Continuous noninvasive in vivo monitoring of intravascular plasma volume and hematocrit changes in response to blood removal and fluid replacement in a rat model

Bin Deng, Evan Kastner, P. Dent, J. Goodisman, and J. Chaiken

a) Department of Chemistry, Syracuse University, Syracuse, New York, 13244
b) Division of Nephrology, SUNY Upstate Medical University, Syracuse, NY, 13210
c) LighTouch Medical Inc, P.O. Box 531, Bryn Athyn, PA, 19009

ABSTRACT

We report a new algorithm and measurement system that permits simultaneous monitoring of the hematocrit and plasma volume fraction of blood within the intravascular space of an optically probed volume of skin. The system involves probing with a near infrared laser and simultaneously collecting the Rayleigh and Mie scattered light as one raw signal and the undifferentiated Raman and fluorescence emission as the second raw signal. Those two physically independent raw signals and six parameters that can be obtained by either direct calculation or empirical calibration permit monitoring of the blood in rat paws. We tested a device based on the algorithm in the context of improving detection of blood loss for people with an early undiagnosed internal hemorrhage via real-time monitoring of signal changes with direct correlation to hematocrit. We performed experiments monitoring rat paw skin in vivo while removing blood, centrally or peripherally, and then adding replacement fluids such as Normocarb and blood. Blood removal itself elicits a predictable and consistent response, decreasing hematocrit and increasing relative plasma volume, that depends on the rate and location of removal, the total amount of blood removed, the location of monitoring, and possibly other factors as yet unknown. Similarly, replacing the blood with whole blood vs. saline consistently produces a rational range of responses. Calibration across subjects and the measurement of absolute hematocrit will also be discussed.

Key Words: noninvasive, in vivo, hematocrit, continuous, hemorrhage, plasma volume, rat model.

1. INTRODUCTION

In trauma and emergency medical situations, as commonly occur in the military and civilian worlds, determining whether a person is bleeding internally and if so the location and rate of that bleeding can be the difference between life and death. In the Golden Hour after injury, insuring the integrity and function of the cardiovascular system is essential but autonomic compensation for the loss of fluid from the intravascular space can delay diagnosis and consequently treatment. Compensation causes changes that begin before the patient is in danger e.g. during a routine blood donation. As bleeding continues, changes in blood composition occur in response to changes in intravascular pressure. Eventually reserve red blood cells (RBCs) and protein from the spleen and liver enter the intravascular space, altering the hematocrit (Hct) and the oncotic balance between interstitial fluids and the blood plasma, and extending the time before circulatory collapse. The goal of this research was to determine if pressure and compositional changes can be detected noninvasively and in vivo in a rat model.

In this paper we focus on monitoring relative changes in Hct and plasma volume that accompany blood removal and fluid replacement in a rat model in the hope that we may learn how to use them as “vital signs” to diagnose, locate and treat internal hemorrhage in humans. We reported a novel algorithm to monitor Hct and plasma volume...
noninvasively a few years ago\textsuperscript{3,4} and we will briefly review the algorithm below. We then report experimental
details specific to rats since all of our previous \textit{in vivo} results have involved only humans\textsuperscript{5}. The results of blood
removal-fluid replacement experiments are shown in terms of the variation in plasma volume and Hct in response to
specific stimuli.

2. THE ALGORITHM

We model the skin as being composed of three phases\textsuperscript{6}, static tissue that deforms when mechanical stress is applied,
and blood that itself is composed of 2 phases i.e. red blood cells and plasma that both move when mechanical stress
is applied. The distribution of scattering centers and fluorophores is considered spatially homogeneous, the specific
nature of the cells comprising the static tissue is considered irrelevant, and the scattering contribution of the RBCs is
dominant. As a consequence there is a region beginning from the outermost skin surface i.e. the stratum corneum
and extending beneath the surface to a certain depth where there is capillary blood, interstitial fluid and tissues that
are viable. We believe that incident near infrared (NIR) light that penetrates \textit{just below} the specific depth where
the tissues begin to have capillaries and are viable makes only a small relative contribution to the net amount of light
remitted from the exact same surface location place as the incident light. Thus the remitted light has sampled dead
tissues on the surface and viable tissues containing only capillaries. Working with volar side fingertip ridged skin
and NIR incident laser light at e.g. 830 in humans is similar to the experiments we report herein because both sites
involve ridged skin with a similar texture and thickness. Given the scattering and absorption coefficients from
published values\textsuperscript{7,8} measured \textit{in vitro} shown in Table 1, we believe the propagation of remitted light generated as
described occurs in the single scattering limit.

