Dual-DMD hyperspectral spatial frequency domain imaging (SFDI) using dispersed broadband illumination with a demonstration of blood stain spectral monitoring

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Abstract: Spatial frequency domain imaging (SFDI) is a widefield diffuse optical measurement technique capable of generating 2D maps of sub-surface absorption and scattering in biological tissue. We developed a new hyperspectral SFDI instrument capable of collecting images at wavelengths from the visible to the near infrared. The system utilizes a custom-built monochromator with a digital micromirror device (DMD) that can dynamically select illumination wavelength bands from a broadband quartz tungsten halogen lamp, and a second DMD to provide spatially modulated sample illumination. The system is capable of imaging 10 wavelength bands in approximately 25 seconds. The spectral resolution can be varied from 12 to 30 nm by tuning the input slit width and the output DMD column width. We compared the optical property extraction accuracy between the new device and a commercial SFDI system and found an average error of 23% in absorption and 6% in scattering. The system was highly stable, with less than 5% variation in absorption and less than 0.2% variation in scattering across all wavelengths over two hours. The system was used to monitor hyperspectral changes in the optical absorption and reduced scattering spectra of blood exposed to air over 24 hours. This served as a general demonstration of the utility of this system, and points to a potential application for blood stain age estimation. We noted significant changes in both absorption and reduced scattering spectra over multiple discrete stages of aging. To our knowledge, these are the first measurement of changes in scattering of blood stains. This hyperspectral SFDI system holds promise for a multitude of applications in quantitative tissue and diffuse sample imaging.

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1. Introduction

Spatial frequency domain imaging (SFDI) is a noninvasive, non-contact diffuse optical imaging modality that enables wide-field imaging of optical absorption and reduced scattering in biological tissue [1]. Absorption coefficients at multiple wavelengths can then provide absolute concentrations of oxy- and deoxy-hemoglobin, and other tissue chromophores. SFDI has been utilized in a range of biomedical applications, both in the clinical and preclinical settings. SFDI has found particular utility for clinical applications related to skin conditions, in part due it’s relatively superficial depth sensitivity, less than about 5 mm [2]. SFDI has been used to examine burn wounds [3–6], as well as nonmelanoma skin cancer, and skin photodamage in humans [7,8]. It has been used to monitor hemodynamics and scattering changes longitudinally in small animal tumor models [9,10], to quantitatively characterize surgically resected cancerous breast tissue [11–13], and its feasibility for monitoring palpable breast lesions has also been described [12]. Recent work has expanded the processing of SFDI data to calculate the absorption, scattering, and thickness of layered media [13]. This processing has been applied to isolate the effect of melanin from underlying hemodynamics [14] as well as measure the location of melanin in normal skin.
and benign nevi [16]. Furthermore, detailed quantification of these features in the epidermis and dermis, including oxy- and deoxy-hemoglobin concentrations, oxygen saturation, scattering properties, melanin content, and epidermal thickness, has been demonstrated in real time [17].

Past SFDI systems have used individual monochromatic sources such as LEDs or laser diodes, which limits the number of wavelengths that can be incorporated. The use of additional wavelengths can assist in quantification of chromophores such as methemoglobin, myoglobin, and hemichrome, which can be important biomarkers for various applications such as burn wound monitoring and forensic pathology [18, 19]. A variety of techniques have previously been developed to enable hyperspectral SFDI imaging. For example, some prior systems have used broadband light from a tungsten halogen lamp filtered in the illumination [20] or detection [21] pathway using a liquid crystal tunable filter (LCTF). One limitation of this technique is that LCTFs have limited light throughput (i.e. <50% transmission), often leading to long exposure times and slow acquisition rates (~1 measurement/minute). An alternative strategy used a computed tomography imaging spectrometer to collect spectrally separated images of multiple wavelengths simultaneously. This strategy offered high acquisition speeds of 6 s for 32 wavelengths, but reduced the resolution of the images to only 156 × 116 pixels for a 1–2 cm field of view [22]. Other systems have employed simultaneous detection with hyperspectral linescan cameras, enabling the acquisition of hundreds of wavelengths between 400 and 900 nm [23]. However, linescan cameras require motorized scanning to obtain 2D images, which limited the field-of-view to around 10 mm x 10 mm per acquisition. More recently, an SFDI instrument was designed using a supercontinuum laser source, with wavelength selection performed by a sliding slit that allows only a narrow wavelength band from dispersed light to pass [24]. This instrument was able to collect up to 1000 spectral bins between 580 and 950 nm, but required mechanical scanning, and the system had limited accuracy for reduced scattering coefficient (~25% errors for high values of \( \mu_s' \)).

