Calibration and analysis of a multimodal micro-CT and structured light imaging system for the evaluation of excised breast tissue

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Abstract

A multimodal micro-computed tomography (CT) and multi-spectral structured light imaging (SLI) system is introduced and systematically analyzed to test its feasibility to aid in margin delineation during breast conserving surgery (BCS). Phantom analysis of the micro-CT yielded a signal-to-noise ratio of 34, a contrast of 1.64, and a minimum detectable resolution of 240 μm for a 1.2 min scan. The SLI system, spanning wavelengths 490 nm to 800 nm and spatial frequencies up to 1.37 mm⁻¹, was evaluated with aqueous tissue simulating phantoms having variations in particle size distribution, scatter density, and blood volume fraction. The reduced scattering coefficient, μ' and phase function parameter, γ, were accurately recovered over all wavelengths independent of blood volume fractions from 0% to 4%, assuming a flat sample geometry perpendicular to the imaging plane. The resolution of the optical system was tested with a step phantom, from which the modulation transfer...
function was calculated yielding a maximum resolution of 3.78 cycles per mm. The three dimensional spatial co-registration between the CT and optical imaging space was tested and shown to be accurate within 0.7 mm. A freshly resected breast specimen, with lobular carcinoma, fibrocystic disease, and adipose, was imaged with the system. The micro-CT provided visualization of the tumor mass and its spiculations, and SLI yielded superficial quantification of light scattering parameters for the malignant and benign tissue types. These results appear to be the first demonstration of SLI combined with standard medical tomography for imaging excised tumor specimens. While further investigations are needed to determine and test the spectral, spatial, and CT features required to classify tissue, this study demonstrates the ability of multimodal CT/SLI to quantify, visualize, and spatially navigate breast tumor specimens, which could potentially aid in the assessment of tumor margin status during BCS.

Keywords: structured light imaging, micro-computed tomography, breast conserving surgery, spatial frequency domain imaging

1. Introduction

Breast conserving surgery (BCS) is common treatment for localized breast cancer, being less invasive than a full breast removal and having similar long term survival rates when combined with radiation therapy (Fisher et al 2002). However, 20–40% of patients undergoing BCS endure follow-up re-excision procedures due to an incomplete original resection (Pleijhuis et al 2009), as determined 1–2 days after the surgery through histopathology. Current standard of care includes intraoperative frozen section pathology and touch-prep cytology. Despite seeming diagnostic value, these procedures are extremely resource intensive to process and analyze just a small subset of the specimen, thus limiting widespread adoption (St John et al 2017). With the high incidence of breast cancer, a critical need exists for surgical guidance tools to aid in assessing tumor involvement at the margin of a resected breast specimen during a BCS procedure in order to limit painful and costly re-excision procedures.

A plethora of biomedical devices have been proposed for this task, including electrical impedance spectroscopy (Kaufman et al 2016), photoacoustic tomography (Wong et al 2017), optical coherence tomography (Erickson-Bhatt et al 2015), non-linear microscopy (Tao et al 2014), dark field-confocal microscopy (Laughney et al 2012), and Raman spectroscopy (Wang et al 2016), among others. However, all of these methods either require exogenous dyes, chemically processing or fixing of tissue, or combing through point sampling volumes with a probe. But, a recently demonstrated imaging method, broadly referred to as high spatial frequency structured light imaging (SLI), has shown label free sensitivity to changes in freshly resected breast morphology over a large field of view (FOV) (\(\sim 10^4 \text{ cm}^2\)) (McClatchy et al 2016).

This approach, originally termed spatial frequency domain imaging (SFDI), was first demonstrated by Cuccia et al to spatially map diffuse optical properties (Cuccia et al 2005, 2009) and later applied to optical tomography (Bélanger et al 2010), spectroscopic imaging (Gioux et al 2011, Laughney et al 2013), and fluorescence imaging (Sibai et al 2015). The key advantage of structured illumination is the ability to tune depth sensitivity with the spatial modulation frequency. While the contrast at low spatial frequencies is dictated by absorption features
The contrast of sub-diffusive high spatial frequency images is dictated by both scattering intensity and the angular distribution of scattering events, or phase function, as photon propagation is constrained to superficial volumes with minimal volumetric averaging over tortuous photon path-lengths (Bodenschatz et al. 2014, Krishnaswamy et al. 2014). The phase function arises from the underlying physical properties of tissue ultrastructure (Bartek et al. 2006, Rogers et al. 2009, Radosevich et al. 2015). Recent studies have used sub-diffusive structured light imaging to quantify angular scattering distributions through the phase function parameter $\gamma$, which is related to the size-scale distribution of scattering features (Kanick et al. 2014, Bodenschatz et al. 2015). This phase function parameter, along with the reduced scattering coefficient $\mu_r'$, was used to cluster benign and malignant breast tissue pathologies in freshly resected human breast specimens (McClatchy et al. 2016) and morphologies within murine tumors (McClatchy et al. 2017), as different tissue morphologies with unique densities and size-scale fluctuations manifest unique light scattering properties.

