SEGMENTATION, CO-REGISTRATION, AND CORRELATION OF OPTICAL COHERENCE TOMOGRAPHY AND X-RAY IMAGES FOR BREAST CANCER DIAGNOSTICS

BY

JONATHAN SUN

THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Bioengineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2012

Urbana, Illinois

Adviser:

Professor Stephen Boppart
ABSTRACT

In this thesis, x-ray imaging is evaluated and compared with optical coherence tomography (OCT). It is shown that each individual modality provides information about a sample through different physical characteristics. The preclinical variant of x-ray imaging is explored in the context of breast cancer detection and diagnosis because their clinical use could be augmented by OCT. After comparing them, each modality showed its specialized usage. Micro-CT is useful in detecting microcalcifications, the most apparent target of the screening mammogram. OCT can be utilized in the specific cases where high resolution is desired and penetration depth does not need to be over a few millimeters. In the case of a needle probe, OCT could provide images of the vicinity of the probe tip, allowing for better localization and identification of abnormal tissue. In the intraoperative case, OCT can be utilized to evaluate tumor margins and lymph nodes that have been resected in order to decrease the chance of tumor reoccurrence and the need for additional operations. In all types of tumor surgeries, the evaluation of lymph nodes is important to help stage the cancer, and real time feedback of lymph node status could prove to be extremely helpful to the surgeon. OCT, with its scattering based image formation, can provide morphological data about a sample that can augment a surgeons screening and diagnosing abilities when used in conjunction with more traditional imaging modalities. Furthermore, an automated segmentation algorithm would be able to identify tumor areas within the operating room, allowing the surgeon to isolate and remove all tumor tissue.
ACKNOWLEDGMENTS

The work presented in this thesis has been made possible through the support of many people. First and foremost, I would like to thank Dr. Stephen Boppart for his help and patience that he has given me over these past few years. His guidance has led me to realize my own goals in research and education. I would also like to thank Dr. Steven Adie who has helped me on every aspect of this work, always challenging me to better understand the reasoning behind every answer. Furthermore, I would like to thank Dr. Jay Yeh and Dr. Phil Newmark for their support in my high school and undergraduate careers, without which I would never have cultivated a love for research. Lastly, I would like to thank my family, friends, and everyone in the Biophotonics Imaging Lab for their love and support.
# Table of Contents

CHAPTER 1: INTRODUCTION...........................................................................................................1  
  1.1 Biomedical Imaging...........................................................................................................1  
  1.2 Breast Cancer.....................................................................................................................2  
  1.3 Optical Coherence Tomography........................................................................................4  
  1.4 Statement of Work............................................................................................................6  

CHAPTER 2: THEORY.......................................................................................................................8  
  2.1 Contrast in Imaging............................................................................................................8  
  2.2 Optical Contrast.................................................................................................................8  
  2.3 X-Ray Contrast.................................................................................................................10  

CHAPTER 3: HARDWARE AND METHODOLOGY............................................................................13  
  3.1 Optical Coherence Tomography System.........................................................................13  
  3.2 X-Ray Hardware...............................................................................................................22  
  3.3 Methodology...................................................................................................................25  

CHAPTER 4: RESULTS AND DISCUSSION.......................................................................................27  
  4.1 Comparing Systems.........................................................................................................27  
  4.2 Image Analysis................................................................................................................30  
    4.2.1 Two-Dimensional Image Correlation......................................................................30  
    4.2.2 Three-Dimensional Correlation..............................................................................34  
    4.2.3 Optimizing Segmentation Methods........................................................................36  
    4.2.4 Automated Segmentation......................................................................................42  
  4.3 Impact..............................................................................................................................46  

CHAPTER 5: CONCLUSIONS AND FUTURE WORK.........................................................................47
CHAPTER 1

INTRODUCTION

1.1 Biomedical Imaging

Advances in modern medicine are linked closely to technological developments in supporting fields such as engineering or the biological sciences. Biomedical imaging has been utilized by researchers and physicians to visualize structures and function that are inherent in the complex human body. There have been several techniques developed to provide morphological imaging, allowing for added insight into the native structure of the area of interest. In 1895, Roentgen invented x-Ray imaging [1], where structure below the surface could be visualized by measuring the x-ray absorption based upon density along the path from source to detector. X-ray images are still acquired today as a commonly used inexpensive method for imaging internal structure of the human body. Because of the high attenuation of x-rays in bone, x-rays are commonly used to visualize bone injuries. Additionally, screening mammograms remain one of the most common diagnostic imaging tools for detecting breast cancer. X-ray computed tomography (CT) is a method used to create a cross-sectional view of a sample [2]. In order to do this, x-ray projections are taken at various angles around the sample and then processed using back-projection algorithms to infer 3-D structure. Clinical x-ray CT, although declining in popularity for breast imaging with increased MRI usage, still has a wide variety of applications in imaging different parts of the body [3]. On the preclinical level with small animals and samples, this method is capable of achieving micron level resolution and is known as micro-CT [4].
Other commonly used imaging modalities in the clinic include ultrasound imaging and magnetic resonance imaging (MRI). In ultrasonography, sound waves are used to detect boundaries in a sample with differing acoustic impedance [5]. The ultrasound image is formed by producing a sound wave and interpreting the backreflection of that pulse to form an image. In MRI, cross-sectional images are obtained by aligning molecule spin states with varying magnetic fields. There have been a few studies comparing OCT and micro-CT [6-7], but none in the context of imaging breast cancer.

1.2 Breast Cancer

Breast cancer is the most common form of non-skin cancer affecting women in the United States. As medicine evolves, new treatment options have developed, with a combination of surgery, radiation therapy, chemotherapy, and hormone therapy being prescribed on an individual basis [8]. However, early detection still remains a critical factor for successful treatment and increased patient survival rates [9-10].

Currently, the standard practice for screening for breast cancer includes physical exam and x-ray mammography. There is ongoing debate about the effectiveness of screening mammography for younger age groups because dense breast tissue can make accurate interpretations challenging. The accuracy of a reading depends on the training and interpretation of a mammogram. The false negative rate for mammography is in the range of 10-25% [11], while the false positive range is between 55-85% [12-15]. While the false positive readings can lead to further tests that may be more expensive, inconvenient, and cause anxiety for the patient, they are not potentially life threatening. On the other hand, false negative
readings may cause a growing tumor to be overlooked for several years, delaying treatment and ultimately worsening the prognosis.

Figure 1.1 The role of imaging in different imaging modalities in the process of breast cancer diagnosis and treatment. The screening process is done with physical exam and x-ray mammography looking for suspicious areas. Breast MRI or ultrasound may be ordered to look at a suspicious lesion if the breast is too dense or is not imaged well with x-ray. A breast biopsy is taken to obtain tissue for histological examination and for tissue diagnosis. During treatment by surgery, intraoperative x-ray imaging may be performed using a digital specimen radiography machine to image resected tissue. This can also be achieved by using a clinical OCT system. The sample is then sent to pathology for histological and immunohistochemical analysis.
The sensitivity and specificity of the screening process for breast cancer has been improved with the aid of different imaging modalities. In high risk women who are known carriers of gene mutations (such as BRCA1 and BRCA2), MRI has been shown to be cost-effective as it provides an accurate reading for the additional cost [16]. Furthermore, in younger women with dense breasts, whole-breast ultrasound is useful as a complement to x-ray mammography. Other complementary imaging methods include nuclear medicine imaging techniques using radioactive tracers such as positron emission tomography or scintimammography and a ductogram using contrast agents to enhance the mammogram.