Here, we describe a new hyperspectral SFDI instrument that implements a novel dual digital micromirror device (DMD) illumination pathway (referred to as “dDMD-SFDI”) to select specific wavelengths from a spectrally broad quartz tungsten halogen (QTH) light source. This allows for rapid switching of arbitrary illumination wavelengths within the instrument spectral bandwidth. The system does not require any mechanical scanning components. In this manuscript, we first describe the design of the instrument, followed by a characterization of the system through measurements of spectral resolution, optical property accuracy, and optical property precision. Lastly, we demonstrate the instrument’s ability to acquire hyperspectral SFDI data longitudinally by measuring spectral changes in blood exposed to air over 24 hours. Blood stain aging but has been investigated with various forms of reflectance spectroscopy as a means to assist investigators determine when a crime has been committed, but has yet been explored using SFDI [16,25,26].

2. Instrument design

The dDMD-SFDI system used a custom Czerny-Turner monochromator incorporating a DMD instead of an output slit to enable rapid wavelength switching (Fig. 1). The light source was a 1000 W QTH Lamp (Newport 66295-1KQ). We measured the power of the lamp after it was turned on and found that it reached 99% of its final value after 50 minutes. For all of the experiments in this manuscript we let the lamp warm up for at least 50 minutes prior to collecting data. Light from the lamp was focused onto a manually adjustable slit (Thorlabs VA100) which could be opened or closed to balance spectral bandwidth with optical throughput. Light from the slit was collimated by a concave mirror with a focal length of 50 mm and directed onto a 600 line/mm, 750 nm blazed diffraction grating (Thorlabs GR50-0608) which spectrally dispersed the light. The grating was mounted on a kinematic mount that enabled the angle to be adjusted. Light from the grating was focused by a second 50 mm focal length concave mirror onto a DMD. Different wavelengths of light could be selected by selecting different columns of this DMD. The
extremely broad QTH spectrum (400–4,000 nm) was dispersed by the grating over a larger area than the width of the DMD, so rapid switching could only be accomplished over the 140 nm bandwidth spanning the DMD’s active area. Additional wavelength bands could be selected by adjusting the angle of the grating. Monochromatic light from the DMD was directed to an off-axis parabolic mirror which expanded the beam to fill a second DMD used to spatially pattern the light. After passing through a linear polarizer, the DMD was imaged onto the sample using an achromatic lens. Light reflected from the sample was passed through a second linear polarizer set 90 degrees from the first to eliminate spectral reflection. The polarizers (Thorlabs, LPNIRE100-B) were anti-reflection coated for the 600–1100 wavelength band and featured an average of 45% transmission of unpolarized light over this range. The extinction ratio in the near-infrared band used in this work was between 1450 and 2000. Finally, the sample was imaged by a scientific CMOS camera (Andor Zyla).

The optical components were placed in a custom built enclosure with only small entry and exit apertures to reduce light leakage. For experiments in the near infrared range, a long pass filter was used to block wavelengths below 600 nm to reduce stray light, and a short pass filter was used prior to the exit aperture to block wavelengths above 900 nm. Additionally, physical barriers were placed in the monochromator to block higher diffraction orders and prevent light leakage around components.
The entire system was controlled using a LabView interface based on software modified from the openSFDI project [27]. Pre-defined patterns were loaded into the wavelength selection DMD’s onboard memory which could be selected using the controller’s USB interface. The spatial modulation DMD was attached to the host computer’s HDMI port and treated by the control system as a second monitor. 8-bit sinusoidal patterns could then be displayed on the DMD and projected onto the sample. This configuration allowed the wavelength to be selected and pattern changed at a rate of 60 Hz, though the exposure time needed to achieve an image with a suitable signal to noise ratio (∼200 ms) was typically the limiting factor in acquisition speed.

3. Methods

3.1. Spectral resolution

Spectral resolution of the dDMD-SFDI system was a function of both slit width and wavelength selection DMD column width. Both of these parameters were independently varied while recording the resultant illumination spectrum at the imaging plane with a spectrometer (Avantes AvaSpec-HS2048) at a center wavelength of 700 nm. Recorded spectra were fit to a Gaussian curve, as defined in Eq. (1) below:

\[
y = ae^{-\left(\frac{\lambda - \mu}{\sigma}\right)^2}
\]

In Eq. (1), \(y\) is light intensity as a function of wavelength \(\lambda\), \(a\) is a parameter that defines the amplitude of the signal, \(\mu\) is a parameter that defines the center location, and \(\sigma\) is a parameter that describes the width of the curve. The full width at half maximum (FWHM) was then computed using the relationship defined in Eq. (2):

\[
FWHM = \sigma \cdot 2\sqrt{\ln(2)}
\]

Spectral resolution, which was defined as the FWHM in nm, was characterized for three slit widths (0.37 mm, 0.5 mm, 1 mm), with DMD column width being varied from 11 to 200 pixels at each slit width.

