In-silico and in-vitro investigation of a photonic monitor for intestinal perfusion and oxygenation

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Abstract: The quantification of visceral organ oxygenation after trauma-related systemic hypovolemia and shock is critical to enable effective resuscitation. In this work, a photoplethysmography-based (PPG) sensor was specifically designed for probing the perfusion and oxygenation condition of intestinal tissue with the ultimate goal to monitor patients post trauma to guide resuscitation. Through Monte Carlo modeling, suitable optofluidic phantoms were determined, the wavelength and separation distance for the sensor was optimized, and sensor performance for the quantification of tissue perfusion and oxygenation was tested on the in-vitro phantom. In particular, the Monte Carlo simulated both a standard block three-layer model and a more realistic model including villi. Measurements were collected on the designed three layer optofluidic phantom and the results taken with the small form factor PPG device showed a marked improvement when using shorter visible wavelengths over the more conventional longer visible wavelengths. Overall, in this work a Monte Carlo model was developed, an optofluidic phantom was built, and a small form factor PPG sensor was developed and characterized using the phantom for perfusion and oxygenation over the visible wavelength range. The results show promise that this small form factor PPG sensor could be used as a future guide to shock-related resuscitation. © 2017 Optical Society of America

OCIS codes: (170.1470) Blood or tissue constituent monitoring; (170.7050) Turbid media

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1. Introduction

Trauma is the leading cause of death for people aged 1-44 year(s) in the United States [1]. The standard of care for injured patients is to control bleeding and administer volume resuscitation [2, 3]. The goal of resuscitation is to restore tissue perfusion and to correct the imbalance between oxygen supply and tissue oxygen demand. Oxygen delivery must often be increased substantially above normal levels in order to resolve accrued tissue oxygen “debt” from the period of shock. Currently, resuscitation is often guided by systemic indicators such as blood pressure, urine output, and heart rate. After these parameters are normalized, up to 85% of patients are still in “compensated shock” with associated tissue acidosis [3]. Serum lactic acid and other markers are used clinically but do not permit organ-specific assessment. The compensated shock state can lead to multiple organ dysfunction syndrome (MODS), the leading cause of death in surgical intensive care units [4]. Tissue perfusion or acidosis and oxygen consumption are better criteria to guide adequacy of resuscitation and to predict the development of MODS [5]. In particular, the small intestine has been demonstrated to be the organ most sensitive to hemorrhagic shock, and it is therefore the ideal target for monitoring [6]. However, there is no gold standard that is used to monitor intestinal perfusion. Basic methods used in surgical suites include palpation of intestinal vascular pulses, and injection of fluorescent dyes and subsequent examination for “abnormal” dye distributions. However, these methods do not provide quantitative results and are not applicable to guide resuscitation from shock. Abnormalities of blood flow and perfusion can, most often, be associated with an absolute decrease in flow from “normal” levels. However, in some situations, flow may be within the “normal” range but be inadequate due to tissue stress. Thus, perfusion and flow measurements coupled with a measure of oxygen consumption to ensure the oxygen supply meets tissue demands may provide a more comprehensive monitoring alternative than measuring just flow.

Abdominal injury associated with significant hemorrhagic shock generally requires staged management with two operations. Initial operation is for “damage control” to address major bleeding sources, and resuscitation continues in the post-operative period until complete [7]. Repeat operation for definitive management of injuries typically occurs within 48 hours. In some patients, the abdominal incision is not closed after the initial operation due to increased abdominal pressure [8], and the intestine may be available for visual inspection. The staged operation paradigm for severe abdominal trauma allows for short-term implantation of an intestinal sensor at the time of initial operation, use of the sensor to guide resuscitation...
between procedures, and device removal at the time of re-operation when resuscitation has
been completed.

The focus of this work is to optically characterize intestinal tissue in-silico using a Monte
Carlo model and two different intestinal phantom designs, build a microfluidic-based three
layer in vitro system based in-part on the in-silico design, construct an optimized PPG-based
sensor designed to be placed on the surface of the small intestinal tissue, and develop/refine
measurement and signal processing methods to produce a complete monitoring system. This
will result in a miniature, photonics-based intestinal perfusion and oxygenation monitoring
system specifically optimized for intestinal use enabling to improve strategies for
resuscitation from trauma and hemorrhagic shock.

