Analyzing near infrared scattering from human skin to monitor changes in hematocrit

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ABSTRACT

The leading preventable cause of death, world-wide, civilian or military, for all people between the ages of 18-45 is undetected internal hemorrhage. Autonomic compensation mechanisms mask changes such as e.g. hematocrit fluctuations that could give early warning if only they could be monitored continuously with reasonable degrees of precision and relative accuracy. Probing tissue with near infrared radiation (NIR) simultaneously produces remitted fluorescence and Raman scattering (IE) plus Rayleigh/Mie light scattering (EE) that noninvasively give chemical and physical information about the materials and objects within. We model tissue as a three-phase system: plasma and red blood cell (RBC) phases that are mobile and a static tissue phase. In vivo, any volume of tissue naturally experiences spatial and temporal fluctuations of blood plasma and RBC content. Plasma and RBC fractions may be discriminated from each other on the basis of their physical, chemical and optical properties. Thus IE and EE from NIR probing yield information about these fractions. Assuming there is no void volume in viable tissue, or that void volume is constant, changes in plasma and RBC volume fractions may be calculated from simultaneous measurements of the two observables, EE and IE. In a previously published analysis we showed the underlying phenomenology but did not provide an algorithm for calculating volume fractions from experimental data. Here we present a simple analysis that allows continuous monitoring of fluid fraction and hematocrit (Hct) changes by measuring IE and EE, and apply it to some experimental in vivo measurements.

Keywords: Noninvasive, hematocrit, blood, hemorrhage, fluorescence, Rayleigh, Mie, Raman

INTRODUCTION

There is considerable motivation for developing methods to perform blood and other tissue analyses noninvasively and in vivo in humans. In order to obtain the concentration of an analyte one must measure an analytical signal specific to that analyte and determine the volume from which the signal originated. We have previously reported1,2 using Raman spectroscopy to specifically monitor glucose while using the simultaneous autofluorescence (IE) to estimate the probed volume. Combined with the tissue modulation technique we have described before1,3, we can localize the measurement to plasma glucose i.e. as opposed to interstitial fluid or the total of the two. By maintaining our focus on probing for fingertip skin capillary bed plasma glucose, if we are successful, we can meet the current ISO standard of care4 defined by fingerstick devices. The work presented5 here represents an extension of that approach in order to produce improved estimates of probed volume that are internally consistent with respect to physical optics, anatomy, homeostasis and general physiological principles.
This improved model attempts to account for all the light that is remitted when tissue is probed by monochromatic light e.g. a laser or possibly a light emitting diode (LED) with regard to absorption and propagation effects in the context of a particular geometry and anatomy. Whenever such light is directed into e.g. skin tissue as shown in Figure 1, some light is remitted at the same wavelength i.e. elastically scattered (EE) or at different wavelengths i.e. inelastically scattered (IE) as shown in Figure 2. The processes that underlie the production of IE and EE are fundamentally different and therefore each can be taken as an independent measurement. Note that with only one incident light source, as opposed to pulse oximetry, there need be only one volume defined by the incident and collection optics that produces IE and EE in a consistent manner regardless of the tissue composition. Furthermore, fluorescence involves an essentially zero background measurement as opposed to absorption in which a small change must be measured on a large baseline signal. This inherent signal to noise advantage of fluorescence detection may provide an advantage over algorithms employing only absorption measurements i.e. photoplethysmography and oximetry.

![Diagram of apparatus and layout for measuring scattering.](image)

Figure 1. Schematic diagram of apparatus and layout for measuring scattering.

On the basis of their relative mobility when the tissue is compressed, the specific tissue we intend to probe can be thought of as being comprised of three phases: plasma, red blood cell (RBC) and static tissue. If the total probed volume is assumed to be constant then these phase fractions must sum to a constant. Based on the idea that our probing geometry produces signal predominantly from the epidermis above the superficial dermal plexus so that we only probe capillary blood, we assume that all light propagation occurs in the single scattering limit and that the distribution of the three phases is homogeneous in the tissue volume. This situation can be modeled using the radiation transfer equation (RTE) to obtain reasonable agreement with actual spatial scans of the surface of volar side fingertip skin with constant probe geometry while IE and EE are collected simultaneously. The success of that approach, in which the contributions of EE and IE from all the phases were calculated and summed linearly, together with empirical observations, led us to consider the situation where we have constant spatial position but time varying tissue composition due to e.g. cardiac driven blood movement.
In this paper we first give a few experimental details before presenting the model and algorithm for the calculation of changes in plasma, red blood cell (RBC) and static tissue volume fractions from EE and IE. We give some idea of how to calibrate this model while comparing with known physiological responses. We then show some interesting applications of the algorithm to measurements of human volar side fingertip skin in vivo and discuss the strengths and weaknesses of this approach.