Although SLI can generate wide-field images of the specimen surface, it is unable to provide high resolution (<1 mm) depth contrast or a tomographic reconstruction through the specimen volume. Micro-computed tomography (CT) is a promising technique for intraoperative visualization of breast specimens, which can be manufactured in a shelf shielded, mobile form factor. By reconstructing x-ray absorption in three dimensions (3D) thereby resolving overlaying features, micro-CT increases low contrast resolution relative x-ray projection imaging, and thus, yields a more accurate delineation of the tumor core extent and proximity to the margin in surgical breast specimens (Tang et al. 2013, 2016). Furthermore, micro-CT and SLI can be symbiotic as the former inherently loses image quality at the tissue-air interface, as the latter becomes the most sensitive.

In this manuscript, a novel multimodal imaging system, utilizing volumetric micro-CT and superficial SLI, is introduced and analyzed for future application in evaluation of excised breast tissues. Image quality and accuracy of both imaging modalities are assessed, as well as the co-registration of the two image systems. Accuracy of the optical property inversion method is validated on comprehensive sets of flat phantoms perpendicular to the imaging plane. As a proof-of-principle, an ex-vivo breast specimen is imaged during gross examination, yielding visualization of the tumor extent in 3D with co-registered structured light images and scatter parameter maps. Further work is needed to develop classification algorithms, at which point the clinical value of this multi-modality system can be tested.

2. Methods

2.1. Multimodal micro-CT and multi-spectral SLI system

The multimodal SLI/micro-CT system was created from a modified and re-purposed IVIS SpectrumCT, which is a pre-clinical bioluminescence imaging and micro-CT system (PerkinElmer Inc., Hopkington, MA). The SLI addition consisted of the native IVIS charged coupled device (CCD) camera (Andor iKon, Andor Technologies Ltd., Belfast, UK) and a retrofitted digital light projector (DLP) (CEL5500 Fiber, Digital Light Innovations Inc., Austin, TX) with a 3.6X telephoto lens. Due to size constraints within the imaging cabinet, the DLP is rotated $22^\circ$ with respect to the CCD, as shown in the schematic of the SLI system depicted in figure 1(a). Light was delivered through an optical fiber to the DLP, was focused onto the digital mirror device (DMD) creating the structured light patterns, and was then focused on the specimen. The DLP was connected to an external virtual monitor which controls the illumination pattern at a frame rate of 60 Hz. The illumination source was a supercontinuum.
laser (SuperK Blue, NKT Photonics, Denmark) with a tunable line filter (SuperK Varia, NKT Photonics, Denmark) to select the wavelength band. At the shelf stage height used for imaging, the diameter of the circular illumination field of the DLP was \( \sim 14 \text{ cm} \) with a projection resolution of 168 \( \mu \text{m} \), while the imaging field was \( \sim 26 \text{ cm} \times \sim 26 \text{ cm} \) with a sampling resolution of 126 \( \mu \text{m} \). Only a subset of the CCD corresponding to \( \sim 13 \text{ cm} \times \sim 13 \text{ cm} \) covering the illumination field was read out to minimize acquisition time. The self-shielded micro-CT of the PerkinElmer system was a cone-beam CT in a ‘pancake’ geometry, with the specimen rotating through 360° on a turntable as shown in figure 1(b).

2.2. Data acquisition and processing

For the multimodal acquisition, micro-CT and SLI data were sequentially acquired with separate software packages. The specimen was placed on the rotation stage shown in figure 1(b), and the micro-CT scan was natively acquired and reconstructed through the PerkinElmer Living Image software. A 1 mA current was used and the tube peak kilovoltage (kVp) and exposure time were set to either 25 kVp and 200 ms/exposure or 50 kVp and 100 ms/exposure, yielding 2.4 min and 1.2 min total scan times for the low and high energy settings, respectively. The filtered back-projection reconstruction time was \( \sim 2 \text{ min} \) for a reconstructed FOV of 12 cm \( \times \) 12 cm \( \times \) 3 cm with 150 micron cubic voxels.

After the micro-CT scan was completed, an SLI acquisition occurred with a custom LabView routine (National Instruments, Inc., Austin, TX). For all scans in this study, seven wavelengths were acquired, \( \lambda = [490, 550, 600, 650, 700, 750, 800] \text{ nm} \), with a 15 nm bandwidth, and at each wavelength, 18 one-dimensional sinusoidal projections were recorded at 6 spatial frequencies, \( f_x = [0.05, 0.15, 0.61, 0.78, 0.92, 1.37] \text{ mm}^{-1} \) with 3 phase offsets, \( \phi = [0, 120, 240]^\circ \). Although previous research has shown sensitivity of the rotation angle of the modulation pattern to oriented features (DAndrea et al 2010, Konecky et al 2011), in this protocol only one rotation angle was considered to minimize the time of a SLI scan, which was \( \sim 8 \text{ min} \) for both acquisition and saving. After specimen scanning, an 8 in. \( \times \) 8 in. silicone
TiO$_2$ phantom and a 10 in. × 10 in. 99% reflectance standard (Labsphere, Inc., Sutton, NH) were imaged for calibration.

