Use of a coherent fiber bundle for multi-diameter single fiber reflectance spectroscopy

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Abstract: Multi-diameter single fiber reflectance (MDSFR) spectroscopy enables quantitative measurement of tissue optical properties, including the reduced scattering coefficient and the phase function parameter γ. However, the accuracy and speed of the procedure are currently limited by the need for co-localized measurements using multiple fiber optic probes with different fiber diameters. This study demonstrates the use of a coherent fiber bundle acting as a single fiber with a variable diameter for the purposes of MDSFR spectroscopy. Using Intralipid optical phantoms with reduced scattering coefficients between 0.24 and 3 mm$^{-1}$, we find that the spectral reflectance and effective path lengths measured by the fiber bundle (NA = 0.40) are equivalent to those measured by single solid-core fibers (NA = 0.22) for fiber diameters between 0.4 and 1.0 mm ($r ≥ 0.997$). This one-to-one correlation may hold for a 0.2 mm fiber diameter as well ($r = 0.816$); however, the experimental system used in this study suffers from a low signal-to-noise ratio for small dimensionless reduced scattering coefficients due to spurious back reflections within the experimental system. Based on these results, the coherent fiber bundle is suitable for use as a variable-diameter fiber in clinical MDSFR quantification of tissue optical properties.

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1. Introduction

White light reflectance spectroscopy provides non-invasive measurement of tissue optical properties, which can yield diagnostic information about tissue microstructure and physiology [1–4]. Reflectance spectra contain the combined effects of all the absorbing and scattering constituents in the optically sampled volume, which makes quantitative analysis of reflectance spectra complicated. To address this challenge, our group has recently developed a semi-empirical model for single fiber reflectance (SFR) spectroscopy based upon an experimentally validated Monte Carlo simulation [5]. SFR spectroscopy utilizes a simple fiber geometry in which a single fiber is used for both delivery of illumination light and collection of reflected light. This compact geometry allows for easy incorporation of SFR into narrow-gauge endoscopic instruments, such as fine needle aspiration (FNA) needles [6, 7], and confines the optically sampled volume to shallow depths [8], which may be useful for investigating precancerous lesions in epithelial tissue. Using an empirical model for the effective photon path length, we have shown that SFR can be used to quantify the tissue absorption coefficient ($\mu_a$) to investigate tissue chromophores without a priori knowledge of the tissue scattering properties [9].

Recently, we have demonstrated that acquiring successive co-localized SFR measurements with different fiber diameters enables quantification of the reduced scattering coefficient, $\mu_g = (1 - g_1)\mu_s$, as well as the phase function parameter $\gamma = (1 - g_2)/(1 - g_1)$, where $\mu_s$ is the tissue scattering coefficient and $g_1$ and $g_2$ are the first two Legendre moments describing the tissue phase function. Quantification of $\mu_g$ and $\gamma$ is achieved through application of a semi-empirical model for SFR in the absence of absorption ($R_{\text{SF}}^{0}$) [10, 11]:

$$R_{\text{SF}}^{0} = \eta_{\text{lim}} \left( 1 + 0.63 \gamma^2 e^{-2.31 \gamma \mu_s d_f} \right) \frac{(\mu_s d_f)^{0.57 \gamma}}{2.31 \gamma^2 + (\mu_s d_f)^{0.57 \gamma}}.$$  \hspace{1cm} (1)

In Eq. 1, $d_f$ is the fiber diameter and $\eta_{\text{lim}}$ is the diffusion limit of the maximum detectable signal determined by the fiber numerical aperture (NA) and the index of refraction of the sample medium ($n_{\text{med}}$) [12]. For NA = 0.22 and $n_{\text{med}} = 1.37$, $\eta_{\text{lim}}$ is approximately 2.7%. The model in Eq. 1 was developed from Monte Carlo simulations modeling tissue with a Modified Heney-Greenstein (MHG) phase function, $\mu_s = [0.4 - 4]$ mm$^{-1}$, $g_1 = [0.8 - 0.95]$, and $\gamma = [1.4 - 1.9]$. Using Eq. 1, $\mu_s$ and $\gamma$ can be solved for directly by acquiring multiple SFR measurements at the same location (assuming constant $\mu_s$ and $\gamma$ with different $d_f$) and solving Eq. 1 for each $d_f$ simultaneously. Because $\mu_s$ can be quantified from an SFR measurement independent of the scattering properties, Eq. 1 can be used to extract tissue scattering properties even in samples with strong absorption by first removing absorption effects from the measured spectrum [9, 11, 13].

