Dual wavelength optical tweezers for confocal Raman spectroscopy

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Abstract

We describe the use of dual optical tweezers to manipulate micron-size particles in and out of the focus of a confocal Raman microscope. One of the beams excites the Raman spectrum while the second tweezers improves the sensitivity of the technique and also allows for the manipulation of the environment of the trapped objects. We concentrated on optimising the alignment of both trapping and Raman excitation beams and on the background subtraction method. Even at the low trapping/excitation powers used a single living cell could be trapped and monitored for over 2 h without incurring damage.

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Optical tweezers utilise the force of radiation pressure to trap and manipulate micron and sub-micron sized particles whose refractive index differs from that of the environment [1,2]. Since 1984 radiation forces have been used to perform spectroscopy on trapped particles, aerosols and living cells (for example [3–13]). The important advantage of such techniques is the ability to perform spectroscopy on a single object of micron size. A greater signal to noise ratio from the trapped object can be achieved when background signals from the cover slip and immersion oil are minimised by manipulating the particle inside a sample holder. In most previous studies the same beam has been used to trap an object and excite either fluorescence or Raman scattering. Such a technique does not allow one to perform spatially resolved spectroscopy of the trapped objects or to take the background for a given position of the trapping beam. A second trapping beam can both
improve the sensitivity of the optical tweezers Raman spectroscopy system and allow one to manipulate the micro-environment of the object.

Recently novel setups consisting of two separate laser beams for trapping and Raman spectra excitation were proposed in [14,15]. The system investigated in [14] consisted of two independent microscopes: one to trap an object and another to excite the Raman spectra and collect scattering light. The functionality of the system was demonstrated by measurements of resonance Raman spectra of red blood cells. In [15] two optical beams of different frequencies were also used for trapping and spectroscopy. It was shown that the set-up which included only one microscope has a better signal to noise ratio and also obviated problems arising from replacing the condenser.

In this Letter, we develop a variant of dual frequency Raman microspectroscopy combined with optical tweezers that includes only one microscope system. In particular, we concentrated on optimising the alignment of both trapping and Raman excitation beam and on the background subtraction method. Our system allows us to achieve a high signal to noise ratio such that at <10 mW input power the Raman spectrum of a trapped polystyrene bead can be monitored in real time and hence used to align our confocal system with greater accuracy. The power of our 1064 nm trapping beam is about 50 times less than that used in [14] and the laser excitation power required is five times less than that used in [15]. These conditions together with using IR wavelengths for trapping allow us to study living cells for long periods of time, thus kinetics for biochemical processes can be obtained. Here, we show results for non resonant Raman spectra where living yeast cells are trapped and spectra recorded for over 2 h with no detrimental effects to the cell.

The main parts of our experimental set-up are as follows (Fig. 1). A diode laser (CrystaLaser) operating at 785 nm was used for excitation of Raman spectra with an average power of <10 mW at the sample. Micro-particles or cells were trapped inside a custom-made holder using a 100-µm thick fused silica cover slip (UQG Optics). The holder was placed on an inverse Olympus IX 51 microscope equipped with a 100× oil immersion objective NA = 1.25 (Edmund Optics) and non fluorescent oil (Cargille, Type DF). Back scattered light collected by the objective passed through a holographic notch filter (NF) (Kaiser) and a confocal system formed by lenses L1 and L2 and a 100 µm pinhole, before finally being focussed by a cylindrical lens onto the spectrometer slit. The spectrometer was a SpectraPro 2500−i from Acton containing a 600 lines/mm grating (750 nm blaze wavelength) and incorporating a Princeton Instruments Spec-10 CCD, cooled to −100 °C. A 1064 nm Nd:YAG laser operating at less than 2.0 mW of power at the sample was used as the auxiliary laser tweezers. By adjusting mirror M and varying the distance between lenses L3 and L4 we manipulated a trapped object along all three coordinates. A CCD camera (JAI) attached to the microscope provided optical images during experiments.

We found that the optimum alignment for Raman spectra of a trapped object differs greatly to that of an object flat on a surface. The system was optimised for Raman spectra from a trapped spherical object by using a 5-µm diameter polystyrene bead. First, the optical systems were aligned in such a way that the trap could be achieved at
the same place inside the sample holder for both beams. Then the bead was trapped using the 785 nm laser and the Raman spectrum was recorded in real time. The signal to noise ratio for our system was large enough to be able to align the system using a 0.2 s or even 0.1 s accumulation time (Fig. 2(a)). By adjusting the position of the bead inside the sample holder and the orientation of the pinhole we optimised the signal to noise ratio for the trapped bead. A sample spectrum of polystyrene taken for 10 s is shown in Fig. 2(b).