Since the volume percent occupied by blood\textsuperscript{9} in relatively well perfused fingertip skin i.e.3-5\% of the total is still
quite small, the skin in that region should be considered optically thin despite the presence of the strongly scattering
RBCs. Note that the Hct of capillary blood is much smaller than in blood sampled from any larger vessels, i.e. 10\%-30\%
is a realistic full range for capillary blood. It is easy to estimate a volume percent or concentration range over
which the production of fluorescence is linear in fluorophore concentration. The production of fluorescence has long
been known to be linear in concentration in optically thin materials\textsuperscript{10} and we expect the production of Raman
scattered light to be the same in that respect.

When one probes fingertip skin\textsuperscript{11} with NIR laser light a typical spectrum of the remitted light is given in Figure 1.
There is always light which is of the same wavelength as the incident light and light that is

<table>
<thead>
<tr>
<th>phase</th>
<th>absorption coefficient (cm(^{-1}))</th>
<th>scattering coefficient (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>r=RBC</td>
<td>4.5</td>
<td>300</td>
</tr>
<tr>
<td>p=plasma</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>t=static tissue</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 1. Absorption and scattering coefficients for the three phases comprising skin from \textit{in vitro} measurements.

shifted to longer wavelengths. We refer to the unshifted light as being elastic emission (EE) and the wavelength
shifted light as being inelastic emission (IE). We note that there is obviously Raman scattered light in addition to
fluorescence but we shall use the entire IE as indicated in Figure 1 without trying to resolve them. First we note
that a number of arbitrary baseline correction algorithms exist in the literature but there really is no actual
knowledge about how to separate the Raman from the fluorescence. For purposes of applying the Hct
plasma volume algorithm we assume that the EE and IE can be used exactly as shown in Figure 1 and
note that for all practical purposes obtaining the EE and IE as shown could be easily accomplished with filters and single channel detectors, forgoing any need for CCD cameras and spectrographs.

Figure 1. inset: raw typical single 20 msec frame of CCD output using 200 mW of 830 nm excitation, bottom: same frame showing sections of emission used to estimate inelastic emission (IE, ≈ 500-1750 cm\(^{-1}\)) and elastic emission (EE, -30 - +10 cm\(^{-1}\))

At any Raman shift the fluorescence and the Raman are mutually independent measures of the chemical composition of the probed volume. Figure 1 shows that the Raman scattered emission is a small contribution to the total IE but if the Raman, the fluorescence or the EE should change, the “apparent” plasma and RBC volumes would change. For example the fluorescence changes when light is first incident on tissue and becomes stable in a few minutes or less, allowing subsequent use of the IE as a measure of the overall chemical composition of a probed tissue volume. But when monitoring is first begun, the hematocrit appears to increase and the plasma volume to decrease because the fluorescence is being bleached by the probing laser itself. What is most important is that the physical processes that produce the IE be independent of the processes that produce the EE to insure that the equations below can be inverted.

The EE is determined completely by the absorption loss experienced by the incident light and the physical scattering characteristics of the materials in the probed volume. The absorption loss is disconnected from the IE because the fluorescence quantum yield is independent of the absorption and scattering coefficients. Thus the EE is much more connected with the physical processes that determine where and how light propagates in the tissues but not with how IE is produced.
In order to model the propagation of light in the probed volume using the radiation transfer equation\(^6,12\) (RTE) in the single scattering limit we assume that the tissues are spatially homogeneous and that there are no voids. Moreover, when we apply external mechanical pressure to the tissue, or if the heart beats, sending a pressure wave throughout the intravascular volume, the blood moves but no voids are developed. These assumptions can be summarized by the following equations as well as the definition of Hct.

