Whole blood reflectance for assessment of hematologic condition and detection of angiographic contrast media

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We present simple whole blood reflectance analyses in the range 500–900 nm, using intact whole blood to simultaneously quantify hematocrit and oxygen saturation from a single spectral reading. We applied these results for the development of an intravascular catheter-based reflectance sensing system to detect and remove contrast media injected during angiography so as to reduce the risk of complications associated with the injected contrast media. We further tested the practicality of the optical detection of angiographic contrast media in a pilot animal study in vivo. We successfully demonstrated the feasibility of real-time in vivo contrast detection and removal during angiography. Our simple method for the detection and removal of angiographic contrast media will facilitate the development of intravascular optical sensing systems. © 2009 Optical Society of America

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1. Introduction
The spectral properties of light reflected from whole blood have been relatively unexplored, although reflectance spectra from bulk tissue including blood vessels have been intensively investigated for the development of optical sensing systems to noninvasively monitor hematocrit (HCT) and oxygen saturation [1,2]. Whole blood reflectance measurements also can be used to develop an intravascular sensor for the detection of radiographic contrast agents mixed in blood during cardiac catheterization procedures, which increased in number by 39% between 1979 and 2002 in the U.S. This increase in procedures has consequently led to increased administration of radiographic contrast agents [3]. Contrast media injected during angiography can cause serious complications such as contrast-induced nephropathy (CIN). CIN is most commonly defined as acute renal dysfunction occurring within 24–72 h of exposure to intravascular radio-opaque contrast media. This type of contrast media can cause a hypoxic state and renal cell toxicity as well as affecting the physical properties of blood, such as viscosity, cell shape, and osmotic pressure, thus necessitating immediate dialysis in serious conditions [4–6]. As a result, CIN has been identified as the third most common form of hospital-acquired renal failure, which, in turn, is associated with increased in-hospital mortality, a poor long-term survival rate, and increased costs [7–10]. Thus, it is critical for cardiologists to be able to remove the contrast medium before it enters the systemic circulation in order to reduce the risk of CIN. However, the current treatment for CIN is mainly symptomatic, involving systemic administration of...
pharmacologic agents, often resulting in limited success [11]. The concept of removal of contrast medium from the coronary sinus before it escapes the injected site and enters the systemic circulation has been suggested [12–14]. Yet previous attempts to remove contrast from the coronary sinus required lumen occlusion, lacking automaticity as well as active contrast detection. In this respect, it has been imperative to develop a real-time intravascular sensor to monitor the kinetics of contrast media during angiography. In fact, catheters with fiber-optic-based intravascular sensors were already researched in the early 1960s for monitoring oxygen saturation and cardiac output [15–19]. However, these initial developments were focused on the development of oxygen saturation sensors using blood reflectance measurements [20–25].

Although the optical properties of whole blood, such as scattering and absorption coefficients, were investigated by several investigators [26–30], there is only limited information available about the reflectance properties of whole blood for the development of intravascular sensing systems. Zdrojowski and Pisharoty reported experimental results of optical transmission and reflection with blood [31] and showed that blood reflectance signals changed as a function of HCT in a parabolic pattern. Johnson experimentally showed different light diffusion patterns of blood at various concentrations and provided an optical diffusion model for whole blood [32]. Reynolds et al. developed a modified optical diffusion theory for whole blood and applied it toward the design of fiber-optic catheters [33]. Steinke and Shepherd further developed a diffuse reflectance model for whole blood [34] and showed various relative reflectance patterns of blood as a function of fiber geometry, optical wavelength, oxygen saturation, and HCT [35]. In addition, they also evaluated the scattering coefficient of whole blood by comparing the experimental results obtained from Mie simulations [36].

