INTERFEROMETRIC SYNTHETIC APERTURE MICROSCOPY (ISAM)
RECONSTRUCTION AND CHARACTERIZATION IN A HIGH NUMERICAL APERTURE SYSTEM

BY
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THESIS
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Optical coherence microscopy (OCM) is an imaging modality that is capable of visualizing structural features of biological samples at high resolution based on their scattering properties. Interferometric synthetic aperture microscopy (ISAM) is a newer technique that can overcome the typical dependence between lateral resolution and depth-of-focus of an optical coherence tomography (OCT) imaging system by offering spatially invariant resolution within the whole 3D data set, including regions that are outside of the focal region. Both OCM and ISAM have many potential research and clinical applications. By combining OCM and ISAM, it is possible to visualize an entire 3D volumetric data set with the high resolution normally available only at the focus. Therefore, this combination will yield more detailed information from the observed sample than OCM alone. This combination will also improve the feasibility of the ISAM technique for wider research and clinical applications. This thesis presents the experimental validation and characterization of ISAM applied to high numerical aperture OCM optical imaging. The validation includes the image reconstruction of a tissue phantom containing nanoparticles both for OCT and ISAM, and system characterization includes quantitative assessment of the confocal parameter, point spread function, and phase stability measurements. Several potential applications also are examined as a part of this thesis.
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<tr>
<td>2D</td>
<td>Two-dimensional</td>
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<td>3D</td>
<td>Three-dimensional</td>
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<td>BS</td>
<td>Beam splitter</td>
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<td>CCD</td>
<td>Charge-coupled device</td>
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<td>CT</td>
<td>Computed tomography</td>
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<td>DG</td>
<td>Diffraction grating</td>
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<td>DM</td>
<td>Dichroic mirror</td>
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<td>DOF</td>
<td>Depth-of-field</td>
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<td>F</td>
<td>Filter (emission)</td>
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<td>FFT</td>
<td>Fast Fourier transform</td>
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<td>FOV</td>
<td>Field-of-view</td>
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<td>FWHM</td>
<td>Full width at half maximum</td>
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<td>HWP</td>
<td>Half-wave plate</td>
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<tr>
<td>iFFT</td>
<td>Inverse fast Fourier transform</td>
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<td>ISAM</td>
<td>Interferometric synthetic aperture microscopy</td>
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<tr>
<td>M</td>
<td>Mirror</td>
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<td>MMF</td>
<td>Multi-mode fiber</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>NA</td>
<td>Numerical aperture</td>
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<td>OBJ</td>
<td>Objective lens</td>
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<td>Acronym</td>
<td>Description</td>
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<tr>
<td>OCDR</td>
<td>Optical coherence-domain reflectometry</td>
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<td>OCM</td>
<td>Optical coherence microscopy</td>
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<td>OCT</td>
<td>Optical coherence tomography</td>
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<td>P</td>
<td>Polarizer</td>
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<td>PCF</td>
<td>Photonic crystal fiber</td>
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<td>PDMS</td>
<td>Polydimethylsiloxane</td>
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<td>PMT</td>
<td>Photomultiplier tube</td>
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<tr>
<td>PSF</td>
<td>Point spread function</td>
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<td>SD-OCM</td>
<td>Spectral-domain optical coherence microscopy</td>
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<tr>
<td>SD-OCT</td>
<td>Spectral-domain optical coherence tomography</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<td>TD-OCM</td>
<td>Time-domain optical coherence microscopy</td>
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<td>TS</td>
<td>Translation stage</td>
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1.1 Optical Coherence Tomography (OCT)

Optical coherence tomography (OCT) is a technology developed for high-resolution and noninvasive cross-sectional imaging for medical and biological applications [1]. OCT utilizes low-coherence interferometry to generate a two-dimensional (2D) or three-dimensional (3D) image of optical scattering properties of tissue and other biological samples. It is analogous to ultrasound imaging, where OCT utilizes low-coherence back-reflected light waves to probe the object while ultrasound imaging uses back-reflection of acoustic waves. This technology is important to medical and biological applications because it allows in situ imaging of the sample with high resolution on the order of a few microns. Therefore, OCT has been applied to various branches of medicine such as ophthalmology, cardiology, gastroenterology, oncology, dentistry and dermatology, to name only a few [2]. However, due to limited OCT penetration depth, on the order of 1 - 2 mm [3], OCT is applied mainly to the easily accessible portions of the body such as the eye and skin. To access internal parts of the body, OCT is coupled with endoscopic or needle probes [4].

It is instructive to compare OCT with current modalities for medical imaging. Figure 1.1 shows this comparison based on resolution and penetration depth. In general, this plot reveals that there is a tradeoff between the resolution and penetration depth, meaning that higher resolution imaging suffers low penetration depth. OCT fills the space between conventional confocal microscopy and high-frequency ultrasound imaging. OCT is able to image more
morphological detail in dense, high-scattering tissue, compared to confocal microscopy, because it detects only light that has been scattered once [5].

1.1.1 Principle of OCT

OCT has its roots in the early development of white light interferometry that led to optical coherence-domain reflectometry (OCDR) [7]. Originally OCDR was employed for finding faults or breaks in optical fiber cable, but soon it was employed to probe optical reflections in biological tissue [8-9]. Tomographic imaging of biological tissue can be generated by applying lateral beam scanning [1].

A simplified block diagram of a typical spectral domain OCT (SD-OCT) system is presented in Figure 1.2. Broadband light from the source, e.g. Ti:sapphire laser, is divided by beam splitter into sample and reference arm. The recombination of light from the reference arm
Figure 1.2: A simplified spectral-domain optical coherence tomography (SD-OCT) system.

and back-scattered light from the sample arm produces the interference pattern. This interference of the light between the two arms can only occur when the two path lengths are within the source coherence length or axial resolution, typically around 5 – 10 µm. The interference pattern is then spectrally decomposed by the grating and is detected by the CCD camera.

In SD-OCT, the broadband light is dispersed onto a CCD camera where each pixel detects signal from all depth positions within the sample without the need to change the optical delay in the reference arm mirror, as is necessary in time-domain OCT systems. The inverse Fourier transform of the spectrally separated interference pattern provides the depth information, which creates a single A-scan or single column of data within an OCT image [10]. A galvanometer mirror in the sample arm scans the beam laterally, in the $x$ direction, across the
sample to create a 2D cross-sectional OCT image, or B-mode image, which is composed of a sequence of laterally displaced A-scans. Similarly, the light can be scanned laterally in the other direction, the \( y \) direction, and a sequence of 2D cross-sectional images can be generated to form a 3D image.

OCT as a noninvasive imaging modality depends on intrinsic variations in the optical properties of tissues to distinguish tissue components. It uses the spatial variation of the scattering properties of tissue microstructure as contrast. In addition, any physical properties changing the amplitude, phase, or polarization can be used as contrast to form images [3]. To generate specific molecular contrast, some researchers have proposed and demonstrated various extrinsic contrast agents such as magnetic nanoparticles and microspheres to broaden the range of applications of OCT [11-12].

The bandwidth and center wavelength of the light source are chosen based on the optical properties within the tissues, considering both the scattering and absorption properties. There is a preferred spectral range for optical imaging in tissue called the “biological window,” generally in the range of 700 – 1400 nm, where attenuation from scattering is more dominant than absorption. In the spectral region less than 700 nm, light will be absorbed by the tissue, particularly by melanin and hemoglobin, whereas in the spectral region greater than 1400 nm, light will be absorbed predominantly by the water in the tissue. Also as a general rule, within the biological window, the greater the center wavelength used, the greater the penetration within the tissue due to reduced scattering [3].
1.1.2 Performance of OCT

