A low-cost, portable, and quantitative spectral imaging system for application to biological tissues

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Abstract: The ability of diffuse reflectance spectroscopy to extract quantitative biological composition of tissues has been used to discern tissue types in both pre-clinical and clinical cancer studies. Typically, diffuse reflectance spectroscopy systems are designed for single-point measurements. Clinically, an imaging system would provide valuable spatial information on tissue composition. While it is feasible to build a multiplexed fiber-optic probe based spectral imaging system, these systems suffer from drawbacks with respect to cost and size. To address these we developed a compact and low cost system using a broadband light source with an 8-slot filter wheel for illumination and silicon photodiodes for detection. The spectral imaging system was tested on a set of tissue mimicking liquid phantoms which yielded an optical property extraction accuracy of 6.40 ± 7.78% for the absorption coefficient ($\mu_a$) and 11.37 ± 19.62% for the wavelength-averaged reduced scattering coefficient ($\mu_s^*$).

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References and links


1. Introduction

A common surgical procedure for breast cancer patients is breast conserving surgery (BCS), where the physician attempts to remove the tumor mass with a sufficient margin of normal tissue. A 2-mm margin of normal tissue is an accepted criterion for clear margins [1]. Unfortunately, between 20 and 70% of patients who undergo BCS require additional surgery due to incomplete removal of the disease during the first operation [2–11]. The heterogeneous appearance of breast cancer to the naked eye creates a challenge for surgeons to intra-operatively identify tumor regions to ensure complete removal of the disease [12]. Two common tools used for intra-operative margin assessment are touch-prep or imprint cytology and frozen section [13]. In experienced hands and with proper resources, these tools can reduce the re-excision rate to as low as 20% [6,14,15]. However, many institutions may not have pathologists readily available or the necessary resources to support these specialized techniques [16]. Thus, in most cases, pathologic margin assessment is performed after the surgery is completed. In the event that a positive margin is detected, the patient must be called in for an additional re-excision surgery. There is a critical unmet need for a fast, automated, and reliable intra-operative device that can reduce the re-excision rate of BCS.

Optical techniques provide a unique, non-destructive approach to characterizing the biological composition of tissue. Diffuse reflectance spectroscopy (DRS) is one such technique which, when coupled with appropriate light transport models, is capable of extracting quantitative information about tissue absorption and scattering, both of which reflect underlying tissue composition [17–22]. Zhu et al. characterized the underlying sources of optical contrast in malignant and non-malignant breast tissues that can be exploited for intra-operative margin assessment and her results are very consistent with other similar studies reported in the literature [21,23–25]. She found a statistically significant decrease in beta-carotene concentration and a statistically significant increase in wavelength-averaged reduced scattering coefficient in malignant over non-malignant tissues.

Our group recently developed and clinically tested an optical spectral imaging system based on DRS to quantitatively image ex vivo breast tumor margins for the detection of residual disease. The core instrumentation has been previously documented in detail [17,24,25]. To briefly summarize, the system consists of a Xenon arc lamp, spectrograph, and a 2D CCD camera. Traditionally, a single-channel fiber-optic probe is used for single-point spectroscopy. However, for the margin assessment application, our single-channel probe was replaced with an imaging probe comprised of 8 independent channels, each containing its own set of illumination and collection fibers. Each measured diffuse reflectance spectra was analyzed using an inverse Monte Carlo model previously developed by our group, to extract beta-carotene concentration, oxy-hemoglobin concentration, de-oxy hemoglobin concentration, and a wavelength-averaged reduced scattering coefficient [26,27]. The performance metrics of the margin assessment device is described in a recent publication by Bydlon et al [28]. The imaging probe and instrument were tested in a 100-patient study to evaluate the clinical utility of this device for detecting positive tumor margins and the results from the first 48 patients are reported in a publication by Wilke et al. [29]. Wilke et al. showed that there were statistically significant differences in beta-carotene concentration, the wavelength-averaged reduced scattering coefficient and hemoglobin concentration with malignancy. The optical contrast observed was consistent with that previously observed by Zhu et al. on breast tissue biopsies [24]. In addition, Wilke et al. used a 2-parameter predictive model based on ratios of beta-carotene:scattering and total hemoglobin:scattering to classify positive and negative tumor margins with sensitivity and specificity of 80% and 67%, respectively.
respectively. The sensitivity of this optical spectral imaging device is on par with that of frozen section pathology [29]. While our clinical studies have demonstrated feasibility of optical spectral imaging for the detection of positive tumor margins, the system’s large physical footprint (2 m x 1.5 m x 1 m) and significant cost (~$55,000) potentially limit its widespread clinical utility. In addition, the number of channels in the clinical system is limited by the number of collection fibers that can be imaged by the CCD (while minimizing cross-talk between adjacent channels) and thus multiple placements (and increased time) are required to fully survey breast tumor margins, which can be as large as 10-20 cm².