\[ I = \phi_s + \phi_p + \phi_r \quad \text{(no voids)} \]
\[ 0 = d\phi_s + d\phi_p + d\phi_r \quad \text{(rare spatial paths)} \]
\[ \text{Hct} = \frac{1}{\mu} \int (\phi_s + \phi_p) \quad \text{(definition)} \]

Where, \(\phi_s, \phi_p, \text{and} \phi_r\) denote the volume fractions of RBCs, plasma and static tissue respectively. We previously published comparisons using this model to spatial scans of skin\(^3\). The observed characteristics of the measurements were reproduced suggesting that the model would also be accurate if we modeled temporal changes at one location over time e.g. cardiac pulse, external mechanical pressure, Valsalva maneuver. Using the RTE in the single scattering limit, and as is known to be true for quantitative fluorescence measurements\(^10\) in optically thin samples, the EE and IE are given by the bilinear forms.

\[ \text{EE} = \partial_1 + \partial_2 \phi_p + \partial_3 \phi_r \]
\[ \text{IE} = \partial_4 + \partial_5 \phi_p + \partial_6 \phi_r \]

We thus have 2 independent equations in 2 unknowns and 2 measureable quantities i.e. EE and IE. Since the equations are independent we know that these equations can be inverted to allow calculation of the plasma volume fraction and the RBC volume fraction and by closure the Hct and the static tissue volume fraction.

\[ \phi_r = a + b \left( \frac{\text{EE}}{\text{EE}_0} \right) + c \left( \frac{\text{IE}}{\text{IE}_0} \right) \]
\[ \phi_p = d + e \left( \frac{\text{EE}}{\text{EE}_0} \right) + f \left( \frac{\text{IE}}{\text{IE}_0} \right) \]

We have inverted equations 4 and 5 and expressed the result in the form of equations 6 and 7 because our immediate medical interest is in monitoring changes in plasma volume and Hct since such changes are the indicators of deviations from homeostasis. Of note is that the intended use of a certain FDA approved device\(^13\), the CRIT-LINE\(^6\), hinged on only being able to monitor changes in plasma volume and Hct of blood as it transited a hemodialysis machine. This knowledge allows medical personnel to better manage fluid removal and replacement to improve outcomes at greater comfort and lower costs. Being able to monitor even such relative changes noninvasively and \textit{in vivo} for all patients is a worthwhile goal.

The quantities EE\(_0\) and IE\(_0\) are the average values of EE and IE and in practice these can be measured at the outset of a monitoring session and then all subsequent changes are relative to the subject’s previous state. They can also be a single set of values obtained by averaging the measured EE\(_0\) and IE\(_0\) values of a large number of subjects and this leads to calibration across individuals and the capacity to monitor absolute Hct. Presently we are only concerned with relative changes and we have published\(^4,5\) at least 3 different approaches to calibration i.e. finding the appropriate values of the parameters a-f. This algorithm can be calibrated easily for humans by comparison with the
FDA approved invasive device and although the procedure for that task will be described in detail in a separate paper, all of the results shown for rats utilized the same parameters a-f, and these were obtained by comparison with the CRIT-LINE using raw human data.

3. EXPERIMENTAL

All procedures performed using animals were conducted under IACUC approval at Syracuse University. The spectroscopic measurements employed a modified commercial Raman Spectrometer (Lambda Solutions, Waltham, MA) and a specially designed probe intended to mimic the type of aperture-orifice that we have described elsewhere\textsuperscript{11} in the context of human monitoring. The probe comprises a 2 mm ID tube with a 2 mm flat lip containing a lens placed near the end of the tube such that when the tube is pressed against the skin, the light is delivered to the skin protruding into the hole at approximately f=2.0. The delivery and collection is symmetric so the collection is also at f=2.0 with an approximate 150 \( \mu \)m diameter spot size. The rest of the optics and filtering is standard for Lambda Solutions probes but there is an additional Raman notch filter (Semrock, Rochester, NY) placed between the collimating lens and the grating to allow adjustment of the EE and IE for optimum dynamic range.

