LIGHT AND ULTRASOUND - ULTRASOUND TAGGING OF LIGHT FOR IMAGING BEYOND THE SCATTERING LIMITS

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Abstract

In optical imaging, the depth and resolution are limited due to scattering. Unlike light, scattering of an ultrasound waves in tissue is negligible. Hybrid imaging methods such as ultrasound modulated optical tomography (UOT) use the advantages of both modalities. UOT tags light by inducing phase change caused by modulating the local index of refraction of the medium. The challenge in UOT is detecting the weak signal. The displacement induced by the acoustic radiation force (ARF) is another ultrasound effect that can be utilized to tag the light. It induces greater phase change, resulting in a stronger signal. Moreover, the absorbed acoustic energy generates heat, resulting in a change in the index of refraction and a strong phase change. Since the speckle pattern is governed by the phase of the interfering scattered waves speckle pattern analysis can obtain information about local displacement and temperature changes in the tissue. We have presented a model to simulate the insonation processes. Simulation results based on fixed-particle Monte Carlo and experimental results show that the signal acquired by utilizing ARF is stronger compared to UOT. The introduced mean irradiance change (MIC) signal reveals both thermal and mechanical effects of the focused ultrasound wave in different timescales. Simulation results suggest that variation in the MIC signal can be used to generate a displacement image of the medium. In addition to the displacement correlated image, the MIC signal can provide images based on the morphology of the tissue. The MIC signal can provide for tumor detection in a healthy tissue.
Chapter 1

Introduction (Optics and Ultrasound)

The challenge in biomedical imaging is to provide images with good contrast and resolution that can help physicians for diagnosis. Most popular biomedical imaging modalities use light or ultrasound or both for imaging. Light provides good resolution and contrast, but the penetration depth is limited [11–14]. Ultrasound can provide images with good penetration depth, however the resolution and contrast is poor compared to optical imaging modalities. Figure 1.1 plots the resolution of different imaging modalities with respect to their penetration depth.

Light has been proven to be a useful tool for medical imaging. It can provide images with high contrast and sub-cellular resolution. However, due to the high scattering of light in tissue, optical imaging depth is limited. Absorption is another factor that can limit the penetration depth of the light [15–18].

Ultrasound provides images based on the mechanical properties of the tissue. An ultrasound wave has the advantage of higher penetration compared to an optical
Figure 1.1: resolution vs. penetration depth for different imaging modalities. CM: Confocal microscopy, OCT: Optical coherence tomography

wave, however, this comes at the cost of losing contrast and resolution. \[19\] \[22\]

Hybrid imaging modalities have been introduced to use the advantages of both good optical contrast and higher penetration of ultrasound waves. Modalities such as Photo Acoustic Tomography (PAT), Ultrasound modulated Optical Tomography (UOT) use both light and ultrasound to provide images beyond the optical scattering limits. \[6\] \[23\] \[32\]

In this work, we introduce a novel method to use the ultrasound wave to tag the light to obtain information about the medium non-invasively for imaging purposes. Acoustic Radiation Force (ARF) speckle pattern analysis employs a similar idea as UOT to tag light and separate the tagged light from the background diffused light. We have shown that the ARF regime can provide a stronger signal compared to UOT. \[33\] \[35\]

Chapter 2 of this work present a review of optical and ultrasound imaging modalities. The advantages and limitations of each imaging modality are discussed. Hybrid imaging modalities such as PAT and UOT are briefly reviewed and the general idea
of the ARF regime is presented.

Chapter 3 discusses different computational modeling techniques that are being used to model the light and tissue interactions. The Finite-Difference Time-Domain (FDTD) technique is compared to the conventional Monte Carlo. At the end of the chapter, fixed-particle Monte Carlo is introduced as an alternative method to reduce the computational expense of FDTD and yet provide for light coherence. The fixed-particle Monte Carlo is used in our work in Chapters 4 and 5 to perform speckle pattern analysis.

Ultrasound tagging of light utilizing the ARF and UOT regimes is discussed in more detail in Chapter 4. The UOT regime utilizes pressure waves to modulate the index of refraction of the medium and ARF regime induce changes in the phase of the scattering waves by applying displacement and temperature rise in the medium. Comparison between the ARF and UOT regimes shows that the signal acquired in the ARF regime is stronger and easier to detect. Phase profile analysis that can be used to model the change in the speckle pattern is presented in this chapter and used in Chapter 5.

The Mean Irradiance Change (MIC) signal is introduced in detail in Chapter 5. Important parameters governing the MIC signal and the insonation processes that induce changes in the MIC signal are discussed. Experimentally acquired MIC signal is presented and explained by the simulation results using the fixed-particle Monte Carlo. At the end of this chapter, the instantaneous change at the beginning of the MIC signal corresponding to the displacement caused by the ARF is used to form a displacement correlated image of the medium.

Chapter 6 shows that the MIC signal is also able to provide images based on the morphology of the medium. Two morphological characteristics of a tumor are used
in simulation to detect a tumor in a healthy tissue. Scatterer size and density in
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Finally, future works have been discussed in Chapter 7. In this work, both sim-
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However in order to move towards clinical applications, an epi-illumination approach
is required. Other potential ideas include utilizing the changes in the MIC signal to
form a thermal correlated image of the medium and utilizing the fixed-particle Monte
Carlo in other speckle pattern analysis methods are discussed.
Chapter 2

Background

2.1 Optical Imaging

As light travels through the tissue it interacts with particles in the tissue. Figure 2.1 shows different types of interactions between light and tissue.

Specular reflection is the part of the light that is reflected from the surface of the tissue due to the index of refraction mismatch and the angle of incidence. As light enters the tissue it undergoes several scattering events. Scattering continues until the light is either absorbed or exits the tissue. The light that exits the tissue is called diffused light. Absorbed light can result in fluorescence emission that can exit the tissue to reach the detection point for fluorescence imaging [15,18]. Different optical imaging modalities utilize different types of light and tissue interaction for imaging. For instance, confocal reflectance microscopy utilizes reflected light coming from the focal plane to form the image. In fluorescence microscopy, image formation is based on absorption of light and the fluorescence emission. Optical coherence tomography utilizes single back-scattered photons for imaging and diffuse optical tomography
Figure 2.1: Different interactions of light and scatterers in the medium. (a) specular reflection; the light that is reflected from the surface of the tissue. (b) scattering; when a photon hits a particle, it scatters and travels in a different direction. (c) absorption; the photon can get absorbed by the particle. (d) Fluorescence; the absorbed photon can generate heat or excite the electrons in atoms and result in fluorescence. The light that multiply scatters in the tissue and exits the medium, can be divided into diffused reflectance (e) or diffused transmission (f).

utilizes diffused light to provide optical properties of the tissue from deeper areas compared to other optical methods [36–39].

Besides scattering, absorption is another factor that can limit the penetration depth of the light. Figure 2.2 shows the absorption coefficient of water, oxygenated and deoxygenated hemoglobin. The therapeutic window shown in Figure 2.2 is defined as the window where absorption in the tissue is low and maximum depth of penetration in tissue can be reached.

In this chapter, several optical and ultrasound modalities will be briefly reviewed with their advantages and disadvantages. Hybrid imaging modalities are modalities that use the advantages of both light and ultrasound for imaging. Our work as an
Figure 2.2: Absorption coefficient of water ($H_2O$), oxygenated (HbO) and deoxygenated (Hb) hemoglobin. Lower absorption of light in the range of 650 nm and 900 nm makes it a popular range for optical imaging and therapeutic purposes. [1]

hybrid imaging modality is briefly introduced at the end of this chapter and later on discussed in more detail in Chapters 4 and 5

2.1.1 Microscopy

Optical microscopy utilizes light to produce a magnified image of the field of view. In conventional wide-field microscopy, the light source illuminates the entire specimen uniformly. The light coming from the specimen is collected by the objective lens to form the image. Since the entire specimen is illuminated by the light source, the image contains both in-focus and background light. In order to enhance image quality, background rejection methods can be utilized. Confocal microscopy is an optical imaging modality that rejects the background light to provide high quality images
of the specimen. On the other hand, fluorescence microscopy utilizes the property of the specimen to collect the light only coming from the part of the specimen that fluoresces, and rejects the background light. \[40\]

Confocal Microscopy

The basic idea of confocal microscopy is to illuminate the area of interest one spot at a time and collect the light coming from that spot. Both illumination path and detection path are focused to a diffraction limited spot, hence it is a confocal system. The collected light provides information about the illuminated spot. To form the image, the spot is scanned over the area of interest. Figure 2.3 show a point scanning confocal microscope. The pinhole in front of the detector is confocal with the focal plane so that it rejects the light coming from above or below the focal plane, i.e. background light to provide optical sectioning \[41–46\].

Although a confocal microscope provides a high quality image, scanning the illumination spot on the specimen is time consuming and the field of view is limited. In order to increase imaging speed, a line-scanning confocal microscope can be used. In a line-scanning confocal system, instead of illuminating a single spot, a light is focused on a line on the specimen. In the detection path, a slit and an array detector replace the pinhole and single point detector. To form the image, the line is scanned in one direction on the specimen. Although this increases imaging speed, a line scanning confocal microscope is only confocal in one direction \[47–52\].

A confocal microscope can be used in both reflection and transmission modes. The resolution is governed by the size of the illuminated spot. In lateral direction the resolution is given by
Figure 2.3: Schematic of a confocal microscope. In the illumination path, the light is focused on the focal plane (in solid green). The focal plane is confocal with the pinhole in front of the detector. The background light coming from above or below the focal spot (in dotted yellow and red) focus on a spot before or after the pinhole, therefore, they get rejected by the pinhole.

\[ r_{\text{lateral}} = \frac{0.6\lambda}{NA}, \quad (2.1) \]

where \( \lambda \) is the wavelength and \( NA \) is the numerical aperture of the system.

In the axial direction, the resolution is defined as

\[ r_{\text{axial}} = \frac{2n\lambda}{NA^2}, \quad (2.2) \]

where \( n \) is the index of refraction of the medium between the objective lens and the specimen.
To obtain a better axial and lateral resolution, a high numerical aperture is required in confocal microscopy. Also, due to high scattering of tissue, depth of imaging in confocal microscopy is limited up to hundreds of microns.

**Fluorescence Microscopy**

Fluorescence microscopy is based on fluorescence or phosphorescence properties of the tissue. Fluorescence is the phenomena where the specimen absorbs light and emit light with a longer wavelength. As shown in Fig 2.4 (a), electrons in the specimen absorb a photon with the excitation frequency $\nu_{\text{ex}}$ and go to a higher energy state. Since the electron is not at its stable state, it goes back to the ground state and releases the energy as a photon with a lower frequency $\nu_{\text{em}}$; ($\nu_{\text{ex}} > \nu_{\text{em}}$) \[53\left[55\right] \).

Figure 2.4: Basic concept of one and two photon fluorescence. When an electron absorbs the energy of a photon, it goes up to a higher energy state. some of the the energy dissipates and when electron returns back to the its ground state, it releases the energy in form of emission. In two photon (or multi-photon) fluorescence, the electron absorbs two (or more) photon simultaneously to go up to a higher energy level.
In order to utilize the fluorescence light and reject background light reflected from/transmitted through the specimen, a dichroic mirror and/or an optical filter can be used. Figure 2.5 shows a schematic of a fluorescence microscope.

![Figure 2.5: Schematic of a fluorescence microscope. The dichroic mirror reflects the excitation light to the specimen. The absorbed light causes fluorescence with a longer wavelength. The fluorescence light passes through the dichroic mirror to reach the detector to form the image.](image)

In Figure 2.5, the excitation light is reflected towards the specimen by the dichroic mirror and the fluorescence light passes through the dichroic mirror and emission filter to the detector.

Multiphoton microscopy is a type of fluorescence microscopy where the specimen absorbs multiple photons (typically two or three photons) to raise the material to the excited state. The energy absorbed is two or three times the energy of the excitation photon \( E = h\nu \), as shown in Figure 2.4 (b). The emitted light has a higher frequency compared to the excitation. In order to acquire multiphoton signal, a high concentration of energy is required, therefore pulsed lasers are utilized. Since
the peak intensity occurs at the focal plane, multiphoton signal is obtained only from the focal plane, \textit{i.e.} no background signal. Hence multiphoton provides optical sectioning \cite{56,57}.