After a suspicious area is identified, a biopsy is taken at the site and sent for a pathological workup for identification of abnormal cells. Currently, x-ray or ultrasound is used to guide the needle to the suspicious site, but this may not be precise enough in some early ductal cases of cancer. If a diagnosis of breast cancer has been made, a treatment plan is prescribed by the doctor with a combination of surgery, radiation therapy, chemotherapy, and hormone therapy.

1.3 Optical Coherence Tomography

Optical coherence tomography (OCT) was first described by Fujimoto in 1991 [19] and in the last two decades has been developed for applications in medicine, biology, and materials testing. OCT can non-invasively visualize optical scattering arising from differences in refractive index within a sample area, such as from scattering particles or boundaries. OCT is a useful form of biomedical imaging because it offers micron-level resolution capable of visualizing individual cells. Additionally, the near-infrared (NIR) light used is not highly absorbed by water or skin pigments, allowing for penetration depths up to a few millimeters deep in tissue. As can
be seen in Figure 1.2, a "biological window" exists for tissue, which has the lowest absorbance within the range of 700-1300 nm [18-19].

![Graph showing absorption and scattering coefficients of hemoglobin, melanin, and water over a range of wavelengths](image)

**Figure 1.2** The "biological window" is in the range of 700-1300 nm, where the low absorption coefficients of hemoglobin, melanin, and water allow the scattering of near-IR light to dominate. [19]

Conceptually, OCT is similar to ultrasound, but uses light instead of sound waves. In both modalities, a wave interacts with boundaries within a sample and is reflected back to be analyzed. From this reflected wave, the morphology of the sample can be determined non-invasively. In OCT however, because electromagnetic waves cannot directly be measured with electronic circuits, an interferometric technique and coherence gating are employed to obtain information about the signal.

OCT has found applications in specialized areas of medicine. Relatively inexpensive compared to many other imaging modalities, OCT is able to provide very high resolution (less than 10 microns) information about an area at limited penetration depth (up to 2 mm). Most notably, in ophthalmology, OCT is a natural choice because of the transparent nature of the
eye, and ocular disease detection has been helped immensely with the ability to look beneath the surface of the retina [20-26]. OCT also has applications in dentistry [27-30] and dermatology [31-33], among many other areas.

Because of its micron level resolution and ability to image detailed tissue structure, OCT has promise as a method to perform an "optical biopsy" on a suspicious tissue site [34-39]. This could be implemented into the core biopsy needle used for diagnosing breast cancer either for improved image guidance or to provide a real-time view of cell and tissue microstructure. OCT has been shown to be able to distinguish different benign and malignant lesions [40-41]. Additionally, it has been shown that positive tumor margins can be accurately identified using OCT intraoperatively when imaging an excised tissue sample [42]. Furthermore, there is ongoing work involving intraoperative lymph node imaging in vivo to provide real time insight into sentinel lymph nodes [43-44]. This work has the potential to be very useful in helping stage cancer earlier by detecting metastases and reactivity in the lymph nodes during surgery.

1.4 Statement of Work

The purpose of this work is to compare OCT to x-ray imaging of resected human breast tissue and correlate different tissue features between the modalities for use in real time intraoperative imaging. X-ray imaging (specimen radiography) is currently used during surgical breast cancer procedures to verify tumor margins, but cannot image tissue in situ. OCT has the potential to solve this problem by providing intraoperative imaging of the resected specimen, and also of the tumor cavity or in situ tissue structures. To this end, it is important to determine similarities and differences between images from each modality, and quantitatively correlate image features that can be seen between the two modalities. OCT and micro-CT
images are automatically segmented using different computational approaches, and
quantitatively compared to determine the ability of these algorithms to automatically find
tumor areas. Furthermore, two-dimensional (2-D) and three-dimensional (3-D) results are
compared. This research, combined with the real time nature of OCT, has the potential to allow
a hand held probe to collect intraoperative OCT data and automatically show a surgeon
possible regions of tumor within tissue which correlate to tumor regions identified previously
on x-ray imaging (mammography or specimen radiography).
CHAPTER 2

THEORY

2.1 Contrast in Imaging

Contrast in imaging revolves around making features within an image distinguishable from one another. In the case of breast imaging, contrast in the images can help distinguish different tissue types and features in order to detect malignancies.

Michelson contrast is defined as:

\[
\frac{I_{\text{max}} - I_{\text{min}}}{I_{\text{max}} + I_{\text{min}}}
\]

with \( I_{\text{max}} \) and \( I_{\text{min}} \) being the highest and lowest luminance. However, the source of the images depends upon the modality being used. In optical imaging, contrast is predominantly formed by backscattering of incident light. In x-ray imaging, contrast is based upon differing absorption and attenuation of incident x-ray beams.

2.2 Optical Contrast

In OCT, contrast is a product of differential scattering of light within a tissue as a result of variable optical properties of tissue. Traditional light scattering is approximated using Rayleigh scattering, which describes the elastic scattering of light by particles much smaller than the wavelength of light:

\[
I = I_0 \left(\frac{1+\cos^2(\theta)}{2R^2}\right) \left(\frac{2\pi}{\lambda}\right)^4 \left(\frac{n^2-1}{n^2+2}\right)^2 \left(\frac{d}{\lambda/2}\right)^6
\]  (2.1)

Where \( R \) is the distance between the particle and the observer, \( \theta \) is the scattering angle, \( n \) is the refractive index of the particle, and \( d \) is the diameter of the particle.
However, this approximation cannot be well applied to tissues because of the larger structures involved. Rayleigh scattering is strongly dependent on the size of the particle and the wavelength of light, and the model breaks down when the particle size becomes larger than about 10% of the wavelength of the incident light. Additionally, Rayleigh scattering is isotropic and identical in both the forward and backward directions. Functionally, Rayleigh scattering contributes to attenuation proportional to $1/\lambda^4$, known as Rayleigh's inverse fourth-power law, with short wavelengths scattered more than long wavelengths [45].

In order to model tissue, scattering theory of particles examines several limiting cases in order to make approximations. The size of particles in comparison to wavelength is important, reaching geometrical optics when the scatterer is large relative to the wavelength [46]. Mie theory is applied in cases where the particle size is larger than can be accounted for in Rayleigh scattering. Mie scattering was first described in 1908 in cases where the wavelength of light is similar to the size of the dielectric sphere of a particle. This type of scattering is approximated:

$$Q = 2 - \frac{4}{p} \sin p + \frac{4}{p^2} (1 - \cos p)$$ (2.2)

where $Q$ is the efficiency factor of scattering, the ratio of the scattering cross section and the geometrical cross section $\pi a^2$. The variable $p$ is the phase delay of the wave passing through the center of the sphere, with $p = 4\pi a(n - 1)/\lambda$. The variable $a$ is the sphere radius, $n$ is the ratio of refractive indices inside and outside the sphere, and $\lambda$ is the wavelength of the light.

