Analysis of pressure, angle and temporal effects on tissue optical properties from polarization-gated spectroscopic probe measurements

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Abstract: Noninvasive optical techniques for tissue characterization, in particular, light scattering properties and blood supply quantification of mucosa, is useful in a wide variety of applications. However, fiber-optic probes that require contact with the tissue surface can present a challenging problem in the variability of *in vivo* measurements due the nature of interactions, for example affects due to variations in pressure applied to the probe tip. We present an *in vivo* evaluation of pressure, angle, and temporal effects on tissue properties for polarization-gated spectroscopy at superficial depths (within 100 to 200 microns of tissue surface) for oral mucosa.

OCIS codes: (300.0300) Spectroscopy; (170.1610) Clinical applications; (170.6935) Tissue characterization; (220.4840) Testing.

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1. Introduction

Noninvasive optical techniques are employed by numerous groups for diagnostic and therapeutic applications *in vivo* [1–5]. For clinical applications, many noninvasive techniques include systems with fiber-optic probes which must be placed in contact with the target tissue to transmit light to and from the tissue. To maintain good contact and eliminate gaps between the probe and tissue interface a gentle pressure is usually applied to these probes. In some applications, like those used in conjunction with endoscopes, the angle between the probe axis and tissue surface is difficult to control precisely. Additionally, in research applications, a technician will operate the system while a physician utilizes the probe. This potentially adds small time delays between initial probe contact and measurement acquisition. These factors may cause variability in the data and therefore impact the optical properties measured. Previous studies have reported that pressure applied to a tissue sample affects the optical properties, in both *ex vivo* and *in vivo* experiments [6–9]. For example, in an *in vivo* animal study on mouse thigh muscle, Reif, et al. [6] established that varying probe pressure affects the reflectance spectrum in a predictable manner. In another *in* vivo animal study of rat heart and liver tissue, Ti, et al. [9] found that applying pressure to the probe induces spectral alterations in diffuse reflectance and fluorescence spectroscopy for both short-term and long-term effects, and the minimum pressure required to induce alterations is tissue-type dependent. In an *in vivo* human study, Nath, et al. [7] found that probe contact pressure had little to no effect on fluorescence spectra when measured from cervical tissue. While insights have been gained through these studies, they have largely focused on a single penetration depth into the target tissue. However, tissue is a heterogeneous multilayered structure and probe-tissue interactions may have differential effects on tissue properties depending on the depth of interrogation.

Depth-selective tissue analysis is possible utilizing polarization-gating fiber-optic probes [10–12]. Polarization-gating interrogates progressively deeper tissue depths through analysis of the differential polarization signal, $\Delta I = I_{\parallel} - I_{\perp}$, co-polarized signal, $I_{\parallel}$, and cross-polarized signal, $I_{\perp}$. Tissue phantom studies demonstrated the average penetration depths of the ΔI, $I_{\parallel}$, and $I_{\perp}$ signals are ~95, 145, and 185μm, respectively [13]. The objectives of this study are to exploit the depth-selective capabilities of polarization-gating probes to map the effects of 1) pressure applied to the probe, 2) the angle between the probe axis and tissue surface, and 3) continuous probe contact for short time scales as a function of depth. All measurements were taken *in vivo* on human lip tissue. This tissue was selected for its *in vivo* accessibility and because it closely simulates the colonic mucosa which aligns with the research interests of our group.

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2. Materials and methods

2.1 Experimental setup

The clinical data acquisition system was used for this experimental setup, which consists of a white light LED (WT&T) for an illumination source; two fiber optic spectrometers for detection (Ocean Optics, USB2000); a polarization-gating fiber-optic probe for transmission of light to and from tissue; and a control computer. Figure 1 illustrates the probe design, described in detail previously [2,13], which consists of three 200μm-core diameter fibers, one serves as an illumination channel and the other two for collection of the co-polarized and cross-polarized signals. Each tissue measurement acquires 3 spectra spanning the wavelength range of 350 to 700nm. The first and last acquisitions have the light source on for use investigating temporal effects, and to record the ambient signal, the source is turned off for the second acquisition. The recorded ambient signal is subtracted from each tissue measurement to account for any external effects not related to the probe illumination, such as room light. The entire time for this acquisition sequence is less than three seconds.