SLI data processing occurred off-line with custom scripts in MATLAB (2016a). Images were demodulated by  
$$I_d = \frac{2}{3} \sqrt{(I_{\phi_1} - I_{\phi_2})^2 + (I_{\phi_2} - I_{\phi_3})^2 + (I_{\phi_3} - I_{\phi_1})^2},$$  
where $I_{\phi_1}, I_{\phi_2}, I_{\phi_3}$ are the three phase offsets for a given spatial frequency and wavelength. Due to minor fluctuations in laser power, phase images were scaled so that each had the same mean over the illuminated area to minimize demodulation artifacts. Also, a median filter with 3x3 pixel kernel was applied to demodulated images to remove noise and any specular reflections arising from minor fluctuations of the tissue surface profile. These specular reflections were mitigated by flattening the tissue surface between two transparent acrylic plates and also using an oblique projection scheme. Optical property maps were calculated with a sub-diffusive model inversion method described in a previous manuscript (Kanick et al 2012a, McClatchy et al 2016). The sub-diffusive model was semi-empirically derived from a series of forward Monte-Carlo simulations utilizing a modified Henyey–Greenstein phase function (Kanick et al 2012b). To summarize briefly, demodulated images were calibrated by the TiO$_2$ phantom as  
$$R_{d_{\text{sample}}} (x, y, f_x, \lambda) = \frac{R_{d_{\text{TiO2}}} (x, y, f_x, \lambda)}{I_{d_{\text{sample}}} (x, y, f_x, \lambda)} I_{d_{\text{TiO2}}} (x, y, f_x, \lambda),$$  
where $R_{d_{\text{TiO2}}} (x, y, f_x, \lambda)$ are sub-diffusive model-predicted reflectance values for the calibration phantom. A non-linear least squares routine determined optical properties by minimizing the difference between measured calibrated reflectance and model-based predicted reflectance over all spatial frequencies and wavelengths. Scattering was assumed to follow a power law such that  
$$\mu_s' = A (\lambda) - B,$$  
where $A, B$ are the scattering amplitude and power, respectively. The phase function parameter $\gamma$ was a free fit variable at each wavelength. For tissue specimens, the absorption coefficient, $\mu_a$, was assumed to be a linear sum of oxygenated hemoglobin, deoxygenated hemoglobin, and $\beta$-Carotene with basis spectra obtained from the Oregon Laser Medical Center (OLMC) spectra database (http://omlc.ogi.edu/spectra/index.html Accessed May 2017).

Color images were also reconstructed from the multi-spectral SLI data. For the 5 wavelengths in the visible spectrum, $\lambda = [490, 550, 600, 650, 700]$ nm, SLI images were transformed into a RGB color-space using the 1931 International Commission on Illumination (CIE) tristimulus values (Smith and Guild 1931). For each illumination band, the $x, y, z$ chromaticity values were calculated and normalized, and multiplied by the demodulated intensity of the specimen and the 99% reflectance standard for each respective wavelength band. The resulting $x, y, z$ image stack was converted to RGB space with the value of the 99% reflectance standard set to be the white point.

2.3. 3D–2D co-registration and visualization

Accurate calibration and co-registration of the 2D image coordinates to the 3D CT coordinates are needed for a multimodal visualization. Camera calibration with the optical system was performed to account for lens and perspective distortion. This was done using the Open Source Computer Vision Library (OpenCV) Camera Calibration and 3D Reconstruction Software (Open Source Computer Vision Library 2016), with which the intrinsic camera matrix and radial and tangential lens distortion coefficients were calculated. Images acquired were corrected using the undistortImage function in the MATLAB Computer Vision System Toolbox (version 2016a). The transformation matrix from the optical image coordinates to the CT coordinates in the XY plane was calculated using procrustes alignment method. Because the optical and micro-CT systems were rigidly fixed, these calibrations were performed once, and were used in all imaging experiments reported here.
With the optical system calibrated, a module was created to co-register and display an acquired CT/optical scan automatically which was integrated into NIRFAST Slicer software (www.dartmouth.edu/~nir/nirfast/) (Dehghani et al 2009, Jermyn et al 2013). A diagram of the data-processing and work-flow is shown in figure 2 for a hemispherical gelatin phantom. The height map was extracted from the CT volume by determining the most superficial CT voxel above a manual threshold, for each \(x, y\) vector of voxels. Next, the optical image was undistorted and then co-registered to CT coordinates through the linear transformation. Finally, for each point on the height map, the scalar value of the nearest optical data point was assigned. With this 3D scalar data, a triangular surface mesh was created in the Visualization Toolkit (VTK) file format, which was then simultaneously rendered with the CT volume in the Slicer environment, shown in the bottom right view.

**Figure 2.** Flow diagram of the multimodal data processing and work-flow is shown for a hemispherical phantom. First, optical and CT data are sequentially acquired with the SLI/CT system. A height map is extracted from the CT and the optical data undergoes a coordinate transformation from image coordinates to CT coordinates, from which a 3D surface mesh is created as a VTK file. Both the VTK file and CT DICOM can be simultaneously rendered in the open source Slicer environment, shown in the bottom right view.