The Mie scattering approximation has been applied to biological tissues to predict the behavior of an incident light wave and how it will interact with the tissue microenvironment. This approximation, however, is limited to spheres of a specific size while tissue is composed of
complex structures and a heterogeneous collection of scatterer sizes. Nonetheless, the approximation allows for an understanding of light interactions in tissue.

One source of noise, speckle, can arise from the coherent nature of the incident light and how it interacts with tissue. When there are multiple scatterers in a tissue that are usually in close proximity, variations in predictable light scattering may occur. Because of the coherence gating nature of low coherence interferometry, traditionally only a single reflection is taken into account in OCT. This occurs when the sample beam is reflected back by a single scatterer in the sample and the optical path length traveled in the sample matches the optical path length of the reference arm. However, when the same beamfront meets two separate reflecting particles in the sample, there is a chance that the optical path lengths can still be matched because of scattering in the tissue. This may result in constructive or destructive interference as the sample beam is collected. This effect gives rise to speckle, a source of noise in OCT.

The signal in OCT rises from reflection of incident light from subsurface particles. A highly scattering sample will attenuate the signal in OCT, as light will not penetrate into deeper areas. Additionally, contrast in the image will be seen in any area where there is a detected signal, whether it is from reflectors in the sample or a source of noise.

2.3 X-Ray Contrast

On the electromagnetic spectrum, the corresponding wavelengths for x-rays range from 0.01 to 10 nanometers. "Soft" x-rays range from about 120 eV to 12 keV and are strongly absorbed by water. In contrast, "hard" x-rays range from 12 keV to 120 keV and penetrate through solid objects, making them useful for diagnostic radiography. In the soft x-ray range, a
photon interacting with an atom will cause an electron to be ejected, known as the photoelectric effect, which is also responsible for x-ray attenuation through absorption.

At higher photon energy levels, the Compton effect is a type of scattering that occurs when x-rays and gamma rays interact with matter. In an inelastic interaction, the x-ray transfers some of its energy to a scattering electron which is ejected from the atom, and scatters at a decreased energy. In the classical electromagnetism model, scattered rays have the same wavelength as the incident ray, but in x-rays, it was found that the scattered rays had a greater wavelength [47-48]. Arthur H. Compton was the first to study and describe this effect in 1923 [49].

Compton derived the relationship between the shift in wavelength and scattering angle:

$$\lambda' - \lambda = \frac{h}{m_e c} (1 - \cos \theta) \quad (2.3)$$

where $\lambda$ is the initial wavelength, $\lambda'$ is the scattered wavelength, $h$ is Planck's constant, $m_e$ is the mass of an electron, $c$ is the speed of light, and $\theta$ is the scattering angle.

Contrast in x-ray imaging is derived through differing attenuation through the tissue. As such, a "shadow" is seen at the imaging plane, with the dark areas of the image coming from dense areas in the sample with high molecular weight or closely packed cells. As a high energy x-ray photon travels through dense tissue, Compton scattering will occur, reducing the photon energy. Through a cascade effect, the x-ray photon will be absorbed along that path and will not reach the film. However, in soft tissue, the attenuation is much less and the projected photons will hit the film.

The attenuation of x-rays has been studied in different types of soft tissue [50-51]. In a study of breast tissue and ductal carcinoma, the linear attenuation coefficients were
determined as seen in Figure 2.1. It is clear that the attenuation of x-rays in fatty tissues is much less than that of fibrous or cancerous tissues, especially at lower energies. However, little differentiation between the fibrous and cancerous tissues.

![Graph showing x-ray attenuation in tissue](image)

**Figure 2.1 X-ray attenuation in tissue.** (Left) The mean values of linear attenuation in three types of breast tissue are compared (A, fat; B, fibrous; C, infiltrating ductal carcinoma). (Right) The ranges of linear attenuation are normalized to the normal fibrous tissue (horizontal, infiltrating ductal carcinoma; vertical, fibrous; stippled, fat) [51].

Contrast in optics and x-ray imaging gives rise to the features that are seen in an image. However, because the contrast mechanisms are different for optical and x-ray imaging, it is important to understand how the physical properties of a sample translate into the image that is seen from each. In this work, the features seen in optical and x-ray imaging will be compared and analyzed.
CHAPTER 3

HARDWARE AND METHODOLOGY

3.1 Optical Coherence Tomography System

OCT is based on the theory of low-coherence interferometry. It can generate micron-level resolution 2D images and 3D volumes with penetration depths of several millimeters [52].

OCT is attractive in the biomedical field because of its non-invasive nature and its relatively low cost compared to other techniques [53-58]. Portable OCT systems can be obtained at a fraction of the cost of other machines such as MRI or x-ray CT scanners.

Low-coherence interferometry uses a broad bandwidth source as the wide spectrum of wavelengths allow relevant distances to be measured [59]. With broader bandwidth sources, the axial resolution increases and more precise distances may be resolved. In order to detect a signal, interferometry is employed. Figure 3.1 shows a basic Michelson interferometer. The quasi-monochromatic beam produced by the source has a bandwidth $\Delta \nu$ that is significantly less than the central frequency $\bar{\nu}$. This beam, $E(t)$, is split into two identical beams by a beamsplitter (BS).
Figure 3.1 Michelson interferometer. A beam from the light source is split by a beam splitter (BS) between a sample arm and reference arm with mirrors (M1 and M2) at lengths $L_s$ and $L_r$ respectively.

One of the beams ($E_r(t)$) is reflected off the reference arm mirror M2, and the other beam ($E_s(t)$) is reflected off mirror M1 in the sample arm after being split by the beam splitter:

$$\frac{1}{2} E(t) = E_r(t) = E_s(t) \quad (3.1)$$

As the sample arm beam interacts with a sample with different refractive indices, a difference in the optical distance will be introduced when the beams recombine at the beamsplitter. Hence, $L_s > L_r$, and so the sample arm acquires an extra delay $\tau = 2(L_s - L_r)/c$ before recombining with the reference arm at the beamsplitter to form a total field:

$$E_{\text{total}} = E_r(t) + E_s(t + \tau) \quad (3.2)$$

The combined beam then travels to an optical detector which measures the time-averaged intensity of the incident beam. The signal at the detector is thus:

$$I_D = \langle |E_{\text{total}}|^2 \rangle = \langle [E_r(t) + E_s(t + \tau)][E_r(t) + E_s(t + \tau)] \rangle \quad (3.3)$$
where \( \langle \cdot \rangle \) denotes time averaging over many optical periods. Defining

\[
I_s = \langle E_s(t + \tau) E_s^*(t + \tau) \rangle \quad \text{and} \quad I_r = \langle E_r(t) E_r^*(t) \rangle
\]

allows Equation (3.3) to be re-written:

\[
I_D(\tau) = I_s + I_r + 2\sqrt{I_sI_r} \Re \{ \gamma(\tau) \}
\]

where \( \gamma(\tau) \), the mutual coherence function, is defined as:

\[
\gamma(\tau) = \frac{\langle E_r(t) E_s^*(t + \tau) \rangle}{\sqrt{I_rI_s}} \quad (3.5)
\]