Using this multi-diameter single fiber reflectance (MDSFR) technique in conjunction with the empirical model for photon path length enables quantitative measurement of $\mu_a$, $\mu_g$, and $\gamma$ of a turbid medium. Quantification of these scattering properties may be diagnostically valuable, as changes in the phase function can be directly correlated to changes in tissue microstructure [14, 15]. Moreover, knowledge of the tissue optical properties can also be used to correct single fiber fluorescence measurements for the effects of tissue scattering [16] and absorption [17].

The most straightforward realization of MDSFR spectroscopy is achieved through successive SFR measurements of a single location with a series of different diameter fibers. However, this technique is time consuming and requires placement of each of the SFR probes on precisely the same tissue location with precisely the same pressure [18–20]. This makes MDSFR technology using a series of single fiber measurements cumbersome and sensitive to measurement artifacts. Therefore, the desired MDSFR system would utilize one fiber probe with a variable effective diameter.
In this study, we achieve a single fiber with a variable effective diameter by selectively coupling light into and out of specific fibers in a coherent fiber bundle. In this manner, an array of closely packed individual fibers is used as an ensemble to approximate a single solid-core fiber, where the size of the array controls the effective single fiber diameter. To determine if the SFR spectra measured in this manner are equivalent to those measured by a single solid-core fiber, SFR spectra were measured for a range of Intralipid scattering phantoms, with and without absorber, using both a coherent fiber bundle and a series of single solid-core fibers.

2. Methods and materials

2.1. Experimental setup

The selective coupling of light into and out of specific fibers in a fiber bundle was achieved using the system shown in Fig. 1(a). The system utilizes a 1.35 m-long coherent fiber bundle (Schott AG, Mainz, Germany) consisting of approximately 18,000 step-index fibers in a hexagonal packing arrangement with a bundle diameter of 1.45 mm. Microscopic image analysis of the fiber bundle revealed the single core size to be 7 µm with center-to-center core spacing of 11 µm. As a result, the packing fraction, defined as the light-guiding area of the bundle divided by the total bundle area, is calculated to be approximately 1/3. The NA of the fiber bundle is not specified by the manufacturer, but was measured to vary from 0.43 ± 0.02 to 0.39 ± 0.02 over the wavelength range of 400 nm to 800 nm, in agreement with published values [21].

![Experimental system used for testing coherent fiber bundle.](image)

Fig. 1. Experimental system used for testing coherent fiber bundle. (a) The illumination pathway consists of a halogen lamp (HL), 400 µm diameter illumination fiber (F1), collimating lens (L1), Glan Thompson polarizer (P1), polarizing beamsplitter (PBS), movable pinhole (PH), fiber bundle (FB), and sample (S). The collection pathway consists of a pair of achromatic lenses (L2 and L3), Glan Thompson polarizer (P2), 3 mm diameter liquid light guide (LLG), and 200 µm coupling fiber (F2) to deliver light to a spectrometer (SM). Shutter control for the halogen lamp and acquisition from the spectrometer are coordinated through a personal computer (PC). (b) Microscopic image of the distal end of the coherent fiber bundle. Green light is the overlayed transmission resulting from a 1.0 mm pinhole at the proximal end. (c) Higher magnification image of the fiber bundle with overlayed transmission from a 0.2 mm pinhole. Scale bars in (b) and (c) are 500 µm and 50 µm, respectively.

To control the effective diameter of the fiber bundle, a pinhole (04PIP, CVI Melles Griot,
Didam, Netherlands) is placed on the surface of the proximal end of the fiber bundle using a custom-built mount. This mount enables different sizes of pinhole to be placed against the fiber bundle with high repeatability, while manual positioning allows translation of the pinhole laterally across the face of the fiber bundle. Under microscopic analysis of the distal end of the fiber bundle, shown in Figs. 1(b) and 1(c), we observed that the pinhole system effectively confined the illuminated region of the bundle to the diameter of the pinhole, with no measurable light coupling into fibers beyond the specified diameter. Using this pinhole system in conjunction with a 10 µm pinhole, illumination could even be confined entirely to a single fiber in the bundle (data not shown).

In this system, white light illumination is delivered from a halogen lamp (HL-2000-FHSA, Ocean Optics, Duiven, Netherlands) to the optical system via a 400 µm diameter step-index multimode fiber. The illumination light is collimated and then polarized by a Glan Thompson polarizer (03 PTO 109, Melles Griot, Didam, Netherlands) before being reflected onto the pinhole and fiber bundle assembly by a polarizing beamsplitter (PBS101, Thorlabs, Dachau, Germany). The distal end of the fiber bundle was polished at a 15 degree angle to reduce back reflections.

