixel}} \left( \frac{(E_s E^*_r + E_s E^*_e e^{j(\phi - \beta)} + E_b E^*_r e^{-j(\phi - \beta)} + E_b E^*_b) E^S_{C_2} E^R_{C_2} X_\ast X^\ast e^{j(x_\ast - x_\ast)} + \right. \\
\left. (E_s E^*_r + E_s E^*_e e^{j(\phi - \beta)} + E_b E^*_r e^{-j(\phi - \beta)} + E_b E^*_b) E^S_{C_2} E^R_{C_2} X_\ast X^\ast e^{-j(x_\ast - x_\ast)} \right) + \\
- \left( (i_{BM,2} - i_{BR,2}) + (i_{BR,2} - i_{D,2}) + (i_{BS,2} - i_{D,2}) \right) / \\
\frac{1}{2} E^R_{C_2} X_\ast \left( \frac{(E_s E^*_r + E_s E^*_e e^{j(\phi - \beta)} + E_b E^*_r e^{-j(\phi - \beta)} + E_b E^*_b) E^S_{C_2} E^R_{C_2} X_\ast X^\ast e^{j(x_\ast - x_\ast)} + \right. \\
\left. (E_s E^*_r + E_s E^*_e e^{j(\phi - \beta)} + E_b E^*_r e^{-j(\phi - \beta)} + E_b E^*_b) E^S_{C_2} E^R_{C_2} X_\ast X^\ast e^{-j(x_\ast - x_\ast)} \right) - \\
\frac{1}{2} \left[ E^R_{C_2} X_\ast + E^R_{C_2} X_\ast \right] \left( \frac{1}{2} \sqrt{\frac{q_B}{h \nu}} A_{\text{pixel}} \right) \left( 1 + \frac{i_{BM,2} - i_{BR,2} - i_{BS,2} + i_{D,2}}{2} \right).
\]

\[
\frac{1}{2} \sqrt{\frac{q_B}{h \nu}} A_{\text{pixel}} \left( \frac{(E_s E^*_r + E_s E^*_e e^{j(\phi - \beta)} + E_b E^*_r e^{-j(\phi - \beta)} + E_b E^*_b) E^S_{C_2} E^R_{C_2} X_\ast X^\ast e^{j(x_\ast - x_\ast)} + \right. \\
\left. (E_s E^*_r + E_s E^*_e e^{j(\phi - \beta)} + E_b E^*_r e^{-j(\phi - \beta)} + E_b E^*_b) E^S_{C_2} E^R_{C_2} X_\ast X^\ast e^{-j(x_\ast - x_\ast)} \right) - \\
\frac{1}{2} \left[ E^R_{C_2} X_\ast + E^R_{C_2} X_\ast \right] \left( \frac{1}{2} \sqrt{\frac{q_B}{h \nu}} A_{\text{pixel}} \right) \left( 1 + \frac{i_{BM,2} - i_{BR,2} - i_{BS,2} + i_{D,2}}{2} \right).
\]

Substitute Taylor Series:
\[ j^3 \left( B_{D,3} - B_{R,3} - B_{S,3} \right) / \sqrt{R_{D,3}}: \]

\[
\left[ \frac{1}{2} \frac{\eta_{q}}{n_0} A_{\text{pixel}} \right] \left( (E^*_s E^*_R + E_s E^*_R e^{j(\phi - \beta)} + E_R E^*_R) E^*_C_{C_1} E^*_R X^*_R X^*_s e^{j(x_r - x_s)} + \\
(-E^*_R E^*_s - E^*_s E^*_R e^{j(\phi - \beta)} - E_R E^*_s) E^*_C_{C_1} X^*_R X^*_s e^{-j(x_r - x_s)}) + \\
- j((i_{BM,3} - i_{D,3}) - (i_{BR,3} - i_{D,3}) - (i_{BS,3} - i_{D,3})) \right] / \left[ \frac{1}{2} E^*_C_{C_1} X^*_s |E^*_R + E^*_R| \sqrt{\frac{\eta_{q}}{n_0} A_{\text{pixel}}} \cdot \left( 1 + \frac{i_{BR,3} - i_{D,3}}{\frac{1}{2} \frac{\eta_{q}}{n_0} A_{\text{pixel}} (E^*_s E^*_R + E^*_s E^*_R e^{j(\phi - \beta)} + E_R E^*_R) E^*_C_{C_1} E^*_R X^*_R X^*_s e^{j(x_r - x_s)}) \right) \right]
\]

\[
\left[ \frac{1}{2} \frac{\eta_{q}}{n_0} A_{\text{pixel}} \right] \left( (E^*_s E^*_R + E_s E^*_R e^{j(\phi - \beta)} + E_R E^*_R) E^*_C_{C_1} E^*_R X^*_R X^*_s e^{j(x_r - x_s)} + \\
(-E^*_R E^*_s - E^*_s E^*_R e^{j(\phi - \beta)} - E_R E^*_s) E^*_C_{C_1} X^*_R X^*_s e^{-j(x_r - x_s)}) + \\
- j((i_{BM,3} - i_{D,3}) - (i_{BR,3} - i_{D,3}) - (i_{BS,3} - i_{D,3})) \right] / \left[ \frac{1}{2} E^*_C_{C_1} X^*_s |E^*_R + E^*_R| \sqrt{\frac{\eta_{q}}{n_0} A_{\text{pixel}}} \cdot \left( 1 + \frac{i_{BR,3} - i_{D,3}}{\frac{1}{2} \frac{\eta_{q}}{n_0} A_{\text{pixel}} (E^*_s E^*_R + E^*_s E^*_R e^{j(\phi - \beta)} + E_R E^*_R) E^*_C_{C_1} E^*_R X^*_R X^*_s e^{j(x_r - x_s)}) \right) \right]
\]

Substitute Taylor Series:

\[
\left[ \frac{1}{2} \frac{\eta_{q}}{n_0} A_{\text{pixel}} \right] \left( (E^*_s E^*_R + E_s E^*_R e^{j(\phi - \beta)} + E_R E^*_R) E^*_C_{C_1} E^*_R X^*_R X^*_s e^{j(x_r - x_s)} + \\
(-E^*_R E^*_s - E^*_s E^*_R e^{j(\phi - \beta)} - E_R E^*_s) E^*_C_{C_1} X^*_R X^*_s e^{-j(x_r - x_s)}) + \\
- j((i_{BM,3} - i_{D,3}) - (i_{BR,3} - i_{D,3}) - (i_{BS,3} - i_{D,3})) \right] / \left[ \frac{1}{2} \frac{\eta_{q}}{n_0} A_{\text{pixel}} \right]
\]

\[
\left[ \frac{1}{2} \frac{\eta_{q}}{n_0} A_{\text{pixel}} \right] \left( (E^*_s E^*_R + E_s E^*_R e^{j(\phi - \beta)} + E_R E^*_R) E^*_C_{C_1} E^*_R X^*_R X^*_s e^{j(x_r - x_s)} + \\
(-E^*_R E^*_s - E^*_s E^*_R e^{j(\phi - \beta)} - E_R E^*_s) E^*_C_{C_1} X^*_R X^*_s e^{-j(x_r - x_s)}) + \\
- j((i_{BM,3} - i_{D,3}) - (i_{BR,3} - i_{D,3}) - (i_{BS,3} - i_{D,3})) \right]
\]
Balanced Mixing, DC Term Subtraction, and Camera Normalization:

\[ E_{BM,DC,Norm.blank} = \sum_{k=0}^{3} j \cdot \frac{BM_{D,k} - BR_{D,k} - BS_{D,k}}{\sqrt{BR_{D,k}}} = \]

\[ \left( \frac{1}{2} \sqrt{\frac{\eta_q}{\nu_D}} A_{pixel} \cdot \left( (E_S E_R^* + E_S E_B^* e^{i(\phi-\beta)} + E_B E_R^* e^{-j(\phi-\beta)} + E_B E_B^*) E_c^S E_R^* X_s X_s^* e^{j(x,x_z)} + \right) \right) \]

\[ \left( E_B E_S^* + E_B E_D^* e^{i(\phi-\beta)} + E_B E_D^* e^{-j(\phi-\beta)} + E_B E_B^*) E_c^S E_R^* X_s X_s^* e^{j(x,x_z)} \right) + \]

\[ \frac{i_{BM,0} - i_{BR,0} - i_{BS,0} + i_{D,0}}{2 \sqrt{\frac{\eta_q}{\nu_D}} A_{pixel}} \]

\[ \frac{1}{e_{C_1}^S X_s + e_{C_1}^S X_s} \left( 1 - \frac{i_{BR,0} - i_{D,0}}{2 \sqrt{\frac{\eta_q}{\nu_D}} A_{pixel} (E_B E_B^* + E_B E_D^* e^{i(\phi-\beta)} + E_B E_B^*) E_c^S e^{j(x,x_z)} X_s X_s^* e^{j(x,x_z)} \right) \]

\[ \left( E_S E_R^* + E_S E_B^* e^{i(\phi-\beta)} + E_B E_R^* e^{-j(\phi-\beta)} + E_B E_B^*) E_c^S E_R^* X_s X_s^* e^{j(x,x_z)} \right) + \]

\[ \frac{i_{BM,1} - i_{BR,1} - i_{BS,1} + i_{D,1}}{2 \sqrt{\frac{\eta_q}{\nu_D}} A_{pixel}} \]

\[ \frac{1}{e_{C_1}^S X_s + e_{C_1}^S X_s} \left( 1 - \frac{i_{BR,1} - i_{D,1}}{2 \sqrt{\frac{\eta_q}{\nu_D}} A_{pixel} (E_B E_B^* + E_B E_B^* e^{i(\phi-\beta)} + E_B E_B^*) E_c^S e^{j(x,x_z)} X_s X_s^* e^{j(x,x_z)} \right) \]

\[ \left( E_S E_R^* + E_S E_B^* e^{i(\phi-\beta)} + E_B E_R^* e^{-j(\phi-\beta)} + E_B E_B^*) E_c^S E_R^* X_s X_s^* e^{j(x,x_z)} \right) + \]

\[ \frac{i_{BM,2} - i_{BR,2} - i_{BS,2} + i_{D,2}}{2 \sqrt{\frac{\eta_q}{\nu_D}} A_{pixel}} \]

\[ \frac{1}{e_{C_1}^S X_s + e_{C_1}^S X_s} \left( 1 - \frac{i_{BR,2} - i_{D,2}}{2 \sqrt{\frac{\eta_q}{\nu_D}} A_{pixel} (E_B E_B^* + E_B E_B^* e^{i(\phi-\beta)} + E_B E_B^*) E_c^S e^{j(x,x_z)} X_s X_s^* e^{j(x,x_z)} \right) \]

\[ \left( E_S E_R^* + E_S E_B^* e^{i(\phi-\beta)} + E_B E_R^* e^{-j(\phi-\beta)} + E_B E_B^*) E_c^S E_R^* X_s X_s^* e^{j(x,x_z)} \right) + \]

\[ \frac{i_{BM,3} - i_{BR,3} - i_{BS,3} + i_{D,3}}{2 \sqrt{\frac{\eta_q}{\nu_D}} A_{pixel}} \]

\[ \frac{1}{e_{C_1}^S X_s + e_{C_1}^S X_s} \left( 1 - \frac{i_{BR,3} - i_{D,3}}{2 \sqrt{\frac{\eta_q}{\nu_D}} A_{pixel} (E_B E_B^* + E_B E_B^* e^{i(\phi-\beta)} + E_B E_B^*) E_c^S e^{j(x,x_z)} X_s X_s^* e^{j(x,x_z)} \right) \]
\[
E_{BM, DC, Norm, blank} = \frac{1}{2} \sqrt{\frac{A_{pg, 0} \eta}{h_0}} \cdot [ (E_S E_S^* + E_SE_b e^{j(\phi - \beta)} + E_BE_B^* e^{-j(\phi - \beta)} + E_B E_B^*) X, X^*e^{j(x_3 - x_r)} ] .
\]

\[
\left( \sqrt{\eta_0 E_{C0}^R E_{C0}^*} \right) \left( 1 - \frac{i_{BM0} - i_{BR0} - i_{BS0} + i_{D0}}{2} \frac{\eta_0}{h_0} A_{pixel} | E_R E_{C0}^R X_r + E_B E_{C0}^R X_r | \right) +
\]

\[
\sqrt{\eta_1 E_{C1}^R E_{C1}^*} \left( 1 - \frac{i_{BR1} - i_{D1}}{2} \frac{\eta_1}{h_0} A_{pixel} (E_R E_{C1}^R X_r + E_B E_{C1}^R X_r) \right) +
\]

\[
\sqrt{\eta_2 E_{C2}^R E_{C2}^*} \left( 1 - \frac{i_{BR2} - i_{D2}}{2} \frac{\eta_2}{h_0} A_{pixel} (E_R E_{C2}^R X_r + E_B E_{C2}^R X_r) \right) +
\]

\[
\sqrt{\eta_3 E_{C3}^R E_{C3}^*} \left( 1 - \frac{i_{BR3} - i_{D3}}{2} \frac{\eta_3}{h_0} A_{pixel} (E_R E_{C3}^R X_r + E_B E_{C3}^R X_r) \right) +
\]

\[
\left( \frac{\eta_0}{h_0} A_{pixel} | E_R E_{C0}^R X_r + E_B E_{C0}^R X_r | \right) \cdot \left( \frac{\eta_1}{h_0} A_{pixel} (E_R E_{C1}^R X_r + E_B E_{C1}^R X_r) \right) \cdot \left( \frac{\eta_2}{h_0} A_{pixel} (E_R E_{C2}^R X_r + E_B E_{C2}^R X_r) \right) \cdot \left( \frac{\eta_3}{h_0} A_{pixel} (E_R E_{C3}^R X_r + E_B E_{C3}^R X_r) \right) \cdot
\]

\[
\left( \frac{\eta_0}{h_0} A_{pixel} | E_R E_{C0}^R X_r + E_B E_{C0}^R X_r | \right) \cdot \left( \frac{\eta_1}{h_0} A_{pixel} (E_R E_{C1}^R X_r + E_B E_{C1}^R X_r) \right) \cdot \left( \frac{\eta_2}{h_0} A_{pixel} (E_R E_{C2}^R X_r + E_B E_{C2}^R X_r) \right) \cdot \left( \frac{\eta_3}{h_0} A_{pixel} (E_R E_{C3}^R X_r + E_B E_{C3}^R X_r) \right) \cdot
\]

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Assuming: \( i_{BM} - i_D << \frac{1}{\frac{\eta}{\Delta t}} A_{\text{pixel}} (E_R E_R^* + E_B E_B^* e^{j(\phi - \beta)} + E_B E_R^* e^{-j(\phi - \beta)} + E_B E_B^*) E_C^R E_C^R X, X^*, \)

\[
E_{BM, DC, Norm, blank} = \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{\Delta t}} \left[ (E_S E_R^* + E_S E_B^* e^{j(\phi - \beta)} + E_B E_B^* e^{-j(\phi - \beta)} + E_B E_B^*) X_s X^*_s, e^{j(\chi_s - \chi_r)} \right].
\]

\[
\begin{pmatrix}
\frac{\sqrt{\eta_0 E_C^S E_C^R}}{E_R E_R^* X_r + E_B E_B^* X_r} + \frac{\sqrt{\eta_1 E_C^S E_C^R}}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{\sqrt{\eta_2 E_C^S E_C^R}}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{\sqrt{\eta_3 E_C^S E_C^R}}{E_R E_C^R X_r + E_B E_C^R X_r} \\
\frac{\sqrt{\eta_0 E_C^S E_C^R}}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{\sqrt{\eta_1 E_C^S E_C^R}}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{\sqrt{\eta_2 E_C^S E_C^R}}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{\sqrt{\eta_3 E_C^S E_C^R}}{E_R E_C^R X_r + E_B E_C^R X_r}
\end{pmatrix}
\]

\[
\left( E_R E_S^* + E_B E_B^* e^{j(\phi - \beta)} + E_B E_B^* e^{-j(\phi - \beta)} + E_B E_B^* \right) X_r X^*_s, e^{j(\chi_s - \chi_r)}.
\]

\[
\begin{pmatrix}
\frac{i_{BM, 0} - i_{BR, 0} - i_{BS, 0} + i_{D, 0}}{2 \sqrt{\frac{\eta}{\Delta t}} A_{\text{pixel}}} + \frac{i_{BM, 1} - i_{BR, 1} - i_{BS, 1} + i_{D, 1}}{2 \sqrt{\frac{\eta}{\Delta t}} A_{\text{pixel}}} \\
\frac{i_{BM, 2} - i_{BR, 2} - i_{BS, 2} + i_{D, 2}}{2 \sqrt{\frac{\eta}{\Delta t}} A_{\text{pixel}}} + \frac{i_{BM, 3} - i_{BR, 3} - i_{BS, 3} + i_{D, 3}}{2 \sqrt{\frac{\eta}{\Delta t}} A_{\text{pixel}}}
\end{pmatrix}
\]

\[
\frac{E_C^S E_C^R}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{E_C^S E_C^R}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{E_C^S E_C^R}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{E_C^S E_C^R}{E_R E_C^R X_r + E_B E_C^R X_r}
\]

\[
\left( E_R E_S^* + E_B E_B^* e^{j(\phi - \beta)} + E_B E_B^* e^{-j(\phi - \beta)} + E_B E_B^* \right) X_r X^*_s, e^{j(\chi_s - \chi_r)}.
\]

\[
\begin{pmatrix}
\frac{E_C^S E_C^R}{E_R E_C^R X_r + E_B E_C^R X_r} - \frac{E_C^S E_C^R}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{E_C^S E_C^R}{E_R E_C^R X_r + E_B E_C^R X_r} - \frac{E_C^S E_C^R}{E_R E_C^R X_r + E_B E_C^R X_r}
\end{pmatrix}
\]

If the detectors were ideal: \( i_{BM} = i_{BR} = i_{BS} = i_{D} \quad \text{and} \quad \eta_0 = \eta_1 = \eta_2 = \eta_3 = 1 \)

\[
E_{BM, DC, Norm, blank} = \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{\Delta t}} \left[ (E_S E_R^* + E_S E_B^* e^{j(\phi - \beta)} + E_B E_B^* e^{-j(\phi - \beta)} + E_B E_B^*) X_s X^*_s, e^{j(\chi_s - \chi_r)} \right].
\]
If the fixed pattern noise was the same in the signal and reference paths: \( \frac{E_C^S}{E_C^R} = E_C \)

\[
E_{BM, DC, Norm, blank} = \frac{1}{2} \sqrt{\frac{\lambda_{norm}}{2\pi}} \left[ (E_S E_R^* + E_S E_B^* e^{i(\phi-\beta)} + E_B E_R^* e^{-i(\phi-\beta)} + E_B E_B^*) X_s X_r^* e^{i(x_s-x_r)} \frac{E_{C_0} + E_{C_1} + E_{C_2} + E_{C_3}}{|E_R X_r + E_B X_r|} + (E_R E_S^* + E_R E_B^* e^{i(\phi-\beta)} + E_B E_S^* e^{-i(\phi-\beta)} + E_B E_B^*) X_r X_s^* e^{-i(x_r-x_s)} \frac{E_{C_0} - E_{C_1} + E_{C_2} - E_{C_3}}{|E_R X_r + E_B X_r|} \right]
\]

If there was no fixed pattern noise: \( E_{C_0} = E_{C_1} = E_{C_2} = E_{C_3} = 1 \)

\[
E_{BM, DC, Norm, blank} = \frac{2 \sqrt{\frac{\lambda_{norm}}{2\pi}}}{|E_R X_r + E_B X_r|} \cdot \left( (E_S E_R^* + E_S E_B^* e^{i(\phi-\beta)} + E_B E_R^* e^{-i(\phi-\beta)} + E_B E_B^*) X_s X_r^* e^{i(x_s-x_r)} \right)
\]

If there were no aberrations in the system: \( X_s e^{ix_s} = X_r e^{ix_r} = 1 \)

\[
E_{BM, DC, Norm, blank} = \frac{2 \sqrt{\frac{\lambda_{norm}}{2\pi}}}{|E_R + E_B|} \cdot \left( (E_S E_R^* + E_S E_B^* e^{i(\phi-\beta)} + E_B E_R^* e^{-i(\phi-\beta)} + E_B E_B^*) \right)
\]

If there was no noise in the laser: \( E_B e^{i(\omega t + \beta)} = 0 \)

\[
E_{BM, DC, Norm, blank} = \frac{2 \sqrt{\frac{\lambda_{norm}}{2\pi}} E_S^*}{|E_R^*|}, \text{ which is proportional to the electric field for the ideal system.}
\]

Therefore, for the ideal system, dividing the original reconstruction by the blank reconstruction:

\[
\frac{E_{BM, DC, Norm, sample}}{E_{BM, DC, Norm, blank}} = Ae^{j\alpha}
\]
A.3 Realistic Assumptions

