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Effect of infrared light on live blood cells: Role of β-carotene

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ABSTRACT

We have utilized Raman tweezers to measure and assign micro-Raman spectra of optically trapped, live red blood cells (RBCs), white blood cells (WBCs) and platelets. Various types of WBCs– both granulocytes, lymphocytes, and their different types have been studied. The Raman bands are assigned to different biomolecules of blood cells. The Raman spectra thus obtained has been enabled detection of β-carotene in these blood cells, the spectral features of which act as a signature that facilitates experimental probing of the effect of 785 nm laser light on different blood cells as a function of incident laser power in the mW range. The spectral changes that we obtain upon laser irradiation indicate that, both haemoglobin as well as the cell membrane sustains damage. In case of lymphocytes and platelets the peaks corresponding to β-carotene showed drastic changes. Thorough analysis of the spectral changes indicates possibility of free radical induced damage of β-carotene in lymphocytes and platelets. Among different blood cells, RBCs have a power threshold of only 10 mW. The power threshold for other types of blood cells is somewhat higher, but always below about 30 mW. These values are likely to serve as useful guides for Raman tweezers based experiments on live cells.

1. Introduction

Knowledge of blood components is of obvious importance in understanding different conditions of the human body; [1,2] they are routinely of utility in monitoring physiological functions by application of diverse methodologies [3–6]. Among non-invasive techniques, optical techniques, like Raman spectroscopy, are appealing since they do not necessitate external labelling and contributions made to the signal of interest by ubiquitous water can be readily identified and dealt with. Raman spectroscopy measurements have, indeed, been carried out for the detection and monitoring of various blood related diseases [7–10]. In fact, Raman spectroscopy combined with optical tweezers - Raman tweezers - has provided an experimentally useful means of carrying out spectroscopy on optically-trapped single live cells [11,12], in order to monitor biochemical changes in red blood cells (RBCs) [12–18], white blood cells (WBCs) [19–21], and platelets [22,23].

Raman tweezers have, in recent years, been successfully applied to study oxidative stress [24], haemoglobin related disorder, and thalassemia in RBCs [16] in addition to studies of other microorganisms [25,26], including stem cells [9,27]. Most experiments have focused on biomolecules of blood cells, such as haemoglobin, lipids, and nucleic acids. In contrast there continues to be a paucity of experimental work on carotenoids – like β-carotene – which are present in erythrocytes [28–30], leukocytes [31–33], platelets [34,35], and blood plasma [36–38]. β-Carotene, known to be an effective antioxidant that quenches singlet oxygen and free radicals, has been investigated for potential anticancer attributes and, more generally, as an agent that acts against oxidative stress [6]. There is some evidence of an inverse relation between high plasma concentrations of β-carotene and chances of various cancers [39,40]. High purity liquid chromatography (HPLC) has been the main tool used to explore the concentration of β-carotene in plasma, red blood cells and blood mono-nuclear cells [34] as well as those that are present in leukocyte and erythrocyte membranes [28,41]. Raman spectroscopy, of course, offers a very distinct advantage for inspecting β-carotene as it can yield information on *live* cells kept under physiological-relevant conditions. Micro-Raman spectroscopy has, indeed, begun to be applied to probe carotenoid levels in human lymphocytes [42]. Sub-populations of lymphocytes (CD4 +, CD8 +), T-cell receptors (γδ +, CD19 +), and natural killer cells (CD16 +) were studied by separating different types of cells by means of fluorescence

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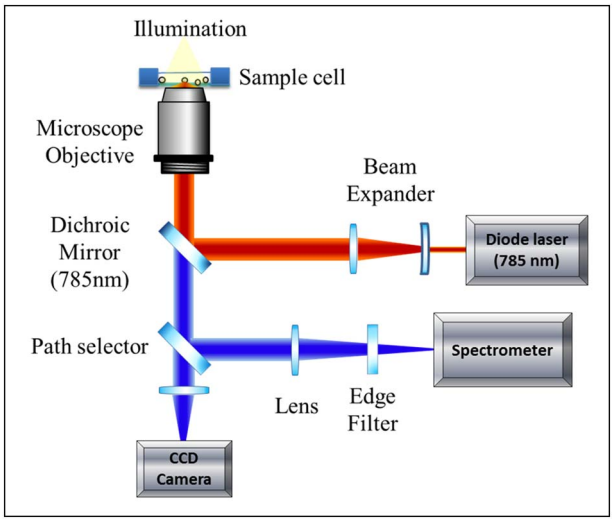

Fig. 1. Schematic diagram of Raman tweezers set-up.

based flow sorting [42]. A high concentration of carotenoids was found in CD4 +. Micro-Raman spectroscopy has also been applied to quantify carotenoid level in lymphocytes from healthy individuals and lung carcinoma patients and to characterize the carotenoids in different leukocytes and, concomitantly, imaging their distributions within these cells [43].

