DEDICATION

To

those who believed in me when I didn’t

“Ask yourself whether the dream of heaven and greatness should be waiting for us in our graves – or whether it should be ours here and now and on this earth”

-Ayn Rand
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<td>A</td>
<td>reduced scattering amplitude</td>
</tr>
<tr>
<td>b</td>
<td>reduced scattering slope</td>
</tr>
<tr>
<td>C</td>
<td>Fourier coefficient matrix</td>
</tr>
<tr>
<td>FFT</td>
<td>fast Fourier transform</td>
</tr>
<tr>
<td>FFT$^{-1}$</td>
<td>inverse fast Fourier transform</td>
</tr>
<tr>
<td>$f_x$</td>
<td>spatial frequency</td>
</tr>
<tr>
<td>H</td>
<td>image resulting from 2D Hilbert transform</td>
</tr>
<tr>
<td>I</td>
<td>raw intensity vector</td>
</tr>
<tr>
<td>O</td>
<td>order of</td>
</tr>
<tr>
<td>R</td>
<td>demodulated reflectance vector</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>wavelength</td>
</tr>
<tr>
<td>$\mu_a$</td>
<td>absorption coefficient</td>
</tr>
<tr>
<td>$\mu_s'$</td>
<td>reduced scattering coefficient</td>
</tr>
<tr>
<td>I</td>
<td>raw intensity</td>
</tr>
<tr>
<td>M</td>
<td>modulated (AC) term in SFDI intensity</td>
</tr>
<tr>
<td>R</td>
<td>demodulated reflectance</td>
</tr>
<tr>
<td>S</td>
<td>spiral phase map</td>
</tr>
<tr>
<td>$\phi$</td>
<td>phase angle</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Meaning</td>
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<td>--------------</td>
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<tr>
<td>AC</td>
<td>alternating current (modulated illumination)</td>
</tr>
<tr>
<td>CCD</td>
<td>charge coupled device</td>
</tr>
<tr>
<td>DAQ</td>
<td>data acquisition</td>
</tr>
<tr>
<td>DC</td>
<td>direct current (planar illumination)</td>
</tr>
<tr>
<td>DMD</td>
<td>digital micromirror device</td>
</tr>
<tr>
<td>DT-MRI</td>
<td>diffusion tensor magnetic resonance imaging</td>
</tr>
<tr>
<td>eGFR</td>
<td>estimated glomerular filtration rate</td>
</tr>
<tr>
<td>FOV</td>
<td>field-of-view</td>
</tr>
<tr>
<td>FPS</td>
<td>frames per second</td>
</tr>
<tr>
<td>GPU</td>
<td>graphics processing unit</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>hematoxylin and eosin</td>
</tr>
<tr>
<td>HSV</td>
<td>hue, saturation, value</td>
</tr>
<tr>
<td>InGaAs</td>
<td>Indium gallium arsenide</td>
</tr>
<tr>
<td>IRI</td>
<td>renal ischemic reperfusion injury</td>
</tr>
<tr>
<td>KIM-1</td>
<td>Kidney Injury Molecule -1</td>
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<tr>
<td>LED</td>
<td>light emitting diode</td>
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<tr>
<td>MSE</td>
<td>multi-frequency synthesis and extraction</td>
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<tr>
<td>NGAL</td>
<td>Neutrophil Gelatinase-Associated Lipocalin</td>
</tr>
<tr>
<td>NIR</td>
<td>near infrared</td>
</tr>
<tr>
<td>PAS</td>
<td>periodic Acid Schiff</td>
</tr>
<tr>
<td>PPG</td>
<td>photoplethysmography</td>
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<tr>
<td>QTSI</td>
<td>quantitative tissue spectral imaging</td>
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<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>sCMOS</td>
<td>scientific-grade complementary metal-oxide semiconductor</td>
</tr>
<tr>
<td>SEER</td>
<td>Surveillance Epidimeology and End Result</td>
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<tr>
<td>SFD</td>
<td>spatial frequency domain</td>
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<tr>
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<td>Meaning</td>
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<td>--------------</td>
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<tr>
<td>SFDI</td>
<td>Spatial Frequency Domain Imaging</td>
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<tr>
<td>SLM</td>
<td>spatial light modulator</td>
</tr>
<tr>
<td>s-MTF</td>
<td>spatial modulation transfer function</td>
</tr>
<tr>
<td>SNR</td>
<td>signal-to-noise ratio</td>
</tr>
<tr>
<td>SOI</td>
<td>scattering orientation index (SOI)</td>
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<tr>
<td>SP</td>
<td>scattering power</td>
</tr>
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<td>SPIFI</td>
<td>Spatial Modulation for Imaging</td>
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<td>SSOP</td>
<td>single snapshot optical properties</td>
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<tr>
<td>StO₂</td>
<td>tissue oxygen saturation</td>
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<tr>
<td>SWIR</td>
<td>short wave infrared</td>
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<tr>
<td>μM</td>
<td>micromolar</td>
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2. K.P. Nadeau, T.B. Rice, A.J. Durkin, B.J. Tromberg, “Multi spatial frequency synthesis and extraction using square wave patterns for quantitative tissue imaging” (in revision, Journal of Biomedical Optics, Manuscript ID: 150118)


5. K.P. Nadeau, A.J. Durkin, B.J. Tromberg, “Advanced demodulation technique for the extraction of tissue optical properties and structural orientation contrast in the spatial frequency domain”, Journal of Biomedical Optics, 56013 (2014)


7. K.P. Nadeau, P. Khoury, A. Mazhar, D.J. Cuccia, A.J. Durkin, “Component and system evaluation for the development of a handheld point-of-care spatial frequency imaging
domain (SFDI) device”, Proc. SPIE 8573, Design and Quality for Biomedical Technologies VI, 857304 (March 13, 2013)

PATENTS
1. K.P. Nadeau, T.B. Rice, S.D. Konecky, A.J. Durkin, B.J. Tromberg, “Spatial Frequency Domain Imaging Using Custom Patterns”, PCT/US15/10201 (International patent filed on 1/5/15, IP has been licensed)

2. K.P. Nadeau, A.J. Durkin, B.J. Tromberg, “Method for Extraction of Spatial Frequency Information for Quantitative Tissue Imaging”, PCT/US15/10278 (International patent filed on 1/6/15, IP has been licensed)

PRESENTATIONS
1. K.P. Nadeau, M.T. Ghijsen, T.B. Rice, E. Kwan, A.J. Durkin, B.J. Tromberg, “Novel information extraction methods and high-speed instrumentation in spatial frequency domain imaging”, OSA/SPIE European Conferences on Biomedical Optics, Munich, Germany, June 2015 (accepted)


Abstract of the Dissertation

High-speed, Quantitative Tissue Spectral Imaging in the Spatial Frequency Domain

By

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Doctor of Philosophy in Biomedical Engineering

University of California, Irvine, 2015

Professor Bruce J. Tromberg, Chair

The goal of this work is to enable high-speed, video-rate (>30 Hz) spatial frequency domain imaging (SFDI). SFDI is a non-contact, quantitative spectral imaging approach for mapping in vivo tissue optical properties, allowing for the determination of chromophore concentrations and structural parameters. Conventional SFDI employs spatially-modulated sinusoidal projection patterns of light to decouple absorption from scattering in the spatial frequency domain. In particular, three projected patterns per wavelength per spatial frequency are used for each timepoint. Typical SFDI imaging rates are currently on the order of seconds, preventing use in cases involving real-time intraoperative guidance and visualization of physiological signals such as heart rate and respiration.

To increase SFDI imaging speed, a 2D Hilbert transform demodulation technique and the use of binary, square wave projection patterns are demonstrated, mitigating bottlenecks related to frame count and pattern projection rate, respectively. The Hilbert demodulation technique allows for optical property and structural orientation mapping using a single frame of data for each spatial frequency, increasing data acquisition speed by threefold. Square wave patterns are
projected one to two orders of magnitude faster than sinusoids using standard SFDI hardware, eliminating the pattern refresh rate bottleneck. This approach is adapted to a real-time, multi-spectral SFDI instrument, using hardware triggering and a digital micromirror device (DMD) to generate square wave patterns having refresh rates faster than the camera exposure time. This system is capable of acquiring oxy/deoxy-hemoglobin, tissue oxygen saturation, and reduced scattering map data at 33 Hz.

Performance of the Hilbert demodulation and square wave pattern techniques are compared to conventional SFDI pattern schemes on tissue-simulating phantoms and an in vivo forearm model, showing agreement in absorption and reduced scattering maps to within 1% of conventional SFDI. The real-time SFDI instrument is applied to a pressure cuff occlusion and paced breathing model on an in vivo forearm, allowing for the acquisition of sub-second physiological signals and hemodynamics.
Chapter 1

Introduction

Quantitative tissue spectral imaging (QTSI) is a non-invasive approach for generating maps of absolute chromophore concentrations and structural parameters beneath the surface of biological tissue \textit{in vivo}. QTSI has a number of applications ranging from neurovascular coupling [1-3], to wound healing [4, 5] to cancer [6, 7]. QTSI typically employs near-infrared (NIR) wavelengths (650-1000 nm), where light is dominantly scattered in biological tissue in order to maximize penetration depth, probing tissue volumes invisible to the naked eye.

To quantify chromophore concentrations and structural parameters, the effects of absorption and scattering must both be accounted for in QTSI measurements. Several methods exist for separating absorption from scattering, including time domain [8] temporal frequency domain [9, 10] and spatial domain [11] techniques. This work, by comparison, focuses on technological advances for a spatial frequency domain approach for QTSI.

1.1 Spatial frequency domain imaging (SFDI)

The acquisition and analysis of light propagation data in the spatial frequency domain (SFD) allows for a non-contact approach to decouple scattering from absorption in biological tissue, and thus perform quantitative analysis [12]. The first implementation of an SFD technique employed a radially-varying periodic wave as a source [13]. This approach acquires diffuse reflectance measurements taken over several millimeters in the field of view, and performs Fourier analysis on this data to determine the absorption and reduced scattering coefficients ($\mu_a$).
and $\mu_s'$) at a single point. Since this technique requires diffuse reflectance measurements taken over a wide area in the field of view, spatial resolution is limited to a centimeter or greater. An alternative approach was developed, employing a periodic point pattern as a source [14]. In this case, a 2D Fourier transform is applied to the entire image, which warrants the determination of optical properties at each pixel in the image, resulting in a scan-free method for generating $\mu_a$ and $\mu_s'$ maps. Additionally, this approach overcomes the limit in spatial resolution associated with the spatial sampling of reflectance data employed in the first technique. However, there are issues associated with using pencil beam patterns. For example, light detected close to a pencil-beam source is likely to saturate an imaging camera, while light detected further is likely to be attenuated, and thus susceptible to noise.

An imaging modality known as spatial frequency domain imaging (SFDI) has been developed, which employs sinusoidal patterns of spatially-modulated light as an excitation source [15, 16]. In a similar fashion to the previous approaches, diffusely reflected light is analyzed in the SFD to decouple $\mu_s'$ from $\mu_a$. The use of sinusoidal patterns of light as a source mitigates the dynamic range issues associated with pencil-beam sources, by providing more uniform illumination to the field of view. SFDI has been used to evaluate absolute changes in tissue chromophores such as oxy/deoxy-hemoglobin, water, and structural parameters such as $\mu_s'$ spectra for a variety of tissue types including skin [5], brain [1], and kidney [17].

1.1.1 SFDI workflow

SFDI instrumentation, data acquisition, and data analysis have been described in detail previously [16]. Structured light is projected onto a sample using a spatial light modulator
(SLM), and a camera detects the diffusely reflected light emitted from the boundary of the sample. Fig. 1.1 shows a block diagram of an SFDI instrument.

**Figure 1.1**: Illustration of an SFDI instrument. Patterned light is projected onto a sample using a light source coupled with a spatial light modulator (SLM) inside a projection unit. The diffusely reflected light is then coupled to a lens and detected by a charge-coupled device (CCD) inside a camera.

In conventional, 3-phase SFDI, three frames of data are acquired at relative modulation phases of 0, 120, and 240 degrees for each AC spatial frequency. These phase-offset images are applied to a simple formula to extract the AC information content pixel-by-pixel, shown below in Eq. 1.1. Here, $R_{AC}$ and $R_{DC}$ represent the demodulated AC and DC reflectance, and $I_{\phi}$ denotes the measured intensity at a given phase angle ($\phi$). We assume that the pattern propagates along the “x” axis, with $\omega_x$ representing angular frequency.

**Equation 1.1**: 3-phase AC component demodulation formula

$$R_{AC}(x, \omega_x) = 2^{1/2} \cdot \left( \frac{1}{3} \cdot \left( [I_{0}(x) - I_{120}(x)]^2 + [I_{120}(x) - I_{240}(x)]^2 + [I_{240}(x) - I_{0}(x)]^2 \right) \right)^{1/2}$$

Where
\[ I_\phi(x, y) = \frac{R_{DC}}{2} + \frac{R_{AC}}{2} \sin(\omega_x x + \phi) \]

A simulation of this demodulation approach using three phase-offset modulation patterns is shown in Fig. 1.2. All data processing and computation used to produce figures in this section was performed using the MATLAB software suite (MATLAB and Statistics Toolbox Release 2011b, The MathWorks, Inc., Natick, Massachusetts).

**Figure 1.2** – (a) Simulation of 3-phase demodulation technique on a turbid sample with uniform scattering and a circular absorbing lesion in the center of the field of view. Images are acquired at relative modulation phases of 0, 120, and 240 degrees. These images are then applied to a 3-phase demodulation formula (Eq. 1.1), which extracts the AC information content from the sample pixel-by-pixel. (b) Plot of reflectance cross-sections taken from the center row (dashed line shown in a) of each AC+DC image, and the resulting AC+DC information envelope (left). Also shown is a cross-section of the demodulated DC (taken from additional planar image) and AC information.
content (right). The DC cross-section is more sensitive than AC to absorption, while the AC cross-section is more sensitive than DC to scattering.

Next, the demodulated intensity data from the sample is calibrated to that of a tissue-simulating phantom having known optical properties. This calibrated diffuse reflectance data at each spatial frequency is then applied to a light transport model such as diffusion or Monte Carlo, from which $\mu_a$ and $\mu_s'$ maps are determined. Finally, these maps are generated at several wavelengths, and are fit to known chromophore spectra. An illustration of the SFDI workflow is shown on Fig. 1.3. The sample of interest in this case is a porcine kidney in a preclinical partial nephrectomy model. This SFDI application will be described in the following section.

Figure 1.3: Illustration of SFDI workflow. (top-left) a minimum of 2 spatial frequencies are employed, consisting of a single DC frame and 3 phase-offset AC frames. (bottom-left) The demodulated spatial frequency images of the sample are calibrated to a tissue phantom having known optical properties. (bottom-right) A light transport model is applied to the calibrated spatial frequency images to determine optical properties. (top-right) By determining
absorption maps at multiple wavelengths are fit to known chromophore spectra to determine absolute chromophore concentrations.