The goal of this paper is to demonstrate the feasibility of designing a low cost, compact optical spectral imaging system for quantitative imaging of tissue optical properties in the visible spectral range that directly addresses the above limitations of our previously developed clinical system. To our knowledge, there is only one group has previously designed a quantitative diffuse reflectance spectral imaging system in the UV-visible wavelength range. This system raster scans the image of a fiber-optic probe (connected to a single channel spectrometer) onto the tissue plane to acquire quantitative images of absorption and scattering contrast [30]. This serial scanning device is considerably slower than a device that images all pixels simultaneously as we propose. The core components of our technology are a silicon photodiode with an aperture in the middle for illumination. Each photodiode serves as a single pixel of the spectral imaging system and can be scaled for different sizes and pixel densities by simply scaling the size and number of photodiodes, respectively. Previously, we demonstrated that a silicon photodiode with a centrally placed optical fiber coupled to a broadband source provides excellent signal-to-noise ratio (SNR) for quantitative measurements of tissue absorption and scattering from diffuse reflectance measurements of tissue mimicking liquid phantoms [31,32]. The goal of this paper is to extend this proof-of-concept device to a multi-pixel quantitative optical spectral imaging system with at minimum, similar performance characteristics to the current clinical spectral imaging system used in the 100-patient clinical study. Specifically, we present the design and construction of this new quantitative spectral imaging system which incorporates a photodiode imaging array and illumination fibers coupled to the center aperture of each photodiode at one end and to a filtered broad band source at the other end, and we present the performance metrics of the system for extraction of quantitative optical properties from tissue-mimicking liquid phantoms.

2. Methods

2.1 System design

Briefly, the current clinical system consists of a Xenon arc lamp, fiber-optic imaging probe, grating-based spectrograph, and cooled 2D CCD as shown in Fig. 1(A). The broadband Xenon source is used to launch light into an illumination fiber bundle. The broadband light exits the illumination fibers from 8 independent channels placed on the tissue surface. Each of the 8 channels consists of 19, 200 µm diameter illumination fibers (NA = 0.22) and 4, 200 µm diameter collection fibers (NA = 0.22). The illumination light is diffusely reflected within the tissue and collected at the probe tip by each of four collection fibers. These collection fibers deliver the collected diffusely reflected light to a spectrograph which separates the broadband light into its wavelength components whose intensity is measured using a 2D CCD (horizontal axis resolves wavelength and vertical axis separates the 8 channels of the imaging probe). A recent publication by our group contains specific details of this clinical system as well as its performance metrics [28]. The current clinical system provided a basic blueprint which heavily influenced the design of the compact optical spectral imaging system.

Figure 1(B) and 1(C) show a schematic of the proof-of-concept compact optical spectral imaging system and the tip of the spectral imaging system that comes in contact with the tissue. Briefly, the design changes implemented in the compact optical spectral imaging system [Fig. 1(B)] involved replacing the spectrograph in the clinical system with a simple 8-
slot filter wheel on the illumination end. Instead of a 2D CCD, silicon photodiodes were used for detection by multiplexing the previous single-pixel design reported in the publication by Lo et al. into a 9-pixel 3x3 matrix [31]. The light source for the compact optical spectral imaging system is a broadband 350-Watt Xenon arc lamp (MAX-302, Asahi Spectra). Light from the Xenon arc lamp was immediately passed through one of eight bandpass filters selected using an 8-slot filter wheel. The resulting monochromatic light was launched into a bundle of 9 optical fibers, each 0.6 mm in diameter (FVP600660710, Polymicro), which deliver the light to the tissue through the centered aperture of each of the nine 5.8 x 5.8 mm silicon photodiodes (S1227-66BR, Hamamatsu) at the distal end of the imaging probe [Fig. 1(C)].

Simultaneous measurements of the diffusely reflected light from all 9 fiber-photodiode pairs can be collected by placing the probe on the surface of the sample. The photocurrent generated by each of the 9 photodiodes was read using a multi-channel transimpedance amplifier (Multiboard, SolGel Technologies GmbH) so that the signal from each photodiode could be read simultaneously. The transimpedance amplifier circuitry was assembled within a small metal housing and powered using a commercial ± 12V power supply. The output voltage (photocurrent converted to voltage via transimpedance amplifier) was read and transmitted to a laptop computer using a USB controlled data acquisition card (NI USB-6210, National Instruments).