Optical techniques offer the advantage of monitoring changes in the hemodynamics of the
tissue without inducing perturbation in the tissue environment. By analyzing the pulsatile and
non-pulsatile components of the PPG signal, information about the perfusion and oxygenation
of the tissue probed can be extracted [9, 10]. Note that the pulsatile components stem from the
arterial pulsations and the non-pulsatile components stem from the optical properties of the
tissue, venous blood, and non-pulsatile arterial blood [9]. Such a system would help assure
the physician that patients are fully resuscitated from shock while eliminating common
complications of “over-resuscitation”, particularly edema. In general, optical spectroscopy
techniques in the red to NIR region are the gold standard for non-invasive monitoring of
hemoglobin oxygen saturation, utilizing a pair of wavelengths, usually between 600 and 700
nm and between 900 and 1000 nm [11, 12]. In particular, for the design of optical pulse
oximeters and heart rate monitors, signal quality can be improved through proper wavelength
selection and proper source to detector separation [13]. Conventional sensors use large source
to detector separations, different applications utilizing a separation of 5 to 7 mm for
monitoring superficial layers of tissue as well as up to 15 mm for deeper probing, as is used
for the monitoring of cerebral activity [14–16]. Many medical monitors use transmission
mode through the finger, and for these or the reflection approaches with the larger source-
detector separations the use of NIR wavelengths maximizes penetration depth to probe deeper
microvasculature. However, in the case of the intestine, the intestinal wall is relatively thin
(~2 mm) [17], and current sensors utilizing NIR light would probe through the wall of the
intestine with measurements impacted by luminal contents or adjacent tissues. Such devices
would also suffer from poor signal and high background when measuring intestinal perfusion
and oxygenation. Specifically, the transparency of the thin intestinal tissue when using the red
to NIR wavelengths leads to high background levels and motion artifact from other
tissues while the relatively low vascular density in the intestine results in a low hemoglobin
signature, compounded by the lower absolute absorbance of hemoglobin in the NIR [19]. In
order to properly select wavelengths and source to detector separations, optical modeling of
the tissue of interest was performed using the Monte Carlo method. This method has been
used to estimate light fluence through different types of tissue [20, 21]. A commonly used
software simulation program developed by Wang et al [22], multi-layer Monte
Carlo(MCML), estimates tissue as homogenous rectangular domains. This estimation of
tissue geometry yields results similar to those seen in experimental cases [23], however,
accurate estimation sometimes relies on the tissue geometry implemented, wherein more
physiologically correct models yield more accurate results [24]. A novel intestinal geometry
was implemented in the simulation presented here and used to guide the generation of the
photronics sensor in both wavelength selection and source-to-detector separation. The results
from the modified simulation were also compared to those generated by the MCML program
for a simplified geometry to guide the creation of an opto-microfluidic phantom used to verify
the performance of the PPG system in-vitro. The initial selection of visible wavelengths was
performed by selecting wavelengths with high differences in the absorption of oxygenated
and deoxygenated hemoglobin, due to the fact that changes in oxygenation would be seen as
more exaggerated differences in the signals collected. Further, selection of the wavelengths
was performed in order to have wavelengths that change absorption in opposite directions with changes in oxygenation to increase contrast in the calculated parameters. By comparing changes in 470 nm, which decreases in absorption with decreases in oxygenation, to changes in 560 nm or 630 nm, which increase in absorption with decreases in oxygenation, the oxygenation changes can be found [19]. Though 630 nm falls in the range of wavelengths used for traditional pulse oximetry due to its comparable depth penetration to NIR wavelengths [11], with the configuration of the sensor, utilizing a short source-to-detector separation of 2 mm, the depth penetration is reduced. The sensor was designed, built, and tested at three wavelengths (470 nm, 560 nm, and 630 nm) to estimate the hemodynamics in the intestinal tissue phantom perfused by mixtures of both oxygenated and deoxygenated blood phantom.

2. Methods and materials

2.1 Monte Carlo modeling

A Monte Carlo simulation based on the MCML program developed by Wang et al [22] was created to guide the optimization of a wireless PPG sensor and development of an in vitro phantom. The use of the Monte Carlo technique in modeling light propagation through tissue has been performed extensively [25–27]. Other methods include solving the Kubelka-Munk equations [28] or using a finite element model utilizing diffusion theory [29]. In this case though, while computation times were increased, the desire for a more accurate model as well as a more detailed description of the spatial information returned led to the choice of using the Monte Carlo method. A model was created to represent the tissue of the small intestine including three layers of combined tissue and blood; an outer muscular layer, a submucosal vessel layer, and a mucosal layer. In the model, the steady-state reflectance was scored as the counts of photons returning back to the surface of the muscular layer, and steady-state transmission was scored as the counts of photons passing through the mucosal layer. Although the photons that pass completely through the tissue may return to surface, for these visible wavelengths, the penetration depth is reduced [30], and light traveling to that depth is sufficiently attenuated that it does not contribute significantly to the returning signal [31]. Traditionally, the intestine is described as a four-layer tissue [32]; the serosa, muscularis, submucosa, and the mucosa. In the model examined, the serosa has been omitted. While an important physiological feature in the generation of mucous for the mobility of the intestine in the abdominal cavity, the serosa consists of a layer of mucous producing cells and connective tissues that attach to the peritoneum, which are thin [33]. Though the serosa does contain vasculature, these include larger arteries and veins, which do not cover the surface of the tissue entirely, and if a sensor were placed over them, would obscure the signals coming from the bulk of the tissue. By proper placement of the sensor away from these vessels, only the thin layer of the serosa would be between the sensor and the tissue. Due to these features, the impact this layer has on the optical signals is reduced as compared impacts of the other three layers selected for the model [18]. Further, in other examinations of light propagation through the intestine, the serosa has been omitted as well, most likely for the reasons mentioned above [31, 34]. A comparative two-model approach was used to determine whether or not the presence of intestinal villi, present on the luminal aspect of the tissue, would affect a noticeable difference in the reflectance PPG signal. Figure 1 shows the two models explored in the Monte Carlo simulations. Widths of the layers as well as the optical properties of the tissue at the wavelengths explored can be found in Table 1.
Fig. 1. The “slab” and villi models explored by the simulations. Dimensions of the layers are shown below in Table 1: the muscularis is shown at the bottom of the tissue in the figure, and the mucosa and villi are shown at the top of the tissue in the figure.