Resolution and penetration depth of a fluorescence microscope are comparable to a confocal microscope and are governed by the NA of the imaging system.

### 2.1.2 Optical Coherence Tomography

Optical Coherence tomography (OCT) is an imaging modality that is based on low coherence interferometry. The illumination light is split into two, a reference arm and a sample arm. The light in the sample arm interacts with the sample and reflects back. The light from the reference arm is also reflected back from the reference mirror. The light in the reference and the sample arms combine to generate the interference \cite{36,37}.

The envelope of the interference pattern varies as the optical path length difference of the light from the two arms changes. The peak of the envelope of the interference corresponds to where the optical path length of the light from the reference arm and the sample arm match. The intensity of the interference can be written as:

$$I_{\text{int}} = \langle E_1^2 \rangle + \langle E_2^0 \rangle + 2 Re \langle \vec{E}_1 \cdot \vec{E}_2 \rangle$$  \(2.3\)

The third term on the right side of Equation \ref{2.3} corresponds to the modulation of the optical path length.

In the case of Time-domain OCT (TDOCT), as shown in Figure \ref{2.6}, light from a low-coherence source with continuous wave (CW) mode is split and then combined to generate the interference pattern. The combined light is detected and processed to find the envelope of the fringe pattern. By scanning the reference mirror an \textit{A-scan}
of the sample is obtained \([36, 37, 58, 59]\).

![Diagram of Time Domain OCT](image)

**Figure 2.6**: Time domain OCT; a) the envelope of the interference pattern is used to find the reflected light from the depth corresponding to the location of the reference mirror. b) In order to acquire an A-scan of the medium, the reference mirror is scanned. \([2]\)

Fourier-Domain OCT (FDOCT) is divided into Spectral-Domain OCT (SDOCT) and Swept-Source OCT (SSOCT). In both methods, the reference arm is fixed and corresponds to the approximate position of the sample. In SDOCT, a broadband source is utilized and the spectral interference pattern is collected simultaneously for all depth using a spectrometer (as shown in Figure 2.7). In SSOCT, the source has a narrow line-width, however it sweeps in wavelength rapidly and the interference pattern can be detected as a function of time. \([60, 62]\)

In FDOCT the spectral frequency contains the whole depth resolved structure of
the sample. In FDOCT, an *A-scan* is obtained by utilizing inverse Fourier transform.

![Diagram of Fourier domain OCT](image)

**Figure 2.7:** Fourier domain OCT. a) Reflection from a mirror, the Fourier transform of the interference signal represents the depth resolved information in the medium. b) In order to acquire an A-scan, a wideband source or a source with a narrow line-width that sweeps in wavelength can be used. [2]

OCT imaging is based on detecting single backscattered photons. The contrast in OCT is based on the optical reflection properties of the tissue (index of refraction). The lateral resolution of an OCT system is a function of the center wavelength of the light source and the Numerical Aperture (NA) of the system, given by
\[ \delta_{\text{lateral}} = 0.37 \frac{\lambda_0}{NA} \]  

(2.4)

where \( \lambda_0 \) is the center wavelength of the source.

On the other hand, the axial resolution of an OCT system is governed by the coherence length of the source, given by

\[ \delta_{\text{axial}} = \frac{2 \ln(2) \lambda_0^2}{\pi \Delta \lambda} \]  

(2.5)

where \( \Delta \lambda \) is the line-width.

And the axial field of view of an OCT is also governed by the wavelength and the NA of the system as

\[ FOV_{\text{axial}} = \frac{0.22 \lambda}{\pi \Delta \lambda} \]  

(2.6)

In order to maintain a long depth of focus, a low NA is used in OCT. Therefore compared to microscopy, the resolution is poor (down to couple of microns), however OCT provides images deeper in the tissue, compared to microscopy methods (up to millimeters).

### 2.1.3 Diffuse Optical Tomography

As discussed, due to high scattering, optical imaging depth is limited. Diffused light is the light that undergoes multiple scattering events and reaches the detection point. Although the diffused light travels a random path to reach the detector, it still contains information about the local optical absorption and scattering coefficient of the medium. Diffuse optical tomography (DOT) utilizes the diffused light and the photon transfer model and diffusion equation as in Equation 2.7 to find the local optical
properties of the tissue \[38,39\].

\[
\left[ \frac{\partial}{\partial t} + \mu_a(r)c - \nabla(D(r)c \nabla) \right] I(r,t) = S(r,t); \quad (2.7)
\]

where \(I(r,t)\) is the photon density field, \(S(r,t)\) represents the light source, \(\mu_a\) is the absorption coefficient, \(c\) is the speed of light in the medium and \(D\) is the diffusion coefficient as:

\[
D = \frac{1}{3(\mu_a + (1 - g)\mu_s)} \quad (2.8)
\]

where \(g\) is the scattering anisotropic factor and \(\mu_s\) is the scattering coefficient.

Each source-detector pair provides a measurement that will be utilized in inverse problem solving techniques to find the local optical properties of the medium. Figure 2.8 shows the diffused light travel pattern between a source and a detector.

![Figure 2.8: Diffused light traveling from the source to the detector.](image)

DOT utilizes multiple source-detector pairs, as shown in Figure 2.9. \(M\) sources and \(N\) detectors provide \(MN\) measurements. Scanning source-detector pairs will provide additional measurements, \(i.e. K\) scans will provide \(MNK\) measurements. Figure 2.9 show a single source-detector pair that scans rotationally to provide more
measurement.

![Figure 2.9: A single source with multiple detector DOT system. Each pair provides a measurement. Rotating the source-detector pairs provides more measurements that can be used in inverse problem solving techniques to find the optical properties of the tissue.](image)

In the time domain, a short pulse of light (in the order of pico-seconds) is sent to the medium. The detector measures the light intensity over time, to record the travel time from the source to the detector, to acquire temporal point spread function, as shown in Figure 2.10. In frequency domain, the amplitude of the source is modulated at a chosen frequency. As light travels through the medium, local absorption and scattering change the amplitude and phase of the light. In order to recover information, the amplitude and the phase of the detected light is measured and utilized in inverse problem solving. [63][64]

Since DOT utilizes diffused light, it provides information from deeper regions (up to around 10cm) of the medium compared to microscopy methods and OCT. However, this is achieved by sacrificing the resolution ($\approx 1\, \text{mm}$) of the imaging system. The resolution of the image in DOT is also affected by the algorithm utilized for inverse problem solving.
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Figure 2.10: Diffused optical tomography. a) time domain; where the time that light travels to reach the detector is recorded to find the temporal point spread function. b) frequency domain; where the amplitude and phase of the diffused light is recorded to be used for inverse problem solving.

2.2 Ultrasound Imaging

Similar to electromagnetic waves where electromagnetic property mismatch affects wave propagation, mechanical property mismatch from one medium to another medium causes back reflection of the incident ultrasound wave. The reflected wave is used in imaging. Since mechanical properties of soft tissue do not vary much, ultrasound waves can travel in the tissue with negligible scattering. Therefore, unlike an electromagnetic wave, an ultrasound wave can penetrate deep in the soft tissue with negligible scattering and provide information at a depth where optical waves can not reach. However, since the contrast in ultrasound imaging is based on the mechanical properties of the tissue, compared to optical imaging, images obtained by ultrasound do not have a good contrast. In addition, the resolution of images obtained by ultrasound wave is not comparable to that of the images obtained by optical imaging modalities. Although by utilizing high ultrasound frequency high resolution images can be obtained, the ultrasound wave penetration depth decreases as the frequency increases.
The basic idea of utilizing an ultrasound wave in imaging is similar to idea of optical coherence tomography. Based on the wave travel time from transducer to the medium, and then back to the detector, the location of the object with a different mechanical property compared to the surrounding tissue can be found. Figure 2.11 shows the concept of utilizing ultrasound for imaging. The ultrasound wave travels to the object and back to the detector, traveling twice the distance between the transducer and the object \((d = 2z)\). By measuring the travel time \((t)\) and considering the speed of the wave in the tissue \(c_{us}\), the location of the object is given by \[z = \frac{c_{us}t}{2}.\] (2.9)

![Figure 2.11: Schematic of an ultrasound imaging system. The transmitted wave travels into the tissue and at each surface where there is an acoustic impedance mismatch, part of the wave is backscattered. The backscattered wave is then used to find the location of each surface and form the image.](image-url)
Doppler ultrasound imaging utilizes the Doppler effect to detect whether the object is moving towards or away from the transducer. In addition, based on the frequency shift, it can acquire the velocity of the object. Figure 2.12 shows a schematic of an ultrasound Doppler imaging system. The ultrasound wave with the frequency \( f_t \) hits the moving object. According to the Doppler effect, the frequency of the received ultrasound wave \( f_r \) is shifted by \( f_d = \frac{2f_t V \cos(\theta)}{c_{us}} \), where \( V \) is the velocity of the object and \( \theta \) is the angle of incidence between the ultrasound wave and the direction of \( V \). The difference is called the Doppler shift frequency \( f_d = f_t - f_r \), which can be used to find the velocity of the object [22,65,66].

![Schematic of a Doppler imaging system](image)

Figure 2.12: Schematic of a Doppler imaging system. The frequency of the transmitted wave \( f_t \) is shifted by the moving particles. The Doppler shift frequency is \( f_d = \frac{2f_t V \cos(\theta)}{c_{us}} \).

Ultrasound Doppler imaging is a useful tool to monitor blood flow. It is used in many diagnosis conditions such as detecting blood clots and monitoring blood valve functioning.

Compared to optical imaging modalities, images obtained by ultrasound waves suffer from low contrast and resolution (frequency dependent, 0.3 – 3mm), however
ultrasound waves can provide images deeper in the tissue (frequency dependent, 3 – 25mm \[21\])

2.3 Hybrid Imaging Modalities

Imaging modalities such as photoacoustic tomography (PAT), ultrasound modulated optical tomography (UOT) are hybrid imaging modalities. PAT utilizes optical absorption as contrast and ultrasound for detection. On the other hand, UOT utilizes deep penetration of the ultrasound wave to encode spatial information in the diffused light \[24\]. Similar to UOT, acoustic radiation force (ARF) with speckle pattern analysis utilizes a focused ultrasound wave to tag the light in a different process.

2.3.1 Photoacoustic Tomography

PAT is based on the photoacoustic effect where a pulsed laser and optical absorption is used to generate a local temperature rise, resulting in a transient thermoelastic expansion. The thermoelastic expansion generates an ultrasound wave that can travel in the tissue with negligible scattering to reach the detection point. Figure 2.13 illustrates the schematic of a PAT system. 

In PAT, contrast is based on optical absorption. Hence it is a useful tool to image materials with high optical absorption in tissue such as blood. PAT is able to provide information about the concentration of blood in soft tissue. For instance, due to different optical absorption of the soft tissue in the brain, PAT can be used to detect brain lesions. \[67\] \[70\]
In cases of cancer in soft tissue, a tumor can generate its own blood vessels (angiogenesis). PAT is proven to be an effective tool to detect tumors with high concentration of blood vessels.

On the other hand, since oxygenated and deoxygenated hemoglobin ($HbO_2$ and $Hb$) are the dominant optical absorbers in tissue, by utilizing multiple optical wavelengths in PAT, total concentration of hemoglobin and hemoglobin oxygen saturation ($SO_2$) can be monitored.