![Diagram](image-url)

Fig. 1. Overview of the two spectral imaging systems. (A) A system schematic of the current clinical spectral imaging system. This figure contains a block diagram of the system as well as a detailed diagram of the probe tip. (B) A system schematic of the compact optical spectral imaging system which details the illumination and collection setup. (C) Photograph of the tip of the 3x3 photodiode array. The numbers represent the pixel numbers, which will be referred to throughout the manuscript.
Spectral measurements were accomplished by cycling through each of the eight bandpass filters and recording individual measurements at each wavelength rather than recording a full spectrum as was done with the clinical system. In the end, the collected data comprised of an 8 wavelength spectral measurement from each of the nine pixels. The impact of measuring with a reduced number of wavelengths on the extraction of optical properties was previously addressed and investigated by Lo et al [31]. It was demonstrated, through phantom experiments and simulations, that using as few as 5 to 8 discrete wavelengths with a FWHM bandwidth of 20 nm was adequate for extracting absorption and scattering properties of phantoms containing polystyrene spheres and hemoglobin. The same methodology was used to identify eight specific wavelengths between 400 and 600 nm (specifically, 400, 420, 440, 470, 500, 530, 570, and 600 nm) each with a band pass FWHM bandwidth of 10 nm. Filters with these specifications (XBPA, Asahi Spectra) were inserted into the 8-slot filter wheel.

In order to facilitate a seamless interface between all the system components (Xenon light source, filter wheel, multi-channel transimpedance amplifier, and DAQ card), a custom LabView GUI was written and executed on the laptop computer. The program was responsible for synchronizing and controlling all system components and storing all collected spectra, analyzed later for extraction of optical properties.

2.2 Theoretical system characterization

2.2.1 Sensing depth simulations

Monte Carlo simulations were carried out to evaluate the system sensing depth and to compare it to that of the clinical system. The sensing depth is a parameter heavily dependent on the probe geometry and optical properties of the sample. To estimate the sensing depth, a full Monte Carlo simulation was performed for the illumination-collection geometry of a single pixel of the compact spectral imaging system and a similar approach was used to obtain the sensing depth of a single channel of the current clinical system. Investigating the sensing depth of a single pixel provides a valid estimate for all 9 pixels of the probe since they all have an identical illumination and collection geometry. In the Monte Carlo simulation, the path of each collected photon was individually tracked, and the deepest point reached in media of each photon was recorded. Initial photon positions were determined by a spatially random uniform distribution over a circular region with a diameter equal to the illumination fiber diameter (600 µm). Photons were propagated through the media characterized by a given set of optical properties. Photons were successfully detected if they escaped the surface of the media within a square region defined by the dimensions of the photodiode (5.8 x 5.8 mm, excluding the circular region occupied by the illumination fiber) and also within the collecting numerical aperture (NA = 0.96) of the photodiode. In Monte Carlo simulations, each photon was assigned a weight of 1 from the point of launch into the tissue. The weight was successively decreased after each scattering event, and the final exit weight was recorded for each successfully collected photon. In addition, the deepest axial position which the photon traveled to was also recorded. The total collected weight of all collected photons was calculated by summing the weights of all collected. Finally, the sensing depth was calculated by finding the exact depth at which 90% of the total collected weight was attained. The sensing depth was heavily dependent on the optical properties chosen for the Monte Carlo simulations. These optical properties were chosen based on clinically measured optical properties of ex vivo breast lumpectomy specimens. In our clinical measurements, the following three different tissue types were encountered (verified by pathology): malignant, normal adipose, and normal fibro-glandular whose median optical properties are documented in Table 1. The reported sensing depth was simulated for all three tissue types at two wavelengths, 450 nm and 600 nm. These two wavelengths were chosen to estimate shallow and deep sensing depths (450 nm was the shortest wavelength used in the clinical system and 600 nm was the longest). Although 450 nm was not actually used in the compact optical spectral imaging system, the purpose of these simulations was to enable a direct comparison.
between the two systems, the only difference being the probe geometry. Therefore, a total of 6 sensing depths for each instrument were reported (2 wavelengths x 3 tissue types).

Table 1. The median (over all measured samples) optical properties of three different tissue types encountered in our clinical studies. The number of samples measured of each tissue type are also shown. These optical properties are reported specifically for the shortest (450 nm) and longest (600 nm) wavelengths acquired in the clinical data.

<table>
<thead>
<tr>
<th>Optical Properties</th>
<th>Malignant (n = 10)</th>
<th>Adipose (n = 323)</th>
<th>Fibro-Glandular (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption Coefficient (\mu_a) (cm(^{-1}))</td>
<td>450 nm</td>
<td>20.34</td>
<td>11.29</td>
</tr>
<tr>
<td></td>
<td>600 nm</td>
<td>1.42</td>
<td>0.55</td>
</tr>
<tr>
<td>Reduced Scattering Coefficient (\mu_s') (cm(^{-1}))</td>
<td>450 nm</td>
<td>9.55</td>
<td>7.44</td>
</tr>
<tr>
<td></td>
<td>600 nm</td>
<td>8.47</td>
<td>6.45</td>
</tr>
</tbody>
</table>