Light reflecting back from the sample emerges from the fiber bundle and is imaged to a 3 mm diameter liquid light guide (Series 2000, Lumatec, Deisenhofen, Germany) by a pair of achromatic doublets (MAP103075-A, Thorlabs). Before entering the liquid light guide, the emitted light first passes through the polarizing beamsplitter, followed by a second Glan Thompson polarizer (NT47-046, Edmund Optics, York, United Kingdom) with its polarization axis parallel to that of the polarizing beamsplitter transmission. This second polarizer serves to further attenuate illumination light that has been back-reflected from the surfaces of the proximal end of the fiber bundle and the pinhole.

The 3 mm core liquid light guide is used for collection to ensure that light from the outer fibers of the fiber bundle can be collected with the same efficiency as light from the inner fibers. Inside the light guide, the collected light becomes spatially mixed to prevent any fiber-position bias in the spectrometer. Light collected by the liquid light guide is coupled into a 200 µm diameter step-index multimode fiber to allow direct connection to a thermoelectrically cooled spectrometer (QE65000, Ocean Optics) with a 200 µm slit. The acquisition and processing of the reflectance spectra, as well as the actuation of the shutter for the halogen lamp, are all controlled by a laptop PC running LabView software.

For measurements by individual solid-core fibers, 0.22-NA bifurcated step-index multimode fibers with \(d_f = [0.2, 0.4, 0.6, 0.8, 1.0] \text{ mm}\) were used as described in Kanick et al. [22], utilizing the same halogen lamp, spectrometer, and data acquisition software described above.

### 2.2. Optical phantom preparation and measurement technique

To determine whether the SFR spectra acquired through portions of a fiber bundle were equivalent to those acquired through single solid-core fibers, a series of 10 mL liquid optical phantoms were constructed using varying concentrations of the scatterer 20% Intralipid (Fresenius Kabi, Bad Homburg, Germany) and an absorber, Evans Blue (Sigma-Aldrich, Vienna, Austria), in 0.9% NaCl solution (Baxter, Utrecht, Netherlands). Using the protocol described in Kanick et al. [22], we created phantoms with \(\mu_s'(800 \text{ nm}) = [0.24, 0.36, 0.48, 0.72, 0.96, 1.2, 2.4, 3.6] \text{ mm}^{-1}\). For each value of \(\mu_s'\), three phantoms were made with varying concentration of Evans Blue, resulting in \(\mu_a(611 \text{ nm}) = [0, 1, 3] \text{ mm}^{-1}\) assuming a specific absorption of Evans Blue of 18 g/L mm\(^{-1}\) at 611 nm. SFR spectra of each phantom were measured using both the coherent fiber bundle as well as a series of single solid-core fibers. The fibers diameters (for the solid-core fibers) and pinhole diameters (for the fiber bundle) were 0.2, 0.4, 0.6, 0.8, and 1.0 mm, resulting in 120 measurements for the fiber bundle and also for the solid-core fibers.
Reflectance measurements were taken with the distal tip of either the fiber bundle or a single solid-core fiber positioned a few millimeters below the surface of the phantom, with approximately two centimeters separating the fiber from the bottom of the container. The containers are 22 mm in diameter and 45 mm in height. For each measurement, the phantom was gently mixed, after which three consecutive spectra were measured and averaged together. Measurement integration times were adjusted for each pinhole and each solid-core fiber to maintain an approximately constant dynamic range.

Measurements for each fiber diameter and each pinhole diameter were calibrated using the calibration procedure described in Gamm et al. [13]. This procedure consists of measuring reflectance spectra from an Intralipid calibration phantom ($I_{\text{cal}}^{\text{meas}}$; $\mu'_s(800 \text{ nm}) = 1.2 \text{ mm}^{-1}$, $\mu_a(611 \text{ nm}) = 0 \text{ mm}^{-1}$) and from water in a dark container ($I_{\text{water}}^{\text{meas}}$). Each experimental measurement ($I_{\text{SF}}^{\text{meas}}$) is then calibrated by normalizing to the calibration phantom and then multiplying by the absolute reflectance simulated for the calibration phantom for the given fiber diameter using an experimentally validated Monte Carlo model ($I_{\text{cal}}^{\text{sim}}$):

$$RSF = \frac{I_{\text{cal}}^{\text{sim}}}{I_{\text{cal}}^{\text{meas}} - I_{\text{water}}^{\text{meas}}} \cdot \frac{I_{\text{meas}}^{\text{SF}} - I_{\text{meas}}^{\text{water}}}{I_{\text{cal}}^{\text{meas}} - I_{\text{water}}^{\text{meas}}}.$$  \hspace{1cm} (2)

