

Raman imaging of floating cells

Caitriona M. Creely, Giovanni Volpe, Gajendra P. Singh

ICFO - Institut de Ciències Fotòniques, Barcelona, Spain

Marta Soler

IBMB - Institut de Biologia Molecular de Barcelona, CSIC - Consell Superior d'Investigacions Científiques, Barcelona, Spain

Dmitri V. Petrov

ICFO - Institut de Ciències Fotòniques, Barcelona, Spain
ICREA - Institució Catalana de Recerca i Estudis Avançat, Barcelona, Spain

Dmitri.Petrov@icfo.es

Abstract: Raman imaging can yield spatially resolved biochemical information from living cells. To date there have been no Raman images published of cells in suspension because of the problem of immobilising them suitably to acquire space-resolved spectra. In this paper in order to overcome this problem the use of holographic optical tweezers is proposed and implemented, and data is shown for spatially resolved Raman spectroscopy of a live cell in suspension.

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1. Raman spectroscopy of single cells

Raman spectroscopy is a powerful spectroscopic technique that permits the identification of molecules due to their vibrational spectra. Some advantages of Raman spectroscopy are that it requires no special dyes or specific excitation wavelengths, is non-destructive technique and non-contaminating. These are some of the reasons as to why this spectroscopic technique has gained popularity in recent years for the study of living cells. The technique has been successfully combined with optical tweezers [1, 2] which permits one to manipulate micron sized objects such as living cells in solution [3]. The tight focus required by both Raman microscopy and optical tweezers makes combining these two techniques straightforward, (see for example [4, 5, 6, 7]). Recently we applied this to quantitatively study the fermentation process in a single yeast cell in real time [8]. The results were supported by previous biochemical assays, demonstrating the usefulness of this technique for real time monitoring of cell biochemistry.

In many cases it may not be just desirable to have time resolved information about biochemical changes taking place inside a cell but also to have information about where these changes are occurring. Raman imaging of cells plots the concentration of various biochemicals as a function of position. The Raman cell images can be more easily interpreted than spectra alone by biomedical researchers already familiar with microscopy. The data are acquired either by scanning the laser back and forth across the sample or by scanning the sample across the laser beam, and taking the Raman spectrum at each point [9]. The values of the intensity at a certain peak or peaks are plotted as a function of position. These images provide more information than conventional microscopy images alone as they contain information on all cell components and different cell components can be visualised by post-processing of data. To this date an example has been demonstrated where a chemical change signalling cell death was detected in a Raman image at an earlier time than was possible with a fluorescence image [10].

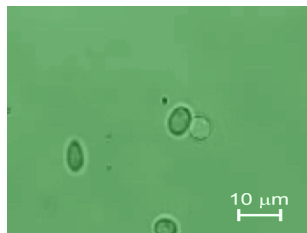


Fig. 1. Frame from a movie showing trapping of a dividing yeast cell (1.95 MB). There is a large movement relative to the trapping point.

Some cell lines grow attached to a surface, and are therefore immobilised for the duration of the experiment, allowing us to image them. However the natural environment for other cells (such as blood cells) is to be in suspension. Optical tweezers can restrict the Brownian motion of these types of cells to a large extent, allowing one to trap a single cell for hours. However the cell will generally rotate in the trap about the trapping axis[11]. The supplementary video for Fig. 1 shows how a dividing cell moves relative to the trapping axis in a single beam trap. In this case Raman imaging cannot be performed because of the movement relative to the excitation beam. Thus, to date, there have been no publications regarding imaging of floating cells.

We propose that a more stable configuration for trapping is to use multiple beams around the periphery of the cell. This should not only allow for immobilising larger or asymmetric cells, but also distribute the trapping power more evenly throughout the volume, limiting the damage to the cell. Holographic Optical Tweezers (HOT) can be used to produce such multiple tweezing sites easily and rapidly and can allow immobilisation, manipulation or controlled rotation of trapped objects[12, 13, 14, 15, 16, 17]. One of the main challenges faced by this system of multiple trapping beams for Raman spectroscopy is that the radiation forces of the Raman excitation beam can affect the controlled movement of the cell provided by the HOT. This excitation beam ideally should produce minimum perturbation of the cell moved by HOT whilst still efficiently exciting the Raman spectra.