Original reconstruction with sample:

\[
E_{BM,DC,Norm,\text{sample}} = \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{h_0}} \left[ A e^{j\alpha} \left( E_S E_R^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_R e^{-j(\phi-\zeta)} + E_N E_N^* X_s X_s^* e^{j(x_s-x_s)} \right) \right]
\]

\[
\left( \sqrt{\eta_0 E_S^* E_0^*} \left( 1 - \frac{i_{\text{r,0}}}{2} A_{\text{pixel}} (E_S E_R^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_R e^{-j(\phi-\zeta)} + E_N E_N^*) E_0^* X_s X_s^* \right) \right) + \\
\left( \sqrt{\eta_1 E_S^* E_C^*} \left( 1 - \frac{i_{\text{r,1}}}{2} A_{\text{pixel}} (E_S E_R^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_R e^{-j(\phi-\zeta)} + E_N E_N^*) E_C^* X_s X_s^* \right) \right) + \\
\left( \sqrt{\eta_2 E_S^* E_C^*} \left( 1 - \frac{i_{\text{r,2}}}{2} A_{\text{pixel}} (E_S E_R^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_R e^{-j(\phi-\zeta)} + E_N E_N^*) E_C^* X_s X_s^* \right) \right) + \\
\left( \sqrt{\eta_3 E_S^* E_C^*} \left( 1 - \frac{i_{\text{r,3}}}{2} A_{\text{pixel}} (E_S E_R^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_R e^{-j(\phi-\zeta)} + E_N E_N^*) E_C^* X_s X_s^* \right) \right)
\]

\[
A e^{-j\alpha} \left( E_S E_S^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_S e^{-j(\phi-\zeta)} + E_N E_N^* X_s X_s^* e^{-j(x_s-x_s)} \right)
\]

\[
\left( \sqrt{\eta_0 E_C^* E_0^*} \left( 1 - \frac{i_{\text{r,0}}}{2} A_{\text{pixel}} (E_S E_R^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_R e^{-j(\phi-\zeta)} + E_N E_N^*) E_0^* X_s X_s^* \right) \right) - \\
\left( \sqrt{\eta_1 E_C^* E_C^*} \left( 1 - \frac{i_{\text{r,1}}}{2} A_{\text{pixel}} (E_S E_R^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_R e^{-j(\phi-\zeta)} + E_N E_N^*) E_C^* X_s X_s^* \right) \right) - \\
\left( \sqrt{\eta_2 E_C^* E_C^*} \left( 1 - \frac{i_{\text{r,2}}}{2} A_{\text{pixel}} (E_S E_R^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_R e^{-j(\phi-\zeta)} + E_N E_N^*) E_C^* X_s X_s^* \right) \right) - \\
\left( \sqrt{\eta_3 E_C^* E_C^*} \left( 1 - \frac{i_{\text{r,3}}}{2} A_{\text{pixel}} (E_S E_R^* + E_S^* E_N e^{j(\phi-\zeta)} + E_N^* E_R e^{-j(\phi-\zeta)} + E_N E_N^*) E_C^* X_s X_s^* \right) \right)
\]
\[ E_{BM, DC, Norm, blank} = \frac{1}{2} \sqrt{\sigma_{n0} \frac{1}{N_{p0}}} \left[ \begin{vmatrix} (E_S E_B^* + E_S E_B e^{i(\phi - \beta)} + E_B E_B^* e^{-i(\phi - \beta)} + E_B E_B^* X_0 X^*_0) e^{i(x_0 - x_0)} \end{vmatrix} + \right. \]

\[ \sqrt{\eta_n E_C^S E_C^R} \frac{E_R E_C^R X_r + E_B E_C^R X_r}{\left(1 - \frac{1}{2} \frac{\eta_n}{\sigma_{n0}} A_{pixel} (E_S E_B^* + E_S E_B e^{i(\phi - \beta)} + E_B E_B^* e^{-i(\phi - \beta)} + E_B E_B^*) E_C^R X_r X^*_0 \right)^{i_{BM,0} - i_{BR,0} - i_{BS,0} + i_{D,0}}} + \]

\[ \sqrt{\eta_r E_C^S E_C^R} \frac{E_R E_C^R X_r + E_B E_C^R X_r}{\left(1 - \frac{1}{2} \frac{\eta_r}{\sigma_{r0}} A_{pixel} (E_S E_B^* + E_S E_B e^{i(\phi - \beta)} + E_B E_B^* e^{-i(\phi - \beta)} + E_B E_B^*) E_C^R X_r X^*_0 \right)^{i_{BM,0} - i_{BR,0} - i_{BS,0} + i_{D,0}}} + \]

\[ \sqrt{\eta_{e0} E_C^S E_C^R} \frac{E_R E_C^R X_r + E_B E_C^R X_r}{\left(1 - \frac{1}{2} \frac{\eta_{e0}}{\sigma_{e0}} A_{pixel} (E_S E_B^* + E_S E_B e^{i(\phi - \beta)} + E_B E_B^* e^{-i(\phi - \beta)} + E_B E_B^*) E_C^R X_r X^*_0 \right)^{i_{BM,0} - i_{BR,0} - i_{BS,0} + i_{D,0}}} + \]

\[ \left. \sqrt{\eta_{e0} E_C^S E_C^R} \frac{E_R E_C^R X_r + E_B E_C^R X_r}{\left(1 - \frac{1}{2} \frac{\eta_{e0}}{\sigma_{e0}} A_{pixel} (E_S E_B^* + E_S E_B e^{i(\phi - \beta)} + E_B E_B^* e^{-i(\phi - \beta)} + E_B E_B^*) E_C^R X_r X^*_0 \right)^{i_{BM,0} - i_{BR,0} - i_{BS,0} + i_{D,0}}} \right] \]
Assuming:

\[ i_R - i_D << \frac{\eta q}{h v} A_{\text{pixel}}(E_R E_R^* + E_R E_B e^{i(\phi - \beta)} + E_B E_R^* e^{-i(\phi - \beta)} + E_B E_B^*) \]

\[ i_{BR} - i_D << \frac{\eta q}{h v} A_{\text{pixel}}(E_R E_R^* + E_B E_B e^{i(\phi - \beta)} + E_B E_R^* e^{-i(\phi - \beta)} + E_B E_B^*) \]

\[ E_{BM,DC,Norm,\text{sample}} = \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{h v}} \left[ A e^{i\alpha} (E_S E_S^* + E_S E_N e^{i(\phi - \zeta)} + E_N E_N^* e^{-i(\phi - \zeta)} + E_N E_S^*) X_s X_s^* e^{i(x_s - x_r)} \right] \]

\[ \left( \sqrt{\eta_0 E_{C_0}^S E_{C_0}^R} + \sqrt{\eta_1 E_{C_1}^S E_{C_1}^R} + \sqrt{\eta_2 E_{C_2}^S E_{C_2}^R} + \sqrt{\eta_3 E_{C_3}^S E_{C_3}^R} \right) \left( \frac{E_R E_C^R X_r + E_B E_C^R X_r}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{E_R E_C^R X_r + E_B E_C^R X_r}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{E_R E_C^R X_r + E_B E_C^R X_r}{E_R E_C^R X_r + E_B E_C^R X_r} \right) \]

\[ \sum_{j=k=0}^{3} \frac{i_{M,j} - i_{R,j} - i_{S,j} + i_{D,j}}{1/2 \sqrt{\frac{A_{\text{pixel}}}{h v}}} [E_R E_C^R X_r + E_N E_C^R X_r] \]

\[ E_{BM,DC,Norm,\text{blank}} = \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{h v}} \left[ (E_S E_S^* + E_S E_B e^{i(\phi - \beta)} + E_B E_B^*) X_s X_s^* e^{i(x_s - x_r)} \right] \]

\[ \left( \sqrt{\eta_0 E_{C_0}^S E_{C_0}^R} + \sqrt{\eta_1 E_{C_1}^S E_{C_1}^R} + \sqrt{\eta_2 E_{C_2}^S E_{C_2}^R} + \sqrt{\eta_3 E_{C_3}^S E_{C_3}^R} \right) \left( \frac{E_R E_C^R X_r + E_B E_C^R X_r}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{E_R E_C^R X_r + E_B E_C^R X_r}{E_R E_C^R X_r + E_B E_C^R X_r} + \frac{E_R E_C^R X_r + E_B E_C^R X_r}{E_R E_C^R X_r + E_B E_C^R X_r} \right) \]

\[ \sum_{j=k=0}^{3} \frac{i_{BM,j} - i_{BR,j} - i_{BS,j} + i_{D,j}}{1/2 \sqrt{\frac{A_{\text{pixel}}}{h v}}} [E_R E_C^R X_r + E_B E_C^R X_r] \]
A.4 Balanced Mixing, DC term Subtraction, and Camera Normalization

Separate Signal and Noise Contributions

\[ E_{BM,DC,Norm,sample} = \frac{1}{2} \sqrt{\frac{A_{pix} q}{h v}} \cdot \left[ A e^{j\alpha} (E_S E_R^* + E_S E_R^* e^{j(\phi - \xi)} + E_N E_R^* e^{-j(\phi - \xi)} + E_N E_N^*) X X_r^* e^{j(\lambda_r - \lambda_r)} \right. \]

\[ \left( \sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*} \right) \]

\[ \frac{A e^{-j\alpha} (E_R E_S^* + E_R E_S^* e^{j(\phi - \xi)} + E_N E_N^*) X X_r^* e^{-j(\lambda_r - \lambda_r)} \right) \]

\[ \left( \sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*} \right) \]

\[ \sum_{k=0}^{3} j^k \frac{i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}}{\sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*}} \]

Signal (Sample):

\[ S = \frac{1}{2} \sqrt{\frac{A_{pix} q}{h v}} \cdot \left[ A e^{j\alpha} (E_S E_R^*) X X_r^* e^{j(\lambda_r - \lambda_r)} \right. \]

\[ \left( \sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*} \right) \]

\[ \left( \sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*} \right) \]

\[ \left( \left( \sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*} \right) \right) \]

\[ \sum_{k=0}^{3} j^k \frac{i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}}{\sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*}} \]

Noise (Sample):

\[ N_s = \frac{1}{2} \sqrt{\frac{A_{pix} q}{h v}} \cdot \left[ A e^{j\alpha} (E_S E_R^* e^{j(\phi - \xi)} + E_S E_R^* e^{-j(\phi - \xi)} + E_N E_N^*) X X_r^* e^{j(\lambda_r - \lambda_r)} \right. \]

\[ \left( \sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*} \right) \]

\[ \left( \sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*} \right) \]

\[ \left( \left( \sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*} \right) \right) \]

\[ \sum_{k=0}^{3} j^k \frac{i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}}{\sqrt{\eta_0 E_{C_0}^* E_{C_0}^*} + \sqrt{\eta_1 E_{C_1}^* E_{C_1}^*} + \sqrt{\eta_2 E_{C_2}^* E_{C_2}^*} + \sqrt{\eta_3 E_{C_3}^* E_{C_3}^*}} \]
Separate Signal and Noise Contributions

\[ E_{BM,DC,Norm, blank} = \frac{1}{2} \sqrt{\frac{A_{DC, N}}{h_{0}}} \left[ (E_S E_R^* + E_S e^{j(\phi - \beta)} + E_B E_R^* e^{-j(\phi - \beta)} + E_B E_R^*) X_r X_r e^{j(\lambda x - \lambda x_r)} \right] \]

\[ \left( \sqrt{\eta_0} E_{C_1} E_{C_2}^* \left[ E_R E_{C_1} X_r + E_B E_{C_1} X_r \right] + \sqrt{\eta_1} E_{C_1} E_{C_2}^* \left[ E_R E_{C_2} X_r + E_B E_{C_2} X_r \right] + \sqrt{\eta_2} E_{C_1} E_{C_2}^* \left[ E_R E_{C_3} X_r + E_B E_{C_3} X_r \right] + \sqrt{\eta_3} E_{C_1} E_{C_2}^* \left[ E_R E_{C_4} X_r + E_B E_{C_4} X_r \right] \right) \]

\[ (E_S E_R^* + E_S e^{j(\phi - \beta)} + E_B E_R^* e^{-j(\phi - \beta)} + E_B E_R^*) X_r X_r e^{-j(\lambda x - \lambda x_r)} \]

\[ \sum_{k=0}^{3} j^k \frac{i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k}}{2} \sqrt{\frac{E_R E_{C_1} X_r + E_B E_{C_1} X_r}{A_{pixel}}} \]

Signal (Blank):

\[ B = \frac{1}{2} \sqrt{\frac{A_{DC, N}}{h_{0}}} \left[ (E_S E_R^*) X_r X_r e^{j(\lambda x - \lambda x_r)} \left( \frac{\sqrt{\eta_0} E_{C_1} E_{C_2}^*}{E_R E_{C_1} X_r + E_B E_{C_1} X_r} \right) + \frac{\sqrt{\eta_1} E_{C_1} E_{C_2}^*}{E_R E_{C_2} X_r + E_B E_{C_2} X_r} \right] + \frac{\sqrt{\eta_2} E_{C_1} E_{C_2}^*}{E_R E_{C_3} X_r + E_B E_{C_3} X_r} \right) + \frac{\sqrt{\eta_3} E_{C_1} E_{C_2}^*}{E_R E_{C_4} X_r + E_B E_{C_4} X_r} \right) \]

\[ (E_S E_R^*) X_r X_r e^{-j(\lambda x - \lambda x_r)} \left( \frac{\sqrt{\eta_0} E_{C_1} E_{C_2}^*}{E_R E_{C_1} X_r + E_B E_{C_1} X_r} \right) + \frac{\sqrt{\eta_1} E_{C_1} E_{C_2}^*}{E_R E_{C_2} X_r + E_B E_{C_2} X_r} \right) + \frac{\sqrt{\eta_2} E_{C_1} E_{C_2}^*}{E_R E_{C_3} X_r + E_B E_{C_3} X_r} \right) + \frac{\sqrt{\eta_3} E_{C_1} E_{C_2}^*}{E_R E_{C_4} X_r + E_B E_{C_4} X_r} \right) \]

Noise (Blank):

\[ N_B = \frac{1}{2} \sqrt{\frac{A_{DC, N}}{h_{0}}} \left[ (E_S E_R^* e^{j(\phi - \beta)} + E_B E_R^* e^{-j(\phi - \beta)} + E_B E_R^*) X_r X_r e^{j(\lambda x - \lambda x_r)} \left( \frac{\sqrt{\eta_0} E_{C_1} E_{C_2}^*}{E_R E_{C_1} X_r + E_B E_{C_1} X_r} \right) + \frac{\sqrt{\eta_1} E_{C_1} E_{C_2}^*}{E_R E_{C_2} X_r + E_B E_{C_2} X_r} \right) + \frac{\sqrt{\eta_2} E_{C_1} E_{C_2}^*}{E_R E_{C_3} X_r + E_B E_{C_3} X_r} \right) + \frac{\sqrt{\eta_3} E_{C_1} E_{C_2}^*}{E_R E_{C_4} X_r + E_B E_{C_4} X_r} \right) \]

\[ (E_S E_R^* e^{j(\phi - \beta)} + E_B E_R^* e^{-j(\phi - \beta)} + E_B E_R^*) X_r X_r e^{-j(\lambda x - \lambda x_r)} \left( \frac{\sqrt{\eta_0} E_{C_1} E_{C_2}^*}{E_R E_{C_1} X_r + E_B E_{C_1} X_r} \right) + \frac{\sqrt{\eta_1} E_{C_1} E_{C_2}^*}{E_R E_{C_2} X_r + E_B E_{C_2} X_r} \right) + \frac{\sqrt{\eta_2} E_{C_1} E_{C_2}^*}{E_R E_{C_3} X_r + E_B E_{C_3} X_r} \right) + \frac{\sqrt{\eta_3} E_{C_1} E_{C_2}^*}{E_R E_{C_4} X_r + E_B E_{C_4} X_r} \right) \]
Substitute: \( \frac{1}{1 + x} \approx 1 - x + x^2 - \ldots \)

\[
\frac{S + N_S}{B + N_B} = \frac{S (1 + \frac{N_S}{S})}{B (1 + \frac{N_B}{B})} = \frac{S}{B} \left(1 + \frac{N_S}{S} - \frac{N_B}{B} - \frac{N_S N_B}{S B}\right) = \frac{S}{B} + \frac{N_S}{B} - \frac{N_B S}{B^2}
\]

Since the noise terms are already near zero, any noise term (N) raised to a power is assumed zero

\[
\frac{S + N_S}{B + N_B} \approx \frac{S}{B} \left(1 + \frac{N_S}{S}\right) (1 - \frac{N_B}{B}) = \frac{S}{B} \left(1 + \frac{N_S}{S} - \frac{N_B}{B}\right) = \frac{S}{B} + \frac{N_S}{B} - \frac{N_B S}{B^2}
\]

Where:

\[
S = \frac{1}{2} \sqrt{\frac{\Delta \text{mu}^2}{h_0}} \left[ \sum \left( q_\text{pixel} \cdot A \right) \cdot \frac{E_B^* E_R}{E_R^* E_R} \cdot X_s X_s^* e^{i(x_i - x)} \right] \cdot \left( \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_R E_R^* X_s} + \frac{\sqrt{q_\text{pixel} E_R^* E_R^*}}{E_R E_R^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_R^* E_B^*}}{E_R E_B^* X_s + E_B E_R^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_R^*}}{E_B E_R^* X_s + E_R E_B^* X_s} \right) + \sum_{j=0}^{\frac{3}{2}} \frac{1}{j!} \frac{1}{\text{mu}_{j-1}^2} \cdot \left[ q_\text{pixel} \cdot A \right] \cdot \frac{E_B^* E_R^*}{E_R^* E_R} \cdot X_s X_s^* e^{i(x_i - x)}
\]

\[
B = \frac{1}{2} \sqrt{\frac{\Delta \text{mu}^2}{h_0}} \left[ \left( E_B^* E_B \right) X_s X_s^* e^{-j(x_i - x)} \cdot \left( \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} \right) + \sum_{j=0}^{\frac{3}{2}} \frac{1}{j!} \frac{1}{\text{mu}_{j-1}^2} \cdot \left[ q_\text{pixel} \cdot A \right] \cdot \frac{E_B^* E_B^*}{E_B^* E_B} \cdot X_s X_s^* e^{-j(x_i - x)} \right]
\]

\[
N_S = \frac{1}{2} \sqrt{\frac{\Delta \text{mu}^2}{h_0}} \left[ \sum \left( q_\text{pixel} \cdot A \right) \cdot \frac{E_B^* E_B^*}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} \right] \cdot \left( \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} \right) + \sum_{j=0}^{\frac{3}{2}} \frac{1}{j!} \frac{1}{\text{mu}_{j-1}^2} \cdot \left[ q_\text{pixel} \cdot A \right] \cdot \frac{E_B^* E_B^*}{E_B^* E_B} \cdot X_s X_s^* e^{-j(x_i - x)}
\]

\[
N_B = \frac{1}{2} \sqrt{\frac{\Delta \text{mu}^2}{h_0}} \left[ \left( E_B^* E_B \right) X_s X_s^* e^{-i(x_i - x)} \cdot \left( \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} + \frac{\sqrt{q_\text{pixel} E_B^* E_B^*}}{E_B E_B^* X_s + E_B E_B^* X_s} \right) + \sum_{j=0}^{\frac{3}{2}} \frac{1}{j!} \frac{1}{\text{mu}_{j-1}^2} \cdot \left[ q_\text{pixel} \cdot A \right] \cdot \frac{E_B^* E_B^*}{E_B^* E_B} \cdot X_s X_s^* e^{-j(x_i - x)} \right]
\]
\[
\frac{S}{B} + \frac{N_S}{B} - \frac{N_B S}{B^2} =
\]
\[
\begin{align*}
\mathbf{A} (E_S E_R^*) X_j X_j^* e^{i(\phi, -\phi)} \\
\mathbf{A}^{-1} (E_S^* E_R^*) X_j X_j^* e^{i(\phi, -\phi)} \\
\mathbf{A} (E_S E_R^*) X_j X_j^* e^{i(\phi - \phi)} \\
\mathbf{A}^{-1} (E_S^* E_R^*) X_j X_j^* e^{i(\phi - \phi)} \\
\mathbf{A} (E_S^* E_R^*) X_j X_j^* e^{i(\phi, -\phi)} \\
\mathbf{A}^{-1} (E_S E_R^*) X_j X_j^* e^{i(\phi, -\phi)} \\
\mathbf{A} (E_S E_R^*) X_j X_j^* e^{i(\phi - \phi)} \\
\mathbf{A}^{-1} (E_S^* E_R^*) X_j X_j^* e^{i(\phi - \phi)}
\end{align*}
\]
Assume all amplitudes are real:

\[ E_S = E_S^*; E_R = E_R^*; E_N = E_N^*; E_B = E_B^* \]

\[
\begin{align*}
\frac{[E_R + E_B]}{[E_R + E_N]} &= \left[ Ae^{i\alpha} (E_S E_R) X_e e^{j(z_x, z_r)} \sum_{k=0}^{3} \sqrt{\eta_k E_{c_k}^S} + Ae^{-i\alpha} (E_R E_S) X_e e^{-j(z_x, z_r)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k E_{c_k}^S} + \frac{1}{4 \pi \varepsilon_0 \varepsilon} \sum_{j=0}^{3} j^k i_{\text{tot}} i_{\text{tot}, \alpha} + i_{\text{tot}, \beta} + i_{\text{tot}, \gamma} \right] + \\
\frac{[E_R + E_B]}{[E_R + E_N]} &= \left[ (E_S E_R) X_e e^{j(z_x, z_r)} \sum_{k=0}^{3} \sqrt{\eta_k E_{c_k}^S} + (E_R E_S) X_e e^{-j(z_x, z_r)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k E_{c_k}^S} + \frac{1}{4 \pi \varepsilon_0 \varepsilon} \sum_{j=0}^{3} j^k i_{\text{tot}} i_{\text{tot}, \alpha} + i_{\text{tot}, \beta} + i_{\text{tot}, \gamma} \right] - \\
\frac{[E_R + E_B]}{[E_R + E_N]} &= \left[ Ae^{i\alpha} (E_S E_R) e^{j(z_x, z_r)} \sum_{k=0}^{3} \sqrt{\eta_k E_{c_k}^S} + Ae^{-i\alpha} (E_R E_S) e^{-j(z_x, z_r)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k E_{c_k}^S} + \frac{1}{4 \pi \varepsilon_0 \varepsilon} \sum_{j=0}^{3} j^k i_{\text{tot}} i_{\text{tot}, \alpha} + i_{\text{tot}, \beta} + i_{\text{tot}, \gamma} \right]
\end{align*}
\]
Assume all noise terms squared equal zero: 

\[ E_N E_N = 0 \quad E_B E_B = 0 \]

\[
\begin{align*}
\frac{E_R + E_B}{E_R + E_N} & = \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S + \sum_{k=0}^{3} \eta_k E_{C_k}^S}{\sum_{k=0}^{3} \eta_k E_{C_k}^S} + \frac{1}{\eta_{E_k}} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S}{\sum_{k=0}^{3} \eta_k E_{C_k}^S} + \frac{1}{\eta_{E_k}} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S}{\sum_{k=0}^{3} \eta_k E_{C_k}^S} + \ldots \\
\frac{E_R + E_B}{E_R + E_N} & = \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S + \sum_{k=0}^{3} \eta_k E_{C_k}^S}{\sum_{k=0}^{3} \eta_k E_{C_k}^S} + \frac{1}{\eta_{E_k}} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S}{\sum_{k=0}^{3} \eta_k E_{C_k}^S} + \frac{1}{\eta_{E_k}} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S}{\sum_{k=0}^{3} \eta_k E_{C_k}^S} + \ldots \\
\end{align*}
\]
Assume laser fluctuation is much less than laser output:

\[
\frac{E_N}{E_S} \approx \frac{E_N}{E_R} \approx \frac{E_B}{E_R} \approx \frac{E_B}{E_S} \approx 0
\]

\[
\frac{|E_R + E_B|}{|E_R + E_N|} \left[ \sum_{k=0}^{3} \sqrt{\eta_k E_{C_{ik}}} + \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k E_{C_{ik}}} + \frac{1}{\sqrt{\eta_k E_{C_{ik}}}} \sum_{k=0}^{3} j \frac{N_{R\alpha} - N_{R\alpha} - i_{R_{ik}} + i_{D_{ik}}}{\sqrt{\eta_k E_{C_{ik}}}} \right] +
\]

\[
\frac{|E_R + E_B|}{|E_R + E_N|} \left[ \sum_{k=0}^{3} \sqrt{\eta_k E_{C_{ik}}} + \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k E_{C_{ik}}} \right] - \left[ \sum_{k=0}^{3} \frac{N_{S_{ik}}}{B^2} \approx \right]
\]

Thus:

\[
\frac{N_S}{B} \approx \frac{N_{B}}{B^2} \approx 0
\]

\[
\frac{S}{B} \approx \frac{|E_R + E_B|}{|E_R + E_N|} \left[ \sum_{k=0}^{3} \sqrt{\eta_k E_{C_{ik}}} + \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k E_{C_{ik}}} + \frac{1}{\sqrt{\eta_k E_{C_{ik}}}} \sum_{k=0}^{3} j \frac{N_{R\alpha} - N_{R\alpha} - i_{R_{ik}} + i_{D_{ik}}}{\sqrt{\eta_k E_{C_{ik}}}} \right] - \left[ \sum_{k=0}^{3} \frac{N_{S_{ik}}}{B^2} \approx \right]
\]
Since the amplitudes are real, the magnitude of the reference plus the noise term is simply equal to the reference plus the noise term:

\[
\left| E_R + E_B \right| = \frac{E_R + E_B}{1 + \frac{E_N}{E_R}}
\]