3.2. Determination of optical properties with SFDI

A calibration phantom with known optical properties was measured prior to all experiments to account for the dDMD-SFDI’s instrument response function. The slit width was fixed at 1 mm and the first DMD’s column width was set to 75 pixels, yielding a spectral resolution of approximately 17.5 nm. For both the sample and calibration acquisition, three phase reflectance images were acquired at two spatial frequencies (0 mm\(^{-1}\) and 0.1 mm\(^{-1}\)) at each wavelength. Although the planar component of illumination (0 mm\(^{-1}\)) can be extracted from the high frequency images [28], the data presented in this manuscript were collected using separate projections for 0 and 0.1 mm\(^{-1}\) frequencies. The absorption and scattering coefficient of each pixel in the image was calculated using standard SFDI processing methods which have been previously described [1]. Briefly, for both sample and calibration image sets, three phase images per spatial frequency per wavelength were demodulated on a pixel-by-pixel basis, producing a single demodulated image for each spatial frequency and wavelength. Each demodulated sample image was then calibrated by dividing the measured sample image reflectance by the corresponding measured calibration image reflectance and multiplying by the expected reflectance for the calibration phantom (computed using a forward model to extract the calibration phantom’s expected reflectance given its known optical properties). This procedure yielded a single calibrated reflectance image per spatial frequency per wavelength. A look-up table (LUT) method was then used to locate the optical property pair \((\mu_a, \mu_s')\) for each pixel that best matched the calibrated reflectance values at the two spatial frequencies for that pixel. LUTs were generated from Monte Carlo simulations as described by Martinelli et al. [29]. The model used to calculate optical properties from
diffuse reflectance ignores surface roughness under the assumption that crossed linear polarizers are sufficient to isolate only diffusely reflected photons. Recent work has shown that surface quality can have a significant impact on diffuse reflectance even in the presence of crossed polarizers which may be one source of error in our experiments [30]. The use of polarized light for illumination and a model that does not consider polarization can also lead to errors [31]. However, both error sources were mainly observed in spatial frequencies greater than 0.1 mm\(^{-1}\) so the effect in this study is likely small. Similarly, our model used assumes that the index of refraction (IOR) of the sample was 1.44. Mismatches in IOR between the model and the actual sample can lead to errors in the extraction of absorption and scattering of about 5% for errors in IOR of 0.05 [32].

3.3. **System accuracy**

To assess system accuracy, SFDI images were acquired on a set of 2.5 cm thick silicone tissue-simulating optical phantoms doped with varying amounts of titanium dioxide nanoparticles and nigrosin dye to alter the scattering and absorption properties respectively [33]. The phantoms were imaged using both the dDMD-SFDI system and a commercial LED-based SFDI system (Modulim, Reflect RS), and extracted optical properties were compared between the two systems. The dDMD-SFDI system was configured to collect images at 731 nm, 811 nm, and 851 nm, which matched wavelengths available on the LED-based system. Measurements were taken on a set of 10 phantoms with optical properties across the three wavelengths in the range of: \(\mu_a = 0.0011\)–0.057 mm\(^{-1}\), \(\mu_s' = 0.56\)–1.8 mm\(^{-1}\). A complete set of measurements was taken with both systems three separate times, with repetitions occurring on different days. We have previously found close agreement in optical property measurements on this phantom set between the Modulim system and three other independently constructed SFDI systems [27].

3.4. **System precision**

To assess system precision, repeated SFDI measurements were performed once per minute for two hours on a phantom at 10 wavelengths between 731 nm and 863 nm. These wavelengths cover a range in the near-infrared commonly used to extract concentrations of hemoglobin. Trends in optical properties over time were observed, and coefficient of variation was quantified for absorption and reduced scattering at each wavelength.

3.5. **Blood stain spectral analysis**

To demonstrate the ability of dDMD-SFDI to track spectral changes longitudinally, a sample of liquid defibrinated bovine blood (Carolina Biological Supply Company) was monitored during exposure to air over 24 hours. We dispensed 1.5 ml of defibrinated bovine blood onto a 3 mm thick white cotton gauze pad with an area of approximately 2 cm\(^2\). The perimeter of the gauze pad was fixed to an optical phantom to prevent motion and provide a diffusive background. At the modulation frequencies used in this study (0 and 0.1 mm\(^{-1}\)) for a highly absorbing sample such as blood, 90% of photons measured will have penetrated less than 3 mm for planar illumination and 2 mm for 0.1 mm\(^{-1}\), so it is unlikely the underlying phantom would influence the optical properties [2]. Immediately following the dispensing of blood, dDMD-SFDI measurements were acquired every 5 minutes for the first 1.5 hours and every 30 minutes for the next 22.5 hours. The same wavelengths listed in Section 3.4 were used here. We also measured the absorption coefficient of gauze soaked in water to ensure that the gauze had no strong spectral features. We found that there was not a strong spectral dependence on wavelength, and that the average absorption coefficient was approximately 0.006 mm\(^{-1}\) in the wavelength range used, which is approximately 1 to 2 orders of magnitude lower than the absorption of blood. Mean absorption and reduced scattering coefficients across a region of interest (ROI) that included nearly the
entire gauze pad area were visualized at each time point. This ROI was 250 pixels x 125 pixels, spanning an area of 18 mm x 9 mm.