### 1.2 A preclinical example: partial nephrectomy

Partial nephrectomies are performed for small renal masses to preserve maximal renal function. During this operation, the renal artery is clamped to prevent the entry of blood to the parenchyma of the kidney, thus minimizing blood loss and providing a clear field of view during surgery [18-20]. Unfortunately, clamping the renal artery causes renal ischemic reperfusion injury (IRI), which is characterized by kidney injury during an extended period of vessel clamping, and reperfusion after release of the clamps. This condition is known to cause permanent renal damage to the entire organ [18, 19]. The lack of an accurate assessment of the severity and extent of renal injury during renal artery clamping is one of the major challenges all urologists face during a partial nephrectomy. A recent U.S. National Cancer Institute Surveillance Epidemeology and End Result (SEER) study showed that the percentage of patients developing any acute or chronic renal injury estimated to be 50.3% after partial nephrectomy, and 82.2% after radical nephrectomy [21]. In order to preserve maximal renal function by minimizing IRI, there is a critical need to study and understand the pathophysiology of IRI.

Currently, there is no mechanism of measuring real-time IRI during partial nephrectomy. The current method for determining IRI utilizes the patient’s post-operative creatinine and estimated glomerular filtration rate (eGFR) to monitor for acute and chronic renal injury. Although these measurements may determine whether or not IRI has occurred after the procedure, they cannot be used during surgery. While hyperspectral imaging approaches have been used by other groups to monitor changes during partial nephrectomy, [22-25] there are certain limitations. For one,
conventional hyperspectral imaging is unable to decouple absorption from scattering in its measurements. Therefore, this imaging technique can only track relative changes in hemodynamics such as tissue oxygen saturation (StO2). Also, while StO2 is an index for tissue metabolism, it has not yet been used directly to track IRI.

We present datasets obtained during arterial occlusion of three porcine kidney models using SFDI, a type of quantitative wide-field functional imaging that employs NIR wavelengths. We demonstrate the ability of SFDI to produce absolute, quantitative values of oxygen saturation (StO2) and the reduced scattering coefficient (μs') of the kidneys during renal arterial occlusion.

1.2.1 Materials and methods

Our SFDI instrument consists of 3 high-power light emitting diodes (LEDs) with peak emission wavelengths of 658, 732, and 850 nm, with a power of <1mW/cm2 delivered to the surface of the tissue for each LED. The LEDs illuminate a digital micromirror device (DMD) (Texas Instruments, DMD Discovery 1100, Texas Instruments, Inc., Dallas, TX). The DMD is used to spatially modulate the LED light, and project sinusoidal intensity patterns onto the kidney. In this set of experiments, we employed two spatial modulation frequencies (0 and 0.2 mm⁻¹). For each spatial frequency, the intensity pattern is projected at three distinct phases (0, 120, and 240 degrees). The diffusely reflected light from the kidney is imaged onto a charge-coupled device (CCD). In order to correct for errors due to the curvature of the kidney, we also performed a profilometry calibration measurement on a sample with known optical properties [26]. To mitigate specular reflection, the projector and camera are equipped with crossed-polarizers.
In order to derive optical property maps, we fit the detected amplitude of the diffusely reflected light from the CCD at each pixel to a semi-infinite, homogeneous, Monte Carlo transport model, employing a fast lookup table approach using cubic spline interpolation on the diffuse reflectance data at two spatial frequencies (0 and 0.2 mm\(^{-1}\)). This method has been validated for separating absorption from scattering over a broad range of \(\mu_s'/\mu_a\) values spanning from \(-0.5-300\) [27]. Once the \(\mu_s'\) and \(\mu_a\) values were separated by SFDI, we calculated hemodynamic parameters from the scatter-corrected \(\mu_a\) values assuming that oxy/deoxy-hemoglobin were the primary absorbers in the sample. From these values, we computed StO\(_2\) from the percent ratio of oxy to total hemoglobin concentration. Data processing in this section was executed using the MATLAB software suite (MATLAB and Statistics Toolbox Release 2010b, The MathWorks, Inc., Natick, MA).

The renal arterial occlusion procedure was performed on 30kg, female Yorkshire pigs. The porcine kidney, ureter and bladder have an anatomical location and function similar to that of the human urinary system [28, 29]. We performed the procedure under general anesthesia after the pigs were intubated. Next, we positioned the pigs in the supine position, and a midline incision was made from the sternum to the pubis to enter the peritoneum. Then we mobilized the intraperitoneal organs away from the kidney to dissect the kidney in the retroperitoneum. We then isolated and dissected the renal artery to prepare for occlusion. Occlusion was performed using an occlusion balloon cuff (Docxs Biomedical, Ukiah, CA) driven by a syringe pump (NE-1000, New Era, Farmingdale, NY), and an ultrasound flow probe (TS-420, Transonic System, Ithaca, NY) was placed downstream to monitor blood flow to the kidney. Our SFDI instrument was then positioned at the bedside next to the pig with the kidney exposed. The entire system was suspended above the kidney via an adjustable arm.
Image acquisition was performed over a period of 80 minutes in increments of approximately two minutes, with each data acquisition lasting approximately 10 seconds. We began acquiring images 10 minutes prior to arterial occlusion in order to establish a baseline for our StO₂ values. Arterial occlusion was established by rapidly inflating the occlusion balloon. Upon full expansion, blood flow was restricted to the kidney, and complete occlusion was confirmed by the flow-meter throughout the study. We continued acquisition for a period of approximately one hour while blood flow was restricted to the kidney, and also for an additional 10 minutes after reperfusion. In order to confirm that renal ischemia injury had occurred in our model, we also extracted tissue samples from the kidney at various time points. We employed a 18 gauge x 25 cm biopsy needle (Maxcore, Bard Medical, Covington, GA), and extracted two core samples from the superior pole kidney 3 just prior to occlusion, 5, 15, 30, 45 and 60 minutes during occlusion, and 10 minutes after reperfusion.

1.2.2 Results and discussion

Our StO₂ and μ₅’ (at 850 nm) datasets are shown in Fig. 1.4. We chose 850 nm for the scattering wavelength since light penetrates tissue deeper here than the other wavelengths available on our system (658 and 732 nm). For each dataset, we examined a region of interest (ROI) located in the top-right portion of the kidneys (Fig. 1.4a). This ROI is 50x50 pixels, which corresponds to a field of view of roughly 1x1 cm. This ROI was chosen to avoid the effects of specular reflection in the center of the kidney, as well as errors due to curvature, which can be seen on the right edge of the kidney in the StO₂ and μ₅’ maps.

Our StO₂ results for the three kidneys are shown in Fig. 1.4b. Here we see an initial mean percentage of approximately 60%. Upon arterial occlusion, the mean StO₂ drops rapidly,
reaching a value of approximately 20% within five minutes. Upon reperfusion, we see a rapid increase in mean StO\textsubscript{2} to 55%, recovering to near the baseline level of 60% within 10 minutes.

SFDI has the ability to decouple \(\mu_s'\) from \(\mu_a\). Using this capability, which in terms of wide-field spectral imaging approaches is unique to SFDI, we are able to generate wide-field \(\mu_s'\) maps, as well as scattering spectrum maps. In this analysis, all \(\mu_s'\) values were taken from three ROI’s, with additional ROI’s located 1 – 1.5 cm to the left and right of ROI shown in Fig 1.4a. Fig. 1.4c shows a plot of the average \(\mu_s'\) for kidney 1 at the three ROI’s, with the scattering spectrum embedded at the top at two time points. Here we see a decrease in the scattering power (SP), which is related to the scattering spectrum decay rate. From the beginning of occlusion (solid line) to the end of occlusion (dashed line) we see a decrease in SP from 1.05 to 0.88.
Figure 1.4: a) Color, StO$_2$, and $\mu_s'$ images of kidney 1 before, during, and after arterial occlusion. b) Average StO$_2$ percentages for three kidneys before (<8 mins), during (8-68 mins), and after (>68 mins) occlusion with standard error bars. c) Plot of mean $\mu_s'$ (850 nm) of kidney 1 at three regions of interest with standard error bars, and $\mu_s'$ spectra 6 mins after occlusion (t=14), and 2 mins before reperfusion (t=66), with corresponding fits and standard error bars.

StO$_2$ values obtained prior to clamping could be informative in terms of the health of the kidney prior to clamping. As mentioned in the introduction, conventional hyperspectral imaging techniques are only able to monitor relative changes in StO$_2$. Since SFDI decouples absorption
from scattering, we are able to derive absolute values of StO$_2$ from our measurements. The health of the kidney prior to surgery may not be well characterized for a given patient. Therefore, baseline, absolute StO$_2$ measurements could be valuable in partial nephrectomy procedures. The mean StO$_2$ for the three kidneys was 58.9\%, with a standard deviation of 3.7\%. This value is lower than what has been reported previously using hyperspectral imaging on a similar model, which is around 70\% \cite{22-25}. Since we use NIR illumination, our mean interrogation depth is deeper than previous techniques. Methods that employ visible light should produce higher StO$_2$ values since they interrogate more superficial, high blood flow regions of the renal cortex.

For each kidney, the amount of total hemoglobin increased during occlusion from beginning to end, with mean beginning and end values of approximately 250 μM and 350 μM respectively. The increase in total hemoglobin during occlusion appeared to be due primarily to deoxy-hemoglobin. In the absence of venous clamping, this is likely the result of retrograde flow of deoxygenated blood into the kidney.

In addition to establishing an accurate baseline for StO$_2$, it may be possible to monitor kidney function with SFDI during IRI. In particular, changes in $\mu_s'$ could potentially be used as a metric for oxidative stress and renal injury. The $\mu_s'$ values at 850 nm for kidneys 1, 2, and 3 increased upon occlusion (0.97, 0.72, and 0.52 mm$^{-1}$ upon occlusion vs. 0.73, 0.63, and 0.48 mm$^{-1}$ for baseline values, respectively). Throughout the course of the occlusion, we saw a significant decrease in $\mu_s'$ in the three kidneys of 0.06, 0.05, and 0.09 mm$^{-1}$. A previous study using SFDI in an occlusion model also shows a decrease in $\mu_s'$ \cite{30}. After reperfusion, the $\mu_s'$ values for each kidney returned to within 0.02 mm$^{-1}$ of the baseline.
The scattering spectrum has been used previously to assess tissue scattering particle composition. In particular, the scattering power (SP) has been related to average scattering particle size [4, 31]. In the case of Rayleigh scattering, SP is 4, and is roughly 1 in the case of Mie scattering. We observed a substantial decrease in the SP in each kidney during occlusion. In particular, from the beginning to end of occlusion, SP decreased from 1.05 to 0.88 in kidney 1 (16% decrease), 1.54 to 1.28 in kidney 2 (17% decrease), and 1.36 to 1.07 in kidney 3 (21% decrease). The decrease in SP during occlusion implies an increase in average scattering particle size. This is likely due to tissue changes such as edema that follow from occlusion and inflammation [32].

Epithelial cells in the kidney are known to succumb to injury and inflammation due to ischemia, which increases the permeability of the cell to its outside environment [32]. This structural alteration on the cellular level may correlate to the decrease in $\mu_s'$ and SP that we see in the three kidneys during occlusion. In order to examine this effect we performed hematoxylin and eosin (H&E) and periodic Acid Schiff (PAS) stains on kidney biopsies. In Fig. 1.5 shows results from samples that were obtained during the experiment at specific time points. Here we demonstrate changes in the proximal tubules, which are known to be highly susceptible to the progression of IRI [32]. The sample shown in Fig. 1.5a was extracted just prior to occlusion, and shows normal proximal tubules. In Fig. 1.5b, there is separation of the brush borders on the epithelial cells, and blurring of the cell borders, which occurred after 15 minutes of occlusion. In Fig. 1.5c, after 60 minutes of occlusion, we see complete detachment of the epithelial cells from the basement membrane, as well as disappearance of the cell borders, which is indicative of an increase in cell permeability, and cellular edema. The average decrease in $\mu_s'$ and SP in the three kidneys during occlusion could be associated with these structural changes.
Figure 1.5: Pathology slides (40×) showing the cross section of proximal tubules from kidney 3 (a) before occlusion (normal), (b) after 15 minutes of occlusion (separation of brush borders, shown by black arrow), and (c) after 1 hour of occlusion (cell borders have disappeared, white arrow, and cells are detached from basement membrane, gray arrow).

In the future, we plan to correlate optical and physiological property changes with serum markers such as creatinine and lactate, as well as with biomarkers of renal injury such as Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Kidney Injury Molecule -1 (KIM-1) [33]. In addition, we will acquire data at 970 nm in order to quantify water content, an indicator of edema and inflammation. We expect that these studies will provide more detailed information on kidney viability and will move us closer toward developing a validated, real-time method for monitoring and predicting kidney IRI.

1.3 Motivation for high-speed SFDI

Current SFDI approaches are limited with respect to the speed at which data can be acquired. This limitation in data acquisition speed in SFDI translates to a limit in the rate at which we can probe temporal dynamics, and amplifies the effects of motion on our measurements [34]. Several
important physiological processes occur on sub-second timescales which we are currently unable to probe using SFDI, including respiration rate, heart rate, and neurovascular coupling.

An example of SFDI motion artifacts are shown in Fig 1.4b in the previous section. Here, low frequency variations are seen in the StO₂ tracing during occlusion. These variations are due to a mismatch between the respiration rate of the model and our data acquisition start point at each timepoint, causing height changes over the course of the acquisition period (>1 second). We implemented a height correction technique in this experiment. However, the height correction data is acquired once per timepoint, and thus any variation in height during the data acquisition period will result in error. Ideally, data acquisition could be performed in real-time to capture this respiration rate signal, and mitigate motion artifacts. Fig. 1.5 shows an image of a phantom with and absorbing feature having spatial distortions due to motion. Spatial localization of absorbing lesions is a key feature of SFDI, and therefore it is critical to mitigate these spatial localization artifacts.

Figure 1.6: SFDI image of circular absorber with (left) no motion in the sample and (right) motion in the sample

In this work, we present techniques for reducing frame count and increasing frame rates for high-speed SFDI, as well as a video-rate, multispectral SFDI platform capable of imaging at 33 frames per second.
Conventional SFDI employs a demodulation method requiring a total of three frames of data for each modulating (AC) spatial frequency [15, 16]. These frames correspond to sinusoidal pattern phase offsets of 0, 120, and 240 degrees, and are combined to extract information content at each AC spatial frequency. The need for having three frames of data taken at each AC spatial frequency limits the speed at which SFDI data can be acquired, and is one of the primary bottlenecks preventing the implementation of a real-time SFDI platform. One component of this work is to find new ways to reduce the data acquisition time of SFDI by using fewer frames of data, while at the same time preserving information content. These techniques will be discussed in Chapter 2.