We have assumed that the amplitude of the noise divided by the amplitude of the signal is approximately zero:

\[
\left| E_R + E_B \right| \approx 1
\]

\[
S \approx \frac{Ae^{j\alpha} e^{j(\chi_s - \chi_r)} \sum_{k=0}^{3} \sqrt{\eta_k} E^S_C \omega + Ae^{-j\alpha} e^{-j(\chi_s - \chi_r)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} E^S_C \omega}{B e^{j(\chi_s - \chi_r)} \sum_{k=0}^{3} \sqrt{\eta_k} E^S_C \omega + e^{-j(\chi_s - \chi_r)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} E^S_C \omega}
\]

\[
\beta = \chi_s - \chi_r
\]

\[
I_{S,\text{Norm}} = \sum_{k=0}^{3} \sqrt{\eta_k} E^S_C \omega
\]

\[
I_{B,\text{Norm}} = \sum_{k=0}^{3} j^k \sqrt{\eta_k} E^S_C \omega
\]

Substituting:

\[
S = \frac{Ae^{j\alpha} Ye^{j\beta} + Ae^{-j\alpha} Ze^{-j\beta} + I_{S,\text{Norm}}}{Ye^{j\beta} + Ze^{-j\beta} + I_{B,\text{Norm}}}
\]

Substitute Taylor Series:

\[
(Ae^{j\alpha} + \frac{Z}{Y} A e^{-j2\beta} + \frac{1_{Y,\text{Norm}}}{Y} e^{-j\beta}) \frac{1}{1 + (\frac{Z}{Y} e^{-j2\beta} + \frac{1_{Y,\text{Norm}}}{Y} e^{-j\beta})} \approx (Ae^{j\alpha} + \frac{Z}{Y} A e^{-j2\beta} + \frac{1_{Y,\text{Norm}}}{Y} e^{-j\beta})(1 - (\frac{Z}{Y} e^{-j2\beta} + \frac{1_{Y,\text{Norm}}}{Y} e^{-j\beta}))(1 - \frac{Z}{Y} e^{-j2\beta} - \frac{1_{Y,\text{Norm}}}{Y} e^{-j\beta})
\]

\[
S \approx Ae^{j\alpha} (1 - \frac{Z}{Y} e^{-j2\beta} \frac{1_{Y,\text{Norm}}}{Y} e^{-j\beta}) + \frac{Z}{Y} A e^{-j2\beta} (1 - \frac{Z}{Y} e^{-j2\beta} \frac{1_{Y,\text{Norm}}}{Y} e^{-j\beta}) + \frac{1_{Y,\text{Norm}}}{Y} e^{-j\beta} (1 - \frac{Z}{Y} e^{-j2\beta} - \frac{1_{Y,\text{Norm}}}{Y} e^{-j\beta})
\]

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Ideally, the amplitude of the noise is equal to 1. Therefore, we set the amplitudes to:

\[ E_{C1}^S = 1 + \Delta_k^S \quad E_{C1}^R = 1 + \Delta_k^R \quad X_s = 1 + \delta_s \quad X_r = 1 + \delta_r \]

\[
I_{S,\text{Norm}} = \frac{1}{\sqrt{\frac{1}{4} \sum_{k=0}^3 j^k E_{S,k}^2 E_{R,k}^2 (1+\delta_s)(1+\delta_r) \eta_k (1+\Delta_k^S)}} \\
I_{B,\text{Norm}} = \frac{1}{\sqrt{\frac{1}{4} \sum_{k=0}^3 j^k E_{S,k}^2 E_{R,k}^2 (1+\delta_s)(1+\delta_r) \eta_k (1+\Delta_k^S)}}
\]

\[
Y = \sum_{k=0}^3 \sqrt{\eta_k} E_k^S = \sum_{k=0}^3 \sqrt{\eta_k} (1+\Delta_k^S) \\
Z = \sum_{k=0}^3 (-1)^k \sqrt{\eta_k} E_k^S = \sum_{k=0}^3 (-1)^k \sqrt{\eta_k} (1+\Delta_k^S)
\]

\[
\Psi = \frac{Z}{Y} = \frac{\sum_{k=0}^3 (-1)^k \sqrt{\eta_k} (1+\Delta_k^S)}{\sum_{k=0}^3 \sqrt{\eta_k} (1+\Delta_k^S)}
\]

Substituting:

\[
\frac{S}{B} \approx A e^{j\alpha} (1 - \Psi e^{-j2\beta} - \frac{1_s}{\sqrt{\gamma}} e^{-j\beta}) + \Psi A e^{-j\alpha} e^{-j2\beta} (1 - \Psi e^{-j2\beta} - \frac{1_s}{\sqrt{\gamma}} e^{-j\beta}) + \frac{1_s}{\sqrt{\gamma}} e^{-j\beta} (1 - \Psi e^{-j2\beta} - \frac{1_s}{\sqrt{\gamma}} e^{-j\beta})
\]

Assuming noise terms squared approximately equal to zero:

\[
\delta_s \delta_r \approx 0; \quad I_{S,\eta} \approx 0; \quad I_{B,\eta} \approx 0; \quad \Psi I_{S,\eta} \approx 0; \quad \Psi I_{B,\eta} \approx 0
\]

\[
\frac{S}{B} \approx A e^{j\alpha} (1 - \Psi e^{-j2\beta} - \frac{1_s}{\sqrt{\gamma}} e^{-j\beta}) + A e^{-j\alpha} (1 - \Psi e^{-j2\beta} - \frac{1_s}{\sqrt{\gamma}} e^{-j\beta}) + \frac{1_s}{\sqrt{\gamma}} e^{-j\beta}
\]

Approximation for Dark Current Terms:

\[
I_{S,\text{Norm}} = \sum_{k=0}^3 j^k \sqrt{\frac{A_{S,k}}{\eta_k}} \sqrt{\eta_k} (1+\Delta_k^S) \\
I_{B,\text{Norm}} = \sum_{k=0}^3 j^k \sqrt{\frac{A_{B,k}}{\eta_k}} \sqrt{\eta_k} (1+\Delta_k^S)
\]

\[
\sqrt{B_S} = \frac{1}{2} E_{C1}^S (1+\delta_s) |E_S + E_N| \sqrt{A_{S,\text{Norm}}} \sqrt{\eta_k} \sqrt{1 + \frac{1_s}{\sqrt{\gamma}} A_{S,\text{Norm}} (E_S E_R^* + E_N E_{\gamma,k}^{(\phi\gamma)}) (E_S E_R^{\phi\gamma} + E_N E_{\gamma,k}^{(\phi\gamma)}) E_{S,k}^* E_{R,k}^* X,X'}
\]

\[
\sqrt{B_R} = \frac{1}{2} E_{C1}^R (1+\delta_r) |E_R + E_N| \sqrt{A_{R,\text{Norm}}} \sqrt{\eta_k} \sqrt{1 + \frac{1_s}{\sqrt{\gamma}} A_{S,\text{Norm}} (E_S E_R^* + E_N E_{\gamma,k}^{(\phi\gamma)}) (E_S E_R^{\phi\gamma} + E_N E_{\gamma,k}^{(\phi\gamma)}) E_{S,k}^* E_{R,k}^* X,X'}
\]
Assuming: 

\[ i_{S,k} \ll \frac{1}{4} \frac{g}{h^2} A_{\text{pixel}} (E_S E_S^* + E_S E_N^* e^{i(\phi-\zeta)} + E_N E_S^* e^{-i(\phi-\zeta)} + E_N E_N^*) E_{C_1} E_{C_2} X, X^* \]

\[ i_{R,k} \ll \frac{1}{4} \frac{g}{h^2} A_{\text{pixel}} (E_R E_R^* + E_R E_N^* e^{i(\phi-\zeta)} + E_N E_R^* e^{-i(\phi-\zeta)} + E_N E_N^*) E_{C_1} E_{C_2} X, X^* \]

\[ \sqrt{BS} = \frac{1}{2} E_{C_1}^S (1 + \delta_s) |E_S + E_N| \cdot \sqrt{\frac{\lambda_{\text{pixel}}}{h^2}} \sqrt{\eta_k} \]

\[ \sqrt{R} = \frac{1}{2} E_{C_1}^R (1 + \delta_r) |E_R + E_N| \cdot \sqrt{\frac{\lambda_{\text{pixel}}}{h^2}} \sqrt{\eta_k} \]

Assuming: 

\[ E_S \gg E_N \quad E_R \gg E_N \]

\[ \sqrt{BS} \approx \frac{1}{2} E_{C_1}^S (1 + \delta_s) E_S \sqrt{\frac{\lambda_{\text{pixel}}}{h^2}} \sqrt{\eta_k} \]

\[ \sqrt{R} \approx \frac{1}{2} E_{C_1}^R (1 + \delta_r) E_R \sqrt{\frac{\lambda_{\text{pixel}}}{h^2}} \sqrt{\eta_k} \]

Thus:

\[ \sqrt{R} \cdot \sqrt{BS} \approx \frac{1}{4} E_{C_1}^S E_{C_1}^R (1 + \delta_s)(1 + \delta_r) E_S E_R \frac{\lambda_{\text{pixel}}}{h^2} \eta_k \]

\[ \sqrt{R} \cdot \sqrt{BS} = \frac{1}{4} E_{C_1}^S E_{C_1}^R (1 + \delta_s + \delta_r) E_R E_S \frac{\lambda_{\text{pixel}}}{h^2} \eta_k \]

\[ \Psi = \frac{Z}{Y} = \sum_{k=0}^{3} \frac{(-1)^k \sqrt{\eta_k} (1 + \Delta_k)}{\sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S)} = \frac{1}{2} \sqrt{\frac{\lambda_{\text{pixel}}}{h^2}} E_S (1 + \delta_s) \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} (1 + \Delta_k^S) \sum_{k=0}^{3} \sqrt{BS_k} \]

Substituting:

\[ I_{S,\text{Norm}} = \sum_{k=0}^{3} j^k \frac{i_{S,k} - i_{S,k} - i_{S,k} + i_{D,k}}{4 \frac{\lambda_{\text{pixel}}}{h^2} E_S (1 + \delta_s + \delta_r) \sqrt{\eta_k} (1 + \Delta_k^S)} \]

\[ I_{R,\text{Norm}} = \sum_{k=0}^{3} j^k \frac{i_{R,k} - i_{R,k} - i_{R,k} + i_{D,k}}{4 \frac{\lambda_{\text{pixel}}}{h^2} E_S (1 + \delta_s + \delta_r) \sqrt{\eta_k} (1 + \Delta_k^S)} \]

\[ I_{S,\text{Norm}} = \sum_{k=0}^{3} j^k \frac{i_{S,k} - i_{S,k} - i_{S,k} + i_{D,k}}{4 \frac{\lambda_{\text{pixel}}}{h^2} E_S (1 + \delta_s + \delta_r) \sqrt{\eta_k} (1 + \Delta_k^S)} = \frac{1}{2} \sqrt{\frac{\lambda_{\text{pixel}}}{h^2}} E_S (1 + \delta_s) \sum_{k=0}^{3} j^k \frac{i_{S,k} - i_{S,k} - i_{S,k} + i_{D,k}}{\sqrt{\eta_k} (1 + \Delta_k^S)} \]

\[ I_{B,\text{Norm}} = \sum_{k=0}^{3} j^k \frac{i_{B,k} - i_{B,k} - i_{B,k} + i_{D,k}}{4 \frac{\lambda_{\text{pixel}}}{h^2} E_S (1 + \delta_s + \delta_r) \sqrt{\eta_k} (1 + \Delta_k^S)} = \frac{1}{2} \sqrt{\frac{\lambda_{\text{pixel}}}{h^2}} E_S (1 + \delta_s) \sum_{k=0}^{3} j^k \frac{i_{B,k} - i_{B,k} - i_{B,k} + i_{D,k}}{\sqrt{\eta_k} (1 + \Delta_k^S)} \]

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Substituting: \[ Y = \sum_{k=0}^{3} \sqrt{\eta_k (1 + \Delta_k^S)} \]

\[
\frac{I_{S,Norm}}{Y} = \frac{1}{2} \sqrt[3]{\frac{A_{pixel}}{\kappa_D}} E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S) = \frac{3}{2} \sqrt[3]{\frac{A_{pixel}}{\kappa_D}} E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S) = \frac{3}{2} \sqrt{\frac{A_{pixel}}{\kappa_D}} E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{BS_k} = I_{S,Norm}
\]

\[
\frac{I_{B,Norm}}{Y} = \frac{1}{2} \sqrt[3]{\frac{A_{pixel}}{\kappa_D}} E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S) = \frac{3}{2} \sqrt[3]{\frac{A_{pixel}}{\kappa_D}} E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S) = \frac{3}{2} \sqrt[3]{\frac{A_{pixel}}{\kappa_D}} E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{BS_k} = I_{B,Norm}
\]
Complex Field Reconstructed by Balanced Mixing, DC Term Subtraction, and Camera Normalization

\[ E_{BM, \text{DC, Norm}} = \Lambda e^{j\alpha} \left[ 1 - \Psi e^{-j2(\chi_s - \chi_r)} - I_{B, \text{Norm}} e^{-j(\chi_s - \chi_r)} \right] + \Lambda e^{-j\alpha} \left[ \Psi e^{-j2(\chi_s - \chi_r)} - \Psi^2 e^{-j4(\chi_s - \chi_r)} \right] + I_{S, \text{Norm}} e^{-j(\chi_s - \chi_r)} \]

Where:

\[ \Psi = \sum_{k=0}^{3} (-1)^k \frac{\sqrt{BS_k}}{\sqrt{\sum_{k=0}^{3} BS_k}} \]

\[ I_{S, \text{Norm}} = \sum_{k=0}^{3} \frac{j^k l_{S,k} \cdot i_{S,k} \cdot \sqrt{i_{D,k}}}{\sqrt{R_k}} \sqrt{\sum_{k=0}^{3} BS_k} \]

\[ I_{B, \text{Norm}} = \sum_{k=0}^{3} \frac{j^k l_{B,k} \cdot i_{B,k} \cdot \sqrt{i_{D,k}}}{\sqrt{R_k}} \sqrt{\sum_{k=0}^{3} BS_k} \]

\( \Lambda e^{j\alpha} \): Magnitude (\( \Lambda \)) and phase (\( \alpha \)) of the sample

\( \chi_s \): Phase of aberrations induced within the signal arm

\( \chi_r \): Phase of aberrations induced within the reference arm

\( BS \): Image of the pure signal beam without the sample in the path

\( R \): Image of the pure reference

\( i \): Dark current noise in an acquired image
A.4.1 Include Wavefront Mismatch Between Sample and Blank Images

Substitute: \( \beta_1 = \chi_s - \chi_r \) (Signal) \( \beta_2 = \chi_s - \chi_r \) (Blank)

\[
S \approx B = \frac{\sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + \sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + 1}{\sum_{k=0}^{3} \frac{j k i_{BM,A}^{-}i_{BS,A}^{-}i_{DS,A}^{-}t_{D,A}}{\sqrt{\eta_k} E^S_{C_k}}}
\]

Substituting:

\[
Y = \sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k}
\]

\[
I_{S,Norm} = \frac{1}{\sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k}} \sum_{k=0}^{3} \frac{j k i_{BM,A}^{-}i_{BS,A}^{-}i_{DS,A}^{-}t_{D,A}}{\sqrt{\eta_k} E^S_{C_k}}
\]

\[
Z = \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} E^S_{C_k}
\]

\[
I_{B,Norm} = \frac{1}{\sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k}} \sum_{k=0}^{3} \frac{j k i_{BM,A}^{-}i_{BS,A}^{-}i_{DS,A}^{-}t_{D,A}}{\sqrt{\eta_k} E^S_{C_k}}
\]

\[
gives: \frac{S}{B} = \frac{\sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + \sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + 1}{\sum_{k=0}^{3} \frac{j k i_{BM,A}^{-}i_{BS,A}^{-}i_{DS,A}^{-}t_{D,A}}{\sqrt{\eta_k} E^S_{C_k}}}
\]

Substitute Taylor Series:

\[
(\sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + \sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + 1) = \frac{1}{1 + \left(\sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + \sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + 1\right)}
\]

\[
S \approx B = \frac{\sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + \sum_{k=0}^{3} \sqrt{\eta_k} E^S_{C_k} + 1}{\sum_{k=0}^{3} \frac{j k i_{BM,A}^{-}i_{BS,A}^{-}i_{DS,A}^{-}t_{D,A}}{\sqrt{\eta_k} E^S_{C_k}}}
\]

Ideally, the amplitude of the noise is equal to 1. Therefore, we set the amplitudes to:

\[
E^S_{C_k} = 1 + \Delta^S_k \quad E^R_{C_k} = 1 + \Delta^R_k \quad X_s = 1 + \delta_s \quad X_r = 1 + \delta_r
\]
Substituting:

\[ \frac{S}{B} \approx A e^{j\alpha} e^{j(\beta_1 - \beta_2)} (1 - \Psi e^{-j\beta_1} - \frac{1}{\sqrt{\lambda}} e^{-j\beta_2}) + \Psi A e^{j\alpha} e^{j(\beta_1 + \beta_2)} (1 - \Psi e^{-j\beta_1} - \frac{1}{\sqrt{\lambda}} e^{-j\beta_2}) \]

Assuming noise terms squared approximately equal to zero:

\[ \delta_s \delta_r \approx 0; \quad I_{S,q} I_{B,q} \approx 0; \quad \Psi I_{S,q} \approx 0; \quad \Psi I_{B,q} \approx 0 \]

\[ \frac{S}{B} \approx A e^{j\alpha} (e^{j(\beta_1 - \beta_2)} - \Psi e^{-j(2\beta_2 - (\beta_1 - \beta_2))} - \frac{1}{\sqrt{\lambda}} e^{-j(\beta_2 - (\beta_1 - \beta_2))}) + A e^{-j\alpha} (\Psi e^{-j(\beta_1 + \beta_2)} - \Psi^2 e^{-j(2\beta_2 + (\beta_1 + \beta_2))} + \frac{1}{\sqrt{\lambda}} e^{-j\beta_2}) \]

Approximation for Dark Current Terms:

\[ I_{S,Norm} = \sum_{k=0}^{\infty} \left| k \right|^2 \frac{\eta_{S,Norm}}{\lambda \eta_k} \sqrt{\frac{A_{pixel}}{h\nu}} \sqrt{\frac{i_{S,k}}{\lambda \eta_k}} \frac{i_{S,k}}{\lambda \eta_k} \]

\[ I_{B,Norm} = \sum_{k=0}^{\infty} \left| k \right|^2 \frac{\eta_{B,Norm}}{\lambda \eta_k} \sqrt{\frac{A_{pixel}}{h\nu}} \sqrt{\frac{i_{B,k}}{\lambda \eta_k}} \frac{i_{B,k}}{\lambda \eta_k} \]

Assuming:

\[ i_{S,k} \ll \frac{\eta_{S,Norm}}{\lambda \eta_k} A_{pixel} E_S E_S^* e^{j(\phi - \zeta)} + E_S E_S^* e^{-j(\phi - \zeta)} + E_N E_N^* e^{j(\phi - \zeta)} + E_N E_N^* e^{-j(\phi - \zeta)} \]

\[ i_{R,k} \ll \frac{\eta_{B,Norm}}{\lambda \eta_k} A_{pixel} E_R E_R^* e^{j(\phi - \zeta)} + E_R E_R^* e^{-j(\phi - \zeta)} + E_N E_N^* e^{j(\phi - \zeta)} + E_N E_N^* e^{-j(\phi - \zeta)} \]

Assuming:

\[ E_S \gg E_N \quad E_R \gg E_N \]

\[ \sqrt{S_B} \approx \frac{1}{2} E_{C_1} (1 + \delta_s) \sqrt{E_S + E_N} \cdot \sqrt{\frac{A_{pixel}}{h\nu}} \sqrt{\eta_k} \]

\[ \sqrt{R} \approx \frac{1}{2} E_{C_1} (1 + \delta_r) \sqrt{E_R + E_N} \cdot \sqrt{\frac{A_{pixel}}{h\nu}} \sqrt{\eta_k} \]
Thus:

\[
\sqrt{R} \cdot \sqrt{BS} \approx \frac{1}{2} E_C^R (1 + \delta_x) E_R \sqrt{\frac{A_{\text{pixel}}}{h\nu}} \sqrt{\eta_k} \cdot \frac{1}{2} E_C^S (1 + \delta_x) E_S \sqrt{\frac{A_{\text{pixel}}}{h\nu}} \sqrt{\eta_k}
\]

\[
= \frac{1}{4} E_C^R E_C^S (1 + \delta_x)(1 + \delta_x) E_R E_S \frac{A_{\text{pixel}}}{h\nu} \eta_k
\]

\[
\sqrt{R} \cdot \sqrt{BS} = \frac{1}{4} E_C^R E_C^S (1 + \delta_x + \delta_r) E_R E_S \frac{A_{\text{pixel}}}{h\nu} \eta_k
\]

\[
\Psi = \frac{Z}{Y} = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} (1 + \Delta_k)}{\sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S)} = \frac{\frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{h\nu}} E_S (1 + \delta_s) \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} (1 + \Delta_k^S)}{\frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{h\nu}} E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S)}
\]

Substituting:

\[
I_{S, \text{Norm}} = \sum_{k=0}^{3} j^k \frac{i_{\text{L},k} - i_{\text{R},k} - i_{\text{A},k} + i_{\text{D},k}}{\sqrt{R_k}}
\]

\[
I_{B, \text{Norm}} = \sum_{k=0}^{3} j^k \frac{i_{\text{B},k} - i_{\text{L},k} - i_{\text{A},k} + i_{\text{D},k}}{\sqrt{R_k}}
\]

Substituting:

\[
Y = \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S)
\]

\[
\frac{I_{S, \text{Norm}}}{Y} = \frac{1}{\sqrt{\frac{A_{\text{pixel}}}{h\nu}} E_S (1 + \delta_s)} \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{h\nu}} E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S) = \frac{3}{2} \sum_{k=0}^{3} \frac{j^k i_{\text{L},k} - i_{\text{R},k} - i_{\text{A},k} + i_{\text{D},k}}{\sqrt{R_k}} = I_{S, \text{Norm}}
\]

\[
\frac{I_{B, \text{Norm}}}{Y} = \frac{1}{\sqrt{\frac{A_{\text{pixel}}}{h\nu}} E_S (1 + \delta_s)} \frac{1}{2} \sqrt{\frac{A_{\text{pixel}}}{h\nu}} E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^S) = \frac{3}{2} \sum_{k=0}^{3} \frac{j^k i_{\text{B},k} - i_{\text{R},k} - i_{\text{A},k} + i_{\text{D},k}}{\sqrt{R_k}} = I_{B, \text{Norm}}
\]
Complex Field Reconstructed by Balanced Mixing, DC Term Subtraction, and Camera Normalization
(assuming fringe pattern of sample image does not match fringe pattern of blank)

\[ E_{BM,DC,Norm} = A e^{j\alpha} \left[ e^{j(\beta_1 - \beta_2)} - \Psi e^{-j(3\beta_2 - \beta_1)} - I_{B,Norm} e^{-j(2\beta_2 - \beta_1)} \right] + A e^{-j\alpha} \left[ \Psi e^{-j(\beta_1 + \beta_2)} - \Psi^2 e^{-j(3\beta_2 + \beta_1)} \right] + I_{S,Norm} e^{-j\beta_2} \]