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### 2.4. Micro-CT, optical, and co-registration phantoms

To quantify image quality of the micro-CT system, a mammography target phantom (Mammo 156 Phantom, Gammex Inc., Middleton, WI) was utilized. This mammography accreditation phantom is composed of acrylic with a wax insert containing various fibers, hemispherical masses, and specs. It has been designed to simulate a compressed breast comprised of 50% glandular and 50% adipose tissue and size of 10.2 cm \(\times\) 10.8 cm \(\times\) 4.2 cm. While the phantom was larger than the micro-CT FOV, surgical specimens are smaller than the entire breast and contain less background tissue; thus, only the wax insert was imaged. Furthermore, if the entire phantom were imaged, partial volume artifacts would have greatly degraded image quality, with a large portion of phantom having been outside of the x-ray beam.
The spatial resolution of the SLI system was analyzed with a step phantom. A sheet of highly absorbing black paper was placed on a 99% reflectance standard creating a sharp edge between a highly reflective and nearly non-reflective surface. The spatial resolution was analyzed by characterizing the response of the imaging system across the edge.

To validate the sensitivity of the SLI system and accuracy of the optical property inversion method, two sets of tissue simulating aqueous phantoms were imaged. In the first set of phantoms, the size-scale distributions of scatterers were varied by selectively titrating various concentrations of Intralipid (IL) and a solution of 140 nm polystyrene spheres. The optical properties of the sphere solution were calculated with Mie theory (Bohren and Huffman 2008), while the optical properties of IL were taken from a previous publication (Michels et al 2008), and a discrete particle model was used to calculate the optical properties of different ratios of each solution (Gélébart et al 1996). The various solutions contained $\gamma \in [0.99 - 2.00]$ and $\mu'_s$ of each phantom was matched at $\lambda = 650$ nm. In the second set of phantoms, absorption and scattering were varied independently with various concentrations of IL and whole porcine blood (Lampire Biologicals, Pipersville, PA). The hemoglobin concentration of the blood was measured at 13.4 g dl$^{-1}$, and to maintain neutral pH, phosphate buffered was used for dilution. Solutions were made with $[0.5, 1, 2]$% IL and $[0, 0.5, 1, 2, 4]$% blood volume fractions (BVF), yielding phantoms with $\mu'_a \in [0.8 \times 10^5]$ over the acquired wavelength range assuming fully oxygenated blood. The influence of blood on the scattering properties were considered to be negligible compared the influence of IL, based upon previous studies measuring the optical properties of blood (Friebel et al 2006, Bosschaart et al 2014). Each phantom set was imaged in a 24-well plate having black, non-transparent walls and 2.5 ml of each solution per well.

Accurate co-registration between the micro-CT and optical imaging system was experimentally validated and quantified with a custom-made phantom containing radio-opaque and optically bright markers. The markers were 1/8 in. acetal pins, the top surfaces of which were coated in commercial liquid paper to increase optical reflection. Two orthogonal lines of 7 markers with approximately 1 cm spacing were set on optically dark paper creating a cross-hair pattern. The co-registration accuracy was measured as the co-localization of the marker positions in the CT and SLI datasets.

2.5. Imaging a surgically resected breast specimen

To demonstrate feasibility of the system to acquire and display a multimodal clinical dataset, a freshly resected breast tissue specimen was scanned. The imaging protocol did not interfere with standard of care posing minimal risk to the patient, and was approved by the Dartmouth Hitchcock Medical Center (DHMC) Internal Review Board (IRB) and the Committee for the Protection of Human Subjects (CPHS). After a breast surgery specimen was received by the Department of Pathology, it was subsequently ‘bread loafed’ and grossly assessed per standard of care. Cut tissue specimens with superficial lesions were imaged with system, which was located in the surgical specimen lab. To maintain the orientation of the specimen through imaging and also provide a flat imaging surface, it was placed in a customized holder, consisting of two 1/8 in. thick, optically-clear acrylic plates, between which the specimen was secured with elastic bands. The silicone calibration phantom, having a refractive index similar to tissue (Ayers et al 2008), was likewise imaged with the acrylic plate. Immediately after imaging, the specimen was cut into cassette sized sections (~1 cm), photographed, and underwent standard histological processing of dehydration, fixation, wax embedding, sectioning and hematoxylin and eosin (H&E) staining. Resulting histology was evaluated by a trained pathologist (W.A.W) and included in the patient’s report.
3. Results

3.1. Micro-CT analysis of mammography target phantom

The micro-CT target phantom analysis resulted in the same minimum detectable objects (MDO) for the low and high energy scans, with only the smallest fiber and smallest speck cluster not clearly visible. This yielded a limiting resolution of $240 \mu m$ for a high-contrast spherical object. To assess image quality of the micro-CT, the signal-to-noise ratio (SNR) and Contrast were evaluated for each kVp scan. A single slice through the largest mass was analyzed, with the mean, $\mu$, and standard deviation, $\sigma$, of the linear attenuation coefficient calculated in a region of interest (ROI) in both the largest mass and in the abutting background. SNR was calculated as $SNR = \mu_{\text{mass}} / \sigma_{\text{background}}$ and Contrast $= \mu_{\text{mass}} / \mu_{\text{background}}$. The 50 kVp scan yielded an SNR of 34 for a 72 sec acquisition, while the 25 kVp scan yielded an SNR of only 23.8, despite compensating for higher attenuation by doubling the exposure time. The 25 kVp did have a slightly higher Contrast of 1.81, compared to 1.64 for the 50 kVp scan. A tabulated summary of the metrics quantifying the micro-CT performance is shown in Table 1.