The performance of an OCT system is measured in general by its resolution in the axial and lateral directions, penetration depth, sensitivity, and its imaging speed [13]. OCT uses a broadband-light source with a low coherence length to obtain better axial resolution and contrast, compared to a small focus and confocal region in conventional confocal light microscopy. The axial resolution of an OCT system is given by the coherence length \( l_c \) of the light source, which depends on the full width at half maximum (FWHM) of the source spectrum and is given by:

\[
\Delta z = \frac{2 \ln 2 \lambda^2}{\pi \Delta \lambda},
\]

where \( \lambda \) is the center wavelength and \( \Delta \lambda \) is the FWHM bandwidth of the optical spectrum assuming it is Gaussian-shaped [3]. Therefore, higher axial resolution \( \Delta z \) can be achieved by a light source with a shorter center wavelength and a broader bandwidth. However, shorter center wavelengths are more scattered inside and outside of the biological window and will have reduced imaging penetration depth. The transverse or lateral resolution of an OCT system is determined by the numerical aperture (NA) of the objective lens and is commonly given as the focused spot size. The lateral resolution \( \Delta x \) is given by [14]:

\[
\Delta x = \frac{4 \lambda}{\pi} \cdot \frac{f}{d},
\]

where \( f \) is the focal length of the objective lens and \( d \) is the beam diameter on the objective lens. A higher-NA objective lens can be employed to focus the beam to a small spot size to enhance lateral resolution, but will subsequently decrease the DOF of the OCT beam. The DOF of the OCT beam is defined as the confocal parameter \( b \) or two times the Raleigh range, \( 2z_R \), and is given by [14]:
\[ b = 2z_R = \frac{\pi \Delta^2}{2\lambda} = \frac{2\pi w_o^2}{\lambda}, \]  

(1.3)

where \( w_o \) is beam radius and \( \lambda \) is center wavelength. An OCT system has typical lateral and axial resolutions around 10 – 30 \( \mu \)m and 1 – 10 \( \mu \)m, respectively.

It is important to maintain a constant resolution within the observed area of the sample because the object is commonly classified based on its apparent morphology. Non-uniform resolution within the observed region may result in misdiagnosis [15]. For example, precancerous cellular changes may not be detected at low resolution [16-17].

To increase (improve) the lateral resolution over a relatively large depth-of-field (DOF), different methods have been proposed, such as applying adaptive optics [18], axicon lenses [19-21], dynamic focusing [22,23], and a small liquid-filled polymer lens [24]. One of the drawbacks of the proposed methods is that they require specific setup and modifications of the imaging systems that may be difficult to implement. A numerical method that requires little or no instrument modification is preferable. A solution of the inverse problem for OCT has been presented [25-26], but this solution is only for a one-dimensional model and does not take into account data acquired at various lateral positions of the light beam.

The typical imaging penetration depth of an OCT system is around 1 – 3 mm, which is dictated by both multiple backscattering and light absorption within biological tissues. The maximum theoretical penetration depth considering hardware limitations in a SD-OCT system is given by:

\[ z_{max} = \frac{\lambda^2}{4n\Delta\lambda} N, \]  

(1.4)
where $n$ is the average refractive index of the medium, and $N$ is the number of pixels in CCD camera [2, 27]; however, the actual imaging penetration depth may be reduced based on the degree of optical scattering within the tissue.

Optical coherence microscopy (OCM) is the term which refers to an OCT system having high lateral resolution. OCM uses a high-NA objective lens and consequently must construct *en face* images rather than cross-sectional images because of the DOF limitation inherent to a tightly focused optical beam. The DOF limitation in OCM can be overcome by using a spectral-domain detection system and by applying a computational method known as interferometric synthetic aperture microscopy (ISAM). This is possible because of the excellent phase stability in spectral-domain detection resulting from a static reference arm mirror and high data acquisition rates [28].

The sensitivity $S$ of an OCT system is defined as the minimum detectable reflectivity or the lowest detectable backscattered optical power and is given by [10, 29]:

$$S = \frac{1}{8} \frac{\eta \lambda P_S}{hc B},$$  \hspace{1cm} (1.5)

where $\eta$ is the quantum efficiency of the detector, $\lambda$ center wavelength, $h$ the Plank’s constant, $c$ the speed of light, $P_S$ the incident optical power on the sample, and $B$ the electrical bandwidth. In SD-OCT, $B$ refers to the A-scan acquisition rate based on $B = 1/(2T_i)$, in which $T_i$ is the camera integration time [29].

The imaging performance of the data acquisition scheme is governed by the dynamic range of the reflectivity or the strength of scattering signal [30]. The achievable dynamic range is limited by the bit depth during the digitization of the signal acquisition [31]. In SD-OCT, the bit
depth of the CCD camera is distributed over a large coherence background, thus decreasing the effective bit depth of the signal, which limits the ability of the OCT system to detect both strong signals near the surface and weak signal from deep in turbid tissue [30].

1.2 Optical Coherence Microscopy (OCM)

OCT and confocal microscopy are two excellent techniques that allow in vivo imaging of tissue microstructure [1,32]. While confocal microscopy can be used to image single cells, one of the drawbacks of this technique is that it is limited to imaging relatively transparent specimens due to degraded image contrast by light scattering. While OCT systems can image deeper in highly scattering tissues, OCT cannot be utilized to image individual cells due to the limited transverse resolution. The technique optical coherence microscopy (OCM) combines the capability of both OCT and confocal microscopy by taking advantage of the high resolution of confocal microscopy and the coherence-gated detection of OCT in order to increase rejection of unwanted scattered light from outside the focus. The improved axial sectioning from the coherence gating enables greater penetration depth and contrast compared to confocal microscopy alone [33]. OCM images are acquired in the en face plane, as in confocal microscopy, and can achieve a lateral resolution on the order of a micrometer. Therefore, OCM is a powerful imaging technique that can image, with high cellular-level resolution, human tissues such as skin [34], gastrointestinal tissue [35], and oral neoplasia [36]. OCM can use contrast methods other than scattering, such as fluorescence [37], quantitative phase [38,39], and spectroscopic properties [40]. Finally, OCM has the potential to be a key technology to enable cellular imaging for internal body applications via endoscopic probes or catheters.
Figure 1.3 shows a comparison of the axial PSF between OCM and confocal microscopy alone. It reveals that OCM has greater rejection of light from outside the focal region compared to confocal microscopy. For a Gaussian beam, the sensitivity of OCM decreases exponentially with distance from the focal plane, whereas the sensitivity will decrease geometrically for a single-mode confocal microscope. In the next section, the principles of OCM will be presented along with its properties such as resolution, SNR and depth of imaging penetration.

![Graph showing comparison of PSF](image)

Figure 1.3: Comparison of experimental point spread function acquired by scanning a mirror through the confocal plane with and without coherence gating, using a 20X and NA = 0.4 objective lens [33].

1.2.1 Principles of OCM

Similar to OCT, OCM uses low-coherence light with broadband bandwidth to illuminate the sample and detects the backscattering light with interferometry, enabling the varying intensity of backscattering from different transverse positions in the sample to be resolved. The
lateral resolution of both OCT and OCM is governed by the diameter of the beam at the focus. OCT uses a low-NA lens which produces a relatively constant lateral resolution over the axial scan. Therefore, OCT with lower-NA will have lower lateral resolution, but will provide a relatively constant beam diameter across a longer DOF. On the other hand, OCM uses a high-NA lens which provides higher lateral resolution, but is limited basically to one plane with a small DOF. High-NA lenses produce more significant distortion of images outside the focal plane, and therefore high-NA lenses are more suitable for acquiring images in en face planes such as in multiphoton and confocal microscopy. The beam geometries for both low and high-NA are presented in Figure 1.4. Furthermore, the confocal parameter of the lens $b$, which is the region near the focus where the beam has a relatively constant diameter, is called the DOF, which is considered in-focus in optical imaging systems.

Figure 1.4: The Gaussian beam geometry for low and high-NA lenses, where $b$ is the confocal parameter and $w_o$ is the radius of the beam at the focus.

OCT acquires 2D cross-sectional images by scanning the beam in a straight line and resolving the scattering depth information at each position (Figure 1.5). On the other hand, OCM collects en face images by scanning the beam in a plane and acquires the en face images limited to only the focal plane with high lateral resolution (Figure 1.5).
Similar to OCT, there are two different detection schemes for OCM: time-domain and spectral-domain detection. Both time- and spectral-domain detection schemes use a low-coherence optical source and an interferometer. In time-domain detection, a photodiode is used as a detector and a moving mirror is installed in the reference arm to produce delay and apply a frequency shift to the light because of the Doppler effect. By scanning the reference mirror, the scattering from various depths can be resolved. In spectral-domain detection, a CCD camera is used as a detector and a fixed mirror is installed in the reference arm. The scattering depth information (A-scan) can be determined by the acquisition of the spectral interference pattern followed by the inverse Fourier transform of the data [41].