To produce a Raman image using HOT the cell is trapped and scanned back and forth across the focus of the stationary excitation beam and in this way builds up an image of the entire cell, requiring no moving mechanical parts during the experiment. In this way a Raman image of a floating cell can be constructed. We show here for the first time a Raman image of a cancer cell in suspension, with movement completely controlled by HOT.

2. HOT with Raman setup

Our Raman system, shown in Fig. 2(a), was fully explained in [7]. In brief, it uses a diode laser (CrystalLaser) operating at 785 nm and focused with a $100\times$ objective into a sample holder placed on an inverse microscope (Olympus IX 51). This laser excites the Raman spectra and the back scattered Raman light is collected with the same objective. This scattered light then passes through a holographic notch filter, confocal system and finally into a spectrometer (Spectra Pro 2500-I, Acton) equipped with a Princeton Instruments Spec-10 CCD cooled to $-70\text{ }^\circ\text{C}$. Raman spectra were recorded over the wavenumber range of $485 - 1608\text{ cm}^{-1}$, with a spectral resolution of 8 cm^{-1} . The acquisition time used for Raman spectra for the HOT set up was 60 s at each point, using 0.1 mW of excitation power at the sample plane.

For this work we needed a configuration of relatively few (1 to 10) rapidly programmable trapping points. The trapping laser used was a $1064\text{ nm Nd} : \text{YAG}$ (Laser Quantum), which was expanded by a telescope (using lenses L_1 and L_2 in Fig. 2(b)) to fit the size of the active area of a liquid crystal Spatial Phase Modulator (SPM) (Holoeye, LC-R 2500) with a resolution of 1024×768 pixels and a pixel size of $19\text{ }\mu\text{m}$. The SPM allows us to alter the phase of the incident wavefront of the incident beam without changing its amplitude which would involve a reduction in the intensity of the beam. However we wanted to exclude the zero-order beam coming from the SPM which would interfere with the trapping, meaning we lose a lot of power as at this trapping wavelength (1064 nm) our SPM is relatively inefficient, with maximal nominal obtainable diffraction efficiency of about 40%. One result of this is that the laser power of the Raman excitation laser had to be decreased many times in order not to compete with the trapping from the HOT. To be able to select the first diffraction order without interference from the other orders, some measures were taken. First, a Fresnel lens was added to the hologram. Secondly, to improve the rejection of the zero order beam we introduced an angle between the first order and zeroth order beams. This was accomplished by adding a linear phase term to the