4. Results

4.1. Spectral resolution

Figure 2 illustrates the effect of slit width and DMD column width on spectral resolution. As expected, the spectral resolution increased when either the slit, or the DMD column width was narrowed. We interpret the fact that as the slit width was reduced from 0.5 mm to 0.37 mm there was almost no improvement in resolution to indicate that the resolution was limited by the DMD column widths in these cases. The fact that DMD column width dominates the resolution is likely due to aberrations in the monochromator leading to a broadened image of the slit on the DMD. These aberrations could be due to small errors in alignment, system geometry, or mirror construction. The relationship between DMD column width and spectral resolution was roughly linear between the 200 pixel and 100 pixel column width, with diminishing effect on resolution once the width was reduced below 100 pixels. The maximum spectral resolution was 12.5 nm when the DMD column width was set to 32 pixels. As most spectral features in the near infrared are fairly broad, the range of spectral resolution values for DMD column widths below 100 pixels are sufficient for many applications. For subsequent studies we used a slit width of 1 mm and a DMD column width of 75 pixels providing a spectral resolution of 17.5 nm.

![Fig. 2. Spectral resolution as a function of DMD column width and slit width. The legend refers to the slit width.](image)

4.2. System accuracy

When compared against the Reflect RS, the dDMD-SFDI system had an average error of 23% for $\mu_a$ and 6% for $\mu_s'$. Figure 3(a) and 3(b) show scatter plots comparing the measured absorption and reduced scattering coefficients from the dDMD-SFDI system and the Reflect-RS across all wavelengths and optical phantoms. Each point on these figures represents the mean value of the three repeat measurements, with horizontal and vertical error bars indicating standard deviation of those three measurements. The relationship between the measurements taken with the two systems was linear and close to the identity line. The standard deviation values across replicates
were generally so small that error bars are not discernible relative to the size of the points. Figure 3(c) and 3(d) show Bland-Altman plots for absorption and reduced scattering coefficients. From these plots, it is evident that there is a trend in absolute error becoming more negative as both absorption and scattering increase indicating that larger values of absorption and reduced scattering coefficient have larger errors than samples in other OP ranges. The dashed lines in the Bland-Altman plots indicate the limits of agreement of the two instruments and show that the dDMD-SFDI system yields statistically identical results to within those bounds. These limits refer to the bounds within which differences between the two systems will fall 95% of the time. These bounds themselves have some degree of uncertainty, however, which is represented by the error bars around the dashed lines in Fig. 3(c) and 3(d). The limits of agreement were $-0.0099 \pm 0.0021$ mm$^{-1}$ for $\mu_a$, and $-0.14 \pm 0.03$ mm$^{-1}$ for $\mu'_s$. The errors we observe in optical property estimation could arise from several different sources. The most significant are likely: lamp instability, noise added during image acquisition, and mismatch between light transport model and experimental conditions. Lamp instability can lead to small changes in illumination power and subsequent errors in extracted diffuse reflectance. Camera shot noise may also lead to errors in optical property extraction. Mismatches between the assumptions of the light transport model used for calculating optical properties and the experimental conditions such as surface roughness or index of refraction can also be an additional source of error [30,32].

![Fig. 3. Scatter plots of (a) $\mu_a$ and (b) $\mu'_s$ comparing the dDMD-SFDI system to the Modulim (Mod.) system. Each point refers to the mean across three repeat measurements, while the error bars refer to the standard deviation across these repeats. Most error bars are not visible, as the standard deviation is so small that the bars do not extend past the displayed size of the point. Bland-Altman plots of (c) $\mu_a$ and (d) $\mu'_s$ are shown below the scatter plots. The dashed lines show the limits of agreement between the two systems, which were $-0.0099$ and $0.0067 \pm 0.0021$ mm$^{-1}$ for $\mu_a$, and $-0.14$ and $0.15 \pm 0.03$ mm$^{-1}$ for $\mu'_s$.](image-url)
4.3. System precision

The dDMD-SFDI system had high precision for both $\mu_a$ and $\mu_s'$, with coefficients of variation across wavelengths ranging from 0.5% to 4.5% for $\mu_a$ and 0.1% to 0.2% for $\mu_s'$. We interpret this to mean that changes above 5% in a longitudinal measurement are unlikely to be caused by instrument drift. Figure 4 depicts the change in $\mu_a$ and $\mu_s'$ for each wavelength throughout the duration of the precision test. Visual inspection of Fig. 4(a) indicates that for each wavelength, there is no clear trend temporally with respect to $\mu_a$. However, as seen in Fig. 4(b), for $\mu_s'$, there is a slight downward trend for wavelengths above 805 nm. We are unsure what would cause this trend in only some of the measured wavelengths. One possibility is lamp instability. Regardless of the source, the slope of the observed trend is less than 0.001 mm$^{-1}$ per hour which would have a negligible impact on measurements for many biomedical applications.