Imaging speed is one of the important factors governing OCM performance where fast acquisition enables the observation of dynamic changes in samples. It is instructive to distinguish the difference between time-domain (TD) and spectral-domain OCM (SD-OCM) when analyzing imaging speed. For TD-OCM, no scanning of the delay mirror is needed because it acquires
scattering from only one depth; however, the reference mirror position still needs to be adjusted when different depths are desired, and to adjust for the varying index of the sample. Also, in TD-OCM, the reference arm mirror is often still modulated to produce the heterodyne frequency for later demodulation of the data, whereas acquisition of the whole 3D volume is needed for SD-OCM to perform imaging at one depth. Some improvements can be made to increase the acquisition speed of spectral-domain OCM; for example, real-time architecture has been added to improve the processing time significantly [42]. This method, however, still needs to handle an enormous amount of 3D data that decreases the imaging speed. Therefore, image acquisition in TD-OCM is relatively fast compared to SD-OCM.

1.2.2 Resolution of OCM

The transverse resolution of OCM, assuming Gaussian beam, is given by:

$$\Delta x \approx \frac{2 \lambda}{\pi NA} \approx \frac{0.64 \lambda}{NA} ,$$

(1.6)

where $\lambda$ is the center wavelength of the beam and $NA$ is the numerical aperture of the objective lens. The confocal parameter $b$, on the other hand, is determined by:

$$b \approx \frac{2 \lambda}{\pi NA^2} .$$

(1.7)

The axial resolution in OCM can be described as the coherence length of the Gaussian beam and for lower NA can be described as:

$$l_c = \frac{2 \ln 2 \lambda^2}{\pi \Delta \lambda} .$$

(1.8)

For confocal microscopy, the axial resolution at the focus assuming Gaussian beam is given by [13]:

12
\[ \Delta z = \frac{1.4 \, n \, \lambda}{NA^2} \quad . \quad (1.9) \]

From the equations above, the transverse or lateral resolution changes inversely with the value of NA, while the axial resolution changes inversely with the square of the NA of the objective lens. A high-NA objective lens is usually used to acquire a high resolution image of the sample and subsequently improve the image quality. A lateral resolution of \(3 - 5 \ \mu \text{m}\) and axial resolution of around \(1 \ \mu \text{m}\) can be achieved by using a typical objective lens with NA values from 0.7 to 1.2 for a confocal microscopy system [13]. In contrast, a recent study reveals that an OCM system for 1060 nm wavelength could achieve resolutions of \(< 4 \ \mu \text{m}\) axial and \(< 2 \ \mu \text{m}\) transverse [43].

### 1.2.3 Signal-to-noise ratio (SNR)

For any electronic measurement system, the signal-to-noise ratio (SNR) is an important determining factor in the performance or quality of the measurement system. The SNR for an imaging system, e.g. CCD camera, is a value that represents the ratio of the ideal light signal to the combined noise signal consisting of the unwanted noise signal from electronic system and inherent variation of the incident photon flux. Therefore, the SNR is an important factor to assess the image quality of the imaging system. The SNR of a time-domain OCM system is described by [8]:

\[
\text{SNR}_{\text{time-domain}} = \frac{1}{2} \frac{\eta P_s R_s}{h \nu B} , \quad (1.10)
\]

where \(\eta\) is the detector quantum efficiency, \(P_s\) is the incident power, \(R_s\) is the reflectivity of the sample, \(h\) is Planck’s constant, \(\nu\) is the optical frequency, and \(B\) is the bandwidth of the signal.

The SNR of a spectral-domain OCM is given by [44]:
\[
\text{SNR}_{\text{spectral-domain}} = \frac{1}{2} \frac{\eta R_s P_s \Delta t}{h\nu},
\]

where \(\Delta t\) is the exposure time of the CCD camera. The typical value for a good measurement system is 90 dB [8]. In addition, an SD-OCM system usually has improvement of 20-30 dB over a TD-OCM system [44].

### 1.2.4 Penetration depth

Although imaging penetration depth is difficult to measure because it depends essentially on optical properties of the tissue, OCM generally has better penetration depth than confocal microscopy [33,35,36]. Initial OCM systems showed an improvement in penetration depth of around 2 – 3 times compared to confocal microscopy [33]. In addition, a newer study showed that the OCM can extend the available imaging depth of confocal microscopy up to several hundred micrometers in highly scattering tissues [35].

### 1.3 Motivation and Outline of the Thesis

OCT and OCM are excellent high-resolution optical imaging methods that can penetrate 2-3 mm in biological tissue, whereas confocal microscopy cannot achieve this penetration. However, penetration of OCM is limited by the working distance of objectives, and by the aberrations, more than by the scattering properties of the sample. One of the limitations of both OCT and OCM is that they need to acquire the image within the confocal parameter limit [1,33]. Therefore, to get a high-resolution 3D image, the focus must be scanned through the entire region of interest within the sample. A new computational imaging method based on OCT systems called interferometric synthetic aperture microscopy (ISAM) was proposed to overcome this problem [28,45-47]. A depth-independent resolution can be obtained within the entire
volume while the focus remains fixed in one depth position. Many ISAM studies such as real-time processing, non-paraxial vector-field modeling, autocorrelation artifact reduction, full field imaging, and partially coherent illumination have appeared in the literature [28,45-54]. However, these ISAM experiments were mostly performed at low-NA. This thesis presents experimental validation of ISAM for a high-NA imaging system, in particular, high-NA OCM. In order to reconstruct a high-NA ISAM image from OCM data, the characterization of such a high-NA OCM system is also presented.

The outline of the thesis is as follows: Chapter 2 discusses ISAM theory. Chapter 3 describes the experimental setup and measurement results and discussion. Finally, Chapter 4 presents the conclusion and future work.
CHAPTER 2

INTERFEROMETRIC SYNTHETIC APERTURE MICROSCOPY

(ISAM) THEORY

Biomedical imaging encompasses a wide variety of medical imaging modalities used to acquire internal anatomic images of tissues and organs to provide biochemical and physiological analysis [55]. The more common modalities include x-ray computed tomography (CT), magnetic resonance, ultrasound, nuclear, and optical imaging. All of these modalities use absorption, reflection, emission, or transmission of electromagnetic or acoustic waves. Each of these modalities has its own advantages and disadvantages. For example, CT offers good anatomical information of tissues, but suffers from low contrast between soft tissues and involves ionizing radiation that limits its applications. Magnetic resonance imaging (MRI) has good penetration depth but has relatively low resolution and is expensive. On the other hand, ultrasound has good penetration depth, a portable and compact system, and is inexpensive. However, it has low resolution, and the requirement of contact between the transducer and tissue precludes many applications.

Optical imaging modalities such as fluorescence, visual endoscopy, multiphoton microscopy, confocal microscopy, optical coherence microscopy, and optical coherence tomography (OCT) are usually easy to implement, economical, nondestructive and appropriate for in vivo tissue imaging and have higher spatial resolution than other imaging modalities. Among these optical modalities, OCT and OCM have a unique place because of their high resolution, low cost, subsurface imaging capability, and potential for real-time imaging.
Current interferometric microscopy techniques cannot visualize objects located outside the confocal region (that is the focus) because their processing does not account for the defocusing and diffraction effects [54]. A model to address the problem has been proposed [25-26], but this solution is valid for a one-dimensional model and does not consider data acquired at various lateral positions of the light beam. ISAM is a new 3D optical microscopy modality that uses a comprehensive model for OCT to enable the reconstruction of objects located outside the focus of the lens [28], something that OCT alone cannot accomplish. Therefore, ISAM provides high-resolution, 3D, optical imaging with the ability to reconstruct object structure from outside the confocal region, effectively extending the DOF while maintaining high, spatially invariant, transverse or lateral resolution.

ISAM provides 3D volume structure of the sample by solving the inverse scattering problem involving scattering, diffraction and beam parameters [28]. Mathematical modeling in ISAM is similar to those of computed tomography, magnetic resonance imaging, and synthetic aperture radar (SAR). For example, SAR combines the back-reflected radar signals from the object into a single image, while ISAM combines data from back-scattered light to offer 3D volume imaging of the sample with spatially invariant resolution at all depths within the volume.