As a result of the quotient on the right-hand side of Eq. 2, the calibrated reflectance spectrum $RSF$ is independent of the fiber NA used during the SFR measurement, i.e., the calibrated spectra are unaffected by the difference between the 0.4 NA of the fiber bundle and the 0.22 NA of the solid-core fibers. Because $I_{\text{cal}}^{\text{sim}}$ has been simulated for a 0.22-NA fiber, the calibrated spectra from the 0.22-NA solid-core fibers represent the absolute percentage of reflected light at each wavelength. In contrast, the calibrated spectra from the 0.4-NA fiber bundle represent the equivalent reflectance, as a percentage of illumination light, that would be measured by a 0.22-NA fiber. Further discussion of the calibration process is provided in Section 4.2.

Measurements acquired with the pinhole-based fiber bundle system exhibited a limited signal-to-noise ratio (SNR). As a result, the signal measured for the calibration phantom with the 0.2 mm pinhole is only slightly larger than that of the background water measurement, causing the denominator in Eq. 2 to be small and thereby increasing the effect of noise on the calibrated SFR. To address this problem, a highly-scattering 30% TiO₂ solid phantom ($\mu'_s(800 \text{ nm}) = 222 \text{ mm}^{-1}$) was used as the calibration phantom for the 0.2 mm diameter pinhole and 0.2 mm solid-core fiber, using the procedure described in Kanick et al. [5]. Accordingly, the simulated reflectance for the TiO₂ phantom was used as $I_{\text{cal}}^{\text{sim}}$ in Eq. 2.

To quantify the correlation between the SFR measurements from the fiber bundle and the individual solid-core fibers, the $R_{\text{SF}}^0$ values for each combination of $d_f$ and $\mu'_s$ were compared at 500, 611, 700, 800, and 900 nm, along with the effective path lengths ($\tau_{\text{SF}}$) at 611 nm. The effective path lengths were calculated using the relationship

$$\tau_{\text{SF}} = \frac{-\ln(R_{\text{SF}} / R_{\text{SF}}^0)}{\mu_a},$$  \hspace{1cm} (3)

using the procedure detailed in Kanick et al. [22]. Here, $R_{\text{SF}}$ is the calibrated SFR measured in the presence of absorption and $R_{\text{SF}}^0$ is the calibrated SFR measured without absorption, as in Eq. 1. In Eq. 3, all terms are evaluated at 611 nm, where the value of $\mu_a$ is known.

To reduce the impact of signal noise on the calculated values, $R_{\text{SF}}$ and $R_{\text{SF}}^0$ were averaged over 50 nm, centered at the measurement wavelength, and the measurement uncertainty was estimated using the data variance to calculate 95% confidence intervals about the mean. $R_{\text{SF}}$ and $R_{\text{SF}}^0$ are slowly varying at the measurement wavelengths and, while the absolute value of the averaged $R_{\text{SF}}$ and $R_{\text{SF}}^0$ may be slightly biased by the size of the averaging window, the comparisons between the fiber bundle and the solid-core fibers will not be affected as the same.
procedure was used for both cases.

3. Results

Figure 2 shows un-calibrated spectra taken with the fiber bundle system using the 1.0 mm pinhole ((b) and (e)) and using the 1.0 mm solid-core fiber ((a) and (d)). Each panel shows measurements taken in water (\(I_{\text{water}}^{\text{meas}}\), red dashed line), representative of the background signal due to back reflections in each system, and measurements taken in three phantoms with the same scattering coefficient but different concentrations of Evans Blue. The spectra calibrated according to Eq. 2 are shown in Figs. 2(c) and 2(f), with the blue lines indicating the single fiber spectra and the black data points representing the fiber bundle spectra. It is observed that the un-calibrated spectra acquired with the fiber bundle system (Figs. 2(b) and 2(e)) display a significant amount of background signal, which can be observed in the water calibration spectra. This signal is thought to arise from reflected illumination from the surfaces of the pinhole and fiber bundle reaching the detector, and it severely limits the signal-to-background ratio (SBR) of the measured fiber bundle spectra. In comparing the un-calibrated spectra from the fiber bundle with those of the solid-core fiber for the \(\mu'_{\text{a}}(800 \text{ nm}) = 3.6 \text{ mm}^{-1}\) phantoms, the magnitude of the useful signal is also three orders of magnitude lower for the case of the fiber bundle system, despite using the same light source and spectrometer. As a result of the low signal-to-noise ratio (SNR), longer integration times were used with the fiber bundle, varying from 4000 ms to 8000 ms, to be compared with integration times of 50 ms to 300 ms used with the solid-core fibers.