Table 1. Optical properties and dimensions of the layers used for the simulations

| Tissue Layer | \( \mu_a(470\text{ nm}, 560\text{ nm}, 630\text{ nm}) \) | \( \mu_s(470\text{ nm}, 560\text{ nm}, 630\text{ nm}) \) | g | N | Thickness |
|--------------|--------------------------------------------------|--------------------------------------------------|---|----|-----------|
| Muscularis   | 1.88, 1.49, 0.71 cm\(^{-1}\)                     | 172.0, 96.00, 64.86 cm\(^{-1}\)                  | 0.9 | 1.4 | 0.0327 cm |
| Oxygenated Blood | 179.33, 176.1, 3.29 cm\(^{-1}\)               | 400.97, 353.16, 293.90 cm\(^{-1}\)               | 0.9 | 1.3 | 0.0033 cm (varied) |
| Deoxygenated Blood | 87.245, 290.46, 27.80 cm\(^{-1}\)               | 400.97, 353.16, 293.90 cm\(^{-1}\)               | 0.9 | 1.3 | 0.0033 cm (varied) |
| Mucosa       | 3.98, 3.18, 1.5 cm\(^{-1}\)                     | 262.79, 167.81, 124.13 cm\(^{-1}\)               | 0.9 | 1.4 | 0.0374 cm |
| Villi        | 3.98, 3.18, 1.5 cm\(^{-1}\)                     | 262.79, 167.81, 124.13 cm\(^{-1}\)               | 0.9 | 1.4 | 0.08 cm |

The luminal surface of the tissue in the villi model was modeled by a two-dimensional squared sine function with spatial frequencies representing the spacing and width of the villi and amplitude representing the height of the villi. The dimensions of the tissue layers were estimated from several histology slides of intestinal tissue, where the relative thickness of each layer was determined [35]. Measurements of intestinal wall thickness from the literature [17] were then applied to these estimates to give the dimensions used for the simulation and the phantom.

The simulated tissue was defined by optical parameters including the absorption coefficient, \( \mu_a \), scattering coefficient, \( \mu_s \), reduced scattering coefficient, \( \mu_s' \), anisotropy, g, and index of refraction, n, determined from Bashkatov et. al. [18]. In the modulation of perfusion in the simulated tissue, Akl et. al [13] utilized a weighted average of the optical properties of blood and tissue. This model of the perfusion of the tissue allows for the simplification of a heterogeneous vascular domain into a homogenous one applicable for these types of simulation programs. While this is an accepted, adopted model, here, a hybrid approach is taken in the variation of perfusion in the tissue. Perfusion in the submucosal vascular layer is modulated by changing the thickness of the submucosal vascular layer, simulating the dilation.
of the submucosal arterioles in response to increased blood flow, while perfusion in the mucosal layer and villi is modulated by altering the weighted average of blood and tissue properties, taking into account that particular capillary beds may have reduced perfusion in the case of trauma [36]. The heterogeneity of perfusion seen in cases of hypovolumic shock, is well mimicked through the use of this heterogeneous method of vessel perfusion. Equations (1) and 2 below detail the modulation of the width of the submucosal layer in response to arterial perfusion and the change in optical properties of the mucosal layer in response to tissue perfusion, respectively. By modeling the submucosal vascular layer as a network of vessels that feed the mucosal layer, the patterns of perfusion observed in-vivo can be accurately modeled. Changes in tissue perfusion, shown below as alterations in the weighting of the optical properties of tissue and blood represent the constriction of vessels leading to the mucosa, reducing the amount of blood in the tissue [36]. This change models the filling and recruitment of non-pulsatile capillaries that are densely present in the mucosa [37].

\[ d = 0.0033 \cdot (1 + \frac{P_{\text{Arterial}}}{100}) \]  

\[ x_a = 0.12 \cdot \frac{P_{\text{Tissue}}}{100} \cdot \left( \frac{SO_2}{100} \cdot x_{\text{Mu,Ar}} + \left(1 - \frac{SO_2}{100}\right) \cdot x_{\text{Mu,Ar}} \right) + \left(1 - 0.12 \cdot \frac{P_{\text{Tissue}}}{100}\right) \cdot x_{\text{Mu,Ar}} \quad (2) \]

In Eqs. (1) and (2), \( d \) is the width of the submucosal layer in centimeters, \( P_{\text{Arterial}} \) is the scaled perfusion of the artery, \( P_{\text{Tissue}} \) is the scaled perfusion of the tissue, \( x \) represents the array of optical properties for each of the layers, and \( O_2 \) is the arterial oxygenation of the blood. At full tissue perfusion, the blood accounts for 12% of the optical properties of the layer, as was used previously [13].