ISAM development to improve its functionality has included rotationally scanned ISAM [46], vector ISAM [48], full-field ISAM [50], and partially coherent ISAM [53]. Early ISAM developments used only a simplified scalar model of Gaussian beam focusing and scattering. A full vectorial model was developed to improve the model by adding the effects of high-angle fields and polarization on scattering and propagation so that ISAM can achieve maximum resolution [45]. Full-field ISAM is able to image an entire en face array of images of the 3D sample simultaneously while the focus is fixed at one plane inside the sample [50].
Rotationally scanned ISAM, used to image luminal tissue structures, such as intravascular imaging, has been implemented by utilizing a fiber-optic catheter as a beam-delivery probe where its aperture was scanned along the catheter direction and in the rotational dimension [46]. Partially-coherent ISAM employs a full-field frequency-scanning illumination with a partial spatial coherence model in order to alleviate multiple scattering artifacts, as in full-field ISAM [53].

All of these ISAM alternative modalities were analyzed theoretically and tested computationally by computer simulation. In addition, Ralston et al. validated ISAM experimentally for low-NA, NA = 0.05, by imaging both a tissue phantom consisting of 1 µm diameter titanium dioxide scatterers and a human breast tissue [28]. The experimental validation revealed that ISAM could recover structure in planes located outside focal regions. High-NA ISAM has been proposed and evaluated theoretically [47]. However, there has been no publication on the experimental validation for high-NA ISAM to date. Therefore, this thesis presents the experimental validation for high-NA ISAM where the NA is ≈ 0.84 and the sample is a tissue phantom consisting copper zinc iron oxide (CuZnFe₄O₄) particles with a mean diameter < 100 nm embedded in PDMS. The next section will discuss the fundamental Gaussian beam, present a brief overview of ISAM theory, and describe the inverse-scattering procedure for ISAM.

2.1 Gaussian Beams

For a Gaussian beam with \( r = (\rho, z) = (x, y, z) \), the field is given by [14]:

\[
g(x, y, z) = A_0 \frac{w_0}{w(z)} \exp \left( -\frac{x^2 + y^2}{w^2(z)} \right) \exp \left( -j kz - jk \frac{x^2 + y^2}{2R(z)} + j\phi(z) \right),
\]  

(2.1)
where the Guoy phase is given by

$$\phi(z) = \tan^{-1}\left(\frac{z}{z_R}\right),$$  \hspace{1cm} (2.2)

the radius of curvature of the wavefront is given by

$$R(z) = z \left[1 + \left(\frac{z_R}{z}\right)^2\right],$$  \hspace{1cm} (2.3)

and the depth-dependent beam waist size is given by

$$w(z) = w_0 \left[1 + \left(\frac{z}{z_R}\right)^2\right]^{1/2}.$$  \hspace{1cm} (2.4)

Figure 2.1 shows the basic parameters for characterizing a Gaussian beam. Rayleigh length or range is defined as a distance along the propagation direction of a Gaussian beam from the waist to the position where the area of the cross-section is doubled [56], whereas the confocal parameter $b$ is twice the Rayleigh range. These parameters are important for characterizing a Gaussian beam.

The relations among these parameters are given as follows [14]:

\textit{Spot size} = 2 \, w_o  \hspace{1cm} (2.5)

\textit{Rayleigh range} = z_R = \frac{\pi w_0^2}{\lambda}  \hspace{1cm} (2.6)

\textit{Beam divergence} = \theta = \frac{\lambda}{\pi w_o}  \hspace{1cm} (2.7)

\textit{Angular divergence} $\Theta = 2 \, \theta = \frac{4}{\pi} \frac{\lambda}{z w_o}$  \hspace{1cm} (2.8)

\textit{Numerical Aperture (NA)} = n \sin \theta.  \hspace{1cm} (2.9)
Figure 2.1: Illustration of Gaussian beam width \( w(z) \); \( w_o \) = beam radius, \( b \) = confocal parameter, \( z_R \) = Rayleigh range, and \( \Theta \) = total angular spread.

Although there is no specific definition for high-NA, moderate-NA, and low-NA in the literature, one can typically define low-NA as \( NA < 0.1 \), moderate-NA as \( 0.1 \leq NA \leq 0.4 \), and high-NA as \( NA > 0.4 \).

The Gaussian beam can be focused by a lens to achieve transverse localization of field or power, as shown in Figures 1.4 and 2.1. The power of the focusing is determined by the NA of the lens. The larger the NA of the lens used, the smaller the focus size will be. Figure 1.4 also illustrates the dependence between the transverse resolution and the depth-of-field or the depth-of-focus, DOF. A beam with a smaller focus or spot size diverges faster than a beam with a wider focus or larger spot size. Qualitatively, the focal size is inversely proportional with the NA, while the DOF is inversely proportional to \( NA^2 \). Consequently, standard OCT systems will demonstrate non-uniform transverse resolution depending on the distance from the focus and the
NA of the lens. The raw axial scan data of OCT will inherently exhibit a trade-off between transverse resolution and DOF. By analyzing the physics of the problem and solving the inverse problem, it is possible to recover images that are comparable with ideal beam collimation [45].

Higher NA lenses are capable of focusing a Gaussian beam to smaller spot size, but they exhibit a greater distortion of features located outside of the focal plane. Therefore, high-NA lenses are more appropriate for en face optical sectioning such as in OCM [33]. In addition, OCT images often exhibit curved or blurred features outside the confocal region due to poor and reduced transverse resolution. Specifically for high-NA lenses, the distortion is greater in the area exceeding the confocal parameter region $b$ or twice of Rayleigh range $z_R$.

2.2 ISAM Theory

A linear model is used as the result of the first Born approximation for scattering to relate the sample susceptibility $\eta$ and the measured signal $S$:

$$ S = K \eta \ , $$

where $K$ is an operator describing the OCT imaging system. The first Born approximation is appropriately sufficient for an OCT system because the influence from multiple scattering will be postponed or delayed by a longer time and may fall outside the coherence time of the reference. The deeper the penetration, the greater the contribution from multiple scattering in the shallow region. The complete derivation of the mathematical model for ISAM has been published [30, 45, 48, 52].

A simplified forward model for in-focus and out-of-focus regions can be written as [30]:

21
\[
S(q_x,q_y,k) \approx k^2 |E_x(\omega)|^2 \int \frac{H(q_x,q_y,k)}{R(z)} \tilde{\eta}(q_x,q_y,z) \exp \left[ i2k_z \left( \frac{q_x}{2}, \frac{q_y}{2} \right) z \right] dz
\]

(2.11)

where \( H(q_x,q_y,k) = H_N(q_x,q_y,k) \), \( H_N(q_x,q_y,k) \) is a transfer function of the system at the focus and \( R(z) = 1 \) for in-focus regions, while for out-of-focus regions \( H(q_x,q_y,k) = H_F(q_x,q_y,k) \), \( H_F(q_x,q_y,k) \) is a transfer function of the system for out-of-focus regions, \( R(z) = kz \), and \( \tilde{\eta}'(q_x,q_y,2k_z \left( \frac{q_x}{2}, \frac{q_y}{2} \right)) \) is the 3D Fourier transform of \( \eta(x,y,z)/R(z) \).

In addition, the transition between these two regions is estimated to occur at one Rayleigh range or \( |z| = \lambda/(\pi NA^2) \) [48].

### 2.3 Inverse Scattering Procedures

The algorithmic procedures for inverse scattering can be outlined as [30,52]:

1. Starting from acquired / raw data \( S(u,v,k) \), apply the transverse Fourier transform to obtain Fourier domain data \( \tilde{S}(q_x,q_y,k) \).
2. Perform a linear filtering which is a multiplication of \( \tilde{S}(q_x,q_y,k) \) and a transfer function in the Fourier-domain to compensate for \( H(q_x,q_y,k) \), the transfer function of the system.
3. Apply Stolt mapping (see Figure 2.2) to re-map the coordinate space of \( \tilde{S}(q_x,q_y,k) \).
4. Apply the inverse Fourier transform to get \( \eta(x,y,z)/R(z) \), which is the attenuated object.
5. The scattering potential \( \eta(x,y,z) \) can be calculated, if necessary, by multiplying with \( R(z) \) to recover the weak signal from points away from the focus.
Figure 2.2 shows the main points of the ISAM calculation procedure. It reveals that the resampling of \( k \) to \( q_z \) using Stolt mapping or similarly mapping from Fourier space data to the Fourier space object is the principle of invariant resolution for all depths. Depending on the system used to acquire the images, it might be necessary to perform preprocessing steps to compensate material dispersion, optical distortions, and phase instabilities in the system.