The measurement process used an improvised platform (base from Lambda Solutions) that allows us to know how the pressure between the probe and the tissue varies in time and whether there is gross motion. Blood is very responsive to externally applied pressure. Data interpretation utilizes the probe-tissue pressure changes during monitoring blood removal and fluid replacement. Having tried ears, tails, belly and front and back paws we chose front paws as the preferred site. The paw is placed between the probe and rubber bulb shown in Figure 2 and the pressure in the bulb is measured by a capacitance manometer. If there is motion of any kind or if there are internal pressure changes, it is recorded in the digital record provided by an AD Instruments Power Amp/data logger. That record, combined with direct observation, allows differentiation between movement and internal pressure fluctuations. An example of the baseline behavior of the plasma volume and Hct for a rat with no blood removal and fluid replacement is shown in Figure 3.

![Lambda Solutions probe mounted on microscope base with probe contacting a latex bulb whose internal pressure can be monitored. The rat paw is placed in between the probe and bulb, palm up.](http://proceedings.spiedigitallibrary.org/)

Figure 2. Lambda Solutions probe mounted on microscope base with probe contacting a latex bulb whose internal pressure can be monitored. The rat paw is placed in between the probe and bulb, palm up.
Figure 3. Typical baseline behavior (20 msec per data point) of plasma volume and Hct and spontaneous variation in probe
depth during course of an experiment in which no blood removal or fluid replacement occurred. The variation in probe
pressure is due to the response of tissue to recover homeostasis i.e. to the state before the probe was applied as well as systemic
changes due to onset of anesthesia and sedation and near the start of monitoring at 33 minutes, bleaching of tissue
autofluorescence. (capacitance manometer is MKS Instruments, Andover, MA)

4. RESULTS

The results of all experiments were qualitatively identical i.e. internally consistent after 54 separate procedures,
some involving baseline measurements and some involving blood removal and fluid replacement, involving 20
different rats. The responses that will be shown below can therefore be considered typical however, as we learned
with human studies,11 better reproducibility results from achieving more reproducible probe placement and pressure.
Since we learned that there is a reproducible baseline behavior as shown in Figure 3 we were able to design a series
of experiments to be executed over a few weeks with a single rat in which a baseline response for that specific rat
could be obtained and then used to help interpret the behavior for the same rat in later blood removal-fluid
replacement experiments. Since the baseline behavior depends on the age and weight of the rat and dosage of the
ketamine-xylazine combination used, having recent rat specific baseline data allowed subtraction of the baseline
responses to better compare quantitatively, the device provided Hct and plasma volume responses to the known
blood removal and fluid replacement. We have termed this subtraction “orthostatic correction” because it involves
the redistribution of blood and other fluids to placing the rat in the monitoring platform immediately after anesthesia
is administered and the animal is adjusting. It must be emphasized that the Hct and plasma volume changes shown
below could all be seen in real time without any such correction and we shall show one such data set below.
Figure 4. Schematic depiction of the site of monitoring and the site of blood removal i.e. the right jugular vein. In Figure 6 the blood is removed from the left carotid artery. Fluid replacement involves either the same whole blood initially removed or NormoCarb, an electrolyte replacement fluid commonly used for fluid replacement during dialysis. (In accordance with Creative Commons License the animal drawing is adapted from http://www.biologycorner.com/resources/rat_circ_vein.gif)

Over a 3 week period, the same rat used to obtain 1) a baseline response i.e. no fluid replacement/removal at all, 2) a response involving major blood loss followed by replacement with whole blood, 3) a response involving major blood loss followed by replacement with Normocarb. Correcting the response of the two replacement experiments using the baseline response allows observations.

Figure 5. Behavior of Hct with fluid replacement using Normocarb after whole blood removal. Annotation gives timescales and amounts of removal and replacement.

According to the results produced by the algorithm some general observations can be made. 1) Removal of whole blood always results in a decrease of Hct and increase of plasma volume. The decrease in Hct depends on the rate of blood removal and the amount removed. 2) Fluid replacement using whole blood i.e. as in Figure 6 results in an
increase in Hct whereas straight Normocarb replacement 3) as in Figure 5 produces a more muted response due to an increase in the number of RBCs into the intravascular space. Note that all observations were visible in real time without the baseline correction.