The mutual coherence function depends on position as well as time, but because \( E_s(t) \)
and \( E_r(t) \) are identical except for a delay, it is necessary only to consider the temporal
coherence since \( \tau \) is the only varying component. The mutual coherence function is a
normalized auto-correlation of the source beam. Along with the quasi-monochromatic nature
of the beam, Equation (3.4) is rewritten:

\[
I_D(\tau) = I_s + I_r + 2\sqrt{I_sI_r} \left| \gamma(\tau) \right| \cos(2\pi\nu\tau)
\]

(3.6)

The magnitude of the autocorrelation of the source beam \( \left| \gamma(\tau) \right| \) amplitude modulates
the signal to form the envelope of an interferogram. The Weiner-Khinchin theorem states that
the Fourier transform of the autocorrelation of a signal is the power spectral density \( S(f) \):

\[
S(f) = \int_{-\infty}^{\infty} \gamma(\tau) e^{-i2\pi f \tau} d\tau \quad (3.7)
\]

The measured full-width at half-maximum (FWHM) of the autocorrelation function is
known as the coherence length of the signal. The coherence length is the maximum difference
in length between the sample and reference arms for which interference will still be detected.
Since it is related to spectral width by a Fourier transform (Equation (3.7)), a larger bandwidth signal leads to a shorter coherence length and increased axial resolution.

In OCT, the mirror in the sample arm of the Michelson interferometer is replaced with the sample. The sample beam is scattered back into the interferometer from interfaces in the sample with different refractive indices as well as from individual scatters. The higher refractive index of the sample increases the optical path length traveled by the sample arm beam, introducing an extra delay with respect to the reference arm. Backscattered photons from within the sample that have an equivalent optical distance (within the coherence length) as the reference arm will be detected. When the distances are not matched, the signal is no longer correlated, their cross-section $|\gamma(\tau)|$ is zero, and no interference fringes are detected. This is known as coherence gating, and proves very useful for probing different depths in a sample. Translating the reference arm mirror back and forth allows a depth-resolved plot of the backscattered intensity within the sample to be generated. The detected intensity is:

$$I_D(\tau) = I_s + I_r + 2\sqrt{I_sI_r}|\gamma(\tau)|\cos(2\pi\nu\tau)$$  

(3.8)

The OCT signal is contained in the $2\sqrt{I_sI_r}|\gamma(\tau)|\cos(2\pi\nu\tau)$ term, and the envelope of this signal provides the backscattered intensity.

Galvanometers are commonly used in the sample arm to scan the beam transversely, allowing a 2D tomogram of the scatterers within the sample to be generated by assembling adjacent axial depth scans (A-scans). Except for the delay line and beam delivery systems, optical fiber is used in a majority of the components of an OCT system as illustrated in Figure
This allows the system to be more compact and robust. There are several other higher-order corrections made in the practical implementation of an OCT system.

Polarization paddles in the sample arm rotate the light backscattered from the sample so that it has the same polarization as the light returned from the reference arm to maximize the interference. Dispersive glass is used in the reference arm to correct for dispersion introduced by the sample or optical fiber length mismatches. Dual-balanced detectors are commonly used to subtract common-mode noise from the OCT signal. The outputs are then sampled by an analog-to-digital (A/D) card which is synchronized with galvanometer movement and delay line scanning by a computer. The digitized signal is filtered and processed to display the backscattered intensity versus depth.
The coherence gating effect localizes scatterers to within a coherence length of the source, determining the axial resolution in OCT. Most OCT sources have roughly Gaussian spectra which can be described by the model:

\[ S(\nu) = \frac{2 \ln(2) / \pi}{\Delta \nu} \cdot \exp \left[ -4 \cdot \ln(2) \cdot \left( \frac{\nu - \nu_0}{\Delta \nu} \right)^2 \right] \]  

(3.9)

In this equation, \( \Delta \nu \) represents the FWHM of the spectrum. Taking the inverse Fourier transform of Equation (3.9), the Weiner-Khinchin theorem (Equation (3.7)) is used to solve for the source autocorrelation:

\[ \gamma'(\tau) = \exp \left[ - \left( \frac{\pi \Delta \nu \tau}{2 \sqrt{\ln(2)}} \right)^2 \right] \exp(-i2\pi\nu\tau) \]  

(3.10)

The coherence length and the axial resolution of OCT \( (R_A) \) are determined by the FWHM of the autocorrelation of the source:

\[ R_A = l_c = \frac{2c \ln(2)}{\pi} \cdot \frac{1}{\Delta \nu} = 0.44 \frac{\lambda_o^2}{\Delta \lambda} \]  

(3.11)

The autocorrelation function \( |\gamma'(\tau)| \) is the ideal point spread function (PSF) of the OCT system if a mirror were used and placed at the focus of the sample arm optics. However, several sources of aberration can change the shape of the ideal point spread function. Significant aberrations cause differences in the dispersion characteristics between the sample and reference arms and this broadens the interference length, lowering (worsening) the axial resolution of the OCT system [61-63].

In the ideal case, the source has a perfectly Gaussian profile since a Gaussian function has the smallest time-bandwidth product. In the case that the source spectrum is not Gaussian,
the transform will have sidelobes, widening the coherence and lowering resolution. This can lead to ghosting effects as scatterers at different depths are seen at scanning distances that are not close to the reference length. These effects can all be digitally compensated to some extent by post-processing technique. It is the number of frequencies incident on the sample that ultimately determines axial resolution with the shape of the spectrum being less important.

Transverse resolution in OCT is determined by the focusing optics in the sample arm. The typical OCT setup uses a lens to focus the beam, creating a Gaussian beam. The spot size is the diameter of the beam at its waist and is denoted \(2w_0\). It can be calculated using a formula derived from Gaussian optics:

\[
R_T = 2w_0 = 2.44 \frac{f \lambda_0}{D}
\]  

(3.12)

In the expression, \(f\) is the focal length of the lens, \(D\) is the diameter of the beam incident on the back aperture of the lens, and \(\lambda_0\) is the central wavelength of the source. The working distance of the lens is the distance from the face of the lens to the focus.

The confocal parameter, \(b\), is the portion of the beam which is considered to be “in focus,” defined as the distance from the focus where the beam diameter has expanded by a factor of \(\sqrt{2}\) times the radius at the beam waist. It is calculated as:

\[
b = \frac{\pi R_T^2}{2\lambda_0} = \frac{2\pi \omega_0^2}{\lambda} = 3\pi\lambda_0 \left( \frac{f}{D} \right)^2 \text{ where } 2\omega_0 = R_T
\]  

(3.13)

Increasing the beam diameter or shortening the focal length can produce a smaller spot size but will also reduce the confocal parameter.

Numerical aperture (NA) is often used to describe how tightly a beam is focused:
\[ NA = n \sin \theta \quad (3.14) \]

where \( n \) is the refractive index of the medium, and \( \theta \) is the acceptance angle of the lens.

