THREE-DIMENSIONAL MODELING AND
RECONSTRUCTION FOR MULTIMODALITY PHASE
MICROSCOPY

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Abstract

Phase microscopy modalities are extensively used to image living transparent biological samples because of their ability to obtain high contrast images without the use of enhancing agents. However when the imaged object is optically thick, that is, when the thickness of the object is larger than the depth of field of the imaging system, the development of relevant image modeling and reconstruction techniques is necessary to extract useful information from the images.

The contribution of this work is toward the development of both a theoretical model and reconstruction techniques for phase images of optically thick objects. In particular, we have developed and tested a generative forward model based on a product of convolutions (POC) approach for these phase images. The POC model is based on the use of the point spread function (PSF) of the optical system and can be used to simulate phase images of any transparent object geometry. We have also developed an initial reconstruction method, specifically a multi-modal boundary-constrained inversion that combines two distinct phase imaging modalities, differential interference contrast (DIC) and optical quadrature microscopy (OQM) modalities. We use DIC to extract 3D information about object morphology and OQM to obtain a quantitative phase image from the entire object. The reconstruction algorithm combines the information from the two modalities to obtain the spatial variation of
the indices of refraction of the imaged objects.

Our methods could be used to improve the analysis of a number of classes of biological and biomedical samples. We use as a prototype application mouse embryo development studies, where the goal is to extract detailed information about the embryo at different development stages. We present results showing validation of the work done to date using simulated and measured multimodality phase images of both synthetic and mouse embryo samples.
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Chapter 1

Introduction

Microscopy techniques for biological applications have experienced a significant growth during the past decades. Advances allow the visualization of structures of biological specimens at high resolution and three-dimensional imaging of cellular functions as they occur in the living cells. Therefore, we are now able to analyze complex living cellular specimens. Living cells tend to be transparent under visible illumination, that is, they do not absorb (or scatter) light significantly [1]. Thus in ordinary brightfield microscope images they offer low contrast or even are invisible. To overcome this difficulty when imaging these samples, researchers have used contrast agents that bind to various biochemical structures in the object and render those structures visible when using a fluorescence microscope [2]. However, this modality is considered to be invasive because an exogenous substance is being added to the sample. There are situations where invasive techniques are toxic or, cause harm to living specimens and thus are unsuitable for those in-vivo applications. Instead phase microscopy techniques are widely used to study live transparent biological samples because of their ability to provide high contrast images without the need for contrast agents.
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[1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14]. Two common phase imaging modalities, phase contrast (PC) and differential interference contrast (DIC) microscopy, have been used extensively to image live cells in a noninvasive manner [1, 3, 5, 4]. However, PC and DIC microscopy are qualitative in terms of optical path-length measurement. Therefore, their potential for quantitative analysis is limited. Extraction of quantitative information from biological samples is important in several biomedical and biological applications. As an example of importance in this thesis, quantitative phase measurements, index of refraction distribution and thickness of live cells can be used for mouse embryo development studies. In response to this need, quantitative phase imaging modalities based on applying optical interferometry have been developed to obtain quantitative information about morphology and dynamic changes of living specimens [5, 6, 7, 8, 9, 10, 11, 12, 13, 14].

As mentioned, phase microscopy has been used extensively to acquire information about unstained transparent biological objects [1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14]. However, there are still many unanswered questions about how the three-dimensional structures of different objects affect the measured phase images. One approach to answer these questions is modeling the microscopy system to interpret and analyze measured images and to gain a better understanding of the underlying imaging process [15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. Common modeling approaches have included the use of linearized models and related simplified approaches to solve the wave equation. Linearized models have generally been based on the first Born approximation [25, 26]. These models have the advantage that they are well understood, simple to implement, and can be used in the application of inversion techniques. However, the Born approximation places strong constraints on the thickness of the object and the maximum refractive index contrast [25, 26]. Other approaches including higher order
Born approximations and direct discretization of Maxwell’s equations involve more computationally complex algorithms [24, 27]. They do overcome the limitations of the Born approach. However these methods usually require extensive numerical computation to simulate the required imaging volume at adequate resolution and in many cases are not computationally suitable for the application of inversion techniques.

Therefore, there is still a need for more computationally tractable robust models for phase microscopy that also allow the application of efficient inversion techniques. In this thesis, a model for phase imaging modalities based on a Product of Convolutions (POC) approach is presented. The model is not more computationally intensive than current state of the art linearized models. At the same time it relaxes the object size and contrast limitations that those models impose. The POC model has been applied for the reconstruction of transparent samples in phase microscopy [28]. A multimodal iterative approach that combines DIC and measured quantitative phase information to reconstruct the refractive indices of an object is also presented in this thesis. The method assumes that object morphology can be extracted from 3D DIC images. Then the POC forward model and the quantitative phase are incorporated in an iterative algorithm to find the refractive indices of the object.

The contributions of this thesis are completed in the context of *in vivo* imaging of mouse embryos at different development stages, which are used for *in vitro* fertilization research.

In the rest of this chapter we first present an overview of phase microscope imaging modalities and relevant related work on modeling and inversion techniques for phase microscopy images. An introduction to the type of biological samples that were used in this work follows. Finally, we discuss the scope and contributions of our work and provide an overview of the remainder of this thesis.
1.1 Phase Microscopy Modalities

Various optical phase microscope modalities that allow the visualization of transparent cellular specimens without staining have been developed \[1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14\]. Each modality utilizes schemes that change the form in which light is transmitted, effectively providing different information about the sample. In this section we provide a description of the phase microscopy techniques that are most commonly utilized for the analysis of live specimens.

1.1.1 Differential Interference Contrast

Differential interference contrast (DIC) was invented in the 1950’s by Georges Nomarski \[1, 3, 4, 5\]. DIC provides amplitude images where their variations are generated from phase shifts induced by the specimen’s index of refraction. DIC works by separating the light source into two beams which take different paths through the sample. After interacting with the sample, the beams are recombined to form the image. Interference occurs when recombining the beams, giving the appearance of a three-dimensional structure that corresponds to the variation of optical path of the sample.

The DIC microscope is equipped with special optics to transform phase differences to amplitude variations \[3, 1\]. The key optical components of a DIC microscope are a polarizer, an analyzer, and two Wollaston prisms arranged as shown in Fig.1.1. The first polarizer ensures the illumination light is polarized at 45 degrees. The polarized light is split spatially by a birefringent Wollaston prism in the back-focal plane of the condenser lens. The lateral separation distance between the two ray components, called the shear, is of the order of the resolution limit of the objective
The two wave components pass through the condenser into different parts of the specimen, where each is either phase advanced or retarded depending on the refractive index and thickness of part of the specimen it travels through. The components are then collected and focused by the objective lens into the second Wollaston prism which is oriented opposite to the first prism. When the second prism is perfectly aligned with the first one (as in Fig. 1.1) the phase shift introduced to the two wave components by the first prism is cancelled by the second prism, and thus any remaining phase difference is due to the specimen. By sliding the second prism along the direction of shear (perpendicular to the optical axis of the microscope) an additional uniform phase difference between the two components, called the bias retardation, is introduced. Then, the waves are combined to form a single beam and passed through the polarizing analyzer. The combined wave entering the analyzer is elliptically polarized. The interference of the two components results in constructive and destructive superposition of the waves giving rise to the dark and bright areas present in the final DIC image. In other words, a DIC microscope converts phase differences to intensity variations.
Nomarski’s DIC is a wavefront shearing interferometer that allows imaging with large illumination aperture. Larger condenser apertures result in higher lateral resolution and better depth discrimination which have contributed much to the popularity of this modality.

However, despite the many advantages of DIC, standard DIC microscopy has some limitations: (1) DIC images are limited to qualitative and morphological applications, because there is not a linear relation between the intensity and quantitative object’s phase values; (2) DIC images are direction sensitive because the object edges are enhanced along the shear microscope direction; (3) each 2D image is collected by focusing the microscope at a plane of the object is not a representation of that 2D slice of the object, due to the out-of-focus information from other planes.

1.1.1.1 Extension of DIC Microscopy

As mentioned, one of the major disadvantages of DIC microscopy is the limited amount of quantitative information that can be extracted about the imaged sample. Several techniques based on an extension of DIC microscopy have been developed to overcome this limitation [5, 29, 30, 31, 32, 33, 34, 35]. An example of these techniques is the work presented by Cogswell and Sheppard [29], in which a confocal reflected-light DIC microscope is developed to provide an optical sectioning imaging system based on DIC microscopy. In their work, a comparison study between the frequency transfer properties of a conventional DIC microscope and a confocal one for reflection optics is presented. The effect of the shear and the bias on the performance of conventional and confocal DIC microscopy was demonstrated for the cases of imaging both a weak phase specimen and a specimen with a constant phase gradient. This is the first study that quantifies the effect of these system parameters
on the DIC image. Their theoretical comparison of frequency transfer as well as their experimental comparison of DIC images showed that conventional DIC microscopes do not have the same optical-sectioning capabilities as confocal DIC microscopes.

Contributions have also focused on addressing the nonlinearity of DIC intensity images with the object’s phase and the single direction of shear dependence. An approach to solve these limitations, consists of a modified version of DIC that is able to collect multiple images from different shear directions. The set of collected images is then used in conjunction with reconstruction algorithms to compute the object’s phase [30, 31]. Arnison et al. [30] has shown that two DIC images with orthogonal shear directions are necessary and in some cases sufficient to reconstruct object’s phase.

Additions to these contributions include: (1) an iterative phase estimation method developed for reflection DIC, which incorporates the use of an atomic force microscope [32]; (2) a method that applies noniterative deconvolution with an approximate modulation transfer function (MTF) to phase-modulated DIC images [5]; (3) results from a quantitative method, employing phase-shifting techniques that use the Abel transform to numerically integrate linear phase gradients of rotationally symmetric objects with high accuracy [33, 34]. A spiral phase integration (SPI) has been also applied to phase shifted DIC techniques, to obtain gradient information for reconstruction of quantitative phase measurements [35]. The linear phase imaging technique is also implemented using a standard DIC microscope altered to allow controlled phase shifting, a low noise CCD camera, and post-processing in Matlab. The results presented confirm the linear proportionality of intensity to phase in these images.


1.1.2 Quantitative Phase Microscopy

Quantitative phase microscopy modalities we refer to in this section are those that combine the useful qualitative attributes of DIC imaging approaches with the additional advantage of quantitative representation of the specimen’s phase [6, 7, 8, 9, 10, 11, 12]. Images provide phase values that cycle between $-\pi$ to $\pi$, in other words are phase "wrapped". The images of samples with phase values greater than $2\pi$ contain discontinuities at every multiple of $2\pi$ and must be unwrapped to facilitate the extraction and interpretation of the phase information. The unwrapping process requires appropriate sampling and that the noise levels on the sampled signal are low. Appropriate sampling is defined by Nyquist theorem, which requires two pixels of wrapped phase for the quickest $2\pi$ phase change produced by the sample. In the case of one dimensional signals the unwrapping process is easily solved by adding or subtracting $2\pi$ at every discontinuity. However, in the case of two dimensional quantitative phase images, determining appropriate boundaries between the discontinuities along horizontal and vertical directions require the use of more sophisticated unwrapping algorithms. Several algorithms have been developed to execute such unwrapping task [36]. They provide unwrapped phase images, but also require several minutes to unwrap a single typical $640 \times 480$ two dimensional image. Solutions to speed up unwrapping algorithms are being developed, however it is beyond the scope of this thesis to discuss in detail about these approaches. In this work, the $L^p$ norm unwrapping algorithm is used to unwrap the collected quantitative phase images. In Figure 1.2 we show an example of the wrapped and unwrapped images of one of the samples that are used in this work. The figure shows an image of a Poly-MethylMethAcrylate (PMMA) bead immersed in oil, which was collected with the optical quadrature microscopy (OQM) used for our experimental evaluation (OQM...
Figure 1.2: Quantitative phase image of a PMMA bead in oil. (a) Wrapped phase image, (b) unwrapped phase image, and (c) profile taken along the x axis at the middle of the bead.

will be discussed later in this Chapter). The original wrapped phase image is shown in Figure 1.2(a) and Fig. 1.2(b) shows the resulting unwrapped phase image. A plot through the center of the bead shows the phase discontinuities at every multiple of $2\pi$ is shown in Figure 1.2(c).