Further steps were taken to mimic the particulars of the operation of the sensor, on both the side of the tissue and the sensor. A simulation of the countercurrent exchange of blood in the mucosal layer [37] was implemented as an oxygenation gradient from the base to the tip of the layer. This was achieved by varying the optical properties of the tissue. By altering the proportion of oxygenated and deoxygenated optical absorption that was attributed to the tissue to represent perfusion, the tissue was given a difference in the oxygenation at different depths into the tissue. As found in the literature, a 25% oxygenation gradient was implemented from the arterial layer to the luminal aspect of the mucosal layer [38]. Implementation of this gradient is performed through the calculation of the saturation of the hemoglobin along the traveled path through the tissue. Equation (3) details the method for determining the average oxygenation of the tissue over the travelled path.

\[ \overline{PO_2} = \frac{1}{z_v \cdot s} \int_{d_i}^{d_s + z_v} \overline{PO_2}_{\text{avg}} \cdot (1 - 0.25 \cdot \left(\frac{z}{l}\right))dz \quad (3) \]

In Eq. (3), \( PO_2 \) bar is the average oxygenation over the path, \( s \) is the length of the path, \( z_v \) is the directional cosine representing travel along the z-axis through the layers of tissue, \( d_i \) is the depth into the mucosal layer, and \( l \) is the total width of the mucosal layer. Solving this equation yields the Eq. (4), which is dependent upon \( s \), the length of the path travelled.

\[ \overline{PO_2} = PO_2_{\text{avg}} \cdot \left(1 - \frac{d_i}{4l}, \frac{s \cdot z_v}{8l}\right) \quad (4) \]

Following the photon propagation mechanism proposed in MCML, the step size of the photon is dependent upon the optical properties of the tissue being travelled through and is given by Eq. (5) [22].

\[ s = \frac{-\log(N(0,1))}{\mu} \quad (5) \]
In Eq. (5), $N(0,1)$ is a random number from 0 to 1, and $\mu_t$ is the total attenuation coefficient, found as the sum of $\mu_a$ and $\mu_s$. Because $\mu_t$ varies with the oxygenation saturation and not directly with the partial pressure of oxygen, a relation between the two must be introduced. The oxyhemoglobin dissociation curve defines the relationship between the partial pressure of oxygen in the blood and the saturation of the hemoglobin with oxygen. A numerical estimation of this function is given below in Eq. (6) [39].

$$SO_2 = \frac{PO_2^3 + 150 \cdot PO_2}{PO_2^3 + 150 \cdot PO_2 + 23400} \cdot 100$$ (6)

Plugging Eq. (6) into Eq. (2) to solve for $\mu_t$ in terms of PO$_2$ yields a function that can then be plugged into Eq. (5) that gives s as a function of the average PO$_2$. Substituting this result of s into Eq. (4) and solving will yield the average oxygenation over the path length, which allows for the subsequent solving of the path length using the average oxygenation found. Equation (6) was also used to solve for the oxygenation saturation of the hemoglobin at different blood oxygenations in the submucosal vascular layer.

The unmodified MCML simulation models a collimated point source of light, so the initial propagation steps of the photon were altered to allow for the simulation of the LEDs used on the sensor. Photon initial positions were randomly placed in a two-dimensional Gaussian distribution and photon initial direction was randomly distributed across the viewing angle of the LED. Each simulation consisted of 1 million photon packets.

2.2 Development of the tissue phantoms

After reviewing multiple images of colonic vascular structure [40], it was determined that the vasculature of the small intestine could be approximated using a rectangular grid of microchannels, mimicking the semi-regular structure seen in literature. The dimensions of the microchannels were chosen to represent how multiple parallel capillaries are grouped together in each grid line. Rectangular phantom shapes were chosen to represent a section of tissue oriented upon the longitudinal axis of the intestine. Although the curved intestinal geometry was originally considered, due to the short source-to-detector separations proposed and the narrow lateral spread of the photons [41], the possible geometric effects can be seen to be negligible. Three diverging microchannels were fabricated to evenly distribute the fluid across the beginning of the grid from the input, and the exiting fluid was collected at the output by a similar three microchannel configuration as shown in Fig. 2.

Fig. 2. a) CAD drawing of the grid pattern used to represent the submucosal vasculature, b) Zoomed in image of green rectangle in a) showing the details of the grid pattern.

Photolithography transparency masks of the channels with a resolution of 2500 DPI were purchased (CAD/Art services, Bandon, OR). The microchannels were designed to have a width of 50 $\mu$m. SU-8 master molds were produced on silicon wafers using SU-8 2050 photoresist and traditional soft photolithography techniques. Feature height was designed to be 70 $\mu$m. Polydimethylsiloxane (PDMS) (Dow Corning, Sylgard 184, Midland, MI) was used to form the phantom. To be consistent with the Monte Carlo simulations, the phantom consisted of two layers of PDMS, one imprinted with the grid and the other a flat slab. To
complete the three-layer model implemented in the simulation, blood phantom was flowed through the PDMS channels. In order to match the optical properties used in the simulations, the uncured PDMS was mixed with a combination of food dyes (Wilton, Gel Food Color Set, Woodridge, IL) and aluminum oxide particles (Inframat Advanced Materials, alpha-Al₂O₃ powder, 99.99% ultrapure grade, 0.5-1 micron, Manchester, CT), and then sonicated for 5 hours, stirring every hour. The dyes provided the main optical absorbing agent and the aluminum oxide contributed to the scattering. The optical properties were assessed using a single integrating sphere [42] to take transmission and reflection data, and the Inverse-Adding-Doubling program [43] to analyze and calculate the optical coefficients. Once the properties were assessed, it was determined that the optimal volumetric proportion of the dyes was 0.9% red, 0.15% yellow, and 0.05% blue with an aluminum oxide concentration of 9.563 milligrams per milliliter in uncured PDMS for both the upper and lower layer, two tissue layers’ optical properties being similar. After mixing the optical agents into the uncured PDMS, the curing agent was added with a PDMS prepolymer to curing agent volume ratio of 12:1. The solution was then poured over both the raised mold as well as a flat dish, each to a height of ~1mm. The PDMS was then baked at 85°C for 20 minutes to be partially cured, and the two slabs were affixed to each other and baked for an additional 10 minutes to complete the curing process. The patterned PDMS was then affixed to the PDMS in the petri dish via oxygen plasma treatment. After plasma treatment, the phantom was baked at 85°C for 20 minutes and left under pressure overnight to allow for a stronger bond.

