A compact, cost-effective diffuse reflectance spectroscopic imaging system for quantitative tissue absorption and scattering

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ABSTRACT

There is clinical utility for a wide-field, spectroscopic imaging device for quantitative tissue absorption and scattering in a number of applications. We present the design of a compact, cost-effective spectroscopic imaging system, which consists of a broadband source with bandpass filters and a light guide for illumination and an inexpensive array of silicon photodiodes for detection. A single-pixel version of the system was tested in liquid phantoms simulating a wide range of human breast tissue and optical properties can be extracted with absorption and reduced scattering errors of 12.6% and 4.7%, respectively. We show proof-of-concept for performing fast, wide-field spectroscopic imaging with a simple design. The design also allows for scaling and expansion into higher pixel number and density in future iterations of custom device design, which includes in-house photodiode array fabrication processes and integration of on-board current amplifier circuits.

Keywords: diffuse reflectance spectroscopy, spectroscopic imaging, tissue optics

1. INTRODUCTION

Wide-field, quantitative spectroscopic imaging of tissue absorption and scattering can have tremendous impact in many clinical situations, such as pre-cancer and cancer diagnosis, tumor margin assessment, and observing tumor response to therapy. For example, there are still a number of unmet clinical needs in the complex problem of breast cancer management and treatment. Nearly 200,000 women per year in the US undergo breast conserving surgery to remove a breast tumor, but nearly half of these patients must undergo a re-excision surgery due to a positive margin after the initial surgery. Currently, there is no effective tool for surgeons to assure the complete removal of a tumor during breast conserving surgery.

Diffuse reflectance spectroscopy has been previously explored as a method for providing near real-time visual maps of tissue composition in breast lumpectomy margins. Because optical spectral imaging in the visible spectral range is sensitive to the absorption properties of biological molecules, such as hemoglobin and β-carotene in tissue, as well as to tissue scattering, which can be attributed to cell density and morphology, non-destructive and quantitative characterization of tissue composition can be extracted from the intact margin. In a previous clinical study, Bydlon et al. have shown the capability of using optical spectral imaging for effective intraoperative breast margin assessment. The system used in the previous study consists of a broadband source, an 8-channel fiber-optic probe, an imaging spectrograph, a cooled CCD, and a laptop computer for data acquisition and processing. While this conventional optical spectral imaging system shows great potential for the detection of positive margins, it has drawbacks in size, cost, coverage, and clinical practicality and utility. It is also not easily scalable or customizable to have a higher pixel density for improving spatial resolution as well as to have more pixel numbers to survey a larger region of interest.

In this manuscript, we present a prototype of a compact and cost-effective quantitative optical spectral imaging system specifically for the application to breast tumor margin assessment, but also with the potential for use in other applications for other organ sites in which quantitative tissue spectral imaging would be useful. The new imaging design described here is built upon our previously developed systems that consisted of fiber-based illumination strategies. We explain alternative illumination and detection strategies that are different from those previous systems and validate the new design with liquid phantom studies. The imaging system is smaller, less costly, has higher pixel density and faster scans over a larger surveillance area than the other systems, and has high potential to be clinically translatable for tumor margin assessment.
2. MATERIALS AND METHODS

2.1 System design

The design of the compact spectroscopic imaging system is essentially a modification of various components of our previous benchtop system. As shown in Figure 1, the light source of the compact spectral imaging system is a 350W Xenon arc lamp (MAX-302, Asahi Spectra) with a built-in 8-slot filter wheel, which contains the bandpass filters: 400, 420, 440, 470, 500, 530, 570, and 600 nm, each with a 10-nm FWHM (XBPA, Asahi Spectra). The source differs from our previous clinical system’s larger 400W Xenon arc lamp (JY Horiba), for which all the wavelengths from 350-700 nm were recorded for spectral analysis. The detection component of our compact spectroscopic imaging system is an array of 16 individual silicon photodiodes with a 2.4x2.4 mm active area (S1227-33BR, Hamamatsu), each with a hole of approximately 1 mm in diameter mechanically drilled in the center of the active area, arranged in a 4x4 matrix. The detection component differs from our previous system, which utilized the more sophisticated, thus more expensive, imaging spectrograph and cooled CCD camera.