**EXPERIMENTAL**

Experimentation with human test subjects is preceded by obtaining informed consent in accordance with our Crouse Memorial Hospital IRB approved protocol. We probe the volar side of human fingertips using 200mW of continuous-wave 830-nm laser excitation (Sacher Lasertechnik) that impinges on the skin at 53° to the normal, through a 2.1-mm aperture in a 0.635-μm-thick sheet of spring steel. Pressed against the spring steel under external control using only the force needed to maintain optical registration with the collection system, the skin (i.e. soft matter) extrudes a small dome <20 μm into the aperture. The RTE model must consider this profile and its effect on the angle of incidence and propagation of the incident and secondary light to obtain even qualitatively accurate results. The remitted light is collected at a ∼f 2.1, and a Semrock “Razor Edge” filter is used to simultaneously reduce the Rayleigh scattered light and adjust the dynamic range of the EE and IE as indicated in Fig. 2. A careful measurement gave ∼1 μW total power (i.e., IE + EE reaching the input of the spectrograph slits). When integrated, but without inserting the Razor Edge filter, the EE is approximately five to six orders of magnitude greater than the IE. After being dispersed in the spectrograph, the light is detected by a −45°C Critical Link MityCCD-E3011-BI CCD camera (not shown). A single 20-ms frame as used for all the data in this paper is shown in Fig. 2.
In order to obtain reproducible results we have developed a position detector pressure monitor\(^3\) (PDPM) that can ensure reproducible placement of the skin over the aperture and constant pressure in real time during the measurement cycle. Also, the adjustment of the Semrock filter is crucial in that saturation of the CCD must be avoided. Not only will saturation damage the pixels but there is a very bad artifact involving correlated changes in apparent EE and IE that can occur and confound the algorithm. Adjusting this filter to have the Rayleigh line in the range of \(\approx 15000\) counts/frame-pixel is acceptable.

**RESULTS - THE MODEL AND ALGORITHM**

The volume fractions for RBCs, plasma and static tissue sum to unity implying that there are no voids. This is summarized in equations [1] and [2] using \(\phi\) for each of the volume fractions, i.e. RBCs, plasma and static tissue in the probed volume.

\[
1 = \phi_r + \phi_p + \phi_s \quad [1]
\]

\[
0 = d\phi_r + d\phi_p + d\phi_s \quad [2]
\]

We use the RTE to propagate the incident light from air into a three phase, three layer medium in the single scattering limit and with the phases, i.e. the RBCs, plasma and static tissue distributed homogeneously in the probed volume. Since we were able to obtain agreement with experiment by summing contributions to IE and EE linearly we can write:

\[
Hct = \phi_r / (\phi_r + \phi_p) \quad [3]
\]

\[
EE = \partial_1 + \partial_2 \phi_p + \partial_3 \phi_r \quad [4]
\]

\[
IE = \partial_4 + \partial_5 \phi_p + \partial_6 \phi_r \quad [5]
\]

The 6 parameters can be determined using the RTE and published scattering and absorption coefficients but presently our interest is better served by noting that since we have 2 linear equations linking 2 measured quantities, EE and IE, to the 2 volume fractions, \(\phi_r\) and \(\phi_p\), for each frame, it is possible to invert equations 4 and 5 to obtain 6 and 7.

\[
\phi_r = a + b \left( \frac{EE}{EE_0} \right) + c \left( \frac{IE}{IE_0} \right) \quad [6]
\]

\[
\phi_p = d + e \left( \frac{EE}{EE_0} \right) + f \left( \frac{IE}{IE_0} \right) \quad [7]
\]