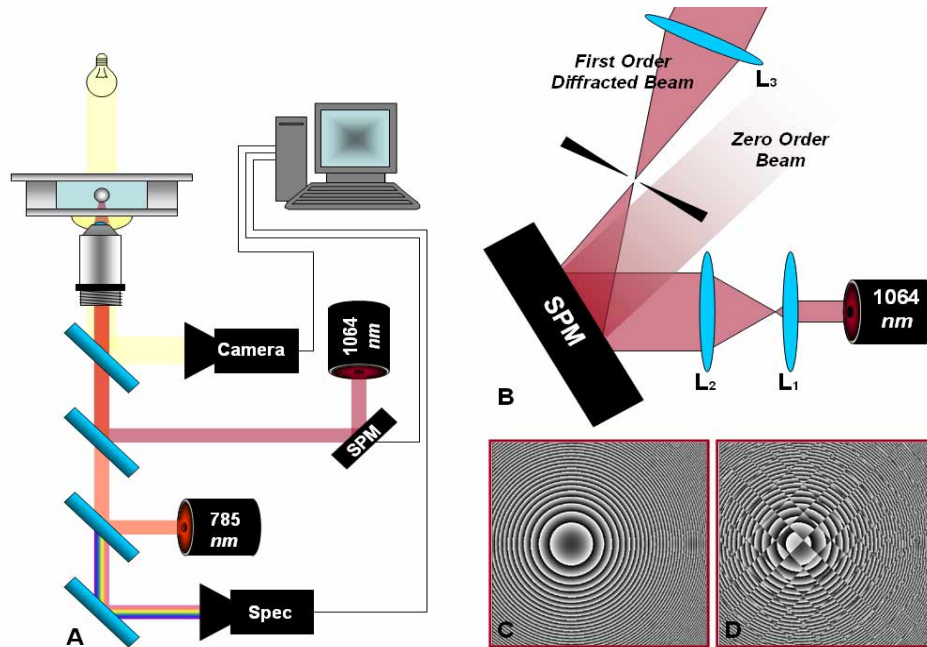


Fig. 2. Set up for HOT combined with Raman spectroscopy (a). A more detailed view of the SLM arrangement (b) and some examples of holograms used to produce one (c) and four (d) trapping points respectively at the focal plane.

created hologram, thus creating an off-axis Fresnel lens. Fig. 2(c) and Fig. 2(d) show samples of holograms used to provide one and four beam at the focal plane respectively.

The setup up was aligned such that the 1st order diffracted beam produced via the SPM was focused through the same microscope objective and aligned so that its focal plane was inside the sample holder. The full trapping power measured at the focal plane was 2 mW. The trapping laser was used to move a living cell back and forth across the focus of the laser beam for Raman excitation and a spectrum was recorded at each position. The cell was not observed to rotate during the duration of our experiments. The analysis of the spectra thus acquired was automated by a custom-made program based on MatLab 7. The program integrated cosmic ray removal, Savitsky-Golay smoothing, background subtraction, spectra plotting and image plotting for peaks into a single graphical interface. For each image a band of interest was selected and the intensity plotted as a function of position.

The cells used were Jurkat Clone E6-1, T-lymphocytes cultured in RPMI in suspension with 10% FBS under standard conditions. Jurkat cells are a cell line derived from human T-cell leukaemia and used to determine the mechanism of differential susceptibility to anti-cancer drugs and radiation. We chose them for this trapping and imaging experiment as they are larger than the cells we normally trap in suspension, ca 10 – 15 μm in diameter. MatTek glass bottom culture dishes were used as sample holders (Cat no. P35G-0-10-C). Dishes were sterilised overnight in pure ethanol and subsequently UV irradiated before cells were added. The movie which accompanies Fig. 3(a) shows 1 and 4 beams created and moved by the SPM at the focus of our microscope. Figure 3(b) shows a still from a movie where one of the Jurkat cells is being moved by HOT tweezers in a controlled manner.

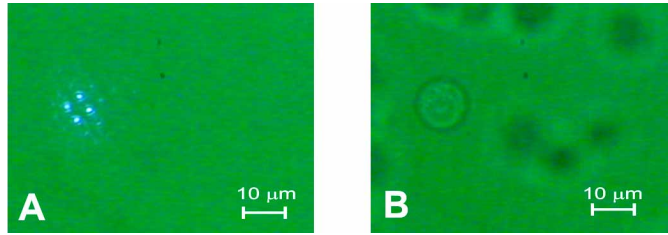


Fig. 3. (a) Still from a movie showing one and four beams created and moved by SLM control (1.6 MB). (b) Still from movie where Jurkat cell is being moved by HOT (2.19 MB)