The phase of a specimen is related to its index of refraction distribution. For example, assuming transmission microscopy, a uniform (with a single index of refraction) transparent (non-scattering) specimen with index of refraction $n$ and thickness $d$ that is immersed in a medium with index of refraction of $n_0$, has a phase $\phi$ defined by

$$\phi = \frac{2\pi}{\lambda} (n - n_0)d,$$  \hspace{1cm} (1.1)

where $\lambda$ is the wavelength of the illumination light. To provide a general idea of the sensitivity of the quantitative phase techniques, phase values in the order of two decimal places can be accurately measured using the most popular quantitative phase techniques. Below we review some of those quantitative phase image techniques.
1.1.3 Optical Quadrature Microscopy

The optical quadrature microscope (OQM) is an interferometer, where the light (coherent light source) that has interacted with the specimen (signal path) mixes with a reference wavefront (reference path) to produce an interference pattern. This pattern consists of the amplitude of the signal multiplied by the cosine of the phase difference between the signal and the reference (which is known) [9]. Figure 1.3 shows a layout of the OQM system.

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. Phase and amplitude images of the sample are generated by applying a reconstruction algorithm to images from the 4 CCD cameras. Assuming we have an
optically transparent sample with a single index of refraction, its thickness can be extracted from the collected phase image and can be easily analyzed by using equation 1.1. However, for a more complex object structure, the interpretation of the phase images need to be done using more rigorous analysis. For example, an object that contains two regions with the same total phase, but different refractive indices or different thickness, can be misinterpreted by considering the phase values only. Thus, prior information about the sample or the application of image processing algorithms will be required to analyze these type of images.

QOM has been used to image mouse embryos images and has been shown to be non-toxic to developing embryo samples, with 2-cell embryos developing to the blastocyst stage after extended imaging exposure [37]. 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 with high accuracy beyond the 8-cell stage [38].

1.1.4 Quantitative Phase-amplitude Microscopy

Quantitative phase-amplitude microscopy (QPM) is based on mathematically derived information about specimen phase modulating characteristics [6]. The implementation of QPM involves the calculation of a phase map from a triplicate set of images captured under standard bright field microscopy. A computational algorithm is applied to the analysis of an in-focus image and a pair of equidistant positive and negative out-of-focus images. The mathematical processes involved have been described in detail elsewhere, but essentially the procedure entail calculation of the rate of changes of light intensity ("transport of intensity" equation) between the three images in order to determine the phase shift induced by the specimen. Both the image acquisition
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and the computational process for QPM can be performed by commercially available hardware software.

1.1.5 Digital Holographic Phase Microscopy

Digital holographic phase microscopy (DPHM) is one of the most established techniques for quantitative phase imaging [11]. It consists of a digital image sensor camera that records a hologram. In general, the hologram is the interference between scattered light from the specimen and the reference plane wave recorded at the Fourier(pupil) plane. The subsequent reconstruction of the object wave is carried out by a computational model (typically on the Fresnel approximation). An advantage of DPHM is that from a single hologram collected at pupil plane, an image for various depths can be computed by applying mathematical reconstruction methods. Other digital holography methods include phase reconstruction from amplitude images at different distances [39] and phase-shifting interferometry [40].

1.1.6 Fourier Phase Microscopy

Fourier phase microscopy (FPM) demonstrated by Popescu et al. [41], uses a programmable phase modulator in the Fourier plane to introduce phase differences between scattered and unscattered light waves. For quantitative phase determination, it records four interferograms with different phase differences and obtains the phase distribution of the sample as in phase-shifting interferometry. However, it requires a sophisticated device for phase modulation, and necessitates at least four interferograms for phase determination.
1.1.7 Hilbert Phase Microscopy

Hilbert phase microscopy (HPM) is similar to DPHM, but it permits direct observation of the specimen because the interference signal is detected at the image plane [42]. In order to obtain quantitative phase information, HPM utilizes the Fourier fringe analysis that enables the measurements of the phase and the amplitude in the spatial frequency domain. Compared to FPM, HPM is faster due to its "single shot" nature, but requires external phase stabilizer because of the separate reference and measurement beam paths.

1.1.8 Phase Shifting Interferometry

Phase shifting interferometry (PSI) is a well known technique in the development of interferometer imaging systems [13, 43]. In this technique, the phase is calculated using a minimum of three images, each having a constant phase that has been introduced to one path of the interferometer. The phase shift must be between 0 and $\pi$ to satisfy Nyquist sampling. The most common PSI technique consists in mounting one of the mirrors of the interferometer on a piezoelectric transducer (PZT) to induce the phase shift. Many techniques acquire the phase-shifted images simultaneously to reduce the error that may be introduced by sample movement.

1.1.9 Tomographic Phase Microscopy

Tomographic phase microscopy (TPM) is a technique for quantitative, 3D index of refraction measurements of cellular organisms with no need for sample perturbation or immersion in special media. The setup is based on a Mach-Zehnder interferometer. A galvanometer-mounted tilting mirror is used to vary the angle of illumination of the
sample, which is positioned between the oil-immersion condenser and objective lenses. Quantitative phase images are then collected at different illumination angles [44, 14]. Finally, the images are processed with a backpropagation algorithm to reconstruct a 3D index of refraction map of the sample (cells or tissue).

1.2 Related Work on Modeling and Reconstruction for Phase Microscopy

There are a variety of different computational models that are used for the design of new phase microscopy modalities or for the validation of the information captured by the imaging system. Simpler computational models can be inverted under some limiting assumptions, to reveal specific information about the imaged sample. On the other hand robust models that are more flexible imposing restrictions about the sample properties (thickness, geometry and index of refraction variation), require extensive computational calculations and their application to inversion techniques are limited in some cases by their computational complexity. In this section we review several basic computational modeling and reconstruction approaches for phase microscopy.

1.2.1 Projection Model

The projection model assumes that light rays propagate straight through the sample neglecting refraction and diffraction. The model assumes there are only phase changes introduced to the propagated light [26, 45]. The phase projection model provides the optical path difference (OPD) of the light propagated through the object. The total OPD is the integral of the index of refraction difference between the object and the
medium \( n_\delta(x, y, z) \) over a distance \( d(x, y) \), which is the total physical thickness of the object along the optical axis:

\[
OPD(x, y) = \int_0^{d(x,y)} n_\delta(x, y, z) \, dz. \tag{1.2}
\]

Although this projection model is basic, it may be used for the modeling and reconstruction of 2D images of quantitative phase microscopy due to its simplicity and speed of execution when implemented in a simulation of an optical system.

### 1.2.2 Ray-tracing Model

The basic ray-tracing model is based solely on geometrical optics to describe how light propagates. The ray-tracing model consists of the application of elementary geometry and the law of refraction; Snell’s law: \( n_1 \sin \theta_1 = n_2 \sin \theta_2 \). The light rays bend where the index of refraction changes. In other words, light being propagated through a sample that is immersed in a uniform medium, refracts at the index of refraction variations introduced by the sample [26]. Figure 1.4 illustrates this situation.

The use of ray-tracing to model light propagation through an optical system takes into consideration the effect of the lens and internal reflection of the imaged sample. As a consequence of these effects, the collected images exhibit a bright boundary at the edges of the imaged sample. This boundary is known as the Becke line. The appearance of the Becke line is related to the focus position of the sample. If the sample is in-focus, the line coincides with the edges of the sample. If the sample is moved out-of-focus, the Becke line moves forward or toward the edges of the sample. This information can be used to model how light propagates at different positions of the optical system through an imaged sample. This model has been used to simulate phase microscopy and unlike the projection model, simulations can be
Figure 1.4: Illustration of a basic ray tracing model for an object with index of refraction of $n_s$ immersed in a medium with index of refraction of $n_m$ (a) for $n_s = n_m$ rays travel straight through the sample (rays don’t refract) and (b) for $n_s \neq n_m$ rays refract at every index of refraction change.

obtained for different focal planes. Kagalwala and Kanade report a computational model of DIC microscopy for reconstruction of 3D samples [22]. The model is a polarized ray-tracing algorithm that includes an approximation of the wave aberration due to diffraction by the lens and the object. The model is then used in an iterative nonlinear optimization to reconstruct the refractive index of the sample. Results and comparisons of simulated to measured images for binary phase objects are presented and have shown that both images share similar content. Results for the reconstruction approach were provided as well.

1.2.3 First Born Approximation Models

The first Born approximation based models work well to account for the diffraction and refraction restrictions imposed by the previously mentioned models. The first Born approximation has been extensively used for modeling microscope imaging systems. Theoretical formulations have been developed for two-dimensional and
Let $u(\vec{r})$ be the total electric field that is being propagated in a scattering medium that satisfies the wave equation

$$\left[ \nabla^2 + k_0^2(\vec{r}) \right] u(\vec{r}) = -o(\vec{r}) u(\vec{r}),$$

(1.3)

where $\vec{r}$ is the three dimensional position and $o(\vec{r}) = k_0^2[n^2(\vec{r}) - 1]$. $k_0$ represents the wave number of the medium and $n(\vec{r})$ is the index of refraction at position $\vec{r}$.

Now, we assume that the total electric field is expressed as

$$u(\vec{r}) = u_0(\vec{r}) + u_s(\vec{r}).$$

(1.4)

where $u_0(\vec{r})$ is the incident field, that corresponds to the electric field without any inhomogeneities and $u_s(\vec{r})$, is the scattered field attributed to the inhomogeneities. Thus the incident field $u_0(\vec{r})$ solves the homogeneous wave equation

$$\left[ \nabla^2 + k_0^2(\vec{r}) \right] u_0(\vec{r}) = 0.$$ 

(1.5)

Substituting equations (1.4) and (1.5) in (1.3), we obtain an expression of the wave equation for the scattered field:

$$\left[ \nabla^2 + k_0^2(\vec{r}) \right] u_s(\vec{r}) = -u(\vec{r}) o(\vec{r}).$$

(1.6)

A solution of (1.6) can be expressed in terms of the Green’s function $g(\vec{r})$, which represents the solution to the wave equation for single point scatter [26]. Thus, under the assumption that the total field is the summation of shifted and scaled version of $g(\vec{r})$, the scattered field is given by

$$u_s(r) = \int g(\vec{r} - \vec{r}^\prime) o(\vec{r}^\prime) u(\vec{r}^\prime) d\vec{r}^\prime.$$ 

(1.7)
Substituting (1.4) in (1.7), the scattered is now written as

$$u_s(r) = \int g(\vec{r} - \vec{r}')o(\vec{r}')u_0(\vec{r}')d\vec{r}' + \int g(\vec{r} - \vec{r}')o(\vec{r}')u_s(\vec{r}')d\vec{r}'. \quad (1.8)$$

The first Born approximation assumes that $u_s(\vec{r}) \ll u_0(\vec{r})$, thus the contribution from the second integral can be ignored [26, 25]. Thus under such assumptions the scattered field in the first Born approximation the scattered field $u_s(\vec{r}) \approx u_B(\vec{r})$ as

$$u_B(\vec{r}) = \int g(\vec{r} - \vec{r}')o(\vec{r}')u_0(\vec{r}')d\vec{r}'. \quad (1.9)$$

Thus, the scattered electric field is presented as a 3D convolution of the object function by the incident field with the Green’s function. We will provide more details about the derivations of the first Born approximation to model the image formation process of an optical system in Chapter 2.

The application of the first Born approximation to model the image formation process on microscopy systems results in a convolution between the point spread function (PSF) of the microscope and the object function. This representation has made the first Born approximation models very popular and desirable for the application of reconstruction techniques. This modeling approach has contributed to the development of a considerable amount of accurate models, especially for DIC microscopy.

Cogswell and Sheppard [29] developed a spatial frequency transfer theory image formation model for DIC microscopy assuming coherent illumination. The model was developed as a part of a comparison study between the frequency transfer properties of a conventional DIC microscope and a confocal DIC for reflection optics. In their model, the effect of the shear and bias components, which are characteristics of DIC were included to analyze the optical-sectioning capabilities of DIC microscopy. Theoretical and experimental results were shown to predict imaging of a weak phase specimen. An extension of Cogswell and Sheppard’s model from coherent to partially
coherent illumination was presented by Preza et al. [21]. A comparison to DIC modeling using coherent, incoherent and partially coherent illumination was presented as well. Preza’s model has also been extended for modeling and reconstruction of “shear-varying” DIC imaging systems. In such cases iterative reconstruction algorithms have been applied to reconstruct quantitative phase information from DIC images. This approach works extremely well with thin objects, but as the authors pointed out, the model loses accuracy for the case of thick objects.