![Fig. 4. Results of a drift test to measure system precision for (a) $\mu_a$ and (b) $\mu_s'$. In each figure, values of $\mu_a$ and $\mu_s'$ are vertically offset for each wavelength to more easily visualize individual time traces. Horizontal dashed lines for each wavelength trace represent the mean value for that wavelength across the entire 2-hour drift test. The scale bars in the bottom left corner of each figure define the y-axis spacing. The coefficient of variation for $\mu_a$ ranged between 0.5 and 4.5% while the coefficient of variation for $\mu_s'$ was between 0.1 and 0.2%.](image)

4.4. Blood stain spectral analysis

Measurements on the bovine blood stain revealed changes in the spectral features of the stain within 24 hours of deposition. Figure 5(a) shows how the absorption spectrum changed over time. Initially, $\mu_a$ was 0.064 mm$^{-1}$ at 731 nm and gradually increased with wavelength to 0.10 mm$^{-1}$ at 863 nm. These absorption coefficients then uniformly dropped over the next 1.73 hr, after which they rose again, with $\mu_a$ rising more at shorter wavelengths than longer wavelengths. This resulted in a spectral “flattening” around 9.73 hr, at which time $\mu_a$ at all wavelengths was roughly 0.09 mm$^{-1}$. $\mu_a$ at shorter wavelengths then rose more relative to longer wavelengths, resulting in a final $\mu_a$ of 0.13 mm$^{-1}$ at 731 nm, which gradually decreased to 0.086 mm$^{-1}$ at 863 nm. Figure 5(b) shows the scattering spectra over time, exhibiting less spectral change. There was a decrease in $\mu_s'$ as wavelength increased, as is typical for most diffuse media, and, in general, all $\mu_s'$ values increased slightly throughout the duration of the 24-hour experiment. These time dynamics are visualized in Fig. 6, which shows time traces of $\mu_a$ and $\mu_s'$ at all wavelengths.

Examples of wide-field spatial maps of $\mu_a$ and $\mu_s'$ are shown in Fig. 5(c) and 5(d), respectively. These are maps of the blood sample and a portion of the background phantom at the final time point. A median filter with a 3 x 3 kernel size was applied to these images, with any “not a number” (NaN) values ignored. These NaN values were an artifact of processing, and occur when...
**Fig. 5.** a) Absorption spectra of a blood stain at different time points. Semi-transparent red spectra show the absorption spectra for every other time point. Four time points are specified according to the legend on the far right. b) $\mu_a$ spectra. c) A median filtered spatial map of $\mu_a$ at 731 nm at the final time point. The scale bar is equal to 5 mm. d) $\mu_s'$ map at 731 nm. e) DC image at 731 nm, highlighting the blood and phantom ROI locations used to quantify mean optical properties in parts (a) and (b), as well as Visualization 1 and Visualization 2.

**Fig. 6.** Time traces of (a) $\mu_a$ and (b) $\mu_s'$ of the blood stain for 24 hours following its deposition onto a gauze pad. The traces display the mean values across an ROI that covers nearly the entire blood sample. Measurements were taken every 5 minutes for the first 1.5 hours, and every 30 minutes from then on. A 5-point moving average filter was used to smooth all time traces.
the estimated diffuse reflectance does not lie within the Monte Carlo-generated LUT. Spatial variation of both optical properties was evident in these images. Threads from the gauze pad appear to be visible in both maps as well, which is made evident by a comparison of spatial map structure to a DC image of the sample shown in Fig. 5(e). Visualization 1 shows changes in the absorption spectrum and changes in the spatial map of $\mu_a$ at 731 nm throughout the 24-hour experiment. This video also visualizes changes in the phantom optical properties over time. The phantom changes are minimal, confirming that spectral changes observed in blood are not a product of system drift or changes in environmental conditions. Visualization 2 shows these changes for the scattering spectrum and the spatial map of $\mu_s'$ at 731 nm. Both videos also display changes in the DC image at 731 nm over time, with the ROI locations for the blood sample and background phantom labeled.

5. Discussion

We described here a flexible dual-DMD hyperspectral SFDI system for biological imaging. The spectral resolution of the system can be finely tuned by varying both slit width and DMD column width. DMD column width can be quickly and easily modified entirely through software, making this system suitable for rapid imaging across a wide spectral bandwidth. Since there is a tradeoff between spectral resolution and optical power, the ability to tune this parameter gives the system utility for applications with diverse sample properties. Based on the attenuation of the sample of interest, and the width of the spectral features of interest, the resolution-power tradeoff can be readily optimized for a given application. Unlike some prior multispectral and hyperspectral SFDI systems which sacrifice image resolution for acquisition speed [22], the dDMD-SFDI system is capable of imaging 10 wavelength bands in 30 s while maintaining high spatial resolution (330 $\times$ 470 pixels after 2 $\times$ 2 binning). While the dDMD-SFDI system can only switch wavelengths rapidly within a 140 nm band, it utilizes no moving parts, and offers increased scattering accuracy for relatively high scattering samples compared to a previous scanning slit based device [24]. The performance is most similar to prior LCTF-based devices, but utilizes lower-cost components, has the potential to utilize a broader wavelength range by rotating the grating, and has the flexibility to illuminate with multiple wavelengths simultaneously which can improve rejection of ambient light [34].