Where:

\[ \Psi = \sum_{k=0}^{3} \frac{(-1)^k \sqrt{BS_k}}{\sum_{k=0}^{3} \sqrt{BS_k}} \]

\[ I_{B,Norm} = \sum_{k=0}^{3} \frac{I_{BS_k} e^{-i(\beta_2 - \beta_1)} + i_0}{\sum_{k=0}^{3} \sqrt{BS_k}} \]

\[ I_{S,Norm} = \sum_{k=0}^{3} \frac{I_{BS_k} e^{-i(\beta_2 + \beta_1)} + i_0}{\sum_{k=0}^{3} \sqrt{BS_k}} \]

\( Ae^{j\alpha} \): Magnitude (\( A \)) and phase (\( \alpha \)) of the sample

\( \chi_s \): Phase of aberrations induced within the signal arm

\( \chi_r \): Phase of aberrations induced within the reference arm

\( \beta_1 \): Wavefront mismatch between signal and reference arms for image of sample

\( \beta_2 \): Wavefront mismatch between signal and reference arms for image of blank

\( BS \): Image of the pure signal beam without the sample in the path

\( R \): Image of the pure reference

\( i \): Dark current noise in an acquired image
A.4.2 Balanced Mixing, DC term Subtraction, Camera Normalization, and No Blank

\[ E_{BM, DC, Norm, sample} = \frac{1}{2} \sqrt{\frac{\text{power}}{\text{hor}}} \left[ A e^{j\alpha} (E_S E_R^* + E_S E_N^* e^{j(\phi - \zeta)} + E_N E_R^* e^{-j(\phi - \zeta)} + E_N E_N^*) X_s X_s^* e^{j(x_s - x_r)} \right]. \]

\[ \left( \begin{array}{c}
\sqrt{\eta_0 E_{C_0}^S E_{C_0}^R} \\
\eta_0 E_{C_0}^S E_{C_0}^R
\end{array} \right) \left( 1 - \frac{i_{r,0} - i_{s,0} + i_{d,0}}{\frac{\text{power}}{\text{hor}}} A_{\text{pixel}} (E_SE_R^* + E_SE_N^* e^{i(\phi - \zeta)} + E_N E_R^* e^{-i(\phi - \zeta)} + E_N E_N^*) X_s X_s^* \right) + \\
\sqrt{\eta_0 E_{C_0}^S E_{C_0}^R} \\
\eta_0 E_{C_0}^S E_{C_0}^R
\end{array} \right) \left( 1 - \frac{i_{r,1} - i_{d,1}}{\frac{\text{power}}{\text{hor}}} A_{\text{pixel}} (E_SE_R^* + E_SE_N^* e^{i(\phi - \zeta)} + E_N E_R^* e^{-i(\phi - \zeta)} + E_N E_N^*) X_s X_s^* \right) + \\
\sqrt{\eta_0 E_{C_0}^S E_{C_0}^R} \\
\eta_0 E_{C_0}^S E_{C_0}^R
\end{array} \right) \left( 1 - \frac{i_{r,2} - i_{d,2}}{\frac{\text{power}}{\text{hor}}} A_{\text{pixel}} (E_SE_R^* + E_SE_N^* e^{i(\phi - \zeta)} + E_N E_R^* e^{-i(\phi - \zeta)} + E_N E_N^*) X_s X_s^* \right) + \\
\sqrt{\eta_0 E_{C_0}^S E_{C_0}^R} \\
\eta_0 E_{C_0}^S E_{C_0}^R
\end{array} \right) \left( 1 - \frac{i_{r,3} - i_{d,3}}{\frac{\text{power}}{\text{hor}}} A_{\text{pixel}} (E_SE_R^* + E_SE_N^* e^{i(\phi - \zeta)} + E_N E_R^* e^{-i(\phi - \zeta)} + E_N E_N^*) X_s X_s^* \right) \]
Assuming: \( i_R - i_D << \frac{1}{2} \frac{n_q}{h_0} A_{\text{pixel}} (E_R E_r^* + E_R E_N^* e^{i(\phi - \zeta)} + E_N E_r^* e^{-i(\phi - \zeta)} + E_N E_N^*) E_C^R E_C^r X_r X_r^* \)

\[
E_{\text{BM,DC, Norm, sample}} = \frac{1}{2} \sqrt{\frac{n_q}{h_0}} \frac{A_{\text{pixel}}}{|E_{\text{X}} + E_{\text{Y}}|} \left[ A e^{-i\alpha} (E_S E_r^* + E_S E_N^* e^{i(\phi - \zeta)} + E_N E_r^* e^{-i(\phi - \zeta)} + E_N E_N^*) X_r X_r^* e^{i(x, -x_r)} \right] + \\
\left( \sqrt{\eta_0 E_C^S E_C^S} \right) \left( \sqrt{\eta_1 E_C^S E_C^S} \right) \left( \sqrt{\eta_2 E_C^S E_C^S} \right) \left( \sqrt{\eta_3 E_C^S E_C^S} \right) \\
\left( \sqrt{\eta_0 E_C^S E_C^S} \right) \left( \sqrt{\eta_1 E_C^S E_C^S} \right) \left( \sqrt{\eta_2 E_C^S E_C^S} \right) \left( \sqrt{\eta_3 E_C^S E_C^S} \right) \\
A e^{-i\alpha} (E_r E_r^* + E_r E_N^* e^{i(\phi - \zeta)} + E_N E_r^* e^{-i(\phi - \zeta)} + E_N E_N^*) X_r X_r^* e^{-i(x, -x_r)} \\
\left( \sqrt{\eta_0 E_C^S E_C^S} \right) \left( \sqrt{\eta_1 E_C^S E_C^S} \right) \left( \sqrt{\eta_2 E_C^S E_C^S} \right) \left( \sqrt{\eta_3 E_C^S E_C^S} \right) \\
\frac{i_{M,0} - i_{D,0} - i_{S,0} + i_{D,0}}{2} + \frac{j}{2} \sqrt{\eta_0} A_{\text{pixel}} \left| E_C^R E_C^r X_r + E_r E_C^r X_r \right| \\
\frac{i_{M,2} - i_{D,2} - i_{S,2} + i_{D,2}}{2} + \frac{j}{2} \sqrt{\eta_0} A_{\text{pixel}} \left| E_C^R E_C^r X_r + E_r E_C^r X_r \right|
\]

Substitute Summations for Efficiency Terms:

\[
E_{\text{BM,DC, Norm, sample}} = \frac{1}{2} \sqrt{\frac{n_q}{h_0}} \frac{A_{\text{pixel}}}{|E_{\text{X}} + E_{\text{Y}}|} \left[ A e^{-i\alpha} (E_S E_r^* + E_S E_N^* e^{i(\phi - \zeta)} + E_N E_r^* e^{-i(\phi - \zeta)} + E_N E_N^*) X_r X_r^* e^{i(x, -x_r)} \right] + \\
\sum_{k=0}^{3} \frac{(-1)^k \sqrt{\eta_k} E_k^S e_k^S}{E_k^S} + \\
\sum_{k=0}^{3} \frac{\eta_{M,k} - i_{D,k} - i_{S,k} + i_{D,k}}{4} \sqrt{\eta_k} E_k^S X_r \left| E_r + E_N \right|
\]

Assume all amplitudes are real and:

\[
E_{\text{BM,DC, Norm, sample}} = \frac{1}{2} \sqrt{\frac{n_q}{h_0}} \frac{A_{\text{pixel}}}{|E_{\text{X}} + E_{\text{Y}}|} \left[ A e^{-i\alpha} (E_S E_r^* + E_S E_N^* e^{i(\phi - \zeta)} + E_N E_r^* e^{-i(\phi - \zeta)} + E_N E_N^*) X_r X_r^* e^{i(x, -x_r)} \right] + \\
\sum_{k=0}^{3} \frac{(-1)^k \sqrt{\eta_k} E_k^S e_k^S}{E_k^S} + \\
\sum_{k=0}^{3} \frac{\eta_{M,k} - i_{D,k} - i_{S,k} + i_{D,k}}{4} \sqrt{\eta_k} E_k^S X_r \left| E_r + E_N \right|
\]

\[
E_{\text{BM,DC, Norm, sample}} = \frac{1}{2} \sqrt{\frac{n_q}{h_0}} \frac{A_{\text{pixel}}}{|E_{\text{X}} + E_{\text{Y}}|} \left[ A e^{-i\alpha} (E_S E_r^* + E_S E_N^* e^{i(\phi - \zeta)} + E_N E_r^* e^{-i(\phi - \zeta)} + E_N E_N^*) X_r X_r^* e^{i(x, -x_r)} \right] + \\
\sum_{k=0}^{3} \frac{(-1)^k \sqrt{\eta_k} E_k^S e_k^S}{E_k^S} + \\
\sum_{k=0}^{3} \frac{\eta_{M,k} - i_{D,k} - i_{S,k} + i_{D,k}}{4} \sqrt{\eta_k} E_k^S X_r \left| E_r + E_N \right|
\]

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Assume noise terms squared equal zero:

\[
E_{BM,DC,Norm,\text{sample}} = \frac{1}{2} \sqrt{\frac{A_{\text{mol,q}}}{\hbar \nu}} \frac{1}{e_{\text{ex}} + e_{\text{ex}}} \left[ Ae^{i\alpha} (E_x E_R + E_x E_N e^{i(\phi - \zeta)} + E_N E_R e^{-i(\phi - \zeta)}) X_s e^{i(\chi - \chi_s)} \sum_{k=0}^{3} \sqrt{\eta_k E_{C_k}^S} + Ae^{-i\alpha} (E_R E_x + E_x E_N e^{-i(\phi - \zeta)} + E_N E_R e^{i(\phi - \zeta)}) X_s e^{-i(\chi - \chi_s)} \sum_{k=0}^{3} \sqrt{\eta_k E_{C_k}^S} \right] + \sum_{k=0}^{3} j^k \sqrt{\eta_k E_{C_k}^S} X_s |E_R + E_N| \]

Substitute: \[
\sqrt{R_k} = \frac{1}{4} \frac{A_{\text{mol,q}}}{\hbar \nu} \sqrt{\eta_k E_{C_k}^S} X_s |E_R + E_N| \]

Separate signal terms and noise terms:

\[
S = \frac{1}{4} \frac{A_{\text{mol,q}}}{\hbar \nu} \frac{E_x E_R + E_x E_N}{e_{\text{ex}} + E_{\text{ex}}} X_s e^{i(\chi - \chi_s)} \left[ Ae^{i\alpha} \sum_{k=0}^{3} \sqrt{\eta_k E_{C_k}^S} + Ae^{-i\alpha} e^{-j(\phi - \zeta)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k E_{C_k}^S} \right] + \sum_{k=0}^{3} j^k \frac{i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}}{\sqrt{R_k}} \]

\[
N = \frac{1}{4} \frac{A_{\text{mol,q}}}{\hbar \nu} \frac{E_x E_R - E_x E_N}{e_{\text{ex}} + E_{\text{ex}}} X_s e^{i(\chi - \chi_s)} \left[ Ae^{i\alpha} (E_x e^{i(\phi - \zeta)} + E_R e^{-j(\phi - \zeta)}) \sum_{k=0}^{3} \sqrt{\eta_k E_{C_k}^S} + Ae^{-i\alpha} (E_R e^{i(\phi - \zeta)} + E_x e^{-j(\phi - \zeta)}) e^{-j(\chi - \chi_s)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k E_{C_k}^S} \right] \]

Rearrange Terms:

\[
\frac{1}{4} \sqrt{\frac{A_{\text{mol,q}}}{\hbar \nu}} \frac{E_x E_R X_s e^{i(\chi - \chi_s)}}{E_R + E_N} = \frac{1}{4} \sqrt{\frac{A_{\text{mol,q}}}{\hbar \nu}} \frac{E_x E_R X_s e^{i(\chi - \chi_s)}}{E_R (1 + \frac{E_N}{E_R})} = \frac{1}{4} \sqrt{\frac{A_{\text{mol,q}}}{\hbar \nu}} \frac{E_x E_R X_s e^{i(\chi - \chi_s)}}{(1 + \frac{E_N}{E_R})} \]

Assuming the laser fluctuation is much less than the laser output:

\[
\frac{E_N}{E_R} \approx 0 \]

\[
\frac{1}{4} \sqrt{\frac{A_{\text{mol,q}}}{\hbar \nu}} \frac{E_x E_R X_s e^{i(\chi - \chi_s)}}{E_R + E_N} \approx \frac{1}{4} \sqrt{\frac{A_{\text{mol,q}}}{\hbar \nu}} \frac{E_x E_R X_s e^{i(\chi - \chi_s)}}{E_R + E_N} ; \quad \frac{1}{4} \sqrt{\frac{A_{\text{mol,q}}}{\hbar \nu}} \frac{E_N}{E_R + E_N} \approx 0 \]

Substituting back into the signal and noise terms leaves only the signal term:

\[
S = \frac{1}{4} \sqrt{\frac{A_{\text{mol,q}}}{\hbar \nu}} \frac{E_x X_s e^{i(\chi - \chi_s)}}{E_R + E_N} \left[ Ae^{i\alpha} \sum_{k=0}^{3} \sqrt{\eta_k E_{C_k}^S} + Ae^{-i\alpha} e^{-j(\phi - \zeta)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k E_{C_k}^S} \right] + \sum_{k=0}^{3} j^k \frac{i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}}{\sqrt{R_k}} \]

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Since the amplitude of the fixed pattern noise and aberrations within the system are ideally equal to 1:

\[ X = 1 + \delta \quad ; \quad E_C = 1 + \Delta \]

\[
S = \frac{1}{2} \sqrt{ \frac{A_{\text{det}}}{hv} } E_S (1 + \delta_s) e^{i(\chi_s - \chi_e)} \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta^S_k) \left[ A e^{j\alpha} + A e^{-j\alpha} e^{-j(\chi_e - \chi_s)} \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} (1 + \Delta^S_k) \right] + \sum_{k=0}^{3} i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k} \]

Let:

\[
Y = \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta^S_k) \quad \text{and} \quad Z = \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} (1 + \Delta^S_k) \quad \text{and} \quad I_{BM,\text{Norm}} = \sum_{k=0}^{3} j^k i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k} \]

\[
S = \frac{1}{2} \sqrt{ \frac{A_{\text{det}}}{hv} } E_S (1 + \delta_s) Y \left[ A e^{j\alpha} + A e^{-j\alpha} \frac{Z}{Y} e^{-j(\chi_e - \chi_s)} \right] + I_{BM,\text{Norm}}
\]

As shown on pages 187-188:

\[
\sqrt{R} \cdot \sqrt{BS} = \frac{1}{4} E_{c_x}^S E_{c_y}^R (1 + \delta_s) (1 + \delta_r) E_R E_S \frac{A_{\text{det}}}{hv} \eta_k
\]

\[
\sqrt{BS} = \frac{1}{2} E_{c_x}^S (1 + \delta_s) E_S \sqrt{ \frac{A_{\text{det}}}{hv} } \sqrt{\eta_k}
\]

Substituting:

\[
\frac{1}{2} \sqrt{ \frac{A_{\text{det}}}{hv} } E_S (1 + \delta_s) Y = \sum_{k=0}^{3} \sqrt{BS_k}
\]

\[
\frac{1}{2} \sqrt{BS} \sum_{k=0}^{3} (-1)^k \sqrt{BS_k} \approx \frac{1}{2} \sqrt{ \frac{A_{\text{det}}}{hv} } E_S (1 + \delta_s) \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} (1 + \Delta^S_k)
\]

\[
\frac{1}{2} \sqrt{BS} \sum_{k=0}^{3} \sqrt{BS_k} \approx \frac{1}{2} \sqrt{ \frac{A_{\text{det}}}{hv} } E_S (1 + \delta_s) \sum_{k=0}^{3} (-1)^k \sqrt{\eta_k} (1 + \Delta^S_k) = \frac{Z}{Y} = \Psi
\]

\[
S = \sum_{k=0}^{3} \sqrt{BS_k} \cdot e^{i(\chi_e - \chi_s)} \left[ A e^{j\alpha} + A e^{-j\alpha} \Psi e^{-j(\chi_e - \chi_s)} \right] + I_{BM,\text{Norm}}
\]
Complex Field Acquired by Balanced Mixing, DC Term Subtraction, Camera Normalization, and No Blank

\[ E_{BM,DC,Norm,NB} = \left[ Ae^{j\alpha} + Ae^{-j\alpha} \Psi e^{-j2(\chi_s - \chi_r)} \right] e^{j(\chi_s - \chi_r)} \sum_{k=0}^{3} \sqrt{BS_k} + I_{BM,Norm} \]

Where:

\[ I_{BM,Norm} = \sum_{k=0}^{3} \left( \frac{i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}}{\sqrt{R_k}} \right) \]

\[ \Psi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{BS_k}}{\sum_{k=0}^{3} \sqrt{BS_k}} \]

\( Ae^{j\alpha} \): Magnitude (A) and phase (\( \alpha \)) of the sample

\( \chi_s \): Phase of aberrations induced within the signal arm

\( \chi_r \): Phase of aberrations induced within the reference arm

\( BS \): Image of the pure signal beam without the sample in the path

\( R \): Image of the pure reference beam

\( i \): Dark current noise in an acquired image
A.5 Balanced Mixing and DC Term Subtraction

Balanced Mixing and Subtraction of DC Terms (Sample):

\[ E_{BM,DC,sample} = \sum_{k=0}^{3} j^k (M_{D,k} - R_{D,k} - S_{D,k}) = \]

\[ \left[ \frac{1}{4} \sum_{k=0}^{3} A_{pixel} \left( Ae^{-j\theta} (E_S E_R^* + E_S E_N e^{j(\phi-\zeta)} + E_N E_N^* e^{-j(\phi-\zeta)} + E_N E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \right. \right. \]

\[ \left. \left. Ae^{-j\theta} (E_R E_S^* + E_R E_N^* e^{j(\phi-\zeta)} + E_N E_S^* e^{-j(\phi-\zeta)} + E_N E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \right. \right. \]

\[ \left. \left. (i_{M,0} - i_{D,0} - i_{S,0} - i_{D,0}) \right] + \right. \]

\[ \left[ \frac{1}{4} \sum_{k=0}^{3} A_{pixel} \left( Ae^{-j\theta} (E_S E_R^* + E_S E_N e^{j(\phi-\zeta)} + E_N E_N^* e^{-j(\phi-\zeta)} + E_N E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \right. \right. \]

\[ \left. \left. Ae^{-j\theta} (E_R E_S^* + E_R E_N^* e^{j(\phi-\zeta)} + E_N E_S^* e^{-j(\phi-\zeta)} + E_N E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \right. \right. \]

\[ \left. \left. j((i_{M,1} - i_{D,1}) - (i_{R,1} - i_{D,1}) - (i_{S,1} - i_{D,1})) \right] + \right. \]

\[ \left[ \frac{1}{4} \sum_{k=0}^{3} A_{pixel} \left( Ae^{-j\theta} (E_S E_R^* + E_S E_N e^{j(\phi-\zeta)} + E_N E_N^* e^{-j(\phi-\zeta)} + E_N E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \right. \right. \]

\[ \left. \left. Ae^{-j\theta} (E_R E_S^* + E_R E_N^* e^{j(\phi-\zeta)} + E_N E_S^* e^{-j(\phi-\zeta)} + E_N E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \right. \right. \]

\[ \left. \left. (i_{M,2} - i_{D,2}) - (i_{R,2} - i_{D,2}) - (i_{S,2} - i_{D,2}) \right] + \right. \]

\[ \left[ \frac{1}{4} \sum_{k=0}^{3} A_{pixel} \left( Ae^{-j\theta} (E_S E_R^* + E_S E_N e^{j(\phi-\zeta)} + E_N E_N^* e^{-j(\phi-\zeta)} + E_N E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \right. \right. \]

\[ \left. \left. Ae^{-j\theta} (E_R E_S^* + E_R E_N^* e^{j(\phi-\zeta)} + E_N E_S^* e^{-j(\phi-\zeta)} + E_N E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \right. \right. \]

\[ \left. \left. - j((i_{M,3} - i_{D,3}) - (i_{R,3} - i_{D,3}) - (i_{S,3} - i_{D,3})) \right] \right. \]

\[ \frac{1}{4} A_{pixel} \left[ \left( Ae^{-j\theta} (E_S E_R^* + E_S E_N e^{j(\phi-\zeta)} + E_N E_N^* e^{-j(\phi-\zeta)} + E_N E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} \right) \right. \]

\[ \left. \left. (\eta_0 E_S^* E_R^* + \eta_E E_R^* E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \eta_1 E_S^* E_R^* E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} \right) \right. \]

\[ \left. \left. + \eta_2 E_S^* E_R^* E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} + \eta_3 E_S^* E_R^* E_N^* \sum_k D_{k,s} X_k X_k^* e^{j(\xi-X) + \xi)} \right) \right. \]

\[ \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \]}
Separate into Signal and Noise Terms:

\[
S = \frac{1}{4 \pi \mu_0} \left[ (AE^{\prime\prime + A} (E_S E_R^*) X_s X_s^* e^{i(k - x)} (\eta_0 E_{c_0}^S E_{c_0}^R + \eta_1 E_{c_1}^S E_{c_1}^R + \eta_2 E_{c_2}^S E_{c_2}^R + \eta_3 E_{c_3}^S E_{c_3}^R) + \\
AE^{-i\alpha} (E_S E_R^*) X_s X_s^* e^{-i(k - x)} (\eta_0 E_{c_0}^S E_{c_0}^R - \eta_1 E_{c_1}^S E_{c_1}^R + \eta_2 E_{c_2}^S E_{c_2}^R - \eta_3 E_{c_3}^S E_{c_3}^R) \right] + \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})
\]

\[
N_S = \frac{1}{4 \pi \mu_0} \left[ (AE^{\prime\prime + A} (E_S e^{i(\phi - \zeta)} + E_N e^{i(\phi - \zeta)} + E_N^* X_s X_s^* e^{i(k - x)} (\eta_0 E_{c_0}^S E_{c_0}^R + \eta_1 E_{c_1}^S E_{c_1}^R + \eta_2 E_{c_2}^S E_{c_2}^R + \eta_3 E_{c_3}^S E_{c_3}^R) + \\
AE^{-i\alpha} (E_S e^{i(\phi - \zeta)} + E_N e^{i(\phi - \zeta)} + E_N^* X_s X_s^* e^{-i(k - x)} (\eta_0 E_{c_0}^S E_{c_0}^R - \eta_1 E_{c_1}^S E_{c_1}^R + \eta_2 E_{c_2}^S E_{c_2}^R - \eta_3 E_{c_3}^S E_{c_3}^R) \right)
\]