In Bellair’s work [7], a 3D optical transfer function (OTF) was presented based on Streibl’s approach [15] for predicting 2D phase images of a thick object. This method was applied for simulating quantitative phase amplitude microscopy (QPAM) images of thick objects. Such QPAM images are obtained by leveraging the changes introduced in intensity images with small defocus. The phase image predictions were obtained under the assumptions of the first Born approximation. As with Preza’s models, these approaches worked well for thin objects, but have significant limitations when the objects are optically thick or strongly heterogeneous.

1.2.4 First Rytov Approximation

The first Rytov approximation is another solution to the scattered field, that uses the scattered field from Born approximation and is valid under slightly different restrictions [26, 25]. In this approximation the total field is represented as a complex phase function, \( u(\vec{r}) = e^{\phi(\vec{r})} \), where \( \phi(\vec{r}) \) is the total phase and is expressed as the sum of the phase of the incident field \( \phi_0(r) \) and the phase of the scattered field \( \phi_s(\vec{r}) \). Under the assumption that \( (\nabla \phi_s(\vec{r})^2 + o(\vec{r}) \approx o(\vec{r}) \), the first Rytov approximation defines \( \phi(\vec{r}) = \frac{u_B(\vec{r})}{u_0(\vec{r})} \). Thus, the size of the object is not part of the restrictions of this approximation. The only restrictions are based on the variations of the index of
refraction.

The Rytov approximation can be seen as an improvement of the first Born approximation for modeling image formation in quantitative phase imaging [46, 47]. Its use has been reported for the validation of quantitative phase [46] and tomography imaging systems [25, 14]. The application of the first Rytov approximation in image reconstruction has been reported as well. In tomography, the first Rytov approximation is commonly used with back propagation algorithms to reconstruct 3D object properties [48, 49].

Algorithms for phase retrieval based on the first Born and Rytov approximations have been presented [47, 32]. Gureyev shows by a theoretical approach and numerical simulations that, despite the differences in their formulation, the two approximations deliver fairly similar results when used for optical phase retrieval in the near and intermediate fields [47]. The algorithms are applied to derive explicit solutions to phase-retrieval problems in quantitative phase-contrast imaging and tomography.

1.2.5 Higher Order Born and Rytov Approximations Models

Higher order Rytov and Born approximations provide more robust models than those based only in the first approximation. These higher order approximations provide a solution to the wave equation assuming multiple scattering [25, 26]. The solution is usually presented as a special case of the Born or Rytov series to relax the restrictions than the first order approximations impose to the size and index of refraction of the sample.

The higher order Born and Rytov approaches include two type of models: 1. models based on the extension of the first Born or Rytov approximation and 2. hybrid models, which combine the two approximations and holds the restrictions of one of the
approximations while relaxing the restrictions imposed by the other [48, 23, 50, 51]. As an example of hybrid models, Mark’s approach combines the Born and Rytov series [50]. Such hybrid model introduces a parameter that varies for specific cases of the Born and Rytov series and provides a linearized approach that is used for the application of inversion techniques. The reconstruction of the index of refraction of a cylindrical object for tomography imaging is also presented in Mark’s work.

Distorted-wave Born approximation (DWB) and the distorted Rytov approximation (DWR) are derived under the assumption that the index of refraction of the immersion medium is not constant, in contrast to it being assumed for the derivation of the first Born approximation. In other words, the first order approximation assumes that the solution to the wave equation is expressed as a perturbation of a known solution to a simpler equation (homogeneous wave equation); and the distorted-wave approach assumes that the known solution is already perturbed relatively to some simple model. Detail of the DWB and DWR derivations are found in [23].

The use of DWB and DWR approaches is very popular for modeling and reconstruction in OCT and optical diffraction tomography (ODT) imaging systems [52, 53, 51]. For example, in Cheng’s work, a DWB based index of refraction reconstruction method for OCT is compared and contrasted to reconstructions obtained using the filtered backpropagation algorithm (FBP algorithm) [52]. The DWB based inversion algorithm does take into account multiple scattering within the known background. It yields reconstructions much superior to those obtained using the FBP and first order approximations algorithms.
1.2.6 Finite Different Time Domain Model

The finite difference time domain (FDTD) model provides a numerical solution to the Maxwell’s equations in the time domain. The approach consists of a discretization of these equations in space and time, resulting in a set of finite-difference equations [27]. The finite-difference equations are stepped in time and the electric and magnetic field components at each grid point are alternately updated. To prevent artificial reflections along the edges of the grid, an appropriate boundary condition must be employed.

To obtain accurate results for a given wavelength, the grid spacing must be less than the wavelength, typically $\lambda/10$ or smaller. At each grid point, the permittivity and conductivity of the medium is specified. The object is constructed by assigning permittivity values to each object structure. A range of values may be assigned to a particular structure if that structure is inhomogeneous.

In a standard FDTD model, a plane wave is propagated throughout the grid until a steady state solution is obtained. At this point, the electric and magnetic field values are known on the entire grid which lies in the near field. To compute the scattering pattern in the far field, a near field to far field transformation is required. For two-dimensional FDTD this can be accomplished by using the free space Greens function, and integrating over a surface completely surrounding the object [24].

FDTD has been used in a wide range of applications that include simulation of a propagating wave entering the skin and backscattering toward certain direction, scattering from frequency dependent material and light scattering from cells [54, 24]. In Dunn’s work, the FDTD method is applied to compute 3D scatter patterns of cells that contain several organelles [24]. This same approach is used by Hogenboom et al. [55] to model wide-field microscope images. This work also provides modeling of 3D images, consisting of a FDTD computer model to calculate the image at pupil
plane and the Fresnel-Kirchhoff integral to propagate the field at each image plane (z positions). Comparison of a simulated phase image to a measured image was performed.

All previous applications of the FDTD method have reported a high performance of the model. However FDTD requires that the entire computational domain be gridded, and the grid spatial discretization must be sufficiently fine to resolve both the smallest electromagnetic wavelength and the smallest geometrical feature in the model, very large computational domains can be developed, which results in very long solution times. For this reason, the application of FDTD models for inversion techniques is still limited. However there is undergoing research in parallel processing approaches to make the 3D problem more tractable.

1.2.7 Piecewise Constant Based Models and Inverse Solutions

Piecewise constant based models have recently received attention for certain ill-posed inverse problems [56, 57]. Their advantages include implicit imposition of relevant constraints and reduction in the number of unknowns important for ill-posed problems. Its application has been widely reported in tomographic imaging systems [58, 56]. The assumption of a finite number of unknowns and knowledge of the boundary location of the object properties that are being reconstructed makes 3D reconstruction a more tractable problem for tomography imaging reconstruction. Details regarding the application of piecewise constant based models, specifically to find the conductivities and shape parameter in electrical impedance tomography, may be found in [56].
1.2.8 Split-Step Beam Propagation Method

The Split-Step Beam Propagation Method (BPM), also known in the acoustic wave propagation modeling field as the parabolic approximation [26, 59, 60, 61], is a simplified solution to the wave equation, that essentially decomposes the field into a superposition of plane waves. These individual plane waves are propagated through a finite predetermined distance through a wave guide until the point where the field needs to be determined has arrived.

Let’s start with the Helmholtz equation for the electric field $u(\vec{r})$:

$$[\nabla^2 + k_0^2 n^2(\vec{r})]u(\vec{r}) = 0,$$

where $k_0$ is the wave number for free space and $n$ is the index of refraction and then express the electric as

$$u(\vec{r}) = u(x, y, z) = A(x, y, z) \exp(-jn_\delta k_0 z)$$

where $n_\delta(x, y, z)$ is the difference between the refractive indices of the sample and the immersion medium. Equation (1.11) provides a separation of $u(x, y, z)$ in two parts: a slowly varying factor $A(x, y, z)$ and a rapidly varying phase factor $\exp(-jn_\delta k_0 z)$. It is assumed that the wave propagates along $z$ axis with a slowly variation of the phase profile along $x$ and $y$ axis.

Inserting $A(x, y, z) \exp(-jn_\delta k_0 z)$ into the Helmholtz equation (1.10) gives

$$\frac{\partial^2 A}{dz^2} - 2jn_\delta k_0 \frac{\partial A}{dz} + \frac{\partial A^2}{dx^2} + \frac{\partial A^2}{dy^2} + (n^2 - n_\delta^2 k_0^2 A) = 0.$$  

The assumption that $|\frac{\partial^2 A}{dz^2}| << |2n_\delta k_0 \frac{\partial A}{dz}|$, leads to the BPM approximation:

$$\frac{\partial A}{dz} = -\frac{j}{2n_\delta k_0} \left( \frac{\partial A^2}{dx^2} + \frac{\partial A^2}{dy^2} \right) - \frac{j}{2n_\delta} (n^2 - n_\delta^2) k_0 A.$$
The equation (1.13) is discretized and arranged in a simple way, such that the electric field is computed along the propagation direction ($z$).

BPM method is commonly used for the analysis of wave propagation through complex structures (for example Y-Coupler structures) [62], but the BPM has also been applied to model the impulse response of optical systems [12].

### 1.2.9 Other Approaches

Other contributions have focused on the use of multimodality microscopy imaging [38, 63]. These methods have been used for data visualization and to extract the object’s properties. Among the approaches, the use of confocal and fluorescence microscopy in conjunction with phase microscopy or multimodality phase microscopy are of particular importance to this work. The multimodality information is usually applied to extract morphological or quantitative information about live biological samples, that is generally addressed by applying methods based on segmentation and edge detection algorithms to DIC images [38, 64, 65, 66]. For example, a method that combines information of DIC and quantitative phase images (OQM images) to count the number of cells of mouse embryos is reported in [38]. This method extracts the OPD from OQM images and cells boundaries from DIC images to build a cells model.

Other approaches have used confocal reflectance confocal microscopy and quantitative phase images to extract index of refraction values in the imaged object. Confocal images are used to extract 3D morphology [63].
CHAPTER 1. INTRODUCTION

1.3 Mouse Embryo Development

The study of embryo development is important in human reproduction research. Identifying normal and abnormal embryo development is important for the development of new techniques that reduce birth defects and improve the chance of a successful pregnancy [67, 68]. In assisted reproduction techniques such as in vitro fertilization (IVF), clinicians often transfer multiple embryos to increase the chances of including at least one viable embryo that will produce a successful pregnancy. However, this approach often results in multiple pregnancies [69, 70]. Multiple pregnancies increase the risk of complications that include prematurity, low birth weight, congenital malformations, and infant death.

Mouse embryos are one of the most appropriate animal models to understand more about human embryo development. Mouse embryos have several similarities with human embryos; for example: their diameters stay approximately the same size (70-100 \( \mu m \) in diameter) through preimplantation development, early mouse embryo development is very similar to human development and they share similar genetic properties. Development time is slightly different between mouse and human embryos [67, 68, 71].

Mouse embryos have been imaged with OQM and DIC microscopy which have proven to be non-toxic for embryo development. Figure 1.5 shows a mouse embryo

Figure 1.5: DIC images of different development stages of a mouse embryo. Embryo development starts from a single cell in day 1 (a) to a full blastocyst at day 5 (e).
at different development stages. The mouse embryo development process starts with an unfertilized egg (oocyte), the embryo is fertilized at day 1 (zygote, Fig. 1.5(a)), then the nucleus of the zygote divides (cleaves, see Fig. 1.5(b)) into two nuclei, and each single cell divides into two cells on day 2 (Fig. 1.5(c)). The cells continue to divide to reach the 8-cell embryo stage during the beginning of day 3 and a morula (embryo stage after 8-cell) toward the end of day 3, where the mouse embryo contains between 9 and 30 cells and begins compaction (see Fig. 1.5(d)). After compaction, the embryo enters the blastocyst stage on day 4. At this stage the embryo cells are distributed in two regions, the trophectoderm (set of cells forming the outer layer of a embryo) and the inner cell region (cells inside the primordial embryo that will eventually give rise to the definitive structures of the fetus). The portion of the inner structure of the embryo that does not contains cells is called the blastocoele cavity (see Fig. 1.5(e)). 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 [72].