There are 6 parameters (a, b, c, d, e and f) for which we must obtain numerical values. This can be done using constraints based on empirical data or assumptions. First, consistent with Jacques\(^10\) and our own geometrical approach for very well perfused skin, we assume that \(\phi_r = 0.004\) and \(\phi_p = 0.036\) on average and that \(EE_{ave} = EE_0\) and \(IE_{ave} = IE_0\). A cursory examination of other parameter sets suggests that we can exercise some flexibility in choosing these values because we are interested in monitoring deviations from the “normal” values. This gives 2 constraints: \(a+b+c = 0.004\) and \(d+e+f = 0.036\) according to [6] and [7]. The absolute values of \(\phi_r\) and \(\phi_p\) are now not significant, only deviations from the values 0.004 and 0.036.
Additional constraints can be obtained from changes in EE and IE that can be correlated with known physiology. From [6] and [7] we find:

\[ \Delta \phi_r = b \left( \frac{\Delta EE}{EE_0} \right) + c \left( \frac{\Delta IE}{IE_0} \right) \]  
\[ \Delta \phi_p = d \left( \frac{\Delta EE}{EE_0} \right) + f \left( \frac{\Delta IE}{IE_0} \right) \]  

We note that normally with each cardiac pulse\(^{11}\) about 75 ml of 0.45 Hct blood is injected into the arterial side of the 4000 ml total supply. Uniformly distributed this would result in a 1.9\% transient increase in blood volume, i.e. in both \( \phi_r \) and \( \phi_p \) assuming constant Hct. Thus, we can assume that with each pulse \( \phi_r \) and \( \phi_p \) increase by 0.000076 and 0.000684 respectively. A typical experiment produces a \( \approx 2.08\% \) and a 2.63\% change in EE and IE respectively with each cardiac pulse, i.e. systolic EE and IE minus diastolic EE and IE. These two conditions can be inserted into [12] and [13] to generate two more constraints on the values of the parameters a-f.

\[ b = \frac{0.000076 - 0.0263c}{-0.0208} \quad e = \frac{0.000684 - 0.0263f}{-0.0208} \]

These equations constitute two additional constraints.

\[ b = -0.00365 + 1.264c \quad e = -0.0329 + 1.264f \]

Thus there are various approaches involving empirical observation of IE and EE modulation, associated with known physiological effects, to obtaining constraints on the 6 parameters and we have explained this in greater detail elsewhere. However, for our present purposes the above examples yield 4 constraints on the 6 parameters and we can determine the remaining 2 parameters by minimizing the standard deviation of the Hct from the mean over some time range of measurement. That is, we fit [6] and [7] to measured data using the 4 constraints and varying the two parameters while minimizing the deviation of the hematocrit for the time period of the "calibration" data. This is justified because normally Hct is constant under homeostasis\(^{12}\). The mean value will be \( \approx -0.1 \) since the reference values of \( \phi_r \) and \( \phi_p \) are 0.004 and 0.036 respectively. Again, we emphasize that the assumed values are not important – what matters is the deviation of the calculated \( \phi_r \) and \( \phi_p \) from the reference values.

**RESULTS-COMPARISON WITH EXPERIMENT**

For one demonstration we used a manual blood pressure cuff as a tourniquet to induce hemoconcentration. After determining the test subject’s blood pressure and pulse rate using an automatic cuff, IE and EE were collected with no applied tourniquet, beyond that needed to maintain mechanical registration of the tissue with respect to the optical system, in order to define homeostasis. The applied pressure was maintained by the PDPM at 35±10 g-force/cm\(^2\), the lowest we could maintain (on that day), for 60 seconds. The tourniquet brought the pressure to its final planned value within 5-7 cardiac pulses, by a single increase in pressure (in several pumps on the manual bulb), at 60 seconds. The final pressure was chosen to be just above the systolic pressure in order to insure venous occlusion, which can be confirmed with a stethoscope. Once complete occlusion was established, it was maintained without modification for 60 seconds. Thus at 120 seconds from establishing homeostasis the tourniquet was released as quickly as possible. The associated changes in EE and IE can be seen in Figure 3.
Both scattering intensities decrease gradually after the tourniquet is applied, and rebound quickly to their original values when the tourniquet is released. The decrease is about 10% for EE and about 20% for IE. Data from the first 50 seconds were used to calculate EE₀ and IE₀. The two free parameters in the algorithm were determined by minimizing the standard deviation in the calculated Hct over the same time period. The resulting RBC and plasma volume fractions, and the Hct, are shown in Figure 4. Both volume fractions rise gradually during the period over which the tourniquet is applied, showing that the main effect is trapping blood in the irradiated volume. When the tourniquet is released, both volume fractions drop quickly to their pre-tourniquet values. The Hct, because of our parameterization, is much more constant, changing by only 0.5%, but also increases on application of the tourniquet and decreases on release. The increase, which is a little sharper than the increases in volume fractions, shows that relatively more red blood cells are trapped than plasma. Interestingly, the Hct shows some post-tourniquet effects. After release, the Hct drops to its original value, but then increases gradually to a value about 0.5% higher. By 200 seconds, it has leveled off; our measurements do not go far enough to determine how long it takes to come down again.