Characterization experiments demonstrated that the dDMD-SFDI agreed well with a commercial SFDI system. $\mu_s'$ had a mean percent error of 6%. The mean percent error for $\mu_a$ was higher at 23%, which is likely due to the lower absolute value of $\mu_a$ which results in small absolute errors translating to large percentages. The precision of the dDMD-SFDI system demonstrated coefficients of variation ranging from 0.5% to 4.5% for $\mu_s$, and 0.1% to 0.2% for $\mu_s'$. This level of precision indicates that physiological effects that produce changes in optical properties as low as 5% are measurable with this system.

The blood stain experiment validated the ability of the dDMD-SFDI system to monitor spectral features of a biological sample longitudinally. As shown in Fig. 5(a), the blood stain absorption spectrum initially resembled that of oxyhemoglobin. The absolute values of $\mu_a$ are somewhat low for a blood soaked piece of cotton gauze. This is likely due to the fact that, on average, bovine blood has less hemoglobin than human blood (10 mg/dL vs. 15 mg/dL) [35,36], and the measured optical properties are a combination of blood and gauze with a possible small contribution from the underlying phantom. We also note that the relatively high blood absorption compared to scattering create conditions that border on, or potentially fall outside of, the diffusive regime, which, had the diffusion model of light transport been used to recover optical properties, could have led to errors in the quantification of both $\mu_a$ and $\mu_s'$ [37]. However, as a Monte Carlo-based inverse model was employed, this limitation of the diffusion model did not apply, suggesting that the high absorption of blood contributed minimally to the potentially underestimated $\mu_a$ values.
During the drying experiment we assume that the gauze and phantom optical properties are static and all of the observed change is due to the blood.

It is likely that the initial decrease in $\mu_a$ across all wavelengths during the first 1.73 hr was due to the evaporation of water. Following this monotonic decline, $\mu_a$ uniformly increased across all wavelengths until the $\sim$9 hr mark. This may reflect the conversion of oxyhemoglobin to methemoglobin, whose extinction spectrum is similar to oxyhemoglobin at the NIR wavelengths investigated, but with a higher magnitude of absorption. After 9 hours the blood underwent a significant and rapid spectral shift. We observed a rise in $\mu_a$ at the shorter wavelengths which is consistent with the denaturation of methemoglobin to hemichrome, whose extinction spectrum has higher absorption at 731 nm that decreases as wavelength increases [19]. The conversion from HbO$_2$ to metHb to hemichrome has been previously described with diffuse reflectance spectroscopy measurements, although time scales investigated were on the order of days as opposed to hours [19].

Unlike reflectance spectroscopy, the dDMD-SFDI system allows changes in optical scattering to be explicitly measured. To our knowledge, this is the first demonstration of isolating and quantification of reduced scattering changes in a blood stain over time. In this experiment, we noted that the reduced scattering spectrum increased for the first 9 hours which supports our hypothesis that early spectral changes are due to the evaporation of water. After 9 hours there is a shift in the reduced scattering spectrum which becomes noticeably flatter at shorter wavelengths. We believe this effect may be caused by the gauze fibers squeezing more tightly together once the blood dries. The reduced scattering coefficient may be an important new source of contrast in blood age estimation, potentially helping to increase sensitivity to early time points changes when combined with absorption-based metrics.

6. Conclusion

We presented here dDMD-SFDI as a versatile and quantitative diffuse optical imaging tool. The dynamic wavelength tunability offered by the dDMD-SFDI system lends itself to the exploration and optimization of wavelength choice for a given application. As an initial demonstration of the utility of dDMD-SFDI, we tracked hyperspectral changes in both absorption and reduced scattering longitudinally through measurements of a defibrinated blood stain over a 24-hour period. This revealed both absorption and reduced scattering spectral changes through multiple discrete stages of aging. Going forward, dDMD-SFDI has potential utility for many biological applications that require quantitative optical property and chromophore extractions at a multitude of wavelengths.

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References
1. D. J. Cuccia, F. Bevilacqua, A. J. Durkin, F. R. Ayers, and B. J. Tromberg, “Quantitation and mapping of tissue optical properties using modulated imaging,” J. Biomed. Opt. 14(2), 024012 (2009).
2. C. K. Hayakawa, K. Karrobi, V. E. Pera, D. M. Roblyer, and V. Venugopalan, “Optical sampling depth in the spatial frequency domain,” J. Biomed. Opt. 24(07), 1–14 (2019).
3. J. Q. M. Nguyen, C. Crouzet, T. Mai, K. Riola, D. Uchitel, L.-H. L. Liaw, N. Bernal, A. Ponticorvo, B. Choi, and A. J. Durkin, “Spatial frequency domain imaging of burn wounds in a preclinical model of graded burn severity,” J. Biomed. Opt. 18(6), 066010 (2013).
4. T. T. A. Nguyen, J. C. Ramella-Roman, L. T. Moffatt, R. T. Ortiz, M. H. Jordan, and J. W. Shupp, “Novel application of a spatial frequency domain imaging system to determine signature spectral differences between infected and noninfected burn wounds,” J. Burn Care Res. 34(1), 44–50 (2013).
5. D. J. Rohrbach, N. C. Zeitouni, D. Muffoletto, R. Saager, B. J. Tromberg, and U. Sunar, “Characterization of nonmelanoma skin cancer for light therapy using spatial frequency domain imaging,” Biomed. Opt. Express 6(5), 1761–1766 (2015).
6. J. B. Travers, C. Poon, T. Bihi, B. Rinehart, C. Borchers, D. J. Rohrbach, S. Borchers, J. Trevino, M. Rubin, H. Donnelly, K. Kellawan, L. Carpenter, S. Bahl, C. Rohan, E. Muenich, S. Guenther, H. Hahn, A. Rhein, M. Darst, N. Moudsisicas, E. Cates, and U. Sunar, “Quantifying skin photodamage with spatial frequency domain imaging: statistical results,” Biomed. Opt. Express 10(9), 4676–4683 (2019).

