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Capillary-scale direct measurement of hemoglobin concentration of erythrocytes using photothermal angular light scattering

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ABSTRACT

We present a direct, rapid and chemical-free detection method for hemoglobin concentration ([Hb]), based on photothermal angular light scattering. The iron oxides contained in hemoglobin molecules exhibit high absorption of 532-nm light and generate heat under the illumination of 532-nm light, which subsequently alters the refractive index of blood. We measured this photothermal change in refractive index by employing angular light scattering spectroscopy with the goal of quantifying [Hb] in blood samples. Highly sensitive [Hb] measurement of blood samples was performed by monitoring the shifts in angularly dispersed scattering patterns from the blood-loaded microcapillary tubes. Our system measured [Hb] over the range of 0.35–17.9 g/dL with a detection limit of ~0.12 g/dL. Our sensor was characterized by excellent correlation with a reference hematology analyzer ($r > 0.96$), and yielded a precision of 0.63 g/dL for a blood sample of 9.0 g/dL.

1. Introduction

Measuring the mass concentration of hemoglobin ([Hb]) is a common and vital task in blood donor screening and diagnosis of blood-related diseases. Hemoglobin (Hb) is the crucial component in blood that is responsible for carrying and transporting oxygen to the organs while moving through the circulatory system. The [Hb] therefore serves as a critical indicator of the oxygen-carrying capacity of the blood. One of the representative blood disorders determined by hemoglobin concentration is anemia. The lower limit of [Hb] considered normal differs by race and age, but in general, men and women with [Hb] less than 13 g/dL and 12 g/dL, respectively, are classified as anemic (Beutler and Waalen, 2006). Significant alterations in [Hb] may also be suggestive of hepatobiliary disease, metabolic changes, neurological disorders, cardiovascular disorders, or endocrine disorders (Mokken et al., 1992). The [Hb] also serves as an indicator of oxygen homeostasis; thus, monitoring [Hb] is important in pregnant women with severe hemorrhage and patients with considerable blood loss (Rosenblit et al., 1999).

Owing to its importance, various [Hb] detection methods including hemoglobin cyanide, hematocrit, light-scattering, and spectrophotometric methods have been developed. In the cyanide methemoglobin method, chemicals such as potassium cyanide (KCN) are employed to destroy the lipid bilayers of red blood cells, and subsequent cyanization of the released hemoglobin produces cyanide hemoglobin. As the cyanide hemoglobins exhibit high light absorption at a specific wavelength (i.e., 540 nm), [Hb] can be measured using a colorimetric analysis of light passing through the blood sample (Van Kampen and Zijlstra, 1961). This method provides a simple and accurate determination of [Hb], but the use of toxic chemicals such as KCN and dimethyllaurylamine oxide may pose health and environmental problems. The hematocrit method measures the volume of erythrocytes in comparison to the total volume of blood. It, however, requires a relatively large volume of blood (50–100 μL) to perform centrifugal separation (Billett, 1990). The [Hb] detection technique based on light-scattering determines the volume and hemoglobin concentration of red blood cells based on theoretical models of bloods, but its performance is highly dependent on the accuracy of such models (Tycko et al., 1985). Spectrophotometric methods have also been utilized to measure [Hb], but require large sample volumes and reference measurements at multiple wavelengths for the accurate determination of absorption at each wavelength (Kuenstner et al., 1994; Zwart et al., 1981).

Recently, [Hb] detection methods based on the photothermal

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(PT) response of Hb have been developed. The PT response of Hb molecules arises mainly from the presence of iron oxide in the Hb, as noted in previous publications (Lapotko and Lukianova, 2005; Lapotko, 2006; Lapotko et al., 2002; Wittenberg et al., 1970). Kwak et al. utilized this PT effect of Hb molecules to perform direct measurement of [Hb] in blood samples by measuring the photothermal temperature increase in blood samples under 532-nm light (Kwak et al., 2010). This method operates without the use of any chemicals, but requires a dedicated sensor chip patterned with a platinum resistance temperature detector (Pt RTD). An all-optical [Hb] detector has also been developed based on spectral-domain optical coherence interferometry (SD-OCR) (Yim et al., 2014). This method measures [Hb] by quantifying the optical path-length alterations of a blood-containing chamber under the illumination of a PT excitation light source. It demonstrated superior performance in anemic blood samples compared with the HemoCue® sensor (HemoCue® 201+, HemoCue®, Sweden) (Kim et al., 2015). The photothermal SD-OCR sensor provides direct and accurate measurement of [Hb], but its interferometric arrangement may present challenges in terms of stability and miniaturization.

Here, we present a novel all-optical Hb sensor based on photothermal angular light scattering (PT-AS). Our device, termed the PT-AS sensor, enables simple, direct, and rapid (<5 s) measurement of [Hb] with an extremely small volume of blood. The operating principle of our PT-AS sensor is similar to that of backscattering interferometry (BSI), explored by Bornhop and others (Bornhop, 1995; Sørensen et al., 2006; Tarigan et al., 1996). The BSI represents a simple optical sensor for measuring the refractive index (RI) of a sample solution inside a microcapillary tube. In our PT-AS sensor, we employ the high RI sensitivity of angular light scattering to measure RI changes due to the photothermal effect of Hb molecules under the illumination of a PT excitation light source. We describe the implementation and performance of the PT-AS sensor and demonstrate its utility as a Hb detector by quantifying [Hb] in blood samples at various Hb concentrations. The sensor's relative accuracy is assessed by comparing its measurements with the reference measurements made by a hematology analyzer (ADVIA 2120i, Siemens AG, Germany). Its precision is also quantified by evaluating standard deviation and coefficient of variation from twenty repeated measurements of the same blood sample.