Figure 2.2: Illustration of the ISAM calculation procedure adapted from [57]. The Stolt mapping for the resampling of \( k \) to \( q_z \), mapping from Fourier space data to the Fourier space object, is the essential process to perform focal plane resolution for out-of-focus locations. The raw spectral data is the data acquired by the OCM system.
This chapter briefly discussed the Gaussian beam characteristics, ISAM theory and its calculation procedure. The experimental setup, results, and their explanations are the topic for the next chapter.
CHAPTER 3

EXPERIMENTAL SETUP, RESULTS, AND DISCUSSIONS

The main purpose of this chapter is to present the experimental validation of ISAM for a high-NA optical imaging system, which is OCM. Spectral-domain OCM was employed for the experimental setup. Characterization of OCM includes measurements of the confocal parameter, point spread function (PSF), lateral and axial resolution, and phase stability. High-NA ISAM reconstructions used images from a tissue phantom consisting of a number of copper zinc iron oxide (CuZnFe₄O₄) particles with a mean diameter of < 100 nm that were uniformly suspended in polydimethylsiloxane (PDMS) with the weight ratio between the particle and PDMS being 1:943. Section 3.1 presents the experimental setup, Section 3.2 discusses the characterization of the OCM, and finally Section 3.3 discusses the high-NA ISAM reconstruction.

3.1 Experimental Setup

The OCM system used to acquire the images is an integrated OCM-MPM microscope system shown schematically in Figure 3.1. For OCM operation, the light from a mode-locked Ti:sapphire laser passes the beam splitter - 1 (BS-1) and enters into a photonic crystal fiber (PCF), from Crystal Fibre A/S with LMA-8, for spectral broadening. The broad spectrum light then passes BS-1 and is split by BS-2 to the reference arm and the sample arm. The spectral interference pattern of the broadband light returning from the sample and reference arms is detected by the CCD camera.

The sample arm of the OCM system consists of a pair of scanning mirrors (XY galvos), a telescope (≈3x), objective lens, and translation stage. This sample arm arrangement enables
acquisition of images either by scanning the beam across the sample using XY galvos or by scanning the sample through a stationary beam using a translational stage. Using the XY galvos (Micromax 671, Cambridge Technology), it is possible to collect several images per second, even though it will introduce loss of SNR when operating at higher speeds. On the other hand, moving a sample with the translational stage takes around 1-2 minutes, but reduces some of the aberrations caused by scanning the beam across the objective.

The telescope consists of two lenses and has a magnification \( \approx 3x \). It is installed to expand the beam size so that the beam will fill the back aperture of the objective lens. The length of the telescope or the distance between the two lenses is \( \approx 20 \text{ cm} \).
The objective lens (XLUMPlanFI, Olympus) has a focal length of 9 mm, working distance 1.8 mm, NA = 0.95, magnification 20x, and is designed for water immersion. It is attached to piezo-scanning equipment (MIPOS 500, Piezosystems Jena) in order to enable high-resolution axial focus positioning.

The translational stage (VP-25XA, Newport) is capable of moving in x, y, and z directions and its travel range in each direction is 2.54 mm. It has a maximum speed of 25 mm/s, minimum linear incremental motion of 0.1 µm, and resolution 0.005 µm.

The light source is a high-power, widely tunable Ti:sapphire laser (Mai-Tai HP, Spectra-Physics). The laser has a relatively narrow bandwidth \(\approx 10\) nm. OCM systems require that the laser to have a broad spectrum because the axial resolution of the OCM system is inversely proportional to the bandwidth of the source. To generate the preferred broadband light, the output of the Ti:sapphire laser is fed into the photonic crystal fiber to perform spectral broadening by super-continuum generation. The Ti:sapphire beam is coupled using a 0.4 NA aspheric lens into the PCF (LMA-8, Crystal Fibre A/S) which has a length of 2 m, NA = 0.1, and mode field diameter (MDF) = 6 µm. Figure 3.2 shows the pump spectrum and the output of the PCF after spectral broadening for three different center wavelengths, where the insets depict the combined spectra incident on the sample. Because the coherence length of a beam is inversely proportional to the bandwidth and proportional to the square of the center wavelength, the FWHM of the coherence lengths in air are 4.7 µm, 3.7 µm, and 4.6 µm, respectively [58].

A tissue phantom which is used to mimic the optical and elastic characteristics of tissue [59] is used as the sample to be imaged. To fabricate the tissue phantom, 10 g of non-crosslinking PDMS or silicone was mixed with 2.5 g of cross-linking General Electric RTV 615 A, 0.25 g of
Figure 3.2: Comparison of pump spectra from a Ti:sapphire (dashed-line) and the output spectra from the PCF (solid-line) at (A) 750 nm, (B) 850 nm, and (C) 920 nm center wavelength, where their FWHMs of the broadened spectra are 52, 86 nm, and 82 nm, respectively [58].

curing agent General Electric RTV 615 B, and 0.0135 g of CuZnFe$_4$O$_4$ particles providing a tissue phantom with a concentration of CuZnFe$_4$O$_4$ particles to silicone of 1060 µg/g. This solution was sonicated (Cole-Palmer 8891) for 30 minutes. Finally, this tissue phantom was cured 10 hours in an oven at 80 °C.

3.2 OCM Characterizations

The performance of the OCM system is determined by several parameters such as sensitivity, signal-to-noise ratio (SNR), imaging speed, resolution, phase stability, and penetration depth. The performance of the system will subsequently determine the image quality.

The sensitivity of an OCT system is the ratio between the power of the signal and the power of the noise and is given by:
\[ Sensitivity = 10 \log \left( \frac{\sigma^2}{P} \right) + 10 \log(A) \quad , \] (3.1)

where \( P \) is the peak of the OCT signal, \( \sigma^2 \) is the variance of the noise and \( A \) is the attenuation of the neutral density filter, where the signal will double-pass through the filter. The sensitivity can be determined by putting a mirror in the sample arm and attenuating the incident/reflected light by a neutral density filter. The measured sensitivity of the OCM system used for this experiment was \( \approx 92 \) dB [58]. The minimum sensitivity of 80 dB is required for the OCT system for imaging in nontransparent tissue [31]. In addition, SD-OCT has better sensitivity than TD-OCT over 20 – 30 dB [29,44,60].

The SNR of the OCM system can be evaluated using equations (1.10) and (1.11). The imaging speed mostly depends on the type of the OCM system, time- or spectral-domain detection, and also the type of the detector used. The penetration depth is determined mainly by the type of tissues imaged and the center wavelength [3]. The next section will present the methods to determine confocal parameter, PSF, and phase stability.

3.2.1 Confocal parameter measurement

The confocal parameter of a Gaussian beam can be determined theoretically by measuring the intensity of the beam along its optical path. From equation (2.1), the intensity \( I(\rho,z) \) of a Gaussian beam can be derived as [14]:

\[ I(\rho,z) = I_0 \left( \frac{w_0}{w(z)} \right)^2 \exp \left( - \frac{2\rho^2}{w^2(z)} \right) , \] (3.2)

where at the beam axis (\( \rho = 0 \)) the intensity reduces to:
\[ I(0, z) = I_0 \left( \frac{w_o}{w(z)} \right)^2 = \frac{I_0}{1 + \left( \frac{z}{z_R} \right)^2}. \] 

Equation (3.3) reveals that the intensity will reach half of the peak intensity (FWHM) at position \( z = \pm z_R \), where \( z_R \) is Rayleigh range.

The confocal parameter of a Gaussian beam can be measured by putting a mirror in the sample arm and recording the intensity of the reflected light. By recording the position of the mirror and the intensity of the reflected light, we can plot the intensity of the reflected light over the optical axis and determine the confocal parameter.

The more common convention for this method is to use \((1/e^2)\) rather than FWHM. The former convention produces \( z_R \) at position \( z \) where intensity reaches \((1 - e^{-2}) = 0.865\) of the maximum intensity, while the latter one produces \( z_R \) at position \( z \) where intensity reaches 0.5 of the maximum intensity.