2.2.2 Cross-Talk simulations

Another important parameter assessed using Monte Carlo simulations was the cross-talk between adjacent pixels. For optical property extractions, each pixel was treated as an individual illumination and collection pair. Any diffusely scattered light collected which originated from an adjacent pixel’s illumination fiber was considered cross-talk. Monte Carlo simulations were used to quantitatively assess the amount of cross-talk a pixel would receive from an adjacent pixel’s illumination fiber. To simulate the cross talk, the collection area was defined by the size of the active area for a given pixel (5.8 x 5.8 mm square). For the illumination area, in addition to launching photons from the location of the pixel’s central illumination fiber, they were launched from the location of adjacent pixels’ illumination fiber (assuming 8 mm center-to-center pixel spacing). With this illumination-collection geometry, and given a set of optical properties, we simulated the amount of cross-talk due to photons arriving from adjacent pixels. Cross-talk was simulated for the central pixel (#5) because this represented the worst case scenario as it is surrounded by 8 adjacent pixels. Again, the simulations were performed using the optical properties of the three tissue types encountered in our clinical studies (Table 1) but this time only at 600 nm. This was the only wavelength chosen for this simulation since absorption is at its lowest, which is considered the worst case scenario for cross-talk. Cross-talk was calculated in a similar manner for the clinical system for the purposes of comparison. The illumination area, collection area, and center-to-center pixel spacing were set based on the probe specifications of the clinical system. However, since the 8 channels of the clinical system are arranged in a 2x4 configuration, the pixel experiencing the worst case scenario for cross-talk only had 5 adjacent pixels. The final value of cross-talk was reported as a percentage by dividing the number of cross-talk photons by the number of signal photons.

2.3 Experimental system characterization

2.3.1 Signal-to-Noise ratio (SNR)

Experimental measurements were required to fully characterize the system. The signal-to-noise ratio (SNR) was an important metric to quantify the precision of the experimental measurements. In order to measure the SNR, a liquid phantom was constructed with known optical properties (wavelength-averaged \(\mu_a = 7.00\) cm\(^{-1}\) and \(\mu_s' = 14.84\) cm\(^{-1}\)). The imaging probe was placed at the surface of the phantom and 15 repeated measurements were collected. The SNR was calculated by taking the mean and dividing by the standard deviation of these measurements. This SNR measurement from the compact spectral imaging system was compared to the previously measured SNR by Lo et al. [31]. The SNR reported in that study was measured on a benchtop diffuse reflectance spectroscopy system with instrumentation identical to the clinical spectral imaging system. The SNR measurement with that system on a
liquid phantom with similar optical properties (wavelength-averaged $\mu_a = 7.50 \text{ cm}^{-1}$ and $\mu_s' = 16.00 \text{ cm}^{-1}$) provided a sufficient benchmark for comparison for the compact spectral imaging system.

2.3.2 System drift

The overall drift of the system was also measured in order to determine any change in system measurement over a period of time. The probe was placed on a Spectralon 99% reflectance standard (SRS-99-010, Labsphere Inc) and diffuse reflectance measurements were recorded every 5 minutes over a span of 40 minutes. The entire system (including the probe) was not adjusted or modified during the course of the experiment so changes in measurements strictly represented drift caused by the system. The drift was quantified by dividing the range by the mean of the measurements acquired over this time window. This ratio yielded a percentage that represented the drift of each pixel of the system over the 40-minute period. The drift measurements were taken with both the compact optical spectral imaging system and the clinical system to compare the two systems.

2.4 Tissue mimicking phantom study

To test the optical property extraction accuracy and robustness of the compact optical spectral imaging system, a tissue mimicking phantom study was designed. The liquid phantoms consisted of hemoglobin (H0267, Sigma Co.) as the absorber and polystyrene spheres (07310-15, Polysciences, Inc.) as the scatterer. Hemoglobin was chosen due to its distinct absorption spectral features and biological significance in tissue measurements. The exact wavelength-dependent absorption coefficients ($\mu_a$) of hemoglobin were determined using a spectrophotometer (Cary 300, Varian). Polystyrene spheres were used due to their well-defined size and density, meaning the reduced scattering coefficient ($\mu_s'$) could be accurately estimated using Mie Theory. Prahl’s Mie scattering program was used to perform this task, using the manufacturer’s specified sphere diameter (1.025 µm), density (2.62%), and refractive index (1.6) [33]. With knowledge of the optical properties of the constituents, the liquid phantoms could be constructed with the desired optical properties by varying the absorber and scatterer concentrations.

Table 2 documents the expected optical properties of the 14 phantoms made for this phantom study, constructed using hemoglobin and polystyrene spheres. The optical property range ($\mu_a$ and $\mu_s'$) was chosen to match those of the previous phantom studies used to characterize the clinical system to enable a direct comparison between the accuracy of the two systems [31,34].