Resolution in PAT images is governed by the frequency of the ultrasound transducer. Photoacoustic microscopy utilizes high frequency (about $50 MHz$) to improve the resolution (about $50 \mu m$) compared to PAT (about $700 \mu m$). However, the penetration depth is limited for high frequency ultrasound wave (about $50 mm$), while PAT can provide images from deeper regions (about $5 cm$). [67,69]
2.3.2 Ultrasound Modulated Optical Tomography

UOT utilizes pressure waves to modulate the index of refraction of the medium, hence the phase of the diffused light. Although diffused light can not be used for imaging directly, it still contains the information about the optical properties of the tissue. UOT utilizes a focused ultrasound wave to tag the light that travels through the ultrasound focal spot. Figure 2.14 illustrates the idea of UOT. [23,24,28]

![Figure 2.14: Schematic of an UOT system. The diffused light that travels through the ultrasound focal spot is tagged by the ultrasound wave. The intensity of the diffused light in time domain does not reveal any information, however in frequency domain, besides the optical frequency, the two sidebands represent the tagged light.][3]

Although the intensity of the diffused light in the time domain does not provide any information, in the frequency domain the signal shows the optical frequency and two sidebands that are the result of modulating the index of refraction with the ultrasound wave. Sidebands represent the light that traveled through the ultrasound focal spot and can provide information about the local optical properties of the medium at the ultrasound focal spot. In addition, sidebands can be utilized to send the light back in the medium and focus the light deep in the medium beyond the optical scattering
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limit, as shown in Figure 2.15. Figure 2.15: Finite difference time domain simulation result. Sidebands in UOT are separated from the background light and have been sent back to the medium. The sidebands focus on the focal spot without scattering in the medium.

UOT makes it possible to image beyond the scattering limit, however, the signal provided by UOT is weak and suffers from low signal-to-noise ratio (SNR). UOT is further discussed in Chapter 4, Section 4.2.

2.3.3 Acoustic Radiation Force with Speckle Pattern Analysis

In order to increase the signal strength in UOT, acoustic radiation force (ARF) can be utilized. Similar to UOT, in the ARF regime, a focused ultrasound wave is used to tag the light that passes through the ultrasound focal spot. In the ARF regime, the change induced to the optical path length of the tagged light is the result of particles’ displacement due to ARF. Figure 2.16 illustrates the difference between UOT and ARF regime.
Figure 2.16: Comparison between UOT and ARF regimes to tag light with an ultrasound wave. In UOT (b) the change in the optical path length is due to the changes in the index of refraction caused by fluctuation in pressure and particle displacement is in the form of vibration, however in ARF regime (a) the change in the optical path length is due to unidirectional displacement caused by the ARF and the change in the index of refraction caused by the temperature rise.

In the case of utilizing long ultrasound exposure time, temperature rise can also cause a change in the local index of refraction and a change in the optical path length of the light. Compared to UOT, where changes in the optical path length occurs in the time scale of the ultrasound wave period, in ARF regime the change in the optical path length occurs in a longer time scale which makes the detection easier.

Monitoring the interference pattern of the diffused light i.e. speckle pattern, can obtain information about the displacement caused by the ARF, hence the mechanical properties of the medium. Utilizing ARF to tag the diffused light is discussed in further detail in Chapter 4 Section 4.3. Speckle pattern analysis has many applications that are discussed in the Section 2.4.
2.4 Speckle Pattern Analysis

A speckle pattern is the result of interference of scattered waves. Scattered waves with the same wavelength can interfere constructively or destructively (as in Figure 2.17(a)) to generate a seemingly random bright and dark pattern called speckle pattern (shown in Figure 2.17(b)).

![INTERFERENCE](Image)

Figure 2.17: a) Two waves can interfere constructively or destructively. b) The result of interference of diffused light with different phases, known as the speckle pattern.

Equation 2.10 shows a solution to the wave equation.

\[ E = \alpha e^{j(\omega t + \theta)}, \]

where \( j \) is the imaginary quantity equal to the square root of minus one, \( \alpha \) is the amplitude, \( \omega \) is the angular frequency and \( \theta \) is the phase of the wave.

As mentioned, the phase of the interfering wave is governed by the optical path length of the wave that is the product of the physical distance the light travels and the index of refraction of the medium.
\[ \theta_i = \frac{2\pi}{\lambda} OPL_i, \quad OPL = nl, \quad (2.11) \]

where \( \lambda \) is the wavelength of the wave, \( l \) is the distance traveled by light and \( n \) is the index of refraction.

Although the speckle pattern looks random, the pattern is governed by the phase and amplitude of the interfering waves. Hence, the speckle pattern contains information about the location of scattering particles and the index of refraction of the medium \[71\]. Speckle pattern analysis can provide information about the change in the location of particles (particles motion) in the medium or change in the local index of refraction, for instance due to the temperature change.

Speckle pattern analysis has many applications. The speckle pattern generated by reflected light from a surface reveals information about the roughness of the surface \[72\]. Analysis of speckle displacement caused by surface deformation is used in surface strain measurement \[73\]. In fluid mechanics, speckle is utilized for fluid velocimetry. Similarly, in biomedical imaging, laser speckle contrast analysis is a method to monitor blood flow. Monitoring skin perfusion \[74\], retinal blood flow visualization \[75\] and brain imaging are other applications of laser speckle analysis \[7\]. In addition, speckle pattern analysis, can be a useful method to monitor displacement induced by the ARF, and it can acquire mechanical and thermal properties of the medium.

In this section different methods of analyzing speckle pattern are discussed.

### 2.4.1 Speckle Contrast Imaging

Moving scattering particles change the optical path length and therefore the phase of the interfering waves. The change in the interference pattern appears on the
detector as fluctuations in the intensity. Analyzing the temporal or spatial variations of the speckle pattern can be used to quantify the motion of scatterers. For instance, quantifying the spatial blurring of the speckle pattern caused by blood flow results in 2D maps of blood flow with high spatial and temporal resolution. (Figure 2.18) \[76,77\]

Figure 2.18: a) Raw speckle image from the thin skull of a rat, showing a grainy pattern in which it is possible to discern some spatial variation in the speckle contrast, and b) when the spatial speckle contrast is estimated from a 7 × 7 window of pixels, the blood vessels on the surface of the brain become apparent with high spatial resolution. \[7\]

The term spatial speckle contrast that is used to quantify the blurring of the speckle pattern is defined as the ratio of the spatial standard deviation to the mean intensity, \[78\]

\[
K_s = \frac{\sigma_s}{<I>},
\]

(2.12)

where \(\sigma_s\) is the spatial standard deviation and \(<I>\) is the mean intensity.

Similar to Equation 2.12, a temporal speckle contrast \(K_t\) can be defined. Utilizing spatial speckle contrast provides high temporal resolution, but low spatial resolution. On the other hand temporal speckle contrast provides high spatial resolution but at the expense of losing temporal resolution. The value of the speckle contrast is between
0 and 1, where 1 represents areas with no blurring, therefore no motion. A value of 0 indicates areas with high blurring and therefore fast movement of particles.

Laser speckle contrast imaging has many biomedical applications mostly based on high resolution blood flow imaging. Skin perfusion, retinal flow imaging and applications in brain imaging such as brain functional activation imaging.

2.4.2 Diffuse Correlation Spectroscopy

Diffuse Correlation Spectroscopy (DCS) employs a near-infrared coherent light source for illumination. DCS collects the speckle pattern generated by light that is multiply scattered by tissue. Moving particles cause changes in the phase of the interfering waves and fluctuations in the speckle pattern. The normalized electric field correlation function is defined as, \[ g_1(\tau) = \frac{<E^*(r,t)E(r,t+\tau)>}{<|E(r,t)|^2>} \] (2.13)

In practice, it is easier to monitor the fluctuation in the intensity compared to the electric field.

\[ g_2(\tau) = \frac{<I(r,t)I(r,t+\tau)>}{<I(r,t)>^2}, \] (2.14)

By measuring the temporal intensity auto-correlation of the speckle pattern \( g_2(\tau) \), DCS can quantify the motion of the particles i.e. red blood cells. \[80,81\]. The index of blood flow (BFi) is obtained by the temporal decay of the intensity auto-correlation function, as in Figure 2.19 (c). The absolute blood flow is proportional to the BFi.
Figure 2.19: (A) Schematic for blood flow monitoring in a homogeneous, semi-infinite turbid tissue. Blood cell (e.g., red particles at time $t$ and light-red particles at time $t + \tau$) motion induces temporal fluctuations in the scattered light intensity, $I(t)$, at the light detector (B). These intensity fluctuations are characterized by the normalized intensity autocorrelation function ($g_2(\tau)$). (C) The decay of the intensity autocorrelation function curves is related to tissue blood flow. \[8\]

### 2.4.3 Mean Irradiance Change Signal

In this work, we have introduced a novel method in speckle pattern analysis to recover information about particle movements and changes in the local index of refraction. The mean irradiance change (MIC) signal is defined as the spatial average of the difference image, where the difference image is defined as the absolute value of the changes in the speckle pattern compared to the baseline image (Figure 2.20). Assuming that particles are fixed at their location, and the light source is stable with no drift in the wavelength, the phases of the interfering waves do not change, hence the speckle pattern does not change.

Perturbations induced in the medium can change the location of particles or the local index of refraction of the medium. The MIC signal monitors the changes in the speckle pattern compared to the state before applying the perturbation and it can reveal information about the displacement or temperature change inside the medium. Later in Chapter 5 imaging utilizing the MIC signal is discussed in more detail.
2.5 Conclusion

In this chapter, different imaging modalities have been reviewed. In general, optical imaging modalities provide better resolution and contrast, however, ultrasound imaging provides deeper penetration. In optical imaging modalities, microscopy utilizes a high numerical aperture to provide a good resolution, but it comes with a cost of limited penetration. On the other hand, OCT uses a low numerical aperture to penetrate deeper, but the resolution is lower compared to microscopy. To image deeper in the tissue, DOT utilizes the diffused light but the DOT images suffers from low resolution.

Unlike light, ultrasound can travel in the tissue with negligible scattering. Hybrid imaging modalities such as PAT, UOT and ARF with speckle pattern analysis are introduced to use the good optical contrast and low scattering of the ultrasound waves. Both UOT and ARF utilize ultrasound to tag light and separate the light coming from the ultrasound focal spot from the background light. The ARF regime employs speckle pattern analysis to recover information from the tagged light.
Speckle contrast imaging, DCS and MIC signal imaging utilize the variation in the speckle pattern to obtain information in the diffused light. We have introduced the MIC signal as a novel tool to obtain mechanical and thermal properties of the tissue. The MIC signal imaging is discussed in detail in Chapter 5.
Chapter 3

Fixed-Particle Monte Carlo

In order to model the light and tissue interactions, different computational modeling methods have been introduced. One approach is to treat light as an electromagnetic wave and solve Maxwell’s equations to find the solution of the electromagnetic wave interacting with the tissue. Finite-Difference Time-Domain (FDTD) is an algorithm that solves Maxwell’s equations in time and space \[82,83\]. Another approach is to treat light as photons that travel in the tissue. Photons hit the particles in the tissue and can scatter or get absorbed by particles. For instance, the Monte Carlo algorithm is based on random processes where light as photons or packs of photons interacts with tissue \[84,86\].

Each of these algorithms has advantages and disadvantages. Choosing the proper algorithm depends on the application. In this chapter, FDTD, conventional Monte Carlo and fixed-particle Monte Carlo will be discussed. We will use the fixed-particle Monte Carlo algorithm to model the light-tissue interactions in Chapters 4 and 5.
3.1 Finite-Difference Time-Domain (FDTD)

FDTD is one of the most popular techniques that nowadays is being used to find the solution to electromagnetic problems. The main reason why FDTD is popular and successful is that it is simple and can be implemented easily even for three dimensional problems. FDTD finds the solution to the Maxwell’s equations governing electromagnetic waves. Maxwell’s equations can be written as Ampere’s law,

\[
\frac{\partial E}{\partial t} = \frac{1}{\epsilon} \nabla \times H,
\]

(3.1)

and Faraday’s law,

\[
\frac{\partial H}{\partial t} = \frac{1}{\mu} \nabla \times E.
\]

(3.2)

where \( E \) represents electric field, \( H \) represents magnetic field and \( \epsilon \) and \( \mu \) represents electrical permittivity and magnetic permeability and \( \nabla \times \) represent curl operation.

The FDTD method discretizes time and space to approximate spatial and temporal derivatives in the Maxwell’s equations. The approximation is based on the central difference as,

\[
\left. \frac{df(x)}{dx} \right|_{x=x_0} \approx \frac{f(x_0 + \frac{\delta}{2}) - f(x_0 - \frac{\delta}{2})}{\delta}.
\]

(3.3)

where \( \delta \) is the discrete step size. Note that there are higher orders of \( \delta \) on the right side of the Equation 3.3 that have been neglected assuming \( \delta \) is very small.