Balanced Mixing and Subtraction of DC Terms (Blank):

\[
E_{BM,DC,blank} = \sum_{k=0}^{3} j^k (BM_{D,k} - BR_{D,k} - BS_{D,k}) =
\]

\[
\left[ \frac{1}{2 \pi} A_{pixel} ((E_S^* + E_S^* e^{i(\phi - \beta)}) + E_R^* e^{-i(\phi - \beta)} + E_S^* E_R^*) e_{c_0}^S e_{c_0}^R X_s X_s^* e^{i(k - x)} + \\
(E_S^* + E_R^* e^{i(\phi - \beta)} + E_S^* e^{-i(\phi - \beta)} + E_R^* E_S^*) e_{c_0}^S e_{c_0}^R X_s X_s^* e^{-i(k - x)}) + \\
((i_{BM,0} - i_{D,0}) - (i_{BR,0} - i_{D,0}) - (i_{BS,0} - i_{D,0})) \right]
\]

\[
\left[ \frac{1}{2 \pi} A_{pixel} ((E_S^* + E_S^* e^{i(\phi - \beta)}) + E_R^* e^{-i(\phi - \beta)} + E_S^* E_R^*) e_{c_0}^S e_{c_0}^R X_s X_s^* e^{i(k - x)} + \\
(-E_S^* - E_S^* e^{i(\phi - \beta)} - E_R^* e^{-i(\phi - \beta)} - E_S^* E_R^*) e_{c_0}^S e_{c_0}^R X_s X_s^* e^{-i(k - x)}) + \\
j((i_{BM,1} - i_{D,1}) - (i_{BR,1} - i_{D,1}) - (i_{BS,1} - i_{D,1})) \right]
\]

\[
\left[ \frac{1}{2 \pi} A_{pixel} ((E_S^* + E_S^* e^{i(\phi - \beta)}) + E_R^* e^{-i(\phi - \beta)} + E_S^* E_R^*) e_{c_0}^S e_{c_0}^R X_s X_s^* e^{i(k - x)} + \\
(E_S^* + E_R^* e^{i(\phi - \beta)} + E_S^* e^{-i(\phi - \beta)} + E_R^* E_S^*) e_{c_0}^S e_{c_0}^R X_s X_s^* e^{-i(k - x)}) + \\
-((i_{BM,2} - i_{D,2}) - (i_{BR,2} - i_{D,2}) - (i_{BS,2} - i_{D,2})) \right]
\]

\[
\left[ \frac{1}{2 \pi} A_{pixel} ((E_S^* + E_S^* e^{i(\phi - \beta)}) + E_R^* e^{-i(\phi - \beta)} + E_S^* E_R^*) e_{c_0}^S e_{c_0}^R X_s X_s^* e^{i(k - x)} + \\
(-E_S^* - E_S^* e^{i(\phi - \beta)} - E_R^* e^{-i(\phi - \beta)} - E_S^* E_R^*) e_{c_0}^S e_{c_0}^R X_s X_s^* e^{-i(k - x)}) + \\
j((i_{BM,3} - i_{D,3}) - (i_{BR,3} - i_{D,3}) - (i_{BS,3} - i_{D,3})) \right]
\]
\[ \frac{1}{4 \mu_0 \hbar} \left[ (E_x E^*_y + E_y E^*_x) + E_x E^*_y e^{i(\theta - \phi)} + E_y E^*_x e^{-i(\theta - \phi)} \right] X_x X^*_y e^{i(X - X')} \cdot \left( \eta_0 E^*_c \eta^*_o + \eta_1 E^*_c \eta^*_1 + \eta_2 E^*_c \eta^*_2 + \eta_N E^*_c \eta^*_N \right) + \\
\frac{1}{4 \mu_0 \hbar} \left[ (E_x E^*_y + E_y E^*_x) + E_x E^*_y e^{i(\theta - \phi)} + E_y E^*_x e^{-i(\theta - \phi)} \right] X_x X^*_y e^{i(X - X')} \cdot \left( \eta_0 E^*_c \eta^*_o + \eta_1 E^*_c \eta^*_1 + \eta_2 E^*_c \eta^*_2 + \eta_N E^*_c \eta^*_N \right) + \\
\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k}) \]

Separate into Signal and Noise Terms:

\[ B = \frac{1}{4 \mu_0 \hbar} \left[ (E_x E^*_y) X_x X^*_y e^{i(X - X')} \cdot \left( \eta_0 E^*_c \eta^*_o + \eta_1 E^*_c \eta^*_1 + \eta_2 E^*_c \eta^*_2 + \eta_N E^*_c \eta^*_N \right) + \\
(E_x E^*_y) X_x X^*_y e^{i(X - X')} \cdot \left( \eta_0 E^*_c \eta^*_o - \eta_1 E^*_c \eta^*_1 + \eta_2 E^*_c \eta^*_2 - \eta_N E^*_c \eta^*_N \right) + \\
\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k}) \] 

Divide the image of the sample by the blank:

\[ \frac{S + N_S}{B + N_B} \approx \frac{S}{B} \left(1 + \frac{N_S}{B} \right) = \frac{S}{B} \left(1 + \frac{N_S}{B} - \frac{N_B N_S}{B^2} \right) = \frac{S}{B} + \frac{N_S}{B} - \frac{N_B S}{B^2} \]

Since laser fluctuations are small compared to laser output:

\[ \frac{N_S}{B} \approx \frac{N_B S}{B^2} \approx 0 \]
\[
S = \frac{A e^{i \alpha} (E S E_R^*) X_S X_r e^{i (x R - x_S)} (\eta_0 E_{C_0}^s E_{C_1}^{R^*} + \eta_1 E_{C_1}^s E_{C_1}^{R^*} + \eta_2 E_{C_2}^s E_{C_1}^{R^*} + \eta_3 E_{C_3}^s E_{C_1}^{R^*}) +}{B (E S E_R^*) X_S X_r e^{i (x R - x_S)} (\eta_0 E_{C_0}^s E_{C_0}^{R^*} + \eta_1 E_{C_1}^s E_{C_1}^{R^*} + \eta_2 E_{C_2}^s E_{C_2}^{R^*} + \eta_0 E_{C_1}^s E_{C_2}^{R^*}) +}
\]

\[
\begin{align*}
S &= (E R E_S^*) X_S X_r e^{i (x R - x_S)} (\eta_0 E_{C_0}^s E_{C_0}^{R^*} - \eta_1 E_{C_1}^s E_{C_1}^{R^*} + \eta_2 E_{C_2}^s E_{C_1}^{R^*} - \eta_3 E_{C_3}^s E_{C_1}^{R^*}) + 4 \frac{\hbar \nu}{\eta_{\text{morn}}} \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \\
&= (E R E_S^*) X_S X_r e^{i (x R - x_S)} (\eta_0 E_{C_0}^s E_{C_0}^{R^*} - \eta_1 E_{C_1}^s E_{C_1}^{R^*} + \eta_2 E_{C_2}^s E_{C_1}^{R^*} - \eta_3 E_{C_3}^s E_{C_1}^{R^*}) + 4 \frac{\hbar \nu}{\eta_{\text{morn}}} \sum_{k=0}^{3} j^k (i_{B,m,k} - i_{R,B,k} - i_{S,B,k} + i_{D,k})
\end{align*}
\]

Substitute Summations:

\[
\eta_0 E_{C_0}^s E_{C_0}^{R^*} + \eta_1 E_{C_1}^s E_{C_1}^{R^*} + \eta_2 E_{C_2}^s E_{C_2}^{R^*} + \eta_3 E_{C_3}^s E_{C_3}^{R^*} = \sum_{k=0}^{3} \eta_k E_{C_k}^s E_{C_k}^{R^*}
\]

\[
\begin{align*}
\eta_0 E_{C_0}^s E_{C_0}^{R^*} - \eta_1 E_{C_1}^s E_{C_1}^{R^*} + \eta_2 E_{C_2}^s E_{C_2}^{R^*} - \eta_3 E_{C_3}^s E_{C_3}^{R^*} &= \sum_{k=0}^{3} (1)^k \eta_k E_{C_k}^s E_{C_k}^{R^*} \\
&= \sum_{k=0}^{3} \eta_k E_{C_k}^s E_{C_k}^{R^*}
\end{align*}
\]

Assume All Amplitudes Are Real:

\[
\begin{align*}
S &= (E S E_R^*) X_S X_r e^{i (x R - x_S)} \sum_{k=0}^{3} \eta_k E_{C_k}^s E_{C_k}^{R^*} + A e^{-i \alpha} (E S E^*) X_S X_r e^{i (x R - x_S)} \sum_{k=0}^{3} (1)^k \eta_k E_{C_k}^s E_{C_k}^{R^*} + 4 \frac{\hbar \nu}{\eta_{\text{morn}}} \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \\
&= (E S E_R^*) X_S X_r e^{i (x R - x_S)} \sum_{k=0}^{3} \eta_k E_{C_k}^s E_{C_k}^{R^*} + (E R E_S^*) X_S X_r e^{i (x R - x_S)} \sum_{k=0}^{3} (1)^k \eta_k E_{C_k}^s E_{C_k}^{R^*} + 4 \frac{\hbar \nu}{\eta_{\text{morn}}} \sum_{k=0}^{3} j^k (i_{B,m,k} - i_{R,B,k} - i_{S,B,k} + i_{D,k})
\end{align*}
\]

Divide Numerator and Denominator by:

\[
\begin{align*}
&= \frac{A e^{i \alpha} + A e^{-i \alpha} e^{-i 2 (x R - x_S)} \sum_{k=0}^{3} (1)^k \eta_k E_{C_k}^s E_{C_k}^{R^*}}{1 + e^{-i 2 (x R - x_S)} \sum_{k=0}^{3} (1)^k \eta_k E_{C_k}^s E_{C_k}^{R^*}} + e^{-i (x R - x_S)} \sum_{k=0}^{3} \frac{j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})}{4 \frac{\hbar \nu}{\eta_{\text{morn}}} (E S E_R^*) X_S X_r} + e^{-i (x R - x_S)} \sum_{k=0}^{3} \frac{j^k (i_{B,m,k} - i_{R,B,k} - i_{S,B,k} + i_{D,k})}{4 \frac{\hbar \nu}{\eta_{\text{morn}}} (E R E_S^*) X_S X_r}
\end{align*}
\]
Substitute Taylor Series:

\[
\frac{S}{B} = \left( A e^{i\alpha} + A e^{-i\alpha} e^{-j2(x-x_c)} \right) \sum_{k=0}^{3} \left( \sum_{l=0}^{3} (-1)^l \eta_k E_{C_k}^R E_{C_k}^S \right) \left( \sum_{l=0}^{3} \frac{j^k}{l!} \left( i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k} \right) \right) + \left( A e^{i\alpha} \right) \left( e^{-j2(x-x_c)} \right) \sum_{k=0}^{3} \left( \sum_{l=0}^{3} (-1)^l \eta_k E_{C_k}^R E_{C_k}^S \right) \left( \sum_{l=0}^{3} \frac{j^k}{l!} \left( i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k} \right) \right) + \left( A e^{-i\alpha} \right) \left( e^{-j2(x-x_c)} \right) \sum_{k=0}^{3} \left( \sum_{l=0}^{3} (-1)^l \eta_k E_{C_k}^R E_{C_k}^S \right) \left( \sum_{l=0}^{3} \frac{j^k}{l!} \left( i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k} \right) \right) + \left( e^{-j(x-x_c)} \right) \sum_{k=0}^{3} \left( \sum_{l=0}^{3} (-1)^l \eta_k E_{C_k}^R E_{C_k}^S \right) \left( \sum_{l=0}^{3} \frac{j^k}{l!} \left( i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k} \right) \right)
\]
Ideally, the amplitude of the aberrations is equal to 1, therefore substitute: \( E_C = 1 + \Delta \); \( X = 1 + \delta \)

\[
\begin{align*}
\mathbf{A} e^{j\alpha} \left[ 1 - e^{-j 2(\chi_x - \chi_r)} \frac{\sum_{k=0}^{3} (-1)^k \Delta_k^s (1 + \Delta_k^s)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^s)(1 + \Delta_k^o)} - e^{-j 4(\chi_x - \chi_r)} \frac{\sum_{k=0}^{3} (-1)^k \Delta_k^o (1 + \Delta_k^o)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^o)(1 + \Delta_k^o)} \right] +
\end{align*}
\]

Multiply through and let squared noise terms \( (\Delta^2, \delta^2) \) equal zero:

\[
\begin{align*}
\mathbf{A} e^{j\alpha} \left[ 1 - e^{-j 2(\chi_x - \chi_r)} \frac{\sum_{k=0}^{3} (-1)^k \Delta_k^s (1 + \Delta_k^s)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^s)(1 + \Delta_k^o)} - e^{-j 4(\chi_x - \chi_r)} \frac{\sum_{k=0}^{3} (-1)^k \Delta_k^o (1 + \Delta_k^o)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^o)(1 + \Delta_k^o)} \right] +
\end{align*}
\]
Substituting:

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S)} \]

\[ I_S = \frac{\sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})}{\frac{1}{4} A_{\text{pinq}} E_S E_R (1 + \delta_s + \delta_r) \sum_{k=0}^{3} \eta_k (1 + \Delta_k^S + \Delta_k^R)} \]

\[ I_B = \frac{\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\frac{1}{4} A_{\text{pinq}} E_S E_R (1 + \delta_s + \delta_r) \sum_{k=0}^{3} \eta_k (1 + \Delta_k^S + \Delta_k^R)} \]

\[ Ae^{j\alpha} \left( 1 - \Phi e^{-j^2(x_s - x_r)} - I_B e^{-j(x_s - x_r)} \right) + Ae^{-j\alpha} \left( \Phi e^{-j^2(x_s - x_r)} - \Phi^2 e^{-j^4(x_s - x_r)} - I_B \Phi e^{-j^3(x_s - x_r)} \right) + I_S e^{-j(x_s - x_r)} \]

Assume noise terms squared equal zero:

\[ I_S I_B \approx 0; \quad I_S \Phi \approx 0; \quad I_B \Phi \approx 0 \]

\[ Ae^{j\alpha} \left( 1 - \Phi e^{-j^2(x_s - x_r)} - I_B e^{-j(x_s - x_r)} \right) + Ae^{-j\alpha} \left( \Phi e^{-j^2(x_s - x_r)} - \Phi^2 e^{-j^4(x_s - x_r)} \right) + I_S e^{-j(x_s - x_r)} \]

As shown on page 188:

\[ \sqrt{R} \cdot \sqrt{BS} = \frac{1}{4} E_S E_R E_{\text{pinq}} (1 + \delta_s + \delta_r) E_S E_R \frac{A_{\text{pinq}}}{h\nu} \eta_k \]

\[ I_S = \frac{\sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ I_B = \frac{\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S)} \]

\[ \approx \frac{1}{2} (1 + \delta_s + \delta_r) E_R E_S A_{\text{pinq}} \sum_{k=0}^{3} (-1) \eta_k (1 + \Delta_k^S + \Delta_k^R) \]

\[ \approx \frac{3}{3} \sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k} \]
Complex Field Reconstructed by Balanced Mixing and DC Term Subtraction

\[ E_{BM,DC} = A e^{ja} \left[ 1 - \Phi e^{-j2(\chi_s - \chi_r)} - I_B e^{-j(\chi_s - \chi_r)} \right] + A e^{-j\alpha} \left[ \Phi e^{-j2(\chi_s - \chi_r)} - \Phi^2 e^{-j4(\chi_s - \chi_r)} \right] + I_S e^{-j(\chi_s - \chi_r)} \]

Where:

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{BS_k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ I_S = \frac{\sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ I_B = \frac{\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

- \(A e^{ja}\): Magnitude (A) and phase (α) of the sample
- \(\chi_s\): Phase of aberrations induced within the signal arm
- \(\chi_r\): Phase of aberrations induced within the reference arm
- \(BS\): Image of the pure signal beam without the sample in the path
- \(R\): Image of the pure reference beam
- \(i\): Dark current noise in an acquired image
A.5.1 Include Wavefront Mismatch Between Sample and Blank Images

Substitute: \( \beta_1 = \chi_s - \chi_r \) (Signal) \( \beta_2 = \chi_s - \chi_r \) (Blank)

\[
S_B = \frac{\text{Ae}^{i\alpha}(E_S E_R) X_s X_r e^{i\beta_1} \sum_{k=0}^{1} \eta_k E_{C_k}^S E_{C_k}^R + \text{Ae}^{-i\alpha}(E_R E_S) X_s X_r e^{-i\beta_1} \sum_{k=0}^{1} (-1)^k \eta_k E_{C_k}^R E_{C_k}^S + 4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})}{(E_S E_R) X_s X_r e^{i\beta_2} \sum_{k=0}^{1} \eta_k E_{C_k}^S E_{C_k}^R + (E_R E_S) X_s X_r e^{-i\beta_2} \sum_{k=0}^{1} (-1)^k \eta_k E_{C_k}^R E_{C_k}^S + 4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})}
\]

Divide Numerator and Denominator by: \( (E_S E_R) X_s X_r e^{i\beta_2} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R \)

\[
S = \frac{\text{Ae}^{i\alpha} e^{j(\beta_1 - \beta_2)} + \text{Ae}^{-i\alpha} e^{-j(\beta_1 + \beta_2)} \sum_{k=0}^{3} (-1)^k \eta_k E_{C_k}^R E_{C_k}^S + e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_S E_R) X_s X_r} + e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_R E_S) X_s X_r} \sum_{k=0}^{3} \eta_k E_{C_k}^R E_{C_k}^S + e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_S E_R) X_s X_r} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{1 + e^{-j2\beta_2} \sum_{k=0}^{3} (-1)^k \eta_k E_{C_k}^S E_{C_k}^R + e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_S E_R) X_s X_r} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R + e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_R E_S) X_s X_r} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R} \}
\]

Substitute Taylor Series:

\[
S = \left\{ \frac{\text{Ae}^{i\alpha} e^{i(\beta_1 - \beta_2)} + \text{Ae}^{-i\alpha} e^{-i(\beta_1 + \beta_2)} \sum_{k=0}^{3} (-1)^k \eta_k E_{C_k}^R E_{C_k}^S + e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_S E_R) X_s X_r} + e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_R E_S) X_s X_r} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R + e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_S E_R) X_s X_r} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{1 - e^{-j2\beta_2} \sum_{k=0}^{3} (-1)^k \eta_k E_{C_k}^S E_{C_k}^R - e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_S E_R) X_s X_r} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R - e^{-j\beta_2} \frac{\sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R}{4 \frac{\text{E}^{\text{pixel}}}{\lambda \mu_0} (E_R E_S) X_s X_r} \sum_{k=0}^{3} \eta_k E_{C_k}^S E_{C_k}^R} \right\}.
\]
$S = \left( A e^{i\alpha} e^{i(\beta_1 - \beta_2)} + A e^{-i\alpha} e^{-j(\beta_1 + \beta_2)} \frac{\sum_{k=0}^{3} (-1)^k \eta_k E^R_{C_k} E^S_{C_k}}{\sum_{k=0}^{3} \eta_k E^S_{C_k} E^R_{C_k}} + e^{-j\beta_2} \frac{1}{\sum_{k=0}^{3} \eta_k E^S_{C_k} E^R_{C_k}} \right) \frac{1}{\frac{\sum_{k=0}^{3} \eta_k E^S_{C_k} E^R_{C_k}}{\sum_{k=0}^{3} \eta_k E^S_{C_k} E^R_{C_k}}}.$

$$\left( 1 - e^{-j2\beta_2} \frac{1}{\frac{3}{\sum_{k=0}^{3} \eta_k E^S_{C_k} E^R_{C_k}}} \right)\frac{1}{\frac{3}{\sum_{k=0}^{3} \eta_k E^S_{C_k} E^R_{C_k}}} - e^{-j2\beta_2} \frac{1}{\frac{3}{\sum_{k=0}^{3} \eta_k E^S_{C_k} E^R_{C_k}}} \right)$$

$$A e^{i\alpha} \left( e^{i(\beta_1 - \beta_2)} - e^{-j(\beta_1 + \beta_2)} \frac{\sum_{k=0}^{3} (-1)^k \eta_k E^R_{C_k} E^S_{C_k}}{\sum_{k=0}^{3} \eta_k E^S_{C_k} E^R_{C_k}} + e^{-j(2\beta_1 + \beta_2)} \frac{1}{\frac{3}{\sum_{k=0}^{3} \eta_k E^S_{C_k} E^R_{C_k}}} \right)$$

$$A e^{-j\alpha} \left( e^{-j(\beta_1 + \beta_2)} - e^{-j(3\beta_1 - \beta_2)} \frac{\sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta^R_k) (1 + \Delta^S_k)}{\sum_{k=0}^{3} \eta_k (1 + \Delta^S_k) (1 + \Delta^R_k)} \right)$$

Ideally, the amplitude of the aberrations is equal to 1, therefore substitute: $E_C = 1 + \Delta$ ; $X = 1 + \delta$

$$A e^{i\alpha} \left( e^{i(\beta_1 - \beta_2)} - e^{-j(3\beta_1 - \beta_2)} \frac{\sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta^R_k) (1 + \Delta^S_k)}{\sum_{k=0}^{3} \eta_k (1 + \Delta^S_k) (1 + \Delta^R_k)} + e^{-j(2\beta_1 + \beta_2)} \frac{1}{\frac{3}{\sum_{k=0}^{3} \eta_k (1 + \Delta^S_k) (1 + \Delta^R_k)}} \right)$$

$$A e^{-j\alpha} \left( e^{-j(\beta_1 + \beta_2)} - e^{-j(3\beta_1 + \beta_2)} \frac{\sum_{k=0}^{3} (1)^k \eta_k (1 + \Delta^S_k) (1 + \Delta^R_k)}{\sum_{k=0}^{3} \eta_k (1 + \Delta^S_k) (1 + \Delta^R_k)} \right)$$

$$\left( e^{-j2\beta_2} \frac{1}{\frac{3}{\sum_{k=0}^{3} \eta_k (1 + \Delta^S_k) (1 + \Delta^R_k)}} \right)$$

$$\left( 1 - e^{-j2\beta_2} \frac{1}{\frac{3}{\sum_{k=0}^{3} \eta_k (1 + \Delta^S_k) (1 + \Delta^R_k)}} \right)$$

$$\left( e^{-j2\beta_2} \frac{1}{\frac{3}{\sum_{k=0}^{3} \eta_k (1 + \Delta^S_k) (1 + \Delta^R_k)}} \right)$$

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Multiply through and let squared noise terms \((\Delta^2, \delta^2)\) equal zero:

\[
\begin{align*}
\mathcal{A} e^{j\alpha} & \begin{pmatrix} e^{i(\beta_1 - \beta_2)} - e^{-j(3\beta_2 - \beta_1)} \frac{\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\sum_{k=0}^{3} j^k (\Delta_{BM,k} - \Delta_{BR,k} - \Delta_{BS,k} + \Delta_{D,k})} & \frac{1}{4} \frac{\Delta_{\max}}{h^2} \frac{1}{E_s} E_R (1 + \delta_x + \delta_y) \sum_{k=0}^{3} \eta_k (1 + \Delta_{BM,k}^x + \Delta_{BM,k}^y) \end{pmatrix} + \\
\mathcal{A} e^{-j\beta_2} & \begin{pmatrix} e^{-j\beta_2} - e^{j\beta_2} \frac{\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\sum_{k=0}^{3} j^k (\Delta_{BM,k} - \Delta_{BR,k} - \Delta_{BS,k} + \Delta_{D,k})} & \frac{1}{4} \frac{\Delta_{\max}}{h^2} \frac{1}{E_s} E_R (1 + \delta_x + \delta_y) \sum_{k=0}^{3} \eta_k (1 + \Delta_{BM,k}^x + \Delta_{BM,k}^y) \end{pmatrix}
\end{align*}
\]

Substituting: \(\Phi = \frac{\sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_{BM,k}^x + \Delta_{BM,k}^y)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_{BM,k}^x + \Delta_{BM,k}^y)}\)

\[
I_s = \frac{\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\frac{1}{4} \frac{\Delta_{\max}}{h^2} \frac{1}{E_s} E_R (1 + \delta_x + \delta_y) \sum_{k=0}^{3} \eta_k (1 + \Delta_{BM,k}^x + \Delta_{BM,k}^y)}
\]

\[
I_B = \frac{\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\frac{1}{4} \frac{\Delta_{\max}}{h^2} \frac{1}{E_s} E_R (1 + \delta_x + \delta_y) \sum_{k=0}^{3} \eta_k (1 + \Delta_{BM,k}^x + \Delta_{BM,k}^y)}
\]

\[
\mathcal{A} e^{j\alpha} \left( e^{j(\beta_1 - \beta_2)} - \Phi e^{-j(3\beta_2 - \beta_1)} - I_B e^{-j(2\beta_2 - \beta_1)} \right) + \mathcal{A} e^{-j\beta_2} \left( e^{-j\beta_2} - e^{j\beta_2} \right)
\]