The origins and effects of the weak SBR and SNR observed for the fiber bundle are discussed in more detail in Section 4.1.

Despite the disparity in signal levels, the calibrated spectra acquired with the fiber bundle system match well with those acquired with the solid-core fibers, as illustrated in Figs. 2(c) and 2(f). It is worth noting in Fig. 2(f) that both systems display slightly increasing reflectance values with increasing wavelength for low dimensionless reduced scattering coefficients (\(\mu'_{d'/f}\)), which is due to the increased probability of back-scattering at higher wavelengths in Intralipid [23]. In comparing the calibrated reflectance for the two systems at 500, 611, 700, 800, and 900 nm, shown in Figs. 3(a-e), we observe Pearson correlation coefficients of \(r = 0.997 - 0.999\) for effective fiber diameters down to 0.4 mm. Though a few values for the 0.4 mm effective fiber diameter appear to lie off of the correlation line by more than the 95% confidence interval, these data correspond to the lowest signal levels and are thus more susceptible to systematic error, which could arise from small differences in the measurement systems not corrected for during calibration, as discussed in more detail in Section 4.1. These systematic errors will not be reflected in the error bars.

The SNR of the spectra acquired with the 0.2 mm pinhole is insufficient to conclude correlation to the 0.2 mm diameter solid-core fiber. In the comparison plot in Fig. 3(f), the fiber bundle with the 0.2 mm pinhole and the 0.2 mm solid-core fiber are compared for the three highest scattering coefficients. While the correlation coefficient of 0.816 suggests that the strong correlation observed for the 1.0 - 0.4 mm pinholes will also be observed for the 0.2 mm case, future experiments using a system with an improved SNR would be required to prove this correlation.

The measured effective path lengths also correlate well for effective diameters of 1.0 - 0.4 mm. Nearly all values fall on the correlation line, with the exception of several measurements of the \(\mu_{a} = 1 \text{ mm}^{-1}\) phantoms acquired with the 0.4 mm effective fiber diameter. As in Fig 3(d), these data represent the measurements with the weakest SNR of the plotted data set and are therefore more susceptible to systematic errors. The measured path lengths for the 0.4 mm effective fiber diameter using the \(\mu_{a} = 3 \text{ mm}^{-1}\) phantoms are less sensitive to small systematic errors due to the greater difference between \(R_{SF}\) and \(R_{0}^{SF}\), and these data lie on the correlation
Fig. 2. Representative SFR spectra collected by the coherent fiber bundle with a 1.0 mm pinhole (black data points) and by the 1.0 mm diameter solid-core fiber (blue lines). (a), (b) Uncalibrated SFR spectra, normalized by integration time, collected for the μ′₉₀₀ = 3.6 mm⁻¹ phantoms for the single solid-core fiber and the fiber bundle. Red dashed lines correspond to the water calibration spectra, consisting of back-reflected light not originating from the sample. (c) Overlay of the data in (a) and (b) after calibration. (d), (e), and (f) correspond to (a), (b), and (c), respectively, for the μ′₉₀₀ = 0.24 mm⁻¹ phantoms. The integration times used during acquisition were 50 ms and 4000 ms for the solid-core fiber and the fiber bundle, respectively.

This confirms that the fiber bundle is indeed still behaving as single solid-core fiber when using the 0.4 mm pinhole. If the μₐ = 1 mm⁻¹ data for the 0.4 mm effective fiber diameter are excluded, the correlation coefficient of the remaining data set is 0.971.

4. Discussion

4.1. Performance of the experimental system

The calibrated reflectance spectra and measured path lengths for the fiber bundle are equivalent to those of the single solid-core fibers to within the measurement accuracy of the current experimental system for effective diameters of 1.0—0.4 mm. Based on the equivalence of the magnitude and shape of the calibrated reflectance spectra and of the magnitude of the effective path lengths, the selectively-illuminated fiber bundle can be deemed equivalent to the individual solid-core fibers for the purposes of single fiber reflectance spectroscopy over the broad range of dᵢ = 1.0—0.4 mm and μₛ₉₀₀ = 0.24—3.6 mm⁻¹, which is representative of reduced scattering coefficients found in tissue [24–26].