Figure 3.3 shows the intensity of the reflected light along the optical axis and its fitted Gaussian curve over the measured data. It reveals that the maximum intensity at position \( z = 20.26 \mu m \) and its 0.865 maximum intensities are at \( z_1 = 18.00 \mu m \) and \( z_2 = 22.52 \mu m \) and its 0.5 maximum intensities are at \( z_3 = 15.35 \mu m \) and \( z_4 = 25.17 \mu m \). Therefore, the former convention \((1/e^2)\) produces the confocal parameter \( b = z_2 - z_1 = 4.52 \mu m \), while the FWHM convention produces the confocal parameter \( b = z_4 - z_3 = 9.82 \mu m \). Using equation (1.3) and with \( \lambda = 800 \text{ nm} \), then the former convention estimates the beam radius \( w_o = 0.76 \mu m \) and the spot size = \( 2w_o = 1.52 \mu m \), while the latter estimates the beam radius \( w_o = 1.12 \mu m \) and the spot size = \( 2w_o = 2.24 \mu m \). Previous measurements revealed that for \( \lambda = 800 \text{ nm}, \Delta \lambda = 60 \text{ nm}, b = 2.20 \mu m, l_c = 4.7 \mu m, \text{beam radius}w_o = 0.53 \mu m \) and spot size = \( 2w_o = 1.06 \mu m \) [61].
Figure 3.3: Intensity of reflected light over the optical axis for the confocal parameter measurement. The blue dots are the measured intensity and the solid red line is the fitted Gaussian curve.

The OCM system for the experiment uses a pinhole as a spatial filter to improve the quality of the beam by allowing the desired beam to pass and blocking the beam containing undesired structure from aberrations and imperfect optics. However, it is not straightforward to choose the right pinhole, which is determined by the focal length of the lens, the diameter and quality of the input beam, and the wavelength. The effect of the pinhole should also be considered in the calculations. Considering the pinhole, the lateral and axial resolutions of a confocal microscope can be calculated as [62]:

\[
\text{Position z [microns]} \quad 0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25 \quad 30 \quad 35
\]

\[
\text{Intensity [arbitrary unit]} \quad 0.0 \quad 0.2 \quad 0.4 \quad 0.6 \quad 0.8 \quad 1.0 \quad 1.2
\]
where $\lambda$ is the center wavelength of the light, $n$ is the refractive index of immersion liquid, and $NA$ is the numerical aperture of the objective lens. Equations (3.4) and (3.5) are valid for pinhole size / diameter greater than 1 AU, where 1 AU is given by [62]:

$$1 \text{ AU} = \frac{1.22 \lambda}{NA}.$$  

Since the pinhole diameter in the OCM system is 15 $\mu$m and 1 AU = 1.116 $\mu$m for $\lambda$ = 800 nm and NA = 0.84, equations (3.4) and (3.5) can be used. Considering these, the lateral and axial resolutions can be calculated as $\Delta x = FWHM_{lateral} = 0.489 \mu$m and $\Delta z = FWHM_{axial} = 2.388 \mu$m. Then, the confocal parameter can be calculated using equation (1.3) and equation $\Delta x = 2 w_o = 0.489 \mu$m with the result $b = 0.47 \mu$m.

If we compare the confocal parameter results of the four calculations, the former convention ($1/e^2$) gives $b = 4.52 \mu$m, the second convention (FWHM) gives $b = 9.82 \mu$m, equation (3.4) gives $b = 0.47 \mu$m, and the previous measurements gave $b = 2.20 \mu$m [61]. In the next section, an experimental PSF measurement using subresolution particles gives $b = 1.10 \mu$m, which is the best method to determine the resolutions and other related parameters of the OCM system.

### 3.2.2 Point spread function (PSF) measurement

Before PSF measurements can be made, lateral and axial calibration of the OCM system must be performed. The lateral calibration is determined by the field-of-view (FOV) of the microscope,
which can be controlled by both the galvanometers and the translational stage (TS). The galvanometers change the FOV by steering the beam based on their input voltage, while the TS changes the FOV by moving the sample. Table 3.1 shows the corresponding FOVs and the input voltages of the galvanometers. The FOV is measured by recording the displacement of the sample by the TS, which corresponds to the displacement of the image from the initial- to the end-pixel.

Since the input voltage used for the measurements is 0.25 V and an *en face* image contains 256 x 256 pixels, lateral calibration in the x or y direction is \((\frac{22}{256})\) µm/pixel \(\approx 0.086\) µm/pixel. The axial calibration can be determined by moving the sample in the z direction and recording the corresponding movement in the cross-sectional image. By moving the sample 3.6 µm in the z direction with the TS, the corresponding image of the mirror surface moves 3 pixels in the cross-sectional image. Given this, the axial calibration is \(\approx 1.2\) µm/pixel.

Table 3.1: Input voltage of the galvanometers and corresponding FOVs of the OCM system.

<table>
<thead>
<tr>
<th>Input Voltage [V]</th>
<th>Field of View or FOV [µm x µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>22 x 22</td>
</tr>
<tr>
<td>0.5</td>
<td>47 x 47</td>
</tr>
<tr>
<td>1</td>
<td>95 x 95</td>
</tr>
<tr>
<td>2</td>
<td>185 x 186</td>
</tr>
<tr>
<td>2.5</td>
<td>232 x 232</td>
</tr>
<tr>
<td>3</td>
<td>278 x 278</td>
</tr>
</tbody>
</table>
The PSF can be measured more precisely by imaging a subresolution particle and measuring the full width at half maximum (FWHM) of the intensity variation in a desired direction. The intensity variation is usually fitted by a Gaussian function to determine the FWHM which is the resolution in that direction. A tissue phantom containing a uniform distribution of copper zinc iron oxide (CuZnFe$_4$O$_4$) particles, each having mean diameter < 100 nm, can be used to measure the PSF of the OCM system.

Figure 3.4 shows the PSF of the OCM system by imaging a single subresolution scattering in an *en face* and cross-sectional image. By fitting a Gaussian curve, the PSFs in the *en face* plane are 0.80 µm in the y direction and 0.70 µm in the x direction. Theoretically, the PSF in the x and y directions should be the same since the dimension of the particle is 100 nm, which is less than the lateral resolution of the OCM system. The PSF for x and y are different due to possible aberrations in the non-ideal OCM system. The lateral resolution $\Delta x$, therefore, is

![Figure 3.4: 3D PSF of the OCM system for a subresolution scattering particle. A single particle is viewed in an: (A) en face image and a (B) cross-sectional image.](image-url)
roughly the average of these two PSFs, which is \( \approx 0.75 \ \text{µm} \). In contrast, the PSF in the cross-sectional plane is 2.31 µm which also called the axial resolution \( \Delta z \) or coherence length \( l_c \).

After measuring the lateral resolution which is 0.75 µm and operating at center wavelength \( \lambda = 0.8 \ \text{µm} \) and applying equations (2.5)-(2.9), other parameters of the OCM system can be calculated such as:

- beam radius \( w_o = 0.375 \ \text{µm} \) and spot size = 2 \( w_o = 0.75 \ \text{µm} \),
- Rayleigh range \( z_R = 0.55 \ \text{µm} \) and confocal parameter \( b = 2 z_R = 1.10 \ \text{µm} \),
- Beam divergence \( \theta = 38.91^\circ \) and angular divergence \( \Theta = 2 \theta = 77.81^\circ \), and
- numerical aperture \( \text{NA} = 0.84 \).

The OCM system has a transverse resolution of \( \Delta x = 0.75 \ \text{µm} \), while its spatial sampling interval or step size of the galvos is \( (22 \ \text{µm} / 256 \ \text{pixel}) = 0.09 \ \text{µm} \). Based on the sampling theorem, it is required that the step size be at least half of the resolution. For en face image, the transverse step size is 0.09 µm and half of the transverse resolution is 0.375 µm, so that it is spatially oversampling. However, for cross-section image, the axial step size is \( (3.6 \ \text{µm} / 3 \ \text{pixel}) = 1.2 \ \text{µm} \), while the half of the axial resolution is \( (2.31 \ \text{µm} / 2) = 1.16 \ \text{µm} \). Therefore, it is spatially under-sampled since the axial step size is larger than half of the axial resolution. In order to oversample, the data are usually zero-padded in order to satisfy the sampling theorem.