<table>
<thead>
<tr>
<th>Phantom #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_a$ (cm$^{-1}$)</td>
<td>0.50</td>
<td>1.00</td>
<td>1.50</td>
<td>2.00</td>
<td>2.50</td>
<td>3.00</td>
<td>3.50</td>
</tr>
<tr>
<td>$\mu_s'$ (cm$^{-1}$)</td>
<td>20.53</td>
<td>20.09</td>
<td>19.66</td>
<td>19.22</td>
<td>18.78</td>
<td>18.34</td>
<td>17.91</td>
</tr>
<tr>
<td>[Hb] (µM)</td>
<td>2.28</td>
<td>4.57</td>
<td>6.85</td>
<td>9.14</td>
<td>11.42</td>
<td>13.71</td>
<td>15.99</td>
</tr>
<tr>
<td>Phantom #</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>$\mu_a$ (cm$^{-1}$)</td>
<td>4.00</td>
<td>4.50</td>
<td>5.00</td>
<td>5.50</td>
<td>6.00</td>
<td>6.50</td>
<td>7.00</td>
</tr>
<tr>
<td>$\mu_s'$ (cm$^{-1}$)</td>
<td>17.47</td>
<td>17.03</td>
<td>16.59</td>
<td>16.15</td>
<td>15.72</td>
<td>15.28</td>
<td>14.84</td>
</tr>
<tr>
<td>[Hb] (µM)</td>
<td>18.28</td>
<td>20.56</td>
<td>22.84</td>
<td>25.13</td>
<td>27.41</td>
<td>29.70</td>
<td>31.98</td>
</tr>
</tbody>
</table>

To measure the diffuse reflectance of each of these phantoms, the probe tip was placed flush in contact with the surface of the liquid phantom. The liquid phantom was created in a container with dimensions of $11 \times 7 \times 1.6 \text{ cm}$ (L x W x D). Its length and width were large enough to accommodate the entire face of the distal end of the probe. The depth of the
phantom was sufficiently large enough to simulate a semi-infinite media (roughly 8 times the simulated sensing depth). The liquid phantom was continuously stirred using a magnetic stir bar over the course of the phantom study to ensure homogeneity. Light at each wavelength was successively launched into the illumination fibers and the diffuse reflected light was measured with all nine photodiodes. A separate calibration measurement was taken, after all the phantom measurements, on a Spectralon 99% reflectance standard (SRS-99-010, Labsphere Inc.) with the same measurement procedure. The collected spectrum of liquid phantom was divided by that of the reflectance standard to obtain the calibrated diffuse reflectance spectrum, correcting for wavelength-dependent instrument throughput and the spectral shape of the source.

2.5 Monte Carlo inverse model of diffuse reflectance

The collected diffuse reflectance spectrum was processed using a fast scalable inverse Monte Carlo model previously developed by our group [26,27]. Our model can quickly generate optical properties ($\mu_a$ and $\mu_s'$) for a given wavelength-dependent diffuse reflectance and specific probe geometry [35]. Previous studies demonstrating the extraction accuracy and robustness of the model have been reported [24–26,34].

The forward Monte Carlo model assumes the measured diffuse reflectance spectrum is dependent on the two optical properties, absorption ($\mu_a$) and reduced scattering coefficients ($\mu_s'$). The absorption coefficient is a function of the wavelength-dependent molar extinction coefficient of the absorber and its concentration. The reduced scattering coefficient is described using Mie Theory and is dependent on the size and density of the scatterer. In the inverse Monte Carlo model, an initial guess of the absorber concentration, scatterer size and density is input into the forward model, producing a modeled diffuse reflectance spectrum. These guesses for the optical properties are iteratively updated until the residual sum of squared errors between the experimentally measured and Monte Carlo modeled diffuse reflectance is minimized. The final optical properties that generate the modeled spectrum which most closely matches the measured spectrum are designated as the extracted values.

3. Results and discussion

3.1 System performance

The compact spectral imaging system was assembled and constructed according to the desired specifications listed in the previous section. With the exception of pixel 8, all pixels appeared to be functional once construction was completed. Unfortunately, the central illumination fiber for pixel 8 was broken during assembly. The individual illumination fiber was already permanently fixed in the center of the detector at the distal end and bundled with the remaining illumination fibers at the proximal end. While polishing the proximal end of the illumination bundle, the illumination fiber separated in the middle resulting in no optical output from pixel 8. Replacing the individual illumination fiber at this stage was not possible, so data was not measured or presented for pixel 8. The results of our simulation and experimental system analysis for the remaining pixels of the compact optical spectral imaging system are presented below.

Figure 2(A) shows a photograph of the compact optical spectral imaging system alongside the clinical system in Fig. 2(B). Scale bars are displayed to show the reduction in footprint between the new and original systems. Both the size and cost of the compact optical spectral imaging system were significantly reduced compared to the clinical system. More importantly, the compact optical spectral imaging system can be more easily expanded to a larger and denser imaging array with mature semiconductor technology. The key physical parameters of the compact optical spectral imaging system were compared to those of the clinical spectral imaging system, as shown in Table 3.
Fig. 2. System photographs to compare physical size (A) Photograph of the modified 3x3 compact optical spectral imaging system compared to the (B) Photograph of the clinical system. The same laptop is pictured in both system photographs in order to compare system scale.