1.4 Contributions of this Thesis

The key contribution of this work is to introduce a computational image reconstruction approach for multimodality phase microscopy. The approach emphasizes two areas: Building the imaging system model and developing an algorithm for the image reconstruction. In this thesis, a phase microscopy model for thick transparent objects used to reconstruct the object’s properties is presented. The model enables the use of 3D DIC images and quantitative phase microscopy to reconstruct an index of refraction map of a thick transparent object. The major contributions of this dissertation
1. The development of an imaging forward model for three-dimensional phase imaging under coherent illumination:


2. The evaluation of that three-dimensional imaging model with thick phantom specimens:


3. A comparison of the model with the most commonly used existing models for imaging thick heterogeneous live biological samples:


4. The reconstruction and identification of the morphology of blastocyst stage mouse embryos using 3D DIC images:

5. The application of inversion techniques to reconstruct the index of refraction of thick heterogeneous objects from simulated quantitative phase images:


6. A methodology to reconstruct an index of refraction map from phase images of thick heterogeneous samples:


1.5 Organization of this Thesis

Chapter 2 discusses the theory behind PSF modeling for transmission quantitative phase microscopy images. A theoretical expression for the total field at image plane is provided for all the models: beam propagation method, first Born approximation, first Rytov approximation and the POC model. Experiments that include simulated and measured images are also reported to understand the strengths and weaknesses of the models. Chapter 3 describes a local entropy based segmentation approach to extract
3D morphology information about a blastocyst mouse embryo. The segmentation approach was applied to 3D DIC images. Validation of the extracted morphology is performed by using the POC forward model and measured quantitative phase images of the embryo. Chapter 4 describes an iterative boundary based approach to reconstruct the index of refraction distribution of thick transparent objects by using multimodality phase images. Finally Chapter 5 provide the conclusions and proposed future directions for extending this work.
Chapter 2

Phase Imaging Model

In this chapter, we describe the formulation of the POC model and show how it relates to the BPM and first Born and Rytov approximations models. We start with a definition of the object model that will be used in the imaging model. Second, we present a brief theoretical description of the first Born and Rytov approximations and then, the details behind the POC model are explained. Finally, we present the derivation of the total field at the image plane for the BPM model. All models provide an expression for the total electric field at the image plane and will be presented using the PSF. We will begin with a calculation of the perturbed field at the image plane using the first Born approximation, and then define the first Rytov approximation at the image plane, which is based on the perturbed field generated by the first Born approximation. We report on comparisons using both computer simulations and measured images. The simulations are performed using thick heterogeneous objects; cases where the Born and Rytov models have well-known limitations.

We also apply the POC approach to model 3D DIC images. The model is based on a 3D PSF that contains optical parameters that are characteristic (shear and bias
CHAPTER 2. PHASE IMAGING MODEL

retardation) of a DIC system. The model is verified by comparing simulated images to measured DIC images. The results show that the POC model captures the behavior of measured images along the optical axis.

2.1 3D Transparent Object Model

We model a 3D transparent object as a stack of parallel transparent slices. Each m’th slice is defined in terms of its index of refraction $n(x, y, z_m)$ and thickness $\Delta z$. A cartoon of our object representation is shown in Fig. 2.1. Observe that the illumination light source propagates perpendicularly through each slice.

A similar object model is presented in [19, 20]. In that approach, the object is also imaged in transmission mode, and refraction, reflection and diffusion effects are neglected. The object is described as a stack of translucent parallel slices, each defined in terms of the transmission and absorption coefficients.

In our approach, the 3D physical object is modeled optically in terms of the index of refraction alone. The index of refraction within the object is expressed in the form $n(x, y, z) \approx n_0 + n_\delta(x, y, z)$, where $n_\delta(x, y, z)$, is the difference between the index of refraction of the object and the immersion medium $n_0$. The optical behavior of every object point is modeled by an exponential complex phase function

$$f(x, y, z) = \exp(jkn_\delta(x, y, z)\Delta z) \quad (2.1)$$

where $k = 2\pi/\lambda$ is the wave vector in free space for wavelength $\lambda$. As noted, absorption and backscatter are neglected.


**2.2 Phase Microscopy Imaging Model**

The imaging system model uses a simple optical system approach as shown in Fig. 2.2. It is composed of a 3D object, a light source, and a lens. The light source is modeled as a coherent plane wave. The plane immediately preceding the lens is referred to as the pupil plane. The image formation model is as follows: light propagates along the optical axis and interacts with the object. After this interaction, the transmitted light is spatially truncated in the pupil plane to represent the finite extent of the imaging lens, and the truncated light then propagates to the image plane.

This image formation process is modeled using a theoretical model of the PSF $h(x, y, z)$. The PSF of an optical system can be modeled in several different forms depending on the system used [16, 15, 17]. We assume the use of coherent transmission optical systems along with a PSF model based on Fresnel-Kirchhoff diffraction [26, 73].

---

Figure 2.1: Cartoon of three-dimensional object description. The object is discretized along the optical axis and the light propagates perpendicularly through each slice. For illustration, a section of seven interior slices, each of thickness $\Delta t$, is shown expanded in the bottom part of the figure.
CHAPTER 2. PHASE IMAGING MODEL

2.2.1 The First Born Approximation

The first Born approximation calculates the total electric field as a superposition of the fields from all of the slices, assuming there is no interaction between them [25]. It models the total electric field at the image plane $z_I$, as the sum of an unperturbed incident field $U_0(x, y, z_I)$, which is the field that propagates through a homogeneous medium, and a perturbed field $U_p(x, y, z_I)$ produced by the heterogeneities in the medium:

$$U(x, y, z_I) = U_0(x, y, z_I) + U_p(x, y, z_I).$$  \hspace{1cm} (2.2)

The total electric field $U(x, y, z_m)$ incident at an object plane $z_m$ is

$$U(x, y, z_m) = U_0(x, y, z_m) + U_p(x, y, z_m),$$  \hspace{1cm} (2.3)

where $U_p(x, y, z_m)$ corresponds to the perturbed field that precedes $z_m$. Thus, the expression for the propagated field through an object slice with thickness $\Delta z$ and location $z_m$ is:

$$U(x, y, z_m + \Delta z) = (U_0(x, y, z_m) + U_p(x, y, z_m)) \exp(jkn_\delta(x, y, z_m)\Delta z).$$  \hspace{1cm} (2.4)

The first Born approximation assumes an optically weak object, which means that $|n_\delta(x, y, z)| << n_0$. Thus the perturbed field is small when compared to the incident

Figure 2.2: Optical transmission system illustration. The light is propagated to image plane using a PSF model defined in terms of the NA of the objective lens. Coherent illumination is assumed.
CHAPTER 2. PHASE IMAGING MODEL

field and its interaction with the preceding object slices can be neglected. Then the total field at \( z_m + \Delta z \) can be expressed in terms of the interaction of the unperturbed incident field with the object slice function:

\[
U(x, y, z_m + \Delta z) \approx U_0(x, y, z_m) \exp(jk\delta(x, y, z_m)\Delta z). \tag{2.5}
\]

The exponential object function can be expanded with a first order Taylor series approximation with respect to \( \Delta z \) giving the following result:

\[
U(x, y, z_m + \Delta z) = U_0(x, y, z_m) + jkU_0(x, y, z_m)n_\delta(x, y, z_m)\Delta z, \tag{2.6}
\]

where \( jkU_0(x, y, z_m)n_\delta(x, y, z_m)\Delta z \) is the perturbed field from the single slice between \( z_m \) and \( z_m + \Delta z \). The perturbed field from the complete object at the image plane \( U_p(x, y, z_I) \) is the coherent sum of the perturbed fields from all of the slices through the object propagated to the image plane. This perturbed field can be expressed as a 3D convolution between \( U_p(x, y, z) \) and the PSF \( h(x, y, z) \) [21]. Thus the expression for the perturbed field at the image plane is:

\[
U_p(x, y, z_I) \equiv U_B(x, y, z_I) \approx \sum_{m=-N}^{m=N} \Delta z \int_{r,s} U_0(\tilde{x}, \tilde{y}, z_m)(jk\delta(\tilde{x}, \tilde{y}, z_m))h(x - \tilde{x}, y - \tilde{y}, z_I - z_m) d\tilde{x} d\tilde{y} \tag{2.7}
\]

where \( \Delta z \) is the thickness of each slice and \( r, s \) define the intervals over which we consider the transverse coordinates \( (x, y) \). The incident field at the image plane also can be calculated using the PSF:

\[
U_0(x, y, z_I) = \int_{r,s} U_0(\tilde{x}, \tilde{y}, z_0)h(x - \tilde{x}, y - \tilde{y}, z_I - z_0) d\tilde{x} d\tilde{y}, \tag{2.8}
\]

where \( z_0 \) is the in-focus plane. Thus, the first Born approximation model calculates the total electric field at the image plane by substituting Eq. 2.7 and Eq. 2.8 into Eq. 2.2.
2.2.2 The First Rytov Approximation

The first Rytov approximation is derived under the assumption that the phase change of the perturbed or scattered field over one wavelength is small [25]. The validity conditions for the first Rytov approximation are slightly different and somewhat less restrictive than the first Born approximation conditions.

The total electric field at the image plane can be represented as a complex phase function:

$$U(x, y, z_I) = \exp(\phi(x, y, z_I)), \quad (2.9)$$

where the total complex phase $\phi(x, y, z_I)$ is the sum of the incident complex phase $\phi_0(x, y, z_I)$ and the perturbed complex phase $\phi_p(x, y, z_I)$. The incident electric field $U_0(x, y, z_I) = \exp(\phi_0(x, y, z_I))$, provides the incident phase function, and the perturbed complex phase is calculated by

$$\phi_p(x, y, z_I) = \frac{U_B(x, y, z_I)}{U_0(x, y, z_I)}, \quad (2.10)$$

where $U_B(x, y, z_I)$ is the perturbed field calculated by the first Born approximation. Thus, the total electric field at the image plane can be obtained

$$U(x, y, z_I) = U_0(x, y, z_I) \exp\left(\frac{U_B(x, y, z_I)}{U_0(x, y, z_I)}\right). \quad (2.11)$$

Equation 2.11 leads to the Rytov approximation imaging model:

$$U(x, y, z_I) = U_0(x, y, z_I) \prod_{m=-N}^{N} \left(1 + \frac{\Delta z \int \int \int_U \left(U_0(\tilde{x}, \tilde{y}, z_m) (jkn(\tilde{x}, \tilde{y}, z_m) h(x-\tilde{x}, y-\tilde{y}, z_I-z_m)) d\tilde{x} d\tilde{y}\right)}{U_0(x, y, z_I)}\right) \quad (2.12)$$

by use of Eq 2.7 and a first order approximation with respect to $\Delta z$ for the exponential term representing the perturbed electric field.
2.2.3 Product of Convolutions Model (POC)

The POC model defines the phase of the total electric field at the image plane as the sum of the phase of the incident field at the detector in the absence of the object \(\phi_0(x, y, z_I)\), and the phase introduced by the object at the image plane \([74]\). We will define this phase function as \(\phi_p^{POC}(x, y, z_I)\) to differentiate it from the perturbed phase \(\phi_p(x, y, z_I)\) defined in Eq. (10) for the first Rytov approximation. For the POC model, the complex phase function \(\phi_p^{POC}(x, y, z_I)\) is the sum of the phase from each object slice after it is propagated to the image plane by a 2D convolution with the PSF.

Let \(g(x, y, z_m; z_I)\) be the representation at the image plane for an object slice \(f(x, y, z_m)\) located at the plane \(z_m\):

\[
g(x, y, z_m; z_I) = \int \int_{r,s} \exp(jkn_\delta(\bar{x}, \bar{y}, z_m)\Delta z)h(x - \bar{x}, y - \bar{y}, z_I - z_m)\ d\bar{x}\ d\bar{y}. \tag{2.13}\]

The POC model assumes that each \(g(x, y, z_m; z_I)\) can be expressed as an exponential complex function:

\[
g(x, y, z_m; z_I) = \exp(\phi_p^{POC}(x, y, z_m; z_I)). \tag{2.14}\]

An expression for the total object phase at the image plane is computed in terms of the phase of each object slice as seen at the image plane. Therefore, from the assumption that each object slice contributes to the total object phase, we can write an expression for the total phase object at the image plane:

\[
\phi_p^{POC}(x, y, z_I) = \sum_{m=-N}^{m=N} \phi_p^{POC}(x, y, z_m; z_I), \tag{2.15}\]

and equivalently an expression for the total object at the image plane:

\[
g(x, y, z_I) = \prod_{m=-N}^{m=N} \int \int_{r,s} \exp(jkn_\delta(\bar{x}, \bar{y}, z_m)\Delta z)h(x - \bar{x}, y - \bar{y}, z_I - z_m)\ d\bar{x}\ d\bar{y}. \tag{2.16}\]
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This approach is similar to a projection approach, in which the total phase is calculated as a ray-casting projection along the optical axis [13, 45]. However, the POC model includes interaction along the lateral axes and diffraction effects in the optical system through the convolution between each object slice and a 2D slice of the PSF.