After this measurement was made the 1064 nm tweezers were switched on and used to trap and then move the particle out of the Raman laser beam waist in order to take a background measurement under the exact same experimental conditions (see Fig. 2(c)). The final back subtracted data is seen in Fig. 2(d). All background subtractions for trapped objects were made in the same way. The spectra were processed by background subtraction and subsequent Savitsky–Golay smoothing. After the system was aligned for polystyrene beads, measurements were made on living cells using the same procedure for the background subtraction.

The Saccharomyces cerevisiae yeast cell is widely used as a model for fundamental studies of cell processes including cell stress response, as many fundamental cellular processes are conserved from yeast to human cells and corresponding genes can often complement each other. Many disease-related genes have been identified from studies of this organism, forming the basis for understanding more complex cell-signalling events [16,17]. Cell processes such as stress response have been monitored by biochemical methods but not on a single cell basis. To be able to monitor dynamic processes such as mitosis or metabolic changes in single cells a method must be available to immobilise and if necessary manipulate a single cell over a period of time relative to the length of the cell cycle.

For our experiments yeast cells (S. cerevisiae) were grown in synthetic defined media (SDC) with complete supplements under standard conditions. For Raman measurements the cells were diluted further in SDC such that a single cell could be trapped with no other cells in the surrounding medium. This medium was used to ensure cell viability over the long time scale of our experiments, providing nutrients for the cell to live on. Raman spectra of single, budding live yeast cells were recorded every 3 min for 180 s accumulation time, for a total of 150 min in the same optical trap. After the experiment the buds were seen to have grown appreciably, indicating the normal growth of the cell in the optical trap. The resolution for our experiments was 8.5 cm$^{-1}$ as estimated from the polystyrene spectrum by measuring the
FWHM of the 1001.4 cm$^{-1}$ peak. This spectrum also served as a Raman shift frequency standard for our experiments using literature values [18].

The monitoring of the fission yeast *Saccharomyces pombe* using Raman microspectroscopy was reported in [19]. In this study the cell was adhered on a glass slide and the Raman spectra recorded the spectral changes due to cell mitosis. However, reference [9] cites some problems associated with acquiring Raman spectra from cells in this manner including larger background and fluorescence from the glass slide, and showed how optically trapping the cell at some distance from the slide could avoid such problems. Another experimental consideration is that trapping over long periods of time must not damage the cell. Here, we report living yeast cells trapped by near-IR light for over 2 h. The spectra are shown in Fig. 3 with tentative peak assignments as indicated in [9,20,21].

Changes can be easily seen in the spectra after different times, for example the ratio of peak intensities of adenine to phenylalanine changes drastically between 3 and 126 min. Xie and Lie [9] report a large change in the ratio of $I_{1004}/I_{946}$ from 1.33 to 12.5 after heat treating cells which they suggest is a measure of the increase of the amount of proteins in the $\alpha$ helical state. By comparison the peak intensities from our background subtracted data show a ratio of $I_{1002}/I_{943}$ that increases only slightly from 1.12 to 1.40 after 126 min of trapping indicating that there is little change in protein conformation during the experiment. The comparatively large increase in the 1004 cm$^{-1}$ peak seen after heating yeast cells as reported in Xie, indicated that the phenylalanine side chain was more exposed after protein denaturation. Although the intensity of the phenylalanine peak in our experiment increases slightly as time progresses it does not do so to the same extent. This is some indication that for our set up localised heating of the cell due to trapping for long periods of time is not taking place on an appreciable scale.

Another example of information inferred from changes in the Raman peak intensities is characterising the level of RNA translation in a cell by computing the ratio of a peak for DNA to a protein peak [22]. The relative intensity of a Raman line is crucial when comparing processes from different cells. In each case the environment of the cell will be slightly different, for example in the distance from the substrate. To accurately perform comparative kinetic studies on different cell samples background subtraction must account for these sample to sample differences. Fig. 4 illustrates the sizeable effect background subtraction has on the Raman peak intensities. Unlike the background spectrum of the buffer seen in Fig. 2 the background spectrum of the SDC shows peaks in this region due to its composition of amino acids and other nutritional supplements. Thus, background subtraction will have an effect on the ratio
of the peak intensities. In Fig. 4 we see that peak positions are the same in both cases however the peak intensities differ greatly for certain bands. Our background subtraction method takes into account the distance from the substrate, the spectrum of the substrate and the spectrum of the medium at the same time. In this case one can exclude variations in the microenvironment between samples as reasons for differences in spectra between individual cells.

Our system for optical tweezers with Raman spectroscopy requires only one microscope system while incorporating two laser beams of different wavelengths. The use of dual optical tweezers resolved the problem of acquiring the correct background spectrum for each sample. Furthermore a relatively short accumulation time and low power was needed to achieve well resolved spectra. An investigation is currently under way to monitor the effect of chemically induced stress on live yeast cells.

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