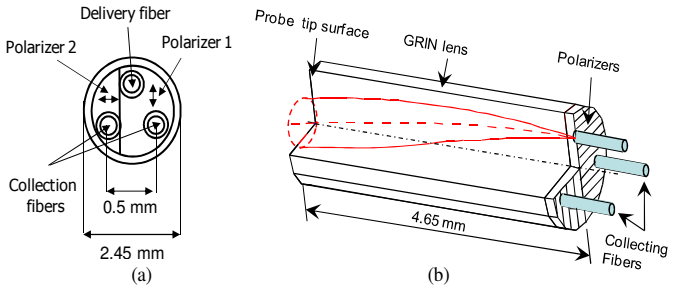

Fig. 1. (a) Schematic diagram (*frontal view*) of the polarization-gating fiber optic probe tip. (b) Schematic (*side view*) of the probe tip, which comes in contact with the sample, showing the fibers, thin-film polarizers and GRIN lens.

Experiments were performed on the inner, mucosal surface of the lower lips of 4 volunteers. For all measurements, the volunteer manipulated the probe to ensure contact with the lip tissue while a technician acquired the data. Prior to measurements, the inner surface of the lip was moistened to ensure water or saliva was present at the interface between the probe tip and tissue surface. In each experiment, measurements were normalized to our perceived ideal operating conditions which included applying a gentle pressure and the probe axis normal to the tissue surface. From each tissue measurement, five parameters were extracted from the algorithm used in previous *in vivo* analysis [2,13–16]: (1) total hemoglobin (Hb) concentration, (2) oxygen saturation, (3) packaging length scale (PLS) which is proportional to blood vessel diameter, (4) shape parameter, m, which describes the distribution of length scales, and (5) total scattering intensity. The parameter m characterizes the shape of the refractive index correlation function and is used to model the distribution of scattering length scales. Changes in m lead to changes in the power law that describes the reduced scattering coefficient dependence on wavelength. For the Whittle–Matérn model of refractive index distribution, values of parameter $m < 1.5$ correspond to a mass fractal, $1.5 < m < 2$ corresponds to a stretched exponential, and $m = 2$ corresponds to an exponential correlation function [17]. Each parameter (except total scattering intensity) was calculated at 2 depths from the tissue surface: the differential polarization spectra, ΔI, (average penetration depth ~100μm) and cross-polarization spectra, $I_{\perp}$, (average penetration depth ~200μm).

Exclusion criteria were determined to indicate poor contact between the probe tip and tissue surface. One criterion is low reflected signal, defined as any calculated Hb concentration value more than 2.5 standard deviations from the mean for ideal operating conditions (i.e. gentle pressure). Another criterion is signal-to-noise ratio (SNR = mean/standard deviation of the signal intensity) below a threshold of 33. The SNR threshold ensured that variation in calculated Hb concentration due to noise was 3 times less than the

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inter-measurement variability in ideal operating conditions. For the temporal study, exclusion of an outlier was determined based on its placement in the time sequence. A point at the beginning of the time sequence was assumed to have poor contact for the initial measurement and the starting point for that particular set was adjusted. These sets will have less than 20 measurements per tissue site. A single, random point in the sequence was simply excluded and the remaining data analyzed. Any points near the end of the sequence (i.e. in the last 10 seconds) were not excluded because this may be an effect of continuous contact.

To demonstrate that the probe can accurately measure the reduced scattering coefficient of a sample, we conducted a series of measurements on liquid suspensions of polystryene microspheres (Duke Scientific) in water. The scattering coefficient was varied from 6 – 36.5 cm^{-1} by altering the concentration of microspheres and was calculated from Mie theory. In addition, suspensions with two different sizes of microspheres, 0.52 μm and 0.87 μm, were made corresponding to anisotropy values of 0.75 and 0.89 respectively. For each scattering coefficient and anisotropy value, measurements were taken by inserting the probe into the sample and recording the total reflected intensity collected by the probe. The relative reflectance was then determined as the ratio of the absolute reflectance of the phantom over the absolute reflectance of a polytetrafluoroethylene reflectance standard (Ocean Optics). The relative reflectance as a function of the reduced scattering coefficient is shown in Fig. 2(a). The overlap between the two different anisotropy values suggests that the relative intensity is a function of the reduced scattering coefficient only rather than the scattering coefficient or anisotropy factor independently. The functional relationship is observed to be linear with an R^2 of 0.99.