Assume noise terms squared equal zero: \(I_s I_B \approx 0; \quad I_s \Phi \approx 0; \quad I_B \Phi \approx 0\)

\[
\mathcal{A} e^{j\alpha} \left( e^{j(\beta_1 - \beta_2)} - \Phi e^{-j(3\beta_2 - \beta_1)} - I_B e^{-j(2\beta_2 - \beta_1)} \right) + \mathcal{A} e^{-j\beta_2} \left( e^{-j\beta_2} - e^{j\beta_2} \right)
\]

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As shown on page 188:

\[
\sqrt{R} \cdot \sqrt{BS} = \frac{1}{4} E^S_C E^R_C (1 + \delta_s + \delta_r) E_R E_S \frac{\lambda_{\text{max}}}{\lambda_0} \eta_k
\]

\[
I_s = \frac{\sum_{k=0}^3 j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})}{\sum_{k=0}^3 \sqrt{R_k} \sqrt{BS_k}}
\]

\[
I_B = \frac{\sum_{k=0}^3 j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\sum_{k=0}^3 \sqrt{R_k} \sqrt{BS_k}}
\]

\[
\Phi = \frac{\sum_{k=0}^3 (-1)^k \eta_k (1 + \Delta^S_k + \Delta^R_k)}{\sum_{k=0}^3 \eta_k (1 + \Delta^S_k + \Delta^R_k)} = \frac{1}{\frac{1}{2} (1 + \delta_s + \delta_r) E_R E_S \frac{\lambda_{\text{max}}}{\lambda_0}} \sum_{k=0}^3 (-1) \eta_k (1 + \Delta^S_k + \Delta^R_k) \approx \frac{3}{\sum_{k=0}^3 \sqrt{R_k} \sqrt{BS_k}}
\]
Complex Field Reconstructed by Balanced Mixing and DC Term Subtraction
(assuming fringe pattern of sample image does not match fringe pattern of blank)

\[ E_{BM,DC} = A e^{j\alpha} \left[ e^{j(\beta_1 - \beta_2)} - \Phi e^{-j(3\beta_1 - \beta_3)} - I_B e^{-j(2\beta_2 - \beta_3)} \right] + A e^{-j\alpha} \left[ \Phi e^{-j(\beta_1 + \beta_2)} - \Phi^2 e^{-j(3\beta_1 + \beta_3)} \right] + I_s e^{-j\beta_2} \]

Where:

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{BS_k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ I_s = \frac{\sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ I_B = \frac{\sum_{k=0}^{3} j^k (i_{BM,k} - i_{BR,k} - i_{BS,k} + i_{D,k})}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ A e^{j\alpha} \]: Magnitude (A) and phase (\( \alpha \)) of the sample

\[ \beta_1 \]: Wavefront mismatch between signal and reference arms for image of sample

\[ \beta_2 \]: Wavefront mismatch between signal and reference arms for image of blank

\[ BS \]: Image of the pure signal beam without the sample in the path

\[ R \]: Image of the pure reference beam

\[ i \]: Dark current noise in an acquired image
A.5.2 Balanced Mixing, DC Term Subtraction, and No Blank

\[ E_{BM,DC,\text{sample}} = \sum_{k=0}^{3} j^k (M_{D,k} - R_{D,k} - S_{D,k}) = \]

\[
\frac{1}{4} A_{\text{pixel}} \left[ \begin{array}{c}
\eta_{0} E_{C_0}^S E_{C_0}^R + \eta_{1} E_{C_1}^S E_{C_1}^R + \eta_{2} E_{C_2}^S E_{C_2}^R + \eta_{3} E_{C_3}^S E_{C_3}^R \\
\eta_{0} E_{C_0}^S E_{C_0}^R - \eta_{1} E_{C_1}^S E_{C_1}^R - \eta_{2} E_{C_2}^S E_{C_2}^R - \eta_{3} E_{C_3}^S E_{C_3}^R \\
\end{array} \right] \]

Substitute Summation for Dark Current Terms:

\[
\frac{1}{4} A_{\text{pixel}} \sum_{k=0}^{3} j^k (M_{D,k} - R_{D,k} - S_{D,k})
\]
Substitute Summations for Efficiency Terms:

\[
\frac{1}{4\delta_{\mu q}} \left[ (Ae^{j\alpha} (E_x E_R^* + E_y E_N e^{j(\phi - \zeta)} + E_x E_y e^{-j(\phi - \zeta)} + E_N^*) X_x X_y e^{j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} + \\
Ae^{-j\alpha} (E_y E_R^* + E_y E_N e^{j(\phi - \zeta)} + E_x E_y e^{-j(\phi - \zeta)} + E_N^*) X_x X_y e^{-j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} + \\
\sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \right]
\]

Assume all amplitudes are real and:

\[
\frac{1}{4\delta_{\mu q}} \left[ (Ae^{j\alpha} (E_x E_R^* + E_y E_N e^{j(\phi - \zeta)} + E_x E_y e^{-j(\phi - \zeta)} + E_N^*) X_x X_y e^{j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} + \\
Ae^{-j\alpha} (E_y E_R^* + E_y E_N e^{j(\phi - \zeta)} + E_x E_y e^{-j(\phi - \zeta)} + E_N^*) X_x X_y e^{-j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} + \\
\sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \right]
\]

Assume noise terms squared equal zero:

\[
\frac{1}{4\delta_{\mu q}} \left[ (Ae^{j\alpha} (E_x E_R^* + E_y E_N e^{j(\phi - \zeta)} + E_x E_y e^{-j(\phi - \zeta)} + E_N^*) X_x X_y e^{j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} + \\
Ae^{-j\alpha} (E_y E_R^* + E_y E_N e^{j(\phi - \zeta)} + E_x E_y e^{-j(\phi - \zeta)} + E_N^*) X_x X_y e^{-j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} + \\
\sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \right]
\]

Separate signal terms and noise terms:

\[
S = \frac{1}{4\delta_{\mu q}} \left[ (Ae^{j\alpha} (E_x E_R) X_x X_y e^{j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} + Ae^{-j\alpha} (E_y E_R) X_x X_y e^{-j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} + \\
\sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \right]
\]

\[
N = \frac{1}{4\delta_{\mu q}} \left[ Ae^{j\alpha} (E_x E_N e^{j(\phi - \zeta)} + E_y E_R e^{-j(\phi - \zeta)}) X_x X_y e^{j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} + \\
Ae^{-j\alpha} (E_y E_N e^{j(\phi - \zeta)} + E_x E_R e^{-j(\phi - \zeta)}) X_x X_y e^{-j(x_r - x_r) \sum \eta_k E_{C_1}^S E_{C_1}^R} \right]
\]
\[ S = \frac{1}{4} \frac{\Delta}{ht} E_s X_s X_r e^{j(\chi_s - \chi_r)} \left[ A e^{j\alpha} \sum_{k=0}^{3} \eta_k e_{C_k}^R e_{C_k}^S + A e^{-j\alpha} e^{-j2(\chi_s - \chi_r)} \sum_{k=0}^{3} (-1)^k \eta_k e_{C_k}^R e_{C_k}^S \right] + \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \]

\[ N = \frac{1}{4} \frac{\Delta}{ht} E_n X_s X_r e^{j(\chi_s - \chi_r)} \left[ A e^{j\alpha} (E_s e^{j(\delta_s - \delta_r)} + E_r e^{-j(\delta_s - \delta_r)}) \sum_{k=0}^{3} \eta_k e_{C_k}^R e_{C_k}^S + \right. \]
\[ \left. A e^{-j\alpha} (E_r e^{j(\delta_s - \delta_r)} + E_s e^{-j(\delta_s - \delta_r)}) e^{-j2(\chi_s - \chi_r)} \sum_{k=0}^{3} (-1)^k \eta_k e_{C_k}^R e_{C_k}^S \right] \]

Since the amplitude of the fixed pattern noise and aberrations within the system are ideally equal to 1:

\[ X = 1 + \delta; \quad E_c = 1 + \Delta \]

\[ S = \frac{1}{4} \frac{\Delta}{ht} E_s X_s X_r (1 + \delta_s + \delta_r) e^{j(\chi_s - \chi_r)} \left[ A e^{j\alpha} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S) + A e^{-j\alpha} e^{-j2(\chi_s - \chi_r)} \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S) \right] + \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \]

\[ N = \frac{1}{4} \frac{\Delta}{ht} E_n X_s X_r (1 + \delta_s + \delta_r) e^{j(\chi_s - \chi_r)} \left[ A e^{j\alpha} (E_s e^{j(\delta_s - \delta_r)} + E_r e^{-j(\delta_s - \delta_r)}) \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S) + \right. \]
\[ \left. A e^{-j\alpha} (E_r e^{j(\delta_s - \delta_r)} + E_s e^{-j(\delta_s - \delta_r)}) e^{-j2(\chi_s - \chi_r)} \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S) \right] \]

Assume noise terms squared or multiplied by another noise term equal zero:

\[ S = \frac{1}{4} \frac{\Delta}{ht} E_s X_s X_r (1 + \delta_s + \delta_r) e^{j(\chi_s - \chi_r)} \left[ A e^{j\alpha} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S) + A e^{-j\alpha} e^{-j2(\chi_s - \chi_r)} \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S) \right] + \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \]

\[ N = \frac{1}{4} \frac{\Delta}{ht} E_n X_s X_r (1 + \delta_s + \delta_r) e^{j(\chi_s - \chi_r)} \left[ A e^{j\alpha} (E_s e^{j(\delta_s - \delta_r)} + E_r e^{-j(\delta_s - \delta_r)}) \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S) + \right. \]
\[ \left. A e^{-j\alpha} (E_r e^{j(\delta_s - \delta_r)} + E_s e^{-j(\delta_s - \delta_r)}) e^{-j2(\chi_s - \chi_r)} \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S) \right] \]
With a wavelength of 633nm and pixels that are 7μm on a side:
\[ \frac{A_{\text{pixel}} q}{\hbar \nu} = 2.5 \times 10^{-11} \text{ m}^2 \text{e}\]

Including the 1/4th creates an effective noise term:
\[ \frac{1}{4} \frac{A_{\text{pixel}} q}{\hbar \nu} = 6.3 \times 10^{-12} \text{ m}^2 \text{e} \]

Thereby allowing us to assume:
\[ \frac{1}{4} \frac{A_{\text{pixel}} q}{\hbar \nu} E_N \approx 0 \Rightarrow N \approx 0 \]

Substituting leaves only the signal term:
\[
S = \frac{1}{4} \frac{A_{\text{pixel}} q}{\hbar \nu} E_S E_R (1 + \delta_x + \delta_y) e^{j(x-x_s)} \left[ A e^{j\alpha} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S) + A e^{-j\alpha} e^{-j2(x-x_s)} \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S) \right] + \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})
\]

Rearrange terms:
\[
S = \frac{1}{4} \frac{A_{\text{pixel}} q}{\hbar \nu} E_S E_R (1 + \delta_x + \delta_y) \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S) \left[ A e^{j\alpha} + A e^{-j\alpha} e^{-j2(x-x_s)} \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S) \right] + \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})
\]

As shown on page 188:
\[
\sqrt{R} \cdot \sqrt{BS} = \frac{1}{4} E_C^S E_C^R (1 + \delta_x)(1 + \delta_y) E_R E_S \frac{A_{\text{pixel}} q}{\hbar \nu} \eta_k
\]

\[
\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k} = \frac{1}{4} \frac{A_{\text{pixel}} q}{\hbar \nu} E_S E_R (1 + \delta_x + \delta_y) \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S)
\]

\[
\sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{BS_k} \approx \frac{1}{4} \frac{A_{\text{pixel}} q}{\hbar \nu} E_S E_R (1 + \delta_x + \delta_y) \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S) \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S)
\]

Let:
\[
\Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{BS_k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}}; \quad I_{BM} = \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k})
\]

\[
S = \sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k} e^{j(x-x_s)} \left[ A e^{j\alpha} + A e^{-j\alpha} \Phi e^{-j2(x-x_s)} \right] + I_{BM}
\]


Complex Field Acquired by Balanced Mixing, DC Term Subtraction, and No Blank

\[ E_{BM,DC,NB} = \sum_{k=0}^{3} \sqrt{R_k} \sqrt{B S_k} \cdot e^{j(\chi_r - \chi_s)} \left[ A e^{j\alpha} + A e^{-j\alpha} \Phi e^{-j2(\chi_r - \chi_s)} \right] + I_{BM} \]

Where:

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{B S_k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{B S_k}} \]

\[ I_{BM} = \sum_{k=0}^{3} j^k (i_{M,k} - i_{R,k} - i_{S,k} + i_{D,k}) \]

\( A e^{j\alpha} \): Magnitude (A) and phase (\( \alpha \)) of the sample

\( \chi_s \): Phase of aberrations induced within the signal arm

\( \chi_r \): Phase of aberrations induced within the reference arm

\( BS \): Image of the pure signal beam without the sample in the path

\( R \): Image of the pure reference beam

\( i \): Dark current noise in an acquired image
Balanced Mixing with Image of Sample:

\[
E_{BM,\text{sample}} = \frac{1}{4} \lambda_{w0} \left( A e^{i \alpha} (E_R e^R + E_N e^R) e^{i(\phi - \chi)} + E_N e^R e^{-i(\phi - \chi)} + E_R e^N e^{i(\chi - \tau)} + E_N e^N e^{-i(\chi - \tau)} \right). 
\]

\[
(\eta_0 E^S_{C0} e^R + \eta_1 E^S_{C1} e^R + \eta_2 E^S_{C2} e^R + \eta_3 E^S_{C3} e^R) + \frac{A^2 (E_R e^R + E_N e^R) X, X, \cdot (\eta_0 E^S_{C0} e^R + \eta_1 E^S_{C1} e^R + \eta_2 E^S_{C2} e^R + \eta_3 E^S_{C3} e^R)}{(i M_0 - i M_2 + j(i M_1 - i M_3))} 
\]

Assume all amplitudes are real and separate signal and noise terms (put dark current terms in signal so 2nd and 3rd terms of Taylor Series for signal/blank will still go to zero by multiplying by \( \frac{E_R e^R}{E_N e^R} \)):

\[
S = \frac{1}{4} \lambda_{w0} \left( A e^{i \alpha} (E_S e^R) X, X, e^{i(\chi - \tau)} \right) (\eta_0 E^S_{C0} e^R + \eta_1 E^S_{C1} e^R + \eta_2 E^S_{C2} e^R + \eta_3 E^S_{C3} e^R) + \frac{A^2 (E_S e^R e^{i(\phi - \chi)} + E_N e^R e^{-i(\phi - \chi)} + E_N e^R) X, X, \cdot (\eta_0 E^S_{C0} e^R + \eta_1 E^S_{C1} e^R + \eta_2 E^S_{C2} e^R + \eta_3 E^S_{C3} e^R)}{(i M_0 - i M_2 + j(i M_1 - i M_3))} 
\]

\[
N_S = \frac{1}{4} \lambda_{w0} \left( A e^{i \alpha} (E_S e^R e^{i(\phi - \chi)} + E_N e^R e^{-i(\phi - \chi)} + E_N e^R) X, X, e^{i(\chi - \tau)} \right) (\eta_0 E^S_{C0} e^R + \eta_1 E^S_{C1} e^R + \eta_2 E^S_{C2} e^R + \eta_3 E^S_{C3} e^R) + \frac{A^2 (E_S e^R e^{i(\phi - \chi)} + E_N e^R e^{-i(\phi - \chi)} + E_N e^R) X, X, \cdot (\eta_0 E^S_{C0} e^R + \eta_1 E^S_{C1} e^R + \eta_2 E^S_{C2} e^R + \eta_3 E^S_{C3} e^R)}{(i M_0 - i M_2 + j(i M_1 - i M_3))} 
\]

Let \( E^R_{C^0} = 1 + \Delta^R \) and \( E^S_{C^0} = 1 + \Delta^S \):

\[
(\eta_0 E^S_{C0} e^R + \eta_1 E^S_{C1} e^R + \eta_2 E^S_{C2} e^R + \eta_3 E^S_{C3} e^R) = \eta_0 (1 + \Delta^S_0 + \Delta^R_0 + \Delta^R_0 \Delta^S_0) + \eta_1 (1 + \Delta^R_1 + \Delta^R_1 \Delta^S_1) + \eta_2 (1 + \Delta^R_2 + \Delta^R_2 \Delta^S_2) + \eta_3 (1 + \Delta^R_3 + \Delta^R_3 \Delta^S_3) 
\]

\[
\sum_{k=0}^{3} \eta_k (1 + \Delta^R_k + \Delta^R_k \Delta^S_k) 
\]

\[
\eta_0 E^R_{C0} e^R - \eta_1 E^R_{C1} e^R + \eta_2 E^R_{C2} e^R - \eta_3 E^R_{C3} e^R = \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta^R_k + \Delta^R_k \Delta^S_k) 
\]

\[
\eta_0 E^S_{C0} e^S + \eta_1 E^S_{C1} e^S - \eta_2 E^S_{C2} e^S - \eta_3 E^S_{C3} e^S = \sum_{k=0}^{3} (j)^k \eta_k (1 + 2 \Delta^S_k + \Delta^S_k \Delta^R_k) 
\]

\[
\eta_0 E^R_{C0} e^R + \eta_1 E^R_{C1} e^R - \eta_2 E^R_{C2} e^R - \eta_3 E^R_{C3} e^R = \sum_{k=0}^{3} (j)^k \eta_k (1 + 2 \Delta^R_k + \Delta^R_k \Delta^S_k) 
\]

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Substituting:
\[ S = \frac{1}{\pi} \sum_{k=0}^{3} \eta_k (1 + \Delta^R_k + \Delta^S_k + \Delta^R_k \Delta^S_k) + \]
\[ \operatorname{Re} \left[ E_x E_y e^{j(\theta - \varphi)} + E_x E_y e^{-j(\varphi - \theta)} + E_x E_y e^{-j(\theta - \varphi)} + E_x E_y e^{j(\varphi - \theta)} \right] \]
\[ \operatorname{Re} \left[ E_x e^{j(\theta - \varphi)} + E_x e^{-j(\varphi - \theta)} \right] X_x X_x^* e^{j(x - z_r)} \]

Balanced Mixing with Image of Blank:
\[ E_{BM, blank} = \frac{1}{4} \sum_{k=0}^{3} \left( (E_x E_y^* + E_y E_x^*) e^{j(\varphi - \theta)} + (E_x E_y^* - E_y E_x^*) e^{-j(\varphi - \theta)} + (E_x^* E_y + E_y^* E_x) e^{j(\varphi - \theta)} + (E_x^* E_y - E_y^* E_x) e^{-j(\varphi - \theta)} \right) \]
\[ \left( \eta_1 E_{c_1}^S E_{c_0}^R + \eta_1 E_{c_1}^S E_{c_1}^S + \eta_2 E_{c_2}^S E_{c_2}^R + \eta_2 E_{c_2}^S E_{c_1}^R \right) + \]
\[ (E_x^* E_y + E_y^* E_x) X_x X_x^* \left( \eta_1 E_{c_1}^S E_{c_0}^S + \eta_1 E_{c_1}^S E_{c_1}^S + \eta_2 E_{c_2}^S E_{c_2}^R + \eta_2 E_{c_2}^S E_{c_1}^R \right) \]
\[ \left( \eta_0 E_{c_0}^R E_{c_0}^S + \eta_0 E_{c_0}^R E_{c_1}^S + \eta_0 E_{c_0}^R E_{c_2}^S - \eta_0 E_{c_0}^R E_{c_3}^S \right) + \]
\[ \left( i_{BM,0} - i_{BM,2} + j(i_{BM,1} - i_{BM,3}) \right) \]

Assume all amplitudes are real and separate signal and noise terms:
\[ B = \frac{1}{4} \sum_{k=0}^{3} \left( (E_x E_y + E_y E_x) e^{j(\varphi - \theta)} + (E_x E_y^* + E_y E_x^*) e^{-j(\varphi - \theta)} + (E_x^* E_y + E_y^* E_x) e^{j(\varphi - \theta)} + (E_x^* E_y^* + E_y^* E_x^*) e^{-j(\varphi - \theta)} \right) \]
\[ \left( \eta_1 E_{c_1}^S E_{c_0}^R + \eta_1 E_{c_1}^S E_{c_1}^S + \eta_2 E_{c_2}^S E_{c_2}^R + \eta_2 E_{c_2}^S E_{c_1}^R \right) + \]
\[ (E_x^* E_y + E_y^* E_x) X_x X_x^* \left( \eta_1 E_{c_1}^S E_{c_0}^S + \eta_1 E_{c_1}^S E_{c_1}^S + \eta_2 E_{c_2}^S E_{c_2}^R + \eta_2 E_{c_2}^S E_{c_1}^R \right) \]
\[ \left( \eta_0 E_{c_0}^R E_{c_0}^S + \eta_0 E_{c_0}^R E_{c_1}^S + \eta_0 E_{c_0}^R E_{c_2}^S - \eta_0 E_{c_0}^R E_{c_3}^S \right) + \]
\[ \left( i_{BM,0} - i_{BM,2} + j(i_{BM,1} - i_{BM,3}) \right) \]

\[ N_B = \frac{1}{4} \sum_{k=0}^{3} \left( (E_x E_y + E_y E_x) e^{j(\varphi - \theta)} + (E_x E_y^* + E_y E_x^*) e^{-j(\varphi - \theta)} + (E_x^* E_y + E_y^* E_x) e^{j(\varphi - \theta)} + (E_x^* E_y^* + E_y^* E_x^*) e^{-j(\varphi - \theta)} \right) \]
\[ \left( \eta_1 E_{c_1}^S E_{c_0}^R + \eta_1 E_{c_1}^S E_{c_1}^S + \eta_2 E_{c_2}^S E_{c_2}^R + \eta_2 E_{c_2}^S E_{c_1}^R \right) + \]
\[ (E_x^* E_y + E_y^* E_x) X_x X_x^* \left( \eta_1 E_{c_1}^S E_{c_0}^S + \eta_1 E_{c_1}^S E_{c_1}^S + \eta_2 E_{c_2}^S E_{c_2}^R + \eta_2 E_{c_2}^S E_{c_1}^R \right) \]
\[ \left( \eta_0 E_{c_0}^R E_{c_0}^S + \eta_0 E_{c_0}^R E_{c_1}^S + \eta_0 E_{c_0}^R E_{c_2}^S - \eta_0 E_{c_0}^R E_{c_3}^S \right) + \]
\[ \left( i_{BM,0} - i_{BM,2} + j(i_{BM,1} - i_{BM,3}) \right) \]
Substituting summations:

\[
B = \frac{A}{\pi} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R + \Delta_k^S) + (E_k E_s) X_s X_s e^{i(\phi_s - \phi_t)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R + \Delta_k^S) + \\
(E_k E_s) X_s X_s \cdot \sum_{k=0}^{3} (j) \eta_k (1 + 2 \Delta_k^S + \Delta_k^2) + (E_k E_s) X_s X_s \cdot \sum_{k=0}^{3} (j) \eta_k (1 + 2 \Delta_k^S + \Delta_k^2) + \sum_{k=0}^{3} j^k i_{BM,k}
\]

\[
N_B = \frac{A}{\pi} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R + \Delta_k^S) + \\
(E_k E_s) X_s X_s e^{i(\phi_s - \phi_t)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R + \Delta_k^S) + \\
(E_k E_s) X_s X_s e^{i(\phi_s - \phi_t)} \cdot \sum_{k=0}^{3} (j) \eta_k (1 + 2 \Delta_k^S + \Delta_k^2) + \\
(E_k E_s) X_s X_s e^{i(\phi_s - \phi_t)} \cdot \sum_{k=0}^{3} (j) \eta_k (1 + 2 \Delta_k^S + \Delta_k^2)
\]