As mentioned previously, the pinhole-based fiber bundle system used in this study exhibits a large background signal. This signal can be observed in the water calibration spectrum (red
Fig. 3. Correlation between the coherent fiber bundle and the single solid-core fibers for $R_{SF}^0$ and $\tau_{SF}$. (a-e) The values of $R_{SF}^0$ at 500 (♦), 611 (■), 700 (▲), 800 (▼), and 900 (●) nm for both the fiber bundle and the solid-core fibers are plotted against each other for $d_f = 1.0-0.2$ mm for $\mu'_s(800 \text{ nm}) = 0.24-3.6$ mm$^{-1}$. Only five representative wavelengths are plotted for graphical clarity. For $d_f = 0.2$ mm, only the data for $\mu'_s(800 \text{ nm}) = 1.2-3.6$ mm$^{-1}$ provided sufficient signal and are plotted. (f) The values of $\tau_{SF}$ are plotted for $d_f = 1.0$ (♦), 0.8 (■), 0.6 (▲), and 0.4 (▼) mm. The nominal absorption coefficient is 3 mm$^{-1}$ for the filled markers and 1 mm$^{-1}$ for the open markers. Error bars represent the 95% confidence interval and the black lines are the lines of perfect one-to-one correlation, shown here to guide the eye.
dashed line) in Figs. 2(b) and 2(e), which consists of the sum of all spurious back reflections in the experimental system that contribute to the signal. This background signal is suspected to originate from back reflection of illumination light from the metal pinhole and the fiber bundle surface and is incompletely rejected by the polarization discrimination system. The reduced efficacy of the polarization-based discrimination system is known to arise from the polarization rotating effect of the lens system used to couple the SFR signal into the liquid light guide [27,28]. While the use of an index-matching fluid at the fiber bundle surface is commonly used to reduce similar back reflections in confocal endoscopy [29–31], the back reflections arising from the metal pinhole cannot be corrected for in this manner. In addition to the significant background signal, the fiber bundle system also provides substantially less useful signal than the single solid-core fibers. This disparity can be observed by comparing the differences between the phantom (blue curves) and water (red curves) spectra acquired by the solid-core fiber in Figs. 2(a) and 2(d) with the differences between the phantom (black curves) and water (red curves) acquired by the fiber bundle in Figs. 2b and 2(e). In the μ′s = 3.6 mm⁻¹ phantom, the maximum useful signal from the 1.0 mm solid-core fiber is 808 counts/ms, compared with only 0.420 counts/ms from the fiber bundle. The useful signal in the fiber bundle system is limited by loss of both illumination and signal light due to polarization, overfilling of the pinhole to achieve uniform illumination of the fibers, poor coupling into the fiber bundle caused by the packing fraction of the bundle and any back reflection at the bundle surface, and coupling of the collected light from a 3 mm 0.5-NA liquid light guide into a 0.2 mm 0.22-NA multimode fiber. The weak signal makes the measurements more sensitive to noise. Additionally, the combination of weak useful signal with a strong background signal makes the measurements more sensitive to small changes in the system transmission or illumination properties after calibration, such as drift in spectrometer gain due to temperature changes or variations in lamp output. These factors can introduce systematic errors into the measurements, particularly for small signal levels, i.e., for small reduced dimensionless scattering (μ′d/μ) values.

To numerically illustrate the limitations imposed by the low signal and high background levels for the 0.2 mm effective diameter fiber, the useful signal measured by the fiber bundle with the 0.2 mm pinhole on the μ′s = 3.6 mm⁻¹, μa = 0 mm⁻¹ phantom was 0.008 counts/ms while the background signal was 0.479 counts/ms, yielding a signal-to-background ratio (SBR) of 0.016. For comparison, the measured useful signal and background signal for the 0.2 mm solid-core fiber and the same phantom were 44.499 counts/ms and 4.611 counts/ms, respectively, yielding an SBR of 9.651.