3. Results and discussion

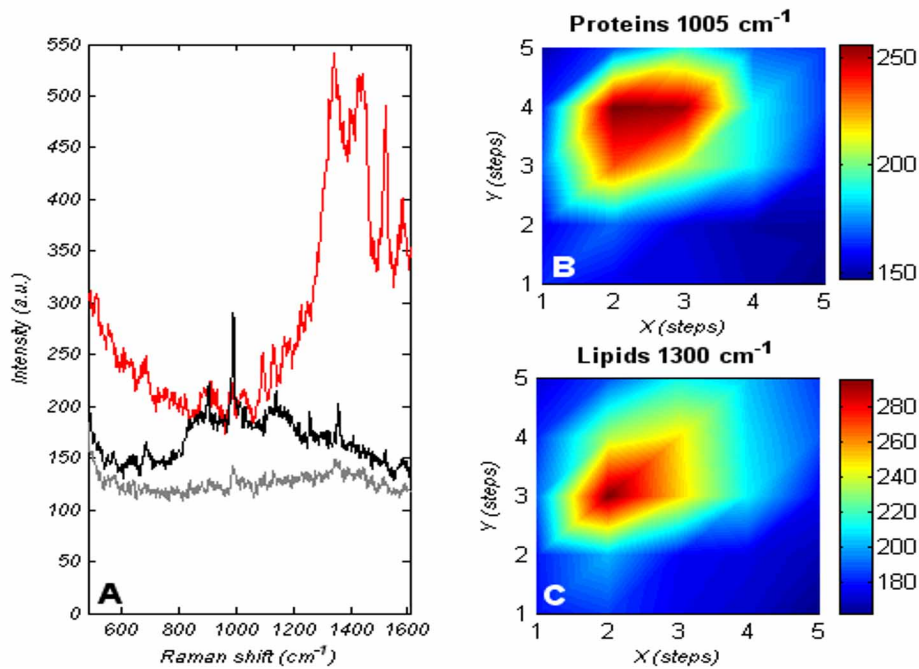


Fig. 4. (a) shows spectra from this cell acquired for 60 s inside the cell (black) and outside (grey) compared with spectrum from a cell imaged on the surface (red) with 15 s acquisition. Raman images of single floating Jurkat cell with different bands imaged (b) and (c). Each step was 3 μm .

An asymmetric Jurkat cell was trapped using the HOT and scanned back and forth across the Raman excitation beam in a 5×5 step scan (stepsize 3 μm) and a spectrum was taken at each point. To show the advantage of using optical tweezers for Raman spectroscopy we compare a single spectrum taken of this cell trapped far from the surface with HOT with one taken when the cell was attached to the surface. Figure 4(a) shows the comparison of spectra taken of the floating cell (grey: background and black: inside the cell) and compared to the signal taken of a HeLa cell grown attached to the surface and which was imaged directly on the glass substrate (red line). The large broad bands seen in the spectrum of the attached cell arise from the glass.

These bands are absent in the spectrum of the floating cell as we are trapping cells at a distance of $10\ \mu\text{m}$ from the surface.

Two separate Raman images of the Jurkat cell are shown in Fig. 4(b) and Fig. 4(c) obtained by plotting the intensity of the Raman bands assigned to specific chemicals such as proteins (phenylalanine) $1005\ \text{cm}^{-1}$ and lipids $1300\ \text{cm}^{-1}$ [18]. Despite the fact that the resolution is not high due to the large step size used we can clearly see different distributions of the chemicals inside the cell as we would expect. The cell membrane mainly consists of lipids and we can see that the protein distribution is within the border of the lipid distribution for this reason.

4. Conclusions

This initial study demonstrates the feasibility of Raman imaging of floating cells using HOT to immobilise and manipulate the cells relative to the exciting beam. Some issues have to be addressed however. The trapping effect of the Raman excitation laser competes with that of the HOT as mentioned in the experimental section. At present in our setup the $785\ \text{nm}$ laser used for Raman excitation overfills the objective which leads to very efficient trapping, which, although some beam power is lost, is a suitable configuration for trapping and exciting with the same beam. This is not the case for HOT imaging where we wish to trap and excite with different beams, however by adapting the optical setup, so that the Raman beam is larger at the focal plane there will be a two-fold beneficial effect. The trapping efficiency will decrease thus we can use a higher power for excitation. This means that the acquisition time at each step can be decreased many times, allowing for faster imaging. Our current setup could be adapted to be able to switch between two geometries. One where the Raman beam overfills the objective for time-resolved Raman spectroscopy of attached cells where the trapping of the excitation beam will not have an unwanted effect, and one where the Raman beam is larger at the focus so as not to compete with the HOT beam for imaging of floating cells. It has been previously shown that 3D controlled rotation is possible by utilising the SPM to create multiple points and rotating using Laguerre-Gaussian beams and we wish to exploit this to be able to image the cell from any chosen angle [19].

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