To mimic blood in different oxygenation states, two different blood phantoms were developed using a combination of different food dyes (4 Food Colors, Adams Extract, Gonzales, Texas) and tie dyes (Tulip One-Step Dye Kits - Purple, Fresno, CA), one to represent fully oxygenated blood, and the other to represent deoxygenated blood. Specifically, the dyes mixed for the oxygenated blood phantom had volumetric proportions of 0.149% red food dye, 0.078% purple tie dye, 0.0000626% blue food dye in distilled water. The volumetric proportions of dyes for the deoxygenated blood were 0.0323% red food dye, 0.644% purple tie dye, 0.0353% yellow food dye, and 0.00316% blue food dye in distilled water. The relationship between the two phantoms was assessed by comparing the percent difference in absorption at the wavelengths of interest between the two fluid phantoms to the spectra of oxygenated and deoxygenated hemoglobin. The results of this assessment are in Table 3. Absorbance measurements were performed on a Tecan spectrometer (Tecan Inc, M200Pro, Mannedorf, Switzerland) in addition to the measurements performed on the single integrating sphere.

2.3 Development of the PPG sensor boards and data acquisition

To obtain a small form factor for eventual implantation, two commercial PPG sensor boards (ADPD103-based development boards, Analog Devices Inc., Norwood, MA), were modified to test three different wavelengths. The green, red, and infrared LEDs initially installed on each of the boards were removed and the boards were modified to facilitate attachment of the selected LED wavelengths. On the first board two blue (OSRAM Opto Semiconductors, LB T67C, Sunnyvale, CA) and two green (OSRAM Opto Semiconductors, LP E675, Sunnyvale, CA) LEDs were installed onto the board. On the second board two blue and two red (OSRAM Opto Semiconductors, LS T676, Sunnyvale, CA) LEDs were installed onto the board. Source-to-detector separation was guided by results of the simulations. In both units, the LEDs were positioned such that one of each wavelength was located on both the left and right of the photodetector and the order chosen to best normalize the tissue illumination (See Fig. 7).

A data acquisition unit (Analog Devices Inc. Norwood, MA) provided an interface to the modified sensor boards via a 25-mil spaced ribbon cable and enabled sensor LED drive control for each of the two LED wavelengths. PC readout of the detected optical signal was accomplished using either USB or Bluetooth connectivity. A time division multiplexed drive
method as implemented in the sensor hardware enabled wavelength separation without the need for optical filters. A PC-resident software tool was also provided by ADI enabling data acquisition setup, control, PPG waveform visualization, and data storage. Following sensor PCB customization, use of this ADI system supported rapid sensor characterization studies under various drive conditions, including individual LED drive current levels and signal averaging.

2.4 In-vitro measurement system

An in-vitro system was constructed to simulate the pulsatile waveform through the intestinal optofluidic tissue-mimicking phantom. A 1 Hz simulated cardiac flow waveform was generated in LabVIEW (National Instruments, Austin, TX) and used to drive an M6 pump (Vici/Valco, CP2-4841-100M1, Houston, TX). This waveform was consistent with the flow seen in the superior mesenteric artery, which feeds the small intestinal vasculature [44]. The pump pushed optically blood-mimicking fluid through the three inputs of the phantom, was circulated in a closed loop fluidic system, and collected at the three outputs. The input waveform generated by LabVIEW was scaled linearly to alter both the average perfusion of the fluid through the phantom as well as the pulsatile wave perfusion by adjusting the DC and AC magnitudes of the pulse amplitude. The frequency of the pulsatile waveform could also be varied. Perfusion levels were modified by applying a linear scaling factor to the input waveform magnitude.