4.2. Calibration procedure

Despite the poor SNR and SBR of the pinhole-based fiber bundle system, the calibration procedure is able to correct for almost all the differences in transmission, collection, and back reflection properties to produce calibrated spectra that can be compared with those of the solid-core fibers. This direct comparison is possible because the calibration procedure also eliminates the effect of the difference in NA between the 0.4-NA fiber bundle and the 0.22-NA solid-core fibers. During calibration, the water spectrum is measured in a large dark container so that the only signals contributing to the spectrum originate from internal reflections inside the experimental system. After subtracting the water spectrum from an un-calibrated SFR spectrum, the remaining signal (I) is a function only of the illumination (L) and round-trip transmission (T) properties of the system, the collection efficiency of the fiber as a function of (NA/n)² [5], and the tissue response (f), which is some function of the tissue absorption and scattering properties and the fiber diameter:
\[
I - I_{water} \equiv \hat{I} \propto L \cdot T \cdot \left( \frac{\text{NA}}{n} \right)^2 f_{\text{meas}}^{\text{SF}},
\]

where each term is a function of wavelength. It is important to note that here the optical response of the tissue, \( f \), is independent of both the fiber NA and the index of the tissue medium. Monte Carlo simulation of the NA-dependence of SFR found that the SFR sensitivity to tissue optical properties does not change with NA over the range \(0.1 \leq \text{NA} \leq 0.4\) and is scaled by a factor of \((\text{NA}/n)^2\) to within an average residual of 5% [5]. Consequently, the NA and \( n \) only affect the magnitude of the collected signal in this regime and can be separated from the portion of the signal that is a function of tissue optical properties. As a result, the calibrated spectra of Eq. 2 can be rewritten as

\[
R_{\text{SF}} = \frac{f_{\text{cal}}^{\text{SF}}}{f_{\text{cal}}^{\text{meas}}} \propto \left( \frac{\text{NA}_{\text{sim}}}{n_{\text{cal}}} \right)^2 \frac{f_{\text{cal}}^{\text{sim}}}{f_{\text{cal}}^{\text{meas}}} \left[ \frac{L \cdot T \cdot \left( \frac{\text{NA}_{\text{exp}}}{n_{\text{sample}}} \right)^2 f_{\text{meas}}^{\text{SF}}}{L \cdot T \cdot \left( \frac{\text{NA}_{\text{exp}}}{n_{\text{cal}}} \right)^2 f_{\text{cal}}^{\text{meas}}} \right],
\]

where \( I_{\text{sim}} \) has been modeled for \( \text{NA}_{\text{sim}} = 0.22 \), \( n_{\text{cal}} = 1.33 \), and spectrally independent transmission and illumination of unity. Equation 5 can be simplified to

\[
R_{\text{SF}} \propto \left( \frac{f_{\text{cal}}^{\text{sim}}}{f_{\text{cal}}^{\text{meas}}} \right) \left( \frac{\text{NA}_{\text{sim}}}{n_{\text{sample}}} \right)^2 f_{\text{meas}}^{\text{SF}}.
\]

As illustrated in Eqs. 5 and 6, the calibration procedure eliminates the effects of the illumination and transmission characteristics of the experimental system. Importantly, the influence of the experimental NA is also removed, enabling direct comparison of calibrated data acquired by fibers with different NAs. In Eq. 6, the quantity \( (f_{\text{cal}}^{\text{sim}}/f_{\text{cal}}^{\text{meas}}) \) is equal to one if the optical properties of the calibration sample have been modeled accurately. The remaining terms represent the calibrated reflectance spectra, where \( (\text{NA}_{\text{sim}}/n_{\text{sample}})^2 \) scales the calibrated spectra to absolute percent reflectance for a 0.22-NA fiber in the index of the experimental media, \( n_{\text{sample}} \). For the 0.22-NA solid-core fibers, the calibrated data are absolute measurements. For the 0.4-NA fiber bundle, the calibrated spectra are the percent reflectance that would be measured by an equivalent 0.22-NA fiber. Note that for measurements in tissue, \( (\text{NA}_{\text{sim}}/n_{\text{sample}})^2 \) approaches \( n_{\text{lim}} \) and, after removing the effects of absorption, \( f \) can be modeled by the last two terms of Eq. 1 to extract quantitative values of \( \mu' \) and \( \gamma \) independently of \( \text{NA}_{\text{exp}} \).

The strong correlation observed between the fiber bundle and the solid-core fibers, despite the two-fold difference in NA, seems to support the simulation results, which found that changes in NA do not affect the sensitivity of an SFR measurement to tissue properties over \(0.1 \leq \text{NA} \leq 0.4\). In other words, the form and the fitted coefficients of Eq. 1 are unaffected by the fiber NA in this range. For NAs below 0.1, an increased dependence on single-scattering back-scatter events may arise and this relationship may no longer hold.