Although all the studies listed above are helpful for understanding the fundamental properties of blood optics, they lack information about whole blood reflectance spectra in the visible to near-infrared range and their subsequent dependency on HCT and oxygen saturation. To facilitate the development of intravascular sensors, the following question has to be addressed: What is the optimal range of the wavelength for reflectance measurements of arterial and venous blood to detect foreign contrast media as well as to monitor HCT and oxygenation? Indeed, the spectral reflectance measurements of whole blood have been studied under narrowband wavelength measurements [27] in separated and undiluted blood [26, 29]. In addition, diffusion-approximation-based modeling of whole blood reflectance is not straightforward because of the high anisotropy of whole blood [37, 38], and thus it is difficult for biomedical engineers to estimate practical and realistic spectral data in designing intravascular optical sensing systems.

Therefore, in this paper, we report reflectance spectra of intact whole blood at various levels of HCT and oxygen saturation. We show that such broadband reflectance spectra ranging from 500 to 900 nm can be used to monitor HCT and oxygen saturation simultaneously. We further demonstrate the feasibility of an integrated approach of angiography contrast detection and removal including (1) in vivo contrast detection using catheter-based reflectance sensing via blood dilution by contrast and (2) contrast removal using an automatic aspiration system at the downstream site of the injection during angiography.

2. Material Preparation and Methods

A. Blood Samples

We obtained blood samples, following the preclinical protocols for the evaluation of cardiovascular medical devices at the Interventional Cardiovascular Research Laboratory, Division of Cardiovascular Medicine, Stanford University Medical Center (Stanford, California). Blood was collected from 10 ovine subjects and 7 porcine subjects after their procedure but immediately before their scheduled euthanasia under direction of the Veterinary Service Center of Stanford University. Blood samples were heparinized immediately after collection to prevent coagulation, kept at room temperature during the tests, and were then refrigerated for storage. For spectroscopic reflectance measurements, the sample dimension was approximately 40 mm (diameter) by 40 mm (height); the probe was securely positioned by a probe holder, and the distal tip of the probe was immersed approximately at the center of the samples. We also gently stirred the blood sample before every measurement to prevent blood cell sedimentation. To vary HCT levels, we mixed normal saline (0.9% sodium chloride) with the blood. For completely deoxygenated blood samples, we used sodium dithionite (Na2S2O4) and measured oxygen saturation levels with an oxygen meter (Unisense OX-10, Aarhus, Denmark). We mixed a sufficient amount of sodium dithionite with saline to completely consume the oxygen and to set the sensor value to 0% oxygen saturation. To measure 100% oxygen saturation, we performed measurements with whole blood that was exposed to air. HCT is defined as the proportion of blood volume occupied by red blood cells, and oxygen saturation is the proportion of hemoglobin that is bound to oxygen.

B. Experimental Setup

Our experimental setup including the optical reflectance probe is shown in Fig. 1(a). We measured reflectance spectra with a specially designed fiber-optic-based probe (Ocean Optics R400-7-UV-VIS, Dunedin, Florida). The probe consists of six surrounding fibers serving as illumination channels and one central fiber serving as a collection channel. The core diameter of all fibers was 400 μm, and the separation distance between the collection and illumination fibers was 487 μm. The core and the
cladding were made of pure silica and fluorine-doped silica, respectively, with a numerical aperture of 0.22. We mounted the optical probe with a probe holder to maintain the same probe depth in the sample. We illuminated samples with a tungsten-halogen lamp (Ocean Optics LS-1) coupled with a Teflon disc (Ocean Optics OF2-LS) to reduce the intensity by half and with a balancing filter (Ocean Optics BG34) to enhance the blue and red regions. We reduced the incident intensity of the light source by using the Teflon disc because the original intensity was high for the spectrometer dynamic range, and we attenuated the intensity near 600 nm by using the balancing filter to relatively enhance the blue and red regions. We recorded spectral signals with a spectrometer (Horiba Jobin Yvon VS140, Edison, New Jersey), which can cover visible to near-infrared ranges. All collected data were transmitted to a personal computer and were analyzed by using the software provided by the spectrometer manufacturer. We measured the spectral response of the light source and the spectrometer by using a diffuse reflectance standard (Ocean Optics OF2-LS) to reduce the intensity by half and with a balancing filter (Ocean Optics BG34) to enhance the blue and red regions. We recorded spectral signals with a spectrometer (Horiba Jobin Yvon VS140, Edison, New Jersey), which can cover visible to near-infrared ranges. All collected data were transmitted to a personal computer and were analyzed by using the software provided by the spectrometer manufacturer. We measured the spectral response of the light source and the spectrometer by using a diffuse reflectance standard (Ocean Optics OF2-LS). To compensate for the spectral response profile of the system, we normalized the raw reflectance spectrum from the sample by the spectral response profile of the system: \[ I(\lambda) = I_{\text{sample}}(\lambda)/I_{\text{ws}}(\lambda), \]