3.2.3 Phase stability measurement

The phase stability of the OCM system is an important factor for performing ISAM reconstructions. In spectral domain OCT/OCM, the Fourier transform of the spectral density
defines an axial scan (A-scan). Phase instability results from galvanometer and translational stage vibrations, thermal changes, and jitter within the OCM system or the sample object. ISAM reconstruction requires multiplexing of the A-scans where each A-scan usually has a different relative phase drift than the others. Fortunately, each A-scan is relatively phase stable for spectral-domain OCT/OCM [63] due to simultaneous integration of each wavelength of the spectrum. When acquiring 2D scans, the phase in adjacent A-scans is relatively stable due to the fast acquisition time, typically $\approx 34 \mu s$ for a 29 kHz line-rate CCD camera [54]. On the other hand, when acquiring a 3D scan, the phase instability is increased due to the increase in acquisition time and the multi-axis beam scanning that is required. Therefore, adjacent A-scans in one 2D image (cross-section) are expected to have smaller phase variation than those of one 3D image. In general, ISAM reconstruction requires that the phase variation between adjacent A-scans is less than $\lambda/4$ or 1.57 radian [30].

Figure 3.5 shows the relative phase between adjacent A-scans from a rough surface for three different galvanometer positions. It reveals that the phase fluctuations between adjacent scans with the galvanometer unplugged and off are $\approx 300$ mrad, while for the galvanometer that is powered on they are $\approx 500$ mrad. In this experiment, the beam remains fixed at one position for 10 seconds while 20,000 scans are acquired.

ISAM requires that all axial scans of a 3D acquisition to be relatively phase stable in order to reconstruct an object. Ralston et al. proposed a method to achieve a stable 3D acquisition using a reference reflector such as a microscope coverslip [63]. Using a coverslip as a reference, the measured phase fluctuations between adjacent scans were reduced greatly to $\approx 3$ mrad.
Figure 3.5: Phase fluctuation of the OCM system for three different galvanometer states. “Galvo off” state means the signal cable to the galvo is plugged in but the galvo is off, whereas “Galvo unplugged” means the signal cable to the galvo has been unplugged and the galvo is off.

3.3 ISAM Reconstruction

ISAM measurements and reconstructions are performed using the OCM system illustrated in Figure 3.1. Using voltage input of 0.25 V on the galvanometer, a volume roughly 22 μm x 22 μm (transverse) x 300 μm (axial) was imaged using the above OCM system.

After acquiring the 3D image from the OCM system, the image must be pre-processed before performing the ISAM reconstruction. The pre-processing includes coherence gate curvature correction [64], background subtraction, computational translation of the focus of the image to the DC position, and phase corrections. Coherence gate curvature is caused by path length difference due to the transverse scanning mechanism in high-NA spectral-domain optical coherence imaging systems [64]. Phase correction uses a cover slip as the reference as in Ref. [65].

Figure 3.6 depicts a series of en face images from ISAM (1st row) and OCT (2nd row) of the tissue phantom for various positions: below, at, and above the focus. The focus of the objective was located 10 μm below the coverslip or surface of the tissue phantom, meaning that
Figure 3.6: *En face* ISAM (1st row) and OCT (2nd row) images of a tissue phantom containing of copper zinc iron oxide (CuZnFe₂O₄) nano-particles embedded in PDMS (silicone). The vertical and horizontal axes represent the pixel number, and for the value of Z, the minus sign represents below focus and the positive sign represents above focus. Each image dimension is 47 μm x 47 μm.

Only a 10 μm range, or about 17 Raleigh ranges, above the focus was available for imaging, and greater distances were available below the focus. The distance range of the *en face* planes to the focus (which is at z = 0) is from z = -38.4 μm to z = +9.6 μm, where the minus sign indicates planes below the focus. The measurements revealed that at the focus both ISAM and OCT have similar image qualities, whereas at out-of-focus regions, the OCT images show blurring.
(reduction of transverse resolution). These images show that the ISAM reconstruction has resolved the scatterers along a range of depths over 17 times the Rayleigh range or \( \approx 9.4 \, \mu m \) above the focus and over 70 times the Rayleigh range or \( \approx 38.4 \, \mu m \) below the focus, where one Rayleigh range \( z_R \) is \( 0.55 \, \mu m \). These results show that ISAM can reconstruct the particle structure for over 87 Rayleigh ranges or \( \approx 47.8 \, \mu m \). This is remarkable since two times the Rayleigh range is called the DOF, which is considered in-focus in optical imaging systems.

Figure 3.7 shows the cross-sectional image of the tissue phantom for both ISAM (top) and OCT (below) where the focus at pixel number 8 corresponds to the location 10 \( \mu m \) below the cover slip or surface. The OCT image shows that point scatterers located outside of the confocal range are not resolved, while the ISAM reconstruction shows all points resolved. Consider 2 scatterers where scatterer 1 at pixel number 18 is \( \approx 12 \, \mu m \) below focus, and scatterer 2 at pixel 40 is \( \approx 38.4 \, \mu m \) below focus. The ISAM reconstruction shows that both scatterers are clearly displayed, whereas the OCT image shows both scatterers are blurred. In addition, the second scatterer is located 38.4 \( \mu m \) below focus or \( \approx 70 \) times the Rayleigh range. This number agrees with the maximum distance that can be recovered from the en face images. The maximum distance that an en face ISAM image can be recovered is determined by the noise floor of the OCM system or the SNR of the back-reflected signal.

Previously, Ralston et al. [47] reported a theoretical evaluation of ISAM reconstruction for a high-NA imaging system with characteristics of wavelength \( \lambda = 800 \, nm \), bandwidth \( \Delta \lambda = 320 \, nm \), \( NA = 1.0 \), and SNR = 35 dB to image an object volume of 50 wavelengths in depth, and 20 x 45 wavelengths in the transverse directions and consisting of randomly distributed point scatterers located inside and outside of the scanning boundary. This theoretical OCM system had
Figure 3.7: Cross-sectional ISAM and OCT images of a tissue phantom containing CuZnFe₄O₄ nano-particles embedded in PDMS (silicone), where the image dimensions are 62.4 µm (vertical axis) x 22 µm (horizontal axis), and the focus is at pixel number 8, or z ≈ 10 µm below the top surface, on the vertical axis.

A resolution \( \Delta z = 0.88 \) µm, lateral or transverse resolution \( \Delta x = 0.51 \) µm, and Rayleigh range \( z_R = 1.03 \) µm. The report revealed that ISAM could reconstruct point scatterers up to \( \approx 10 \) wavelengths or \( \approx 8 \) Rayleigh ranges both below and above focus [47].
The experimental measurement data show that ISAM can reconstruct the particle at a position $\approx 17$ Rayleigh ranges above the focus and $\approx 70$ Rayleigh ranges below the focus while theoretical simulations revealed that ISAM could reconstruct particles at positions $\approx 8$ Rayleigh ranges below and above the focus [44]. The difference may stem from the SNRs of both systems. The theoretical simulation data used SNR = 35 dB [47], where the SNR was defined as the “dynamic range” of the data collected from the brightest pixel value to the noise floor. The experimental data have a brightest pixel value of 116310 and noise floor of 19, so the SNR is 37.9 dB, almost a 3 dB improvement over the theoretical simulation data.

To qualitatively compare en face ISAM and OCM images, such as those shown in Figure 3.6, average lateral PSF FWHMs can be compared. A method for measuring the average lateral PSF FWHM of an image consisting of multiple scatterers has been reported [66], where the transverse Fourier transform of the amplitude of the image was performed. A Gaussian curve was fitted to the average Fourier transform and the bandwidth of this Gaussian is related to the reciprocal of the FWHM of the PSF. In this thesis, a similar procedure was used to measure an average lateral PSF FWHM: The transverse Fourier transform of the amplitude of the image was performed, and the Gaussian curve was fitted to the average Fourier transform after the DC part of the spectrum was averaged to the values of its nearest pixels. The inverse Fourier transform was then performed to the Gaussian curve and the result is the average PSF of all scatterers from the original image. This method is applied to calculate the average lateral PSF of the ISAM images, while the average lateral PSF of the OCM images must be calculated manually due to the overlapping signals from nearby scatterers.