Yee’s cube shown in Figure 3.1 illustrates the implementation of the FDTD algorithm.

1. Temporal and spatial derivatives in Ampere’s and Faraday’s laws are replaced
by their central difference approximation

2. Equations are solved to find the magnetic fields at the next time step utilizing the fields in the current or past time.

3. Equations are solved to find the electric fields at the next time step utilizing the fields in the current or past time.

4. The found fields in steps 2 and 3 are used as the current fields and steps 2 and 3 are repeated to find the electromagnetic fields for the next step. This process continues for the desired duration.

Figure 3.1: Yee’s cube illustrates the algorithm to discretize space and time to find the electric and magnetic fields. Note that the values for the electric field and the magnetic field are separated by half a temporal and spatial step size.

In order to demonstrate the implementation of the FDTD algorithm, in one dimension, Equations 3.1 and 3.2 are replaced with:
CHAPTER 3. FIXED-PARTICLE MONTE CARLO

\[
\frac{E_{x}^{n+\frac{1}{2}}(k) - E_{x}^{n-\frac{1}{2}}(k)}{\Delta t} = \frac{1}{\epsilon} \frac{H_{y}^{n}(k + \frac{1}{2}) - H_{y}^{n}(k - \frac{1}{2})}{\Delta z},
\]  

(3.4)

and

\[
\frac{H_{y}^{n+1}(k + \frac{1}{2}) - H_{y}^{n}(k + \frac{1}{2})}{\Delta t} = -\frac{1}{\mu} \frac{E_{x}^{n+\frac{1}{2}}(k + 1) - E_{x}^{n+\frac{1}{2}}(k)}{\Delta z}.
\]  

(3.5)

Equations 3.4 and 3.5 imply that the values calculated for the electric and the magnetic fields are separated half a step in time and space \(i.e.\) the electric field is calculated only for times \((n + \frac{1}{2})\Delta t\) and at points \((K + L)\Delta z\) where \(m, l \in \mathbb{Z}\). Similarly the magnetic field is calculated only for times \((n + m)\Delta t\) and at points \((K + \frac{1}{2}l)\Delta z\) where \(m, l \in \mathbb{Z}\).

Since FDTD is based on approximation, to ensure that the solution obtained by FDTD is correct, it is important to pick the value of \(\delta\) in Equation 3.3 \(i.e.\) temporal and spatial step size, so that the approximation is valid.

In order to ensure an accurate representation of the solution, the spatial step size is required to be at least a tenth of the wavelength as,

\[
\Delta z \leq \frac{\lambda}{10},
\]  

(3.6)

where the wavelength \(\lambda\) to be used to calculate the spatial step size has to be the smallest wavelength in the simulation. In case dielectrics are present in the medium, the change in the wavelength should be considered as well.

According to stability conditions, the wave can not propagate more than one spatial step size in one temporal step size. Therefore, in the case of one dimension we have:
\[ \Delta t \leq \frac{\Delta z}{c_0}, \quad (3.7) \]

where \( c_0 \) is the speed of the wave propagation in the medium.

In general, the Courant stability condition indicates that the temporal step size should be

\[ \Delta t \leq \frac{\Delta z}{c_0 \sqrt{d}}, \quad (3.8) \]

where \( d \) represents the dimension of geometry of the problem and it can be 1, 2 or 3 for one, two or three dimension, respectively.

The temporal step size is commonly chosen as \( \Delta t = \frac{\Delta z}{2c_0} \) to ensure stability in the simulation.

Although FDTD solves Maxwell’s equations and yields a solution to the wave propagation problem, it requires a large amount of memory and computation time; therefore FDTD is computationally expensive. The size of the domain to be modeled is limited by the spatial step size and the available computational memory. If the dimension of the medium is exceedingly larger than the wavelength, then techniques such as ray tracing or Monte Carlo can be more efficient. However FDTD has the advantage of providing coherence, as it treats the light as an electromagnetic wave. Figure 3.2 shows FDTD simulation results to model the interaction of light with an object in the medium in an OCT imaging system. Since FDTD provides coherence information, the reflected light can be mixed with a reference arm to provide OCT measurements.
Figure 3.2: OCT simulation utilizing FDTD. The pulse of wave propagates in the medium. Red rectangle at the bottom represents the source and the black line represents the detector. Objects with different index of refraction (shown in different colors) scatter some of the incident wave back to the detection point. The detected wave is utilized for OCT imaging.

3.2 Monte Carlo

Unlike FDTD, Monte Carlo treats light as photons. The Monte Carlo technique is based on a random process for light propagation in the tissue. The distance that a photon travels between two scattering events and the scattering angle are chosen randomly based on probability distributions governed by the optical properties of the tissue.

There are many different methods to implement the Monte Carlo technique. In this section we are going to discuss the conventional Monte Carlo and fixed-particle Monte Carlo. In this work, fixed-particle Monte Carlo is utilized as it can provide the coherence required for the speckle pattern analysis. In Chapters 4 and 5, simulation results based on fixed-particle Monte Carlo are presented.


3.2.1 Conventional Monte Carlo

In conventional Monte Carlo, a photon or a pack of photons is sent inside the tissue and is tracked until either the photon is absorbed or diffused to be detected. This process repeats for many photons. As the number of the photons tracked by the Monte Carlo increases, the distribution of the photon paths provides a better approximation of light propagation in the medium. 

\[ \mu_t = \mu_a + \mu_s, \]  
(3.9)

where \( \mu_a \) and \( \mu_s \) are absorption coefficient and scattering coefficient of the tissue, respectively.

The first step in Monte Carlo ray tracing technique is to find the step size. The step size is defined as the distance the photon travels to the next interaction with the tissue. The step size that the photon travels is exponentially distributed. The distribution is a function of the total attenuation coefficient of the medium given as,

\[
p(s) = \frac{e^{-\mu_t s}}{\mu_t}.
\]  
(3.10)

Utilizing Equation 3.10, the probability distribution function for a particular step, \( s_1 \) is

\[
F(s_1) = \int_0^{s_1} p(s)ds = \int_0^{s_1} \frac{e^{-\mu_t s}}{\mu_t}ds = 1 - e^{-\mu_t s_1}.
\]  
(3.11)

By picking a random value \( (x) \) for \( F(s_1) \) and solve it to find \( s_1 \), we have
\begin{align*}
  s_1 &= \frac{-ln(1 - x)}{\mu_t} = \frac{-ln(x)}{\mu_t}.
  \tag{3.12}
\end{align*}

Since \( x \) is a uniformly distributed random number between 0 and 1, the distribution of \( 1 - x \) is also a uniformly distributed random number between 0 and 1, and can be replaced by \( x \) on the right side of the Equation \( 3.12 \). Therefore, the random number \( x \) provides the random step size that the photon travels to the next interaction with the tissue.

After each step, the weight of the photon decreases with the step size and the absorption of the medium. The weight is used to determine whether the photon is terminated or continues traveling in the medium. In order to perform the termination process and yet conform to the law of conservation of energy, the Roulette method is utilized. After each step, the weight of the photon is compared with a predefined threshold. If the photon weight drops below the threshold, then a random number is picked to decide whether a chance (a predefined number, typically 0.1) should be given to the photon or not. If the random number is less than the chance, then the weight of the photon increases by a factor of \( \frac{1}{\text{chance}} \). The value of 0.1 for the chance variable implies that 1 out of 10 photons is given a second chance and its weight is increased by a factor of 10 to continue traveling in the tissue.

If the photon passes the termination process, a scattering event occurs. Therefore the trajectory of the photon changes. The new trajectory can be defined by finding the scattering angles, deflection angle (\( \theta \)) and azimuthal angle (\( \phi \)). Assuming cylindrical symmetry, the azimuthal angle is uniformly distributed in the 0 to \( 2\pi \) span as

\[ \phi = 2\pi x, \tag{3.13} \]
where \( x \) is a random number from 0 to 1 with uniform probability distribution.

To find the deflection angle \((\theta)\), typically the *Henyey-Greenstein* function is used.

\[
p(\theta) = \frac{1}{4\pi} \frac{1 - g^2}{(1 + g^2 - 2g\cos(\theta))^2},
\]

(3.14)

where \( g \) is the anisotropy factor defined as the average of \( \cos(\theta) \) over all directions as,

\[
g = \langle \cos(\theta) \rangle.
\]

(3.15)

If \( g = 0 \), scattering is isotropic. Positive values of \( g \) imply more forward scattering and negative values of \( g \) imply more backscattering.

Using Equation (3.14) we can solve for \( \cos(\theta) \). For \( g \neq 0 \) we have

\[
\cos(\theta) = \frac{1 + g^2 - \left( \frac{1 - g^2}{(1 - g + 2gx)^2} \right)^2}{2g}.
\]

(3.16)

And for the case where \( g = 0 \) we have

\[
\cos(\theta) = 2x - 1,
\]

(3.17)

where \( x \) is a random number from 0 to 1 with uniform probability distribution.

Therefore, by using Equations (3.16) and (3.17) and picking a random number \( x \), the deflection angle \( \theta \) can be obtained.

Once the new direction of the photon is found and the weight of the photon is updated, the process repeats till the photon is terminated or exits the tissue. Figure 3.3 represents the flowchart of the conventional Monte Carlo method.

Conventional Monte Carlo with an adequate number of photons provides a good
approximation of the light and tissue interaction. Compared to FDTD, Monte Carlo is faster and can be applied to a larger geometries. However, since Monte Carlo treats light as photons, it can not provide coherence [85, 86]. In order to obtain coherence with Monte Carlo, fixed particle Monte Carlo can be employed [88].

### 3.2.2 Fixed-Particle Monte Carlo

The main difference between fixed-particle and conventional Monte Carlo is the process to find the distance between two scattering events. In fixed-particle Monte Carlo, particles are fixed at their location and scattering events occur only at the location of the particles [88]. Therefore it provides the optical path length and the phase of scattering paths that can be used for coherence simulation [89, 91]. We can list the algorithm of the fixed-particle Monte Carlo step by step:
Shooting a New Photon

In conventional Monte Carlo each photon sees the medium as a new random medium. However, in fixed-particle Monte Carlo, the medium is the same for all photons i.e. if two photons travel in the same direction at the same location, they will hit the same particle.

The process starts with shooting a photon inside the medium within the region where the medium is being illuminated. In practice, this is governed by the beam size on the medium. After the photon enters the medium, based on the position vector and the direction of the photon, the algorithm determines whether the photon hits any particle or not. If the light does not hit any particle, it counts as the transmitted light without scattering, otherwise scattering occurs.

Scattering Angle

In the case of collision, a new scattering direction is chosen randomly based on the angular weighted function. As mentioned earlier, in convention Monte Carlo, the Henyey-Greenstein function is typically used. However other probability distribution functions can be used. The scattering regime to be used depends on the size of the particle. The size parameter is defined as

\[ x = \frac{2\pi r n}{\lambda} \]  

(3.18)

where \( r \) is the radius of the particle, \( n \) is the index of refraction of the medium and \( \lambda \) is the wavelength of the light.

In case where \( x \ll 1 \), the Rayleigh scattering regime describes the scattering events and can provide the probability distribution function to be used to find the
scattering angle ($\theta$). For very large values of $x$, the geometric optics regime provides the scattering angle probability distribution function. Otherwise, the Mie scattering is utilized.

After each collision, the weight function of the photon is updated to determine whether the photon is absorbed or it can continue traveling through the medium. In the case of scattering, the process continues with finding the new scattering direction. One method to find the next scattering particle is to use the inner product between the vector that describes the photon trajectory and the vector from the center of the current particle to the surrounding particles, as shown in Figure 3.4. The angle between the photon trajectory ($\vec{s}$) and the vector between the two particles ($\vec{d}$) is:

$$\alpha = \cos^{-1} \left( \frac{\vec{s} \cdot \vec{d}}{|\vec{s}| |\vec{d}|} \right).$$

Hence, if $dsin(\alpha) < r$, then scattering occurs at the location of the particle.