To illuminate the sample, an optical light guide directs the 8 wavelengths of light into a reflective aluminum probe housing, where the light travels in free space, and out through the holes of the backside of each individual photodiode onto the sample. Using Monte Carlo simulations, we have previously modeled similar probe geometries in fiber-based systems and found that the sensing depth for each pixel is approximately 1.5-2.0 mm, which is important for determining a negative, or clear and cancer-free, margin. A multi-channel transimpedance amplifier (Multiboard, SolGel Technologies GmbH) was used to read photocurrent generated by each photodiode, and the output voltage was transmitted to a computer with a USB-controlled data acquisition card (NI USB-6210, National Instruments).

A summary of the system schematic and this new “back-illumination” strategy through the central apertures of the photodiodes are illustrated in Figure 1. As shown in two previous studies, the rationale for having a illumination aperture be centrally placed is to maximize the collection of scattered light and the SNR. Furthermore, this strategy eliminates the need for optical fibers, which are typically crucial illumination and collection components in diffuse reflectance systems, such as our previous benchtop system; however, the fibers are costly, unwieldy, and can be fragile once multiplexed for use in a clinical setting. The use of inexpensive photodiodes not only cuts the cost associated with sophisticated detection equipment used in diffuse reflectance spectroscopy, but also further reduces the size of the system.

2.2 Validation of a single channel in tissue phantoms

Due to the inherent problem of accurately characterizing the geometries of all 16 dissimilar, mechanically drilled photodiodes, a smaller single-pixel version of the probe was fabricated and used to perform initial tests and to show the
feasibility of the wavelength-reduced, back-illumination strategy for quantitative spectral analysis. We created 15 liquid phantoms, which consists of hemoglobin (H0267, Sigma Co.) and 1-μm polystyrene spheres (07310-15, Polysciences, Inc.), and spans a range of optical properties similar to those of breast tissue ($\mu_a = 1.3 - 10.3$ cm$^{-1}$, $\mu_s' = 8 - 12$ cm$^{-1}$, averaged over 400-600 nm). For each phantom, 5 scans of diffuse reflectance measurements were recorded and averaged at each of the 8 wavelengths. The diffuse reflectance spectrum was corrected for the wavelength-dependent system response and the throughput of the instrument by normalizing it to that of a Spectralon 99% reflectance standard (SRS-99-010, Labsphere, Inc.), which was measured with the probe in contact with the Spectralon standard. A fast, scalable inverse Monte Carlo model previously developed by our group was used to extract the absorption coefficient ($\mu_a$) and reduced scattering coefficients ($\mu_s'$) of these single-absorber liquid phantoms measured by the proof-of-concept single-pixel probe.9,10

In previous studies by Wilke et al. and Brown et al., it was found that the main sources of optical contrast to delineate tissue types in a breast tumor margin observed in the clinical studies were the ratios of hemoglobin and β-carotene to scattering.2,3 Based on these sources of contrast, a second phantom study with two absorbers, mimicking the absorption of hemoglobin and β-carotene, was performed. A set of 20 phantoms was made with hemoglobin and crocin (17304 Fluka, Fluka) as the two absorbers and 1-μm polystyrene spheres as the scatterer. Crocin was used to simulate β-carotene in breast tissue because the two molecules have very similar absorption spectral features. The optical properties of these two-absorber phantoms mimic breast tissue and are identical to the set of phantoms described in a previously published work by Bender et al., which investigated the robustness of the Monte Carlo model for extracting biological absorption and scattering.8 We then used our fast, scalable inverse Monte Carlo model to extract optical properties as well as hemoglobin and crocin concentrations.