The use of sinusoidal patterns in conventional SFDI presents a data acquisition bottleneck that limits SFDI imaging speed. Current SFDI instruments typically use digital micromirror devices (DMD’s) to project spatially modulated light onto the sample. These DMDs are based on arrays of mirrors. Each of the array elements flickers on and off several times to generate grayscale intensities, resulting in pattern refresh rates typically on the order of hundreds of Hz. High-end scientific-grade complementary metal-oxide semiconductor (sCMOS) cameras have the ability to acquire frames on the order of kHz or greater, exceeding the grayscale pattern refresh rate of DMD’s. In many situations, such as those where the sample is susceptible to motion artifacts (e.g. clinical data acquisition) or high temporal dynamics are required (e.g. neuroscience/cerebral hemodynamics) [6, 7], data acquisition speed is critical. Additionally, certain applications require multiple spatial frequency components, most notably SFD tomography, which relies on the spatial frequency dependence of depth penetration in turbid media [35, 36]. Ideally, multiple AC (non-planar) spatial frequency components could be extracted from a sample simultaneously, although this not possible using sinusoidal patterns. Square wave patterns contain frequency
components at the harmonics of the fundamental frequency, which can be synthesized into each SFDI frame, increasing the amount of spatial frequency information embedded into each frame of data. In Chapter 3, we will discuss the implementation of binary patterns for high-speed SFDI.

To acquire SFDI data at video rates, we developed a hardware-triggered system capable of mapping absolute concentrations of oxy/deoxy-hemoglobin and $\mu'_s$ at 33 frames per second, allowing for the detection of physiological signals such as respiration and heart rates. Using this system, we enable video rate motion tracking and hemodynamic monitoring. Our video-rate SFDI platform will be discussed in Chapter 4.
Chapter 2

Reducing frame count

2.1 Hilbert transform demodulation technique

In this chapter, we first describe a method that attempts to optimize the tradeoff between SFDI data acquisition and image quality. This approach relies on a variant of a 2D Hilbert transform by employing a complex-valued spiral phase function in Fourier space. In this case, only a single frame of data is required for each AC spatial frequency, resulting in an increase in data acquisition speed. Additionally, using the spiral function allows for the extraction of spatial frequency information content from rotated sinusoidal patterns, which are used to elucidate tissue structural orientation in the SFD, measured by the scattering orientation index (SOI) \[37\]. We show agreement between our new technique and conventional, 3-phase SFDI by comparing diffuse reflectance maps taken at several spatial frequencies from a tissue-simulating phantom, $\mu_a$ and $\mu_s'$ maps taken from the volar forearm of a human subject, and SOI maps taken from structural orientation phantoms at various orientation angles. Our results show that SFDI data acquisition speed for mapping $\mu_a$ and $\mu_s'$ can be increased by two-to-threefold using the proposed technique, depending on the number of AC spatial frequencies used. Additionally, in Chapter 2.2, we show that data acquisition speed for probing tissue structural orientation can be increased threefold.

In order to decouple scattering from absorption, at least two spatial frequencies are required. In the case where the minimal number of frames are taken, 0 mm$^{-1}$ (planar illumination) and 0.2
mm\(^{-1}\) are typically used. Since there is no spatial variation in the spatial modulation pattern at 0 mm\(^{-1}\), only one phase is required, so a minimum of four frames (per wavelength) total are needed in 3-phase SFDI, consisting of a single DC frame, and three phase-offset, AC frames. After demodulation, a fast lookup table is employed to determine \(\mu_a\) and \(\mu_s'\). The primary limitation with respect to the implementation of real-time SFDI is data acquisition time. In particular, as we currently practice the technique, the need to acquire three frames of data for each AC spatial frequency limits the speed of SFDI data acquisition. We have developed a technique to address this bottleneck, by reducing the number of frames required for each modulating frequency from three to one.

2.1.1 Materials and methods

The Hilbert transform is a ubiquitous tool in signal processing, with a wide variety of applications in the communication field [38]. The general principle is that a modulating double-sideband signal such as a sine or cosine contains redundant information; only one sideband is needed to extract the modulated information content. Using the Hilbert transform, one can derive a single-sideband expression for this modulated signal with no loss of information. This single-sideband expression allows for the extraction of the demodulated information content and phase map of the modulated signal. Recently, the concept of applying the Hilbert transform using spiral phase functions in 2D Fourier space to demodulate 2D curved patterns in space was developed by Larkin et al. [39, 40]. We have adopted the general concept of this approach, and have applied it to the SFDI workflow.
The modulated reflectance images obtained in SFDI can be described by Eq. 2.1, where \( f_{x,y} \) is the modulating spatial frequency, and \( \Phi_{x,y} \) is the phase.

**Equation 2.1: Spatial frequency domain imaging (SFDI) illumination pattern formula**

\[
I(x, y) = 0.5 \ast R_{DC}(x, y) + 0.5 \ast M(x, y)
\]

Where

\[
M(x, y) = R_{AC}(x, y) \ast \cos\{2\pi f_{x,y} + \Phi_{x,y}\}
\]

The purpose of demodulation is to extract the AC diffuse reflectance term \( R_{AC}(x, y) \) from the detected amplitude \( I(x, y) \). Using Euler’s theorem, a cosine function can be expressed as the sum of two complex exponentials, or sidebands in the frequency domain. As mentioned previously, the Hilbert transform is used to obtain a single-sideband expression for a double side-band function such as a cosine. Since a single-sideband function can be expressed as a complex exponential, demodulation is straightforward. The magnitude of the single side-band expression for SFDI modulation results in the diffuse reflectance we wish to obtain.

Our new SFDI demodulation approach employs a 2D Hilbert transform to SFDI frames by applying a spiral phase function to the image in 2D Fourier space. One unique aspect of this approach is that it can demodulate frames whose modulation patterns are rotated, or arbitrarily oriented. That is, the wavenumber of the modulating pattern can have arbitrary directionality with respect to the lateral imaging axes \((x,y)\). The spiral function is described in Eq. 2.2.

**Equation 2.2: Spiral phase function map**

\[
S(u, v) = \frac{u + iv}{\sqrt{u^2 + v^2}}
\]

Where \( u \) and \( v \) are the lateral coordinates in 2D Fourier space
In order to implement the Hilbert demodulation technique, the following steps are performed:

First, the DC component of the spatially modulated image, which consists of both AC and DC components ($I(x, y)$ from Eq. 2.1), is removed. A 2D FFT is then applied to the resulting AC image ($M(x, y)$ from Eq. 2.1). In 2D Fourier space, the transformed AC image is multiplied by a map generated using Eq. 2.3, having the same dimensions as the AC image. Next, an inverse FFT is applied to this product. The resulting image is similar to the original AC image, except that the modulating “cosine” is now a “sine”, i.e. the phase of the modulating wave is shifted by 90 degrees. Then the magnitude of this “sine” image is taken, which accounts for the complex contribution of the transformed map due to the orientation angle of the modulating wave, shown in Eq. 2.4. The resulting term, $H(x, y)$, represents the Hilbert transform of the original AC image ($M(x, y)$). Finally, $H(x, y)$ is multiplied by the complex unit and added to the AC component of the original AC image. The resulting magnitude is the demodulated AC diffuse reflectance, denoted in Eq. 2.3 by $R(x, y)$.

**Equation 2.3: Reflectance formula using 2D Hilbert transform**

$$R(x, y) = |M(x, y) + iH(x, y)|$$

**Equation 2.4: 2D Hilbert transform map**

$$H(x, y) = |FFT^{-1}[FFT(M(u, v)) * S(u, v)]|$$

A walkthrough of the Hilbert technique is shown in Fig. 2.1 using a simulated DC and AC + DC image. In this simulation the sample is highly reflective, such that the sinusoidal pattern is kept intact as the light reaches the boundary of the sample. In reality, we apply this technique to turbid tissue simulating samples or biological samples, which will be demonstrated in the
experimental results, but this virtual sample was chosen to clearly illustrate the Hilbert
demodulation concept. We expect that the Hilbert technique will yield results comparable in
quality to those shown in Fig. 2.1, so long as the reflected modulation pattern is greater in
amplitude than the camera noise. We expect that this constraint will typically be satisfied when
looking at biological samples.

Here we begin with a DC image at a uniform intensity of one, and an AC+DC image (Eq.
2.1) with an intensity varying from zero to one, with a modulation pattern oriented diagonally.
First, the DC component is removed from the AC+DC image, and an FFT is performed on the
resulting AC image. Next, the spatial frequency map of the transformed AC image is multiplied
with the complex spiral function map. The resulting map is then FFT inverted, and the
magnitude is taken. This “magnitude” image is then multiplied by the imaginary unit, and added
to the initial AC image (before Hilbert transform). The magnitude of this sum results in the
demodulated AC diffuse reflectance, which is uniform at an intensity of approximately 0.5.

It should be noted that the demodulated AC images obtained in Fig. 2.2 and subsequent
figures using the Hilbert technique contain residual ringing artifacts, which are due to the fact
that the spatially projected sinusoidal patterns are cutoff by the boundaries of the image, and are
therefore finite in length. We refer to the degradation in image quality as resulting from ringing
artifacts. In theory, these artifacts should increase in severity as the number of periods in the
modulation pattern in the image decreases. Therefore, one potential strategy to minimizing these
artifacts, particularly at lower spatial frequencies, is to use an SFDI instrument having a large
field of view. Alternatively, a window function such as a Gaussian or Hamming filter could be
applied to the image in post-processing [41], which will be the subject of future work.
Figure 2.1: Simulation of the 2D Hilbert demodulation method on a highly reflecting surface. First, the DC component of the modulated image is removed, and a fast Fourier transform (FFT) is performed on the AC+DC image. The resulting 2D map in Fourier space is then multiplied by a spiral phase function, consisting of a continuous, radially-varying map ranging in value from -1 to +1 in real and imaginary space. An inverse FFT is performed on the map, resulting in an image whose magnitude is the original modulated image phase-shifted by 90 degrees. This image is multiplied by the imaginary unit and added to the original image. The magnitude of this image results in the demodulated diffuse reflectance of the AC component from the original AC+DC image.

We performed a side-by-side comparison of our advanced Hilbert demodulation technique to 3-phase demodulation. To generate the data used to produce the images analyzed in this section, we employed a clinical SFDI system at a wavelength of 658 nm. This system has been described previously [42]. In our first experiment, we compared diffuse reflectance maps obtained on a tissue-simulation phantom at multiple spatial frequencies. This phantom consists of a silicone foundation consisting of India ink as an absorbing agent, and titanium dioxide as the scattering agent. The fabrication technique used to make these phantoms has been described previously [43]. Next, we compared $\mu_a$ and $\mu_s'$ maps extracted from an in vivo human forearm. Finally, we evaluated SOI maps taken on a structural orientation phantom consisting of a silicone-based
bottom layer (described above), and a top layer composed of sections of pleated air filters at various orientation angles. The SOI of these phantoms has been evaluated previously [37].

2.1.2 Results

_Tissue phantom reflectance experiment_

Demodulation in the SFDI workflow allows for the extraction of information content in the SFD, which is used to generate $\mu_a$ and $\mu_s'$ maps. **Fig. 2.2** shows a comparison between 3-phase SFDI and the Hilbert technique of demodulated, diffuse reflectance maps of a homogeneous tissue-simulating phantom. Shown are maps of demodulated reflectance at an AC spatial frequency of 0.2 mm$^{-1}$. Here, only the first phase ($0^\circ$) intensity image is applied to the Hilbert technique, while intensity images at three phases ($0^\circ, 120^\circ, 240^\circ$) are applied to 3-phase demodulation (shown in **Eq. 2.1**). Also shown are average demodulated diffuse reflectance results at five spatial frequencies evenly distributed from 0 – 0.2mm$^{-1}$ taken from the region of interest (ROI) shown in the black box. These spatial frequencies are typically employed in the SFDI workflow, and instrumentation and models have been shown to perform adequately in this range [4, 16]. Here we see good demodulation quality across the entire field of view, with pixel intensity differences between 3-phase and Hilbert generally within 5%. We also show agreement in diffuse reflectance values between the two techniques, with mean reflectance values within 1% within the ROI for spatial frequencies of 0.05, 0.1, 0.15, and 0.2 mm$^{-1}$. It should be noted that, although the sample in this case is homogeneous, the reflectance intensity over the field of view is not. This is due to the inhomogeneity of the light source, which is accounted for during calibration.
Figure 2.2: Demodulated reflectance images at 0.2 mm\(^{-1}\) of a tissue-simulating phantom using (a) conventional, 3-phase SFDI (4 frames), and (b) the advanced, Hilbert-based technique (2 frames). (c) Map of percent difference in demodulated reflectance between 3-phase SFDI and the Hilbert-based technique. (d) Plot of mean diffuse reflectance vs. spatial frequency for the region of interest (ROI), shown in a.

Accurate demodulation, and thus determination of AC information content in the SFD, is a necessary component of SFDI, and is what allows for the generation of \(\mu_a\) and \(\mu'_s\) maps. In the following section, we present \(\mu_a\) and \(\mu'_s\) maps extracted from a volar forearm using the Hilbert demodulation technique, and compare this data directly to \(\mu_a\) and \(\mu'_s\) maps derived using 3-phase demodulation.

**in vivo volar forearm experiment**

Optical property maps of a human volar forearm were calculated using a fast lookup table method, employing spatial frequencies of 0 and 0.2 mm\(^{-1}\). **Fig. 2.3** shows a comparison of \(\mu_a\) and
μ_s' maps of the forearm using the 3-phase (left column) and Hilbert (right column) demodulation techniques. Here we see agreement in optical property values between the two techniques, with the difference in mean μ_a and μ_s' values in the ROI (shown in the black box) being 0.2% and 0.15%, respectively.

**Figure 2.3:** *in vivo* optical property results taken from a human volar forearm. (a) Absorption (μ_a) and (b) reduced scattering (μ_s') coefficient maps derived from 4-frame, 3-phase SFDI (left) and the 2-frame, Hilbert (right) demodulation techniques. For μ_a and μ_s', the difference in optical property calculations over the region of interest (ROI, shown in black box) is 0.2% and 0.15%, respectively.

A key motivation for spatially modulating light in SFDI is to decouple scattering from absorption. Therefore, quantitative optical property mapping is an essential feature of the technique. **Fig. 2.4** demonstrates the ability of our new demodulation technique to produce μ_a and
μ′ maps in biological tissue that agree with the conventional demodulation method, which suggests that we can reduce this new technique to practice. The payoff using the new Hilbert technique is the reduction in frames of data required to derive μ_a and μ_s′, and thus an increase in data acquisition speed. In the case shown in Fig. 2.3, the number of frames reduced using the Hilbert technique over 3-phase demodulation is from four to two, resulting in a twofold increase in imaging speed. However, the payoff in speed increases further if more spatial frequencies are employed, asymptotically approaching a threefold increase.