Tissue phantom models for oxygenation measurements were constructed according to the methods given in Siegel, et al. [12]. In brief, lypholized human hemoglobin (Sigmal-Aldrich) was added to a polystyrene microsphere suspension to create the starting solution. This starting solution had a hemoglobin concentration of 3 g/l, g = 0.86, and μ_s = 200 cm^{-1}. A trace amount of baker's yeast was added to this starting solution and simultaneous measurements were taken with the polarization-gating probe and an oxygen-sensitive microelectrode (Microeletrodes Inc.) as the starting solution gradually deoxygenated. The subroutine reported by Kelman [18] was used to convert the partial pressure of oxygen measured by the electrode into percent oxygenation for comparison with the output of the polarization-gating probe and algorithm. Figure 2(b) compares these simultaneous measurements and demonstrates that the oxygenation values measured by the polarization-gating probe closely matched the oxygenation values determined by the electrode. The average percent error between both techniques was 2% over the physiological range of 35%-100% oxygenation. These results validate the accuracy of oxygenation determination by the polarization-gating probe.

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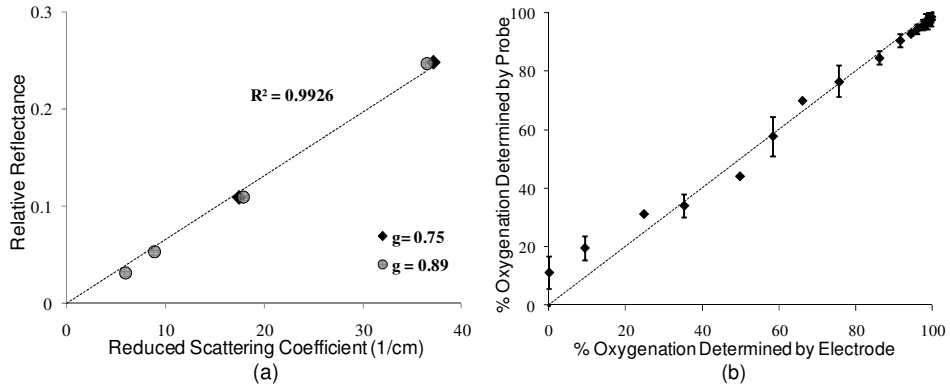

Fig. 2. (a) Relative Reflectance from a tissue scattering phantom collected by the EIBS probe as a function of the reduced scattering coefficient for two different anisotropy factors. (b) The EIBS probe accurately measures percent oxygenation compared to a standard oxygen sensitive microelectrode. Perfect agreement between the probe and electrode oxygenation measurements is indicated by the diagonal line. The average percent error between both oxygenation measurement methods was 2% over the physiological range of 35%-100% oxygenation.

2.2 Pressure study

To study the effect of pressure applied by the probe to tissue, a set of measurements were taken randomly along the bottom lip. A set consisted of 2 measurements, each with different pressure, taken on the same tissue site with a 3 second recovery period between successive measurements. Pressure was manually applied to the probe by the volunteer. For each volunteer, 20 measurements were taken with firm pressure first and 20 with gentle pressure first. The order of applied pressure was randomized. Each spectrum was acquired within 2s of bringing the probe in contact with tissue. From the total 160 tissue sites probed, 5 were excluded due to poor tissue contact, resulting in a total of 155 tissue sites analyzed. Gentle pressure corresponded to the probe lightly touching the tissue surface, while firm pressure was enough to visibly indent the tissue surface. In order to estimate a value for each pressure, individuals applied a similar pressure to that used in experiments onto clay that was placed on a scale. From this, gentle and firm pressures were found to correspond to 0.009 – 0.012 N/mm^2 and 0.15 – 0.2 N/mm^2, respectively.