Because of the shorter scan time, superior SNR, and similar Contrast, subsequent micro-CT scans displayed this point forward were acquired with the 50 kVp, 100 ms/exposure scan settings.

| Exposure time (s) | FibeMDO (mm diameter) | SpeckMDO (mm diameter) | MassesMDO (mm thick) |
|------------------|-----------------------|------------------------|----------------------|
| 144              | 0.54                  | 0.24                   | 0.25                 |
| 72               | 0.54                  | 0.24                   | 0.25                 |

Table 1. Summary of micro-CT performance metrics and size of minimum detectable objects (MDO) in the mammography target phantom.
3.2. Multi-spectral structured light imaging analysis

The spatial resolution of the demodulated intensity as a function of spatial frequency and wavelength was tested by analyzing the response of a step phantom. In figure 3(a), a captured image of the step phantom is shown for $f_x = 0$ mm$^{-1}$ and $\lambda = 490$ nm. The direction of the illumination frequency is at a $68^\circ$ angle with the step, while the $y$-axis of the CCD is parallel, so that the captured image can be averaged along the step. Measured edge responses across the step are shown for each imaged wavelength at $f_x = 0$ mm$^{-1}$ in figure 3(b). A forward difference approximation and subsequently a fast Fourier transform (FFT) was applied to each edge response to calculate the corresponding modulation transfer functions (MTF), shown in figure 3(c). Each MTF was normalized by its maximum value and smoothed with a 5 pixel moving average filter. Edge responses and MTFs are likewise shown in figures 3(d) and (e) respectively, for each imaged spatial frequency at $\lambda = 490$ nm. The limiting spatial resolution is quantified as the point where the MTF reaches 10% of its maximum value. These values are tabulated for the variation in wavelength and spatial frequency in figure 3(f).

For the case of diffuse illumination, a clear trend of increasing spatial resolution from 2.24 cycles mm$^{-1}$ to 3.17 cycles mm$^{-1}$, occurs as wavelength decreases from 800 nm to 490 nm. Although the reflectance standard is near completely reflective, this wavelength dependent behavior is still expected as the lower edge of the phantom will have a higher scattering cross section with shorter wavelength light, and therefore, a shorter transport length resulting in a sharper response. Also, as the spatial illumination frequency increases, a slight increase is
found in spatial resolution, which is also expected as increasing the spatial illumination frequency constrains the light transport to become more superficial and localized, also resulting in a sharper response. Results revealed a maximum spatial resolution of 3.78 cycles mm\(^{-1}\) at \(\lambda = 490\) nm and \(f_s = 1.37\) mm\(^{-1}\).

The sensitivity and accuracy of the SLI system was evaluated with aqueous tissue simulating phantoms, mimicking the expected contrast and background absorption in human breast tissue. Two sets of phantoms were characterized: a first set with variations in particle size scale distribution, and a second set with variations in BVF and IL concentration to alter both absorption and scattering, independently. The recovered values of \(\gamma\) and \(\mu'_s\) for each particle size scale distribution are shown in figures 4(a) and (b), respectively. The magnitude of \(\gamma\) was unique over all wavelengths for each particle size scale distribution, with \(\gamma\) exhibiting a linear relationship with wavelength. The magnitude of \(\gamma\) decreased with the increasing contribution of the more isotropic 140 nm spheres compared to the more forward scattering IL. Likewise, the spectral slope of \(\mu'_s\) also stratified for each particle size scale distribution, with an increasing slope for greater contributions of the 140 nm spheres and a flatter slope for IL. The greatest dynamic range for both \(\gamma\) and \(\mu'_s\) to changes in particle size scale distribution appear to be towards the shorter wavelengths of light. The known versus recovered values of \(\gamma\) and \(\mu'_s\) for each particle size distribution at each measured wavelength are plotted in figures 4(c) and (d) respectively. Error was calculated as \(\text{Error} = 100 \times \frac{\text{recovered} - \text{known}}{\text{known}}\), and the mean and standard deviation were computed over all phantoms at each wavelengths, yielding \((10 \pm 5.4)\%\) for \(\gamma\) and \((5.77 \pm 6.6)\%\) for \(\mu'_s\). The recovered values of \(\gamma\) and \(\mu'_s\) for variations in IL concentration and BVF are shown in figures 4(e) and (f) respectively. As expected, the recovered \(\gamma\) spectra