The total electric field at the image plane $U(x, y, z_I)$ is then calculated as the product of the object function $g(x, y, z_I)$ and the unperturbed incident field $U_0(x, y, z_I)$ at the image plane:

$$U(x, y, z_I) = U_0(x, y, z_I)g(x, y, z_I).$$  \hspace{1cm} (2.17)

2.2.4 Split Step Beam Propagation (BPM) Method

The BPM method discretizes the optical axis just as the POC model does [59, 60, 61]. The object function is defined by equation 2.1) with the assumption of weak scattering and a low index of refraction inside each object slide (similar to the POC model). However, the BPM operates at the object plane; the incident field at each slice is propagated within that slice using the two dimensional Green’s function $G(x, y, z)$ and then is multiplied element by element with the object’s contribution at that slice. Thus, assuming that the object has been discretized in $2N + 1$ planes (as it is assumed above) along the optical axis, the scatter field at the plane $z_{2N+2}$ is given by

$$U_s(x, y, z_{2N+2}) = \int \int_{r,s} U_{2N+1}(\tilde{x}, \tilde{y}, z_0)G(x - \tilde{x}, y - \tilde{y}, z_{2N+2}; z_{2N+1}) \, d\tilde{x} \, d\tilde{y} \times$$

$$\exp(jkn_\delta(x, y, z_{2N+1})\Delta z). \hspace{1cm} (2.18)$$

Once the total object volume has been covered, the field exiting the object is convolved with the PSF of the optical system to propagate it to the image plane. Then the scatter field at the image is given by
\[ U_s(x, y, z_I) = \int \int_{r,s} U_{2N+2}(\tilde{x}, \tilde{y}, z_0) h(x - \tilde{x}, y - \tilde{y}, z_{2N+2} - z_I) d\tilde{x} d\tilde{y}. \] (2.19)

The total field at the image plane is obtained from the product of the scatter field \( U_s(x, y, z_I) \) and the incident field at image plane \( U_0(x, y, z_I) \). The incident field is obtained by using the PSF \( h(x, y, z) \), which is incident field at object plane propagate to the image plane.

### 2.3 Experiments

In this section we compare the POC model to the Born, Rytov and BPM models. First, we provide a numerical evaluation of each image model, identifying the strengths and weaknesses for each, using synthetic images of a simple square object with varying thickness. Second, we simulate images of a spherical homogeneous object using the three models and compare the resulting images to experimental images. Third, we simulate more complex heterogeneous objects and show that the strengths of the POC model make it suitable to simulate images of objects that are similar to experimental images of biological samples. The experimental phase images utilized in the comparisons were generated using the OQM imaging modality [55, 38].

The synthetic phase images were created using the PSF model with the Born, Rytov, BPM and POC models as described in the previous section. The PSF was computed using an illumination wavelength of \( \lambda = 0.633\mu m \) and a 10\times objective lens with a numerical aperture of 0.500. Each set of resultant images was compared to a simulated image using a phase projection model [13, 45]. The phase projection model neglects diffraction and provides the optical path difference (OPD):
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Figure 2.3: OPD profile comparison of a synthetic uniform square object with $n_\delta = 0.035$ taken at the middle of the object. (a) A transverse slice through either object at the center of the glass block, (b) comparison of simulated OPD as a function of position along the x axis at $y = 0\mu m$ for the object with thickness 2$\mu m$ and (c) similar comparison of simulated OPD for object with thickness 35$\mu m$.

$$\text{OPD}(x, y) = \int_0^{d(x,y)} n_\delta(x, y, z) \, dz.$$  \hspace{1cm} (2.20)

The total OPD is the integral of the index of refraction difference between the object and the medium $n_\delta(x, y, z)$ over a distance $d(x, y)$, which is the total physical thickness of the object along the optical axis. This projection will be used as the basis for comparison with the synthesized images.

OQM and simulated images that have phase values greater than $2\pi$ were unwrapped using a 2D $L^p$-norm algorithm [36]. The corresponding OPD images were calculated by dividing the unwrapped phase images by the wavenumber $k$. 

(a)  (b)  (c)
Figure 2.4: OPD comparison from images of uniform square objects as a function of varying thicknesses. The plot shows for each thickness the OPD averaged over the five OPD values closest to the center of the object along a profile taken at the middle of the object.

2.3.1 Numerical Evaluation of the Models Using a Rectangular Solid Object

We simulate two rectangular solid objects to model glass blocks of differing thickness both with an index of refraction of 1.550 and a width of 41 μm, immersed in oil with an index of refraction $n_0 = 1.515$. Thus the difference in index of refraction $n_\delta = 0.035$. The objects have thickness of 2 μm and 35 μm respectively. This experiment demonstrates the accuracy of each model of representing both thin and thick objects. Figure 2.3(a) shows a phase slice through either object geometry at the middle of the glass block. Figures 2.3(b) and 2.3(c) show the profiles of the simulated OPD from the unwrapped simulated phase images at the center of the square object using the projection, POC, BPM, Rytov and Born models. As Fig. 2.3(b) shows, all models are capable of representing with similar fidelity an OPD that matches the projection model for the case of thin objects. However, when the object thickness is larger, as in
Fig. 2.3(c), the OPD representation obtained by the POC and BPM models match the projection model with essentially the same accuracy. The Rytov model shows some degradation in its OPD results, and the Born shows much worse degradation.

Figure 2.4 shows calculations of the OPD values at the center of the square in the xy-plane as a function of the thickness. At each thickness the average of the five OPD values closest to the center of the object was used to provide a better approximation of the central value. It is clearly seen that the POC model obtained the most accurate OPD in cases where the object thickness was large. The Rytov model worked relatively well over this range of object thickness variation, while the limitations of the first Born approximation were again confirmed when the object was optically thick. The POC, BPM, Rytov and Born models had an RMS error of 0.001, 0.003, 0.004 and 0.140 respectively when compared to the projection model.

2.3.2 Comparison of Simulated and Experimental Phase Images from a Uniform Sphere

The second simulated object is an uniform sphere with a radius of 37 $\mu m$ and index of refraction of 1.492 submerged in a uniform medium with index of refraction $n_0 = 1.515$. This provides a difference in index of refraction of $n_\delta = -0.023$. We note that $n_\delta < 0$ induces negative OPD, as the object is immersed in a medium with a higher index of refraction than the background. The optical properties of the simulation were chosen to match those of an experimental poly (methyl methacrylate) (PMMA) bead in oil. A cartoon slice through the object geometry at the middle of the sphere is shown in Fig. 2.5(a). Figures 2.5(b) - 2.5(e) show the OPD images from the simulated unwrapped phase images. Figure 2.5(f) shows the OPD image from a measured OQM phase image of a real PMMA bead which matches the computational model.
Figure 2.5: Simulated and measured OPD images of a PMMA bead in oil. All images are shown with the same scale and contrast. (a) Cartoon of the cross section of the real part of the simulated PMMA bead in oil, (b) Projection model, (c) POC, (d) BPM, (e) Rytov, (f) Born, (g) OQM measured image of a real bead matching the model and (g) plot of the profile of simulated and measured OPD images.
We observe that for Born and Rytov models the maximum OPD values are above −1.00µm and −1.47µm respectively, while the BPM reports a maximum at 1.57 and projection and POC models report a maximum OPD value of −1.67µm. The measured image has a maximum phase value of −1.66µm. These results illustrate that the relative behavior of the models are similar when simulating phase images of a thick PMMA bead as for the thick square object.

2.3.3 Comparison of Synthetic and Measured Phase Images from a Nonuniform Object

A nonuniform object model was created based on previously obtained observations of experimental phase images from one-cell mouse embryos. The object model consisted of a large sphere with a radius of 35µm, containing an off-center smaller sphere with a radius of 12.5µm representing the nucleus (see Fig. 2.6(a)). The center of the smaller sphere was placed 17.5µm to the right of the center of the larger one. The small and large spheres had indices of refraction of 1.37 and 1.35 respectively, again simulating the nucleus and the cytoplasm of the cell [24]. Mitochondria inside the cytoplasm were simulated as small rectangular solid particles, of size 1.5µm × 0.5µm × 0.5µm with index of refraction 1.43, placed randomly inside the larger sphere but outside the smaller one at different orientation angles chosen randomly to be any of the following :\{-\pi/4, 0, \pi/4\} for all \((x, y, z)\) coordinates.

An 9µm wide shell was placed surrounding the larger sphere to simulate the zona pellucida of the embryo, the region surrounding the cytoplasm. The shell had an index of refraction of 1.34, a common value found in literature [24]. The entire object is simulated as being immersed in a medium with index of refraction of 1.33. Figure 6(a) shows a slice through the object geometry at the center of the larger sphere.
Figure 2.6: Measured and simulated OPD images of a 1-cell mouse embryo. All images are shown with the same scale and contrast. (a) Slice of the real part of the synthetic object. (b) Projection, (c) POC, (d) BPM, (e) Rytov, (f) Born, (g) experimental measured OQM image of a typical embryo, (h) profiles taken at the middle of the simulated OPD images and (i) OPD profile taken at the middle of the OQM image.
Figures 2.6(b)- 2.6(f) show OPD images of simulated unwrapped phase images using projection, POC, BPM, Rytov and Born models respectively of the nonuniform object. The OPD of a measured unwrapped phase image from a one-cell mouse embryo again using an OQM is shown in Fig. 2.6(g). The diameter of a mouse embryo is approximately 90$\mu$m, including the zona pellucida, with a corresponding OPD of 2.52$\mu$m.

We observe that simulated images using the Born and Rytov models have maximum OPD values of 0.08$\mu$m and 1.50$\mu$m while the projection, POC and BPM models have maximum OPD values of 2.54$\mu$m, 2.52$\mu$m and 2.45$\mu$m respectively. We plot the profile of the OPD images at the center of the object to better demonstrate our results in Fig. 2.6(h-i). Figure 2.6(h) illustrates the poor accuracy of the Born and Rytov models when comparing these to the projection model, and shows that the POC model gives the best representation of the OPD. The profile of the measured image is shown in Fig. 2.6(i) to illustrate the shape and maximum value of the OPD of the experimental one-cell embryo image.

2.4 Modeling 3D Effects in DIC Imaging of Extended Objects

The product of convolution (POC) model has been used successfully applied to simulate quantitative phase images of thick transparent objects [28]. In previous published work we reported that the POC model is able of accurately simulate 2D DIC images of thick transparent objects [75]. In this section we combine the nonlinearity concepts of the image formation process to model 3D DIC images, specifically the point spread function (PSF) formulation; capturing behavior of measured images along the
optical axis that are not obtained when using first Born approximation models [21]. We verify our model by comparing simulated images to real measured images.

2.4.1 PSF Model for DIC Microscopy

Let $K(x, y, z)$ be the amplitude point spread function of a brightfield configuration to model the DIC PSF, which is defined in [21]. For our simulation, we assume a $45^\circ$ shear orientation to match conditions of measured images. The PSF is defined as

$$h(x, y, z) = (1 - R) \exp(-j\Delta\theta)K(x - \Delta x, y - \Delta y, z) - R \exp(j\Delta\theta)K(x + \Delta x, y + \Delta y, z),$$

(2.21)

where $\Delta x$ and $\Delta y$ are the $xy$-oriented shear and $\Delta\theta$ the bias retardation respectively. $R$ corresponds to the amplitude ratio and $K(x, y, z)$ is the amplitude PSF for transmitted-light optics under coherent illumination.

A simple Gaussian beam model can be used to model the $K(x, y, z)$ function

$$K(x, y, z) = \sqrt{\frac{2P}{\pi w^2}} \exp\left(\frac{x^2 + y^2}{w^2}\right) \exp\left(i\frac{x^2 + y^2}{\lambda\rho}\right) \exp(ikz),$$

(2.22)

where the beam diameter $w = w_0\sqrt{1 + \left(\frac{z}{b}\right)^2}$, $w_0 = \frac{\lambda}{\sin(NA)}$, NA represents the numerical aperture of the objective, the radius of curvature $\rho = z + \frac{\rho_i}{z}$ and $b = \frac{\lambda w_0^2}{\lambda}$ the Rayleigh range. An XZ slice of the imaginary part of $K(x, x, z)$ calculated with equation (2.22), is shown in Fig. 2.7(a). For calculating $K(x, y, z)$ a $10\times$ objective lens with a $NA = 0.75$ were assumed. Figure Fig. 2.7(a) shows symmetry along the $Z$ axis.