2.3 Angle study

To study the effect of the angle between the axis of the probe tip and tissue surface, a set of measurements were performed similar to pressure. Here, a set consisted of 3 measurements taken on the same tissue site using three different angles between the axis of the probe tip and the surface normal: 0-10 degrees, 30-50 degrees, and 60-90 degrees from normal to the surface. Gentle pressure was applied for all measurements. Only 3 volunteers participated in this experiment, and for each volunteer, 10 sets were taken on random tissue sites and the order of angle randomized. From the total 30 tissue sites probed, 3 were excluded due to poor tissue contact, resulting in a total of 27 tissue sites analyzed.

2.4 Temporal study

To study temporal effects regarding the length of contact time between the probe and tissue, and whether this effect depends on applied pressure, a set of measurements were taken randomly on the lip. For a measurement set in this study, 10 successive acquisitions were taken on the same tissue site, without moving the probe. Since two spectra are acquired for each measurement, this produced 20 tissue acquisitions within a 30 second time interval. Throughout the entire sequence, either gentle or firm pressure was applied consistently. From the total 100 tissue sites probed, 8 were excluded due to poor tissue contact throughout the

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entire measurement sequence, resulting in a total of 43 tissue sites analyzed for gentle pressure and 49 tissue sites analyzed for firm pressure.

3. Results

Statistical analysis was done using Microsoft Excel and Stata 9 (StataCorp). To investigate effects of the probe, measurements were normalized according to the study. For the pressure study, measurements were normalized to gentle pressure. Individual differences in pressure applied were accounted for by normalizing parameters by the mean gentle pressure for each volunteer separately, and then compared between individuals in a two-sided Welch's *t* test with a significance level of $\alpha = 0.05$. For the angle study, parameters were normalized by the mean value at 0-10 degrees (probe normal to tissue surface) for each volunteer and then assessed using a single-factor ANOVA analysis with a significance level of $\alpha = 0.05$. For the temporal study, the change over time between the two pressures, gentle and firm, was tested using linear mixed models with pressure and time as the fixed effects, a pressure by time interaction, and a random effect for sets within volunteer with a significance level of $\alpha = 0.05$. To investigate the time point when a change in the parameters becomes significant, each measurement set was evaluated with a paired *t* test between the means at time = 1 second and 5 distinct time points: time = 6, 10, 15, 19, and 30 seconds. The significance level was adjusted to $\alpha = 0.01$ for multiple comparison tests.

Reflectance signals were measured from the inner surface of the bottom lip tissue of 4 volunteers while the probe was held and manipulated by the volunteer. For each study, 5 parameters were quantified: (1) total Hb concentration, (2) oxygenation, (3) PLS, (4) m, and (5) total scattering intensity. The polarization-gating probe provided analysis for 2 depths (ΔI ~100μm and $I_{\perp}$ ~200μm) below the tissue surface. The mean and standard errors of the five parameters extracted from analysis of the pressure and angle experiments are presented in Figs. 3(a) through 3(i) and the temporal experiments are presented in Figs. 4(a) through 4(i). In these figures, panels (a) and (b) show total Hb concentration; panels (c) and (d) show oxygenation; panels (e) and (f) show PLS; panels (g) and (h) show m for superficial (100μm) and deep penetration depths (200μm), respectively; and panel (i) shows the total scattering intensity.

3.1 Pressure

In the pressure study, there were several trends worth noting. For total Hb content with firm pressure applied, there is a statistically significant increase ($p = 0.002$) for ΔI signal [Fig. 3(a)] and a significant decrease ($p < 0.001$) for $I_{\perp}$ signal [Fig. 3(b)]. For oxygenation, no significant change was observed at either depth ($p = 0.80$ for ΔI and $p = 0.21$ for $I_{\perp}$) [Fig. 3(c), 3(d)]. For PLS, ΔI remained constant ($p = 0.99$) [Fig. 3(e)], but $I_{\perp}$ showed a significant decrease in PLS ($p < 0.001$) [Fig. 3(f)]. The m parameter also showed no change for ΔI ($p = 0.15$) [Fig. 3(g)] and a significant increase for $I_{\perp}$ ($p = 0.006$) [Fig. 3(h)]. For total scattering intensity, there is a significant increase ($p < 0.001$) when firm pressure is applied [Fig. 3(i)].

3.2 Angle

In the angle study, there were no significant trends observed ($p > 0.25$ for all parameters) in any parameter between the 3 different angles measured [Fig. 3(a)–3(i)]. This suggests that the angle between the probe tip and tissue surface has no influence on the probe measurements.