![Figure 3.4: Process of finding the next scattering particle. $\vec{s}$ represent the scattering vector, $\vec{d}$ represent the vector between the two particle. The angle $\alpha$ is used to determine whether the light hits the particle or not.](image)
CHAPTER 3. FIXED-PARTICLE MONTE CARLO

Termination

The process of finding the location of the next scattering event i.e. the next particle that the photon hits, continues until the photon does not undergo further scattering and exits the medium or the weight of the photon drops below the threshold. In case of termination, the process is similar to the conventional Monte Carlo method.

For each scattering path the optical path length, weight and position vector of the photon is recorded for further analysis. Figure 3.5 (on the left) shows the step by step algorithm of the fixed-particle Monte Carlo. On the right, the process of decision making for scattering events is illustrated.

Figure 3.5: Fixed-particle Monte Carlo algorithm. On the right, the process starts with tracking a photon in the medium. The photon is tracked till it exits the medium. The information of the diffused photon is recorded for further analysis. On the left, $\theta_i$s are scattering angles obtained by the Mie scattering theorem, $l_i$s are the distance between two particles which light scattered. The optical path length is calculated as the product of the average of the index of refraction between two particles and $l_i$. 
Figure 3.6 represents the geometry of the 2D model. The medium is 1cm $\times$ 1cm, particle size is $r = 10\mu m$ with concentration of 625 particles per $mm^2$. The index of refraction of the particles is $n = 1.46$ to match with the index of refraction of lipid particles in milk that we used in experiments (presented in Chapter 5). The figure also shows examples of scattering paths obtained by the fixed-particle Monte Carlo algorithm.

![Figure 3.6: Geometry of the model and examples of scattering path obtained by fixed-particle Monte Carlo method. Paths that exit the medium from the side toward the detection point contribute in the speckle formation (red and yellow paths). Red paths pass through the acoustic focal area, shown in green, therefore the acoustic wave effect their optical path length. Black path are ignored in our calculations since they exit the medium form other boundaries that do not contribute in the speckle formation.](image-url)
3.2.3 Conclusion

In this chapter we have discussed common approaches to simulate light and tissue interactions based on treating light as a wave or particles. FDTD treats light as a wave and solves Maxwell’s equations to find the solution to the wave propagation in the tissue. Therefore, it is able to provide coherence. However, FDTD is computationally expensive and the area of interest to be used in the simulation is limited due to limitation of computational memory. Also, FDTD is time consuming as it solves equations step by step in the tissue. On the other hand, Monte Carlo is based on particle behavior of the light. In Conventional Monte Carlo all light-tissue interactions are modeled randomly based on probability distribution functions governed by the optical properties of the tissue. Compared to FDTD, conventional Monte Carlo is faster and does not require large computational memory. The results in conventional Monte Carlo is presented as the net distribution of photons. The disadvantage of conventional Monte Carlo is that it can not provide the coherence of the light. To include the ability of coherence modeling to the conventional Monte Carlo, fixed-particle Monte Carlo can be utilized. Unlike conventional Monte Carlo, in fixed-particle Monte Carlo, the scattering events occur only at the location of particles. Hence, the optical path length, and therefore the phase of the scattering paths can be obtained to model coherence. In Chapters 4 and 5 we have utilized the fixed-particle Monte Carlo to model speckle pattern generated by a coherent light source.
Chapter 4

US Tagging of Light

As discussed in Chapter 2, light can provide images with higher contrast and resolution compared to ultrasound. On the other hand, ultrasound can travel deeper in the tissue with negligible scattering. In order to increase the imaging depth with light, the diffused light has to be used. Although the diffused light contains information about the optical properties of its path in the tissue, it is challenging to retrieve that information. The main issue is that it is not determined where the light is coming from. The idea of utilizing light and ultrasound is to tag the light that travels through the ultrasound focal spot and separate the tagged light from the background diffused light at the detector. One approach is to tag the light by modulating the index of refraction of the medium as in ultrasound modulated optical tomography. In addition, acoustic radiation force can also tag the light by applying displacement to the particles in the medium. The basis of both methods is inducing changes in the optical path length of the diffused light to tag the light. In this chapter, we are going discuss processes that UOT and ARF regimes employ to induce changes in the optical path length and compare the two regimes.
4.1 Optical Path Length

In order to illustrate the approach to tag the light by inducing changes in the optical path length, first we need to study the electric field of a wave. The electric field of an electromagnetic wave diffused from the medium is

$$\vec{E} = E_0 e^{j(\omega t - \vec{k} \cdot \vec{r})}$$  \hspace{1cm} (4.1)

where vector $\vec{r}$ is the position vector, $\vec{k}$ is the wave vector, $\omega$ is the angular frequency and $E_0$ is the amplitude of the wave. The wave number is defined as the amplitude of the wave vector,

$$k = \frac{2\pi}{\lambda}$$ \hspace{1cm} (4.2)

where $\lambda$ is the wavelength of the wave. Therefore, Equation 4.1 can be written as

$$\vec{E} = E_0 e^{j(\omega t - \frac{2\pi}{\lambda} x)},$$ \hspace{1cm} (4.3)

and $x$ is the distance that light traveled considering the index of refraction of the medium i.e. optical path length. Optical path length $x$ is defined as the integral of the product of the index of refraction of the medium and the distance it travels over the whole path as

$$x = OPL = \int n dl.$$ \hspace{1cm} (4.4)

where $n$ is the index of refraction of the medium and $l$ is the physical distance that light travels.

Any perturbation applied to the medium that changes the location of particles or
the index of refraction of the medium, changes the optical path length of the diffused light. UOT and ARF regimes utilize ultrasound to apply local perturbations in the medium to change the optical path length of the light. The change in the optical path length can be written as

$$\Delta OPL = \Delta nl + n\Delta l, \quad (4.5)$$

where the first term on the right side corresponds to the change in the optical path length due to the change in the index of refraction. The second term on the right side represents the change in the optical path length due to displacement of the scattering particles.

### 4.2 Ultrasound Modulated Optical Tomography

When the ultrasound wave is applied inside the tissue, the high intensity variation of pressure changes the index of refraction of the tissue, i.e. index of refraction is modulated by the ultrasound frequency ($f_{us}$). There are other processes where the ultrasound wave can induce changes in the optical path length of the diffused light. Vibration caused by the pressure waves changes the optical path length, however at ultrasound frequencies, the amplitude of the vibration displacement is negligible compared to the changes in the index of refraction and is neglected in UOT.

There are various methods to monitor the changes in the optical path length and separate the tagged light. For instance, although the irradiance of the diffused light does not reveal any information, monitoring the frequency of the light provides two sidebands, which represent the modulated signal. Figure 4.1 illustrate light modulation process in UOT. [5][6][23][24]
CHAPTER 4. US TAGGING OF LIGHT

Figure 4.1: Light modulation utilizing a focused ultrasound wave. a) Particles inside the ultrasound focal spot vibrate at their location. The amplitude of vibrations is very low and their effect on the optical path length is negligible. The change in the index of refraction due to the changes in pressure is utilized to modulate the index of refraction. b) The detected light contains two sidebands of $f_{opt} \pm f_{us}$, that represent the tagged light.

Figure 4.2 shows an UOT simulation result utilizing FDTD method. Results show that the two sidebands can be used to focus the light deep in the tissue without scattering.

Although results show that the sidebands can be utilized to obtain local properties of the tissue at the ultrasound focal spot, the signal acquired by the demodulation process is very weak and suffers from low signal-to-noise ratios. In addition to demodulation to acquire the sidebands signals, other methods such as measuring the autocorrelation function and the light intensity spectra have been studied [5].

When an ultrasound wave is applied to the tissue, the speckle pattern contrast generated by the diffused light decreases. The variation in the speckle contrast that is dependent on the ultrasound wave can be utilized for imaging in the tissue. Regardless of the tissue acoustical homogeneity, measuring the variation of the speckle contrast can produce images of the tissue. Figure 4.3 shows the results of utilizing speckle
CHAPTER 4. US TAGGING OF LIGHT

Figure 4.2: The phase conjugate carrier (a), phase conjugate sideband (b), and phase conjugate fit (c) delivery waves in an example titanium oxide phantom. The ultrasound beam is illustrated in green, the medium of random scatterers in gray, and the electric field in red. [6]

contrast to measure the modulated light to detect the presence of two rubber objects buried in thick chicken breast tissue.

4.3 Acoustic Radiation Force

The ARF regime employs different processes to induce changes in the optical path length of the tissue to tag the light. Unlike UOT, in the ARF, the displacement caused by the ultrasound wave is unidirectional and has a larger amplitude. Hence the changes induced to the optical path length are larger. Therefore the resulting signal representing the tagged light is stronger and easier to detect. In addition, in an acoustically absorbing medium, the ultrasound energy absorbed by the medium generates a temperature rise that results in a change in the local index of refraction of the medium. This effect can also be used to tag the light in the ARF regime. Utilizing the ARF regime effects can provide local mechanical and thermal properties of the tissue.
4.3.1 Displacement Effect

Particle displacement is the result of acoustic interaction between an absorbing target with the ultrasound wave. As the wave hits the target, the target absorbs part of the power of the wave. The result of this process is wave momentum transfer to the target, which generates the acoustic radiation force \[ F = \frac{2\alpha \tilde{I}}{c_{US}}, \] (4.6)

where \( \tilde{I} \) is the averaged ultrasound temporal intensity, \( \alpha \) is the absorption coefficient and \( c_{US} \) is the ultrasound wave speed of propagation. And for the displacement we
have:

$$\Delta l(\vec{r}, t) = \frac{2\alpha}{c_{US}} \tilde{I}(\vec{r}) f(t).$$

where $\vec{r}$ is the location vector from the ultrasound focal spot and $f(t)$ represents the time dependence of the displacement.

As shown in Equations 4.6 and 4.7, the force and the displacement are governed by the time averaged ultrasound wave spatial intensity. Moreover, higher ultrasound frequencies generate higher force. Finally, the displacement is governed by the force and the mechanical properties of the tissue ($\alpha$). Figure 4.4 illustrates the process by which displacement changes the optical path length of the scattered light in the tissue. We define the phase profile as the distribution of the phases of the scattering paths. Applying the ARF in the medium changes the phase profile. By monitoring the changes in the phase profiles, the changes in the MIC signal can be obtained.

Figure 4.5 shows the simulation results that illustrate the changes in the phase profile of the diffused light as function of maximum displacement induced by the ARF. Figure 4.5(a) shows the average and the standard deviation of the changes in the phase profile versus maximum displacement. Due to the distortion in the location of particles, some of the scattering paths become longer and some scattering paths become shorter, therefore the average of the changes is about zero. As the distortion increases, the phase profile distribution expands, as can be seen in Figure 4.5(b) and (c).

Monitoring the displacement caused by the ARF has many applications in medical imaging. In many cases of disease, the unhealthy tissue has different mechanical properties from the surrounding healthy tissue. In some cases, manual palpation is utilized by clinicians for tissue stiffness evaluation. However, besides not being
CHAPTER 4. US TAGGING OF LIGHT

Figure 4.4: Change in the optical path length due to displacement induced to the particles inside the ultrasound focal spot. On the left, particles placed randomly in the medium. Some sample scattering paths are shown. particles in green are located inside the ultrasound focal spot. On the right, one of the scattering paths after (solid red) and before (dotted green) applying the displacement.

accurate, this method is limited to the size of the unhealthy tissue and only works for superficial parts and when the unhealthy tissue has considerably high stiffness. To overcome these limitations, ARF can provide remote palpation for tissue evaluation \[94\]. Acoustic Radiation Force Impulse (ARFI) imaging utilizes acoustic radiation force to induce a mechanical perturbation to the tissue and monitor the tissue response to form the image. Two common methods are monitoring the speed of the shear wave generated as the result of ARF perturbation (Shear Wave Elasticity Imaging), and generating a stiffness image of the tissue based on the tissue response to the
Figure 4.5: Phase distribution as a function of maximum displacement induced by the acoustic radiation force. a) The average and the standard deviation of changes in the optical path length. b) Histogram of the change in the optical path length. c) Histogram of the corresponding change in the phase of the scattering paths.

perturbation \cite{95}. The amount of displacement corresponding to the ARF reveals mechanical properties of the target and forms the basis of different modalities of elastography \cite{96,97}.