The results of the sensing depth simulations are shown in Table 4. The reported value (in mm) is a range since sensing depth was calculated at the clinical system’s shortest wavelength (450 nm) and the longest wavelength (600 nm). The comparison between the clinical system and compact optical spectral imaging system show that the sensing depths are very similar. In addition, the probe of the compact optical spectral imaging system appears to surpass the minimum criterion of 2 mm for a clear margin.

Table 3. Side-by-side comparison of key physical system parameters between the clinical system and compact optical spectral imaging system. A noteworthy comparison is the large reduction of footprint in the compact system.

<table>
<thead>
<tr>
<th>Spectral Imaging System</th>
<th>Probe Geometry (One Channel)</th>
<th>Number of Channels</th>
<th>Center-to-Center Distance Between Channels (mm)</th>
<th>Footprint of Entire System (L x W x H) (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical System</td>
<td>19, 0.2 mm diameter fibers</td>
<td>4</td>
<td>10</td>
<td>2 x 1.5 x 1</td>
</tr>
<tr>
<td>Compact System</td>
<td>0.6 mm diameter fiber</td>
<td>9</td>
<td>8</td>
<td>0.35 x 0.3 x 0.2</td>
</tr>
</tbody>
</table>

The cross-talk simulations were also carried out according to the methods described earlier. The results of these simulations are shown in Table 4 as well and as can be seen, the cross-talk in all three tissue types is significantly smaller in the clinical system than the compact spectral imaging system. A higher cross talk is certainly expected for the compact system, considering the collection area of each pixel in the compact spectral imaging system (5.8 x 5.8 mm = 33.6 mm²) is much larger than each pixel in the clinical system (4 x 0.2 mm diameter fibers = 0.125 mm²) and more importantly, there were 8 adjacent pixels in the compact spectral imaging system as opposed to 5 in the clinical system (due to differences in system configuration). In addition, the center-to-center pixel spacing is closer in the compact spectral imaging system (8 mm) than the clinical system (10 mm) which would also lead to increased cross-talk. However, the results of the tissue mimicking phantom study (presented later in this section) demonstrate that this level of cross-talk between pixels did not significantly impact the ability to quantitatively extract optical properties.
Table 4. Comparison of the simulated sensing depth and cross-talk between the clinical system and the compact optical spectral imaging system. The sensing depth was simulated at two wavelengths, 450 nm and 600 nm, while cross-talk was only simulated at 600 nm.

<table>
<thead>
<tr>
<th>System</th>
<th>Tissue type</th>
<th>Sensing depth (mm)</th>
<th>Cross-talk encountered by pixel surrounded by most adjacent pixels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>450 nm</td>
<td>600 nm</td>
</tr>
<tr>
<td>Clinical System</td>
<td>Malignant</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>0.7</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Fibro-Glandular</td>
<td>0.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Compact System</td>
<td>Malignant</td>
<td>0.6</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>0.9</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Fibro-Glandular</td>
<td>0.7</td>
<td>1.9</td>
</tr>
</tbody>
</table>

The SNR was measured on a highly absorbing liquid phantom with optical properties of $\mu_a = 7.00 \text{ cm}^{-1}$ and $\mu'_s = 14.84 \text{ cm}^{-1}$. Measurements were taken and the SNR was calculated at each of the eight wavelengths following the procedure described in the methods section. The SNR is shown in Fig. 3(A), along with the optical power (measured at 400 nm) at the tip of each illumination fiber in Fig. 3(B). As previously mentioned, data could not be collected from pixel 8 due to a broken illumination fiber. The minimum SNR was about 40 dB (at 400 nm) and the maximum was approximately 65 dB (at 600 nm). The SNR for each pixel at 400 nm illumination was comparable to the 32 dB SNR (measured at 405 nm) of the clinical system [31]. The discrepancy of the optical power among pixels [Fig. 3(B)] is due to the packaging of the 9 illumination fibers on the proximal end (lamp side). Light from the Xenon lamp did not couple uniformly into all illumination fibers, causing the differences in optical output at the distal end. Although the output power of pixel 9 was noticeable lower, no physical defects in the fiber or photodiode were visible.

Another performance metric which was measured was the long-term drift of the system. The system drift over 40 minutes was approximately $\pm 2\%$ for the compact optical spectral imaging system and $\pm 3\%$ for the clinical system.

![Signal-to-Noise Ratio of all Pixels](image)

Fig. 3. (A) Signal-to-noise ratio (SNR) for all pixels at three different wavelengths (400, 500, 600 nm). The SNR was calculated by taking 15 repeated measurements on a liquid phantom and dividing the mean by the standard deviation. The optical properties of the phantom used in the SNR measurements were $\mu_a = 7.00 \text{ cm}^{-1}$ and $\mu'_s = 14.84 \text{ cm}^{-1}$. (B) The table on the right indicates the optical power output (at 400 nm) from the illumination fiber of each pixel.