Divide the image of the sample by the blank:

\[
\frac{S + N_S}{B + N_B} \approx \frac{S}{B} (1 + \frac{N_S}{S}) (1 - \frac{N_B}{B}) = \frac{S}{B} (1 + \frac{N_S}{S} - \frac{N_B}{B} - \frac{N_B N_S}{S B}) = \frac{S}{B} + \frac{N_S}{B} - \frac{N_B S}{B^2}
\]

Assuming laser fluctuation is much less than laser output:

\[
\frac{N_S}{B} \approx \frac{N_B S}{B^2} \approx 0
\]
Divide numerator and denominator by $E_s E_B X_s X_e e^{j(x - x_e)}$:

$$S = \frac{A e^{j\mu} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + A e^{-j\mu} e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S)}.$$  

Divide numerator and denominator by:

$$S = \frac{A e^{j\mu} + A e^{-j\mu} e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + A^2 \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + A^2 \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S)}.$$  

Substitute Taylor Series:

$$S \approx \left( A e^{j\mu} + A e^{-j\mu} e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + A^2 \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + A^2 \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + e^{-j2(x - x_e)} \cdot \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) + \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) \right).$$  

$$1 - \left( \sum_{k=0}^{3} \eta_k (1 + \Delta_k^R + \Delta_k^S + \Delta_k^R \Delta_k^S) \right).}
Rearrange to individual terms:
\[
\sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} e^{-j(\lambda_2, \lambda_1)}, \quad \sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} e^{-j\lambda_2} = e^{-j\lambda_2} \left( \sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} \right)
\]

\[
\sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} e^{-j\lambda_2} = e^{-j\lambda_2} \left( \sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} \right)
\]

\[
\sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} e^{-j\lambda_2} = e^{-j\lambda_2} \left( \sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} \right)
\]

\[
\sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} e^{-j\lambda_2} = e^{-j\lambda_2} \left( \sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} \right)
\]

\[
\sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} e^{-j\lambda_2} = e^{-j\lambda_2} \left( \sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} \right)
\]

\[
\sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} e^{-j\lambda_2} = e^{-j\lambda_2} \left( \sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} \right)
\]

\[
\sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} e^{-j\lambda_2} = e^{-j\lambda_2} \left( \sum_{k=0}^{3} \frac{(-1)^k \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^y)(1 + \Delta_k^x)} \right)
\]
From page 187:

\[ \sqrt{BS} \approx \frac{1}{2} \sqrt{\frac{A_{\text{max}}}{h_{\text{B}}}} \sqrt{\eta_k E_S (1 + \delta_s) (1 + \Delta_k^S)} \]

\[ \sqrt{R} \approx \frac{1}{2} \sqrt{\frac{A_{\text{max}}}{h_{\text{D}}}} \sqrt{\eta_k E_R (1 + \delta_r) (1 + \Delta_k^R)} \]

Substitute Summation:

\[ \sum_{k=0}^{3} \sqrt{BS_k} = \frac{1}{2} \sqrt{\frac{A_{\text{max}}}{h_{\text{B}}}} \sum_{k=0}^{3} \sqrt{\eta_k (1 + \Delta_k^S)} \]

\[ \sum_{k=0}^{3} \sqrt{R_k} = \frac{1}{2} \sqrt{\frac{A_{\text{max}}}{h_{\text{D}}}} \sum_{k=0}^{3} \sqrt{\eta_k (1 + \Delta_k^R)} \]

\[ \sum_{k=0}^{3} \sqrt{BS_k} = E_S (1 + \delta_s) \sum_{k=0}^{3} \sqrt{\eta_k (1 + \Delta_k^S)} \frac{3 \sum_{k=0}^{3} \sqrt{BS_k}}{3 \sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ \sum_{k=0}^{3} \sqrt{R_k} = E_R (1 + \delta_r) \sum_{k=0}^{3} \sqrt{\eta_k (1 + \Delta_k^R)} \frac{3 \sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}}{3 \sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]
Substitute Summations:

\[
\begin{align*}
\text{A}e^{j\alpha} & \left( 1 - \frac{\sum_{k=0}^{j} (-1)^k \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j2(\chi_2 - \chi_1)} - \frac{\sum_{k=0}^{j} B_k}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j(\chi_2 - \chi_1)} \right) \\
& \quad - \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} - \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j(\chi_2 - \chi_1)} \frac{\sum_{k=0}^{j} B_k}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} \\
& \quad + \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} - \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j(\chi_2 - \chi_1)} \frac{\sum_{k=0}^{j} B_k}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} \\
& \quad + \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} - \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j(\chi_2 - \chi_1)} \frac{\sum_{k=0}^{j} B_k}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} \\
& \quad + \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} - \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j(\chi_2 - \chi_1)} \frac{\sum_{k=0}^{j} B_k}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} \\
& \quad + \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} - \frac{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j(\chi_2 - \chi_1)} \frac{\sum_{k=0}^{j} B_k}{\sum_{k=0}^{j} \sqrt{R_k} \sqrt{B_k}} e^{-j3(\chi_2 - \chi_1)} \right) 
\end{align*}
\]
Substitute: 

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k \sqrt{BS_k}}}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \]

\[ \Gamma_r = \frac{\sum_{k=0}^{3} j^k R_k}{\sum_{k=0}^{3} \sqrt{BS_k \sqrt{R_k}}} \]

\[ \Gamma_s = \frac{\sum_{k=0}^{3} j^k BS_k}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \]

\[ I_{s,m} = \frac{\sum_{k=0}^{3} j^k i_{M,k}}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \]

\[ I_{b,m} = \frac{\sum_{k=0}^{3} j^k i_{BM,k}}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \]

\[ A e^{ja} \left( 1 - \Phi e^{-j2(x_z - x_r)} - \Gamma_s e^{-j(x_z - x_r)} - \Gamma_r e^{-j(x_z - x_r)} - I_{b,m} e^{-j(x_z - x_r)} \right) + \]

\[ A e^{-ja} \left( \Phi e^{-j2(x_z - x_r)} - \Phi^2 e^{-j4(x_z - x_r)} - \Gamma_s \Phi e^{-j3(x_z - x_r)} - \Gamma_r \Phi e^{-j3(x_z - x_r)} - \Phi I_{b,m} e^{-j3(x_z - x_r)} \right) + \]

\[ A^2 \Gamma_s \left( e^{-j3(x_z - x_r)} - \Phi e^{-j3(x_z - x_r)} - \Gamma_s e^{-j2(x_z - x_r)} - \Gamma_r e^{-j2(x_z - x_r)} - I_{b,m} e^{-j2(x_z - x_r)} \right) + \]

\[ \Gamma_r \left( e^{-j2(x_z - x_r)} - \Phi e^{-j2(x_z - x_r)} - \Gamma_s e^{-j2(x_z - x_r)} - \Gamma_r e^{-j2(x_z - x_r)} - I_{b,m} e^{-j2(x_z - x_r)} \right) + \]

\[ I_{s,m} \left( e^{-j4(x_z - x_r)} - \Phi e^{-j4(x_z - x_r)} - \Gamma_s e^{-j2(x_z - x_r)} - \Gamma_r e^{-j2(x_z - x_r)} - I_{b,m} e^{-j2(x_z - x_r)} \right) \]

Assume noise terms squared equal zero: 

\[ I_s I_b \approx 0; \quad I_s \Phi \approx 0; \quad I_b \Phi \approx 0; \quad I_s \Gamma \approx 0; \quad I_b \Gamma \approx 0; \]

\[ A e^{ja} \left( 1 - \Phi e^{-j2(x_z - x_r)} - \Gamma_s e^{-j(x_z - x_r)} - \Gamma_r e^{-j(x_z - x_r)} - I_{b,m} e^{-j(x_z - x_r)} \right) + \]

\[ A e^{-ja} \left( \Phi e^{-j2(x_z - x_r)} - \Phi^2 e^{-j4(x_z - x_r)} - \Gamma_s \Phi e^{-j3(x_z - x_r)} - \Gamma_r \Phi e^{-j3(x_z - x_r)} \right) + \]

\[ A^2 \Gamma_s \left( e^{-j3(x_z - x_r)} - \Phi e^{-j3(x_z - x_r)} - \Gamma_s e^{-j2(x_z - x_r)} - \Gamma_r e^{-j2(x_z - x_r)} \right) + \]

\[ \Gamma_r \left( e^{-j2(x_z - x_r)} - \Phi e^{-j2(x_z - x_r)} - \Gamma_s e^{-j2(x_z - x_r)} - I_{b,m} e^{-j2(x_z - x_r)} \right) + I_{s,m} e^{-j4(x_z - x_r)} \]
Rearrange Terms:

\[ Ae^{ja} \left( 1 - \Phi e^{-j2(z_r, x_r)} - (\Gamma_S + \Gamma_R) e^{-j3(z_r, x_r)} - I_{B,M} e^{-j(x_r, \phi_r)} \right) + \]

\[ Ae^{ja} \left( \Phi e^{-j2(z_r, x_r)} - \Phi (\Gamma_S + \Gamma_R) e^{-j3(z_r, x_r)} - \Phi^2 e^{-j4(z_r, x_r)} \right) + \]

\[ A^2 \Gamma_S \left( e^{-j(z_r, x_r)} - (\Gamma_S + \Gamma_R) e^{-j2(z_r, x_r)} - \Phi e^{-j3(z_r, x_r)} \right) + \]

\[ \Gamma_R \left( e^{-j(z_r, x_r)} - (\Gamma_S + \Gamma_R) e^{-j2(z_r, x_r)} - \Phi e^{-j3(z_r, x_r)} \right) + I_{S,M} e^{-j(z_r, x_r)} + \]

\[ Ae^{ja} \left( 1 - \Phi e^{-j2(z_r, x_r)} - (\Gamma_S + \Gamma_R) e^{-j3(z_r, x_r)} - I_{B,M} e^{-j(x_r, \phi_r)} \right) + \]

\[ Ae^{ja} \left( \Phi e^{-j2(z_r, x_r)} - \Phi (\Gamma_S + \Gamma_R) e^{-j3(z_r, x_r)} - \Phi^2 e^{-j4(z_r, x_r)} \right) + \]

\[ (A^2 \Gamma_S + \Gamma_R) \left( e^{-j(z_r, x_r)} - (\Gamma_S + \Gamma_R) e^{-j2(z_r, x_r)} - \Phi e^{-j3(z_r, x_r)} \right) + I_{S,M} e^{-j(x_r, \phi_r)} + \]
Complex Field Reconstructed by Balanced Mixing

\[ E_{BM} = Ae^{j\alpha} \left[ 1 - \Phi e^{-j2(\chi_s - \chi_r)} - \Gamma e^{-j(\chi_s - \chi_r)} - I_{B,M} e^{-j(\chi_s - \chi_r)} \right] + \]

\[ Ae^{-j\alpha} \left[ \Phi e^{-j2(\chi_s - \chi_r)} - \Phi \Gamma e^{-j3(\chi_s - \chi_r)} - \Phi^2 e^{-j4(\chi_s - \chi_r)} \right] + \]

\( (\Lambda^2 \Gamma_S + \Gamma_R) \cdot \left[ e^{-j(\chi_s - \chi_r)} - \Gamma e^{-j2(\chi_s - \chi_r)} - \Phi e^{-j3(\chi_s - \chi_r)} \right] + I_{S,M} e^{-j(\chi_s - \chi_r)} \)

Where:

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k \sqrt{BS_k}}}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \quad \Gamma = \Gamma_S + \Gamma_R \quad \Gamma_S = \frac{\sum_{k=0}^{3} j^k BS_k}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \quad \Gamma_R = \frac{\sum_{k=0}^{3} j^k R_k}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \]

\[ I_{S,M} = \frac{\sum_{k=0}^{3} j^k i_{M,k}}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \quad I_{B,M} = \frac{\sum_{k=0}^{3} j^k i_{BM,k}}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \]

\( Ae^{j\alpha} \): Magnitude (A) and phase (\( \alpha \)) of the sample
\( \chi_s \): Phase of aberrations induced within the signal arm
\( \chi_r \): Phase of aberrations induced within the reference arm
\( \eta \): Quantum efficiency of the cameras
\( \Delta^S \): Fixed pattern noise associated with beamsplitters and cameras in signal path
\( \Delta^R \): Fixed pattern noise associated with beamsplitters and cameras in reference path
\( BS \): Image of the pure signal beam without the sample in the path
\( R \): Image of the pure reference beam
\( i \): Dark current noise in an acquired image
A.6.1 Include Wavefront Mismatch Between Sample and Blank Images

Substitute: \( \beta_1 = \chi_s - \chi_r \) (Signal) \( \beta_2 = \chi_s - \chi_r \) (Blank)

\[
\frac{S}{B} = \frac{A e^{i\alpha} (E_S E_R) X_s X_r e^{i\beta_2} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R) + A e^{-i\alpha} (E_R E_S) X_s X_r e^{-i\beta_2} \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R) +}{(E_S E_R) X_s X_r e^{i\beta_2} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R) + (E_R E_S) X_s X_r e^{-i\beta_2} \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R) +}
\]

\[
A^2 E^2_3 X^2 \cdot \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + E^2_3 X^2 \cdot \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + 4 \eta_{pixel} \sum_{j=0}^{3} j^j \eta_{BM,k}
\]

Divide numerator and denominator by \( E_S E_R X_s X_r e^{i\beta_2} \):

\[
\frac{S}{B} = \frac{A e^{i\alpha} e^{i(\beta_1 - \beta_2)} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R) + A e^{-i\alpha} e^{-i(\beta_1 + \beta_2)} \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R) +}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R) + \sum_{k=0}^{3} (-1)^k \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R) + e^{-i\beta_2} \sum_{j=0}^{3} j^j \eta_{BM,k}}
\]

\[
A^2 E^2_3 X^2 \cdot \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + E^2_3 X^2 \cdot \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + \frac{1}{\eta_{pixel}} \sum_{j=0}^{3} j^j \eta_{BM,k}
\]

Divide numerator and denominator by:

\[
\sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)
\]

\[
\frac{S}{B} = \frac{A e^{i\alpha} e^{i(\beta_1 - \beta_2)} + A e^{-i\alpha} e^{-i(\beta_1 + \beta_2)} \sum_{k=0}^{3} \eta_k \frac{(1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)_0}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)} + A^2 E^2_3 X^2 e^{i\beta_2} \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + E^2_3 X^2 e^{-i\beta_2} \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + \frac{1}{\eta_{pixel}} \sum_{j=0}^{3} j^j \eta_{BM,k} e^{-i\beta_2}}{1 + \frac{1}{\eta_{pixel}} \sum_{j=0}^{3} j^j \eta_{BM,k} e^{-i\beta_2}}
\]

Substitute Taylor Series:

\[
\frac{S}{B} = \left( A e^{i\alpha} e^{i(\beta_1 - \beta_2)} + A e^{-i\alpha} e^{-i(\beta_1 + \beta_2)} \sum_{k=0}^{3} \eta_k \frac{(1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)_0}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)} + A^2 E^2_3 X^2 e^{i\beta_2} \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + E^2_3 X^2 e^{-i\beta_2} \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + \frac{1}{\eta_{pixel}} \sum_{j=0}^{3} j^j \eta_{BM,k} e^{-i\beta_2} \sum_{k=0}^{3} \eta_k \frac{(1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)_0}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)} + A e^{i\alpha} e^{i(\beta_1 - \beta_2)} + A e^{-i\alpha} e^{-i(\beta_1 + \beta_2)} \sum_{k=0}^{3} \eta_k \frac{(1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)_0}{\sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)} + A^2 E^2_3 X^2 e^{i\beta_2} \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + E^2_3 X^2 e^{-i\beta_2} \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + \frac{1}{\eta_{pixel}} \sum_{j=0}^{3} j^j \eta_{BM,k} e^{-i\beta_2}}{1 + \frac{1}{\eta_{pixel}} \sum_{j=0}^{3} j^j \eta_{BM,k} e^{-i\beta_2}}
\]

\[
\frac{S}{B} = \left( \frac{e^{-i\beta_2} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R) + E^2_3 X^2 e^{-i\beta_2} \sum_{j=0}^{3} (j)^k \eta_k (1 + 2\Delta_k^s + \Delta_k^r) + \frac{1}{\eta_{pixel}} \sum_{j=0}^{3} j^j \eta_{BM,k} e^{-i\beta_2} \sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)}{1 + \frac{1}{\eta_{pixel}} \sum_{j=0}^{3} j^j \eta_{BM,k} e^{-i\beta_2}} \right)
\]

\[
\sum_{k=0}^{3} \eta_k (1 + \Delta_k^s + \Delta_k^r + \Delta_k^S \Delta_k^R)
\]

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Rearrange to individual terms:
\[ A e^{j\beta} \left( e^{j(\beta_1 + \beta_2)} - e^{-j(3\beta_1 + \beta_2)} \right) \left( \sum_{k=0}^{3} \frac{j^{(1-k)} \eta_1 (1+\Delta_k^3)(1+\Delta_k^3)}{E_{k} \Sigma_{\eta_1 (1+\Delta_k^3)(1+\Delta_k^3)}} \right) = \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu}(1+\Delta_k^3)(1+\Delta_k^3)}{E_{\sum_{k=0}^{3} \eta_{\mu}(1+\Delta_k^3)(1+\Delta_k^3)}} + \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu,\phi}}{E_{\sum_{k=0}^{3} \eta_{\mu,\phi}}(1+\Delta_k^3)(1+\Delta_k^3)} + \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu,\phi}}{E_{\sum_{k=0}^{3} \eta_{\mu,\phi}}(1+\Delta_k^3)(1+\Delta_k^3)} + \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu,\phi}}{E_{\sum_{k=0}^{3} \eta_{\mu,\phi}}(1+\Delta_k^3)(1+\Delta_k^3)} + \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu,\phi}}{E_{\sum_{k=0}^{3} \eta_{\mu,\phi}}(1+\Delta_k^3)(1+\Delta_k^3)} + \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu,\phi}}{E_{\sum_{k=0}^{3} \eta_{\mu,\phi}}(1+\Delta_k^3)(1+\Delta_k^3)} + \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu,\phi}}{E_{\sum_{k=0}^{3} \eta_{\mu,\phi}}(1+\Delta_k^3)(1+\Delta_k^3)} + \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu,\phi}}{E_{\sum_{k=0}^{3} \eta_{\mu,\phi}}(1+\Delta_k^3)(1+\Delta_k^3)} + \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu,\phi}}{E_{\sum_{k=0}^{3} \eta_{\mu,\phi}}(1+\Delta_k^3)(1+\Delta_k^3)} + \frac{\sum_{k=0}^{3} j^{k} \eta_{\mu,\phi}}{E_{\sum_{k=0}^{3} \eta_{\mu,\phi}}(1+\Delta_k^3)(1+\Delta_k^3)} \]
From page 187:

$$\sqrt{BS} \approx \frac{1}{2} \sqrt{\frac{A_{\text{max}}}{h_{1v}}} \sqrt{\eta_k} E_s (1 + \Delta_s) (1 + \Delta_s)$$
$$\sqrt{R} \approx \frac{1}{2} \sqrt{\frac{A_{\text{max}}}{h_{1v}}} \sqrt{\eta_k} E_R (1 + \Delta_r) (1 + \Delta_r)$$

Substitute Summation:

$$\sum_{k=0}^{3} \sqrt{BS_k} = \frac{1}{2} \sqrt{\frac{A_{\text{max}}}{h_{1v}}} \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^s)$$
$$\sum_{k=0}^{3} \sqrt{R_k} = \frac{1}{2} \sqrt{\frac{A_{\text{max}}}{h_{1v}}} \sum_{k=0}^{3} \sqrt{\eta_k} (1 + \Delta_k^r)$$

$$\sum_{k=0}^{3} \sqrt{BS_k} = \frac{E_s X_s \sum_{k=0}^{3} (j^k \eta_k (1 + \Delta_k^s) (1 + \Delta_k^s))}{E_s X_s \sum_{k=0}^{3} \eta_k (1 + \Delta_k^s) (1 + \Delta_k^s)}$$
$$\sum_{k=0}^{3} \sqrt{R_k} = \frac{E_R X_s \sum_{k=0}^{3} (j^k \eta_k (1 + \Delta_k^r) (1 + \Delta_k^r))}{E_R X_s \sum_{k=0}^{3} \eta_k (1 + \Delta_k^r) (1 + \Delta_k^r)}$$

$$\sum_{k=0}^{3} \sqrt{BS_k} = \frac{E_s X_s \sum_{k=0}^{3} (j^k \eta_k (1 + \Delta_k^s) (1 + \Delta_k^s))}{E_s X_s \sum_{k=0}^{3} \eta_k (1 + \Delta_k^s) (1 + \Delta_k^s)}$$
$$\sum_{k=0}^{3} \sqrt{R_k} = \frac{E_R X_s \sum_{k=0}^{3} (j^k \eta_k (1 + \Delta_k^r) (1 + \Delta_k^r))}{E_R X_s \sum_{k=0}^{3} \eta_k (1 + \Delta_k^r) (1 + \Delta_k^r)}$$
Substitute Summations:

\[ Ae^{j\alpha} \left( e^{j(p_1 - p_2)} - \frac{1}{j} \sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{B_k} e^{j(3p_2 - p_1)} - \frac{1}{j} \sum_{k=0}^{3} j^k B_k - e^{-j(2p_2 - p_1)} - \frac{1}{j} \sum_{k=0}^{3} R_k - e^{-j(2p_2 - p_1)} + \frac{1}{j} \sum_{k=0}^{3} j^k \lambda_{k,BM} \right) + \]

\[ Ae^{-j\alpha} \left( \frac{1}{j} \sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{B_k} e^{j(2p_2 + p_1)} - \frac{1}{j} \sum_{k=0}^{3} j^k R_k - e^{-j(2p_2 + p_1)} - \frac{1}{j} \sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{B_k} e^{j(2p_2 + p_1)} - \frac{1}{j} \sum_{k=0}^{3} j^k \lambda_{k,BM} \right) + \]

\[ A^2 \left( \frac{1}{j} \sum_{k=0}^{3} j^k B_k - \frac{1}{j} \sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{B_k} e^{j3p_1} - \frac{1}{j} \sum_{k=0}^{3} j^k B_k - e^{-j2p_1} - \frac{1}{j} \sum_{k=0}^{3} R_k - e^{-j2p_1} + \frac{1}{j} \sum_{k=0}^{3} j^k \lambda_{k,BM} \right) + \]

\[ \frac{1}{j} \sum_{k=0}^{3} j^k R_k \left( e^{-j2p_1} - \frac{1}{j} \sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{B_k} e^{j3p_1} - \frac{1}{j} \sum_{k=0}^{3} j^k B_k - e^{-j2p_1} - \frac{1}{j} \sum_{k=0}^{3} R_k - e^{-j2p_1} + \frac{1}{j} \sum_{k=0}^{3} j^k \lambda_{k,BM} \right) + \]

\[ \frac{3}{j} \sum_{k=0}^{3} j^k \lambda_{k,BM} \left( e^{-j2p_1} - \frac{1}{j} \sum_{k=0}^{3} (-1)^k \sqrt{R_k} \sqrt{B_k} e^{j3p_1} - \frac{1}{j} \sum_{k=0}^{3} j^k B_k - e^{-j2p_1} - \frac{1}{j} \sum_{k=0}^{3} R_k - e^{-j2p_1} + \frac{1}{j} \sum_{k=0}^{3} j^k \lambda_{k,BM} \right) + \]
Substitute:  
\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k R_k \sqrt{BS_k}}{\sum_{k=0}^{3} R_k \sqrt{BS_k}} \]

\[ \Gamma_R = \frac{\sum_{k=0}^{3} j^k R_k}{\sum_{k=0}^{3} \sqrt{BS_k} \sqrt{R_k}} \]

\[ \Gamma_S = \frac{\sum_{k=0}^{3} j^k BS_k}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ I_{S,M} = \frac{\sum_{k=0}^{3} j^k i_{M,k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ I_{B,M} = \frac{\sum_{k=0}^{3} j^k i_{BM,k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ \begin{align*}
\mathcal{A} e^{j \alpha} \left( e^{j(\beta_1 - \beta_2)} - \Phi e^{j(3\beta_2 - \beta_1)} - \Gamma_S e^{-j(2\beta_2 - \beta_1)} - \Gamma_R e^{-j(2\beta_2 - \beta_1)} - I_{B,M} e^{-j(2\beta_2 - \beta_1)} \right) + \\
\mathcal{A} e^{-j \alpha} \left( \Phi e^{-j(\beta_2 + \beta_1)} - \Phi^2 e^{-j(3\beta_2 + \beta_1)} - \Gamma_S \Phi e^{-j(2\beta_2 + \beta_1)} - \Gamma_R \Phi e^{-j(2\beta_2 + \beta_1)} - \Phi I_{B,M} e^{-j(2\beta_2 + \beta_1)} \right) + \\
\mathcal{A}^2 \Gamma_S \left( e^{j \beta_2} - \Phi e^{j3 \beta_2} - \Gamma_S e^{-j2 \beta_2} - \Gamma_R e^{-j2 \beta_2} - I_{B,M} e^{-j2 \beta_2} \right) + \\
\Gamma_R \left( e^{j \beta_2} - \Phi e^{j3 \beta_2} - \Gamma_S e^{-j2 \beta_2} - \Gamma_R e^{-j2 \beta_2} - I_{B,M} e^{-j2 \beta_2} \right) + \\
I_{S,M} \left( e^{j \beta_2} - \Phi e^{j3 \beta_2} - \Gamma_S e^{-j2 \beta_2} - \Gamma_R e^{-j2 \beta_2} - I_{B,M} e^{-j2 \beta_2} \right)
\end{align*} \]