Although the primary benefit of using the Hilbert technique over 3-phase demodulation is the increase in imaging speed, there are additional benefits. In particular, as shown in Fig. 2.2, the Hilbert technique can demodulate rotated, or oriented sinusoidal patterns using only one AC phase. Acquiring reflectance data at multiple sinusoidal pattern orientation angles is used to characterize tissue structural orientation in the SFD. In Chapter 2.2, we show orientation angle and contrast maps on tissue structural orientation phantoms using both the Hilbert and 3-phase demodulation techniques.

2.1.3 Discussion

One of the primary goals of quantitative tissue optical imaging is to separate scattering from absorption as quickly as possible to mitigate motion artifacts, and enable the visualization of dynamic signals. This work introduces the Hilbert demodulation technique, which seeks to increase SFDI data acquisition speed while minimizing losses in information content. Our results suggest that the Hilbert technique is accurate in determining optical properties compared to 3-phase SFDI using one half the number of frames for two frequencies, and approaching one third the number of frames for many spatial frequencies. The tradeoff in image quality using the
Hilbert approach comes primarily from ringing artifacts resulting from the finite length of the spatial modulation patterns. Our results demonstrate that these artifacts have a minor effect on our diffuse reflectance intensity measurements, and thus optical property and SOI calculations. Taken at several wavelengths, these optical property values are used to determine the absolute concentration of chromophores such as oxy/deoxy-hemoglobin, which are direct measures of tissue function. In our in vivo forearm experiment (shown in Fig. 2.3), we obtained optical property values within 1% of 3-phase SFDI. The degree to which this difference in optical property values translates to differences in chromophore concentrations will vary depending on several parameters, including the chromophore spectra, the wavelengths and spatial frequencies used, and the inversion model employed to fit for $\mu_a$ and $\mu_s'$. However, we expect that a 1% margin of error in $\mu_a$ and $\mu_s'$ calculations will be suitable for most applications.

In addition to reducing the number of frames needed, it should also be possible to further increase SFDI data acquisition speed using the Hilbert technique by exploring new modulation hardware that would otherwise be unavailable using 3-phase demodulation. Since we only need a single, arbitrary phase for each AC modulation frequency, we can use a mechanical object such as a printed film in transmission geometry to modulate light. These devices have several benefits over electronic SLM’s such as digital micromirror devices (DMD’s) that are typically used in SFDI. For example, they have no refresh rate, so there is no longer a data acquisition time bottleneck due to this feature. sCMOS cameras have the ability to image at several thousand frames per second. However, scientific-grade digital micromirror devices (DMD’s) have refresh rates on the order of single milliseconds. Therefore, in order to fully utilize these types of high-speed cameras, a DMD may not the preferred modulation tool. Assuming adequate reflectance
from the sample, allowing for millisecond or less frame rates, the use of a mechanical object for modulation could increase data acquisition speed by fivefold or more.

Ultimately, our goal is to acquire, process, and render optical property maps and SOI in real-time. Therefore, in addition to acquiring data, we must also process the data in real-time, which requires a certain amount of computation. In the case of 3-phase demodulation, the mathematical operators in the demodulation formula are not computationally demanding, having linear or near linear computational complexities, denoted in Big O notation by $O(n)$. $n$ in this case represents the number of digits used for each pixel, or simply each pixel since the operation is linear. The fundamental difference between the Hilbert and 3-phase demodulation techniques is the use of a 2D FFT, which is applied to the entire image in the Hilbert technique. The FFT operation currently has a minimal computational demand of $O(n log(n))$ operations [44], where $n$ in this case represents the number of pixels. Assuming an image having $10^6$ pixels (1000x1000), this translates to a computational demand of roughly an order of magnitude greater than the linear, or “simple” operations. The 3-phase formula consists of 12 simple operations for each pixel, whereas the Hilbert algorithm consists of 11 simple operations and two FFT’s, resulting in a computational demand of roughly 31 simple operations for each pixel. Consequently, in theory the Hilbert technique is expected to have approximately three times as much computational demand as the 3-phase technique. By implementing the two techniques on images having the same pixel dimensions and bit depths as those shown in Figs. 2.1-2.3 in MATLAB on a desktop computer with a 3.06 GHz quad-core processor (Core i7-950, Intel Corporation, Santa Clara, CA), we obtained computational times of 0.026 and 0.005 seconds for the Hilbert and 3-phase techniques, respectively, which corresponds to a fivefold increase in computational time. With
proper code optimization, the computational time of the Hilbert technique is not expected to prevent the implementation of a real-time SFDI platform.

In addition to mapping $\mu_a, \mu_s', \text{and SOI using fewer frames of data, the Hilbert demodulation technique has additional potential benefits that are not demonstrated here. One potential benefit to using the technique is an increase in profilometry data acquisition speed. As mentioned previously, the Hilbert transform allows for the determination of the phase angle of a modulated signal. The phase angle is derived by taking the inverse tangent of the ratio of the imaginary to the real component of the sum of the two terms $M(x, y)$ and $H(x, y)$, shown in Eq. 2.1 and Eq. 2.4, respectively. This relationship is shown below in Eq. 2.5. Here, the concept of extracting phase angle is applied to images.

**Equation 2.5: Phase angle formula**

$$ phaseAngle(x, y) = \tan^{-1} \left( \frac{\text{imag}(M(x, y) + iH(x, y))}{\text{real}(M(x, y) + iH(x, y))} \right) $$

Modulated light can be used in the SFDI workflow to correct for artifacts related to surface curvature. Currently, SFDI employs a 3-phase approach to compute phase angle maps, which are used to correct for surface curvature artifacts in reflectance data [26]. Using the Hilbert technique, it should be possible to perform a profilometry measurement using one frame of data instead of three, thus increasing profilometry data acquisition speed by threefold.

**2.2 Structural orientation: Spatial scattering contrast enhancement**
Probing the structural orientation of biological tissue non-invasively is an area of interest in the medical community, and has been made popular by diffusion tensor magnetic resonance imaging (DT-MRI). DT-MRI is most commonly used to probe white matter degradation in neurodegenerative diseases such as multiple sclerosis [45, 46], and has also been used to monitor muscle fibers [47] and collagen [48]. Optical techniques have also been developed to probe tissue structural orientation [49, 50]. However, these approaches only give a qualitative measure of tissue anisotropy and are incapable of wide-field imaging.

Konecky et al. first reported that, by rotating SFDI modulation patterns and acquiring diffuse reflectance maps at several projection angles, we can determine the orientation angle and magnitude of structures in biological tissue [37]. To assess the degree to which the underlying structures are oriented, we use a normalized quantity known as the scattering orientation index (SOI), shown in Eq. 2.6 [37]. Here, the SOI is determined by maximizing, for all projection angles, the reflectance taken at a given angle subtracted by the reflectance taken at the orthogonal projection angle, divided by the sum.

**Equation 2.6: Scattering orientation index (SOI) formula**

\[
SOI = \max \left\{ \frac{|g(\theta) - g(\theta + \pi/2)|}{|g(\theta)| + |g(\theta + \pi/2)|} \right\}
\]

**Fig. 2.4** shows SOI results taken on an *ex vivo* sample consisting of rat tail tendon collagen having known structural anisotropy [51], in a background of isotropically-scattering Intralipid (2%). Here we employ a spatial frequency and wavelength of 0.25 mm\(^{-1}\) and 658 nm, respectively. To visualize orientation, we employ a hue, saturation, value (HSV) map, where the intensity and color of each pixel corresponds to the magnitude and direction of orientation, respectively. HSV space has been used previously in DT-MRI for visualization [52]. Using a
total of 36 projection angles (angular resolution of 5 degrees), we are able to visualize collagen orientation.

![RGB image and Orientation image](image)

**Figure 2.4:** *ex vivo* scattering orientation results on rat tail tendon collagen in a background of 2% Intralipid. (left) a color camera image of the sample and (right) scattering orientation map in hue, saturation, value (HSV) space are shown. The intensity of the HSV map pixel corresponds to anisotropy magnitude, and the color denotes direction of orientation.

As noted in **Chapter 2.1**, the Hilbert demodulation technique has the ability to demodulate rotated SFDI patterns, and is thus applicable for speeding up scattering orientation data acquisition. If we wish to use the Hilbert technique to probe orientation, we must verify that it can produce similar structural orientation contrast as 3-phase SFDI. **Fig. 2.5** shows scattering orientation angle and contrast maps for the 3-phase and Hilbert techniques at an AC spatial frequency of 0.2 mm\(^{-1}\). The tissue structure orientation phantoms used consist of rectangular-shaped, pleated air filters having significant structural orientation, placed on top of a tissue-simulating phantom having minimal structural orientation. The orientation angle of the structure being probed is determined by the angle at which minimum diffuse reflectance is detected. We
see here that the average orientation angle for all three ROI’s (shown in white boxes) is within 1 degree, which is well within our angular resolution of 5 degrees.

**Fig. 2.5** shows SOI maps of tissue structural orientation phantoms using the 3-phase (left column) and Hilbert (right column) demodulation techniques. In general, the SOI values obtained using the Hilbert technique are within 10% of those obtained using 3-phase demodulation. In particular, the mean difference in the SOI is well within 10% for the three ROI’s, and within 2% for two out of three of the ROI’s. This demonstrates an overall agreement in SOI between results obtained using the Hilbert and 3-phase techniques.

---

**Figure 2.5:** Structural orientation results on structural orientation phantoms consisting of air filters with known structural anisotropy. Orientation angle maps derived from demodulated reflectance images using the (a) 3-phase and (b) the Hilbert technique. Scattering orientation index (SOI) maps using (c) 3-phase SFDI and (d) the Hilbert technique. Regions of interest (ROI’s) were analyzed in 3 filters (white boxes). The difference mean orientation angle determined by the left, top, and bottom ROI’s is 0, 1, and 0.75 degrees respectively. The difference in mean
scattering orientation contrast (SOI) in the left, top, and bottom ROI’s between the Hilbert technique and conventional SFDI is 7.8, 1.7, and 0.27% respectively.

Since the characterization of structural orientation in SFDI uses multiple projection angles of sinusoidal patterns, several frames of data are required. In the case shown in Fig. 2.5, the angular resolution in orientation analysis is five degrees. Since the orientation angle has a range of 0 – 180 degrees, 36 projection angles were employed. Using the 3-phase technique, this results in a total of 108 frames, while the Hilbert technique requires only 36 frames (one frame per projection angle). Thus, the Hilbert technique in this example increases imaging speed threefold over 3-phase demodulation.

It is currently unclear how the difference in SOI values between the 3-phase and Hilbert techniques will translate to quantifying structural orientation. The SOI is a qualitative index whose value depends on several parameters such as spatial frequency and wavelength. Moving forward, it will be important to evaluate the Hilbert technique in the context of quantitative orientation mapping, not just SOI. In the short term, there are enhancements that can be made that should improve orientation mapping using the Hilbert technique. For example, since the Hilbert technique uses fewer frames of data, there is more noise intrinsic to the demodulated images. The processing code used to spatially and angularly filter structural orientation data presented in Fig. 2.5 is optimized for 3-phase SFDI, so tailoring this processing code to Hilbert demodulation could improve SOI mapping accuracy. Additionally, the use of a camera having less pixel noise could also improve SNR, and thus potentially improve SOI mapping.

2.3 Reduced scattering extrapolation: Spectral scattering contrast enhancement
This section introduces a technique for determining $\mu_s'$ over a wide wavelength range by employing a power-law model fit to $\mu_s'$ values determined at a small number of discrete wavelength bands (5 in this case). By extrapolating $\mu_s'$, only planar (DC) frames are required for the remaining wavelength bands to determine both $\mu_a$ and $\mu_s'$, thus reducing frame count. In this experiment, we exhibit the $\mu_s'$ extrapolation approach on a preclinical model in the short-wave infrared (SWIR) region. Here, frame count is reduced by threefold over conventional SFDI.

Over the past two decades, optical methods have been widely employed to obtain information about the absorption and scattering content of biological tissues [12]. Recently, spatial frequency domain imaging (SFDI) has been developed to extract spatially resolved tissue properties at multiple wavelengths from reflectance measurements at multiple spatial frequencies [27]. SFDI studies have typically employed wavelengths in the near-infrared (NIR) (650 to 1000 nm).

Expanding the wavelength range of SFDI to include the short-wave infrared (SWIR) can potentially provide increased sensitivity to water [53] and lipids [53, 54], both of which have absorption features throughout the SWIR that are more prominent than those found in the NIR. Thus, SWIR imaging has the potential to complement NIR imaging approaches [55]. SWIR optical sensing of skin has previously been employed to spectrally characterize porcine [56, 57] and human [58, 59]. However, these measurements have largely been performed on ex vivo tissue samples using integrating sphere based techniques. In this letter, we report on integration of SFDI methods, which we have previously described elsewhere [4, 12, 27] with planar illumination and a SWIR camera capable of spectral imaging from 850 to 1800 nm. This combination of SWIR instrumentation and a hybrid SFDI procedure (using data from both structured and uniform planar light) enables us to obtain tissue absorption and scattering spectra well into the SWIR wavelength range.
The outline of this hybrid SFDI technique is as follows: (1) SFDI data from 850 to 1050 nm is employed to extract the tissue reduced scattering coefficient using a Monte Carlo fitting method. (2) The reduced scattering coefficients are fit with a power law to extrapolate the scattering spectrum at SWIR wavelengths. (3) The reflectance data from unstructured illumination and the extrapolated scattering spectrum are employed to solve for the absorption spectrum over the entire wavelength range. The hybrid SFDI method can extract tissue optical properties over an expanded wavelength range (850 to 1800 nm) that has not been heavily explored by SFDI or other tissue optics methods. Here, for a proof-of-principle experiment, we employed this hybrid SFDI procedure to characterize SWIR tissue optical properties in an in vivo rat model of partial-thickness burns. Following the controlled rat burn protocol, detailed in Nguyen et al. [4], partial thickness burns were produced on the lateral side of a shaved 500 g Sprague–Dawley rat using a brass burn comb heated to 100°C. The comb was placed in contact with the skin for 8 seconds, producing four deep partial thickness burn areas, 1×2 cm² in size. Upon conclusion of the experiment, the burn areas were resected and prepared for histological examination.

The imaging setup for the rat burn measurements is similar to that employed previously [4], except with a SWIR camera for detection. The sample was illuminated with multiple spatially modulated patterns of broadband light from a tungsten halogen lamp projected through a digital micromirror device (DMD, Texas Instruments, Dallas, Texas). A Raytheon Vision Systems minSWIR camera was employed to detect reflectance over 850 to 1800 nm. The camera contained an InGaAs detector array and a SB373 CMOS readout, and the lens was attached to a Perkin-Elmer (Waltham, Massachusetts) liquid crystal tunable filter (#131928 LNIR 850 to 1800 nm). The camera integration time was set to 32 ms. Images were acquired preburn and ∼2 hours postburn. At both time points, the data consisted of reflectance images taken in 50 nm steps from
850 to 1800 nm, using three spatial frequencies: 0, 0.05, and 0.1 mm$^{-1}$. With the hybrid SFDI method, we can use both efficiently transmitted unstructured light in the SWIR range and spatially modulated wavelengths in the NIR to extract the absorption coefficient ($\mu_a$) and reduced scattering coefficient ($\mu_s'$) spectra over the entire 850 to 1800 nm range.