Then if we add the Gouy phase term $\psi$ where $\tan(\psi) = z/b$ to the phase of $K(x, y, z)$ in previous equation, we have

$$K(x, y, z) = \sqrt{\frac{2P}{\pi w^2}} \exp\left(\frac{x^2 + y^2}{w^2}\right) \exp\left(i\frac{x^2 + y^2}{\lambda\rho}\right) \exp(ikz) \exp(i\psi).$$

(2.23)
Figure 2.7: XZ section image from a simulated Gaussian beam. (a) Imaginary part of the Gaussian beam without the Gouy phase term. (b) imaginary part of the Gaussian beam with the Gouy phase term.

Figure 2.7(b) shows an XZ slice of $K(x, y, z)$ calculated with equation (2.23) where the symmetry along Z axis with respect to $Z = 0$ does not appears due to the introduction of the Gouy term.

The introduction of the Gouy phase is what makes a Gaussian beam differ from a plane wave with the same optical frequency. The Gouy term changes the phase as the wave passes through the focus when compared with a plane wave.

In the next section we show DIC simulated images for a transparent thick object to visualize the effect of the Gouy phase term.

2.4.2 Simulations

The objective of these experiment is to study the ability of the POC model to mimic DIC images of a thick transparent object. We also analyze the effect of adding the
Figure 2.8: 2D DIC simulated and measured images from a PMMA bead in oil: (a) measured DI image, (b) simulated image without Gouy phase, (c) simulated image with Gouy phase.

Gouy phase to a simple Gaussian model used to calculate the 3D DIC PSF. First we simulate 2D DIC images for a uniform sphere object. Then we calculate a 3D image.

The simulated object is a uniform sphere with a radius of $37 \mu m$ and index of refraction of $1.492$ submerged in a uniform medium with index of refraction $n_0 = 1.515$. This provides a difference in index of refraction of $n_\delta = -0.023$. The optical properties of the simulation were chosen to match those of an experimental poly (methyl methacrylate) (PMMA) bead in oil.

In the 2D DIC image shown in Fig. 2.8(a) from a PMMA bead immersed in oil we can see the effect of the diagonal shift introduced by the microscope system. The directionality of the shift is controlled by the microscope’s shear direction. The phase shift (bias) is chosen, so a small change in phase will cause the largest variation in intensity, making maximum use of the dynamic range of the system. Figures 2.8(b) and 2.8(c) show simulated images using equation (2.21) with equations (2.23) and (2.22)
Figure 2.9: A XZ slice of the PMMA bead image: (a) XZ slice of a measured DIC image, (b) XZ simulated image with POC model introducing the Gouy phase, (c) XZ simulated image with POC model without introducing the Gouy phase.

respectively. A $10 \times 0.75$ NA objective, bias equal to 1.57 radians and a shear of 2 $\mu m$ were assumed.

The comparison of 2D simulated images to a measured DIC image of a thick object shows a good quality agreement of how the model capture patterns that are part of measured DIC images. Now we repeat the previous simulation for the system focused at different planes along the object and compare to measured images. A XZ slice of simulated and measured images are presented in Figures 2.9(a) - 2.9(c).

### 2.5 Final Remarks

The results when calculating the OPD of the simulated images show that the POC model is capable of representing phase images of thick objects without suffering a degradation in accuracy for thick objects and large index of refraction variations, which limit the accuracy that can be obtained from the Born and Rytov models. The
results also show that information about the object shape is detectable in simulated images with Rytov, BPM and POC models. We have illustrated the ability of the POC model to represent the phase image of an inhomogeneous object with reasonable fidelity under real conditions where both the Born and Rytov models failed.

BPM introduces changes in phase along the optical axis as light propagates through
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the object by multiplying fields, as the POC method does; and the exiting field (electric field right after the object) is convolved with the PSF of the optical system to propagate it to the image plane. As consequence of this approach, the diffraction effect from the objective lens is introduced only once, operating on the whole object when calculating the final propagation to the image plane. On the other hand, the POC model introduces the diffraction effect on each slice independently when it is being propagated to the image plane. The total object representation a the image plane is obtained as the product of all the propagated slices.

As mentioned, the BPM method requires plane-by-plane propagation (using function G as shown in Eq. 2.18) to the final plane of the object to then make a final propagation to the image plane. This propagation involves rapid changes of the propagator function that have an impact on the image, specifically at the edges of the object. Since the propagation in the POC model is done independently for each object slice (object plane to image plane), the propagation effect is already considered in the PSF model of the system. Our simulations indicate that there are some differences between the POC and BPM methods in their ability to accurately represent sharp edges of optically thick objects, as well as their relative sensitivity to arbitrary modeling decisions such as the edge of the computational object volume.

The accuracy of the POC and BPM models for predicting phase images depends on the resolution of the discretization routine. Since each object slice is considered to be uniform in z when calculating the image, rapid changes in the index of refraction along the optical axis might produce a highly inaccurate image representation if the z-axis discretization is not fine enough to capture these variations. Another limitation of the POC and BPM models is that the backscattered field is not considered; only the contributions that travel in the forward direction through each slice are taken
into account.

We have developed the modeling approach under the initial assumptions that the imaged objects are transparent samples (phase objects) and that the refraction effect is neglected in the image formation process model. An area to explore in future work would the incorporation of attenuation in the object model and the effect of refraction in the image model.

After validating the capability of the PSF model to represent 2D DIC images of a thick homogeneous object, we observe that the asymmetry along the optical axis is not a characteristic of DIC microscope or the imaged object properties. Including the Gouy phase term in the PSF function allows to us visualize the difference of the waves propagated above and below \( Z = 0 \). This difference affects the calculation of the simulated image when each slice of the object is convolved with a slice of the PSF and then interact with each other to obtain an image from the total object. To calculate 3D images the object moves along optical axis to change the object plane in focus. The asymmetry along the optical axis in the PSF introduces an asymmetric effect in the 3D simulated images. The simulated image has a good agreement with the measured image, capturing the object shape and the appearance along the optical axis. Figure 2.4.2 shows slices above and below of \( Z = 0 \) plane of the the 3D simulated and measured images. We observe that the model captures the inverse effect of the brightness observed in the measured image. As we mentioned above we are able to capture this behavior due to the phase delay introduced by the Gouy phase term. We are able to obtain the same behavior when using a different model for \( K(x, y, z) \) such as those based on Fressnel-Kirchhoff diffraction is the phase delay is introduced correctly.
We have shown that POC method is capable of modeling DIC images of a homogeneous thick transparent object. It has been shown that the introduction of the Gouy phase term to the Gaussian model is necessary to produce accurate models for DIC images. The asymmetry of $xz$ along $z$ axis that is present in measured DIC images, is captured in the simulated images as well.
Chapter 3

Extracting Information from Phase Images

The extraction of 3D morphological information about thick objects is explored in this chapter. We extract this information from 3D DIC images by applying a texture detection method. Texture extraction methods have been successfully used by different applications to study biological samples. A 3D texture image is obtained by applying a local entropy based texture extraction method. The use of this method to detect regions of blastocyst mouse embryos that are used in assisted reproduction techniques such as IVF is presented as an example. Results demonstrate the potential of using texture detection methods to improve morphological analysis of thick samples, which is relevant to many biomedical and biological studies. Fluorescence and OQM phase images are used for validation.
CHAPTER 3. EXTRACTING INFORMATION FROM PHASE IMAGES

3.1 3D Segmentation and Identification in DIC Images

An advantage of DIC microscopy is its applicability at high numerical apertures. Larger apertures result in higher lateral resolution and better depth discrimination. Additionally, DIC provides high contrast of transparent objects and is sensitive to phase changes. These characteristics allow DIC images to be suitable candidates for the application of texture extraction methods.

Texture extraction methods have been successfully applied to wide field and confocal microscope images of biological samples to both delineate and segment regions of interest. The detection of such regions has been the target of several automatic or iterative algorithmic methods [65, 66, 76, 64, 77]. The use of these algorithms varies with respect to the targeted application and microscope modality.
Previous texture detection methods in DIC microscopy images of biological samples have generally focused on the detection of cells or nuclei. Hamahashi et al. [66] present a method for detection of nuclei in the process of cell division using a set of temporal 3D DIC images of *C. elegans* embryos. In these type of embryos the cell’s nuclei can be easily detected by simply looking at the image. The method combines local entropy and object-tracking algorithms. The nuclei are successfully detected by the use of boundary and texture characteristics. However, in the case of thick complex objects such as mouse embryos, nuclear appearance is not as detectable as in *C. elegans* embryos and in many cases it is undetectable by looking at the image.

The current work is focused on extracting information from a dense z-stack of DIC images using a local entropy based texture detection method. Local entropy texture methods have been successfully used to segment 3D DIC images [66]. The success of the method is due in part to its low sensitivity to out-of-focus effects. In this work we use such sensitivity to follow changes in texture within the 3D positions of the different sample regions, rather than trying to delineate or extract a specific region shape. The extracted texture provides information about the object morphology [71, 67, 68]. A set of 3D DIC images of blastocyst mouse embryos is processed. A blastocyst is composed of a spherical encasement known as the zona pellucida that surrounds and contains the inner structure (see Fig 3.1). The inner structure of the blastocyst consists of three primary regions: inner cell, trophectoderm and blastocoele cavity. Characteristics of the inner regions such as size, expansion, and distribution are used by clinicians to evaluate how well developed the embryo is at a particular time post fertilization [78, 79, 72].

We extract DIC image textures using local entropy concepts to segment the inner cell, trophectoderm and blastocoele cavity regions in the embryo. The obtained
results are validated in two distinct ways, leveraging both fluorescence images and OQM phase images [9, 55, 37]. In a direct approach, we compare our results to nuclei locations obtained through fluorescent staining. All nuclei should be clustered together in the inner cell and trophectoderm regions of the embryo. We also use a model-based validation approach computing 2D quantitative phase images from the reconstructed embryo 3D structure. We assign previously published indices of refraction [24, 80] to the regions obtained from the texture analysis. Then a forward model [28] is applied to estimate phase images. The computed phase images are compared to measured OQM phase images. The texture detection procedure and the two validations methods are applied to DIC images of five different blastocyst embryos.
3.2 Methodology

The DIC images consist of 26 slices collected in $5\mu m$ increments along the optical axis $(z)$ from five blastocyst mouse embryos. The resulting z-stack covers $125\mu m$ along the $z$ axis, which is enough to cover the complete volume of the embryo. Each slice is $640 \times 480$ pixels with a pixel resolution of $0.3\mu m$. Figure 3.2(a) shows DIC images of five different blastocyst embryos and Fig. 3.2(b) shows slices at three representative focal planes ($z = -25\mu m$, $z = 0\mu m$, $z = 25\mu m$), of a single embryo. DIC image stacks are processed with the local entropy method to differentiate regions that contain cells (inner cell and trophoderm) from those that do not contain cells (blastocoele cavity and spaces between cells).

A stack of 26 fluorescence images is collected for each of the five blastocyst samples after applying a Hoechst nuclear stain (see Fig. 3.3(b)). The fluorescent dye (Hoechst 33342) binds fluorophores to the nucleus of a cell, giving more contrast to the nuclei in the embryo image. In practice this stain is not feasible for live embryo applications such as IVF because the Hoechst stain permanently modifies the DNA of the nuclei. Therefore, this stack is used for validation purposes only.

OQM phase images are collected before staining (see Figs. 3.5(a) and 6(b)). OQM is a noninvasive technique that provides amplitude and phase of an optically transparent sample [55, 38]. These phase images were unwrapped with a 2D $L^p$-norm algorithm [36]. Measured OQM phase images were used to validate the detected embryo structure from DIC images.
3.3 Detection of Regions Using Local Entropy

Our region detection scheme is based on differentiating smoothness levels of image texture. The smoothness levels in a 3D DIC image are quantified using local entropy [81, 82]. Local entropy is defined by the entropy of a pixel window surrounding a point of interest within the image [83]. For a rough texture, the local entropy values are high, while lower values correspond to smooth texture regions. Because a smooth texture in a blastocyst DIC image is characteristic of the blastocoele, we expect its local entropy to be lower than that in cellular regions, in which a rough texture is indicative of the organelles in the cytoplasm. Our analysis is focused on the distribution of the cells and blastocoele regions in the embryo. Thus we have removed the zona pellucida from the images by applying an edge detector [83] before computing the local entropy images.