Figure 5. In (a), the photograph of CT/Optical co-registration phantom is shown. In (b), the 2D XY plane view of the segmented CT and co-registered optical mesh points is presented, with a zoomed-in view of the center marker in (c). In (d), the same segmented CT and co-registered optical pixels are displayed in 3D space.
Cluster together, independent of both IL concentration and background absorption from BVF. Additionally, the magnitude of the \( \mu' \) values stratify by the various IL concentrations, independent of the BVF. Plots of the corresponding known versus recovered values are presented in figures 4(c) and (d) for \( \gamma \) and \( \mu' \), respectively. The mean error in recovering \( \gamma \) in the presence of various scattering and absorption concentrations was \( \langle 4.8 \pm 3.1 \rangle \% \), while the mean error for \( \mu' \) was \( \langle 12 \pm 7.4 \rangle \% \). These phantom studies experimentally validated the ability of the multi-spectral SLI system to accurately recover and quantify sub-diffusive scatter parameters accurately over a broad range of optical properties, which can be expected for surgical tissues in the visible to NIR wavelength bands.

3.3. Optical and micro-CT co-registration accuracy

Co-registration accuracy between the micro-CT imaging space and optical imaging spaces was evaluated and quantified. A phantom with radio-opaque pegs having an optically bright coating, shown in figure 5(a), was measured with the multimodal system, yielding a micro-CT scan and a co-registered 3D surface mesh of SLI reflectance data. The micro-CT and SLI data were manually thresholded to segment the surface pegs. A top down 2D view of the segmented CT and optical mesh points is presented in figure 5(b). A zoomed in view of the center peg is shown in figure 5(c), where individual locations are shown with an apparent offset of a few points, each being 150 \( \mu \text{m} \) apart. In figure 5(d), the segmented CT and optical mesh points are plotted in 3D, showing the minor error in co-registration resulting in slight misalignment. The co-registration accuracy is quantified for each peg as the Euclidean distance in \( x, y, z \) between the centroids of the CT surface and the optical mesh surface. These results are tabulated in table 2, and shown a mean co-registration accuracy of 620 \( \mu \text{m} \) with little variation across the coordinate space. Furthermore, this implies that in future studies, optical and CT data of breast cancer specimens can be spatially interpreted together within a range 620 \( \mu \text{m} \).

3.4. Visualization of a freshly resected breast tissue specimen

Illustrative multimodal data of a freshly resected breast specimen are shown in figure 6. A photograph of the cut specimen appears in figure 6(a) with tessellated H&E stained histology slides displayed in figure 6(b). Evaluation of the histology from a trained pathologist (W.A.W) revealed a 2.5 cm lesion of invasive and in situ lobular carcinoma with adjacent, benign fibrocystic disease (FCD) surround by background adipose. While the background adipose exhibits a shade of yellow in the photograph, the invasive versus benign areas both appeared in shades of white. The demodulated intensity is shown at \( f_x = [0, 1.37] \text{ mm}^{-1} \) for \( \lambda = 490 \) nm in figures 6(c) and (d) respectively and for \( \lambda = 600 \) nm in figures 6(e) and (f), respectively. For the diffuse images at \( f_x = 0 \text{ mm}^{-1} \), significantly more contrast occurred between the glandular tissue and adipose for \( \lambda = 490 \) nm compared to the longer wavelengths, due to absorption from \( \beta \)-Carotene. However, the superficial high-spatial frequency images at \( f_x = 1.37 \text{ mm}^{-1} \), revealed similar spatial features for both the shorter and longer wavelengths. Contrast was

| Table 2. Optical micro-CT co-registration accuracy. Mean distances between centroids of phantoms for CT points and Image mesh points with varying phantom locations. |
| --- |
| Center phantom (n = 1) | 1 cm from center (n = 4) | 2 cm from center (n = 4) | 3 cm from center (n = 4) | Total (n = 13) |
| \( \Delta C = |C_{CT} - C_{Mesh}| \) (mm) | 0.7 | 0.6 ± 0.2 | 0.6 ± 0.3 | 0.6 ± 0.4 | 0.6 ± 0.3 |

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further quantified over all wavelengths and spatial frequencies. Surfaces of the ratio of the
demodulated intensity between tumor and adipose and between tumor and FCD are shown in
figures 6(h) and (i), respectively, for the areas marked by arrows in figure 6(b). Tumor to adi-
pose had much greater maximum contrast of 5.6 than tumor to FCD, which had a maximum
contrast of 1.6, because of the greater dissimilarity in tissue ultrastructure. For both ratios,
contrast increased with spatial frequency with the maximum contrast occurring at
$f_x = 1.37 \text{ mm}^{-1}$, but at $\lambda = 490 \text{ nm}$ for tumor to adipose and at $\lambda = 700 \text{ nm}$ for tumor to FCD. In fig-
ure 6(j), axial and sagittal slices of the specimen are shown for the 50 kVp micro-CT scan.
A histogram of the reconstructed linear attenuation coefficient values of the breast
tissue specimen is shown in (k), where an apparent bimodal distribution is observed
with separate fat and glandular peaks.