2.3 Proof-of-concept spectroscopic imaging of hemoglobin capillary tubes

At press time for this Proceedings manuscript, we were unable to get sufficient throughput from the corner pixels of the 4x4 spectroscopic imaging device in very dark phantoms. Because we were able to show the feasibility of accurate extractions with a single pixel and with a reduction of wavelengths (using only 8 as opposed to the full spectrum in the previously developed benchtop system), we wanted to still illustrate the idea of spectroscopic imaging with a compact system despite the throughput issues while we continue to work to resolve these problems in the future. To show this qualitatively, we filled four glass capillary tubes with hemoglobin solution and arranged them in a Petri dish as shown in Figure 2. The Petri dish was filled with 20% intralipid to achieve scattering properties similar to that of breast tissue, $\mu_s' = 10$ cm$^{-1}$ over 400-600 nm. Diffuse reflectance measurements were made by placing the probe in contact with the liquid, with the hemoglobin capillary tubes directly under some of the photodiodes. Inverse Monte Carlo model of reflectance was used to extract optical properties. Although we have not, at this point in time, been able to successfully extract optical properties with very high accuracies using the 4x4 detector array, we should still able to see some contrast between pixels with hemoglobin tubes and those without in a hemoglobin extraction map.

![Figure 2. Schematic of coarse 4x4 spectroscopic imaging of hemoglobin-filled capillary tubes](image)

3. RESULTS

3.1 Extraction of optical properties in liquid phantoms

The extracted optical properties in the 15 single-absorber phantoms using a single channel of our back-illumination photodiode-based probe are shown in Figure 3. The average percent error for the absorption and reduced scattering coefficients were 9.4±13.1% and 3.9±3.4%, respectively. These are comparable to the 9.1±11.9% and 5.3±4.1% errors
obtained with our previous benchtop system from a previous study that tested the same range of optical properties. This initial simplified phantom study ensured that optical properties can be extracted with high accuracy using the photodiode and back-illumination strategy.

In addition to extracting optical properties with good accuracy in single-absorber phantoms, we were also able to perform well in 2-absorber phantoms, which consisted of hemoglobin and crocin. Using our fast, scalable inverse Monte Carlo model, we were able to extract hemoglobin and crocin concentrations as well as optical properties with good accuracy. The hemoglobin concentration and crocin concentration extraction errors were 12% and 15%, respectively. The absorption and reduced scattering coefficient errors were 12.6% and 4.7%. Figure 4 shows the extracted ratios of the same sources of contrast (Hb:scattering and Cr:scattering) that were significant in differentiating tissue types for breast tumor margin assessment. Note that in these phantoms, β-carotene is replaced with crocin, which has similar spectral features. This preliminary study is significant because we have shown that using the back-illumination, photodiode-based spectroscopy system, we can accurately extract concentrations of more than one species of absorbers. Although the dominant absorber, by far, in breast tissue is hemoglobin, we have found that β-carotene absorption, while smaller in scale, is also clinically significant. By increasing the bandpasses of each wavelength to 10 nm, and reducing the number of wavelengths required to only 8, we are still able to extract these concentrations.

Figure 3. Absorption and reduced scattering coefficient extractions. All units in cm⁻¹.

Figure 4. Extractions of ratios of hemoglobin and crocin to scattering, which are the two significant sources of contrast in delineating malignant to benign tissues in the breast.
3.2 Spectroscopic imaging of capillary tubes filled with hemoglobin solution