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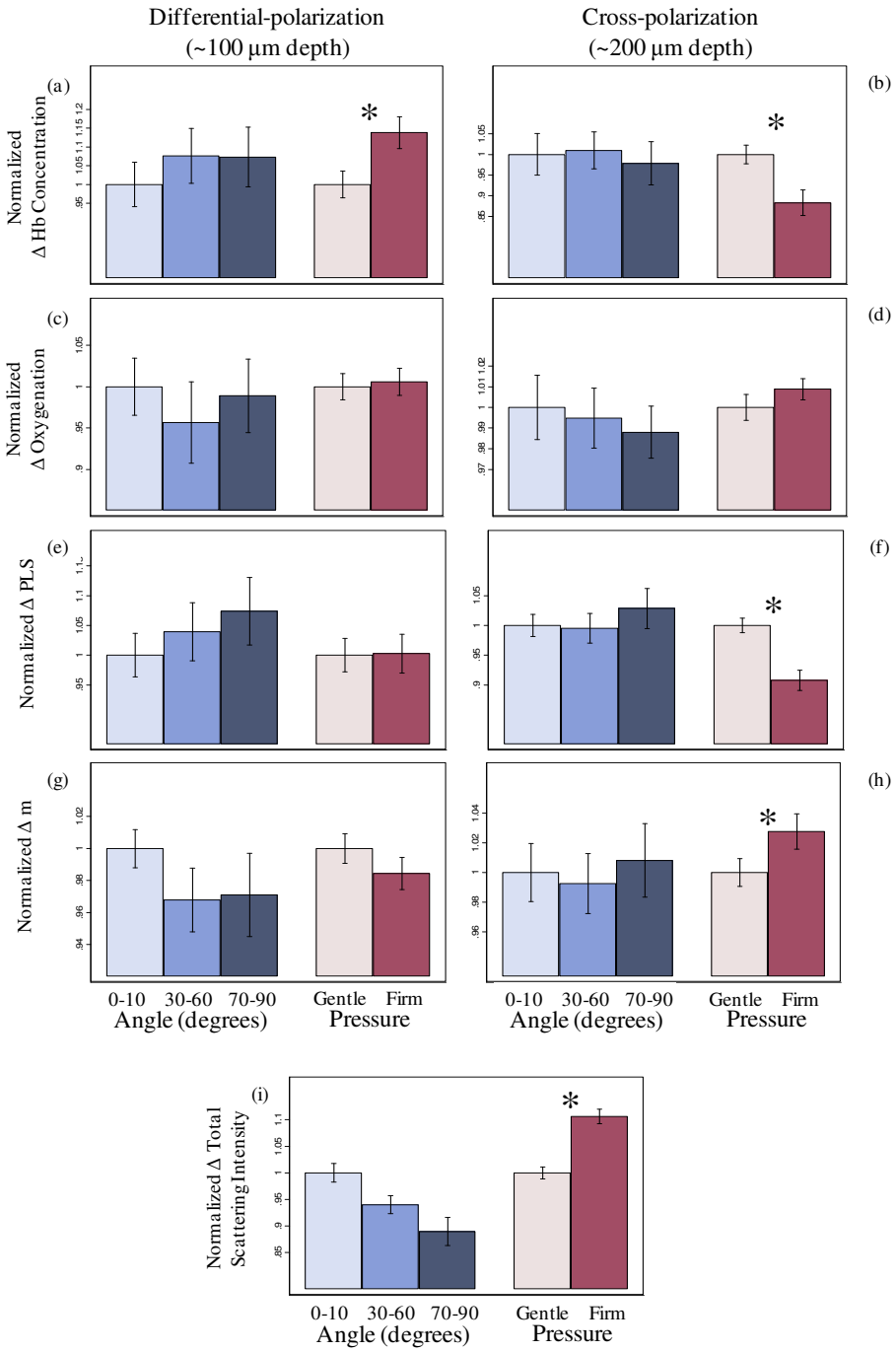

Fig. 3. . Results for pressure and angle experiments. Angle measurements were normalized to the mean value of each parameter for the probe axis normal to tissue. Pressure measurements were normalized to the mean value of each parameter for gentle pressure. Graphs represent the relative change in each parameter. Left panel shows the means and standard errors for (a) total Hb content, (c) oxygenation, (e) PLS, and (g) m from the ΔI signal. The right panel shows the means and standard errors for (b) total Hb content, (d) oxygenation, (f) PLS, and (h) m from the $I_{\perp}$ signal. Panel (i) shows the total scattering intensity. * Notes parameters with statistical significance of $p < 0.05$.