7. Y. Zhao, S. Tabassum, S. Piracha, M. S. Nandhu, M. Viapiano, and D. Roblyer, “Angle correction for small animal tumor imaging with spatial frequency domain imaging (SFDI),” Biomed. Opt. Express 7(6), 2373–2384 (2016).

8. K. Karrobi, A. Tank, S. Tabassum, V. Pera, and D. Roblyer, “Diffuse and nonlinear imaging of multiscale vascular parameters for in vivo monitoring of preclinical mammary tumors,” J. Biophotonics 12(6), e201800379 (2019).

9. A. M. Laughney, V. Krishnaswamy, T. B. Rice, D. J. Cuccia, R. J. Barth, J. Tromberg, K. D. Paulsen, B. W. Pogue, and W. A. Wells, “System analysis of spatial frequency domain imaging for quantitative mapping of surgically resected breast tissues,” J. Biomed. Opt. 18(3), 036012 (2013).

10. A. M. Laughney, V. Krishnaswamy, E. J. Rizzo, M. C. Schwab, R. J. Barth, D. J. Cuccia, B. J. Tromberg, K. D. Paulsen, B. W. Pogue, and W. A. Wells, “Spectral discrimination of breast pathologies in situ using spatial frequency domain imaging,” Breast Cancer Res. 15(4), R61 (2013).

11. D. M. McClatchy, E. J. Rizzo, W. A. Wells, C. C. Black, K. D. Paulsen, S. C. Kanick, and B. W. Pogue, “Light scattering measured with spatial frequency domain imaging can predict stromal versus epithelial proportions in surgically resected breast tissue,” J. Biomed. Opt. 24(7), 1–11 (2018).

12. C. M. Robbins, G. Raghavan, J. F. Antaki, and J. M. Kainerstorfer, “Feasibility of spatial frequency-domain imaging for monitoring palpable breast lesions,” J. Biomed. Opt. 22(12), 1–9 (2017).

13. S. T. Horan, A. R. Gardner, R. B. Saager, A. J. Durkin, and V. Venugopalan, “Recovery of layered tissue optical properties from spatial frequency-domain spectroscopy and a deterministic radiative transport solver,” J. Biomed. Opt. 24(07), 1–11 (2019).

14. R. B. Saager, M. D. Ata Sharif, M. K. M. D. Kristen, and A. J. Durkin, “In vivo isolation of the effects of melanin from underlying hemodynamics across skin types using spatial frequency domain spectroscopy,” J. Biomed. Opt. 21(5), 057001 (2016).

15. R. B. Saager, M. Balu, V. Crosignani, A. Sharif, A. J. Durkin, K. M. Kelly, and B. J. Tromberg, “In vivo measurements of cutaneous melanin across spatial scales: using multiphoton microscopy and spatial frequency domain spectroscopy,” J. Biomed. Opt. 20(6), 066005 (2015).

16. F. Vasefi, N. MacKinnon, R. Saager, K. M. Kelly, T. Maly, N. Booth, A. J. Durkin, and D. L. Farkas, “Separating melanin from hemodynamics in nevi using multimode hyperspectral dermoscopy and spatial frequency domain spectroscopy,” J. Biomed. Opt. 21(11), 114001 (2016).

17. X. Chen, W. Lin, C. Wang, S. Chen, J. Sheng, B. Zeng, and M. Xu, “In vivo real-time imaging of cutaneous hemoglobin concentration, oxygen saturation, scattering properties, melanin content, and epidermal thickness with visible spatially modulated light,” Biomed. Opt. Express 8(12), 5468–5482 (2017).

18. R. B. Saager, R. A. Rowland, M. L. Baldado, G. T. Kennedy, N. P. Bernal, A. Ponticorvo, R. J. Christy, and A. J. Durkin, “Impact of hemoglobin breakdown products in the spectral analysis of burn wounds using spatial frequency domain spectroscopy,” J. Biomed. Opt. 24(07), 1–4 (2019).