4.3.2 Thermal Effect

In an acoustically absorbing medium, the absorbed energy dissipates and converts to heat. As temperature rises, the local index of refraction of the medium decreases. Therefore, the phase of the scattering paths passing through the ultrasound wave focal spot, is tagged. Due to thermal conductivity, the heat spreads in the medium, resulting in cooling the original heated region and heating the surrounding region. Similar to Equation \ref{eq:4.7} for temperature we have:

\[
\Delta T(\vec{r}, t) = \frac{2\alpha}{\rho C_v} \tilde{I}(\vec{r})g(t). \tag{4.8}
\]
where \( \vec{r} \) is the location vector from the ultrasound focal spot, \( \alpha \) is the amplitude absorption coefficient, \( \tilde{I} \) is the spatial-peak acoustic intensity, \( \rho \) is the medium density and \( C_v \) is the medium specific heat.

The change in the temperature due to the acoustic wave absorption is a function of the distance to the focal spot of the focused acoustic wave and time (ultrasound wave pulse width and time for cooling process). In Equation 4.8, the function \( g(t) \) represents the time dependence of the temperature change in the medium. During insonation, we have the temperature rise as [98,99]:

\[
T(r, t) = \frac{2\alpha \tilde{I}}{\rho C_v} e^{-\frac{r^2}{a^2}} \frac{a^2}{4\tilde{\kappa}} \ln(1 + \frac{4\tilde{\kappa}t}{a^2}),
\]

(4.9)

where \( r \) is the radial distance to the ultrasound focal spot, \( \alpha \) is the amplitude absorption coefficient, \( \tilde{I} \) is the spatial-peak temporal-average acoustic intensity, \( a \) is the effective Gaussian radius of the radial spatial intensity profile, \( \tilde{\kappa} \) is the medium thermal diffusivity, \( \rho \) is the medium density and \( C_v \) is the medium specific heat.

Following the heating process, after the ultrasound is turned off, the medium starts to cool. The temperature in the cooling process is [98,99]:

\[
T(t) = \frac{T_{\text{peak}}}{1 + \frac{4\tilde{\kappa}t}{a^2}},
\]

(4.10)

where \( T_{\text{peak}} \) is the temporal-peak temperature reached before cooling.

Figure 4.6 shows the simulation results for the changes in optical path length versus temperature rise in the medium. The rise in the temperature results in a decrease in the index of refraction, therefore the optical path length of the affected scattering paths decreases. Hence, the average change in the optical path length is negative. The two peaks in the changes in the phase distribution represent background
light (zero change in the phase) and tagged light (high number of path with changes in phase).

Figure 4.6: Phase distribution as a function of changes in temperature. a) The average and the standard deviation of changes in the optical path length. b) Histogram of the change in the optical path length. c) Histogram of the corresponding change in the phase of the scattering paths.

4.3.3 Conclusion

Both UOT and ARF regimes utilize a focused ultrasound wave to apply changes in the optical path length of the medium to tag light. In the UOT regime, the change in the index of refraction is due to variation in the pressure that occurs in the time scale of microseconds to nanoseconds based on the frequency of the ultrasound wave. The displacement is in the form of vibration with negligible amplitude. The resulting change in the optical path length is in the nanometer scale. On the other hand, in ARF regime, the displacement is unidirectional and the amplitude is in micrometer scale that occurs in timescale of about milliseconds. The change in the index of refraction due to temperature change occurs in the timescale of seconds. The resulting changes in the optical path length is in the micrometers scale. Hence, the signal corresponding the tagged light is stronger compared to the UOT regime. Also longer timescale of
the process makes the monitoring process easier. Table 4.1 summarizes the difference between UOT and ARF regimes.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta n$</th>
<th>$\Delta l$</th>
<th>$\Delta OPL$ (amplitude)</th>
<th>$\Delta OPL$ (timescale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UOT</td>
<td>pressure</td>
<td>vibration</td>
<td>$\mu m$</td>
<td>$ns - \mu s$</td>
</tr>
<tr>
<td>ARF</td>
<td>temperature</td>
<td>unidirectional</td>
<td>$nm$</td>
<td>$ms - s$</td>
</tr>
</tbody>
</table>

Table 4.1: Comparison between UOT and ARF regimes in ultrasound wave tagging of light.
Chapter 5

Imaging Utilizing MIC Signal

5.1 Mean Irradiance Change (MIC) Signal

Since speckle pattern is the result of interfering waves, any changes in the phase of the waves results in changes in the speckle pattern. As discussed in Chapter 2, many approaches have been introduced to monitor the changes in the speckle pattern to obtain information about the medium. In this work, we have introduced a novel method for speckle pattern analysis. The mean irradiance change signal, is defined as the spatial average of the changes in the speckle pattern. For the electric field of an interference pattern we have

\[ E = \sum_{i=1}^{P} \alpha_i e^{j(\omega t + \theta_i)}, \]

(5.1)

where \( j \) is the imaginary unit, \( P \) is the total number of waves, \( \omega \) is the angular frequency and \( \alpha_i \) and \( \theta_i \) are the amplitude and the phase of the waves.

At the detection point, the intensity detected is obtained by
The MIC signal is defined as the spatial average of the changes in the intensity compared to the intensity at the original state, as

\[ MIC(t) = \langle |I(t) - I_0| \rangle, \]  

where \( I(t) \) is the intensity at time \( t \), and \( I_0 \) is the intensity at the original state.

The original state is defined as the state before applying perturbation to the medium. Since speckle pattern is governed by the location of particles and the index of refraction of the medium, assuming that no perturbation is applied to the medium, the MIC signal should not change with time (\( MIC(t) = 0 \)). However, in practice due to the slight changes in the wavelength of the light source, the speckle pattern varies slightly with time. Therefore the MIC signal has an offset value.

In simulations, the wavelength of the laser and location of particles are assumed to be fixed, so the offset value of the MIC signal is zero. Figure 5.1 presents the MIC signal acquired by simulations. In order to demonstrate the changes due to temperature and displacement, the MIC signal is plotted versus temperature rise for different displacements.

When the displacement is applied to the medium, the normalized MIC signal increases. In the case of a large displacement, the phase profile gets closer to the uniform distribution in 0 to 2\( \pi \) span, and the speckle pattern becomes completely random (Normalized MIC(\( t \)) \( \approx 1 \)), therefore it is not possible to correlate the change in the speckle pattern to the displacement. This is referred to the modulation saturation in the literature. [30]
CHAPTER 5. IMAGING UTILIZING MIC SIGNAL

Figure 5.1: MIC signal acquired with fixed particle Monte Carlo simulations. The MIC signal is plotted as a function of temperature for three cases of no displacement, maximum of 1\(\mu m\) and maximum of 2\(\mu m\).

In addition, when the temperature rises, the phase of the scattering paths decreases. At the point where the phase change reaches 2\(\pi\), phase wrapping occurs. Therefore, the MIC signal shows an oscillation behavior. As the temperature rises the phase profile gets closer to a uniform distribution in the 0 to 2\(\pi\) span, and modulation saturation occurs.

To model the process of tagging light in ARF regime, it is important to study different insonation process that induce perturbation in the medium. Different perturbations in the medium can be monitored by monitoring the MIC signal for imaging purposes.
5.2 Signal Strength

Before utilizing the MIC signal for imaging purposes, it is essential to study the parameters that affect the MIC signal. Important parameters that govern the MIC signal variations are:

1. **Size of the ultrasound focal spot**

   Larger focal spots include more scatterers that can move from their original location. As the size of the focal spot increases, the number of paths affected by the ultrasound wave increases, resulting in a bigger changes in the speckle pattern.

2. **Ultrasound frequency**

   Increasing the ultrasound frequency results in a stronger ARF, therefore larger displacement. Larger displacement leads to a stronger change in the MIC signal and signal to noise ratio enhancement. On the other side, utilizing an ultrasound with higher frequency, decreases the size of the ultrasound focal spot, hence weakens the MIC signal.

3. **Ultrasound pulse width**

   At the beginning of the insonation, particles move to the maximum displacement which induces the rapid change in the MIC signal. Experimental results in Figures 5.5 and 5.6 show that the rapid change at the beginning of the US wave is constant for different ultrasound wave pulse widths. After particles reach their maximum displacement, the absorbed energy which is proportional to the ultrasound pulse width, turn into heat. The temperature increases until it
reaches its equilibrium point due to thermal diffusivity. Unlike the changes corresponding to the ARF excitation, the changes due to ARF relaxation depends on the ultrasound pulse width.

4. **Ultrasound peak pressure**

Higher peak pressure at the focal spot results in greater ARF. As displacement increases, the perturbation in phase distribution increases. Therefore, the ARF induces more changes in the MIC signal. On the other hand, as stated in Equation 4.9, higher peak pressure implies higher temperature rise, hence, the temperature reaches its equilibrium point in a shorter time.

5. **Ultrasound and illumination relative position**

The relative position of the illumination and ultrasound focal spot also plays a role in the MIC signal. In the case where the ultrasound focal spot is well aligned with the illumination, more paths can be tagged by the ultrasound wave as illustrated in Figure 5.2. When the ultrasound focal spot is located at the red area, less scattering path can be tagged compared to case where the ultrasound focal spot is located at the green area.

6. **Depth of Imaging**

As stated, the MIC signal strength is governed by the number of the paths being tagged by the ultrasound wave. As the ultrasound focal spot is moved deeper in the tissue, the number of tagged scattering paths decreases. Figure 5.3 illustrate the MIC signal for a uniform model as a function of depth. Note that the number of backscattered path is governed by the optical properties of the medium. But generally, the MIC signal decreases as the ultrasound focal spot is moved deeper in the medium.
5.3 Insonation Processes

During the insonation, the ultrasound wave travels through and is focused inside the medium. As the ultrasound wave interacts with scattering particles, it generates perturbation in particles’ locations and increase the temperature of the medium. The original state is defined to be the state of the medium before applying the ultrasound wave. After applying the ultrasound wave, we can describe the insonation in four different processes:

**Acoustic Radiation Force Excitation**

At the beginning of the insonation, the ARF induced by the ultrasound wave, causes a perturbation in particles’ locations. As the result of ARF generation in the medium,
Figure 5.3: The MIC signal as a function of the depth of the ultrasound wave, obtained by the simulation. By moving the ultrasound focal spot deeper in the medium, less number of scattering paths are being tagged, therefore the MIC signal drops.

...particles start to move in the direction of the wave propagation. For a tissue mimicking medium, this process typically occurs in timescale of milliseconds. Therefore, at this timescale the temperature rise can be neglected. The displacement continues until the resistance force of the medium become strong enough to cancel the ARF $^{94}$. At this point, particles are assumed to stay fixed at their new location.

**Heating**

After the maximum displacement is reached, the absorbed energy of the ultrasound wave dissipates in from of heat. As a result, the local temperature of the medium increases $^{99,102}$. As shown in Equation 4.9, the amount of temperature rise is a function of the intensity of the ultrasound wave, thermal properties of the tissue and ultrasound exposure time. The temperature rise process requires time and it typically occurs in time scale of seconds. In case of short ultrasound exposure times, the temperature rise is negligible. For longer exposure times ($\approx 1$ seconds or longer),
Acoustic Radiation Force Relaxation

ARF relaxation occurs after the ultrasound is turned off. In the absence of the ARF, particles move back to their original location. Similar to ARF Excitation, this process occurs in the timescale of milliseconds. Therefore, the temperature change during this process can be neglected. This process starts at the end of insonation and ends after particles move back to their original location.

Cooling

After all particles move back to their original location, and in the absence of the ultrasound wave, the local temperature at the ultrasound focal spot decreases due to thermal diffusivity. This process results in a temperature rise in the surrounding area, however, the general temperature of the medium decreases to reach the temperature at the original state. Similar to the heating process, this process occurs in timescale of seconds. The cooling process duration depends on the maximum temperature reached in the heating process as in Equation 4.10

5.4 Methods

To acquire the MIC signal experimentally, an optically scattering - acoustically absorbing tissue mimicking phantom is used. The gelatin based phantom has acoustic properties close to soft tissue. In order to increase optical scattering, milk has been added to the phantom. The phantom contained 100ml water, 25gr gelatin and 5ml milk. Since the ultrasound wave is focused inside the tissue, there is a probability that
the pressure waves generate micro bubbles inside the medium. The process known as cavitation. Since changes in the index of refraction affects the speckle pattern, and the change in the index of refraction between water and air is large, cavitation has a great effect on the speckle pattern. To avoid cavitation, after mixing water, gelatin and milk, the mixture is connected to a vacuum pump for degassing. The water in the tank is also degassed to avoid cavitation in the water. Figure 5.4 shows the experimental setup that is used to record the speckle pattern to acquire the MIC signal.