Oxygenation was altered by mixing the two previously described blood phantoms. Measurements of the PPG signal were collected for 1 minute for each perfusion and oxygenation condition tested. Data processing was performed in MATLAB (Mathworks, Natick, MA). Perfusion and oxygenation conditional sets were separated into 60-second intervals. Mean signal intensity was determined from these sections. Peak-to-peak amplitude measurements were performed for each individual peak after passing the signal through a bandpass filter with a low cutoff frequency of 0.5 Hz and a high cutoff frequency of 10 Hz. In addition, each pulse segment was extracted from the filtered data set and averaged to produce an overall average of the signal in response to the input waveform. Measures of perfusion index, as defined in Eq. (7), where $PI_{\lambda}$ is perfusion index for a particular LED wavelength, and $AC_{\lambda}$ and $DC_{\lambda}$ are the intensities of the signal components with nonzero and zero frequencies, respectively, at a particular LED wavelength, and ratios of the mean signal intensity were calculated to yield indicators of the hemodynamics in the tissue phantom [45,46].

$$PI_{\lambda} = \frac{AC_{\lambda}}{DC_{\lambda}}$$ (7)

The modulation ratio, defined in Eq. (8), where $R$ is the modulation ratio between two LED wavelengths, and $PI_{\lambda_1}$ and $PI_{\lambda_2}$ are the perfusion indices for the two LED wavelengths to be compared, was used to gauge the conditions of oxygenation of the flow in the phantom.

$$R = \frac{PI_{\lambda_1}}{PI_{\lambda_2}}$$ (8)

Average measures of the signal, while not completely independent of perfusion, whether individual mean signal intensities or a ratio of the mean signal intensity, were selected to represent the oxygenation signal. This signal is relatively independent of perfusion, although with increased blood flow the path length of a photon will most likely encounter more blood than in the case where the perfusion is lower [47]. This results in an average decrease in light signal intensity consistent with higher blood volume. The perfusion of the phantom was judged by both the peak-to-peak amplitude height, as well as the perfusion index. The modulation ratio is then used to give a measure of tissue oxygenation.
3. Results and discussion

3.1 Monte Carlo modeling

A comparison of the villi and slab models was performed to assess intensity of reflection as a function of tissue and arterial perfusion as well as to assess any differences for the simulated phantom with villi versus a plain slab geometry, using 560 nm as an example. Figure 3 shows the diffuse reflectance of the two models over a range of arterial and tissue perfusion conditions.

As depicted in Fig. 3, the overall intensity declines as a function of both the arterial and tissue perfusion. Furthermore, the differences in the two model geometries are negligible with less than 1.5% difference across the entire range of perfusions. The relative similarity of the results verifies that the addition of intestinal villi to the tissue domain does not significantly affect the intensity and thus is not required in the design of the in vitro phantom. These differences were also assessed over all conditions explored for each of the other two wavelengths of interest (470 and 630 nm) to determine the necessity of the addition of villi to the phantom. As depicted in Fig. 4, for the lower wavelengths of 470 and 560 nm the results were less than 1.5% but they were slightly higher up to 3.5% for the 630 nm wavelength range. The percent difference shown in Fig. 4 is calculated as an average of each of the cases explored for perfusion and oxygenation. An example of the individual results can be seen in Fig. 3. It was determined that these errors are acceptable given the inherent difficulty of fabricating in vitro phantoms with villi and the fact that the 630 nm wavelength exhibits a deeper penetration depth through intestinal tissue. Although as shown below for real tissue, the depth reached by light at 630 nm is within the thickness of the tissue for the shorter source-to-detector separation chosen, reducing the possible error. For these simulations, the longer mean free path at 630 nm, representing the probabilistic distance a photon will travel before interacting with a scattering particle and defined as the reciprocal of the scattering coefficient, $\mu_s$, combined with the reduced distance to the luminal surface of the tissue results in the photons behaving in a more quasi-ballistic fashion and being lost as transmission [48].
Fig. 4. Percent difference of diffuse reflectance between the villi and slab models for all cases of perfusion and oxygenation examined.

3.2 Development of the tissue phantom

The optical properties of the generated phantom are depicted in Fig. 5. The absorbance coefficient ($\mu_a$) and reduced scattering coefficient ($\mu_s'$) of the phantom are given in 1/cm across the range of wavelengths from 440 to 670 nm.

As can be seen, the particular mix of dyes used in the generation of the tissue phantom matched well with the optical properties determined from Bashkatov et. al [49] of measured tissues, at the wavelengths selected. The numerical data for the optical properties of the phantom components compared to the data used in the simulations is displayed in Tables 2 and 3.
Table 2. Optical properties of tissue phantoms

| Tissue Phantom | \( \mu_a \) (470 nm, 560 nm, 630 nm) | \( \mu_s' \) (470 nm, 560 nm, 630 nm) | \( g \) (470 nm, 560 nm, 630 nm) |
|----------------|----------------------------------|----------------------------------|----------------------------------|
|                | 2.8842, 0.7376, 1.1792 cm\(^{-1}\) | 12.6148, 10.7292, 10.3533 cm\(^{-1}\) | 0.9, 0.9, 0.9 |

Table 3. Optical properties of blood phantoms compared to optical properties used in simulation

| % difference in absorption for oxygenated and deoxygenated | 470 nm | 560 nm | 630 nm |
|-----------------------------------------------------------|--------|--------|--------|
| Blood phantom                                             | 45.73% | 48.78% | 83.44% |
| Simulation                                                | 51.35% | 39.37% | 88.15% |

The completed phantom is shown in Fig. 6(a), alongside a clear phantom for visualization of the channels (Fig. 6(b)).

Fig. 6. a) Fabricated intestinal tissue phantom, b) Clear phantom to show details of channels.

The generated grid structures in the channels match well with the photolithography mask, as well as with the physiological morphology of the vessels.