First, spatially resolved maps of $\mu_a$ and $\mu_s'$ were obtained at the five shortest wavelengths (850, 900, 950, 1000, and 1050 nm) by fitting the spatial frequency-dependent reflectance images pixel-by-pixel to a Monte Carlo model of light transport. The extracted $\mu_s'$ maps at the aforementioned five wavelengths were fit with a power-law model, shown in Eq. 2.7.

**Equation 2.7: Model for reduced scattering coefficient ($\mu_s'$) vs. wavelength ($\lambda$) in tissue**

$$\mu_s'(\lambda) = A(\lambda/\lambda_0)^{-b}$$

where $\lambda$ is the wavelength of the light and $\lambda_0$ is a reference wavelength (set here to 850 nm). This power law is a well-known approximation for bulk tissue scattering [60, 61] and has previously been shown (via comparison with integrating sphere data) to be an accurate model for the scattering coefficient of biological tissue over the 850 to 1800-nm wavelength range [58, 59].

This procedure produced spatial maps of the reduced scattering amplitude $A$ and slope $b$. Second, a power law was employed to extrapolate the reduced scattering spectrum over the 1100 to 1800 nm range. A solution of 2% Intralipid was used as a reference phantom.

### 2.3.1 Results and discussion

The mean $\mu_s'$ spectra over a region of interest were calculated for the rat tissue preburn and postburn (Fig. 2.6). Over this region of interest, the (mean +/- standard deviation) value of the $\mu_s'$ amplitude was calculated to be $A = 1.33 +/- 0.02$ prior to the burn and $A = 1.55 +/- 0.01$ at $\sim 2$
h postburn, while the value of the $\mu_s'$ slope was calculated to be $b = 0.61 +/- 0.02$ prior to the burn and $b = 0.68 +/- 0.01$ at $\sim 2$ h postburn. This result suggests that the scattering parameters $A$ and $b$ have the potential to distinguish between burned and non-burned tissues as we have seen in our NIR rat burn studies.

**Figure 2.6:** Reduced scattering spectra of rat tissue preburn (blue) and postburn (red), with extrapolated (850 to 1800 nm) power-law reduced scattering spectra (solid curves) plotted alongside reduced scattering coefficient values obtained directly by pixel-by-pixel fitting (circles) with a Monte Carlo model for the wavelengths from 850 to 1050 nm. The error bars represent the standard deviation of the extrapolated reduced scattering coefficient over the region of interest. The error bars for the direct-fit scattering coefficient are not pictured, as they are within the diameter of the circles.

Finally, the extrapolated $\mu_s'$ values were employed to extract the $\mu_a$ maps from the unstructured illumination reflectance images. The extracted scattering amplitude $A$ and absorption coefficient $\mu_a$ obtained by this hybrid SFDI method at 1350 nm are shown in **Fig. 2.7** for rat tissue preburn and $\sim 2$ h postburn. We use 1350 nm as a representative example of an SWIR wavelength at which water absorption is notably ($\sim 5\times$) greater than at the 970 nm NIR
absorption peak [62]. The mean preborn and postburn SWIR \( \mu_a \) spectra over the region of interest denoted by the white box in Fig. 2.7 are shown in Fig. 2.8. As Fig. 2.9 suggests, many of the detected SWIR wavelengths (especially those in the 1100 to 1600 nm range) have the potential to provide \( \mu_a \) maps that may provide insight into tissue functional status, such as changes in hydration/edema.

Figure 2.7: (a) Digital color images (preburn and \( \sim 2 \) h postburn) of the region of the rat that was contacted with the burn comb. (b) Map of reduced scattering amplitude \( A \) (preburn and \( \sim 2 \) h postburn), from extrapolated power-law fit to reduced scattering coefficient obtained with SFDI from 850 to 1050 nm, over the region of the rat that was contacted with the burn comb. (c) Map of absorption coefficients at 1350 nm (preburn and \( \sim 2 \) h postburn), over the subregion denoted by the white box in the color images and scattering amplitude maps, obtained by using the extrapolated reduced scattering coefficient and the unstructured illumination reflectance data at 1350 nm.
The multispectral SWIR data illustrate a mean postburn increase of greater than 10% in $\mu_a$ over the 1150 to 1800 nm range. This change is attributed to increased water fraction caused by edema, as previously observed in partial-thickness burns using SFDI in the NIR regime [4]. The SWIR data also show a postburn decrease in $\mu_a$ from 850 to 900 nm, a postburn increase of 16% in the reduced scattering amplitude, and a postburn increase of 11% in the reduced scattering slope.

**Figure 2.8:** (a) Mean extracted absorption coefficients from 850 to 1050 nm for rat tissue preburn (blue) and ~2 h postburn (red) for the region of interest shown in Fig. 2.7. (b) Same as (a) but for 1100 to 1300 nm. (c) Same as (a) and (b) but for 1350 to 1800 nm. For all three panels, the error bars represent the standard deviation of the absorption coefficient over the region of interest.
This proof-of-principle experiment was constrained by several factors. First, at many wavelengths greater than 1050 nm that are associated with water absorption, the signal-to-noise ratio of the collected reflectance at spatial frequencies greater than $f_x = 0 \text{ mm}^{-1}$ was insufficient to determine optical properties by direct fit. Second, the light engine, through which excitation from the source must pass in order to obtain structured illumination, is a source of significant transmission loss through the optical system. Third, the integration of the SWIR camera with the SFDI system was not optimized to maximize SNR for the entire wavelength range (due to proprietary software constraints related to the Raytheon SWIR camera). Future work will involve refinements to the light delivery and detection method over the entire 850 to 1800 nm range, to improve signal-to-noise and enhance precision in the SWIR reflectance and tissue optical property measurements. In this study, we have combined an SFDI system with a SWIR camera that detects light from 850 to 1800 nm. This experiment serves as a proof of principle for two key concepts: (1) integration of SFDI with unstructured illumination and (2) use of this hybrid SFDI technique to measure tissue optical properties over an expanded wavelength range (850 to 1800 nm). As a specific biological example, we used the hybrid SFDI method to quantify in vivo burn related changes in the optical properties of rat skin over this wide wavelength range, which is not typically employed in SFDI experiments. The SWIR range provides two key advantages for enhanced tissue characterization: lower $\mu_s'$ relative to the NIR (enabling a $\sim 20\%$ increase in penetration depth at some wavelengths, such as 1100 nm, as compared to a typical penetration depth in the 650 to 1000 nm range) and increased sensitivity to absorption from water and lipids [63, 64]. These features of the SWIR regime also suggest potential for quantitative, noninvasive, wide-field burn severity analysis, which will be the topic of our future study.
Chapter 3

Increasing frame rate

This chapter describes a method for increasing SFDI data acquisition speed by an order of magnitude or greater by using binary, square wave patterns. To accomplish this, we have developed a multi-frequency synthesis and extraction (MSE) algorithm. Here, custom patterns having known Fourier series coefficients are applied to a sample. The sample acts as a low-pass filter, characterized by the sample’s spatial modulation transfer function (s-MTF), attenuating the spatial frequency components of the diffusely reflected light. By changing the amplitude and phase of the Fourier series components of the pattern, a system of equations is established for each Fourier component at each pixel in the image, and can be solved using a pseudoinverse. MSE has the flexibility of extracting an arbitrary number of frequency components from a sample, assuming the amplitude and phase of each component in the custom pattern is known, and the camera has high enough dynamic range and signal-to-noise ratio (SNR) to extract each component. In this case, we are using square wave patterns having discrete Fourier components at the harmonics of the square wave fundamental frequency.

For SFDI applications where higher AC (modulated) spatial frequencies are required, such as superficial tissue characterization and characterizing scattering contrast [37, 65, 66], square waves having a higher fundamental frequency can be used, such that the higher ordered terms exceed the s-MTF of the sample, and are not present in the reflected light. In sub-surface applications such as SFD tomography where multiple, relatively low AC frequencies are
required, square waves having a lower fundamental frequency can be used, such that a subset of the harmonic terms are preserved.

We show agreement between results obtained using square wave and sinusoidal projection patterns by comparing $\mu_a$ and $\mu_s'$ maps obtained from an in vivo human forearm. In the 1st experiment, we employ a high frequency square wave pattern to extract DC (planar) and single AC (fundamental) spatial frequency components using both conventional SFDI demodulation and MSE. In the 2nd experiment we employ a low frequency square wave patterns to extract multiple spatial frequency components (DC, fundamental, and 2nd harmonics) using MSE. Also shown are multi-frequency depth penetration results on a phantom containing a buried absorbing tube surrounded by a turbid medium. Here, DC, fundamental, and 2nd harmonic components are extracted from two low frequency square wave patterns having different fundamental frequencies. We show agreement to within 1% of conventional SFDI for mapping $\mu_a$ and $\mu_s'$ on an in vivo forearm, and determining reflectance vs. depth on an absorbing tube phantom. Our results imply that SFDI data acquisition speed for mapping $\mu_a$ and $\mu_s'$ and probing buried inclusions can be increased by an order of magnitude or greater with minimal losses in data quality using square wave patterns and MSE.

### 3.1 Materials and methods

In conventional SFDI, two dimensional sinusoidal patterns having a single modulation frequency are projected sequentially at 3 distinct phases (0, 120, and 240 deg). A remitted light image is captured for each phase per spatial frequency. A simple demodulation formula based on square-law detection [15, 67] shown in Eq. 1.1 is applied to the 3-phase detected light. Here,
ω and φ represent the angular frequency and phase angle of the projected pattern, respectively. The SFDI data collection process is repeated for a reference tissue simulating phantom having known optical properties. The data acquired from the reference phantom is used to normalize the sample intensity to account for the s-MTF of the SFDI instrument. Finally, the calibrated reflectance data at multiple spatial frequencies is used to derive the sample’s s-MTF, from which sample (unknown) optical property maps are determined.

Conventional SFDI uses sinusoidal patterns, and thus spatial frequency data is acquired sequentially. Here we present MSE, a new method for extracting spatial frequency information content, and thus capturing the s-MTF of turbid media, including biological tissue. To enable high-speed SFDI data acquisition, we make use of rapidly-generated binary square wave patterns containing multiple spatial frequency components.

3.1.1 Multi-frequency synthesis and extraction (MSE) technique

The goal of MSE is to use custom patterns having multiple spatial frequency components, and extract the attenuated spatial frequency components remitted from the sample. First, custom, multi-frequency illumination patterns are projected onto a sample at different phases, and a camera detects the remitted light. Each spatial frequency component in the custom pattern is simultaneously attenuated by the sample. We can express our series of raw intensity images as a vector (I), which is the product of the Fourier series coefficients of each frame with the reflectance at each spatial frequency component, shown in Eq. 3.2. In this case, we are assuming that our pattern will consist of a linear combination of sinusoids propagating in the “x” direction. We also allow the phase angle (φ) to change as a function of space. Here, C represents the frequency amplitude and phase maps for each projected pattern (i.e. the Fourier coefficient.
matrix). For consistency, we express each frequency component as a real-valued sinusoid, although single complex exponentials (analytical expression) can also be used. \( R \) represents the amplitude attenuation for each frequency component in the reflectance maps. \( k \) and \( p \) are the indices for Fourier component and projected pattern, and \( m \) and \( n \) are the total number of projected patterns and Fourier components, respectively.

**Equation 3.1: Multi-frequency synthesis and extraction (MSE) formula**

\[
I_p(x, y) = C_{k,p}(x, y) \ast R_k(x, y) \rightarrow R_k(x, y) = C_{p,k}(x, y)^{-1} \ast I_p(x, y)
\]

\[
I_p(x, y) = \begin{bmatrix} I_1(x, y) \\ \vdots \\ I_m(x, y) \end{bmatrix} \quad R_k(x, y) = \begin{bmatrix} R_1(x, y) \\ \vdots \\ R_n(x, y) \end{bmatrix}
\]

\[
C_{p,k}(x, y) = \begin{bmatrix} C_{1,1} \sin(\omega_{x,1} + \varphi_1(x, y)) & \cdots & C_{1,n} \sin(\omega_{x,n} + \varphi_1(x, y)) \\ \vdots & \ddots & \vdots \\ C_{m,1} \sin(\omega_{x,1} + \varphi_m(x, y)) & \cdots & C_{m,n} \sin(\omega_{x,n} + \varphi_m(x, y)) \end{bmatrix}
\]

In principle, MSE can be applied to any multi-frequency pattern, assuming that the phase and amplitude of each frequency component are known. Here we are employing binary square wave patterns for the aforementioned projection speed benefit. In general, a square wave pattern in one dimension can be expressed by a Fourier series, shown in \textbf{Eq. 3.3}. Here, \( d \) is the duty cycle of the square wave, denoted as the fraction of high to low intensity values, and \( \omega \) and \( \varphi \) are the angular frequency and phase, respectively. This is an infinite series, however, the low-pass filter nature of biological tissue eliminates higher-ordered harmonics, which can be neglected, assuming they have been sufficiently damped (order of magnitude or greater) relative to the previous terms in the Fourier series. This concept will be demonstrated towards the end of this section.
Equation 3.2: Fourier series of square wave

\[ \text{SquareWave}(x) = \frac{4}{\pi} \sum_{k=1}^{\infty} \left( \frac{1}{k} \right) \sin(\pi d) \cos(k \omega x + k \varphi) \]

\[ = \frac{4}{\pi} \left[ \sin(\pi k d) \cos(\omega x + \varphi) + \frac{\sin(2\pi d)}{2} \cos(2\omega x + 2\varphi) + \frac{\sin(3\pi d)}{3} \cos(3\omega x + 3\varphi) + \cdots \right] \]

**Fig. 3.1** illustrates a cross-section of a two dimensional square wave pattern. The duty cycle of the pattern can be adjusted to change the Fourier coefficients of each harmonic component. After interacting with a sample, the edges of the pattern are blurred, and thus the pattern appears more sinusoidal. In reality, a combination of multiple frequency components is embedded into the pattern.

**Figure 3.1:** Simulation of 1D cross-section of square wave pattern (50% duty cycle) interacting with turbid media. After interacting with a sample, the edge of the square wave is blurred in space. As a result, each frequency component is simultaneously attenuated.