Although Raman tweezers offer obvious advantages in such studies, the inevitable use of a tightly focused laser beam – necessary to achieve optical trapping – is always a nagging point of concern while working on live cells. The mandatory use of a high numerical aperture (NA) objective to achieve sharp focusing of laser light implies that the focused laser intensity can often reach values in the MW cm^{-2} range, even for only mW of laser power. The usually feeble Raman signals make it necessary to use as high a laser power as possible, along with longer acquisition time in order to achieve sufficient signal-to-noise ratios. Both these factors can lead to photo-induced damage. There have been attempts to study and quantify the damage induced by near infrared (785 nm) laser radiation on RBCs [13,44]. Pulse Amplitude Modulation (PAM) technique based photochemical activity measurement has been done on microalga *Trachydiscus minutus* before and after the exposure [45]. The cells were optically trapped with different wavelengths and photodamage was observed for wavelengths 735 nm, 785 nm and 835 nm at a power level of 25 mW [45].

We have now carried out a detailed systematic Raman tweezers study on RBCs, WBCs (granulocytes and lymphocytes) and platelets; we have also explored the potential of Raman tweezers in identifying the presence of carotenoids in blood components. The Raman spectra thus obtained has been enabled detection of β-carotene in these blood cells, the spectral features of which act as a signature that facilitates experimental probing of the effect of 785 nm laser light on different blood cells as a function of incident laser power in the mW range. The spectral changes that we obtain upon laser irradiation indicate that both haemoglobin as well as the cell membrane sustains damage which in the case of RBCs has a power threshold of only 10 mW. The power threshold for other blood cells is relatively higher, but always below about 30 mW.

2. Materials and Methods

2.1. Sample Preparation

Blood was taken from healthy volunteers after obtaining their written, informed consent. Ethical clearance was obtained from the Institutional Ethics Committee, Kasturba Hospital, Manipal (Registration No. IEC 294/2013). The blood was drawn in a vacutainer containing K2-EDTA anticoagulant using the venipuncture method. The collected blood (typically 2 ml) was centrifuged for 5 min at 3000 rpm. Phosphate Buffer Saline (PBS) solution was used to dilute the RBCs for our Raman tweezers experiments.

2.2. Experimental Set-up

A schematic depiction of our experimental set-up is presented in Fig. 1. A 785 nm wavelength laser beam (Starbright Diode Laser, Torsana Laser Tech, Denmark) was used for both trapping and Raman spectroscopy. A dichroic mirror was used to direct the ~8 mm diameter laser beam on to the objective of our inverted microscope (Nikon Eclipse, Ti-U, Japan). A beam expander was used to overfill the 100× oil immersion objective (Nikon, Plan Flour) with high numerical aperture (1.3 NA) so as to obtain a tightly focused spot inside our sample cell. Using the same microscope objective the back scattered light was collected and directed into our Raman spectrometer (Horiba JobinVyon iHR320 with a 1200 grooves/mm grating blazed at 750 nm). This spectrometer had a liquid nitrogen cooled CCD for signal detection. A 785 nm high-pass edge filter (Razor Edge LP02-785RU-25, Semrock, USA) was used to suppress the inevitable Rayleigh scattering signal.

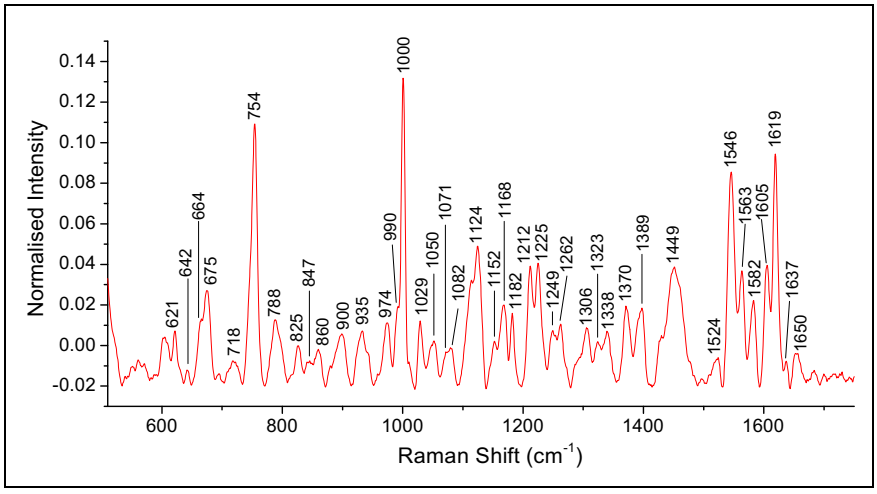

Fig. 2. Raman spectrum of a live optically trapped RBC. 10 mW was the incident laser power and 4 min was the acquisition time.