Figure 3.8 shows the measured lateral PSF FWHM for both ISAM and OCT en face images of CuZnFe$_4$O$_4$ nano-particles embedded in the PDMS tissue phantom as the function of
depth and Rayleigh range for the OCM-ISAM system. The theoretical OCT lateral PSF FWHM for the optical field and squared Gaussian beam profile can be written as:

\[ \Delta x(z) = \sqrt{2 \ln 2} \, w_0 \sqrt{1 + \left( \frac{z}{z_R} \right)^2}, \quad (3.7) \]

where \( w_0 \) is the beam waist radius and \( z_R \) is the Rayleigh range. Equation (3.7) is slightly different from what was previously reported by Ralston et al. [66], who assume that the OCM resolution is the spot size of the Gaussian beam focus. The measured experimental data of the OCM lateral PSF FWHM agree with the theoretical work by Davis et al. [48] showing that

Figure 3.8: Experimental measurements of the lateral PSF FWHM versus distance from focus for experimental ISAM (red curve), experimental OCT (blue curve), and theoretical OCT (green curve). The minus sign indicates below-focus regions and one Rayleigh range \( z_R = 0.55 \, \mu m \).
the effective lateral PSF is actually the Gaussian beam squared, as stated in equation (14) of the paper [48]. The equation states that the PSF is proportional to the multiplication of the illumination and detection patterns. The lateral OCM PSF shows that the OCM system exhibits an aberration, as shown in the asymmetric OCM PSF curve in Figure 3.8.

This ISAM data clearly demonstrate that ISAM reconstructions of OCM images can extend the DOF significantly. This unique extension capability of high-NA ISAM can potentially be used for improved imaging in biological research and medical diagnostics.

This chapter has described the experimental setup and results for the OCM system characterizations and the ISAM reconstruction for a high-NA OCM system. The ISAM reconstruction for the high-NA OCM system is experimental validation that ISAM can be used with phase-stable data from a high-NA OCM system. The next chapter presents the conclusions and the future work.
CHAPTER 4

CONCLUSIONS AND FUTURE WORK

4.1 Summary and Conclusions

This thesis presents several characterizations of OCM and ISAM reconstruction for high-NA optical systems. OCM characterizations include experimentally and theoretically determining the confocal parameter, PSF, and phase stability measurements. The confocal parameter measurement using moving mirror as a sample will produce reasonably accurate results. Better results, however, can be performed by considering the pinhole effect on the measurement. The experimental PSF measurements from an OCM system using subresolution particles, however, show more accurate results. From these PSF measurement results, other optical parameters in the OCM system can be calculated, such as beam radius $w_0$, Rayleigh range $z_R$, beam divergence $\theta$, and numerical aperture NA. Phase stability measurements were performed to evaluate the phase variations and their source in OCM systems.

The ISAM reconstruction results show the experimental validation of ISAM in a high-NA optical imaging system. It reveals that ISAM can reconstruct an image of particles at distances up to $\approx 70$ Rayleigh ranges below the focus and $\approx 17$ Rayleigh ranges above the focus when the scatterer is located at $\approx 17$ Rayleigh ranges below the phase-referencing coverslip.

Both the experimental validation of ISAM and the OCM system characterization show that the combination of OCM and ISAM represents a potentially powerful technique for high resolution 3D cellular-level imaging. The technique can acquire biological and medical information from tissue that cannot be revealed by OCT and OCM alone, or by many other...
microscopy techniques. The potential applications of this technique include imaging engineered
tissues, *in vivo* imaging of human and animal tissue, imaging the human retina, *in vivo* detection
of arterial plaques, and cancer detection in several parts of the body such as the breast, skin,
bladder, and oral cavity [6,17]. The capability of OCM to visualize cellular structure has
potential implications for several clinical applications such as optical biopsy [8] and the
detection of neoplastic changes [63]. With this OCM and ISAM combination, the optical biopsy
and detection of neoplastic changes can likely reveal much more useful information.

### 4.2 Future Work

This section will present some potential future work and possible applications to improve the
performance of the methods developed in this thesis.

#### 4.2.1 Improving the image quality in OCM system

OCM can visualize the structure of biological tissues at high resolution depending on their
scattering properties. It was first introduced by Izatt *et al.* in 1994 and has been used for many
studies to visualize many biological tissues including intact skin [34] and gastrointestinal
tissue [35]. Even though it has been used for 15 years, it has not been as popular as confocal
microscopy for biological and medical imaging. Because both OCM and confocal microscopy
produce *en face* images, their image quality can be compared directly. Even though OCM has
greater penetration depth, most OCM images from intact tissue [36,67-68] exhibit lower quality
than those of commercial confocal microscopy [69,70]. There are several OCM parameters that
can be evaluated to improve image quality such as the optical bandwidth from supercontinuum
generation, the coherence length of the source and spatial filtering using a pinhole [58]. These
parameters can be tuned to optimize the image quality of the OCM system. The broad
bandwidth from supercontinuum generation can be optimized using different photonic crystal fibers or applying other methods to improve the supercontinuum generation reported in the literature. The effect of the coherence length of the optical source can be evaluated to optimize the OCM image quality for a specific tissue. Pinhole size, setup, and positions can be examined as well to optimize the image.

Another method to enhance image quality is to operate the OCM system with higher numerical aperture (NA) optics. The NA of the OCM system in this research is currently \( \approx 0.84 \) and theoretically using a different objective with oil immersion, it is possible to get \( \text{NA} \approx 1.2 \). A higher NA objective will produce higher resolutions that have the potential to get more useful information from the tissue samples.

Other possible methods to increase the image quality lie in the introduction of other contrast properties and mechanisms in addition to scattering. These additional contrast mechanisms include fluorescence [37], quantitative phase [39], and spectroscopic [40], to name a few.

**4.2.2 Enhancing ISAM performance in OCM systems**

Previously, ISAM has been performed experimentally on low-NA optical imaging systems [28] and this thesis confirms experimentally that ISAM can be performed as well on a high-NA system. Since ISAM processing is sensitive to phase fluctuations, a phase stable OCM system will improve the ISAM image quality. The potential sources of phase instabilities include the galvanometer and translational stage vibrations, thermal changes, and jitter from components in the OCM system. In order to reduce the phase instability, careful evaluation and operation of these sources must be performed. For example, one may choose low-vibration galvanometers to
steer the beam, a low-noise translation stage to move the sample, and low-noise electronic components and systems.

ISAM obviates the trade-off between the lateral resolution and DOF for OCT and OCM systems. Different methods to address this limitation have been proposed, such as applying adaptive optics and axicon lenses [18, 19]. A study that characterizes the effect of the axicon lenses or phase masks on ISAM reconstruction offers the potential for improving the DOF even further. However, this study should evaluate the inverse scattering problem for Bessel beams instead of Gaussian beam since the axicon lens or phase masks produces Bessel beams.

Real-time ISAM with an OCM system is another potential tool for visualizing tissue samples for biological and medical applications. Realization of real-time and high resolution 3D data sets from OCM has potential applications in guided surgery, cell biology, and clinical diagnostics. In addition, it has the potential application of real-time in vivo optical biopsies with better resolution and more spatial information throughout the tissue sample compared to conventional OCT.

4.2.3 Imaging biological tissues

In this thesis, high-NA ISAM was used to reconstruct an image of a tissue sample containing a collection of CuZnFe₄O₄ particles having a mean diameter < 100 nm and uniformly embedded in PDMS. The near-future applications of this technique are imaging engineered tissues, human skin, human retina, and cancer detection in several parts of the body such as breast, skin, bladder, and the oral cavity.

Skin cancer imaging is a potential application for high-NA ISAM due to the need for high lateral resolution and large DOF. The fact that melanocytes migrate from the lowermost
“basal” layer of the epidermis, found roughly 100-200 μm under the skin surface [13], while the upper layers of the dermis are located 1-2 mm under the skin surface [71], makes ISAM a suitable modality for imaging these cells and structures. It is also reported that 85% of skin cancer comes from the epithelial layer [72]. One of the critical steps in differentiating cancer cells is nuclear enlargement [73], where the nuclei in the basal cell layer have a mean size around 6 μm [74]. Therefore, high resolution imaging of the entire epidermis is crucial for performing imaging and detection of skin cancer. Finally, in vivo high resolution imaging at a resolution < 5 μm and up to 2 mm below the skin surface would likely identify most skin cancers [75-77].

The other direction for future work is to compare the image quality from this high-NA ISAM reconstruction to standard OCM images of tissues, such as intact skin [34], and oral epithelia for cancer detection [36]. High-NA ISAM reconstructions of these tissues will potentially further reveal the improvements offered by this technique, compared to the previous techniques reported in the literature.
REFERENCES


[55] United States National Library of Medicine, National Institutes of Health