To extract information from a given pattern using MSE, the amplitude and phase of each frequency component in the pattern must be determined. The amplitude coefficients are known from the analytical expression of the pattern itself. However, deriving the phase is non-trivial.
For one, the position of the phase of the pattern field generated will most likely not match what the camera detects. For example, the camera requires a field of view that is smaller than the projected pattern [68], thus the field-of-view of the camera will not match that of the projected pattern. Second, depending on the angle of the camera relative to the projected patterns, sample topography will affect phase angle [26]. We previously developed a technique using a variant of a 2D Hilbert transform to express SFDI images in their analytical form, from which amplitude and phase angle maps can be determined [69]. The phase maps generated using the Hilbert technique also adapt to surface topography. We have integrated the phase mapping capability of the Hilbert technique into MSE.

To demonstrate our new technique, Fig. 3.2 shows results on a simulated sample consisting of an absorbing lesion and a uniform scattering background. This is the simplest case of MSE where the sample filters out all AC frequency components in the square wave except for the fundamental. A damped square wave image, which appears sinusoidal, and a DC image are applied to the Hilbert technique to extract a phase angle map. Next, phase map coefficients are derived by using the phase angle map used in conjunction with the square wave Fourier series expansion. Finally, the Fourier coefficient matrix $C$ is inverted and multiplied by the raw intensity vector $I$ to obtain the reflectance vector $R$. 
Figure 3.2: (a) Simulated workflow of multi-frequency synthesis and extraction (MSE) algorithm on a turbid sample containing a circular absorbing lesion. First, a square wave (duty cycle = 50%) and a DC (planar) image are acquired. Here, the low-pass filtering properties of the sample damp the higher-ordered harmonics of the square wave, such that only the fundamental frequency component is preserved. These images are applied to a 2D Hilbert transform method, from which the phase angle map of the square wave pattern is derived. Using the known Fourier series representation of a square wave (Eq 3.3), the frequency coefficient matrix ($C_k$) is generated. Finally, $C_k$ is inverted and multiplied by the raw data vector ($I$). (b) Extracted spatial frequency intensities from simulation shown in (a), including (i) DC and (ii) fundamental frequencies. (iii) Cross-section of extracted reflectance.
comparing MSE to conventional, 3-phase SFDI.

For simplicity, Fig. 3.2 illustrates MSE for a damped square wave pattern having harmonic components which surpass the s-MTF limit of the sample, such that only a single frequency component remains in the reflected light. However, MSE has the flexibility to extract multiple spatial frequency components from a pattern. In this case, the phase angle for each frequency component cannot be derived directly from the Hilbert technique, since it relies on a single frequency spatial pattern. The fact that these low frequency patterns contain multiple frequency components results in the mapping of a weighted sum of the phase angles, so separating the phases of different frequency components is not possible. To circumvent this issue, we employed a single fundamental frequency sinusoidal pattern having the same fundamental frequency and phase angle as one of the corresponding square wave projection patterns. Here, the known amplitude of each harmonic component is taken from the analytical square wave expression, and the phase angle of each harmonic component and projection pattern (i.e. k and p from Eq. 3.1, respectively) are extrapolated from the sinusoidal pattern phase angle map.

In Chapter 3.3, we demonstrate that $\mu_a$ and $\mu_s'$ can be accurately determined on an in vivo forearm using a square wave pattern by extracting DC and fundamental frequency components, similar to Fig. 3.2. Here, a square wave pattern having a relatively high spatial frequency (0.28 mm$^{-1}$) is phase shifted three times (similar to Eq 1.1) and applied to both MSE and the 3-phase formula. A lookup table based Monte Carlo model is applied to extract optical property maps. Also shown are $\mu_a$ and $\mu_s'$ results using a lower fundamental frequency (0.06 mm$^{-1}$) square wave pattern, from which three spatial frequency components (DC, fundamental, and 2nd, harmonic) are extracted. Here, 7 frames of data are acquired at pattern phases evenly distributed from 0 to 360 degrees. A diffusion model is then used to determine $\mu_a$ and $\mu_s'$. Finally, we exhibit the
potential for MSE to extract data capable of layered or tomographic reconstruction by measuring reflectance vs. depth using two square wave patterns having different fundamental frequencies, and extracting DC, fundamental, and 2nd harmonic components from each.

To generate the data used to produce the images analyzed in this section, we employed a 2nd generation clinical SFDI system (VIS-NIR, Modulated Imaging Inc., Irvine, CA). All data processing and computation used to produce figures was performed using the MATLAB software suite (MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, Massachusetts).

### 3.3 Results

In a preliminary experiment, we show reflectance data taken on a tissue-simulating phantom having known optical properties using square wave patterns having different base frequencies and duty cycles. These results are shown in **Fig. 3.3**. Here, we use both a high and low fundamental frequency square waves to characterize spatial frequency component intensities of the reflected light in the phantom. Using the high frequency pattern (0.28 mm\(^{-1}\)), only the fundamental frequency component is preserved. This enables use of conventional, 3-phase demodulation (**Eq. 1.1**), since the reflected pattern is sinusoidal (shown in **Fig 3.3a**). The reflected light from the low frequency pattern (0.06 mm\(^{-1}\)), by comparison, contains harmonic components which are extracted, shown in **Fig. 3.3b**. The 3\(^{rd}\) harmonic (4\(^{th}\) term) in this case is corrupted by noise.
Figure 3.3: Square wave reflectance results on a tissue phantom having known optical properties ($\mu_s = 0.0177$, $\mu_s' = 1.0023$ mm$^{-1}$ at 659 mm$^{-1}$). (a) A high frequency (0.28 mm$^{-1}$) square wave appears sinusoidal as the light is reflected from the phantom (top). In this case, the DC (planar) and fundamental frequency from the pattern are extracted. (b) The light reflected from a low frequency (0.06 mm$^{-1}$) pattern contains harmonic components, which are extracted using multi-frequency synthesis and extraction (MSE). The 3rd harmonic component is corrupted by noise.

Fitting to the s-MTF requires a minimum of two spatial frequencies, which are used to decouple $\mu_s'$ from $\mu_a$. In the simplest case, we can employ a DC (planar) and a single AC (modulated) component to derive $\mu_a$ and $\mu_s'$ maps, which is demonstrated in the 1st experiment using a square wave pattern at a relatively high fundamental spatial frequency. In the 2nd
experiment, we demonstrate how multiple AC frequency components can be extracted from a sample using a single square wave pattern at several phases having a relatively low fundamental spatial frequency. We then use this data to map $\mu_a$ and $\mu'_s$. In the 3rd experiment, we show reflectance maps taken from a phantom consisting of a buried tube containing an absorbing dye occupying a range of depths surrounded by a background of Intralipid.

*High spatial frequency in vivo optical property extraction*

We performed a side-by-side comparison of optical property maps derived using two spatial frequency components extracted using conventional, 3-phase demodulation (**Eq. 1.1**) and MSE at a modulation frequency of 0.28 mm$^{-1}$, and a source wavelength of 659 nm, shown in **Fig. 3.4.** For conventional SFDI, 3 phase-offset sinusoidal patterns (**Eq. 1**) and a DC frame are taken to extract the DC and AC spatial frequency components. For MSE, 3 phase-offset square wave patterns with a duty cycle of 50% are taken. The 50% duty cycle was chosen to maximize the separation between the fundamental and nearest harmonic component to allow for optimal damping, since 50% duty cycle square waves have no even (i.e. 2$^{nd}$) harmonic components
Figure 3.4: Absorption and reduced scattering ($\mu_a$ and $\mu'_s$) maps generated using (left) sinusoidal patterns and 3-phase demodulation (conventional SFDI), square wave patterns and (middle) 3-phase demodulation and (right) multi-frequency synthesis and extraction (MSE) approaches, using 3 phase-offset square wave patterns at a wavelength of 659 nm. Here we see agreement in $\mu_a$ values derived using square waves in the region of interest (ROI, black box) to within 0.5% and 0.3% for 3-phase and MSE approaches, respectively. $\mu'_s$ values show agreement to within 0.2% and 0.5% for 3-phase and MSE approaches, respectively.

Fig. 3.4 shows agreement in optical property values to within 1% for both $\mu_a$ and $\mu'_s$ employing sinusoidal and square wave illumination using 3-phase demodulation (Eq. 1) and MSE approaches, respectively. These findings imply that, for cases where high spatial frequencies are required, square wave patterns can be employed instead of sinusoids, assuming the harmonic components surpass the s-MTF of the sample. The reason for this is because the higher ordered terms in the square wave pattern (i.e. $3^{rd}$, $5^{th}$, $7^{th}$ etc. harmonics) are highly attenuated relative to the fundamental term, and thus can be neglected in the MSE phase mapping and inversion algorithm. In this case, the conventional 3-phase demodulation equation
(Eq. 3.1) can also be used, since the pattern appears sinusoidal, and thus contains a single AC frequency. In Fig. 3.4 we decouple the effects of the square wave damping from MSE by demonstrating that both 3-phase demodulation and MSE are accurate in deriving $\mu_a$ and $\mu_s'$ on the same dataset.

It should be noted that, for square wave patterns to produce high fidelity optical property maps, the choice of spatial frequency of illumination is non-trivial. In the case above, we chose a fundamental frequency such that the higher-ordered harmonics are essentially eliminated due to the filtering properties of the sample. We found that, for most biological samples, a 50% duty cycle square wave with a fundamental frequency less than 0.25 mm$^{-1}$ will generate higher ordered terms on a typical biological sample ($\mu_a = 0.02$ mm$^{-1}$, $\mu_s' = 1$ mm$^{-1}$). In this case, the intensity of the next (3$^{rd}$ order) terms is roughly an order of magnitude less than the fundamental term. Additionally, since square wave patterns have only two unique intensity values (on or off), the number of projector pixels used to generate a single period of the pattern must be even (for 50% duty cycle), such that the duty cycle is consistent. The pixel length of the projected square wave period should also be divisible by the number of phases used in order to avoid duty cycle changes from phase-shifting the pattern.

To fully utilize MSE, the remitted light in the raw data images may contain multiple spatial frequency components, whereas the 3-phase approach (Eq. 1.1) relies on sinusoidal patterns (1 AC spatial frequency component). We show that this is possible in the next experiment using a pattern having a relatively low fundamental frequency, such that multiple frequency components are preserved, and can thus be factored into the inversion algorithm and extracted.
Low spatial frequency in vivo optical property extraction

In a similar manner to the previous experiment, we compared optical property maps derived using MSE to 3-phase demodulation. In this case, we use a square wave pattern having a relatively low spatial frequency, such that multiple frequency components are preserved. We extracted DC and 3 AC frequency spatial frequency components, and applied the DC, fundamental, and 2nd harmonic components to a diffusion model to determine $\mu_a$ and $\mu_s'$. Since we are using multiple AC frequencies, the accuracy of optical property fitting increases, since there are more s-MTF points along which to fit. These results are shown in Fig. 3.5, where 7 uniformly phase-offset square wave patterns having a fundamental frequency of 0.06 mm$^{-1}$ and a duty cycle of 75% are used to extract the DC, fundamental, 2nd and 3rd harmonic components, corresponding to 0, 0.06, 0.12, and 0.18 mm$^{-1}$, respectively. The 0, 0.06, and 0.12 mm$^{-1}$ component maps are applied to a diffusion model, from which $\mu_a$ and $\mu_s'$ maps are derived. These optical property values are compared directly to those obtained using 3-phase demodulation and sinusoidal patterns at the same spatial frequencies.
**Figure 3.5:** *in vivo* forearm results using square wave patterns and multi-frequency synthesis and extraction (MSE).

(a) Calibrated reflectance maps extracted at DC, fundamental (0.06 mm\(^{-1}\)), 2\(^{nd}\), and 3\(^{rd}\) harmonic frequencies. The 3\(^{rd}\) harmonic image is corrupted by noise. (b) Mean raw and calibrated reflectance values from forearm ROI (black box) at DC, fundamental, 2\(^{nd}\), and 3\(^{rd}\) harmonics. Calibrated reflectance values in the ROI agree with conventional SFDI to within 0.4%, 0.2%, 0.1%, and 5.6%, respectively. (c) \(\mu_a\) and \(\mu_{s}'\) maps derived using diffusion model fit and DC, fundamental, and 2\(^{nd}\) harmonic reflectance maps. Mean \(\mu_a\) and \(\mu_{s}'\) agree to within 0.2% and 1.0%, respectively.

Fig. 3.5 illustrates that higher ordered harmonics can be extracted using a single multi-frequency square wave pattern, and that \(\mu_a\) and \(\mu_{s}'\) values agree with those obtained using conventional, 3-phase demodulation and single-frequency sinusoidal patterns using a diffusion model. The use of multiple AC spatial frequency components increases the accuracy of optical property mapping, and thus quantitation of chromophore concentrations and structural parameters.
It should be noted that the presence of noise related to higher-ordered harmonics increases with the higher-ordered terms (i.e. 3rd harmonic in this case). This is due to the fact that the higher-ordered terms in a square wave pattern have less intensity relative to the lower-ordered terms. In Fig 3.5, we calculate optical properties using the DC, fundamental, and 2nd harmonic terms, and discard the 3rd harmonic term, which is corrupted by noise.

As mentioned in the beginning of Chapter 3, the mean interrogation depth of SFDI patterns in turbid media is dependent on the spatial frequency component; lower spatial frequencies penetrate deeper while higher spatial frequencies probe more superficial layers. Thus, SFD tomography is possible by extracting and analyzing multiple spatial frequency components. For these tomographic reconstructions to be accurate, each spatial frequency component in a given pattern must interrogate the appropriate depth. In the following section, we present results obtained from a tissue simulating phantom having a buried tube containing an absorbing dye. The results show that square wave patterns applied to MSE give similar reflectance maps to those obtained using 3-phase SFDI within a simple tomographic context.

*Depth penetration experiment using buried absorber phantom*

Multiple SFDI spatial frequency components can be extracted and processed to perform 3D reconstructions of buried absorbers [35, 36]. To test our ability to extract the correct depth information using square wave patterns, we applied relatively low spatial frequency square wave patterns (0.06 and 0.09 mm\(^{-1}\)), with a duty cycle of 75%, utilizing the DC, fundamental, and 2nd harmonic spatial frequency components, to a tissue phantom containing a buried absorbing tube oriented diagonally in depth, such that the depth of the tube ranged continuously from 0 to 5 mm beneath the surface. The tube contained a solution of 0.5 g/L of a NIR absorbing dye (NIR746A,
QCR Solutions Corp., Fort St. Lucie, FL). This formulation was chosen such that the $\mu_a$ of the tube mimicked venous blood at 731 nm. The background contained a 1% solution of Intralipid, which has a $\mu_s'$ comparable to human skin [70, 71]. **Fig. 3.6** shows results comparing reflectance maps taken at 731 nm using sinusoidal patterns combined with 3-phase demodulation, and square wave patterns combined with MSE.