We define texture using local entropy in the image as follows. Let $X$ represent the input image and $W$ an $m \times n \times p$ voxel window. The resulting local entropy image $Y$ is given by:

$$Y_{i,j,k} = -\sum_{l=0}^{N-1} P(l) \log_2 P(l),$$

(3.1)

where $N$ is the number of gray levels in $X$ and $P(l)$ is the frequency of occurrence of gray level $l$ in the window $W$. Each output pixel $Y_{i,j,k}$ contains the entropy value of the $W$ neighborhood around the corresponding pixel $X_{i,j,k}$ in the input image.

A stack of DIC images is segmented by using various window sizes ($3 \times 3 \times 3$, $11 \times 11 \times 3$ and $7 \times 7 \times 3$ voxels) to obtain a several local entropy image. We observe that processed image using the smallest window does not provide defined segmented regions. On the other hand the other two windows show well delineated regions. However, the processed image using the largest window offers blurred boundaries.
Figure 3.3: Validation of detected regions using local entropy method in DIC images using fluorescence images. Images comparison at different focal positions. (a) Local entropy images, (b) fluorescence images and (c) Overlay of (a) and (b). Note that scales are incommensurate in (a) and (b). Legend in (c): Brighter gray indicates common regions of the overlapped images.
Thus, for the processing we selected a $7 \times 7 \times 3$ voxel size window. The selection of the size of the window was also based on information provided in published work that shows that sizes windows greater than $3 \times 3 \times 3$ and less than $20 \times 20 \times 10$ voxels are suggested for detecting textures in DIC images [66].

Each 2D local entropy image at $z_k$ is obtained by using the $z_{k-\Delta z}$ to $z_{k+\Delta z}$ images along the $z$ axis. The top and bottom images of the stack were processed with a $7 \times 7$ pixels size window. As a result of this process, a 3D local entropy image is obtained.

### 3.4 Validation

Two validation approaches are used to test the predicted blastocyst embryo structure. In one, we compare the local entropy image with a fluorescence image of Hoechst-stained nuclei. The images were overlapped to identify common regions. In the other validation method we use the local entropy images to create an embryo model and compute a quantitative 2D phase image. Then the 2D phase image is compared to a measured OQM phase image. Results of these validation methods provide information about the accuracy of the local entropy texture method to predict embryo structure from 3D DIC images.

#### 3.4.1 Local Entropy and Fluorescence Images

Three slices of a collected stack of fluorescence images are shown in Fig 3.3(b). The fluorescence images provide the shape and location of the nuclei. This information is not provided by the local entropy images, which instead highlight the cytoplasm with its subcellular components. Nevertheless, since regions with high local entropy values represent those occupied by volume of the cells, they are expected to be larger
than the brighter regions identified as nuclei of the cells in the fluorescence images. To validate the accuracy of the segmented regions, we calculate the percentage of the fluorescent areas that are outside the high local entropy regions. We expect that since the high local entropy areas contain the fluorescent areas, a highly accurate method yields a low percentage value.

Figure 3.4: Block Diagram of Embryo Model and Phase Reconstruction from DIC images.

### 3.4.2 2D Quantitative Phase Image Estimation

The obtained 3D local entropy image is used in conjunction with our recently published image formation forward model presented in Chapter 2 to estimate phase images [28]. A calculated image is compared to the corresponding measured OQM phase image to validate the detected regions with the local entropy method.

The procedure is described in Fig 3.4. First, we compute a histogram for the local entropy images to select those regions with high, medium and low values. Regions with high and medium values are assumed to correspond to cell regions. Since each cell contains organelles with different optical properties, texture variation occurs in each cell. Then, medium and high local entropy values are assumed to be located inside the cells while low values are in the blastocoele cavity. These three regions get assigned three different index of refraction values to build an embryo model. The indices of refraction assigned to the region of cells are obtained from published cell modeling work [24, 80]. The assigned values are 1.37 and 1.35, where the first
corresponds to the cytoplasm and the second to the indices of refraction average of several organelles inside the cells. We assign an index of refraction value of 1.33 to the blastocoel cavity. Finally we add the zona pellucida which has been removed previously from the DIC image to calculate local entropy image. The zona pellucida is assumed uniform, thus its index of refraction is obtained from the measured OQM of the embryo. The zona pellucida has an index of refraction of 1.34.

We generate a phase image of the synthetic embryo described above using a forward model [28] for thick objects. The calculated phase image is compared to a measured OQM phase image. To evaluate the similarities between these images we use the Dice metric[84]. Let $T_k$ denote a measured image set of pixels in a region $k$. Then the Dice metric for region $k$ is defined as

$$2 \frac{|T_k \cap \tilde{T}_k|}{|T_k| + |\tilde{T}_k|},$$

where $|.|$ denotes set size and $\tilde{T}_k$ is the set of pixels of the calculated image. For the validation we choose a region that contains phase values greater than 15 radians. Since phase values greater than 15 radians do not belong to the blastocoel cavity region the comparison is performed for a region that contains cells in both simulated and measured phase images.

### 3.5 Experimental Results

Local entropy image stacks are computed from DIC image stacks of each of the five blastocyst mouse embryos (see Fig. 3.2(a)). The embryos volume morphology is identified from the information contained in the local entropy images. This information is assessed with the two validation methods described in the previous section. Validation results using fluorescence images are presented in Fig. 3.3, which show three slices of: the generated local entropy images Fig. 3.3(a), the fluorescence images Fig. 3.3(b) and an overlay of both Fig. 3.3(c) for embryo 1. We can observe that the high entropy
regions almost completely contain the cell nuclei area. The percentage of the region identified as nuclei in the fluorescence images that is outside the area with high local entropy is equal to 3%. This indicates that most of the inner cell region is detected as a high entropy region. Table 3.1 shows the percentages results from all embryos.

<table>
<thead>
<tr>
<th>Embryo</th>
<th>ERROR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.1: Validation of local entropy segmentation of DIC versus fluorescence images. Error is the fraction of fluorescence area outside the inner cell region segmented from DIC image.

We can observe that most of the fluorescent areas are contained in the high local entropy region. The reported error values are less than 5% for four of the samples (embryos 1-3,5) and 12% for just one case (embryo 4). If we observe embryo 4 in particular, this sample is reaching the blastocyst stage but still has a very small and not well delineated blastocoele cavity with most of the embryo’s area being composed of the inner cell region. Thus, this case serves to show a near to worst case performance of our approach that reports a 12% of error. These results give us confidence that the method is highly accurate when extracting the blastocoele cavity region.

Estimated and measured phase images are compared quantitatively. The comparison and validation is performed using the Dice metric. The Dice metric is applied to a selected region in the image. Although the same criterion and threshold (15 radians) is used for each image, the size and location of the region varies for different embryo images. Computed and measured phase images for embryo 1 and its corresponding selected region are presented in Figures 3.5. We can observe from the
Figure 3.5: Embryo 1. Comparison of estimated phase image from processed DIC images to a measured phase image. Visual comparison: (a) measured and (b) estimated. Selected regions with phase values greater than 15 radians for quantitative comparison using a Dice metric. (c) Region in estimated image and (d) region in OQM image. Legend: bright values inside the ellipse represent the selected region.

images that the estimated phase closely resembles the measured phase. Results of the Dice metric values for the five embryos are shown in Table 3.2. Again, embryo 4 being the most difficult case showed a Dice value of 0.71 while the rest provide values of 0.80 or more. These results show that the 3D object structure generated with the information extracted from DIC images closely resembles the true embryo structure.
CHAPTER 3. EXTRACTING INFORMATION FROM PHASE IMAGES

<table>
<thead>
<tr>
<th>Embryo</th>
<th>DICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.80</td>
</tr>
<tr>
<td>2</td>
<td>0.93</td>
</tr>
<tr>
<td>3</td>
<td>0.92</td>
</tr>
<tr>
<td>4</td>
<td>0.71</td>
</tr>
<tr>
<td>5</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Table 3.2: Results of Dice metric. Dice metric is used to compare calculated and measured phase images in a selected region. The region corresponds to phase values greater than 15 radians.

3.5.1 Final Remarks

This work has explored the extraction of 3D morphological information from DIC images of thick objects. The application of a texture detection method based on local entropy has been presented as an noninvasive technique to analyze the structure of five blastocyst mouse embryos.

An important advantage of applying this method to DIC images is its low sensitivity to image quality, this allows the extraction of information about the morphology of a thick object. In conclusion, detecting the blastocoele and cell regions at the blastocyst stage using the local entropy method contributes to the understanding of the overall embryo morphology. This can positively improve the analysis of embryo development for assisted reproduction techniques such as IVF.

The local entropy algorithm can be enhanced by including a thresholding mechanism to have more control in the tuning of the processed images. For example, we specify three levels: low, medium or high local entropy thresholds to enhance the detection of different regions of interest. Additional work must be completed to provide a more general criterion to select a window size that improves the ease of interpretation of the results. A detailed analysis of the effectiveness of different texture
extraction methods could increase the accuracy of this approach as well.
Chapter 4

Image Reconstruction Using Boundary Detection Constraints

In chapter 2 we have presented a forward model for phase microscopy to predict phase images of thick complex objects [28]. In this chapter, we explore the use of this model to reconstruct an index of refraction map of transparent objects by applying inversion techniques. Inverse solutions based on shape constrained have recently received attention for certain ill-posed inverse problems. Their advantages include implicit imposition of relevant constraints and the reduction in the number of unknowns [56, 57, 85], which is important in ill-posed problems.

Our approach is based on the assumption that the index of refraction inside the object can be approximated as piecewise constant. Boundary locations of all inhomogeneities are extracted from one of the imaging modalities. In particular, we process 3D DIC images to obtain boundary information about the structure of the object by applying image processing techniques on a dense z-stack of DIC images. This information is then incorporated into an iterative shape constrained inversion algorithm to
reconstruct the index of refraction map from an OQM phase image. In the following section we discuss in detail how the inverse solution is obtained.

4.1 Inverse Solution

With our assumption of a piecewise constant index of refraction profile, the inverse problem can be treated as a nonlinear optimization problem to estimate a limited number of parameters. The ability to find the optimal index of refraction values depends upon the definition of the error function and the signal-to-noise ratio (SNR). Let \( \phi(x, y, z) \) be the phase at pixel \((x, y, z)\) computed as a function of a putative set of object indices of refraction encountered during the inverse search and \( \phi_m(x, y, z) \) be the experimentally measured phase. We minimize the average quadratic error cost function \( E = \sum_{x,y,z} (\phi(x, y, z) - \phi_m(x, y, z))^2 \) on a large-scale algorithm available in the MATLAB Optimization Toolbox [86]. Specifically, we used the implementations of \textit{lsqnonlin()} for the large-scale algorithm.

The iterative algorithm involves the computation of an approximate solution to a large linear system using a preconditioned conjugate gradient method. This algorithm is based on the reflective interior Newton method and consists of finding local solutions to the quadratic error function assuming an initial estimate to the solution that is being improved on the trust-region (bounded region). This means that the solution to the minimization problem is the solution to the subproblem inside the trust-region. Details about how to define the initial parameters and the trust regions are described in [87].

We use an iterative approach to estimate a set of refractive index values as illustrated in Fig. 4.1. We start with a set of initial guesses \( n_1, n_2, n_3, ..., n_m \). After
initialization, we assign these values to object regions and run the iterative algorithm to update the index of refraction values. After these steps, if the error cost function is less than a threshold, the algorithm stops; otherwise we iterate all the steps. In the iterative algorithm the images are unwrapped before calculating the error. Since there is always some noise in the data due to measurement noise and numerical error in the unwrapping algorithm, to prevent the inverse solution from giving us unrealistic values, we need to constrain the object region before calculating the error. For example, for the comparison we have selected regions with no overlapping objects. A particular concern is that the unwrapping algorithms could fail when objects overlap each other or present abrupt phase changes. Thus, we selected regions with no overlapping objects for the comparison.
CHAPTER 4. RECONSTRUCTION USING BOUNDARY CONSTRAINTS

4.2 Experiments

We report on using images from both biological and inert objects. First, we provide a numerical evaluation of the reconstruction of the index of refraction values using synthetic images of spherical objects. The objects consist of spheres with different refractive indices submerged into a uniform medium. Second, we apply the reconstruction approach to measured images. The first set of measured data is collected from three overlapping glass beads submerged in a uniform medium. The beads are imaged with both DIC and OQM modalities. A set of 20 slices is collected with an increment of 5µm along z axis. Figure 4.2(a) shows DIC images collected at three z positions (z = −5µm, z = 0µm, z = 10µm). Fig. 4.4(b) shows the unwrapped OQM image.