3.2 Tissue mimicking phantom study results

The set of 14 liquid phantoms described earlier were constructed and their diffuse reflectance spectra were measured using the compact optical spectral imaging system. The acquisition time for a single wavelength was approximately 0.5 seconds and approximately 1 second to
switch between wavelengths. Data was collected simultaneously from all pixels. Therefore, approximately 12 seconds was required to collect the reflectance spectra from a single phantom. Figure 4(A) shows a comparison between the diffuse reflectance spectra measured with the clinical system and each pixel of the compact optical spectral imaging system. The spectra displayed are from phantom #14 and calibrated to a reflectance standard and reference phantom (#4). Overall, there is reasonable agreement of the measured spectra between the clinical and compact optical spectral imaging systems. However, pixel #7 does show considerable deviation from all other measurements, particularly at the shorter wavelengths (400 and 420 nm).

![Figure 4(A)](image)

**Fig. 4. (A) Normalized (to reflectance value at 600 nm) diffuse reflectance spectrum collected from phantom 14 using the clinical and compact optical spectral imaging systems, corrected with a reflectance standard and reference phantom. The plots demonstrated reasonable agreement between the two systems. (B) Measured and modeled diffuse reflectance spectra from the compact optical spectral imaging system. These spectra are not normalized but are corrected with a reflectance standard.**

Examples of the experimentally measured spectrum and final Monte Carlo modeled spectrum for two representative pixels (#5 and #7) are shown in Fig. 4(B). While the measured and modeled spectra are in agreement for pixel #5, a small deviation exists between 400 and 420 nm in pixel #7. The inability to accurately model the spectra stems from the measurement error already described. We believe that this is attributed to a construction defect present in pixel 7. A close-up photograph of pixel 5 and 7 are shown in Fig. 5, which reveals the defect in the illumination fiber of pixel 7. This defect is not present in pixel 5, or in any other pixels (not pictured), leading us to strongly believe this was the cause of the variation seen in pixel 7. While our robust Monte Carlo model is capable of accounting for different probe geometries, one requirement is that light exiting the illumination area must maintain a relatively uniform exit distribution. However, as the fiber is damaged, the illumination becomes non-uniform, as seen in the Fig. 5, and the uniformity assumption is compromised.
Ultimately, the performance metric which would determine the viability of the system is its ability to quantitatively extract optical properties. Simply measuring the diffuse reflectance would not provide any significant insight into the composition of breast tumor margins. From the final modeled spectra the $\mu_a$ and $\mu_s'$ values were extracted and compared to the known expected optical properties of the liquid phantoms using the previously described inverse Monte Carlo model. One phantom (Phantom #4) was also chosen as a reference phantom to put the measured and modeled diffuse reflectance on the same scale prior to the inversions. The choice of this reference phantom was based on the comprehensive reference phantom characterization reported by Bender et al. [34]. The inverse Monte Carlo model was capable of extracting the optical properties of all pixels in <15 seconds. Figure 6 shows a plot of expected versus extracted values for both wavelength-averaged absorption ($\mu_a$) and reduced scattering ($\mu_s'$) coefficient for all pixels excluding pixel 7 and 8. These pixels were excluded due to their previously mentioned defects and inability to accurate measure and extract optical properties. Each circle on the plot represents the optical properties of one target phantom (14 total phantoms) averaged over all wavelengths and all functioning pixels (#1-6, 9).

The percent error between the extracted optical properties and expected optical properties are also calculated and summarized in Fig. 7 individually for each pixel. A single value for error percentage is reported by averaging over all wavelengths and all target phantoms. Most of the pixels are capable of extracting $\mu_a$ and $\mu_s'$ with high accuracy (<8%). However, once again pixel 7 is a clear outlier and shows very poor extraction accuracy. This result was anticipated given the previous discussion on the physical defect of the pixel. Overall, the optical property extractions experimentally confirmed the appropriateness of the modeling.
assumptions, i.e. treating each pixel as an independent source-detector pair. In addition, we demonstrated the feasibility of the 3x3 photodiode geometry design for acquiring quantitative spectral images.

![Pixel Data]

**Fig. 7.** Summary of the optical property ($\mu_a$ and $\mu_s^*$ averaged over all wavelengths) extraction errors for all pixels. The errors shown here are the average errors over all 14 target phantoms. Phantom #4 ($\mu_a = 2.00 \text{ cm}^{-1}; \mu_s^* = 19.22 \text{ cm}^{-1}$) was used as the reference phantom for the inversions.

A comparison of all performance metrics between the clinical system and compact optical spectral imaging system are summarized in Table 5. The uncertainty reported in the optical property extraction column is the standard deviation of error over all pixels (excluding pixel #8) and all phantoms. It is clear that the performance of compact optical spectral imaging system is on par with the clinical system, notably the comparison between the $\mu_a$ and $\mu_s^*$ extraction errors compared in Table 5.

**Table 5.** Comparison of performance metrics between the clinical and compact optical spectral imaging systems. The SNR of the clinical system was measured in a previous study (at 405 nm using a phantom with optical properties of $\mu_a = 7.5 \text{ cm}^{-1}$ and $\mu_s^* = 16 \text{ cm}^{-1}$).