**Figure 3.6:** Multi spatial frequency reflectance results obtained on a phantom containing a slanted absorbing tube ranging in depth from 0 to 5 mm, containing an absorbing dye with a scattering background. (a) Schematic of phantom geometry. (b) Reflectance maps calibrated to a homogenous tissue-simulating phantom are derived for
DC (0 mm$^{-1}$), 0.06, 0.09, 0.12, and 0.18 mm$^{-1}$ using the fundamental and 2$^{nd}$ harmonic components from two square wave patterns. (c) Cross-sections of calibrated reflectance taken from horizontal line in center of image where tube is located. Mean reflectance values along the line agree to within 0.2%, 2.3%, 1.4%, 1.1%, and 2.9% for 0, 0.06, 0.09, 0.12, and 0.18 mm$^{-1}$ respectively.

The results shown in Fig. 3.6 indicate that the multi spatial frequency components extracted using MSE and square wave patterns yield depth penetration reflectance similar to 3-phase demodulation using single frequency patterns for DC, fundamental, and 2$^{nd}$ harmonic components. This implies that MSE and square wave patterns can be used to extract multi-frequency datasets used in SFD tomography.

3.4 Discussion

SFDI has the ability to provide information-rich datasets based on the acquisition and analysis of spatial frequency domain reflectance maps, allowing for quantitative analysis of biological tissue. SFDI data should be acquired as quickly as possible to enable the visualization of dynamic signals and mitigate artifacts related to motion. To maximize data acquisition speed, frame rates should be limited to the camera exposure time. Sinusoidal projection patterns used in conventional SFDI take significantly longer to project than the exposure times of most high-end, scientific-grade cameras, resulting in a data acquisition speed bottleneck. This work introduces the combined use of a new signal processing technique (MSE) and the use of square wave projection patterns, which can be generated faster than frame rates of current high-end cameras.

Our results demonstrate that $\mu_a$ and $\mu_s'$ maps derived using MSE and square wave patterns are essentially identical to those derived from conventional SFDI. In the 1$^{st}$ experiment, we
employed a square wave pattern with higher-ordered harmonic components that exceed the s-MTF limit of a biological sample, a configuration suitable for cases where sensitivity to superficial tissue layers or scattering is emphasized. Here, the fundamental component is left intact, and the reflectance information contained in this pattern is extracted. We obtained $\mu_a$ and $\mu_s'$ maps with agreement to within 1% of conventional SFDI. In the 2nd experiment, we used a square wave pattern having a relatively low fundamental spatial frequency, such that the fundamental and harmonic components are left intact. This configuration is suitable for cases where probing deeper tissue layers or s-MTF fitting accuracy is emphasized. Here we derived reflectance maps at DC, fundamental, and 2nd harmonics, and computed $\mu_a$ and $\mu_s'$ that agree to within 1% of those derived using conventional SFDI. The requirement for optical property fitting accuracy will depend on the chromophores or structural parameters of interest, and the application. However, we expect that a 1% margin of error for $\mu_a$ and $\mu_s'$ will be acceptable for most cases.

In the 3rd experiment, we demonstrated the potential for MSE and square wave patterns to generate SFD tomography datasets. We applied two square wave patterns having relatively low fundamental frequencies to a phantom containing a buried, slanted absorbing tube having a continuum of absorber depths as a function of lateral spatial location. Here, reflectance values obtained along the tube at multiple spatial frequencies using MSE and square wave patterns agree to within 1% of conventional SFDI. Importantly, our ability to control optical path length with spatial frequency is clearly evident as the deeper portions of the tube disappear from view at higher spatial frequencies.

A single snapshot optical properties method (SSOP) has previously been developed, which employs a single sinusoidal pattern to map optical properties, reducing frame count over
conventional SFDI [72]. Here, a one-dimensional Fourier is applied to each row or column in the intensity image, and the spectrum is separated into a DC and AC component. Although this technique reduces data acquisition time by using fewer frames, the approach is limited to single-frequency sinusoidal patterns, resulting in limited pattern refresh rates and spatial frequency information content. It may be possible to combine square wave patterns with SSOP processing to allow for increased pattern projection rates, as well as multi-frequency extraction using a single snapshot.

Our eventual goal is to both acquire and process SFDI data in real-time, and processing requires computation time. In the case of 3-phase demodulation (Eq. 3.1), the mathematical operators have linear or near linear computational complexities, denoted in Big O notation by $O(n)$, where $n$ represents the number of digits used for each pixel, or simply each pixel, since the operation is linear. MSE, on the other hand, consists of a pseudo-inverse and matrix multiplication, which have computational complexities of approximately $O(n^3)$ and $O(nmp)$, respectively, where $n$, $m$, and $p$ represent the number of projections, number of spatial frequencies, and 1 (columns in $I$ matrix), respectively. In the case of the multi-frequency experiments, where 7 projection patterns are used and 3 AC spatial frequencies are extracted, this translates to roughly 124 linear operations per pixel per AC spatial frequency. By comparison, the 3-phase formula consists of 10 linear operations for each pixel. Consequently, MSE is expected to have approximately an order of magnitude more computational demand than the 3-phase formula. Advanced techniques such as parallel processing on graphics processing units (GPUs) could be used to alleviate this increased computation burden.

MSE can accommodate projection patterns having arbitrary spatial frequency content. Thus, there is potential for the use of alternate spatial light modulators (SLM’s). A rotating fan, for
example, contains radially-varying square wave patterns. Such a device would be far less costly than a DMD, and would have no refresh rate. Alternatively, a light source having intrinsic spatial frequency patterns such as an LED array could be employed, eliminating the need for an SLM, potentially reducing the footprint and complexity of SFDI instruments.
Chapter 4

Video rate multispectral SFDI

This chapter describes a video-rate (33 Hz) SFDI platform, whose development relies on the techniques discussed in Chapter 3. This system is characterized by the use of high-speed, binary square wave projection patterns and hardware triggering, allowing for unprecedented speeds in quantitative tissue spectral imaging.

To make an imaging technology clinically-viable, data acquisition speed should be maximized in order to mitigate motion artifacts and probe dynamic signals. Additionally, the images acquired ideally should consist of objective, quantitative information about the tissue to provide objective feedback to the surgeon, clinician, or end-user. Spatial frequency domain imaging (SFDI) is has been developed [15, 27], which employs structured light to separate the effects of absorption from scattering from its measurements, allowing for the mapping of absolute chromophore concentrations such as oxy/deoxy-hemoglobin, as well as structural parameters such as the reduced scattering coefficient ($\mu'$). SFDI is a superior imaging modality over conventional color cameras used in medical imaging due to its quantitative capabilities. However, the technique is currently limited with respect to data acquisition speed; SFDI instruments typically have data acquisition rates resulting in imaging speeds less than 1 frame per second, making it impractical in most clinical contexts where motion is present. Additionally, important physiological signals such as respiration and heart rate are difficult or impossible to detect with these imaging speeds.
Here, we present a new SFDI instrument capable of acquiring raw data at 400 frames per second (FPS). Our instrument acquires 12 frames of raw data per SFDI timepoint, resulting in quantitative oxy/deoxy-hemoglobin and $\mu_\varepsilon$ imaging speeds of 33 Hz. In order to accomplish video-rate SFDI, we employ binary, square wave projection patterns, allowing for camera exposure times (1 ms) faster than sinusoidal pattern refresh rates (2 ms). Additionally, the light source, projector, and camera in our instrument are hardware triggered, eliminating data acquisition bottlenecks due to software lag times. We present results on an in vivo forearm model using our new video-rate SFDI instrument, including motion tracking during voluntary movement, hemodynamics during a pressure cuff occlusion experiment, and detection of respiration and heart rate in $\mu_\varepsilon$ during paced breathing. Our motion tracking results demonstrate sensitivity to 2.5 cm/s lateral motion over 2 seconds by tracking deoxy-hemoglobin levels over a vein in the field-of-view (FOV). We also exhibit video-rate hemodynamics by showing changes in oxy/deoxy-hemoglobin and tissue oxygen saturation during a forearm cuff occlusion occlusion. Finally, we show respiration and heart rate signals taken from a region of interest in the video-rate $\mu_\varepsilon$ signal.

4.1 Materials and methods

A block diagram of the video-rate SFDI instrument is shown in Fig. 4.1(a). This instrument consists of a scientific-grade complementary metal-oxide semiconductor (sCMOS) camera (ORCA-Flash 4.0, Hamamatsu Photonics, Shizuoka, Japan) coupled to a wide-angle lens (Edmund Optics). The camera parameters are set using a dedicated software package (HCImage-Live-4.2, Hamamatsu Photonics). The camera exposure time is set to 1 ms, pixel binning to 4x4, and pixel count to 64x512 to optimize between speed, signal-to-noise ratio (SNR). A multi-
spectral light-emitting diode (LED) light source consisting of individually-addressable near-infrared wavelengths of 655, 731, and 850 nm (LumiBright 2910A-100, Innovations in Optics, Woburn, MA) was driven using LED drivers (PicoBuck, Sparkfun, Boulder, CO). To best match SNR between wavelengths, one 655 nm die, two 731 nm dies, and four 850 nm dies were driven simultaneously. A DC power supply was used to supply power to the instrument (PWS2323, Tektronix, Beaverton, OR. A modular projection unit including a digital micro-mirror device was used (CEL5500-Fiber, Digital Light Innovations, Austin, TX), and a dedicated software package was employed to load and produce spatial frequency patterns (DLI conductor). Crossed-polarizers were employed to mitigate the effects of specular reflection in our measurements.

A timing diagram of the video-rate SFDI instrument is shown in Fig. 4.1(a). To control each hardware component, 5 volt pulses were generated using a data acquisition (DAQ) board operated using the Labview software package (Student Edition 2014). Here, pulses were sent to the projector, LED drivers, and camera to control pattern switching, illumination, and frame acquisition, respectively. Pulse durations were set to 1 ms for the camera, and 2.4 ms for the LED drivers. The trigger for the projector was shared with \( \lambda 1 \) from Fig. 4.1(a).
Figure 4.1: (a) Instrument and (b) timing diagrams for video-rate SFDI instrument. A data acquisition (DAQ) board sends timing pulses to each hardware component. The projector changes patterns every 3 frames, in which time the 3 spectral components (655, 731, 850 nm) of the light source are strobed. The camera snaps an image at each timepoint (c) A color image of the forearm is shown with field of view highlighted (3x8 cm).

The camera frame rate was set to 400 FPS, or a 2.5 ms frame period. This rate is determined based on the time required to expose the camera (1 ms) and read out the data from the camera sensor (~1.4 ms). We also allow for extra time (~0.1 ms) for each frame to avoid cross-talk between wavelengths and pattern load time (0.17 ms), which occurs every four frames. For each timepoint, we employ three AC patterns (using Eq 1.1) consisting of square wave patterns having a fundamental frequency of 0.25 mm$^{-1}$, and a planar (DC) pattern, for a total of four patterns. For each pattern, we acquire frames at 2 wavelengths, for a total of 12 frames per SFDI timepoint. Thus, our resulting SFDI imaging rate is $\frac{400}{12} = 33$ Hz.
To generate optical property maps, we fit the DC and demodulated AC images to a semi-infinite, homogeneous, Monte Carlo transport model, employing a fast lookup table approach using cubic spline interpolation on the diffuse reflectance data at two spatial frequencies. Once the $\mu_s'$ and $\mu_a$ values were separated by SFDI, we calculated hemodynamic parameters from the scatter-corrected $\mu_a$ values assuming that oxy/deoxy-hemoglobin were the primary absorbers in the sample. From these values, we computed tissue oxygen saturation ($StO_2$) from the percent ratio of oxy to total hemoglobin concentration. Data processing was executed using the MATLAB software suite (MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, MA).

The 1$^{st}$ experiment seeks to track motion in our measurement. To accomplish this, we applied an absorbing fiducial marker (i.e. black marker) to the field-of-view, and cross-correlated deoxy-hemoglobin images at each timepoint to a cropped image of the marker, and extrapolated from the marker the Euclidean distance to the vein bifurcation. In the 2$^{nd}$ experiment, we image a pressure cuff occlusion model, and look at hemodynamics (oxy/deoxy-hemoglobin and $StO_2$ tracings). Here, a pressure cuff is applied to the forearm at 200 mm/Hg to induce venous and arterial occlusion to the periphery. To verify that arterial blood flow is occluded, we employ a blood flow sensor located at the index finger (FlowMet, Laser Associated Science, Irvine, CA). In the 3$^{rd}$ experiment, we analyze respiration and heart rate signals during a paced breathing experiment (~ 3s inhale, 5s exhale) in the $\mu_s'$ images by taking a small (10x10 pixel) region of interest (ROI). To eliminate the background, and isolate the changes in the signal due to the dynamic signals, we detrend time intervals to detect changes in $\mu_s'$. 
4.2 Results and discussion

To highlight the capabilities of our video-rate SFDI system, we present results related to tracking motion, probing video-rate hemodynamics in an occlusion model, and detecting respiration and heart rate signals, which occur on timescales shorter than data acquisition rates of current SFDI instruments.

Motion tracking of deoxy-hemoglobin in vein

An important aspect of high-speed imaging is the ability to mitigate motion artifacts by allowing for tracking of clinical movement. Fig. 4.2 shows motion tracking results on deoxy-hemoglobin maps as the forearm is moved laterally (from bottom-left to top-right) over time. In particular, the forearm is moved 25 mm over the course of 2 seconds, or >1 cm per second, from the bottom-left to the top-right in the field-of-view (FOV). In the first half of the experiment, the arm is kept still, and we see little change (<3%) in over the region of interest (ROI) between the control (no motion tracking, ROI is static) and the motion tracking (ROI moves with sample) cases. As voluntary movement occurs, the tracing from the control case decreases to >25% below the original value, as the vein region having higher deoxy-hemoglobin relative to background leaves the ROI. In the motion tracking case, the tracing stays constant to within 3% of the original value.
Figure 4.2: Motion tracking results showing (a) deoxy-hemoglobin concentration maps (left) before and (right) after movement and (b) average deoxy-hemoglobin values over time for motion tracking and control cases.

The ability to image at video rates allows for an increased ability to track motion compared to conventional SFDI, enabling the spatial localization of features in a sample over time, resulting in better spatial localization of absorbers such as oxy/deoxy-hemoglobin. In the next experiment, we show time tracings of oxy/deoxy-hemoglobin and StO₂ acquired at video rates during a pressure cuff occlusion experiment.

Hemodynamics in pressure cuff occlusion model

SFDI has the ability to map both \( \mu_a \) and \( \mu_s' \) at multiple wavelengths, allowing for the determination of chromophore concentrations such as oxy/deoxy-hemoglobin. Fig. 4.3 shows hemodynamic results acquired at 33 Hz on a pressure cuff occlusion model. Here, a 10x10 pixel
region of interest (ROI) in the tissue is averaged to produce the tracings (black box in Fig 4.3a). From 0 to 30 seconds a baseline measurement is taken, where the forearm is unoccluded. Here we see an average StO2 % in the ROI of roughly 67%, and oxy/deoxy-hemoglobin concentrations of approximately 30 μM and 15 μM, respectively. During occlusion, we see a decrease in oxy-hemoglobin to 29 μM, and an increase in deoxy-hemoglobin to 17 μM, resulting in a decrease in StO2 of roughly 5%.