![Experimental layout](image)

**Figure 5.4:** a) Experimental layout. The laser is on one side of the phantom. Scattered light leaves the phantom from the other side, and generates a speckle pattern on the detector. The US wave is delivered to the phantom perpendicular to the laser and induces displacement perpendicular to the laser direction. b) ultrasound excitation. c) Image acquisition timing. An image is taken every 16ms.

The unfocused CW laser (HeNe laser, $\lambda = 632.8\,nm$ with about $0.3\,mm$ waist) beam goes through the phantom and is scattered to generate the speckle pattern at the detector (Basler - acA1920-25um). It is important to have the speckle size larger
than the detecting pixel to be able to see the changes in the speckle pattern. The distance from the phantom to the detector is set so that the size of the speckle \(d\), is approximately 5 times the pixel size on the detector, using

\[
d = \frac{\lambda}{\text{FOV}z}; \tag{5.4}
\]

where \(d\) is the speckle size, \(z\) is distance to the detector, \(\text{FOV}\) represents the field of view where the light is collected from, and \(\lambda\) is the wavelength of the light source.

A focused ultrasound transducer with \(f = 1MHz\), is used to generate the ARF within the phantom. The source focuses to an area with lateral radius about 0.5mm and axial radius about 5mm. Figure 5.4 (b) and (c) show the ultrasound exposure time and imaging system timing. Four different ultrasound exposure times (50ms, 100ms, 1s, 2s) have been used to illustrate both the mechanical and thermal effects of the ultrasound wave. A speckle pattern is recorded every 16ms (62.5 fps).

### 5.5 Experimental Results

For each set of experiments, the time variant MIC signal is recorded utilizing Equation 5.3 and the process shown in Figure 2.20. At any given time, the difference image is obtained by taking the absolute value of the subtraction of the speckle pattern from the speckle pattern at the original state. The average of the difference image is the MIC signal. Figures 5.5 and 5.6 represent the resulting MIC signal for four ultrasound exposure times for different ultrasound intensities.

As discussed earlier, ARF excitation and ARF relaxation processes occur in the timescale of milliseconds. Since an image is taken every 16ms, the displacement effects can be seen in the MIC signal as an instantaneous change in a single frame.
CHAPTER 5. IMAGING UTILIZING MIC SIGNAL

Figure 5.5: Experimentally acquired MIC signal for four ultrasound exposure times (50 ms, 100 ms, 1 s and 2 s) and peak pressure of 2.55 MPa at the focal spot.

On the other hand, the heating and cooling processes occur in a longer timescale, which can be seen in the MIC signal as gradual change.

The instantaneous change at the beginning of the MIC signal represent the ARF excitation process, which is independent of the exposure time and is equal for all four different measurements. The gradual change after ARF excitation represent the heating process which follows by the ARF relaxation process. The change in the MIC signal due to ARF relaxation shows different behavior for short and long exposure times.

The change in the irradiance of the interfering waves can be written as

$$
\Delta I = \left| \sum_{i=1}^{P} \alpha_i e^{i(\omega t + \theta_{(1,i)})} \right|^2 - \left| \sum_{i=1}^{P} \alpha_i e^{i(\omega t + \theta_{(1,i)} + \Delta \theta_i)} \right|^2,
$$

where $\Delta \theta_i$ is the phase change induced to $i$th scattering paths.

$\Delta \theta_i$ is the change induced by both displacement and temperature effects. Therefore, the changes in the MIC signal not only depends on the process, but also depends...
In the case of ARF excitation process, the change induced to the phase profile corresponds only to the displacement. Therefore, as long as the intensity of the ultrasound wave and the mechanical properties of the medium do not change, the change in the MIC signal is equal for all different exposure times. However, in case of ARF relaxation, the phase profile is affected by the temperature rise, which is different for different ultrasound exposure times. Therefore the change in the MIC signal corresponding to the ARF relaxation process is different for different ultrasound exposure times.

**Short Ultrasound Exposure Time**

In case of a very short exposure time (50ms), according to Equation 4.9 the temperature rise is negligible. Therefore the rapid change in the MIC signal for ARF relaxation process is similar to the rapid change for ARF excitation, but in negative
sign (The blue curve in Figures 5.5 and 5.6).

In case of slightly longer exposure time (100 ms), the temperature rise can be seen in the MIC signal (The red curve in Figures 5.5 and 5.6) and as the result, the change in the MIC signal for ARF relaxation has a slightly different value compared to the ARF excitation, but still with a negative sign. Figure 5.7 illustrates this process with simulation results. The insonation process starts at the original state (marked as state #1). During the ARF excitation the MIC signal changes to the state #2 on the figure. During the heating process the MIC signal changes on the red curve to the state #3. At this point, during the ARF relaxation the MIC signal jumps from the red curve to the black curve to state #4. This is a jump towards the original state since the temperature rise is small due to short ultrasound exposure time. The cooling process follows and brings the MIC signal back to the original state (state #1).

**Long Ultrasound Exposure Time**

In case of longer ultrasound exposure times, at the beginning of the ARF relaxation process, the phase profile is different for the 1s and 2s experiments. Therefore the change in the MIC signal for ARF relaxation is different. Unlike short ultrasound exposure experiments, the ARF relaxation corresponds to a change further from the original state. Figure 5.8 illustrate the this process. Starting from state #1, after the ARF excitation the MIC signal jumps from the black curve on the red curve to state #2. During the heating process it changes on the red curve to reach state #3. At this point the ARF relaxation causes a jump in the MIC signal from the red curve on the black curve to state #4 which corresponds to a change further from the original state. After ARF relaxation, the cooling process changes the MIC signal on the black curve to the original state (state #1).
Figure 5.7: Simulation results corresponding to short ultrasound exposure time experiment (50ms). The Process begins at state#1 as the original state followed by ARF excitation (with maximum displacement) that corresponds to the jump to the state#2. From state#2, absorbed energy, gradually generates heat and change the MIC signal to the state#3 which is followed by ARF relaxation that corresponds to the jump to the state#4. After state#4, the MIC signal gradually goes back to the original state as the temperature gradually decreases to the original temperature. In the case of using a 50ms or 100ms pulse width, the ARF relaxation corresponds to an rapid drop in the MIC signal.

5.6 Displacement Correlated Imaging

The MIC signal depends on the mechanical and thermal properties of the medium with different timescales so that different effects can be separated. Specifically, the rapid change corresponding the ARF excitation process is independent of the ultrasound exposure time for exposure times longer compared to the elastic time scale; and if the ultrasound intensity does not change, the MIC signal is correlated to the mechanical properties of the medium. Therefore, the amount of the rapid change at the beginning of the MIC signal can be used to acquire displacement correlated images of the tissue. Figure 5.9 shows the simulation results of the imaging system using the change in the normalized MIC signal for ARF excitation process. The model is made
Figure 5.8: Simulation results corresponding to long ultrasound exposure time experiment (1s). The Process begins at state#1 as the original state followed by ARF excitation (with maximum displacement) that corresponds to the jump to the state#2. From state#2, absorbed energy, gradually generates heat and change the MIC signal to the state#3 which is followed by ARF relaxation that corresponds to the jump to the state#4. After state#4, the MIC signal gradually goes back to the original state as the temperature gradually decreases to the original temperature. In the case of using a 1s or 2s pulse width, the ARF relaxation corresponds to an rapid increase in the MIC signal.

so that for the same amount of ARF, the particles in the red area of the medium move two times more than the particles in the blue area i.e. the blue area is stiffer compared to the red area. The ultrasound focal spot has been scanned through the medium to acquire the knife-edge image.

In the knife-edge images, the transition from the red area to the blue area demonstrate the performance of the imaging system. Faster transition in the image, shows that the imaging system has a better resolution. Figures 5.10 and 5.11 represent the MIC signal transition from the red area to the blue area in the knife-edge images at the depth of 5 millimeters. Utilizing the 90% – 10% criteria [48] to define the resolution of the system, the resolution of the system is measured to be 3.3mm in the
Figure 5.9: Displacement profile of the medium (a and c). Considering that the ARF applied to both regions is the same, particle in the red region move two time more than particles in the blue region. The displacement correlated image of the medium utilizing the rapid change in the MIC signal in ARF excitation process is presented in b and d.

The resolution of the imaging system is governed by the size of the ultrasound focal spot. Increasing the frequency of the ultrasound wave results in a smaller focal spot, therefore, enhances the resolution of the imaging system. However, an ultrasound wave with a smaller focal spot can tag less number of scattering path, resulting in drop in the MIC signal, hence losing contrast. On the other hand, increasing the frequency limits the penetration depth of the ultrasound wave.
5.7 Conclusion

We have introduced the mean irradiance change (MIC) signal as a useful tool to monitor the changes induced to the phase profile of the scattering waves. Ultimately, the MIC signal strength depends on the number of scattering path being tagged and the amount of change applied to the phase profile. Parameters such as the size of the focal spot, ultrasound frequency and location of the ultrasound focal spot affect the MIC signal. During the insonation, each process changes the phase profile in a different way. The change in the phase profile by the displacement (ARF excitation and ARF relaxation) occur in a time scale much shorter compared to the changes induced by temperature rise (heating and cooling), hence they can be separated. We have utilized the simultaneous change in the MIC signal representing the ARF excitation process for imaging. The contrast of the imaging system is based on the
Figure 5.11: Transition from the region with high displacement to the region with low displacement. Red lines show the 90% and 10% for resolution measurement. The resolution is measured to be \( \approx 1 \text{mm} \) in lateral direction.

local mechanical properties of the medium. The knife-edge images acquired from simulation show that the resolution of the imaging system is governed by the size of the ultrasound focal spot.
Chapter 6

Tumor Detection

As normal cells go through rounds of successive mutations, they generate abnormal cells that proliferate without control and form a tumor. Cancer cells can ultimately travel through blood vessels and generate tumors in different locations in the body. In addition, tumors can develop their own blood vessels to grow further [103,104].

Detecting tumors in early stages is a crucial challenge. Light has been proven to play an important role in tumor detection. Different imaging modalities utilize different properties of a tumor to generate images. Different imaging methods have been introduced to detect the presence of a tumor in a healthy tissue non-invasively [13,105,106].

Due to the higher metabolism rate of a tumor compared to the healthy tissue, a tumor draws more blood, therefore has a higher optical absorption. In addition, due to the abnormal shape of the cancerous cells, the scattering coefficient of a tumor is also different from the healthy tissue.

In UOT, the imaging contrast is based on the optical absorption. The MIC signal can monitor both absorption and scattering properties of the medium. In Chapter 5
we showed that in the ARF regime we can utilize the scattering paths to obtain the MIC signal to form an image based on the mechanical properties of the medium.

In addition, the MIC signal can provide images based on the morphology of a tumor. In this chapter, we will discuss the difference in the optical scattering of a tumor compared to the healthy tissue. We will briefly study 2 cases to show that the MIC signal can detect tumors based on the morphology of cancerous cells that changes the optical scattering.

### 6.1 Cancerous Cell Properties

A tumor is the result of multiple rounds of successive mutations from a normal cell. The resulting abnormal cell continues proliferating without control. Also, cancer cells do not go through apoptosis; the process which a damaged cell dies. Cancer cells can develop their own blood vessels to provide the supply to grow more and also can travel through the vessels in the body to generate tumors in different locations. Cancer cells grow in abnormal shapes and compared to normal cells, they have large and variably shaped nuclei as illustrated in Figure 6.1 [104,107,108].

In our simulations, we assume that scattering events only occur at the location of nuclei of the cells. However, it is possible to add other scattering organelles like mitochondria to the model [109,112].