The Weiner-Khinchin theorem (Equation (3.7)) implies that instead of scanning the reference arm and measuring the intensity on the detector for several positions (\( \tau \)), the same information can be acquired by measuring the spectrum of the backscattered signal with a stationary mirror. This method is known as spectral domain OCT (SD-OCT) with a typical set up shown in Figure 3.3. In SD-OCT, the intensity measured by the spectral detector is [64]:

\[
I_D(k) = I_S(k) + I_R(k) + 2\sqrt{I_S(k)I_R(k)} \Re \left\{ \Gamma(k) e^{i[\phi_s(k) - \phi_r(k)]} \right\} \quad (3.18)
\]

where \( k = \frac{2\pi}{\lambda} \) is the wavenumber, \( I_S(k) \) is the power spectral density of the sample arm, \( \phi_s(k) \) is the spectral phase of the sample arm signal, and \( \Gamma(k) \) is the spectral degree of coherence between the sample and the reference arms. The optical detector is only capable of recording the spectral intensity \( I_D(k) \) and not the exact scattering profile, so only the autocorrelation of the scattering profile can be extracted through the Fourier transform of the spectral intensity. However, if the backscattered intensity from the object is small enough, this provides a good estimate of the actual sample scattering profile.

Unlike in time-domain OCT, the scan depth for SD-OCT cannot be adjusted by changing the position of the reference arm mirror. Instead, the maximum scan depth \( \Delta z_{\text{max}} \) is dependent on the wavelength resolution \( \delta \lambda \) of the spectrometer [65]:

\[
\Delta z_{\text{max}} = \frac{\lambda^2}{4\delta \lambda} \quad (3.19)
\]
A line scan camera capable of high acquisition speeds is placed at the Fourier plane of a grating-lens assembly to achieve spectral detection in SD-OCT as seen in Figure 3.2. The lack of moving parts means that the phase stability of SD-OCT is far higher than time-domain systems. SD-OCT can also achieve much higher signal-to-noise than comparable time-domain implementations [66].

A SD-OCT system, as shown in Figure 3.2 [60], was used to image samples in this work. The OCT light source was a Nd:YVO₄-pumped Ti:sapphire laser (KMLabs, Boulder, CO) with a center wavelength of 800 nm. The bandwidth of the source was typically around 105 nm, providing an axial resolution of 4 µm in the sample, and an average power of 10 mW was delivered to the sample. The near-infrared light from the laser was launched into a 50:50 single-mode fiber coupler, splitting the signal between the sample and reference arms.

In the reference arm, the beam was collimated and reflected back into the fiber. In the sample arm, the beam was collimated and launched onto a pair of galvanometer mounted mirrors and into a focusing lens. Two galvanometers were used to provide X and Y directional scanning. A 30 mm focal length lens was used to focus the sample arm light for a lateral resolution of 12 µm. The sample and reference beams were coupled back into the fibers and interfere at the coupler. The output was then collimated again and projected onto a grating. This grating separates out the spectrum of the signal which was focused onto a charged coupled device (CCD) line array and acquired by the computer. The computer synchronized the motion of the galvanometers with the acquisition from the line scan camera, and a 3-D volume was obtained in each scan session.
Figure 3.3 Portable clinical OCT systems. On the left is a commercially-based Bioptigen system and a custom-built system is shown on the right.

Professor Boppart's group currently has two operational clinical OCT systems shown in Figure 3.3. Both of these systems operate at 1300 nm within the biological window and are compact enough to be transported into the operation room. At 1300 nm, these systems offer a greater imaging penetration depth, allowing features to be detected deeper within a sample. This is better for the intraoperative setting, where the exact location of underlying tumor areas is unknown. With removed tumor samples, however, the 800 nm laboratory system provides better resolution and was used for this work.

3.2 X-Ray Hardware

Tomography was first proposed in the early 1900s by the Italian radiologist Alessandro Vallebona as a method to represent a single slice of the body. By moving the x-ray source and film in opposite directions around an axis, a 3-D volume can be reconstructed using the Radon
transform, invented in 1917 [67]. In a special case of the Radon transform, Fritz John derived
the x-ray transform, $Xf$, in 1938 [68]:

$$Xf(L) = \int_L f = \int_R f(x_0 + t\theta)dt$$

(3.20)

defined on the set of all lines $L$, where $f$ is the unknown density function, $x_0$ is an initial point on
the line, and $\theta$ is a unit vector giving direction of line $L$.

An Xradia MicroXCT-400 was used for the x-ray imaging in this study (Figure 3.4) as it
was the system with the operating parameters that were most similar to those in clinical x-ray
systems. The sample was placed in a cuvette and mounted on the stage. The source and
detector were positioned to place the sample in the focus of the x-ray beam. An automatic
stage rotates the sample as the scan progresses and the 3-D volume was reconstructed from
the projections.

![Xradia MicroXCT-400 system](image)

Figure 3.4 Xradia MicroXCT-400 system.
The Xradia MicroXCT-400 system can provide a resolution down to 200 nm. Additionally, the photon energy of the source ranges from 20 keV to 80 keV. This lower x-ray energy range is important for contrast because in soft tissues, the higher energy x-rays will not have much attenuation and pass directly through the sample.

There were three other x-ray systems available: the Xradia MicroXCT-200, the Xradia NanoXCT-100, and the SkyScan 1172. The Xradia MicroXCT-200 was identical to the MicroXCT-400, but designed for smaller samples. The NanoXCT-100 was similarly designed for smaller samples and thus would not accommodate the samples in this study. The SkyScan 1172 was used for some of the first imaging sessions, however, the MicroXCT-400 provided better resolution as well as a source capable of delivering lower x-ray energies.

In the clinical setting, digital specimen radiography systems (Faxitron) are often used (Figure 3.5). This portable system would typically be located immediately outside an operating room and a radiologist can provide real-time feedback on a resected tissue sample. These types of systems use x-ray energies ranging from 10-35 keV.

Figure 3.5 Digital specimen radiography system (Faxitron DX-50 Portable system). Similar to our clinical system, this x-ray based system can collect images in real time during surgery.
Standard x-ray mammography scans use x-ray energies from 24-32 keV, although lower energies may be used to enhance contrast at the cost of penetration depth. The micro-CT system can cover almost all of the typically used x-ray energies used in both x-ray mammography and specimen radiography.

3.3 Methodology

The clinical imaging modalities employed in the medical field are complementary to each other, as each has advantages and disadvantages that can be overcome by using more than one at once. It is widely agreed that multiple image modalities used in conjunction lead to a better understanding of what is being imaged. It is important to determine the limitations and advantages of each modality, as well as determine the areas that a newer technique such as OCT may help in the clinic.

Frozen breast tissue, typically around 5 mm in diameter, was obtained from Carle Foundation Hospital under a protocol approved by the Institutional Review Boards at Carle Foundation Hospital and the University of Illinois. Whenever possible, imaging of a tumor sample was performed on each modality in succession, keeping the orientation and imaging areas correlated. Samples were first imaged with OCT in the open and then transferred to a cuvette for micro-CT imaging, using the bottom of the petri dish and cuvette sides for reference. After imaging, the samples were either placed in formalin for paraffin-embedded histology or flash frozen for frozen section histology. Four samples were imaged with both OCT and micro-CT, with an additional 4 samples that were imaged with only OCT. A total of 2400 OCT images were collected with 1250 being analyzed. A total of 2200 micro-CT images were collected and 760 were analyzed. The remainder of the images were not utilized because they
were at the edges of the data sets and either contained a small portion of the sample or were very noisy.