Figure 4.3: Hemodynamic results on a pressure cuff occlusion model. (a) Example images of (left) oxy-hemoglobin, (middle) deoxy-hemoglobin, and (right) StO2 are shown. (b) Tracings for (left) oxy/deoxy-hemoglobin concentration and (right) StO2 taken from the region of interest.
Video-rate SFDI allows for bandwidths suitable for probing dynamic physiological signals. In particular, 33 Hz imaging rates grants us a sampling bandwidth of 0-16.67 Hz, making it suitable for characterizing signals such as respiration rate, which typically occurs on the order of seconds (<1 Hz), and heart rate, which typically occurs on the order of 100’s of milliseconds (~1-3 Hz). Fig. 4.3 shows respiration and heart rate tracings taken from a small (10x10 pixel) ROI using $\mu_s'$. In Fig 4.4(b) shows the heart rate superimposed on the respiration signal during paced breathing with a corresponding Fourier transform magnitude. Here, respiration rate is shown prominently at ~0.15 Hz (~8 cycles per minute) and heart rate is located approximately at 1.2 Hz (~72 beats per minute).

![Figure 4.3](image1)

**Figure 4.3:** Imaging dynamic signals using $\mu_s'$. (a) A sample $\mu_s'$ image showing a small (10x10 pixel) region of interest from which the tracings are derived. (b) Detrended respiration and heart rate signals are shown with (c) the corresponding FFT.

Imaging speed is critical in medical diagnostics, and the increased information burden posed by the quantitative nature of SFDI makes video-rate imaging elusive. This work presents a
video-rate SFDI instrument, capable of 33 Hz imaging of absolute oxy/deoxy-hemoglobin concentrations and \( \mu_s' \). We have demonstrated the ability of video-rate SFDI to track motion, probe hemodynamics, and image respiration and heart rate. To achieve video-rate (>30 Hz) imaging rates, we employed square wave patterns, allowing for a 1 ms camera exposure time, which is at least twice as fast as the refresh rate of sinusoidal patterns in most DMD’s, including the one used in our system.

Although the instrument presented here is significantly faster than conventional SFDI systems, there are potential improvements that could be made to further increase imaging speed. For example, more powerful light sources could be used to avoid the need for pixel binning (4x4 in this case), which would increase data readout speed by fourfold (~0.4 vs 1.4 ms), and thus imaging speeds to 56 Hz, almost double the rate we demonstrate in this work. Alternatively, instead of increasing imaging speed, additional spatial frequency patterns could be used, such as low-frequency square waves used in conjunction with MSE (described in Chapter 3) to increase information content per timepoint. Another possibility for increasing imaging speed is to use a single pixel detector in conjunction with a time-encoding scheme such as compressive sensing [73, 74] or SPatial frequency modulatIon For Imaging (SPIFI) [75]. This subject will be discussed in Chapter 5.

The hemodynamic tracings shown in Fig. 4.3 show a decrease in oxy-hemoglobin and a decrease in deoxy-hemoglobin during arterial occlusion. Since we are able to image at 33 Hz, the slope of these tracings is well defined. In particular, we can see small variations in oxy-hemoglobin during the occlusion, which may be due to oxygenated blood being delivered from the muscle to the superficial tissue during cramping [76]. The slope from the oxy/deoxy-
hemoglobin curves during occlusion could potentially be used to determine the metabolic rate of oxygen consumption of the tissue [77].

In Fig. 4.4, the $\mu_s'$ data shows time tracings of respiration and heart rate when taking the average over a small ROI over time. We found that the $\mu_s'$ respiration and heart rate signal has higher fidelity than absorption, which is measured by conventional methods such as photoplethysmography (PPG) [78, 79]. Although we have not verified the nature of this signal, our hypothesis is that it is related to an increase in red blood cell flux as arterial blood propagates towards the periphery, increasing $\mu_s'$ due to scattering from the red blood cells. Since higher spatial frequencies which interrogate more shallow tissue depths are more sensitive to $\mu_s'$, it is likely that this improved signal is due to fact that the $\mu_s'$ signal originates in more superficial volumes where the respiration and heart rate signals are more pronounced [79]. This hypothesis will be tested in future studies.
Chapter 5

Future work: Video-rate hyperspectral SFDI using a single element detector

Current SFDI uses a camera to record spatial images that are processed to obtain quantitative tissue properties. The relatively slow response of cameras limits SFDI imaging speed, and SPatial Frequency modulation for Imaging (SPIFI) offers a route to circumvent this camera response speed. SPIFI forms images by imposing high-speed spatiotemporal modulation on an illumination beam that maps modulation frequency to spatial position [75]. Spatial images are rendered through analysis of the electronic signal spectrum generated by light recorded with a single pixel detector. Images can be formed from tissue absorption, scattering, or fluorescent light emission. Our goal is to combine SPIFI with spatial frequency domain imaging (SFDI) and hyperspectral imaging, allowing for video-rate, quantitative tissue spectral imaging with 15-20 spectral bands using a single pixel detector.

Hyperspectral imaging provides rich information for assessing tissue composition and providing depth sectioning capability [24, 80, 81]. In addition to spatial information, the spectrum of broad bandwidth light can be recorded in SPIFI by applying a similar modulation scheme to the source, but in this case by varying modulation frequency with optical wavelength. Combining the spatial with spectral mapping capabilities using SPIFI opens the potential for high-speed (30 Hz) hyperspectral spatial imaging with a single pixel detector.

Fig. 5.1a shows a block diagram of a 2D SPIFI imaging system. Here, a beam of light is focused to both a horizontal and vertical line and transmitted through a series of two spinning disks. These disks are patterned such that a series of temporal frequencies are encoded along each line in as motors rotate the disks. The resulting, two-dimensional modulation pattern can be
expressed by the **Eq. 5.1**, shown below. Here, $D_x$ and $D_y$ represent the spatiotemporal patterns generated by the x modulator and y modulator, respectively. $\kappa_x$ and $\kappa_y$ represent the chirp rates of the frequencies encoded onto the disks.

**Equation 5.1: 2D SPatial frequency modulation For Imaging (SPIFI) illumination pattern**

$$P(x, y; t) = D_x(x; t)D_y(y; t)$$

Where

$$D_x(x; t) = \text{rect}(f_xt)[1 + \cos(2\pi \kappa_xtx)]$$

$$D_y(y; t) = \{\text{rect}(f_yt)[1 + \cos(2\pi \kappa_yty)]\} \otimes \text{comb}(f_yt)$$

The resulting time spectrum for the detected beam in a 2D SPIFI setup is shown in **Fig. 5.1b**. Here, the primary peaks correspond to intensities from columns in the vertical or “y” direction, while the sidelobes correspond to intensities from the x,y coordinate pair for the given column (i.e. a single pixel in the 2D image). The values from these peaks will be used directly to reconstruct 2D images using a single pixel detector. Our goal is to generate 100x120 pixel images at video rates (30 Hz). To this end, we will employ 2 modulation patterns on the “x” disk, rotating at 15 Hz, and 40 modulation patterns on the “y” disk, rotating at 90 Hz.
**Figure 5.1:** (a) Illustration of 2D SPIFI instrument. A beam is focused onto a series of 2 disks, which encode a unique temporal frequency into each pixel in the beam. This time-encoded light is then illuminated onto the sample, and the reflected or transmitted light is detected by a single-pixel photodiode. (b) The resulting frequency spectrum consists of main lobes corresponding to each vertical (y) coordinate, and sidelobes corresponding to each horizontal (x) component.

In addition to imaging, we will integrate hyperspectral capabilities into our single pixel setup. To accomplish this, we will add an additional SPIFI disk into the light path. This combined instrument is shown in **Fig. 5.2**. Here, a line is mapped onto a SPIFI disk, modulating each wavelength band at a unique temporal frequency. To modulate the hyperspectral component at high enough frequencies (MHz) to exceed the spatial (x,y) modulation bandwidth (360 kHz), a resonant galvanometer will be employed. Our goal is to enable 8 MHz maximum modulation rates for the hyperspectral source, allowing for full separation of each x,y, λ combination, assuming 20 spectral bands. This modulated hyperspectral beam will then be input to a 2D SPIFI instrument, which modulates each x and y coordinate pair at kHz rates, utilizing the high bandwidth of the photodiode (MHz to GHz).

**Figure 5.2:** (a) Illustration of combined SPIFI/hyperspectral imaging instrument. A beam is input to the hyperspectral arm, which maps the spectrum from a broadband source onto a line, which is encoded at MHz frequencies by a SPIFI modulator. This beam is then redirected to a 2D SPIFI system, modulating x and y spatial axes.
With this system, we will be able to generate hyperspectral (20 wavelength bands) SFDI images (100x120 pixels) at 30 Hz using a single pixel detector. This instrument will allow for quantitative hyperspectral imaging at unprecedented rates, using cost-effective hardware components.
Chapter 6

Conclusions

The body of this work focuses on techniques for increasing both speed and information content in spatial frequency domain imaging (SFDI), with the goal of making the technique more suitable for clinical situations where mitigating motion and probing dynamic signals is paramount. To achieve these goals, we have described and demonstrated signal processing techniques and new instrumentation.

Demonstrated in this thesis in Chapter 2 are techniques for reducing SFDI frame count while preserving information content. We have presented a new method for extracting spatial frequency information content in SFDI, which employs a 2D Hilbert transform using a spiral phase function in 2D Fourier space. This demodulation technique increases SFDI optical property data acquisition speed by two-to-threefold over conventional, 3-phase demodulation, depending on the number of spatial frequencies used. Additionally, this technique increases tissue structural orientation data acquisition speed by threefold. We have applied this new approach to in vivo volar forearm data, from which $\mu_a$ and $\mu_s'$ maps were derived, showing agreement with 3-phase SFDI. We have also shown that scattering orientation index (SOI) values obtained from a structural orientation phantom using our new approach are comparable to those obtained using 3-phase SFDI. Also presented is a $\mu_s'$ extrapolation approach for enhancing spectral scattering contrast. We have shown results indicating that this approach is suitable for a preclinical burn model, resulting in a threefold increase in speed over conventional SFDI.
Another theme, discussed in **Chapter 3**, in the context of SFDI speed increase is frame rate. We have described and demonstrated a new algorithm (MSE) for extracting images of multiple spatial frequency components using square wave patterns of structured light. By using square wave patterns, SFDI data acquisition speed is increased by an order of magnitude or greater over sinusoidal patterns used in conventional SFDI. We have applied MSE to an *in vivo* forearm model and a tissue-simulating phantom that confirms both the accuracy of optical property reconstructions and the depth sensitivity of the technique.

Finally, in **Chapter 4** we introduce a new video-rate SFDI instrument, capable of imaging oxy/deoxy-hemoglobin and $\mu_s'$ at 33 frames per second. This instrument is used to image to an *in vivo* forearm model, where we demonstrate its ability to track motion, probe hemodynamics at high speeds, and detect sub-second dynamic signals.
References


Appendix A

Processing code

Hilbert demodulation technique

```matlab
function [demod_Image phase_Image] = HilbertDemodulation(mod_image,dc_image,size_fft,DC_factor)

% Inputs:
% mod_image - DC offset, AC modulated image
% dc_image - DC image
% size_fft - size of image after zero-padding
% DC_factor - Ratio of DC image subtracted from DC offset, AC image
% (ideally should be 0.5, but may be higher depending on modulation
% hardware)

% Generate vortex maps in the frequency domain
S = vortexPattern(size_fft);

% Subtract the DC image
mod_image_new = mod_image - DC_factor.*dc_image;

% Take the 2D Fourier transform of the DC offset, AC image
% - zero pad according to size_fft input
F = fft2(mod_image_new,size_fft,size_fft);

% Multiply by the vortex function
F_h = F.*S;

% Inverse fft and take magnitude for demod image
F_inv = ifft2(F_h);

% Compute the demodulated AC amplitude and phase map
demod_Image = abs(mod_image_new + 1j.*abs(F_inv(1:size(mod_image,1),1:size(mod_image,2))));

phase_Image = angle(mod_image_new + 1j.*abs(F_inv(1:size(mod_image,1),1:size(mod_image,2))));

function S = vortexPattern(size_fft)
% Generate vortex pattern for Hilbert demodulation
S = zeros(size_fft,size_fft);

for v = (-size_fft/2)+0.5:(size_fft/2)-0.5
    for u = (-size_fft/2)+0.5:(size_fft/2)-0.5
        S(((size_fft/2)+0.5-v),u+((size_fft/2)+0.5)) = ((u + 1j*v)/sqrt(u^2 + v^2));
    end
end
```
end
S = ifftshift(S);
S(1,1) = 1;

Multi-frequency synthesis and extractions (MSE)

% Initial data separated by 50 pixels
% model by 70
% clear all; close all;

% % Script for executing alternate method for demodulation to achieve
% % spatial freq. synthesis
% load BackgroundData.mat;

rows = 520; % pixels
columns = 696; % pixels

% Compute phaseAngle map
[ImPilot_demod ImPhase] = HilbertDemodulation(double(pilotImage),(1/3).*(mean(rawImages,3)),5001 ,0.5);
disp('H transform done!')

C_k0 = zeros(rows,columns,7);
for i = 1:phase
    % Define phase and source intensity coefficients for each SF component
    % in custom pattern (always relative to max pattern intensity of 1

    % For DC term
    C_k0(:,:,i) = 0.75;

    % For fundamental frequency
    C_k1(:,:,1) = (2/pi).*(sin(3*pi/4)).*(cos(ImPhase));

    % For second harmonic
    C_k2(:,:,1) = (1/2).*(2/pi).*(sin(6*pi/4)).*(cos(2.*ImPhase));

    % For third harmonic
    C_k3(:,:,1) = (1/3).*(2/pi).*(sin(9*pi/4)).*(cos(3.*ImPhase));
end

R_k0 = zeros(rows,columns);
R_k1 = zeros(rows,columns);
R_k2 = zeros(rows,columns);
R_k3 = zeros(rows,columns);
for i = 1:rows
    for j = 1:columns

        tempData(:,1) = squeeze(C_k0(i,j,:));
        tempData(:,2) = squeeze(C_k1(i,j));
        tempData(:,3) = squeeze(C_k2(i,j,:));
        tempData(:,4) = squeeze(C_k3(i,j,:));

        a = pinv(tempData);

        b = [rawImage(i,j,1); rawImage(i,j,2); rawImage(i,j,3);
             rawImage(i,j,4); rawImage(i,j,5); rawImage(i,j,6); rawImage(i,j,7)];

        tempData2 = a*b;

        R_k0(i,j) = tempData2(1,:);
        R_k1(i,j) = tempData2(2,:);
        R_k2(i,j) = tempData2(3,:);
        R_k3(i,j) = tempData2(4,:);
    end
end