Figure 6. In (a), a photograph of the resected breast specimen is shown, with white
lines indicating where the specimen was cut for histological processing. In (b),
tessellated H&E sections of the specimen appear with a malignant region out-lined in
black. Demodulated images of $f_x = [0, 1.37] \text{ mm}^{-1}$ for $\lambda = [490, 600] \text{ nm}$ are presented
in (c)–(f), with the tissue specimen segmented from the background. For the areas of
tumor, FCD, and adipose marked by arrows in (b), surfaces of ratios in the demodulated
intensity between tumor to fat and tumor to FCD over spatial frequency and wavelength
are shown in (h) and (i), respectively. Axial and sagittal cross sections of the linear
attenuation coefficient are displayed in (j) for a 50 kVp micro-CT scan of the specimen.
A histogram of the reconstructed linear attenuation coefficient values of the breast
tissue specimen is shown in (k), where an apparent bimodal distribution is observed
with separate fat and glandular peaks.

Processed optical maps from the raw SLI reflectance data are presented in figure 7. A color
image reconstructed from the visible diffuse images at $f_x = 0 \text{ mm}^{-1}$ is shown in figure 7(a)
with green, black, and red boxes outlining regions of adipose, lobular carcinoma, and FCD,
respectively. In figure 7(b), another color image reconstructed from visible demodulated data
is displayed, but for a high spatial frequency of $f_x = 1.37 \text{ mm}^{-1}$ highlighting the superficial, finer spatial features. In figure 7(c), a map of the scatter slope of $\mu'_s$ is shown, which represents the spectral change in scattering intensity and is related to particle size distribution of scatters. The mean values of $\mu'_s$ versus wavelength are plotted in figure 7(d) for the three regions, with error bars representing the standard deviation within each region. The elevated scatter slope of FCD ($\langle 1.94 \pm 0.21 \rangle$) compared to that of carcinoma ($\langle 1.33 \pm 0.35 \rangle$) can be seen, as well as the characteristically flat scatter slope and low scatter magnitude of adipose ($\langle 0.53 \pm 0.21 \rangle$). Mean values of $\gamma$ versus wavelength are plotted in figure 7(e), showing decreased values for FCD and carcinoma with little wavelength variation and increased values for adipose with an apparent negative linear relationship with wavelength ($r = -.992$). Spatial features of the optical properties can be observed in the various optical property maps. In figures 7(f) and (g), $\mu'_s$ maps and in figures 7(b) and (i) corresponding $\gamma$ maps are shown for $\lambda = 490 \text{ nm}$ and $600 \text{ nm}$, respectively. For both $\mu'_s$ and $\gamma$, maximum contrast between the tumor and adipose is achieved at $\lambda = 490 \text{ nm}$, but with minimal contrast between tumor and FCD. At $\lambda = 600 \text{ nm}$, contrast between tumor and FCD increased for $\mu'_s$ and $\gamma$, while contrast between tumor and adipose decreased.

Superficial SLI and volumetric micro-CT data were simultaneously rendered in the open-source Slicer environment (Fedorov et al 2012). A screen-shot of the multimodal display appears in figure 8. A false colored surface of $\mu'_s(490 \text{ nm})$ is shown in figure 8(a), exhibiting spatial detail in optical scattering intensity. In figure 8(b), the $\mu'_s$ surface is shown co-registered to a maximum intensity projection of the micro-CT volume, which was processed with the Slicer Volume Rendering module to create a segmented semi-transparent object. Fat was made virtually transparent while the tumor mass and spiculations arms were more opaque. An apparent cluster of micro-calcifications, marked by a yellow star, appear as completely non-transparent, having a markedly high radio-density. The sagittal and coronal slices through the co-registered optical surface and micro-CT volume are displayed in figures 8(c) and (d), respectively. Standard navigation tools in the Slicer environment enabled annotation between the different views and planes. In figure 8(e), the diffuse color image is also shown to guide spatial recognition of features in the multimodal data set to how the specimen appears to the naked eye. In figure 8(f), axial micro-CT slices are shown in $0.9 \text{ mm}$ steps below the imaging surface illustrating the overlapping structural features of the tissue. The micro-calcification cluster is shown to be $1.8 \text{ mm}$ below surface and is marked with a yellow star. A corresponding H&E section taken at $1.8 \text{ mm}$ depth likewise shows a cluster of type-II micro-calcifications in figure 8(g).

4. Discussion

Results from both phantom experiments and clinical tissue demonstrated the ability of the micro-CT/SLI system to quantify scattering maps and co-register them with a micro-CT image volume. Target phantom analysis and specimen imaging with the micro-CT demonstrated SNR > 30, and sub-millimeter resolution. Micro-calcifications were detectable during specimen imaging, and while the presence of calcifications is not considered diagnostically relevant, microscopic pleomorphic calcifications can be related to malignancy status (Tse et al 2008). Resolution tests of SLI also yielded sub-millimeter resolution, and tissue simulating phantoms demonstrated accuracy in the model inversion well beyond diffusion limited transport. While a previous study has accurately quantified optical properties in aqueous phantoms over a range of $\gamma \in [1.3 - 1.7]$ and $\mu'_s/\mu_a$ as low as 5.6 with modest accuracy (McClatchy et al...
this study experimentally recovers $\gamma \in [0.99 - 2.00]$ and $\mu'_s$ as low as 0.8 with double the accuracy in $\mu'_s$ recovery. Imaging of the breast specimen demonstrated co-localization of scattering information with a volumetric micro-CT scan. Imaging irregular specimen surfaces may pose a challenge in the future studies aimed at whole specimen imaging; however, if flattening the tissue surface with the custom acrylic holder does not suffice, a recently published modified Lambertian model has shown to effectively mitigate surface curative artifacts in spatial frequency domain imaging applications (Zhao et al 2016).