In this chapter, we focus mainly on two characteristics of a cancerous cell: 1) Cells with larger nuclei (Model #1, shown in Figure 6.2) and 2) Group of cells with higher concentration compared to the healthy tissue (Model #2, shown in Figure 6.3).
Figure 6.1: Cancer cells and normal cells comparison. [10]

<table>
<thead>
<tr>
<th>Normal</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Normal Cells" /></td>
<td><img src="image" alt="Cancer Cells" /></td>
</tr>
<tr>
<td>Large, variably shaped nuclei</td>
<td></td>
</tr>
<tr>
<td>Many dividing cells; Disorganized arrangement</td>
<td></td>
</tr>
<tr>
<td>Variation in size and shape</td>
<td></td>
</tr>
<tr>
<td>Loss of normal features</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6.2: Model #1, blue particles represent normal cells and red particles represent a tumor with cancerous cells with larger size. The particle density of the healthy tissue and the tumor is the same.
Figure 6.3: Model #2, blue particles represent normal cells and red particles represent a tumor with cancerous cells with higher density of particles. The particle size of the healthy tissue and the tumor is the same.

6.1.1 Particle Size

In the fixed-particle Monte Carlo model, the optical path length of each scattering path is recorded to be used as the phase of the corresponding interfering wave. The scattering angle is chosen based on the phase function of the scattering particle. The size parameter governs which scattering regime can be used to find the phase function. The size parameter is defined as:

$$X = \frac{2\pi nr}{\lambda}, \quad (6.1)$$

where $n$ is the index of refraction of the medium, $r$ is the radius of the particle and $\lambda$ is the wavelength of the light.
For a larger particle the phase function changes so that more light scatters in the forward direction. Figure 6.4 shows the scattering function corresponding to different size parameters.

Figure 6.4: Scattering phase function for size parameters, a) \( X = 1 \), b) \( X = 3 \) and c) \( X = 10 \). \( \lambda = 632.8 \text{ nm} \) and \( n = 1.3725 \). Note that the polar graphs are in logarithmic scale so that in the case of \( X = 10 \), forward scattering is about 4 orders of magnitude stronger than backward scattering.

On the other hand, as shown in Figure 3.4, a larger particle has a larger cross-section for scattering light. Hence, as the size of the particle increases, a larger number of scattering events occur at the location of that particle. In other words, a larger particle generally participates in a larger number of scattering paths (higher scattering per particle). Figure 6.5 shows the map of number of scattering events in the medium for Model #1 with large particles.

The ultrasound wave tags the scattering paths that travels through its focal spot, by inducing displacement to the particles. Although a larger particle also changes the scattering phase function, it does not affect the change applied to the optical path length of the tagged scattering path. However, in Model #1, compared to particles with normal size, larger particles have higher scattering per particle. Hence, by applying displacement to a larger particle, a larger number scattering paths are
Figure 6.5: The scattering map of the model with larger particles. The tumor with larger particles scatter more light.

tagged. Therefore, by moving the ultrasound focal spot on the tumor in Model #1 we can apply a larger amount of change in the MIC signal.

6.1.2 Scatterer Density

The number of tagged scattering paths is governed by the number of scatterers affected by the ultrasound wave. There are two scenarios which the number of scatterers affected by the ultrasound wave can change: 1) Changing the size of the ultrasound focal spot and 2) Change in the concentration of particles so that more number of
scatterers lie within the ultrasound focal spot.

Increasing the frequency of the ultrasound wave results in a smaller ultrasound focal spot, therefore a better resolution. However, the enhancement in the frequency comes with the cost of a weaker MIC signal and higher ultrasound wave scattering \cite{93}. On the other hand, different concentration of particles in a medium, such as the presence of a tumor in a healthy tissue (Model #2), results in a change in the MIC signal. Figure 6.6 shows the geometry of Model #2 with an inhomogeneity (in red), representing the tumor of abnormal cells. The elliptical shaded area represents the ultrasound focal spot. As the concentration of the particles changes, the number of tagged scattering paths changes, therefore the resulting MIC signal changes.

![Figure 6.6: Geometry of Model #2. Blue particles represent normal cells and red particles represent cancer cells with higher concentration of particles with the same size. The elliptical shaded area represents the ultrasound focal spot. Higher concentration of particles implies that more particles are affected by the ultrasound wave, results in tagging more scattering paths.](image-url)
6.2 Results

In order to demonstrate the effect of the parameters mentioned in Section 6.1, we have performed three sets of simulations. One case is a homogeneous model, as shown in Figure 6.7 and two cases include inhomogeneity in the model with larger particle size (Model #1, Figure 6.8) and higher concentration of particles (Model #2, Figure 6.9). In each simulation, the ultrasound focal spot is scanned in the medium in the X direction at $Y = 0$. The change in the MIC signal caused by the ARF excitation process for a constant maximum displacement is used for imaging.

6.2.1 Homogeneous Medium

In the case of the homogeneous model, as the ultrasound focal spot is scanned in the X direction ($Y = 0$), the MIC signal increases with the maximum at the center of the medium ($X = 0$). The maximum occurs at the center since the illumination is located at $X = 0$, therefore when the ultrasound focal spot is located at $X = 0$, it tags more scattering paths, resulting in a stronger change in the MIC signal. Since the model is homogeneous the change in the MIC signal as the ultrasound focal spot is scanned in the medium, shows a symmetric behavior as in Figure 6.7.

6.2.2 Inhomogeneity - Larger Particles

In contrast, the change in the MIC signal for Model #1, seen in Figure 6.8, shows an asymmetric behavior. This asymmetric behavior is the result of tagging a larger number of scattering paths at the location of the tumor, hence showing the presence of the tumor in the healthy tissue.
Figure 6.7: Homogeneous model. The ultrasound focal spot is scanned in the direction showed by the red arrow step by step. At each step the change in the MIC signal is recorded and the result is shown on the right. The signal reaches its maximum at $X = 0$ where the illumination is aligned to. Since the model is homogeneous, the MIC signal show a symmetric behavior. Note that the MIC signal is normalized by the value of the MIC signal at $X = 0$ where the illumination and the ultrasound focal spot are well aligned.

6.2.3 Inhomogeneity - Higher Concentration

Similar to the results for Model #1, by scanning the MIC signal in the medium in Model #2, the MIC signal shows an asymmetric behavior. At the location of the tumor, the ultrasound focal spot affects a larger number of scattering paths and therefore generates a bigger change in the MIC signal. As shown in Figure 6.9 compared to the left side of the medium, an increase in the MIC signal can be seen, which shows the presence of a tumor in the medium.
CHAPTER 6. TUMOR DETECTION

Figure 6.8: Inhomogeneous Model #1. The red area contains larger particles compared to the blue area (with the same concentration). On the right, the change in the MIC signal shows an asymmetric behavior as the ultrasound focal spot is scanned through the medium, showing the presence of the tumor in the medium. Note that the MIC signal is normalized by the value of the MIC signal at $X = 0$ where the illumination and the ultrasound focal spot are well aligned.

6.3 Conclusion

In this chapter we studied the performance of the MIC signal with the fixed-particle Monte Carlo to detect a tumor in a healthy tissue, focusing on the optical scattering properties of a tumor based on the two characteristics of a cancerous cell. Results in Figures 6.8, 6.9 and 6.10 show that the MIC signal is able to detect the presence of a tumor based on the size and density of particles in the tumor.

Figure 6.10 shows the results in a single plot. On the left side of the medium, the MIC signal shows the same behavior for all the three models. On the other hand, it is easier to notice the increase in the MIC signal in both Model #1 (green) and Model #2 (red).
Figure 6.9: Inhomogeneous Model #2. The red area contains particles with higher concentration compared to the blue area. On the right, the change in the MIC signal shows an asymmetric behavior as the ultrasound focal spot is scanned through the medium, detecting the presence of the tumor in the medium. Note that the MIC signal is normalized by the value of the MIC signal at $X = 0$ where the illumination and the ultrasound focal spot are well aligned.

Although in both case, the tumor is located at $X = 2mm$, the results show that the change in the MIC signal for Model #1, occurs at $X = 0.5mm$ and in Model #2 at $X = 1.5mm$. This happens because of the higher scattering per particle for the cancerous cells in Model #1, compared to the cancerous cells in Model #2. When the ultrasound focal spot is located at $X = 0.5mm$, although it affects more particles in Model #2, but due to higher scattering per particle of Model #1, a larger number of scattering paths are tagged, therefore a higher change in the MIC signal can be seen. However, as the ultrasound focal spot is scanned at $X = 3mm$, the MIC signal drops faster in Model #1 compared to Model #2. This is due to the shadowing effect. Because of the larger particles in Model #1, particles that are further to the right side of the tumor participate in a smaller number of scattering paths, therefore the
CHAPTER 6. TUMOR DETECTION

Figure 6.10: Simulation results combined. Note that on the left side of the plots, all three cases show the same behavior. However, on the right side of the plot, the two cases of homogeneous and in-homogeneous with larger particles show similar behavior and in the case of in-homogeneous medium with higher concentration, a meaningful change in the MIC signal reveals the presence of the tumor.

In addition to larger particle size and higher concentration, a tumor usually has different mechanical properties compared to the healthy tissue. A stiffer medium results in a considerable change in the MIC signal. Our results demonstrate that the MIC signal is able to detect the presence of a tumor not only based on the mechanical properties, but also based on the morphology of the tumor.
Chapter 7

Future Work

7.1 Epi-illumination

The simulation and experimental results presented in Chapters 4, 5 and 6 are based on trans-illumination approach. However, in practice applying non-invasive trans-illumination imaging in clinical application is challenging.

In trans-illumination approach, the light from the source travels through the tissue and transmitted through (diffusely transmitted) to reach the detector. In other words, the light source and the detector are placed on opposite sides of the tissue. In the epi-illumination method, the light source and the detector are placed on the same side of the tissue and the light that reflects back from the tissue (diffused reflection) is used for imaging. Figure 7.1 illustrates the difference between a trans-illumination system and an epi-illumination system.

Signals obtained in a trans-illumination system contains more information about the tissue since they travel deeper in the tissue to reach the detector. Utilizing epi-illumination with speckle pattern analysis, results in a drop int the MIC signal.
7.2 Temperature Correlated Imaging

In Section 5.6, the instantaneous change in the MIC signal corresponding to the ARF excitation process is used to form an image based on mechanical properties of the tissue. The MIC signal obtained from the long ultrasound exposure time experiment contains more information about the local properties of the tissue. For instance, during the heating process, the MIC signal gradually increases till the point it becomes steady. Assuming that no displacement occurs during the heating process, the point where the MIC signal does not change, corresponds to the point where the medium is in thermal equilibrium; i.e. the heat is diffused to the surrounding tissue and the temperature does not increase further. This can be seen in Figure 7.2 in long exposure time experiments.

The duration of the heating process from the start until the tissue reaches the thermal equilibrium point reflects the local thermal properties of the tissue. Similar to Section 5.6, this time interval can be used to form an image based on the thermal
Figure 7.2: Experimental MIC signal obtained from long ultrasound exposure times. The time interval from the beginning of the heating process to the point where the MIC signal reaches its steady value ($t$), can be utilized to form a thermal property based image.

properties of the tissue.

7.3 Other Applications of Fixed-Particle Monte Carlo

Besides speckle pattern analysis in ARF regime, the fixed-particle Monte Carlo model introduced in Section 3.2.2 can be utilized for other speckle pattern analysis methods. Utilizing the amplitude and the phase of the scattering paths, the speckle pattern can be formed. Similar to the ARF regime, displacement or temperature change in the medium can be modeled and different analysis on the speckle pattern can be performed. For instance, the speckle contrast due to changes applied to the medium such as particle motion can be found utilizing the fixed-particle Monte Carlo.

The analyses presented in Chapters 4 and 5 were under assumption that by moving particles, the scattering paths remain the same. In other words, although the particles are being moved, based on the direction of the photon, it still hits the same particle.
This assumption is valid as long as the particle displacement is smaller than twice the particle size ($d < 2r$). If this condition is fulfilled, speckle pattern analysis simulation can be performed utilizing the fixed-particle Monte Carlo.
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