Three MATLAB algorithms were developed to automatically segment the obtained images, modified from community-developed algorithms. The first was the simplest, applying an amplitude filter to an image based on a user-defined threshold (using the im2bw function on a grayscale image). A spatial frequency filter applied a band-pass filter in the Fourier domain (using the fft function) with a specified bandwidth around a center frequency, and isolated features within that range. A texture-based filter found entropy values (using the entropyfilt function) throughout an image and created a region based on similar features within a user-defined correlation distance. Each filter and segmentation approach was optimized experimentally to obtain a result similar to manual visual segmentation, which served as the gold-standard for comparison. This was done on a training set of 25 images and a Student's t-test was performed to determine statistical significance. Then, for each approach, an algorithm was written to automatically read the images in a data set sequentially (using the imread function), process the image with optimum parameters, and give an area and perimeter value for that image (using the regionprops function). Additionally, the texture-based algorithm had an additional optimization portion as it had large variance in results. These algorithms were then applied to the image data sets and the results analyzed.
4.1 Comparing Systems

In order to analyze the differences between the different imaging modalities, the characteristics of the various systems had to be probed. OCT and micro-CT are both capable of acquiring 3-D data sets, but have different resolutions, field of views, and contrast mechanisms. Real-time acquisition of 3-D OCT volumes is achievable through the use of microelectromechanical (MEMS) scanners as well as using faster cameras. The current speed estimate was based upon a camera scan rate of 1 kHz, which has been superseded by newer systems. Currently, a 96 kHz scan rate camera is in use and along with improved acquisition could, allow for an estimated volume acquisition time of 1 minute for a 3 x 3 x 2 mm$^3$ volume. For a sample of the same size, the micro-CT scanner took 45 minutes to complete. An x-ray mammogram procedure will generally take half an hour to obtain several scans at different angles.

In the clinical mammogram, typical x-ray energies range from 15 keV to 32 keV, depending on the age of the patient and density of the breast. In order to decide what energy to use in this thesis research, a comparison was made of differing x-ray energies on the same sample using the XRadia system. Due to the 20keV lower limit of the x-ray source, Figure 4.1 shows a comparison of 4 different energies incident on a sample.
Figure 4.1 Comparison of 20 keV, 23 keV, 26 keV, and 32 keV x-ray energies used to image three different depths in a normal human breast sample. The darker areas are adipose tissue while the lighter areas are fibrous tissue. The scale is percentage of max intensity.

Analyzing these images, it is found that on average, as source x-ray energy increases, the signal-to-noise ratio (SNR) increases as seen in Figure 4.2. The SNR is defined:

$$SNR_{dB} = 10 \log_{10} \left( \frac{A_{signal}}{A_{noise}} \right)^2 \quad (Eq. \ 4.1)$$

Since there is considerable background signal, the noise signal is obtained by normalizing to the background level. Additionally, the dynamic range is obtained by taking the log scale of the ratio between the maximum and minimum signal strengths.
Figure 4.2 Plot of signal-to-noise ratio (SNR) and dynamic range (DR) versus x-ray energy.

When studying the x-ray energies in Figure 4.2, it is important to note that although the signal-to-noise ratio increased with increasing x-ray energies, the dynamic range stayed relatively constant. This is indicative of the fact that with higher incident energies, greater signal is acquired, while the noise level stays constant. The dynamic range of the sample did not appear to change much with increasing x-ray energy, most likely because the range of energies was relatively small and the sample contained fat and connective tissue that should not attenuate the x-rays much. When imaging the human body, it is often advantageous to use higher energies because of greater penetration, whereas lower energies are useful for better tissue contrast. When using higher energy x-rays, some of the contrast within the soft tissues is lost because these tissues do not attenuate the x-rays as much. Because of this, lower x-ray energies are used to image soft tissue like adipose, and it is useful to employ higher energies for added penetration in harder tissues such as connective tissue or tumors. For the remainder of
the research, an x-ray energy 25 keV was used because this is within the range utilized in the clinical setting.

4.2 Image Analysis

In order to compare the two modalities, it is important to image the same areas with each, and to correlate the ways that different structures appear in each. This, however, proves to be difficult when the sample has to be imaged in the open for OCT and in a cuvette for micro-CT. Additionally, the sample may degrade over time as it is in saline or exposed to the air. The most successful imaging experiments were performed in quick succession.

One of the challenging issues facing the development of OCT is its mode-specific display of different tissue types. When an untrained individual looks at an OCT scan, it is often impossible for them to know what exactly is being displayed, as the black and white nature and speckles may be unfamiliar to them. To this end, it would be advantageous to devise a method to better display images so that different parts are segmented, based on the tissue type. Furthermore, using an automated process, details of an image may be found that might otherwise be missed by a human observer. These methods can also be directly translated into 3-D data sets.

4.2.1 Two-Dimensional Image Correlation

To make correlations between different slices of a sample across modalities, it is important to keep track of the orientation of the sample and the axes of imaging. The OCT scan volumes tend not to cover the entire sample because it has the most limited scan area or field-of-view, which is a tradeoff for better resolution. The following figures show various correlations across modalities.
Figure 4.3 Comparison of OCT and histology images of an infiltrating ductal carcinoma sample. In the OCT image, necrotic regions (blue arrows) can be seen in the intact sample along with microcalcifications (red arrows).

Figure 4.4 Comparison of OCT, micro-CT (30keV x-ray energy), and histology. The dotted blue boxes in the micro-CT and histology images are the approximate scan areas of the OCT images. Tumor structures can be seen in the OCT and histology (green arrows), and microcalcifications can be seen across the modalities (red arrows).
When comparing the different modalities, it can be seen that each modality serves a specialized role in imaging breast cancer. X-ray imaging prominently shows the presence of microcalcifications as seen in Figure 4.4. This is not surprising as x-ray imaging has long been accepted as the preferred screening method for the detection of breast cancer.

OCT, in comparison, can provide morphological information at the cellular level. As seen in Figure 4.18, suspicious structures can be visualized with OCT that are not apparent in the micro-CT scan. Because of the limited field-of-view and imaging depth of OCT, however, OCT applications are limited to very specific uses where the suspicious site has already been identified.

Figure 4.5 En face comparison of OCT and micro-CT of a human breast tumor margin plotted on the same scale. In each image, adipose tissue is seen on the right and the tumor is seen on the left. The x-ray energy used in the micro-CT was 25 keV.
In the OCT image of Figure 4.5, the adipose cells have a characteristic bubble appearance with highly scattering boundaries representing the cell membranes. Also, there is some tumor structure that is visible in on the left. In the micro-CT, some structure can be seen in the adipose region, but there is not much seen in the tumor side in terms of structure aside from a microcalcification on the edge. In Figure 4.6, a higher x-ray energy (40 keV) was used to compare *en face* images of OCT and micro-CT. In this case, more structure can be seen within the tumor region in the micro-CT corresponding to some structure seen by OCT. However, the contrast within the adipose region is decreased.

