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Raman spectroscopy of blood samples for forensic applications

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ABSTRACT

We investigated Raman scattering from human blood as a function of parameters that are relevant for forensic field analysis, such as substrate, sample dilution, individual from which the sample originates, and age of the sample. Peaks characteristic of blood components and in particular the hemoglobin peaks were routinely detected when blood was deposited on substrates that are not strongly luminescent, such as plastic, metal utensils and dry wall. Raman scattering from blood proved quite sensitive and blood samples with a dilution up to 1:250 could be measured for an excitation power of ~2 mW measured at the sample plane. The sensitivity of Raman scattering to diluted blood allowed measurement using blood reconstituted from fabric substrates, thereby alleviating issues related to luminescence and scattering from the substrate. The dependence of Raman scattering on sample age and individual was also investigated. We found that the relative intensities of scattering peaks depended on sample age and history. For example, the relative intensity of oxyhemoglobin peaks decreases after blood has dried. Fresh blood drawn directly from a donor without intermediate storage exhibits also scattering peaks at 1155 and 1511 cm^{-1} which disappear after drying. The origin of these peaks is under investigation. We noticed, however, that they were not found in blood that had been stored for longer than one week in EDTA containers before analysis, thus requiring the use of fresh blood for future studies and validation purposes. The relative intensity of scattering peaks was also found to be somewhat dependent on the donor and, for a same donor, on the day on which blood was drawn.

1. Introduction

Raman spectroscopy is a very sensitive technique that is often used in forensic laboratories, mostly to analyze textile fibers and paints. [1,2] Surprisingly, Raman spectroscopy has seldom been used to analyze blood and body fluids for forensic applications. Recent research by Lednev et al. [3–6] and by De Wael et al. [7,8] has shown, however, that Raman spectroscopy can be employed to discriminate blood from other body fluids [3,4], human blood from feline blood [5] and blood from paint and stains [8]. Thanks to these findings and to the recent availability of hand-held and portable Raman spectrometers [9], Raman field analysis of blood and body fluids may soon become a reality. To this end, several parameters such as influence of substrates, dilution limits and excitation wavelength must be investigated to establish reliable collection protocols. Studies should also be carried out to determine, for example, if Raman scattering from blood depends on the donor, on the clinical condition of the donor, and on the history of the sample. To our knowledge, only one study of this kind has been carried out [6]. This study showed that Raman spectra of blood samples do not depend strongly on the donor. It was also shown that the spectra arise from two main components. The major component is hemoglobin, which yields scattering peaks at 1000, 1368, 1542 and 1620 cm^{-1}, respectively, for excitation at 752 nm. The second component is likely fibrin, which gives rise to peaks at 967, 1248, 1342 and 1575 cm^{-1}, respectively, again for excitation at 752 nm.

In this study, we present data that are relevant to establish field collection protocols such as preferred excitation wavelength, dilution limits and influence of substrates. We show that dilutions of up to 1:250 can be detected using a laser power of a few mW. Thus, Raman scattering has a sensitivity comparable to many presumptive tests for blood, such as the phenolphthalein (Kastle–Meyer), leucomalachite green, Hemastix® and the forensic light source tests. [10,11] These tests are highly sensitive (up to 1:5,000,000 dilutions can be measured in forensic laboratories), but in the field a cotton swab test is generally employed which can only measure dilutions on the order of 1:150. Because of the sensitivity of Raman scattering to diluted blood, a protocol for the reconstitution of blood from fabrics was established which alleviates issues related to luminescence and scattering from substrates. Reconstitution is a destructive procedure, but Raman scattering yields definite answers regarding the identity of a

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sample. Therefore Raman analysis of reconstituted blood can be more advantageous than presumptive techniques. We also analyzed the dependence of Raman scattering on sample age and donor. We found substantial differences between fresh and dry blood and we also found that the spectra of freshly drawn blood differed from the spectra of blood stored in EDTA containers. In addition to the peaks of dry blood, fresh blood analyzed immediately after drawing from a donor exhibited peaks at 1155 and 1511 cm^{-1} which disappeared upon drying. These peaks were evident for up to 1 h after drying, and they could be used to determine the age of blood found at a crime scene. The peaks at 1155 and 1511 cm^{-1} were not noticed in blood that had been kept in EDTA containers for longer than one week. A reduction in the relative intensity of the oxyhemoglobin peaks was also noticed in dry blood. The relative intensities of Raman scattering from dry blood were found to be relatively consistent between donors and between different days for a same donor. Some variations, however, were noticed in the oxyhemoglobin peaks.

Overall, our results indicate that Raman scattering can be safely employed to identify blood deposited on a substrate, even for comparatively high dilutions. However, in our opinion, any analysis based on the relative intensities of Raman peaks should be accompanied by a thorough study of the dependence on individuals.

