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Diffuse reflectance spectroscopy with a self-calibrating fiber optic probe

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Abstract

Calibration of the diffuse reflectance spectrum for instrument response and time-dependent fluctuation as well as interdevice variations is complicated, time consuming, and potentially inaccurate. We describe a novel fiber optic probe with a real-time self-calibration capability that can be used for tissue optical spectroscopy. The probe was tested in a number of liquid phantoms over a relevant range of tissue optical properties. Absorption and scattering coefficients are extracted with an average absolute error and standard deviation of $6.9\% \pm 7.2\%$ and $3.5\% \pm 1.5\%$, respectively.

UV-visible (UV-VIS) diffuse reflectance spectroscopy (DRS) is sensitive to the absorption and scattering properties of biological molecules in tissue and thus can be used as a noninvasive *in vivo* tool to obtain quantitative information about the physiological and morphological properties of human tissue. Potential clinical applications of UV-VIS DRS include precancer detection and cancer diagnostics [1–3], intraoperative tumor margin assessment [4], and monitoring of tumor response to chemotherapy [4], to name just a few examples. Fiber optic probes are commonly used to deliver the illumination light to and collect the diffusely reflected light from the tissue for DRS measurements [5]. For DRS to be used routinely in the clinic, calibration is required to compensate for lamp intensity fluctuations, wavelength-dependent instrument response, interdevice variations, and fiber bending losses during the measurement.

Current calibration techniques typically rely on measurements using power meters, reflectance standards, and/or tissue phantoms, typically after the clinical measurements are completed. In an *in vivo* study of human adenomatous colon polyps, Zonios *et al.* developed a calibration method in which the tissue spectra were divided by the spectrum of a reference phantom made up of a 20% BaSO₄ powder suspension [1]. Utzinger *et al.* [2] and Mirabal *et al.* [3] calibrated the reflectance spectra measured from normal and neoplastic ovarian tissues and cervical tissues by the reflectance spectrum of a solution of polystyrene microspheres. Thueler *et al.* developed a two-step calibration procedure using a spectrally flat reflectance standard and a solid turbid siloxane phantom of known optical properties to obtain absolute reflectance spectra of stomach tissues [6]. Our group has also developed a calibration strategy for UV-VIS DRS [7]. For each instrument and probe combination a spectrum is measured from a reference phantom of known optical properties followed by a measurement from a 99% reflective Spectralon puck (SRS-99-010, Labsphere, Inc.), and this is referred to as a calibrated reference phantom spectrum. Immediately after the tissue spectra are measured a spectrum is collected from the same puck. Calibration is performed by dividing the tissue spectra point by point by that of the puck. Next, the ratio of the calibrated tissue spectra and the calibrated reference spectrum is input into an inverse Monte Carlo model to extract the tissue optical properties [7]. The calibration of the tissue spectrum against a reference phantom is needed to put the experimental and Monte Carlo simulated data on the same scale, while the calibration of the

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tissue and reference phantom spectra to the puck is carried out to account for day-to-day system variations between the time of the tissue measurement and the time of the reference phantom measurement.

There are a number of limitations associated with such calibration methods. First, they cannot correct for real-time system fluctuations, such as variations in the lamp intensity, and thus require at least 30 min for lamp warm up, which is a significant problem in a clinical setting such as the operating room. Second, they can require an additional 10–20 min before or after the clinical measurement for calibration. It is therefore desirable to create a fast, robust, and systematic calibration approach that can be used for correcting tissue optical spectra obtained at different times and with different instruments and probes. In this Letter we present a novel fiber optic probe with self-calibration capability for performing UV-VIS DRS. The probe has a built-in calibration channel that can be used to record the lamp spectrum and instrument–fiber responses concurrently with tissue measurements. We also demonstrate that combined with a one-time single-reference phantom measurement the self-calibrating probe can provide instrument-independent optical properties.