![Micro-CT and OCT images](image_url)

**Figure 4.6** *En face* micro-CT and OCT images of a human breast tumor margin plotted on the same scale. In this comparison, there are some corresponding structures that can be seen in the tumor region on the right by both micro-CT and OCT (yellow arrows). On the left, there are some fibrous extensions that can be seen in both the OCT and micro-CT (green arrows). The x-ray energy used for the micro-CT was 40 keV.
4.2.2 Three-Dimensional Correlation

The following correlations between imaging modalities were performed with the commercial software Amira and manually registering different features. One method that was employed to compare modalities was to compare the 3-D volumes of a sample and match up features found in cross-sections of the sample. In this way, the rough orientation of the sample was obtained across methods and features within the sample can be correlated by looking at slices. This, however, assumed that the sample was in the same shape between modalities. In Figure 4.7, 3-D volumes of micro-CT and OCT were compared on the same scale and orientation.

Figure 4.7 Human breast tumor margin showing 3-D volumes of micro-CT (top) and OCT (bottom). Note the microcalcifications (black arrows) that appear bright in the micro-CT data.
on the adipose tissue on the right. The micro-CT volume is visualized using a direct volume rendering algorithm while the OCT volume is visualized using a surface mesh algorithm.

Furthermore, by filtering these volumes, features in the samples can be isolated. In the case of micro-CT, the microcalcifications that are visible and screened for in mammography are readily apparent, as seen in Figure 4.8. Although the attenuation of x-rays is not significantly different between fibrous tissue and cancerous tissue, there are three separate tissue types that are readily differentiable: fatty tissue, tumor tissue, and microcalcifications.

![Figure 4.8 Microcalcifications (blue arrow) localized in 3-D using micro-CT imaging of a human breast tumor margin.](image)

Although a similar method can be employed with OCT images, this is not as informative because the source of signal from OCT is based on scattering rather than absorption. The large energies involved with x-rays can only be scattered by the Compton interactions with dense tissues (microcalcifications). However, in OCT, optical scattering occurs whenever there is a
difference of refractive index. Because of this, the signal is not solely from the dense calcifications, although these can be identified as well. Figure 4.9 shows an OCT volume filtered to show only highly intensity signal in order to visualize highly scattering particles.

Figure 4.9 OCT volume visualizing highly scattering particles in a human tumor specimen along a tumor margin (yellow line). On the left is adipose tissue in which the highly scattering boundaries are apparent. On the right is the tumor within which highly scattering particles can be seen to possibly be microcalcifications (red arrow).

Different x-ray energies were utilized in this portion so that more features could be highlighted in heavily tumor rich tissues. However, in the typical clinical setting, x-ray energies will not go that high. As such, the data sets utilized for the automated segmentation study utilized an x-ray energy of 25 keV.

4.2.3 Optimizing Segmentation Methods

It is important that the observer be able to orient different data sets so that a specific area of interest can be seen across the modalities. To do this, the orientation, scale, and fields-of-view must be matched. Three MATLAB algorithms were utilized to automatically segment
images, modified from community-developed algorithms. Each of the algorithms made a binary decision, with the white areas in the following figures delineating tumor areas. In the following figures, an array is shown with varying parameters. The optimal setting was selected by visual inspection and is shown by a box selecting the image.

The first method used an intensity filter to segment out tumor areas as seen in Figure 4.10 (OCT) and Figure 4.11 (micro-CT). For OCT, threshold values were optimized to 20%, based upon the value that highlighted the tumor area best while keeping areas in the adipose minimized. For micro-CT, 25% was found to be the optimal threshold value, as almost the entirety of the tumor region was segmented without much of the adipose area.

Figure 4.10 OCT images with threshold settings from 5% - 30% are shown. Setting threshold values at 20% of maximum intensity yielded the best results by visual selection (box). The image dimensions were 3 x 1.5 mm and the tumor area is shown on the left.
Figure 4.11 Micro-CT images with threshold values from 15% - 40%. Setting the threshold values to 25% of maximum intensity yielded the best results by visual selection (box). The tumor portion is on the right side of each image.

Intensity filtering yields better segmentation results for micro-CT because contrast is based upon absorption within the tissue, so the adipose regions had less signal than the tumor region. In the scattering-based OCT image, there are portions of adipose tissue that are seen due to scattering from the adipocyte cell membranes. As such, the intensity approach was not ideal for OCT image processing. This method was the fastest, however, taking 0.7 sec per OCT image and 0.1 sec per micro-CT image.

The second method of segmentation employed was spatial frequency filtering. This method was chosen because tumor areas are more dense and have different spatial frequency content in images compared to adipose tissue. This algorithm applied a band-pass filter in the Fourier domain and returned a filtered image. In order to optimize this algorithm, both the center frequency and bandwidth had to be optimized. Figures 4.12 and 4.13 show this
optimization for OCT and micro-CT, where arrays of images are shown, with the center frequency increasing in the columns from left-to-right, and the bandwidth increasing in the rows, from the top to the bottom. For both OCT and micro-CT, the spatial frequency filter was able to isolate the tumor area. The spatial frequencies were determined by multiplying the number of pixels by the frequency variable and dividing by the size of the image in mm. The algorithm took 0.95 sec per OCT image and 1.8 sec per micro-CT image.

Figure 4.12 Optimization of spatial frequency filtering of an OCT image with tumor on the left.

The center frequencies vary in the range 73.4 - 75.8 cycles/mm from left-to-right, and the bandwidth varies in the range 1.4 - 2.6 cycles/mm from top to bottom. The optimal configuration has center frequency 74.9 cycles/mm and bandwidth 2.2 cycles/mm (box).
Figure 4.13 Optimization of spatial frequency filtering for a micro-CT image with tumor on the right. The center frequencies vary in the range 18.4 - 32.2 cycles/mm from left-to-right, and the bandwidth varies in the range 6.3 - 15.7 cycles/mm from top to bottom. The optimal configuration has center frequency 23.01 cycles/mm and bandwidth 12.3 cycles/mm (box). The last method used a texture-based algorithm, as the entropy and texture of tumor and adipose regions should be different. This approach was optimized around the variables of kernel size and correlation distance as seen in Figures 4.14 and 4.15. This method yielded the best qualitative results for processing OCT images, as it was able to best isolate the tumor region. However, the parameters were highly sensitive to specific images and needed to be optimized for each image, often giving erroneous results if not recalibrated for each image. Furthermore, when applied to micro-CT images, this algorithm had trouble distinguishing borders of tumor regions, as the texture of the micro-CT images are universally grainy. The n1 variable is the width and height of the kernel size, increasing from top to bottom. The n2 variable is the square of the correlation distance, increasing from left-to-right. This algorithm took 2.3 sec per OCT image and 2.3 sec per micro-CT image.
Figure 4.14 Optimization of texture-based filtering for an OCT image with tumor on the left. The columns show increasing correlation distance from left-to-right and the rows show increasing kernel size from top to bottom. The optimum configuration is selected visually (box).