Figure 6 shows the sizes of the observed fluctuations as a function of the accumulated amount of blood removed at least as much as the amount actually removed at a single moment. That is, removing an additional mL after 2.4 mL removed produces a different Hct response than the first mL removed. In humans we expect Stage 2 compensation to begin after removing about 15% of the available blood supply which corresponds to about 2.4 mL for a 225 g Sprague Dawley rat. Since we use one set of a-f parameters obtained from human data and the EE0 and IE0 are set for each rat at the beginning of each monitoring run, we do not necessarily accept the actual numerical values of the Hct change or the plasma volume change due to blood loss at this point only because it cannot be independently confirmed. Presently we can report that the observed changes are very small and reproducible.

Figure 6. Behavior of Hct with fluid replacement using whole blood replacement after whole blood removal. Annotation gives timescales and amounts of removal and replacement. The graph shows the baseline corrected percent change of Hct as a function of the accumulated amount of blood removed.

Figure 7 documents removal of blood from a large vein at bleeding rates of roughly 15-20% of total blood (about 16 ml) in 2-3 minutes and the results clearly show that the effects are discernable from normal background homeostatic variations. The plasma relates to blood pressure since the intravascular space must be filled in order to support pressure. Stage 1 compensation for blood loss includes constriction of the vascular bed maintaining the internal pressure when the intravascular plasma volume is reduced by blood loss. Thus the apparent plasma volume increases since the available intravascular volume itself is decreased.

Figure 7. The real-time raw behavior of Hct and plasma volume to blood loss removal from carotid artery with no fluid replacement is shown without orthostatic correction.
In Figure 8 we show the result of infusing 2 mL Normocarb without blood removal. Direct observation of nearly contemporaneous urination showed that the Normocarb was being removed from the intravascular space almost as fast as we could inject it. Nevertheless there is a clear response that we assume involves the flushing of fluorescent materials from the intravascular space into the interstitial space.

Figure 8. Response of Hct and plasma volume to 2 injections of Normocarb (2 mL each) without prior blood removal.

5. DISCUSSION

The new algorithm clearly presents a new approach to monitoring blood noninvasively and in vivo. We have previously shown\(^5\) that hemoglobinometers, oximeters and photoplethysmographic devices in general also produce a response for the kinds of stimuli we employ and we expect that they would also produce responses to the bleeding and fluid replacement employed in this study. Such devices are all based on Twersky\(^{14}\) algorithms whereas the new algorithm is completely different, providing an opportunity to improve our capacity to detect, locate and treat internal hemorrhage.

First the Twersky algorithm assumes a relationship between the hemoglobin (Hgb) concentration and the hematocrit, that is, the Hgb concentration (in g/dL) is 35 times the Hct, which is not necessarily justified, particularly in cases where homeostasis is not in force. Thus the algorithm is calculating a number that increases with both Hct and Hgb but may not be physically related to either exclusively. This idea is consistent with the fact that a strong correlation between the noninvasive measurement of either Hct or Hgb using Twersky and the invasive finger stick Hgb i.e. HemoCue or conventional centrifuge Hct measurement is well established. But to our knowledge in the literature, credible approaches\(^{15,16}\) to either noninvasive measurement produces Hgb or Hct that has ≈8.3 % error compared to either conventional measurement. This is apparently not sufficiently sensitive, accurate or precise to provide the type of information that medical practitioners need for our intended use\(^{17}\). Conventional measurements\(^{18}\) are often less precise and accurate than either the noninvasive measurements due to sampling error and errors in sample handling.

Why is the present algorithm more sensitive? Except for probing geometry, one channel of the algorithm described in this paper i.e. the EE is identical to one channel of nearly any photoplethysmographic device. Most photoplethysmographic devices work in a trans-illumination geometry in which the effects of multiple scattering
cannot be avoided. In the approach described herein we avoid multiple scattering by choosing an excitation geometry that minimizes collecting light that has penetrated too deeply into the tissue. This gives the present approach an advantage that is analogous to the advantage in vitro nephalometry enjoys over in vitro turbidometry. Nephalometry allows a roughly 2-3 order of magnitude decrease in the detection limit for any directly comparable turbidometric approach. For all turbidometric cases we are measuring a relatively small change on a large background signal that is comparable with the shot noise for the background. There is no background in nephalometry and there is no signal at all unless scattering occurs.