where \( I_{\text{sample}} \) is the intensity of raw reflectance signal of sample, \( I_{\text{ws}} \) is the intensity of the spectral response of the system, and \( \lambda \) is the wavelength of light.

C. Probe and System Performance Validation

We first tested the experimental setup including the probe, using aqueous suspensions of polystyrene microspheres (Bangs Laboratories, Inc., Fishers, Indiana) of various diameters ranging from approximately 3 to 6 μm. The purpose of these experiments was to validate probe performance and to ensure proper calibration of the instrument. We calculated the optical properties of the microsphere suspensions by using Mie theory \([39,40]\). The optical thickness of the aqueous suspensions was 3. We compared spectral distributions of the scattered signals with those simulated by using Mie theory. In Mie simulations, we considered the numerical aperture of the probe fibers (i.e., scattering angle \(-25^\circ\) to \(+25^\circ\)) and size distribution of the microspheres. We convoluted the spectra of the Mie simulation by the spectral point spread function of the spectrometer, using a Gaussian function with FWHM of 6 nm. Figure 1(b) shows a representative reflectance spectrum obtained from the suspension of microspheres distributed in size with the mean diameter of 5.43 μm and a standard deviation of 0.02 μm (the scattering coefficient = 1.67 mm\(^{-1}\) and the anisotropy factor = 0.87 at 600 nm). Figure 1(b) reveals that the probe is able to capture a unique characteristic such as the high-frequency oscillation pattern resulting from the interference of light backscattered from the uniform dielectric sphere. The reflectance spectrum measured by our probe was in excellent agreement with that of Mie simulation \(R^2 = 0.91\), supporting the accuracy of our whole blood reflectance experiments.

D. Algorithm Development for Quantification of HCT and Oxygen Saturation

To test the possibility of estimating both HCT and oxygen saturation simultaneously from one reflectance spectral reading, we developed a simple algorithm. First, we used the wavelength of 773 nm because this isosbestic point provided a HCT value independent of oxygen saturation as a function of HCT. After calculating a HCT value, we used the slope of the curve near 773 nm that depended mainly on oxygen saturation such that the spectral slope is \[ \text{slope} = \frac{I(780 \text{ nm}) - I(766 \text{ nm})}{780 \text{ nm} - 766 \text{ nm}}. \]

E. Applicability of Reflectance for Detecting Contrast Media

We performed a simple study to test the feasibility that our simple reflectance sensing technique can
be used to detect the presence of contrast media mixed in blood. Our hypothesis was that foreign angiographic contrast media simply dilute whole blood and thus change the local HCT level similarly to the way a saline solution dilutes whole blood. We evaluated the reflectance spectra of different concentrations of a representative contrast medium (Visipaque, GE Amersham Health, UK) mixed with ovine whole blood. We also compared the reflectance spectra of the contrast medium with those obtained from different concentration of the saline solution mixed in blood.

F. In Vivo Contrast Detection and Removal Test Using Catheter-Based Sensing and Automatic Aspiration System in a Pilot Animal Study