Figure 4.15 Optimization of texture-based filtering for a micro-CT image with tumor on the right. The columns show increasing correlation distance from left-to-right and the rows show increasing kernel size from top to bottom. The optimum configuration is selected visually (box).
4.2.4 Automated Segmentation

In order to determine effectiveness of each segmentation algorithm, a training set was performed with n=25 images, comparing area and perimeter of segmented areas as compared to a gold-standard of visual segmentation by a user (myself). Figure 4.16 shows the comparison of segmented areas for each method, after being normalized by the area determined by visual segmentation. Although only the spatial frequency filter applied to the micro-CT images is shown to be statistically similar (p > 0.05) to the area segmented by visual inspection, it was shown to be closest to visual approximations of tumor area in OCT as well. Subsequently, the spatial frequency algorithm was used in the automated portion of this study as the normalizing quantity. The intensity-based filter found a much larger area than visual inspection because it did not account for noise in the images. The texture-based filter was not able to find a tumor area approximately 25% of the time, and also had limitations with the micro-CT images, resulting in large error bars and smaller segmented areas.
Figure 4.16 Average area of tumor found by each segmentation method, normalized to visual segmentation (n = 25). A Student’s t-test comparing the mean of areas found in each method with the mean obtained by visual inspection was performed. The p-values are shown on the bottom in the table. Note, because the data is normalized, areas closer to 1 most closely approach the area determined by visual segmentation.

Additionally, the perimeter of automatically segmented areas was compared to visually-segmented areas and the comparison is shown in Figure 4.17. The overall OCT perimeter of the intensity method was large because many small areas were isolated, each contributing to the perimeter measurement. For the spatial frequency method, the perimeter was also larger than through visual inspection because there was more detail in choosing the boundaries of the area. Finally, the texture-based method was closest to visual inspection because the algorithm forms one continuous region based on similar characteristics.
Figure 4.17 Average perimeter of tumor found by each method normalized to visual segmentation (n = 25). A Student's t-test comparing the mean of perimeters found in each method with the perimeter obtained by visual inspection was performed. p values are shown on the bottom in the table. Note, because the data is normalized, perimeters closer to 1 most closely approach the perimeter determined by visual segmentation.

Finally, these algorithms were applied on a larger scale on several data sets, including images where tumor regions were larger and smaller. In each of these images, the intensity and texture-based algorithms were normalized to the areas found by the spatial frequency filter. This was done because the spatial frequency algorithm was closest to visual segmentation in the training set. In figure 4.18, it can be seen that intensity-based
segmentation consistently found larger areas than those identified by the spatial frequency algorithm, as seen before. However, the texture-based method performs better for OCT than micro-CT. This is consistent with previous findings, but the texture-based method has the additional advantage of finding smaller numbers of isolated areas. In the future, a combination of methods may be useful for automatically identifying areas of tumor so that real-time segmentation of OCT can be used in the operating room without the need for training to read and interpret OCT images. Additionally, these algorithms can be expanded to segment 3-D datasets to isolate tumor volumes.

Figure 4.18 Areas normalized to spatial frequency area results (n = 1250 for OCT, n = 760 for micro-CT). A Student's t-test comparing the mean of area found in each method with the area obtained by the spatial frequency filter was performed. The p-values are shown on the bottom in the table.
4.3 Impact

Because of the limited penetration depth and high resolution of OCT, the applications for the technique in breast cancer detection and diagnosis are specialized for certain clinical situations. The use of OCT depends largely upon prior knowledge obtained through other means, whether it is a physical evaluation or another imaging modality. Automating registration of OCT with other modalities would allow for this to be done without worrying that the OCT scans are being taken in an unrelated area. In the case of breast cancer screening, a mammogram may detect a suspicious area. The features from x-ray imaging methods are correlated to OCT image features so that OCT can be used in real time in the operating room.

Intraoperatively, OCT may be used to locate and evaluate tumor margins or lymph nodes. Currently, there is considerable interest in finding a method to evaluate tumor margins, as positive margins found by histological analysis result in re-operations and have been linked to a large recurrence rate for cancer. One such method is the use of x-ray specimen radiography. However, there has yet to be found a method that can accurately and effectively determine in real time whether there are tumor cells left behind following surgery, aside from waiting for histological analysis. OCT can provide morphological data of the structure of a sample, and since positive margins are typically determined to be within the imaging depth of OCT (1-2 mm), this application is well suited for the OCT technology. Furthermore, with the use of automated segmentation methods, tumor regions can be identified and brought to the attention of the surgeon immediately without having to be viewed by a radiologist.
CHAPTER 5

CONCLUSIONS AND FUTURE WORK

In this thesis, x-ray images, OCT images, and corresponding histology of breast tumors and tissue were analyzed and compared using both visual segmentation and several automatic algorithmic segmentation methods. OCT may have the potential to be used to image fine tissue structure in the operating room. However, it is important to understand how features seen in x-ray images such as screening mammography or specimen radiography appear in OCT, so real-time OCT can be correctly interpreted.

It has been shown that each individual modality provides information about the sample in a different way through different types of tissue contrast. Although micro-CT is generally used for laboratory-based studies, it was utilized in the context of breast cancer detection and diagnosis. This lab-based micro-CT machine was used because it could achieve a resolution comparable to OCT while utilizing x-ray energies similar to mammography or specimen radiography.

Micro-CT is useful in detecting very dense areas in a tissue that absorb the x-rays, and in particular, microcalcifications. This shows that x-rays are aptly chosen to be a screening method for breast cancer as these microcalcifications are often indicative of tumor cell secretions. X-ray energy plays an important part in determining the amount of contrast that can be seen. However, fundamentally, it is difficult to distinguish fibrous tissue from developing tumor because of similar linear attenuation coefficients. As such, the three types
that x-ray is good at distinguishing between are soft fatty tissue, fibrous tissue or tumors, and microcalcifications within the tumors.

OCT can show the cellular level morphology of the area being imaged. Although limited by penetration depth and field of view, OCT can differentiate adipose tissue from connective tissue and can also identify some tumor structures and microcalcifications. OCT can be utilized in the specific cases where high resolution is desired and penetration depth does not need to be over a few millimeters. In the intraoperative setting, OCT can be utilized to evaluate tumor margins that have been resected in order to decrease the chance of tumor reoccurrence and the need for additional operations. Additionally, a handheld probe could be used so that a surgeon could image tumor margins, the tumor cavity, and many lymph nodes in situ.

With access to patient data, correlations could possibly be made between the clinical imaging from x-ray mammography, localizing the tumor, and tracking development. When the time for surgery arrives, clinical OCT can be used to image the tumor structure and margins and be compared to the corresponding histopathology. In such a way, knowledge can be gained about how a tumor looks in situ in addition to using different whole breast imaging modalities. This can be used to visualize tumor architecture during surgery in comparison to when sectioned for histology.

Furthermore, a successfully automated program for registering OCT images with other modalities would prove invaluable to the diagnosis of disease. OCT could be used to identify and view structure around a site of interest. There could be minute details that are picked up by the automated segmentation that would be missed by other methods, leading to earlier suspicion, diagnosis, and treatment.
In the future, the automated segmentation algorithms should be improved and combined so that they can accurately and consistently isolate out regions of interest within an imaged sample. Automated co-registration algorithms can also be developed to further correlate findings between OCT and x-ray imaging. Because of the real-time nature of the OCT technology, a fast, efficient method would be ideal for intraoperative imaging. Once optimized, this algorithm should be tested with a clinical system for viability as an aid during surgery.
REFERENCES


"Improved signal-to-noise ratio in spectral-domain compared with time-domain optical

1983.

[68] F. John, "The ultrahyperbolic differential equation with four independent variables,"