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3.3 Temporal

In the temporal study, the change in parameters was first compared between the two pressure groups over time using a random effects mixed model including an interaction. For 3 parameters, total Hb content, oxygenation, and PLS, there was a statistically significant difference between gentle and firm pressure over time (interaction $p < 0.001$) for both ΔI and $I_{\perp}$ [Fig. 4(a)–4(f)]. For m, there was a significant difference over time for ΔI (interaction $p < 0.001$), but not for $I_{\perp}$ (interaction $p = 0.09$) [Fig. 4(g), 4(h)]. For total scattering intensity, there was also a statistically significant difference over time (interaction $p = 0.02$) [Fig. 4(i)].

Next, the time point after initial contact when a change in the parameters becomes significant was evaluated between the means at 5 distinct time points: time = 6, 10, 15, 19, and 30 sec. When gentle pressure was applied, there was no time point within the 30 seconds of continuous contact evaluated in this study that showed a statistically significant change from the first tissue measurement. When firm pressure was applied, some parameters showed a significant change within 6 sec, the first time point tested for significance. For total Hb content, the change is significant within 10 sec for ΔI ($p = 0.005$) and within 6 sec for $I_{\perp}$ ($p = 0.007$). For oxygenation, the change is significant within 6 sec for both the ΔI ($p = 0.008$) and $I_{\perp}$ ($p < 0.001$). There is no significant change within the 30 second interval for PLS and m at either depth, or for total scattering intensity. The means and standard errors of the five parameters extracted from the analysis of the temporal experiments are presented in Figs. 4(a) through 4(i).

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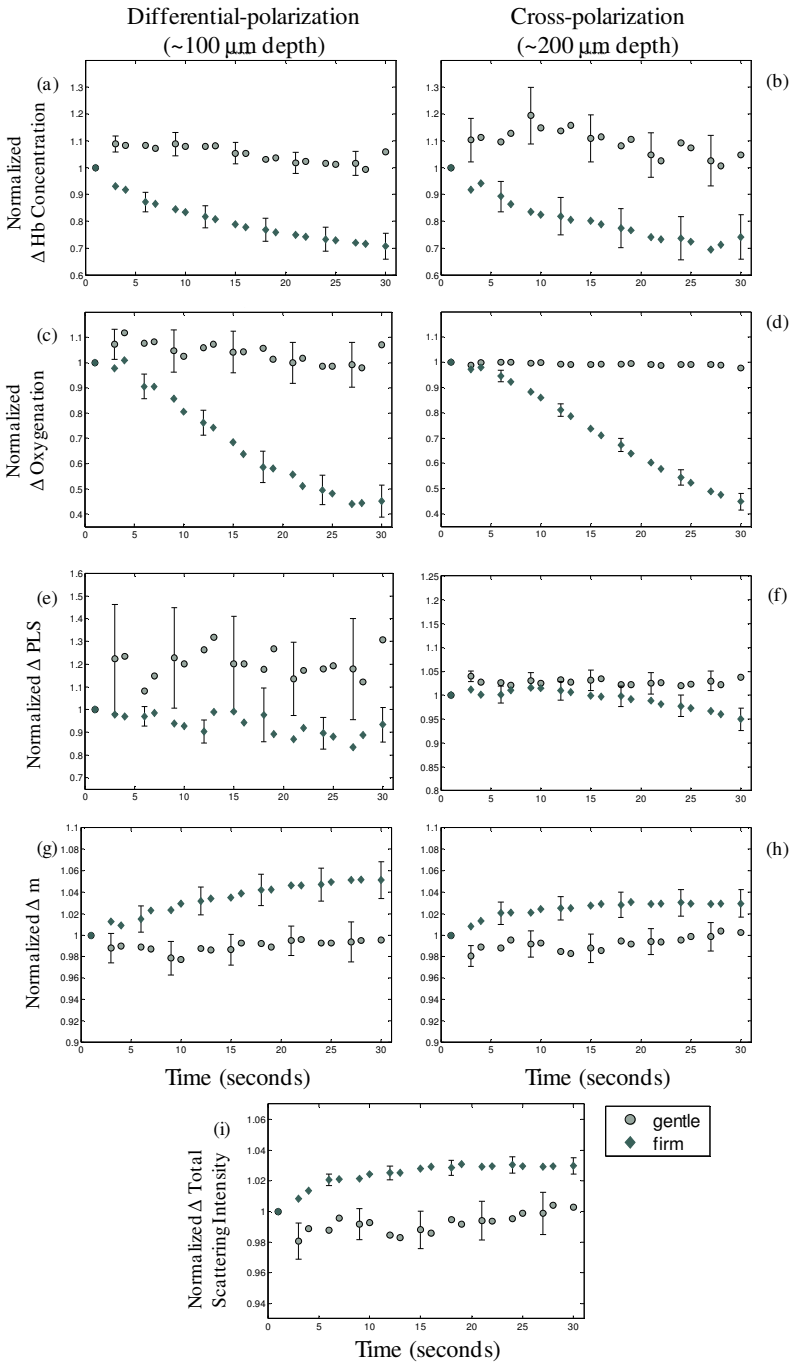