Using the spectra of oxygenated and deoxygenated hemoglobin, comparisons of the absorbance at the wavelengths of interest were calculated and the ratios between the absorbance measurements were used to develop the blood phantoms. The sensor, utilizing LEDs for illumination, will sense across a bandwidth of wavelengths within an individual nominal LED wavelength. For this reason, in matching absorbance ratios between oxygenated and deoxygenated blood, the spectra was weighted using the intensity spectra of the LEDs and the mean absorbance of these weighted spectra were found and used for the ratio comparison. By using this method, the phantom absorbance was matched to an average across the LED bandwidth and not just at the wavelengths of interest, to produce a more accurate representation of the operation of the sensor.

3.3 Customization of the PPG sensor boards

Results from the Monte Carlo simulation were used to guide the optimization of the photonic sensor. The distribution of diffuse reflectance exiting the tissue was examined for each of the wavelengths to determine the source to detector separation that would yield the highest signal intensity. Figure 7 contains the normalized intensity of diffuse reflectance at the surface of the tissue as a function of distance from the source.
The intensity of diffuse reflectance can be seen to peak just below 1 mm of source to detector separation. To maximize the reflectance signal received back to the sensor, the photodetector and LEDs should be brought as close to this optimal distance as possible. In the augmentation of the sensor board, the LEDs closest to the detector were moved as close as was allowed by the geometry of the board to a distance of about 2 mm. Figure 8 shows the optimized configuration of the sensor board for both the green/blue and red/blue LED configurations. For a given LED wavelength pair, one LED was placed at the minimum 2 mm distance and, while not ideal, the second one was placed as close as possible for this board configuration at 6 mm which still allowed for experimental results showing the concept.
With relatively close source-to-detector separation, ~2mm as compared to the higher separations of up to several centimeters [50] utilized by current NIR sensors, the fluence depth of photons can be shortened, reducing background noise from excess probing depth. Equations (9) and 10 [13, 51] were used to quantify this reduction in probing depth. While these equations apply to the estimation of fluence depth in a single homogeneous material, a conservative estimation of the depth reached by the photons was performed for the intestinal domain of interest as a weighted average of the difference optical properties of the multilayer, non-homogenous domain. Equation (9) is used to calculate the average depth of probing ($Z_{R\lambda}$)
for particular optical properties (\(\mu_a, \mu'_s\)) and source to detector separation (\(r\)). Equation (10) describes the probability distribution (\(P_{z,r}\)) of the depths reached by photons.

\[
Z_{r,b} = \frac{0.476 r^{0.5}}{(\mu_a \mu'_s)^{0.25}} \quad (9)
\]

\[
P_{z,r} = 8(3 \mu_a \mu'_s)^{0.5} \cdot \frac{Z}{r} \cdot e^{-\frac{\mu_a \mu'_s \mu_z^2}{r^2}} \quad (10)
\]

Using these equations, the depth fluence for each of the wavelengths used herein was calculated as seen in Fig. 9.

Fig. 9. Probability distribution of fluence depth through the tissue. Estimating an intestinal wall thickness of ~2mm, most of the light incident on the tissue (>99%) will not pass through to the lumen of the intestine for these visible wavelengths. A comparison to 800 nm has been added to show the differences in the depth penetration of light between visible and NIR wavelengths.

From these conservative estimates, the spacing of the LEDs is appropriate for reducing noise in the detected signal due to excess probing depth, wherein most of the light incident on the tissue (>99%) will not pass through to the lumen of the intestine. While the multi-layer, non-homogenous tissue will produce different effects on the light passing through it, these estimates show the benefits of using visible wavelengths over red and NIR wavelengths for this application.

3.4 In-vitro phantom experiment results

Following the optimization of the sensor, the phantom and flow system were utilized to test the performance of the sensor. Figure 10 shows a sample of the time domain PPG signal for each of the wavelengths, as well as the frequency domain signal produced by the Fourier transformation. To quantify the performance of the sensor, the signal-to-background ratio (SBR) was calculated. Here, we compare the highest peak seen in the power spectral density plot attributed to the pulse signal against the highest peak seen not attributable to the pulse signal to define the SBR. While there are peaks observed outside of the fundamental frequency of 1 Hz, these represent signal harmonics, and are not considered noise peaks.
The difference in signal quality at each of the wavelengths can be seen in Fig. 10. The signal from the 630 nm LED, originally selected for its large percent difference in the absorbance of oxygenated and deoxygenated hemoglobin, is seen to contain the PPG waveform, but with lower amplitude and with lower SBR. The lower absolute absorbance of the oxygenated hemoglobin makes the use of this wavelength difficult when the vessel bed being probed is less dense, as in the case of the intestine. Not only is this wavelength noisier for in-vitro phantom experiments without motion artifact, the increased probing depth may also provide less utility for in-vivo experiments where a deeper probing would lead to increased motion artifact, for instance due to breathing.

Each of the different oxygenation and perfusion conditions investigated in the Monte Carlo model was also explored using the phantom flow system. Figure 11 summarizes the peak-to-peak amplitudes observed in the signals for each of the wavelengths for different perfusion conditions. The comparison of these AC signals is useful in both the quantification of SBR, examining the variability of the sensor’s performance to symmetric pulses, as well as determining the ability of the sensor to differentiate between different perfusion conditions.
The peak-to-peak amplitude measurements are correlated with changes in perfusion, though not as well with changes in oxygenation. This separation of variables is helpful in the determination of perfusion conditions across different oxygenation conditions.