The measured effective photon path lengths also appear to be unaffected by the difference in NA between the solid-core fibers and the fiber bundle. This trend was confirmed in the Monte Carlo simulations, which predict the path length to be unaffected by NA in the range of \(0.1 \leq \text{NA} \leq 0.4\) to within an average residual < 5% (data not shown). Close scrutiny of the calibrated data suggests that the fiber bundle slightly but consistently under-predicts the solid-core fiber effective path length. While this trend could be indicative of a systematic difference in the behavior of the fiber bundle, future measurements using a system with greater SNR and SBR will be required to investigate this trend.
4.3. Considerations for clinical development

A critical difference between the liquid phantoms used in this study and biological tissue is the heterogeneity of optical properties found in tissue. As mentioned in Section 1, the accuracy of MDSFR measurements acquired through a series of solid-core fibers can be degraded by heterogeneity in \( \mu'_s \) due to minor variations in fiber placement between measurements. In contrast, MDSFR through a single fiber bundle allows the bundle to be held in place while the diameter is varied, eliminating the effects of lateral variations in \( \mu'_s \). Using the fiber bundle, it may also be possible to directly measure the degree of \( \mu'_s \) heterogeneity by acquiring MDSFR measurements through fibers off of the main axis of the fiber bundle, resulting in a lateral shift in the measurement position at the sample. In layered tissue, it is possible for the different fiber diameters used in an MDSFR measurement to probe different tissue layers because the effective photon path length for SFR is proportional to \( df \). Further investigation is required to determine the effect of layered tissue structures on MDSFR measurements.

MDSFR measurements in tissue also encounter absorption from a variety of tissue chromophores. For determining optical properties in the presence of absorption, the absorption effects are separated out using a background scattering model with a power-law scattering dependence on wavelength [9, 11, 13], so that Eq. 1 can be applied. Because this step is performed for each \( df \), spatial heterogeneities in \( \mu_a \) do not affect MDSFR accuracy. Using this method, MDSFR may even be able to utilize the difference in effective photon path lengths to investigate the depth-dependent distribution of chromophores.

Clinical development of fiber bundle-based MDSFR will require several improvements to the system used in this study. First, the amount of back-reflected light must be reduced while increasing the coupling of illumination light into the fiber bundle. These changes will improve the SNR and SBR, thus enabling reliable measurements at lower dimensionless reduced scattering coefficients and reducing integration times. Additionally, the current system relies on manual replacement of the pinhole to change effective diameters. In a clinical system, an automated means of changing effective diameter would be desired to reduce measurement time.

The aforementioned challenges could be addressed by replacing the pinhole with a spatial light modulator, such as a digital micromirror array, imaged to the bundle surface. This configuration would allow index matching at the proximal face of the fiber bundle, which may help reduce back reflections. Furthermore, the use of a micromirror array would enable fast selection of the effective diameter as well as selective positioning of the effective fiber center to enable investigation of the heterogeneity of the sampled region.

Alternatively, a fiber bundle with a fewer number of larger-core fibers could be used such that each fiber in the bundle could be bifurcated for direct connection to the light source and spectrometer, as is done with traditional solid-core SFR measurements. Though the system would require a complex fiber tree, an all-fiber system could nearly eliminate back reflections and greatly increase light coupling into and out of the fiber bundle. The use of a small number of large core fibers may influence how well the bundle approximates a single solid-core fiber, however, and this effect would need to be investigated.

5. Conclusion

We have presented the use of selective light coupling into and out of portions of a coherent fiber bundle to achieve a variable-diameter fiber for MDSFR spectroscopy. From comparison of the reflectance spectra measured from liquid phantoms, the fiber bundle can be considered equivalent to solid-core fibers of varying diameters for the purposes of quantifying tissue optical properties for effective fiber diameters in the range of 1.0—0.4 mm and tissue optical properties in the range of \( \mu'_s(800 \text{ nm}) = 0.24—3.6 \text{ mm}^{-1} \) and \( \mu_a(611 \text{ nm}) = 0—3 \text{ mm}^{-1} \).

The observed equivalence between the calibrated spectra acquired by the 0.22-NA solid-
core fibers and the 0.4-NA fiber bundle demonstrate that differences in magnitude between SFR spectra arising from differences in NA are removed during calibration. Furthermore, these findings support Monte Carlo simulations that predict that SFR sensitivity to tissue optical properties and effective photon path length are both insensitive to changes in fiber NA over the range \(0.1 \leq NA \leq 0.4\).

The pinhole-based optical system used to investigate the coherent fiber bundle in this study is hampered by poor rejection of spurious back reflections and low signal levels. These challenges can be addressed through improvements to the optical system. Further development of the fiber-bundle system is currently underway to facilitate clinical MDSFR spectroscopic measurements and enable investigation of smaller effective diameters.

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