In the single-pixel proof-of-concept probe, the wavelength-averaged SNR on a reflectance standard was 74±3 dB because the light guide is directly aligned to the hole of the single-pixel detector, which in turn experiences only modest losses of light in free space. The 16-channel back-illuminated spectral imaging system has a measured SNR of 55±25 dB averaged over all pixels. The higher standard deviation of the 16-channel device comes from the much lower throughput of the corner pixels, which experiences higher losses of light because they are not directly aligned to the light guide. For instance, the corner pixels have only a power output of less than 10 µW at 400 nm. By comparison, the single-pixel probe, which we have used to measure and extract optical properties with high accuracies, had a power of more than 40 µW at 400 nm. Furthermore, because the 4x4 back-illuminated detectors have been mechanically drilled, none of the 16 pixels are identical. The mechanical drilling of the photodiodes prevents us from accurately modeling the probe geometry. While it is possible to optimize and model a single drilled detector, it becomes very cumbersome and impractical when the array expands for spectroscopic imaging. Nonetheless, we can illustrate the idea of spectroscopic imaging with a compact system despite the throughput issues in the corner pixels and some modeling issues while we continue to work to resolve these problems in the future. As shown in Figure 5, there is clearly some contrast between the detectors of row 3 and other rows. Refer back to Figure 2 in the methods section to compare this extraction map and the experimental setup.

![Figure 5. Extractions of hemoglobin concentration of capillary tubes aligned in Intralipid using the compact spectroscopic imaging system.](image)

From Figure 5, we can see that with no hemoglobin tubes directly below the detectors in row 3, the extracted absorption coefficients is low, and thus the extracted hemoglobin concentration is also very close to 0 µM. We also expected row 2 to have a much higher hemoglobin extraction because there were 2 capillary tubes below those pixels, and the extracted map also shows this. The problem, again, lies in the corner pixels, which seemed to give us systematically lower extracted hemoglobin concentrations. We cannot definitively attribute this to SNR even though the signal is indeed lower, and thus impacts the accuracies of the extractions. However, most of the extracted hemoglobin concentrations were actually under-estimated for all pixels because we were not able to fully characterize all pixels by modeling them accurately. Despite these problems, we were able to show some proof-of-concept imaging qualitatively and are still working towards quantitative spectroscopic imaging.

4. CONCLUSIONS

In this Proceedings manuscript, we have presented a strategy for creating a cost-effective, compact optical spectroscopic imaging system for quantifying tissue absorption and scattering. The rationale behind our design is twofold. First, we hypothesized that it is unnecessary to scan across all wavelengths in the broadband visible range to extract physiological parameters. Similar to other systems operating on the principle of frequency-domain photon migration in the near
infrared that uses only a few wavelengths to extract hemoglobin concentrations,\(^1\) we use only 8 wavelengths from a broadband source and filter wheel to do the same. The illumination and detection schemes are also novel and unique compared to previous spectroscopy systems in that the spectograph and CCD are replaced by an inexpensive array of silicon photodiodes. By placing the detector directly at the tissue surface, we have also improved the collection efficiency, thus reducing the cost associated with expensive and sophisticated CCD cameras. The back-illumination strategy with light guided through holes of each photodiode obviates the need for unwieldy optical fiber bundles. Through phantom studies using a single-pixel version of the probe, we have shown that this new system is capable of accurately extracting the optical properties of breast tissue. We have also shown qualitatively that optical contrast can be seen using a two-dimensional 4x4 array of photodiodes. The main challenges with our current proof-of-concept compact spectroscopic imaging system are (1) the inability to accurately model the probe geometry due to the mechanically drilled apertures (with irregular edges and center positions) in the thick detectors, and (2) the relatively low throughput in the corner pixels caused by the thick absorptive apertures and non-reflective backing plate. Though at press time of this manuscript we have not performed quantitative spectroscopic imaging, we have recently acquired the access and ability to fabricate thin-film photodiodes in-house. This is significant in that it obviates the need to mechanically drill a commercial detector and allows us to very accurately model the geometry for our next probe. Through preliminary simulations, we have also found that our custom thin-film detectors with a central aperture will have 5-7 times more throughput out of the corner pixels compared to the commercial bulk detectors in our proof-of-concept system. While we continue to further improve throughput and extraction accuracy, we will have not only the capability of making quantitative measurements over a large surveillance area on tissue, but also have a clinically translatable device (easy to use, cost-effective, and approximately 100X more compact), which can fulfill unmet needs in breast cancer treatment, among other applications.

REFERENCES