The experimental setup employs a UV-VIS spectrometer and a fiber optic probe (Fig. 1). The spectrometer consists of a 450 W xenon lamp, an imaging spectrograph with a slit width of 0.6 mm, and a CCD camera. The illumination arm of the probe consists of two 600 μm fibers (A), one for illuminating the sample (the largest fiber in B) and the other for internal calibration. Eight 200 μm fibers collect the diffusely reflected light from the sample, and two 200 μm fibers collect the illumination light reflected by the reflective rod (D) (ten fibers in C) and transmit it to the same imaging spectrograph via a reflective optics coupler with a 1:1 magnification. The center-to-center distances between the sensing illumination fiber and the eight collection fibers range between 0.7 and 1.2 mm. The calibration illumination and collection fibers are terminated inside the rigid part of the probe tip (D). All the fibers are made of the same materials and have the same NAs for an identical bending response. The collected diffuse reflectance and calibration beams are diffracted and projected onto different areas of the CCD camera and recorded by the computer. The CCD chip has 1024 (x axis) $\times$ 256 (y axis) pixels with a pixel size of $26 \times 26 \mu\text{m}^2$. Incident light is wavelength diffracted along the x axis. Pixels 70–95 along the y axis are binned for the calibration channel, while pixels 105–150 are binned for the sensing channel. There is the equivalent of one collection fiber spacing between the self-calibration and sensing areas on the CCD with no measurable cross talk.

To evaluate the self-calibration capability of the probe neutral density (ND) filters of various attenuations (OD=0.6, 0.3, 0.1, and 0.03) were inserted into the light path between the lamp and the illumination adaptor to simulate variations in lamp intensity, which is the major source of drifts during warm up. The tip of the probe was placed in front of an aluminum mirror at a fixed distance and angle and the instrument configurations were unchanged throughout the experiment. A reflectance spectrum from 350–600 nm was measured from the mirror as well as from the calibration channel concurrently for each ND filter. An additional spectrum was also obtained without an ND filter at the beginning and end of the experiment. Figure 2(a) shows the raw spectra measured from the mirror for no attenuation and different levels of attenuation. There is a small but distinct difference between the two scans without the ND filter, which may be attributed to lamp drift during the experiment. Figure 2(b) shows the ratio of the reflectance spectrum of the mirror and the self-calibration channel for each level of attenuation. As can be seen, the variation in the illumination intensity caused by the ND filters is minimized to less than $\pm 3\%$. This small variation is likely due to spatial variations in the light illumination intensity introduced by the insertion of the ND filter.

Liquid phantoms were used to evaluate the performance of the probe for measuring tissue optical properties. The phantoms contained variable concentrations of hemoglobin (Hb)

(H0267, Sigma Co.) as the absorber and 1 mm polystyrene spheres (07310-15, Polysciences, Inc.) as the scatterer [7]. The phantoms were obtained through 17 successive titrations of the Hb from 1.01–31.91 μM . The number of scatterers was fixed, but the scattering coefficient decreased slightly with successive dilution owing to the titration of Hb. This produced a total of 17 phantoms with an overall absorption coefficient (μ_a) range of 0–37.2 cm^{-1} and a reduced scattering coefficients (μ_s) range of 11.2–22.3 cm^{-1} over the wavelength range of 350–600 nm. A reflectance spectrum from 350–600 nm was measured with an integration time of 25 ms from each of the 17 phantoms. A second spectrum was also measured from phantom 8 with an integration time of 20 ms to simulate a reference measurement from a different day as would be the case in clinical applications. The whole phantom study took about an hour. Immediately after all phantom measurements two calibration spectra were obtained from a 20% Spectralon puck (SRS-20, Labsphere, Inc.), with an integration time of 25 ms (referred to as the target Spectralon) and 20 ms (referred to as the reference Spectralon), respectively.