Based on the results obtained from the optical probe by using the fresh blood specimens, we further investigated the practicality of the optical detection of angiographic contrast media in a pilot animal study in vivo. We used a catheter equipped with a fiber-optic probe (Catharos Medical Systems Sentinel catheter, Campbell, California) for the in vivo contrast detection and removal test as shown in Fig. 2. The Sentinel catheter was an aspiration catheter (10-French) consisting of a central aspiration lumen, an integrated fiber-optic probe, and an expanding basket tip. The inset in Fig. 2 illustrates the integrated fiber-optic probe inside the basket. The integrated fiber-optic probe consisted of two unjacketed optical-grade plastic fibers (outer diameter 500 μm, core diameter 500 μm, numerical aperture 0.51, Edmunds Optics, Barrington, New Jersey). One was used for delivering light and illuminating blood at the wavelength of 627 nm from a light source (Super Bright LEDs, St. Louis, Missouri), and the other was used for detecting backscattered light from blood samples and for collecting to a photodetector (Opto Diode Corp., Silicon Photodiodes, Newbury Park, California). The fibers were tied together at the tip to fix their orientation toward the blood flow and were positioned at the flow bypass zone as shown in Fig. 2. The center-to-center distance between the fibers was close to their diameter because they are unjacketed fibers. The centering basket was able to help the fiber bundle to maintain its position at the center of the lumen at all time. The flow bypass zone allowed the blood to pass through under normal conditions when aspiration was not activated. Once the contrast was injected and reflectance signals were changed, the aspiration system was activated, and the blood-contrast mixtures were selectively removed through the catheter. The aspiration line and the fiber-optic probe extended extracorporeally to a control module. For this study, the control module was built, consisting of a microprocessor unit and a variable speed pump. The microprocessor unit was used to process the signals from the fiber-optic probe and to determine the timing to activate and deactivate the pump. The variable speed pump was used to withdraw the blood–contrast mixture. The centering basket expanded itself and supported the entire system at the center of the lumen area when deployed. Blood converged along the membrane toward the fiber-optic probe. Under normal conditions, blood passed by the system through the bypass zone but, upon detection, the control module automatically activated the aspiration system to withdraw contrast–blood mixture through the catheter.

Because the probe used in the in vivo experiments was custom built (Catharos Medical Systems Sentinel catheter), we also compared its performance with reflectance spectral shapes measured by the commercially available probe (Ocean Optics R400-7-UV-VIS). We evaluated reflectance spectral shapes obtained by both probes, using a strong absorption medium and a scattering medium: (1) an ovine blood and (2) an aqueous suspension of polystyrene microspheres (Bangs Laboratories, Inc.). In the aqueous suspension of the microspheres, the mean diameter of the microspheres was 5.43 μm, and the standard deviation was 0.02 μm. The scattering coefficient was 3.62 mm−1, and the anisotropy factor was 0.87 at 600 nm, which were relatively close to the scattering properties of whole blood [26,41].

We delivered the system to the coronary sinus of canine subjects in vivo by inserting the Sentinel catheter into the canine left internal jugular vein and then deploying at the coronary sinus. During this procedure, contrast agent media were also injected multiple times through coronary arteries. The coronary sinus is the venous site of the heart where all the blood flows from coronary arteries converge before entering the right atrium for systemic circulation. Therefore, the coronary sinus is the optimal site...
for detecting and removing injected contrast media where the contrast concentration is highest due to blood convergence. The device can be deployed to capture the mixture before escaping the injected organ. In this pilot in vivo study, we used five canine subjects. The animals were anesthetized according to the existing protocols of Stanford University Animal Care and Experimental Cardiology Department. In addition, we used a spectrometric absorbance assay to determine the contrast removal rate, which is the amount of contrast media in the blood—contrast aspirant solution as described by Michishita and Fujii [12]. Our variability tests determined the accuracy of the assay to be >95%.