<table>
<thead>
<tr>
<th>System</th>
<th># wavelengths between 400 and 600 nm</th>
<th>Drift</th>
<th>SNR @ $\lambda = 400$ nm (dB)</th>
<th>Absorption ($\mu_a$) and Scattering ($\mu_s^*$) Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical System</td>
<td>81</td>
<td>± 3%</td>
<td>32</td>
<td>Mean $\mu_a$ Error 9.03%</td>
</tr>
<tr>
<td>Compact System</td>
<td>8</td>
<td>± 2%</td>
<td>40</td>
<td>Mean $\mu_s^*$ Error 6.04% ± 7.78%</td>
</tr>
</tbody>
</table>

**4. Conclusions**

The major advantage of the compact optical spectral imaging system is the significant reductions in both cost and footprint compared to the traditional clinical system. These reductions were accomplished by replacing two specific components in the current clinical system with simpler, cheaper, and more compact components. Rather than using a cooled CCD (20x20x20 cm, ~$20,000), the new system utilized silicon photodiodes and appropriate readout circuitry (20x10x15 cm, ~$1000). The second component targeted was the grating-based spectrograph (50x50x20 cm, ~$12,000) with a filter wheel with 8 slots for band pass filter (20x20x5 cm, ~$900). Replacing these two components resulted in over a 10x size reduction and over a 15x cost reduction.

For the compact optical spectral imaging system design presented here, a Xenon arc lamp was used, the same type used in the current clinical systems. With experimental proof of our 8 wavelength system, most of the broad spectral output of the Xenon lamp is not needed.
Alternatively, multiple low-cost discrete wavelength light sources, such as light-emitting diodes (LEDs), could replace the Xenon arc lamp. LEDs are available in various visible wavelengths, and are considerably cheaper and smaller than an arc lamp. This modification would have the potential of further decreasing the footprint of the system.

In addition to replacing the light source, other light delivery strategies can also be explored. For the probe presented here, optical fibers are still required for delivery of illumination light. However, optical fibers are fragile and cumbersome, important limitations for clinical use. Individually fixing an optical fiber in the center of each photodiode is a cumbersome procedure, particularly when considering a larger pixel array. One possible strategy for light delivery would be to use a robust large aperture fiber bundle which would terminate a short distance behind the photodiode array, rather than breaking into individual fibers for each pixel. Light exiting from the fiber bundle would travel through free space and pass through the holes drilled in the photodiode detectors. If LEDs are used, then the LEDs themselves may be fixed behind the photodiode array, completely eliminating any optical fibers at the tissue interface. These strategies are analogous to shining a flashlight through a mask, although the actual implementation requires further investigation.

One area of interest is to further explore the imaging aspect of our system. While exploring the system’s imaging capabilities, the spatial resolution is a natural parameter of interest. The spatial resolution of our system is equivalent to the pixel spacing, so in this particular probe design, ~8 mm. The pixel spacing defines the relative spatial location of each measurement with respect to each other. When reconstructing an image, each region of optical properties will be spaced according to this distance. In order to improve the spatial resolution, the probe would be redesigned with smaller pixel spacing. However, this would not be possible using the same 5.8 x 5.8 mm photodiodes used in this paper. As the photograph of the probe tip in Fig. 1(C) shows, the photodiodes are already packed as close as physically possible. Increasing the number of pixels or pixel density in the imaging probe could be implemented simply by increasing the number of silicon photodiodes or using smaller photodiodes, respectively. Undoubtedly, this would increase the spatial resolution of the system, but may impact other important system parameters. Moving each pixel closer together may result in increased cross talk. One possible solution would be to use an alternated illumination pattern (i.e. take two separate measurements and light every other pixel, so the effective cross-talk distance is twice the pixel distance). Cross-talk may also be used to our advantage, as with greater cross-talk the measurements become an image reconstruction problem. This problem is similar to the one faced in the heavily researched field of Diffuse Optical Tomography (DOT), where a volume of optical properties is reconstructed from reflectance collected from multiple source and detector pairs. Analyzing reflectance data collected with our compact spectral imaging system using a DOT algorithm is certainly feasible and would address cross-talk issues. Reducing pixel size can also impact sensing depth. Larger sensing depths could be achieved by using a ring shaped detector with an inner radius slightly larger than the illumination radius, resulting in a ring of “dead area” between the illumination and collection area. This would alter the source-detector separation distance and allow deeper penetrating light to be collected.

The compact optical spectral imaging system presented here experimentally demonstrates progress towards developing a clinically viable spectral imaging system for breast tumor margin assessment. Our group has demonstrated that our scalable Monte Carlo model is capable of quantitatively imaging and extracting optical properties, which contain valuable information on tissue composition which is significant in discerning malignant from benign breast tissue. While these measurements can be acquired using our current clinical system, redesigning the system as described in this manuscript could increase the widespread clinical viability of quantitative spectral imaging through increased portability and speed and decreased cost.
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