The second set of DIC images consists of 25 slices collected in 5µm increments along the optical axis (z) from a blastocyst mouse embryo number one from Chapter 3. The resulting z-stack covers the complete volume of the embryo. Figure 4.3 shows 25 DIC slices of a single embryo. A quantitative phase OQM image was collected as well (see right side of Fig. 3.5(a)). The phase image was unwrapped with an 2D $L^p$-norm algorithm [36].

4.2.1 Extraction of the Object’s Boundaries

We apply image processing techniques to extract the morphology of the imaged sample. The selection of the technique to be applied depends on the type of samples. We define two groups of samples: uniform and nonuniform objects. In uniform objects, intensity changes in DIC images occur only at the boundaries of the objects, due to the DIC imaging mechanism. Thus, the object boundaries can be easily visualized
CHAPTER 4. RECONSTRUCTION USING BOUNDARY CONSTRAINTS

Figure 4.2: Collected DIC images of glass beads in oil. (a) DIC images at three different focal planes and (b) processed DIC images using an edge detection algorithm in DIC images and detected with a simple edge detection algorithm. Figure 4.2(b) show three slices of the processed DIC images with an edge detection algorithm for the three glass beads in oil, described above.

For nonuniform objects, the morphology detection scheme is based on differentiating smoothness levels of the texture of the image. The smoothness levels in a 3D DIC image of a mouse embryo are quantified using local entropy [81] (applying the approach presented in Chapter 3). Local entropy is defined by the entropy of a pixel window surrounding a point of interest within the image [81]. For a rough texture, the local entropy values are high, while lower values correspond to smooth texture regions. In our example blastocysts consist of two regions: a blastocoele with smooth texture and cellular regions with rough texture. We expect local entropy to be lower in the blastocoele region than that in cellular regions in which the rough texture is indicative of the organelles in the cytoplasm. The uniform ring (zona pellucida) that surrounds the embryo volume is extracted by applying an edge detector before computing the local entropy images.
Figure 4.3: Visualization of 25 2D DIC images. Each slice was collected with an interval of 5 microns along the \( z \) axis. In the figure, the \( z \) position increases row-wise from top left to bottom right. The figure at the top left corresponds to the slice taken at \( z = -60\mu m \) and the figure at the bottom left of the image corresponds to the slice at \( z = 60\mu m \).

4.2.2 Results

4.2.2.1 Numerical Evaluation of the Reconstruction of Synthetic Images of Spherical Objects

We have simulated OQM phase images of transparent spheres with different radii placed at different positions (\( z \) overlap). Each object has five slices along the optical
axis. Five experiments of increasing complexity are conducted. The reconstruction of the indices of refraction is performed first for a scene composed of one sphere. Then we added from one to four more spheres to the scene to increase the number of indices of refraction to be reconstructed. The five spheres are assumed to have an index of refraction of $n_1 = 1.02$, $n_2 = 1.03$, $n_3 = 1.04$, $n_4 = 1.05$ and $n_5 = 1.06$; and the immersion medium has a index of refraction of 1.0. Figure 4.5 shows $xy$ slices for each of the five different objects.

The reconstruction approach (Figure 4.1) assumes that the object boundaries are known and the index of refraction $n_1$, $n_2$, $n_3$, $n_4$ and $n_5$ are unknown. The algorithm initializes with set of refractive indices equal to $n_1 = 1.07$, $n_2 = 1.09$, $n_3 = 1.08$, $n_4 = 1.01$ and $n_5 = 1.1$. The unknowns are found in less than 25 iterations for all the five objects.
4.2.2.2 Refractive Indices Reconstruction of Three Glass Beads in Oil from Measured Phase Images

We used the information extracted from DIC images to provide the boundary of the imaged objects (from applying an edge detector algorithm). For the glass beads in oil the edges provide the localization of the center of each bead along \( x, y \) and \( z \) axes. Since the edges are clearly visualized and the beads have spherical shape, the centers and the radii of the bead can be calculated. The prediction of the object morphology is obtained analytically from these parameters using the sphere volume equation.

The goal is to find the three unknown indices of refraction \( n_1, n_2, \) and \( n_3 \) of the three beads. The three glass beads have the same index of refraction of \( n_0 = 1.5380 \) and the uniform medium where the beads are submerged was filled with an index of refraction of \( n_0 = 1.515 \). We consider two cases for the reconstruction: case 1: initialization of the algorithm assuming that \( n_1 = n_2 = n_3 = 1 \) and case 2: initialization with three different indices \( n_1 = 1.0100, \) \( n_2 = 1.0200 \) and \( n_3 = 1.030 \). The reconstructed values in case 1 were \( n_1 = n_2 = n_3 = 1.5380 \), and for case 2 the obtained values were \( n_1 = 1.5380, \) \( n_2 = 1.5378 \) and \( n_3 = 1.5381 \). In both cases, the algorithm converged in less than 20 iterations. We can see from these results that the reconstructed indices of refraction closely resemble the true values. The results show that the proposed method is able to reconstruct the indices of refraction of spherical objects using DIC.
and OQM images.

4.2.2.3 Refractive Indices Reconstruction of a Blastocyst Mouse Embryo from Measured Phase Images

In this experiment the goal is to reconstruct the refractive indices of the three regions of a blastocyst mouse embryo. The three regions represent high, medium (region of cells) and low local entropy (blastocoele cavity) that are obtained by applying the local entropy based approach to a 3D DIC image of the embryo (more details are provided in the Chapter 3 of this thesis). The regions were defined by using a histogram of the local entropy image. A thresholding shaped based method is applied to the histogram to select two thresholds $th_1$ and $th_2$ to segment the local entropy image in three regions. (see Figure 4.7). The location of the thresholds is selected manually at the locations where the histogram exhibits points of inflection. For the case where two distribution are overlapped (as the case of $th_2$), the threshold location is placed at the middle of the two maximum values of the distributions. For this embryo the thresholds locations were obtained by averaging the obtained thresholds of values of the histograms of the local entropy images obtained from DIC images of five blastocyst mouse embryos (see Fig. 3.2(a)) that were used in the segmentation approach presented in Chapter 3.

Figure 4.6 shows an overly of the DIC and segmented images. For the reconstruction approach the initial refractive indices values are: $n_1 = 1.1$, $n_2 = 1.2$ and $n_3 = 1.3$. The algorithm converged at iteration 36. The obtained index of refraction values were equal to $n_1 = 1.33$, $n_2 = 1.35$ and $n_3 = 1.38$. From information provided in Chapter 3 the predictable index of refraction values are $n_1 = 1.33$, $n_2 = 1.35$ and $n_3 = 1.38$. The total root mean squared error for the reconstructed indices is 0.0058.
In order to observe the behavior of the method for a different set of initial index of refraction values, we perform the same experiment changing the order of the initial set of assigned values such that: $n_1 = 1.3$, $n_2 = 1.1$ and $n_3 = 1.2$. The obtained solution is equal to $n_1 = 1.33$, $n_2 = 1.35$ and $n_3 = 1.38$ and the convergence occurs at iteration 36 as well.

4.2.3 Final Remarks

In this work we introduced a constrained boundary method that combines DIC and OQM phase modalities to reconstruct the morphological structure and indices of refraction of thick transparent objects. The algorithm presented in Fig. 4.1 is capable of finding the indices of refraction of several spherical objects from simulated and measured images. The results obtained so far are promising. Although we have been able to recover a good approximation of the morphological structure and index of refraction of biological samples as in the case of mouse embryos, we still need to extract more information about the internal components of these samples to validate a reconstruction of the indices of refraction. As future work, we are considering experiments increasing the complexity of the object’s structure. In addition we would like to complete our on-going analysis of the limitations and advantages of the method.
Figure 4.6: Visualization of detected regions using local entropy method in DIC images. An overlay of the local entropy (after thresholding) and DIC images. Each slice was collected with an interval of 5 microns along the $z$ axis. In the figure, the $z$ position increases row-wise from top left to bottom right. The figure at the top left corresponds to the slice taken at $z = -60 \mu m$ and the figure at the bottom left of the image corresponds to the slice at $z = 60 \mu m$. 

Figure 4.7: Histogram of the 3D local entropy image of all five embryos. We have selected the threshold based on the distribution shapes. These thresholds (th1 and th2) divide the local entropy values in three groups. These groups are used to define high, medium (cells regions) and low (blastocoele region) local entropy regions for validation purposes.
Chapter 5

Conclusions and Suggested Future Work

In this thesis, we have presented a model-based approach to improve the ability to extract information from phase images: an index of refraction reconstruction method for three-dimensional DIC images and quantitative phase images. Toward this end, the emphasis of the work presented in this dissertation has been in two areas: 1. model development for three-dimensional phase imaging; and 2. development of a reconstruction method for multimodality phase imaging. In this chapter we summarize the main results of the dissertation, review its contributions, and discuss directions for future work.

5.1 Modeling Phase Imaging

In Chapter 2, a general model for three-dimensional phase imaging under coherent illumination (POC) is derived for the case of imaging thick transparent objects.
Because in practice, the analysis of thick objects is required in several biological applications, our model is tested by simulating thick complex objects. Thus, the major contribution of this chapter is the development of a forward model that works well for thick complex objects.

One of the primary benefits of the POC model is its ability to model phase images of thick transparent objects that have similar characteristics to biological samples, without extensive computational calculations. The POC model is based on the use of the PSF of the optical system and the object’s phase changes; both PSF and object models are combined in a nonlinear way to model a phase image.

The future work we suggest for this area is to extend the model to allow the use of coherent and incoherent illumination, specially considering possible modifications to the PSF. This extension would allow the use of POC for other imaging scenarios such as DIC images under partially coherent illumination.

5.2 Model Validation and 3D Segmentation

In Chapter 3, the validation of the developed POC model has been performed using a local entropy based segmentation method. Three-dimensional DIC images of mouse embryos at blastocyst stage are segmented to extract morphology information about the embryo. Then, the morphology is used in conjunction with the POC model to estimate a quantitative phase image. The main contribution of this approach is the introduction of an image processing technique for a real biological application. The model has been tested with a real thick biological sample as well. Extensions to this work include the use of image processing algorithms and the POC model to extract morphological information about other biological samples; mouse embryo at different
5.3 Reconstruction Approach for Multimodality Phase Microscopy

Application of reconstruction techniques to phase microscopy to improve the ability of extracting information from phase images was presented in Chapter 4. Additional information has been extracted from the images without modifying the hardware of the optical system. The approach consists of: (a) the application of reconstruction techniques to phase images and (b) the extraction of the index of refraction and morphology distribution of the imaged objects. In other words, we have presented an inversion solution by applying an iterative reconstruction algorithm that uses a constrained-boundary-based method. The method works under the assumption of prior knowledge about the object’s boundaries. Thus, the numbers of unknowns and computational calculations can be reduced.

We have presented a constrained boundary method that combines DIC and OQM phase modalities to reconstruct the refractive index of thick transparent objects. The method consists of an iterative approach that initializes the reconstruction assuming the morphology of the object can be extracted from DIC images. Experiments for thick objects that have variations of the index of refraction and morphology distribution along the z axis have been performed to study the accuracy of the method. The main contribution of this approach is the introduction of a noninvasive method to extract quantitative information about thick transparent objects by using DIC, which is the one of the most common modalities to image biological samples. Future work includes the application of the reconstruction approach to different biological
samples. Sensitivity to errors in the geometric model is a relevant analysis that might be conducted as well.

5.4 Analysis of 3D Quantitative Phase Images

Quantitative phase images provide valuable information about the imaged sample, however 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. An approach to analyze phase images from thick samples with phase changes greater than $2\pi$ is to convert them to optical path difference (OPD). The OPD image provides a numerical values (in $\mu m$) relative to the physical thickness and index of refraction of the different structures that form the object. It is important to note that ambiguities also exist between the index of refraction and thickness of the sample. From the definition of OPD, an object with a certain index of refraction and thickness will appear the same as another object with half the thickness and twice the index of refraction. The use of the 3D quantitative phase images has been also limited by the ambiguities introduced by the unwrapping algorithms. As future work we propose an analysis of the behavior of unwrapping algorithms in simulated and measured 3D phase images. Finally, the study of possible solutions by modifying the unwrapping algorithms after identifying the cases where the algorithms fail is also proposed.
Bibliography