The DC signal intensity was measured as a function of the oxygenation of the blood in the vessels. Figure 12 shows the comparison of the mean value of the signal across different oxygenation conditions.

Here, the absolute absorbance of the blood within the vessels affects the signal collected at the sensor. With a greater absolute difference in extinction coefficient, the 560 nm signal is the most telling of oxygenation changes, followed by the 470 nm LED, which is slightly less but on the same order of magnitude. The 630 nm wavelength is an order of magnitude less in
its difference signal with oxygenation. For this reason, the use of shorter visible wavelengths may be more effective in the probing of the thinner small intestinal tissue. Further, as expected the green and the red signal increase with oxygenation while the blue decreases with oxygenation. To give a more clinical measure of the hemodynamic conditions in the phantom, the perfusion index was calculated for each of the wavelengths, shown in Fig. 13.

![Fig. 13. Perfusion indices for each of the wavelengths used. Stronger trends are present in the blue and green signals where only a weak trend is present in the red signal, with the dependence upon oxygenation seen to be minimal.](image)

While all wavelengths show an increase in the perfusion index with an increase in flow, the red signal again shows a weaker correlation than the other two wavelengths. The calculation of the modulation ratio, a relatively holistic measure of tissue hemodynamics dependent primarily on oxygenation, was determined for the 470 nm and 560 nm signals and for the 470 nm and 630 nm signals from each type of sensor board configuration. Figure 14 shows the calculation of the ratio for both sensor boards over a range of oxygenations.
Fig. 14. The modulation ratio between the 470 nm and 560 nm signals and between the 470 nm and 630 nm signals, changes in the ratio can be seen to be more of a function of oxygenation than of perfusion.

As seen above, the changes in the modulation ratio reflect changes in oxygenation; however, it is more difficult to discern oxygenation differences at higher perfusion rates. This may be due to the dependence of the reflectance signal on the particular arrangement of vessels in the phantom [47]. In the case of low perfusion or occluded vessels, the modulation ratio has been reported to increase [13]. While the oxygenation of the blood left in the tissue will decrease in cases of lower perfusion, decreased perfusion alone will also produce an effect on the modulation ratio. This combined effect of both oxygenation and perfusion can help to further assess the condition of the tissue. Given that the shorter wavelengths (470nm and 560nm) produce signals with higher SNR than the red wavelength (630nm), it was determined that in order to gather more reliable data, a visible wavelength PPG system should be used instead of a conventional red-IR system if probing the small intestine. The architecture and how thin the small intestinal wall is allows these shorter wavelengths to perform successfully. Furthermore, these wavelengths show similar—if not more dramatic differences in absorbance between oxygenated and deoxygenated blood than NIR-IR wavelengths [13] (Table 4).

Table 4. Ratio of absorbance between oxygenated and deoxygenated blood for different wavelengths

| Wavelength | 470 nm | 560 nm | 630 nm | 800 nm | 900 nm | 1000 nm |
|------------|--------|--------|--------|--------|--------|---------|
| μₐ – HbO₂ (cm⁻¹) | 179.3  | 176.1  | 3.29   | 4.63   | 6.50   | 5.48    |
| μₐ – Hb (cm⁻¹)  | 87.2   | 290.5  | 27.8   | 3.84   | 4.15   | 1.12    |
| Ratio of μₐ’s   | 0.48   | 1.65   | 8.45   | 0.83   | 0.64   | 0.20    |

The combination of a more shallow probing depth and dramatic differences in absorbance between oxygenated and deoxygenated blood in visible wavelengths leads to the conclusion that a PPG system utilizing these wavelengths will be able to assess both perfusion and oxygenation changes within the small intestinal wall.

4. Conclusion

In this work, the development and performance of a PPG sensor was modeled and optimized for the examination of intestinal tissue. Monte Carlo modeling was used to optimize both selections of wavelength as well as the source to detector separation. The optimized sensor
was tested using a novel intestinal phantom to verify its performance. From these results, the applicability of this optical sensor for the assessment of intestinal hemodynamics was demonstrated. Utilizing the visible wavelengths of 470 nm, 560 nm, and 630 nm, with a short source-to-detector separation of 2 mm, penetration depth through the relatively thin tissue of the intestinal wall is decreased and excess probing of the lumen of the intestine can be seen to be reduced. Theoretically validated by the Monte Carlo simulations and the phantom experiments alike, this sensor has the capability to discern different states of oxygenation and perfusion in the tissue probed. While previous devices designed to probe deeper into tissue have used red and NIR wavelengths, in this application, the use of visible blue and green wavelengths were observed to be superior. The relatively thin tissue (~2 mm) paired with a lower vascular density makes the use of these usually highly attenuated wavelengths an effective mode of PPG signal transduction. The 630 nm wavelength, while still providing information, is more prone to noise than either the 470 nm or 560 nm wavelengths.

**Funding**
National Institutes of Health (NIH) (5R21EB020398-02)

**Acknowledgements**
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

**Disclosure**
The authors maintain no financial interest or conflict of interest concerning this work.