3. Results and Discussions

A. Reflectance Spectra of Whole Blood

As discussed, we present, for the first time to our knowledge, intact whole blood reflectance spectra. Indeed, broadband reflectance spectra of whole blood only (e.g., vessel and skin) have been relatively unexplored. Figure 3 shows the reflectance spectra of porcine blood measured by using the standard probe. As is clearly shown in Fig. 3(a), arterial, venous, and completely deoxygenated blood have distinct reflectance spectra. The maximum reflectance intensity appeared at 650 nm for arterial blood, 720 nm for venous blood, and 800 nm for completely deoxygenated blood. The isosbestic point was 773 nm, which is close to that of human blood [26,42]. We further changed the HCT level by adding the necessary amount of saline to dilute the blood by approximately 5% in each step. We measured the HCT level at each step by using a microcapillary HCT centrifuge, which is the gold standard for HCT. Finally, we obtained reflectance spectra as the HCT level was varied as shown in Fig. 3(b). To provide reference data of the wavelength used in the Sentinel system, the reflectance intensity at 627 nm was extracted from the spectrum and is displayed in the inset graph as HCT varied. It clearly shows that the reflectance signal is affected by HCT variations and leveled off as the HCT level was closer to the normal level (i.e., 44%). Such parabolic shapes were also shown in previous work by other research groups [33–35]. As shown in Figure 3, the reflectance intensity was extremely low when the wavelength was less than 600 nm. Because the absorption coefficient of blood rapidly increases as the wavelength approaches the UV region [26,29], the reflectance intensity from 500 to 600 nm was significantly affected by the absorption coefficient [43].

B. Simultaneous Quantification of both HCT and Oxygen Saturation

Under meaningful physiologic conditions, it is highly probable that both HCT and oxygen saturation vary at the same time. Therefore, it is essential to simultaneously quantify both from a single reflectance reading. Figure 4(a) shows reflectance spectra measured by using the standard probe as oxygen saturation varies while HCT is fixed to 42%. It further provides detailed trends of reflectance changes near the isosbestic point, which suggest that the slope of the curve centering at 773 nm is affected by oxygen saturation. A more detailed view of slope changes near the isosbestic point is shown in Fig. 4(b). The straight lines in Fig. 4(b) are linear fittings of reflectance data at each oxygenation level, which clearly show that slope change is a function of oxygen saturation. As a result, the intensity at 773 nm can serve as a measure of the HCT level as shown in Fig. 4(c), while the slope can estimate the oxygen saturation level as shown in Fig. 4(d). Therefore, we are able to estimate HCT and oxygen saturation levels simultaneously from a single spectra reading.

![Fig. 3. (a) Whole blood reflectance spectra: porcine whole blood samples of HCT = 44% (arterial blood, oxygen saturation 99%; venous blood, oxygen saturation 70%; deoxygenated blood, oxygen saturation 0%). Each blood sample of different oxygen saturations has its own distinctive reflectance spectral shape. (b) Whole blood reflectance spectra at various HCT levels of porcine arterial blood samples: the reflectance spectra are shifted up as the HCT level increases, and the spectral signals are saturated as the HCT level reaches the normal HCT level of 44%. Inset, changes in the reflectance intensity at 627 nm.](image-url)
C. Reflectance Measurement with Varying Concentration of Contrast Medium

We investigated how the presence of the contrast media can change whole blood reflectance spectra to quantify HCT levels when the contrast media of angiography were mixed in blood. Figure 5(a) shows reflectance spectra measured using the standard probe, by varying HCT levels of ovine arterial blood diluted with a representative contrast medium (Visipaque, GE Amersham Health). We clearly showed that the contrast medium did not change the overall spectral shape of the reflectance spectra, indicating that it affected mainly the HCT levels. The specific reflectance intensity change at the wavelength of 627 nm is shown in Fig. 5(b). The reflectance intensity was reduced as the concentration of the contrast medium increased (i.e., as blood was diluted). The reflectance intensity of the saline mixture showed the similar intensity change as the angiographic contrast medium, supporting our hypothesis. The most important finding is that when a contrast medium is mixed with blood, it rapidly dilutes the blood. As a result, the local HCT level significantly decreases, and thus the reflectance signal drops. Although our results were obtained in a pilot animal study, we successfully demonstrated that contrast media can also be detected by our whole blood optical reflectance measurement. The previous work by Michishita and Fujii [12] also showed that almost all x-ray contrast media are waterlike transparent liquids containing iodine molecules and that they have absorptions only in the UV range of 200–300 nm. Therefore, it will be feasible that a reflectance-type intravascular sensing system can be used to detect the presence of the injected contrast media in vivo before they escape to other sites during angiography.