Fig. 3 EE/EE₀ and IE/IE₀ versus time for application of tourniquet at $t \sim 60$ s and release at $t \sim 120$ s. Prebleaching was performed before data collection. Plasma and RBC volume fractions and Hct, derived from this data, are shown in Fig. 14.
For direct comparison, three test subjects exercised the tourniquet maneuver using a blood pressure cuff pumped above systolic pressure for 2 min four times in a week time span and conventional Hct determination technique was employed. For each subject 2 to 4 data points were taken both before and after the maneuver each day, which yielded a total number of 61 data points, 30 before and 31 after. The average Hcts before applying tourniquet were 0.3794, 0.3715 and 0.4055, while the averages after were 0.4020, 0.3811, and 0.4138 respectively. The statistics shows a clear trend of increased Hct after the tourniquet is applied. To support the statement in a more statistically meaningful way, the null hypothesis
is tested by splitting the raw data into two sets, 30 before and 31 during application, without differentiating test subjects and the dates on which the experiments are done. The result yields a p-value of 0.039, which is significant at >90% confidence to reject the null hypothesis that assumes no Hct change due to the tourniquet. Moreover, within any set of personal Hct values the behavior is much more consistent, thus the statistic just quoted tends to underestimate the Hct change on a single person basis. Therefore, we used personal average Hct as a normalizer for each individual, and instead of using raw Hct, the relative change of Hct was used to test the null hypothesis. A p-value of 0.0219 resulted even more strongly supporting the probability that Hct increases with the tourniquet applied as described in Figure 4.

Another demonstration involves the Valsalva maneuver\textsuperscript{13}. The Valsalva maneuver refers to the test subject attempting to push air through any closed orifice. When executing the Valsalva maneuver the test subject must constrict the chest muscles and clench the abdominal muscles causing them to occupy a greater volume of the abdominal cavity. This expansion impedes the venous return of blood to the heart thereby decreasing net cardiac output and simulating central hypovolemia. The calculated change in apparent Hct using our algorithm is shown in Figure 5.

![Figure 5. Typical LighTouch Hct changes observed when executing the Valsalva maneuver for this set of parameters a-f. The test subject initiated the maneuver at about 135 seconds and released at about 185 seconds. The EE0 and IE0 were calculated from the prebleached data earlier in the experiment. All parameters a-f were the same as for Figure 4 which corresponds to a different test subject on a different day.]

On a separate experiment we followed the Valsalva maneuver using photoplethysmography as can be seen in Figure 6. These signals were obtained by monitoring test points 9 and 10 (TP9 and TP10) inside a Nellcor 200 pulse oximeter. TP9 is a red LED (660) signal and TP10 is the NIR (947 nm) signal. These two photopleth signals are also responsive to the Valsalva maneuver which in this case began at 195 seconds and ended at 260 seconds.
Figure 6  Typical photoplethysmography signals obtained using a Nellcor 200 pulse oximeter during execution of a Valsalva maneuver that began at about 195 seconds and was released at about 260 seconds.

We also show the ratio of the TP9 and TP10 signals in order to suggest the response of a pulse oximeter (spO2) to central hypovolemia.

Finally for comparison we show the textbook response of pulse rate and systolic blood pressure to a Valsalva maneuver in Figure 8. The response of the pulse rate can be clearly seen in the photopleth response as well as the LighTouch Hct response.
DISCUSSION

Much work remains to be done with regard to whether we are actually calculating plasma and RBC volumes in the sense we seek e.g. as volume normalizers to perform Raman spectroscopic noninvasive blood and tissue chemical analysis i.e. glucose. All tests that we been able to execute have produced results consistent with the expectation we are actually calculating these volumes and thereby Hct but there are other more strenuous tests that will produce more unequivocal results. For example we are currently planning experiments in blood banks, dialysis centers, emergency rooms and trauma centers in order to monitor the response to actual blood loss. We are also currently executing a rat study to monitor the Hct during blood loss.