Fig. 4. Results for temporal experiments which include acquisition of 20 spectra within 30 seconds on the same tissue site. For each tissue site, measurements were normalized to the first measurement in the sequence, corresponding to initial probe contact. Either gentle or firm pressure was applied consistently throughout the measurement sequence. Graphs represent the relative change in each parameter with the applied pressure. Left panel shows the means and standard errors for (a) total Hb content, (c) oxygenation, (e) PLS, and (g) m from the ΔI signal. The right panel shows the means and standard errors for (b) total Hb content, (d) oxygenation, (f) PLS, and (h) m from the $I_{\perp}$ signal. Panel (i) shows the total scattering intensity.

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4. Discussion

In this study, we investigated the influence of applying pressure to the probe, the angle between the probe tip and tissue surface, and temporal dependence on short contact durations. Firm pressure applied to the probe affects several parameters measured. At the deeper depth measured, the results are consistent with our expectations. As the tissue is compressed from firm pressure, the blood vessels are also compressed which forces some blood out of the area under the probe tip, as observed with a decrease in PLS and reduction in total Hb content. Yet, the superficial depth does not have the same trend. We hypothesize that the epithelial layer may have a different elasticity than the lamina propria and that applying firm pressure to the probe compresses the most superficial layer. This may bring some deeper vessels closer to the tissue surface, within the range of the average penetration depth measured by the delta-polarization signal and account for the observed increase in total Hb. We expected firm pressure to decrease oxygenation due to a reduction in fresh blood supply to the area. In this pressure study, however, a single measurement does not provide the sufficient time scale to observe these changes. This is supported in Fig. 4(c), 4(d) which shows significant oxygenation changes do not occur until more than 5 seconds after initiating probe contact. It is suggested that the observed decrease in temporal oxygenation results from a combination of reduced blood supply and continued oxygen consumption by the tissue underneath the probe tip. Since there is less blood supply, the trapped blood under the probe will deoxygenate over time as the oxygen continues to diffuse into the tissue from the only available blood. Consistent with the observation by Reif et al [6], the change in total Hb content is less than 20% for both depths. Although the total Hb content change is near 20% and below the intra-patient and inter-patient variability (~40% for each) observed in our *in vivo* colonoscopy clinical studies [2,13], it is significant. This suggests that pressure could influence the measured parameters and may be largely responsible for the intra- and inter-patient variability since different endoscopists likely apply varying pressures to the probe. This effect needs further investigation to understand differences in utilization of the probe between endoscopists and the subtle differences occurring within the mucosal layers of tissue.