D. In Vivo Detection and Removal of Contrast Media Mixed in Blood

For the comparison of the performance of the two probes, we evaluated the reflectance spectra of the ovine blood sample and the scattering suspension sample measured by using the two probes. The
similarity between the reflectance spectral shapes of the blood was quantified by using the root mean square error (RMSE), which measures the overall estimation accuracy in the spectral shapes, while the oscillatory patterns in the scattering suspension were measured by correlation coefficient ($R^2$). Figures 6(a) and 6(b) show that the reflectance spectral shapes measured by using the probe of the Sentinel catheter are significantly similar, although there are minor differences at the longer wavelength region: RMSE = 0.0097 for the whole ovine blood, and $R^2 = 0.952$ for the scattering suspension of microspheres, respectively. These results indicate that the different configuration of the probe of the Sentinel catheter may minimally affect reflectance spectral shapes of whole blood because of the high anisotropic properties of whole blood [26], supporting our direct applications of the in vitro results to these in vivo experiments.

As mentioned previously, we hypothesize that blood is diluted by injected contrast and the dilution causes a change in the HCT level, which in turn affects the reflectance intensity and enables us to detect the presence of the contrast medium mixed in blood. In the in vivo test using five canine subjects, the Sentinel catheter was inserted into the canine left internal jugular vein and successfully deployed at the coronary sinus of the canine subjects. The illustration of the deployed system is shown in Figure 7(a). The contrast agent (Visipaque) was injected multiple times through the coronary arteries during the procedures. Because of the physical size of the subjects, the injection volume was limited to 5 cm$^3$ per each injection. A typical example of the in vivo contrast detection signal measured by using the custom-designed Sentinel catheter at 627 nm is shown in Fig. 7(b). The Sentinel system successfully registered the reflectance signal changes resulting

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Fig. 5. (a) Whole blood reflectance spectra at different HCT levels resulting from different concentrations of a representative contrast medium for angiography (i.e., Visipaque) in ovine arterial blood: the contrast medium does not change the overall spectral shape of the reflectance spectra, indicating that it affects mainly the HCT levels. (b) Reflectance intensity at 627 nm of different HCT levels for the contrast medium (Visipaque) and the saline solution mixed with ovine arterial blood: the error bar indicates the minimum and maximum values of measurement at each data point.

Fig. 6. Comparison of spectral shapes of (a) the ovine whole blood sample and (b) the scattering suspension of the microspheres. The spectral shapes obtained by using the two probes are significantly similar in both samples.
from contrast injections. The detection signals were highly reproducible and significantly dropped from the preinjection stage (i.e., the baseline signal). The example in Fig. 7(b) shows a 79% drop from the baseline after 5 s and is far beyond the baseline noise level, which was distinctive enough to activate the aspiration system automatically. The average reduction of detection signal compared with the baseline from 20 measurements was 80.13% (ranging from a minimum of 56.18% to a maximum of 91.40%).

Aspiration of the blood–contrast mixture from the canine coronary sinus was initiated at a signal drop when the reflectance signal reached at 15% of the baseline and was terminated when the signal increased to 10% of the baseline. The changes in the aspiration status are illustrated in Fig. 7(b). We collected 15 specimens from the aspiration of the blood–contrast mixture from four canine subjects. Using the conventional spectrometric absorbance assay, the average contrast removal rate was 60% ± 10.7% of the total contrast injection volume, showing that a significant volume of the contrast was removed.

4. Conclusion

We, for the first time to our knowledge, measured the fresh whole blood reflectance spectra in the region of 500–900 nm for various hematologic conditions. Because most of the previous studies have been focused on noninvasive sensing techniques for bulk tissue containing blood vessels, whole blood reflectance measurements for intravascular applications have been relatively unexplored thus far. Our results can be potentially significant for the development of in vivo intravascular or intracardiac optical sensing systems. More important, we implemented our simple findings in a prototype of a catheter-based reflectance sensing system. Although it was at a preclinical stage, we successfully demonstrated the feasibility of real-time in vivo contrast detection and removal of angiographic contrast media in the animal study. Our approach and findings may potentially reduce the risk of complications such as CIN caused by contrast media. Our whole blood spectral data and catheter-based sensing system design with simple algorithms may facilitate the development of optical intravascular devices.

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