The IE is even more immune to background noise since it is so easy to separate from the probing light. The absence of a background for either the EE or the IE causes the present algorithm to be intrinsically more precise and accurate in the same sense that fluorescence is superior to absorption based measurement systems. The present approach uses a single light source to probe the tissue and the Twersky based approach necessarily uses 2 light sources and their mutual overlap in the tissue volume is not necessarily unchanged as Hct and plasma volume change. Fundamentally, the absorption based approaches i.e. Twersky are ideal for oximetry (SPO2) because of the existence of convenient isosbestic points for oxy-deoxy Hgb. They are less successful for Hgb or Hct measurement because the net probed volume absorption is dominated by hemoglobin. As suggested by Table 1 the absorption measurement approach is not sufficiently sensitive to the blood fluid i.e. the water based phase, plasma. Even at 947 nm the absorption of water in blood plasma cannot apparently be differentiated from interstitial water based fluids.

The interstitial fluids do not fluoresce to the same extent as blood plasma. Plasma contains more substances than interstitial fluid. The latter composition is dominated by the presence of small organic molecules and electrolytes. These additional primarily protein components appear to contribute a greater fluorescence per unit volume to plasma. Therefore the IE has a significant contribution from plasma in addition to that from hemoglobin. The relative contributions of these two sources can be adjusted somewhat by choice of excitation wavelength but the effect of Hgb oxygenation is noticeable if the 805 nm isosbestic point is not used. In this study 830 nm provided a good balance between plasma fluorescence and hemoglobin fluorescence but a small oxygenation “defect” was observed.

While a more complete description of these results is beyond the scope of this article and a complete presentation will appear in the peer reviewed literature, the approach presented here apparently obtains sufficient IE contribution from both plasma and hemoglobin so that physically, the calculated volumes do correspond to the intended materials. Also, the scattering and absorption contribution from plasma is very small relative to either the RBCs or the static tissue so the movement of plasma also affects the net EE but in a manner exactly opposite of RBCs. We note that we are presenting other data in this Proceedings from a human study used to obtain the specific parameters a-f employed in this study. Furthermore, in vitro validation of this algorithm has also been accomplished successfully using quartz spheres suspended in a density and fluorescence adjusted aqueous fluid phase. For these reasons we suggest that perhaps the most important aspect of this algorithm is that the changes it detects have a much clearer physical interpretation than do changes monitored using photoplethysmographic algorithms.

6. CONCLUSIONS

We have presented data from blood removal and fluid replacement experiments involving a rat model and a new algorithm intended to calculate relative Hct and plasma volume changes. The data suggests that the algorithm actually calculates the intended physical parameters and that bleeding and fluid replacement may be observed with sufficient sensitive and stability to allow timely diagnosis, location and treatment of hemorrhage. More study is required but this algorithm may be a significant improvement over photoplethysmographic technologies.
7. ACKNOWLEDGEMENTS

Numerous conversations with Dr. C. M. Peterson throughout this study, as well as with Dr. George Shaheen in the early stages were very helpful. Dr. Robert Quinn of the SUNY Upstate Medical University provided invaluable assistance with designing, performing and interpreting rat based experiments. Prof. Julie Hasenwinkle of the Syracuse University Department of Chemical and Biomedical Engineering provided specialized tools and materials for use with the rats. Prof. Sri Narisipur of the Upstate Medical University Nephrology Department lent the AD Instruments Power Amp and data logger used in this study. The expert assistance of Critical Link, LLC and Lambda Solutions, Inc is gratefully acknowledged. In particular, the probe used to contact the rat skin was designed and fabricated by Dr. Yongwu Yang of Lambda Solutions according to specifications provided by LighTouch. This research was supported by TATRC-USAMRAA Contract W81XWH-11-0188. LighTouch Medical, Inc. and Syracuse University also supported this research.

8. REFERENCES