19. R. H. Bremmer, A. Nadort, T. G. van Leeuwen, M. J. C. van Gemert, and M. C. G. Aalders, “Age estimation of blood stains by hemoglobin derivative determination using reflectance spectroscopy,” Forensic Sci. Int. 206(1-3), 166–171 (2011).

20. A. Mazhar, S. Dell, D. J. Cuccia, S. Gioux, A. J. Durkin, J. V. F. M. D. and B, and J. Tromberg, “Wavelength optimization for rapid chromophore mapping using spatial frequency domain imaging,” J. Biomed. Opt. 15(6), 061716 (2010).

21. R. H. Wilson, K. P. Nadeau, F. B. Jaworski, R. Rowland, J. Q. M. Nguyen, C. Crouzet, R. B. Saager, B. Choi, B. J. Tromberg, and A. J. Durkin, “Quantitative short-wave infrared multispectral imaging of in vivo tissue optical properties,” J. Biomed. Opt. 19(8), 086011 (2014).

22. J. R. Weber, A. J. Durkin, B. J. Tromberg, D. J. Cuccia, W. R. Johnson, D. W. Wilson, G. H. Bearman, M. Hsu, A. Lin, and D. K. Binder, “Multispectral imaging of tissue absorption and scattering using spatial frequency domain imaging and a computed-tomography imaging spectrometer,” J. Biomed. Opt. 16(1), 011015 (2011).

23. R. P. Singh-Moon, D. M. Roblyer, I. J. Bigio, and S. Joshi, “Spatial mapping of drug delivery to brain tissue using spatial frequency-hyperspectral domain imaging,” J. Biomed. Opt. 19(9), 096003 (2014).

24. M. Torabzadeh, P. A. Stockton, G. T. Kennedy, R. B. Saager, A. J. Durkin, R. A. Bartels, and B. J. Tromberg, “Hyperspectral imaging in the spatial frequency domain with a supercontinuum source,” J. Biomed. Opt. 24(07), 1–9 (2019).

25. G. Edelman, V. Manti, S. M. van Ruth, T. van Leeuwen, and M. Aalders, “Identification and age estimation of blood stains on colored backgrounds by near infrared spectroscopy,” Forensic Sci. Int. 220(1-3), 239–244 (2012).

26. G. Zadora and A. Menzyk, “In the pursuit of the holy grail of forensic science – Spectroscopic studies on the estimation of time since deposition of bloodstains,” TrAC, Trends Anal. Chem. 105, 137–165 (2018).

27. M. B. Applegate, K. Karrobi, J. P. A. Jr, W. M. Austin, S. M. Tabassum, E. Aguêrounon, K. Tilbury, R. B. Saager, S. Gioux, and D. M. Roblyer, “OpenSFDI: an open-source guide for constructing a spatial frequency domain imaging system,” J. Biomed. Opt. 25(01), 1–13 (2020).

28. J. Vervander and S. Gioux, “Single snapshot imaging of optical properties,” Biomed. Opt. Express 4(12), 2938–2944 (2013).
29. M. Martinelli, A. Gardner, D. Cuccia, C. Hayakawa, J. Spanier, and V. Venugopalan, “Analysis of single Monte Carlo methods for prediction of reflectance from turbid media,” Opt. Express 19(20), 19627–19642 (2011).
30. S. Nothelfer, F. Bergmann, A. Liemert, D. Reitzle, and A. Kienle, “Spatial frequency domain imaging using an analytical model for separation of surface and volume scattering,” J. Biomed. Opt. 24(07), 1–10 (2018).
31. J. West, N. Bodenschatz, A. Brandes, A. Liemert, and A. Kienle, “Polarization influence on reflectance measurements in the spatial frequency domain,” Phys. Med. Biol. 60(15), 5717–5732 (2015).
32. N. Bodenschatz, A. R. Brandes, A. Liemert, and A. Kienle, “Sources of errors in spatial frequency domain imaging of scattering media,” J. Biomed. Opt. 19(7), 071405 (2014).
33. A. E. Cerussi, R. Warren, B. Hill, D. Roblyer, A. Leproux, A. F. Durkin, T. D. O’Sullivan, S. Keene, H. Haghany, T. Quang, W. M. Mantulin, and B. J. Tromberg, “Tissue phantoms in multicenter clinical trials for diffuse optical technologies,” Biomed. Opt. Express 3(5), 966–971 (2012).
34. M. B. Applegate and D. M. Roblyer, “High-speed spatial frequency domain imaging with temporally modulated light,” J. Biomed. Opt. 22(7), 076019 (2017).
35. W. G. Zijlstra and A. Buursma, “Spectrophotometry of hemoglobin: absorption spectra of bovine oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, and methemoglobin,” Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol. 118(4), 743–749 (1997).
36. H. H. Billett, “Hemoglobin and Hematocrit,” in Clinical Methods: The History, Physical, and Laboratory Examinations, H. K. Walker, W. D. Hall, and J. W. Hurst, eds. (Butterworth, 1990).
37. S. L. Jacques and B. W. Pogue, “Tutorial on diffuse light transport,” J. Biomed. Opt. 13(4), 041302 (2008).