We observed that firm pressure has some impact on total scattering intensity and the shape of the index correlation function, quantified by the parameter m. As stated above, we hypothesize that the probe tip compresses the tissue. This would bring the scatterers closer together, allowing two smaller scatterers to act as one larger scatter, leading to an increase in the total scattering intensity. Additionally, compressing the tissue leads to a change in the distribution of scattering length scales, measured by m. Future studies are needed to further investigate these effects to gain a better understanding of the observed changes in m and total scattering intensity. Future studies should also aim to verify that a controlled, gentle pressure applied to the probe can reduce variability in the clinical setting. This can be accomplished with a sensor on the probe tip that controls or measures the pressure applied.

We observed that changing the angle between the probe tip and tissue surface did not show a significant effect for any parameter. This indicates that the tissue conforms to the surface of the probe lens when it is in good contact with the target tissue. Although a slight trend can be observed for total scattering intensity, with an ANOVA p-value = 0.26, it is not statistically significant (all other parameters range from $p = 0.5$ to $p = 0.9$). It is possible that changing the probe angle could potentially distort the tissue under the probe or introduce changes in optical coupling that could be responsible for the observed trend. Since the data show less than 10% change, we can assume that the parameters measured are not dependent on the angle.Although the probe was designed to be used with the probe axis normal to the tissue surface, when it is delivered through the accessory channel of an endoscope, this is often difficult to achieve for every *in vivo* measurement. These results confirm that precise normal incidence to the tissue is not required and that angle is not an important source of variability in our clinical studies.

From the temporal analysis, we observed that within a short time scale, continuous contact between the probe and tissue affects the parameters measured. The 30 second time scale was

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chosen to represent probable delays for the clinical setting that occur between the time the physician places the probe in contact with tissue and the time the technician starts data acquisition. For gentle pressure, there are no significant differences in the parameters at any time point in this scale. For firm pressure, the rapid decrease observed in total Hb content and oxygenation in as little as 6 seconds from initial probe contact results from reduced blood supply and continued oxygen consumption, as discussed above. Also similar to the pressure study, PLS is not affected at the more superficial depth and only slightly decreases with pressure at the deeper depth. This further supports the hypothesis that firm pressures may compress the more elastic epithelial layer and collapse deeper vessels resulting in the reduced blood flow. These results suggest that temporal trends can yield information about oxygen consumption of the interrogated tissue. In the case of a controlled, known pressure, *in vivo* temporal measurements could correlate decreasing oxygenation with metabolic demand for potential diagnostic applications.

These studies demonstrate that firm pressure impacts the data collected and needs to be addressed in the clinical setting. A proper protocol can be developed to include maintaining good contact between the probe tip and tissue surface with application of gentle pressure. Conversely, we realize that gentle pressure is subject to interpretation and even more difficult to assess if utilizing the probe through an endoscope. The temporal study revealed that changes due to pressure can occur in as little as 6 seconds. Thus, there is an urgent need for a robust mechanism to minimize and control any delay between probe contact and data acquisition. One potential solution is implementation of a sensor to automatically trigger data acquisition when contact is detected between the probe and target tissue. This will eliminate the need for a technician and ensure the delay between tissue contact and acquisition is consistent, potentially reducing variability previously encountered from pressure or temporal effects. We expect to further expand this study in a clinical setting to more relevant tissues, such as colon polyps or other lesions, coinciding with development of the sensor.

One limitation to this study is that the volunteer manipulated the probe during measurements. While this allowed the volunteer to maintain good contact with the lip tissue and apply a more consistent pressure throughout measurements, this does not replicate the clinical scenario in which a physician uses the probe on a patient. Future studies should aim to investigate the effects of pressure and time when the probe is manipulated by another user, as well as with the probe used through the accessory channel of an endoscope. Furthermore, future studies should confirm that variability can be reduced in our clinical *in vivo* data when either the pressure applied to the probe or the time delay between contact and acquisition is controlled. Additionally, other sources of variability need to be explored, such as colonic distension.

Another limitation of allowing the volunteer to manipulate the probe is that the pressure applied was subjective and each volunteer may interpret ‘gentle’ and ‘firm’ differently. We can assume that the gentle pressures represent the range of pressures applied in the clinical setting, and the firm pressures, which visibly indented the surrounding tissue, are greater than pressures utilized by physicians in our clinical studies. Eliminating pressure and temporal effects would simplify the use of our device in mutli-center clinical trials and in future applications.

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