2. Materials and methods

2.1. Sample preparation

Blood was collected from eight volunteers of both genders and various ethnicities. In most cases, blood was drawn from donors and immediately measured at different times after collection. For dilution and reconstitution studies a few ml of blood were drawn from a volunteer, collected in acid (EDTA) Vacutainers® and stored at 4 °C until use. For analysis, approximately 100 μl of blood was placed on a substrate. Generally, the substrate was a microscope slide coated with commercial aluminum foil. For comparison purposes, silicon wafers and uncoated microscope glass slides were also employed. To simulate blood from the crime scene, one or more 100 μl aliquots of neat blood were placed on commercial fabrics such denim, flannel, or cotton, on dry wall and eating utensils such as plastic forks and allowed to dry. Reconstituted blood was prepared by excising an approximately 3 mm^2 portion of the stained fabric. The excised portion was placed in a 2 ml centrifuge tube with a small amount of water (typically 500 μl). The tube was capped and flick-mixed for approximately 1 min. 100 μl of the reconstituted blood was pipetted from the tube and allowed to dry on the aluminum foil.

2.2. Raman spectroscopy

A Horiba Jobin Ivon HR 800 Raman Spectrometer was used for all measurements. This spectrometer is coupled to an Olympus confocal microscope and is equipped with a liquid nitrogen-cooled CCD detector (Synapse). The excitation sources were a He–Ne laser emitting at 632.8 nm with a power of 30 mW and a diode-pumped solid state (DPSS) laser emitting at 532.11 nm with a power of 50 mW. The laser power at the sample plane was measured with a power meter (Thor Labs) to be 9 mW at 632.8 nm and 2 mW at 532.1 nm. The lower power of the 532.1 nm laser is due to a different optical path. The DPSS laser had a higher divergence than the 632.8 nm laser and it was mounted about 1.4 m away from the spectrometer. The 532.8 nm laser was mounted about 10 cm from the sample. The excitation light was focused on the sample with a 10× or a 50× objective. In the initial stages of the experiments, measurements were repeated several times at regular time intervals to ensure that the incident light was not altering the samples. These experiments ensured that Raman analysis was non-destructive at the powers employed for the measurements. All spectra presented here were not manipulated except for the subtraction of a constant background and removal of cosmic rays. This simplified analysis was deliberate, since in field applications time and simplicity of analysis are of the essence.

3. Results and discussion

The goal of our experiments was to outline optimal procedures and to pinpoint potential pitfalls for the use of Raman spectroscopy for forensic examination of blood.

3.1. Excitation wavelength

Fig. 1 reports spectra of blood samples excited by red light (632.8 nm, lower spectrum) and, respectively, by green light (532.1 nm, upper spectrum). The laser power was measured at the sample position and it was about 9 mW for the 632.8 nm laser and about 2 mW for the 532.1 nm laser. Excitation with 532.1 nm light yielded peaks that were in overall agreement with those reported by previous experiments focusing on Raman scattering from blood. The frequencies of the Raman peaks are reported in Fig. 1 and their attribution can be found in standard reference works such as [12,13]. For the purposes of this paper, the most relevant peaks are those at 1365 and 1636 cm^{-1}, which are attributed to oxyhemogobin, and, respectively, to the ferrous low spin band. Illumination with red light yielded a scattering signal that was barely distinguishable from the background. The higher scattering cross-section of the 532.1 nm excitation is in agreement with measurements recently reported in [8] and is due to the different excitation mechanisms. Both 532.1 and 632.8 nm wavelengths scatter resonantly by coupling with electronic transitions of blood porphyrins. However, green light couples to the highly intense Soret band (located between 410 and 450 nm in human blood). Red light couples to an absorption at 625 nm. This absorption arises from porphyrin–Fe charge transfer and it is more than 100 times less intense than the Soret band. It is not surprising that the Raman scattering cross-section scales with the absorption cross-sections.

3.2. Dilution

For forensic applications, a determination of the maximum sample dilution for which measurements can be carried out is very

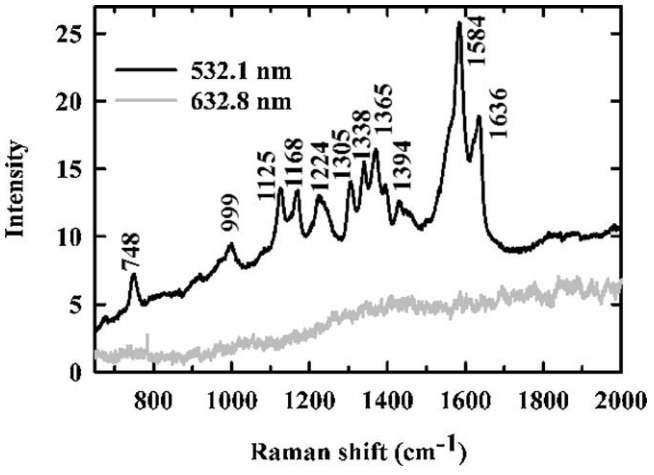

Fig. 1. Dependence of Raman scattering on excitation wavelength.