Characteristics of blood fluorescence spectra using low-level, 457.9-nm excitation from Ar^+ laser

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We measured the fluorescence spectra of the whole blood, the red blood cell (RBC) and the hemoglobin using 457.9-nm Ar^+ laser excitation. It was found that the fluorescence spectra of the whole blood and the RBC have much similarities in the intensity, the emission peaks and the emitting region, and abundant peaks can be found. But for the hemoglobin, fluorescence could only be found in the wavelength range 580−650 nm. It was concluded that in the wavelength range of 650−850 nm, the fluorescence spectra were emitted by the new fluorophores generated by the breakdown of some weak bonds on the RBC membrane, such as the C−C bond and the C−N bond. In the wavelength range of 590 − 650 nm, the fluorescence spectra are mainly emitted by the hemoglobin, but the hemoglobin solution of cracked RBC has a strong quencher effect on the fluorescence spectrum. The experimental result and the theoretical analysis are meaningful for the medical diagnostics and the therapy.

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Both non-imaging and imaging fluorescence *in vivo* oncological applications of light-induced fluorescence spectra (LIFS) have been proved to be helpful for detection of early-stage lesions when conventional diagnostic tools do not provide needed urgency and accuracy. The techniques have potential to provide optical biopsy without removing tissue. The use of optical spectroscopy for tissue diagnosis is a new approach in medical diagnostics[1]. Complementary to other existing techniques, fluorescence may reveal valuable diagnostic information regarding tissue composition and pathology[2].

On the other hand, many diseases can be cured with the help of low-level laser therapy (LLLT)[3]. Although some scholars had investigated it from many aspects[4,5], the mechanism is not yet raveled. Studies of fluorescence spectra provide information on the chemical and physical nature of the environment. In this way the information regarding specific sites on proteins, nucleic acids, and biological membranes can be obtained and related to their function[6].

The purpose of this study was to evaluate the LIFS characteristics of normal blood cell, and focus on the development of their respective spectral characteristics and relations by their respective laser-induced fluorescence spectra. Because shorter wavelength of exciting light results in more abundant fluorescence spectral peaks and less damage of blood cells, excitation source used in our experimental study was a continuous wave (CW) argon laser with the wavelength of 457.9 nm and power density of 25.7 mW/cm^2.

Figure 1 shows a schematic diagram of the experimental apparatus. The experimental apparatus recording autofluorescence of blood cells *in vitro* has been described previously in detail[7]. The excitation source was a CW 457.9-nm argon laser here, which has a power density of 25.7 mW/cm^2 and an irradiated spot of 0.7 cm^2.

Spectroscopy is performed with the specimen in air. The resulting autofluorescence is focused onto the slit of a spectrometer (WGD-3, with a 1200-g/mm grating, 2.5-nm/mm dispersion, and 0.5-nm resolution). A photomultiplier (PMT) R955, made by Hamamatsu Corp. in Japan, is placed in the exit slit of the spectrometer. Spectra are calibrated by using He-Ne laser. In this paper, the fluorescence spectral intensities are measured with the same gain of PMT, normalized with respect to laser excitation energy, and reported in arbitrary units.

All the blood specimens that are from the same healthy white female mouse are in 5% heparin anticoagulant. Some of the blood sample is diluted into 6% blood liquor by physiological saline water and the rest is centrifugated to obtain red blood cell (RBC). Some of the obtained RBC is diluted into 3% RBC by physiological saline and the rest is diluted into 3% hemoglobin liquor by distilled water.

All samples were measured without any extrinsic dyes. Our experiments were carried out within 2 hours after getting the blood with same parameters of measurement. The environmental temperature is 24 °C, and all the measurements are repeated more than three times to improve the accuracy.