A Monte Carlo inverse model was employed to extract the μ_a and μ_s of the liquid phantoms from the reflectance spectra, assuming that the absorbers and their extinction coefficients are known *a priori* [7]. In the first inversion the reference phantom 8 spectrum divided by the reference Spectralon spectrum was used as a reference, and phantoms 1–17 spectra divided by the target Spectralon spectrum were used as targets. In a second inversion the Spectralon spectrum was replaced by the self-calibration spectrum simultaneously recorded with each phantom spectrum. Figure 3 shows the Spectralon-calibrated and self-calibrated reflectance spectra of phantoms 1, 7, and 17 with wavelength averaged (μ_a, μ_s) of (0.24, 17.98), (1.73, 16.99), and (7.50, 13.12) cm^{-1} , respectively. The three intensity valleys at 415, 540, and 575 nm in all spectra are the Soret band, α band, and β band (569 nm) of oxygenated hemoglobin, respectively. The absorption characteristics of the α and β bands in phantom 1 are hardly noticeable owing to the low Hb concentration in this phantom. The self-calibrated and Spectralon puck calibrated spectra for all phantoms show excellent overlap.

Figures 4(a) and 4(b) show the extracted versus expected μ_a and μ_s for all measured wavelengths for all phantoms using both calibration techniques. An average absolute error of $6.9\% \pm 7.2\%$ for μ_a and $3.5\% \pm 1.5\%$ for μ_s were achieved using the self-calibration approach, where the standard deviations were calculated across all the 17 phantoms. These errors are comparable to those obtained using the traditional Spectralon puck-based method ($6.8\% \pm 5.1\%$ for μ_a and $5.6\% \pm 2.9\%$ for μ_s).

In summary, we have demonstrated the feasibility of performing DRS using a self-calibrating fiber optic probe. The reference spectrum collected by the built-in calibration channel concurrent with the tissue measurement can be used to replace calibration measurements that need to be performed immediately after or before the tissue spectra. In addition, collecting a reference spectrum with a sample measurement could save 30 min of valuable clinical time by accounting for variations in intensity due to lamp warm up. The probe can also be used to remove system drift that occurs during the experiment. This approach can also be adopted into other optical modalities, such as fluorescence and Raman spectroscopy. The probe could be an important step toward instrument-independent optical spectroscopy.

Acknowledgments

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References

1. Zonios G, Perelman LT, Backman V, Manoharan R, Fitzmaurice M, Dam JV, Feld MS. Appl Opt 1999;38:6628. [PubMed: 18324198]

2. Utzinger U, Brewer M, Silva E, Gershenson D, Blast RC Jr, Follen M, Richards-Kortum R. *Lasers Surg Med* 2001;28:56. [PubMed: 11430444]
3. Mirabal YN, Chang SK, Atkinson EN, Malpica A, Follen M, Richards-Kortum R. *J Biomed Opt* 2002;7:587. [PubMed: 12421125]
4. Bigio IJ, Bown SG. *Cancer Biol Ther* 2004;3:259. [PubMed: 15107613]
5. Utzinger U, Richards-Kortum RR. *J Biomed Opt* 2003;8:121. [PubMed: 12542388]
6. Thueller P, Charvet I, Bevilacqua F, St Ghislain M, Ory G, Marquet P, Meda P, Vermeulen B, Depeursinge C. *J Biomed Opt* 2003;8:495. [PubMed: 12880356]
7. Palmer GM, Ramanujam N. *Appl Opt* 2006;45:1062. [PubMed: 16512550]

Fig. 1.
 (Color online) Block diagram of the DRS system and self-calibrating fiber optic probe (A, illumination adaptor; B, distal end of the probe; C, collection adaptor; and D, termination of the calibration fibers).

Fig. 2.

(Color online) Correction for illumination intensity fluctuations: (a) raw spectra from the mirror for different levels of attenuation by an ND filter and no attenuation, (b) ratio of the mirror and self-calibration spectra under different attenuation conditions.

Fig. 3.
(Color online) Raw spectra of phantom 1, 7, and 17 divided by the Spectralon puck spectrum (solid curve) and by the self-calibration spectrum (circles).

(a)

(b)

Fig. 4.
(Color online) Extracted versus expected phantom optical properties: (a) absorption coefficient, (b) reduced scattering coefficient.