2. Materials and method

2.1. PT-AS experimental setup

Fig. 1 depicts a schematic of our PT-AS sensor. Light from a 650-nm laser pointer (LP-4, Lasmac, Korea) illuminated a blood-containing microcapillary tube (TSP100245, Polymicro Technologies, USA). The light was then scattered, producing an angularly-dispersed scattering pattern. The inner diameter of the tube was 100 μm, and the probe beam size from the light source was 2.8 mm. Therefore, effective probe volume was estimated to be ~22 nL. A commercial webcam (C525, Logitech, USA) was employed to record the scattering pattern at a frame rate of 30 fps. The lens in the webcam was removed to directly measure the scattering pattern. A low-cost plastic long pass filter (Red optical cast plastic color filter #43-945, Edmund Optics, USA) was installed in front of the webcam to filter all but the 650-nm probe light.

A 532-nm, 100-mW diode-pumped solid-state (DPSS) laser (MGL-lll-532, Continuous wave, CNI Laser, China) was employed as the PT excitation light source, as hemoglobin exhibits high absorption at 532 nm. The beam diameter of the PT light was set to 4.0 mm, so that the entire probe volume was heated by the PT light. The optical chopper in the PT light path was inserted to enable intensity modulation at 2 Hz. This operating condition was set to achieve both rapid and highly sensitive [Hb] measurements. A detailed description of the operating condition is given in Section 2.4.

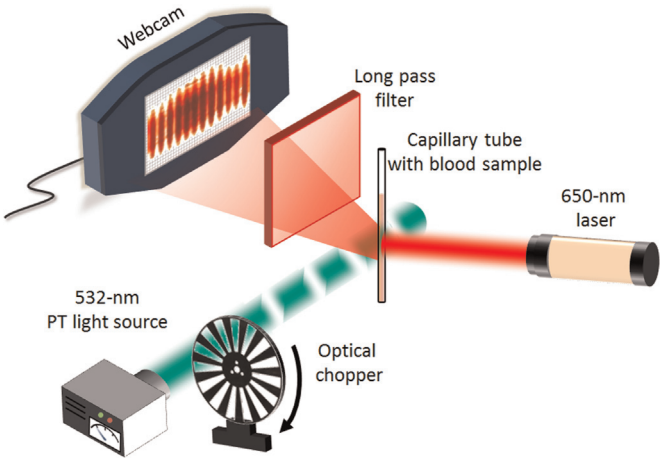

Fig. 1. Schematic of the PT-AS sensor for rapid and direct [Hb] measurement. A 650-nm probe light from a commercial laser pointer illuminates a capillary tube filled with a blood sample. The interference of the scattered light from the tube then produces a distinct scattering pattern on a complementary metal-oxide semiconductor (CMOS) webcam. Upon illumination with the 532-nm PT excitation light source on the tube, the Hb molecules absorb the PT light energy and generate heat, which leads to RI changes in the blood sample. These RI changes result in a shift in the scattering pattern on the CMOS detector. The optical chopper enables the modulated illumination of the PT light. A long-pass filter is inserted in the path of the probe light to detect only the 650-nm light.

2.2. PT-AS measurement method

The operating principle of our PT-AS sensor is similar to that of the BSI described in earlier publications (Bornhop, 1995; Markov et al., 2002). A probe light is directed to a glass microcapillary tube filled with a sample solution. The probe light is scattered by the tube, and the interference of the scattered light produces a distinct pattern on a detector array as a function of angle. This scattering pattern is known to be highly sensitive to the RI and physical size of the tube, and thus has been extensively utilized to measure the variation in RI of solutions and chemical reactions inside the tubes (Bornhop et al., 2007; Sørensen et al., 2006). The analytical expression for the angular scattering pattern can be found in Ref. Tarigan et al. (1996). Unlike the setups employed by others, a forward-scattering configuration was employed in our case, as it provided a sufficiently strong scattering signal that could be detected by a low-cost commercial webcam. However, the backscattering setup can also be utilized.

For our PT-AS sensor, a transparent microcapillary tube was filled with a blood sample for measurement. Upon illumination with the PT excitation light source, the Hb molecules absorb the PT light energy and produce thermal energy. The resultant increase in temperature alters the RI of the blood sample, which shifts the scattering pattern on the detector (Fig. S1). The measurement of this phase change in scattering pattern can be carried out in either continuous or intensity-modulated illumination of PT excitation light. In practice, however, external disturbances such as vibration may affect the phase measurement. Therefore, we employed the intensity-modulation scheme, which enabled the highly sensitive measurement of phase changes with an improved signal-to-noise ratio (SNR). The intensity modulation was enabled by an optical chopper (optical chopper system with chopper wheel, MC1F2, Thorlabs, USA) in the PT beam path.

The signal processing procedure for the PT-AS sensor output is