The direct comparison of the LighTouch response to the photoplethysmographic signals and their ratio clearly shows that we are monitoring the optical effects of subsurface blood movement. Both LighTouch and Nellcor signals demonstrate the pulse rate modulation and the effects of systolic blood pressure variation although it should be noted that the considerable motion defect of the Nellcor signals and their ratio is not shown. Although the PDPM of the LighTouch maintains the tissue motionless and isobaric during experimentation, in actual commercial use the LighTouch approach could also be implemented using a similar device-human interface i.e. finger clip and then we suspect a motion defect would likely be observed as well. We note that the ratio of TP9 and TP10 i.e. spO2 would seem to provide somewhat less of a visible response to the Valsalva maneuver than either the LighTouch or simple photopleth signals.

The raw EE and IE signals are obtained using a LighTouch instrument that is intended for other purposes so a direct comparison of signal to noise between the Nellcor and the LighTouch is inappropriate at this time. Nevertheless there has been considerable experimentation over decades with spO2 and photoplethysmographic devices with the intent to obtain early indications of internal hemorrhage and all such devices have proven less than adequate. It should be noted that both spO2 and noninvasive hemoglobinometers all rely on the original Twersky algorithm in which the Hct must be assumed to be
constant in order to obtain meaningful results. And although these devices can provide *absolute* Hct to about \( \pm 8.3\% \) this is apparently not sufficiently sensitive to provide medical practitioners appropriate information to detect internal hemorrhage when there is no external injury. Perhaps the nature of the information provided by the LighTouch device is different from that provided by Twersky based devices since according to our model the LighTouch device should in fact be sensitive to Hct changes and it would be changes in this quantity early in the autonomic compensation period that might give the earliest indicators of treatable internal hemorrhage. This is very important since the leading preventable cause of death, world-wide, civilian or military, for all people between the ages of 18-45 is undetected internal hemorrhage\(^{18,19}\).

Presently perhaps the most confounding issue with respect to applying this model and algorithm is the photobleaching of the autofluorescence and the variability of the autofluorescence across test subjects. Although we deal with this explicitly elsewhere\(^ {20} \) it is clear that once a specific test subject has been bleached then the LighTouch Hct algorithm is potentially quite useful for monitoring percent relative changes in Hct and fluid volumes as they respond to normal physiological factors or other stresses i.e. blood loss due to hemorrhage as it progresses to circulatory collapse. Since it only takes a few 10s of seconds to accomplish most prebleaching using the same laser power as used for the measurements, this limitation is not too serious.

On the other hand we are pleasantly surprised to report that one set of parameters a-f produces an algorithm that provides identical qualitative responses for any number of different test subjects spanning a wide variety of skin tones and textures. It appears that it will be possible to empirically calibrate the LighTouch device to produce quantitatively accurate Hct changes but again this will depend on the extent of photobleaching and inherent autofluorescence levels to indicate the degree of precision and accuracy achievable. Nevertheless, percent relative changes in Hct may be accessible and given the advantages of continuous noninvasive monitoring over invasive point of care testing this can still be a valuable tool\(^ {21} \) for health care professionals. Empirical calibration has long been the case for oximeters but since photoplethysmographic devices cannot be normalized\(^ {15} \) the LighTouch Hct device would seem to be potentially more useful than photoplethysmographic devices for a variety of applications.

**CONCLUSIONS**

We have described and demonstrated a completely new technology for noninvasively measuring plasma and RBC volumes in human skin *in vivo* in addition to Hct. This approach has the weakness that autofluorescence and photobleaching effects can produce systematically biased results but it has an inherent advantage over all photoplethysmographic devices in that it can be empirically normalized, calibrated and parameterized. Present results show that a range of people spanning many different skin tones and textures can use it based on a single calibration and parameterization. This device may allow discovery of internal hemorrhage in people without external injury by revealing small fluctuations in Hct during the autonomic compensation period.

**ACKNOWLEDGEMENTS**

This research was supported by LighTouch Medical, Inc. and the Telemedicine and Advanced Technical Research Center (TATRC) at Fort Detrick. All software and electronics were produced by John Fayos, Dave Rice, and Dave Stehlik of Critical Link, LLC. Machine shop fabrication was by Lou Buda, Charlie Brown, Phil Arnold and Les Schmutzler. Assistance with the design and optimization of our optical system by Rebecca J. Bussjager is gratefully acknowledged.
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