The whole blood, the RBC and the hemoglobin have concentrations of 6%, 3% and 3%, respectively. All of the samples are excitated by 457.9-nm light from a low-intensity CW, Ar^+ laser. The fluorescence spectra in the wavelength range of 550 − 850 nm were measured

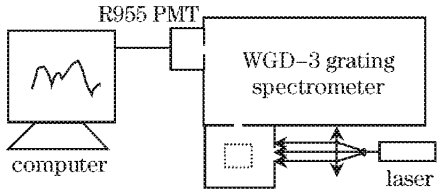

Fig. 1. Schematic diagram of the experimental apparatus.

with the setup shown in Fig. 1. The experimental results were shown in Fig. 2. It is obvious from the figure that the fluorescence spectra of the whole blood and the RBC are very similar in spectral intensity, emission peaks and emitting region, and there are abundant and strong peaks at 616, 666, 691, 708, 739, 752, 766, 800, 812 and 844 nm. But in fluorescence spectrum of the hemoglobin there are only two weak peaks at 629 and 666 nm, respectively, and the spectral intensity is almost zero in the wavelength range of 650 – 850 nm.

Pigment group, ambipolar phospholipidand other chromophores with conjugate double bond structure on RBC membrane can immediately emit fluorescence in the case of laser excitation, so their emitting is the majority of blood fluorescence spectra. A peak with strong intensity can be found in the spectra of the RBC and the whole blood (see Fig. 2). Because the energies of normal C–C bond and C–N bond are only 2.55 and 2.13 eV, respectively[8], some macromolecules containing such low energy bonds on RBC membrane easily absorb photons when excited by polarized and coherent laser, taking place optical breakdown and generating unpaired-electrons. New fluorophores due to the combination of unpaired-electrons with the chains and groups of other macromolecules emit fluorescence under laser excitation, which is small part of blood fluorescence spectra[9].

In addition, we think that the very weak 666-nm peak in the fluorescence spectrum of the hemoglobin is due to remainder RBC membrane in the hemoglobin solution obtained by cracking RBC.

Normally, a cell has two kinds of photon receptors: the cytochrome and the molecule with chromophore. Some scholar presented that low-level laser-blood interaction is the process of cytochrome absorbing photons to make mitochondrion from ADP into ATP[10]. On the other hand, clinic experiment in intravascular LLLT indicated that low-level laser can not only act on cytochromes but also interact on molecules with chromophore in cells, which may be biostimulation effect of LLLT. It may be the explanation of the abundant and strong fluorescence spectral peaks of RBC excited by 457.9-nm laser at low-intensity.

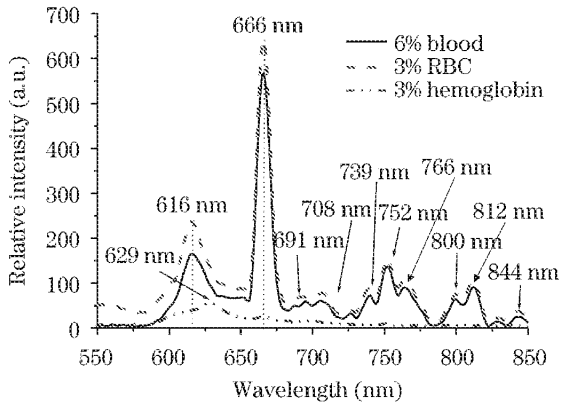

Fig. 2. Fluorescence spectra of blood, RBC and hemoglobin, obtained by using 457.9-nm excitation from a low-intensity CW, Ar^+ laser.

Figure 2 shows that there is a fluo rescence peak in the spectra from 590 to 650 nm. Although the fluorescence intensity of the hemoglobin is obviously lower than that of the RBC, and the position of its peak is red-shifted, it is the highest of hemoglobin fluorescence spectra. Therefore, the blood fluorescence spectra in the wavelength range of 590 – 650 nm are mainly due to fluorophores in hemoglobin. The mechanisms are: 1) Resonance energy transfer between the fluorophores in hemoglobin obtained by cracking RBC and ambient changed may results in fluorescence quenching[11]. It may be used to diagnose some diseases in hemolysis. 2) The red-shift of the spectral peak results from the difference of absorption spectra of hemoglobin[12] and RBC[13].

In addition, Ar^+ laser-induced fluorescence spectral distributions of blood cells do not change with the concentration of blood cells except for the fluorescence intensity in our experiment.

Indeed, many other factors could affect the fluorescence spectra of blood, such as impurities, eradicators, and so on. We have tried our best to avoid them in the experiments.

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