The preliminary data suggests SLI and micro-CT are likely to be complementary, as the micro-CT lacks contrast between benign and malignant glandular tissues but provides a 3D reconstruction of the tumor core within a specimen, whereas SLI offers enhanced superficial contrast between more subtle morphological changes. Sensitivity to the surface of tissue is substantiated by a current consensus report which states that ‘no tumor on ink’ is appropriate

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**Figure 7.** A summary of reconstructed SLI data of the segmented tissue specimen is shown. Color images, reconstructed from multi-spectral SLI data at $f_x = [0, 1.37]$ mm$^{-1}$ appear in (a) and (b), respectively. An image of the spectral slope of $\mu'_s$ is presented in (c). Black, red, and green boxes in (a) outline regions of lobular carcinoma, fibrocystic disease, and adipose. The means within each region of $\mu'_s$ and $\gamma$ are plotted as a function of wavelength in (d) and (e), respectively, with error-bars representing the standard deviation within the region. Maps of $\mu'_s$ are shown in (f) and (g) and maps of $\gamma$ appear in (h) and (i) for $\lambda = 490$ nm and 600 nm, respectively.
for early stage invasive breast cancer (Buchholz et al 2014). With this work flow, the micro-CT could guide selection of surfaces to be optically imaged and provide the proximity of suspicious lesions to the tumor mass. Additionally, contextualizing SLI measurements with the subsurface proximity of the tumor-core, as calculated from the micro-CT, could potentially aid in interpretation of the multimodal data.

Future development of this technology will require creation of a robust classification model to determine margin status based on the multimodal data. A previous study has shown optical scattering parameters of freshly resected human breast tissue recovered with subdiffusive structured light are sensitive to changes in tissue morphology (McClatchy et al 2016). However, with model based inversion methods, fitting time is a limitation. While look-up-table approaches could provide a substantial improvement, a direct calculation of optical properties from an empirically fitted function has demonstrated faster inversion times without the need for a look-up routine (Angelo et al 2016). A classification model based on raw multi-spectral reflectance over multiple spatial frequencies may overcome the computational barrier. Previous research has used raw spatial frequency images captured at multiple rotation angles to map the alignment of avian muscle tissue (Konecky et al 2011). This technique could potentially be useful in determining the degree of stromal alignment in breast specimens, which could be related to disease progression (Provenzano et al 2006, Conklin et al 2011). Furthermore, spatial and spectral analysis of raw reflectance images of excised breast

Figure 8. Multimodal display of the resected breast specimen using NIRFAST Slicer. In panel (a), the $\mu_s'$ surface is shown, while in panel (b), the $\mu_s'$ surface is co-registered to the MIP volume of the micro-CT is shown. In panels (c) and (d), the sagittal and coronal planes of the co-registered $\mu_s'$ surface and micro-CT volume are presented. In panel (e), a diffuse reconstructed color image of the specimen in the acrylic holder is shown. In (f), various axial slices of the micro-CT are displayed in 0.9 mm increments below the imaging surface. In the MIP volume and in the micro-CT slice at 1.8 mm below the imaging surface, micro-calcifications appear as a cluster of white specs, which are marked with a yellow star. In (g), an H&E section taken at 1.8 mm depth corroborate the presence of micro-calcifications. A cluster of type-II calcifications can be seen and are marked with yellow stars.
tissue have offered great diagnostic potential in a dark-field confocal microscopy configuration, where scatter contrast was enhanced similarly to high-spatial frequency SLI (Laughney et al 2012, Pardo et al 2017). With future development of a classification model validated with histopathology, the technology described here can be evaluated in the clinical theater.

5. Conclusions

In this manuscript, a novel multimodal imaging system combining superficial SLI and volumetric micro-CT was systematically evaluated in phantoms, and as a proof-of-principle, a freshly excised human breast specimen was scanned. To the authors knowledge, these results appear to be the first demonstration of SLI or SFDI combined with a standard medical tomography system for imaging excised tumor specimens. System performance quantified through the phantom experiments suggests that the contrast, resolution, SNR, and co-registration accuracy needed for surgical guidance can be obtained within a time frame meeting clinical work flow requirements. The preliminary breast specimen imaging further suggests that the system could aid in visualization of a resected specimen. Further studies in a large cohort of resected specimens are needed to determine the optimal spatial, spectral, and tomographic features required for assessment of margin status before the system can be clinically evaluated.

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