Assume noise terms squared equal zero:  
\[ I_S I_B \approx 0; \quad I_S \Phi \approx 0; \quad I_B \Phi \approx 0; \quad I_S \Gamma \approx 0; \quad I_B \Gamma \approx 0; \]

\[ \begin{align*}
\mathcal{A} e^{j \alpha} \left( e^{j(\beta_1 - \beta_2)} - \Phi e^{j(3\beta_2 - \beta_1)} - \Gamma_S e^{-j(2\beta_2 - \beta_1)} - \Gamma_R e^{-j(2\beta_2 - \beta_1)} - I_{B,M} e^{-j(2\beta_2 - \beta_1)} \right) + \\
\mathcal{A} e^{-j \alpha} \left( \Phi e^{-j(\beta_2 + \beta_1)} - \Phi^2 e^{-j(3\beta_2 + \beta_1)} - \Gamma_S \Phi e^{-j(2\beta_2 + \beta_1)} - \Gamma_R \Phi e^{-j(2\beta_2 + \beta_1)} \right) + \\
\mathcal{A}^2 \Gamma_S \left( e^{j \beta_2} - \Phi e^{j3 \beta_2} - \Gamma_S e^{-j2 \beta_2} - \Gamma_R e^{-j2 \beta_2} \right) + \\
\Gamma_R \left( e^{j \beta_2} - \Phi e^{j3 \beta_2} - \Gamma_S e^{-j2 \beta_2} - \Gamma_R e^{-j2 \beta_2} \right) + I_{S,M} e^{-j \beta_2}
\end{align*} \]
Rearrange Terms:

\[ Ae^{ja\left(e^{j(\beta_1 - \beta_2)} - \Phi e^{-(j(3\beta_2 - \beta_1))} - (\Gamma_S + \Gamma_R)e^{-j(2\beta_2 - \beta_1)} - I_{B,M}e^{-j(2\beta_2 - \beta_1)}\right)\} +
\]

\[ Ae^{-ja\left(\Phi e^{-j(\beta_2 + \beta_1)} - \Phi(\Gamma_S + \Gamma_R)e^{-j(2\beta_1 + \beta_1)} - \Phi^2 e^{-j(3\beta_2 + \beta_1)}\right)\} +
\]

\[ A^2\Gamma_S\left(e^{-j\beta_2} - (\Gamma_S + \Gamma_R)e^{-j2\beta_2} - \Phi e^{-j3\beta_2}\right) + I_{S,M}e^{-j\beta_2}
\]

\[ \Gamma_R\left(e^{-j\beta_2} - (\Gamma_S + \Gamma_R)e^{-j2\beta_2} - \Phi e^{-j3\beta_2}\right) + I_{S,M}e^{-j\beta_2}
\]

\[ Ae^{ja\left(e^{j(\beta_1 - \beta_2)} - \Phi e^{-(j(3\beta_2 - \beta_1))} - (\Gamma_S + \Gamma_R)e^{-j(2\beta_2 - \beta_1)} - I_{B,M}e^{-j(2\beta_2 - \beta_1)}\right)\} +
\]

\[ Ae^{-ja\left(\Phi e^{-j(\beta_2 + \beta_1)} - \Phi(\Gamma_S + \Gamma_R)e^{-j(2\beta_1 + \beta_1)} - \Phi^2 e^{-j(3\beta_2 + \beta_1)}\right)\} +
\]

\[ (A^2\Gamma_S + \Gamma_R)\left(e^{-j\beta_2} - (\Gamma_S + \Gamma_R)e^{-j2\beta_2} - \Phi e^{-j3\beta_2}\right) + I_{S,M}e^{-j\beta_2}
\]

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Complex Field Reconstructed by Balanced Mixing
(assuming fringe pattern of sample image does not match fringe pattern of blank)

\[ E_{BM} = Ae^{j\alpha} \left[ e^{j(\beta_1 - \beta_2)} - \Phi e^{j(3\beta_2 - \beta_1)} - \Gamma e^{j(2\beta_2 - \beta_1)} - I_{S,M} e^{j(2\beta_2 - \beta_1)} \right] + \]
\[ Ae^{-j\alpha} \left[ \Phi e^{-j(\beta_2 + \beta_1)} - \Phi \Gamma e^{-j(2\beta_2 + \beta_1)} - \Phi^2 e^{-j(3\beta_2 + \beta_1)} \right] + \]
\[ (A^2 \Gamma_S + \Gamma_R) \left[ e^{-j\beta_2} - \Gamma e^{-j/2\beta_2} - \Phi e^{-j3\beta_2} \right] + I_{S,M} e^{-j\beta_2} \]

Where:

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{R_k \sqrt{BS_k}}}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \quad \Gamma = \Gamma_S + \Gamma_R \quad \Gamma_S = \frac{\sum_{k=0}^{3} j^k BS_k}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \quad \Gamma_R = \frac{\sum_{k=0}^{3} j^k R_k}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \]

\[ I_{S,M} = \frac{\sum_{k=0}^{3} j^k i_{M,k}}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \quad I_{B,M} = \frac{\sum_{k=0}^{3} j^k i_{BM,k}}{\sum_{k=0}^{3} \sqrt{R_k \sqrt{BS_k}}} \]

\( Ae^{j\alpha} \): Magnitude (A) and phase (\( \alpha \)) of the sample
\( \beta_1 \): Wavefront mismatch between signal and reference arms for image of sample
\( \beta_2 \): Wavefront mismatch between signal and reference arms for image of blank
BS: Image of the pure signal beam without the sample in the path
R: Image of the pure reference beam
i: Dark current noise in an acquired image
A.6.2 Balanced Mixing and No Blank

Balanced Mixing with Image of Sample:

\[ E_{BM} = \frac{1}{4} \sum_{n=0}^{N-1} \left( A e^{i \alpha} (E_S E_R^* + E_S E_N e^{i(\theta - \phi)} + E_N E_R e^{-i(\theta - \phi)} + E_N E_N^*) X_s X_s^* e^{i(\chi - X_s)} \right) . \]

\[ \mathcal{N} (E_S^R E_C^* + \eta_1 E_C^R E_{C_1}^* + \eta_2 E_C^R E_{C_2}^* + \eta_3 E_C^R E_{C_3}^*) + \]

\[ A e^{-i \alpha} (E_S E_R^* + E_S E_N e^{i(\theta - \phi)} + E_N E_R e^{-i(\theta - \phi)} + E_N E_N^*) X_s X_s^* e^{-i(\chi - X_s)} . \]

\[ \mathcal{N} (E_S^R E_C^* - \eta_1 E_C^R E_{C_1}^* - \eta_2 E_C^R E_{C_2}^* - \eta_3 E_C^R E_{C_3}^*) + \]

\[ A^2 (E_S E_S^* + E_S E_N^* e^{i(\theta - \phi)} + E_N E_N^* e^{-i(\theta - \phi)} + E_N E_N^*) X_s X_s^* + \]

\[ (E_R E_R^* + E_R E_N e^{i(\theta - \phi)} + E_N E_N^*) X_s X_s^* \sum_{k=0}^{3} j^k \eta_k E_C^R E_{C_i}^* + \sum_{k=0}^{3} j^k i_{M,k} \]

Substitute summations for efficiency and dark current:

\[ E_{BM} = \frac{1}{4} \sum_{n=0}^{N-1} \left( A e^{i \alpha} (E_S E_R^* + E_S E_N e^{i(\theta - \phi)} + E_N E_R e^{-i(\theta - \phi)} + E_N E_N^*) X_s X_s^* e^{i(\chi - X_s)} \sum_{k=0}^{3} \eta_k E_C^R E_{C_i}^* + \right) \]

\[ A e^{-i \alpha} (E_S E_R^* + E_S E_N e^{i(\theta - \phi)} + E_N E_R e^{-i(\theta - \phi)} + E_N E_N^*) X_s X_s^* \sum_{k=0}^{3} (1 - i^k) \eta_k E_C^R E_{C_i}^* + \]

\[ A^2 (E_S E_S^* + E_S E_N^* e^{i(\theta - \phi)} + E_N E_N^* e^{-i(\theta - \phi)} + E_N E_N^*) X_s X_s^* \sum_{k=0}^{3} j^k \eta_k E_C^R E_{C_i}^* + \]

\[ (E_R E_R^* + E_R E_N e^{i(\theta - \phi)} + E_N E_N^*) X_s X_s^* \sum_{k=0}^{3} j^k i_{M,k} \]

Assume all amplitudes are real and noise terms squared are equal to zero:

\[ E_{BM} = \frac{1}{4} \sum_{n=0}^{N-1} \left( A e^{i \alpha} (E_S E_R^* + E_S E_N e^{i(\theta - \phi)} + E_N E_R e^{-i(\theta - \phi)} + E_N E_N^*) \sum_{k=0}^{3} \eta_k E_C^R E_{C_i}^* + \right) \]

\[ A e^{-i \alpha} (E_S E_S^* + E_S E_N^* e^{i(\theta - \phi)} + E_N E_S e^{-i(\theta - \phi)} + E_N E_N^*) X_s X_s^* \sum_{k=0}^{3} (-1)^k \eta_k E_C^R E_{C_i}^* + \]

\[ A^2 (E_S E_S^* + E_S E_N^* e^{i(\theta - \phi)} + E_N E_N^* e^{-i(\theta - \phi)} + E_N E_N^*) X_s X_s^* \sum_{k=0}^{3} j^k \eta_k E_C^R E_{C_i}^* + \]

\[ (E_R E_R^* + E_R E_N e^{i(\theta - \phi)} + E_N E_N^*) X_s X_s^* \sum_{k=0}^{3} j^k i_{M,k} \]

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Separate signal and noise terms:

\[
S = \frac{1}{2} \frac{A_{\text{wavelength}}}{W} \left( A e^{j\alpha} (E_s E_h) X, X, e^{j(x-x_i)} \sum_{k=0}^{3} \eta_k E^S_{C_i} E^R_{C_i} + A e^{-j\alpha} (E_s E_h) X, X, e^{-j(x-x_i)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k E^R_{C_i} E^S_{C_i} + \right) \\
A^2 (E_s E_h) X, X, \sum_{k=0}^{3} j^k \eta_k E^S_{C_i} E^R_{C_i} + (E_s E_h) X, X, \sum_{k=0}^{3} j^k \eta_k E^R_{C_i} E^S_{C_i} \right) + \sum_{k=0}^{3} j^k i_{M,k}
\]

\[
N = \frac{1}{2} \frac{A_{\text{wavelength}}}{W} E_N \left( A e^{j\alpha} (E_s e^{j(\phi-\zeta)} + E_h e^{-j(\phi-\zeta)}) X, X, e^{j(x-x_i)} \sum_{k=0}^{3} \eta_k E^S_{C_i} E^R_{C_i} + \right) \\
A e^{-j\alpha} (E_s e^{j(\phi-\zeta)} + E_h e^{-j(\phi-\zeta)}) X, X, e^{-j(x-x_i)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k E^R_{C_i} E^S_{C_i} + \right) \\
A^2 (E_s e^{j(\phi-\zeta)} + E_h e^{-j(\phi-\zeta)}) X, X, \sum_{k=0}^{3} j^k \eta_k E^S_{C_i} E^R_{C_i} + (E_s e^{j(\phi-\zeta)} + E_h e^{-j(\phi-\zeta)}) X, X, \sum_{k=0}^{3} j^k \eta_k E^R_{C_i} E^S_{C_i} \right)
\]

Since the amplitude of the fixed pattern noise and aberrations within the system are ideally equal to 1:

\[
X = 1 + \delta \; ; \quad E_C = 1 + \Delta
\]

\[
S = \frac{1}{2} \frac{A_{\text{wavelength}}}{W} \left( A e^{j\alpha} (E_s E_h)(1+\delta)(1+\delta) e^{j(x-x_i)} \sum_{k=0}^{3} \eta_k (1+\Delta^S)(1+\Delta^R) + A e^{-j\alpha} (E_s E_h)(1+\delta)(1+\delta) e^{-j(x-x_i)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k (1+\Delta^R)(1+\Delta^S) + \right) \\
A^2 (E_s E_h)(1+\delta)(1+\delta) \cdot \sum_{k=0}^{3} j^k \eta_k (1+\Delta^S)(1+\Delta^R) + E_s E_h (1+\delta)(1+\delta) \cdot \sum_{k=0}^{3} j^k \eta_k (1+\Delta^R)(1+\Delta^S) \right) + \sum_{k=0}^{3} j^k i_{M,k}
\]

\[
N = \frac{1}{2} \frac{A_{\text{wavelength}}}{W} E_N \left( A e^{j\alpha} (E_s e^{j(\phi-\zeta)} + E_h e^{-j(\phi-\zeta)}) (1+\delta)(1+\delta) e^{j(x-x_i)} \sum_{k=0}^{3} \eta_k (1+\Delta^S) + \right) \\
A e^{-j\alpha} (E_s e^{j(\phi-\zeta)} + E_h e^{-j(\phi-\zeta)}) (1+\delta)(1+\delta) e^{-j(x-x_i)} \cdot \sum_{k=0}^{3} (-1)^k \eta_k (1+\Delta^R) + \right) \\
A^2 (E_s e^{j(\phi-\zeta)} + E_h e^{-j(\phi-\zeta)}) (1+\delta)(1+\delta) \cdot \sum_{k=0}^{3} j^k \eta_k (1+\Delta^S) + (E_s e^{j(\phi-\zeta)} + E_h e^{-j(\phi-\zeta)}) (1+\delta)(1+\delta) \cdot \sum_{k=0}^{3} j^k \eta_k (1+\Delta^R) \right)
\]

With a wavelength of 633nm and pixels that are 7\(\mu\)m on a side:

\[
\frac{A_{\text{wavelength}}}{W} = 2.5 \times 10^{-11} \; \text{m}^2 \cdot \text{W}^{-1}
\]

Including the 1/4\(^{th}\) creates an effective noise term:

\[
\frac{1}{4} \frac{A_{\text{wavelength}}}{W} E_N \approx 0 \implies N \approx 0
\]

Thereby allowing us to assume:

\[
\frac{1}{4} \frac{A_{\text{wavelength}}}{W} E_N \approx 0 \implies N \approx 0
\]
\[
S = \frac{1}{\mu_0} \left( A e^{j\alpha} e^{j(x_i - x_r)} \sum_{k=0}^3 \eta \left( 1 + \Delta_k \right) \left( 1 + \Delta_0 \right) + \sum_{k=0}^3 \eta \left( 1 + \Delta_0 \right) \left( 1 + \Delta_k \right) \right) + \sum_{k=0}^3 \left( -1 \right)^k \eta \left( 1 + \Delta_0 \right) \left( 1 + \Delta_k \right)
\]

\[
A^2 \left( E_s E_s \right) \left( 1 + \delta_s \right) \left( 1 + \delta_r \right) + \sum_{k=0}^3 \left( -1 \right)^k \eta \left( 1 + \Delta_0 \right) \left( 1 + \Delta_k \right)
\]

From page 187:
\[
\sqrt{BS} \approx \frac{1}{2} \sqrt{\frac{A_{m0}q}{h_0}} \sqrt{\eta_s \left( 1 + \Delta_s \right) \left( 1 + \Delta_0 \right)}
\]
\[
\sqrt{R} \approx \frac{1}{2} \sqrt{\frac{A_{m0}q}{h_0}} \sqrt{\eta_r \left( 1 + \Delta_r \right) \left( 1 + \Delta_0 \right)}
\]

Substitute Summation:
\[
\sum_{k=0}^3 \sqrt{BS_k} = \frac{1}{2} \sqrt{\frac{A_{m0}q}{h_0}} E_s \left( 1 + \delta_s \right) \sum_{k=0}^3 \sqrt{\eta_k \left( 1 + \Delta_k \right)}
\]
\[
\sum_{k=0}^3 \sqrt{R_k} = \frac{1}{2} \sqrt{\frac{A_{m0}q}{h_0}} E_r \left( 1 + \delta_r \right) \sum_{k=0}^3 \sqrt{\eta_k \left( 1 + \Delta_k \right)}
\]

\[
\sum_{k=0}^3 \left( -1 \right)^k \sqrt{BS_k} \sqrt{R_k} = \frac{1}{4} \sqrt{\frac{A_{m0}q}{h_0}} E_s \left( 1 + \delta_s \right) \sum_{k=0}^3 \left( -1 \right)^k \eta_k \left( 1 + \Delta_k \right) \left( 1 + \Delta_0 \right)
\]

\[
\sum_{k=0}^3 j^k BS_k = \frac{1}{4} \sqrt{\frac{A_{m0}q}{h_0}} E_s \left( 1 + \delta_s \right) \sum_{k=0}^3 j^k \eta_k \left( 1 + \Delta_k \right) \left( 1 + \Delta_0 \right)
\]

\[
\sum_{k=0}^3 j^k R_k = \frac{1}{4} \sqrt{\frac{A_{m0}q}{h_0}} E_r \left( 1 + \delta_r \right) \sum_{k=0}^3 j^k \eta_k \left( 1 + \Delta_k \right) \left( 1 + \Delta_0 \right)
\]

Substitute Summations:
\[
S = \left( A e^{j\alpha} e^{j(x_i - x_r)} \sum_{k=0}^3 \sqrt{BS_k} \sqrt{R_k} + A e^{-j\alpha} e^{-j(x_i - x_r)} \sum_{k=0}^3 \left( -1 \right)^k \sqrt{BS_k} \sqrt{R_k} \right) + A^2 \sum_{k=0}^3 j^k BS_k + A^2 \sum_{k=0}^3 j^k R_k + \sum_{k=0}^3 j^k i_{M,k}
\]

\[
S = e^{j(x_i - x_r)} \sum_{k=0}^3 \sqrt{BS_k} \sqrt{R_k} \left( A e^{j\alpha} + A e^{-j\alpha} \sum_{k=0}^3 \left( -1 \right)^k \sqrt{BS_k} \sqrt{R_k} \right) + A^2 e^{-j(x_i - x_r)} \sum_{k=0}^3 \sqrt{BS_k} \sqrt{R_k} + e^{j(x_i - x_r)} \sum_{k=0}^3 \sqrt{BS_k} \sqrt{R_k} + \sum_{k=0}^3 j^k i_{M,k}
\]

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Let:

\[ I_M = \sum_{k=0}^{3} j^k i_{M,k} \]

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{B_k} \sqrt{R_k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{B_k}} \]

\[ \Gamma_s = \frac{\sum_{k=0}^{3} j^k B_k}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{B_k}} \]

\[ \Gamma_r = \frac{\sum_{k=0}^{3} j^k R_k}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{B_k}} \]

\[ S = \sum_{k=0}^{3} \sqrt{B_k} \sqrt{R_k} e^{j(\chi_s - \chi_r)} \left( A e^{j\alpha} + A e^{-j\alpha} \Phi e^{-j2(\chi_s - \chi_r)} + A^2 \Gamma_s e^{-j(\chi_s - \chi_r)} + \Gamma_r e^{-j(\chi_s - \chi_r)} \right) + I_M \]
Complex Field Acquired by Balanced Mixing and No Blank

\[ E_{BM,\text{NB}} = \sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k} \ e^{j(z_r - z_s)} \left[ A e^{j\alpha} + A e^{-j\alpha} \Phi e^{-j2(z_r - z_s)} + (A^2 \Gamma_s + \Gamma_r) e^{-j(z_r - z_s)} \right] + I_M \]

Where:

\[ I_M = \sum_{k=0}^{3} j^k i_{M,k} \]

\[ \Phi = \frac{\sum_{k=0}^{3} (-1)^k \sqrt{BS_k} \sqrt{R_k}}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ \Gamma_s = \frac{\sum_{k=0}^{3} j^k BS_k}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ \Gamma_r = \frac{\sum_{k=0}^{3} j^k R_k}{\sum_{k=0}^{3} \sqrt{R_k} \sqrt{BS_k}} \]

\[ A e^{j\alpha} : \text{Magnitude (A) and phase (} \alpha \text{) of the sample} \]

\[ \chi_s : \text{Phase of aberrations induced within the signal arm} \]

\[ \chi_r : \text{Phase of aberrations induced within the reference arm} \]

\[ BS : \text{Image of the pure signal beam without the sample in the path} \]

\[ R : \text{Image of the pure reference beam} \]

\[ i : \text{Dark current noise in an acquired image} \]
References


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