Raman Spectroscopy on Floating Cells

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Research into the biochemical changes that take place inside single living cells has huge potential in many fields of science and industry. However, the current techniques used in molecular and cell biology are not applicable to single cells and are unable to monitor changes *in situ*. Non-biochemical methods such as fluorescence or confocal microscopy require the use of fluorophores and dyes, enabling visualization of only a small number of organelles at any one time.

Raman spectroscopy can be used to identify different biochemical constituents, such as nucleic acids, proteins and lipids, and has the advantage of being able to acquire all this information simultaneously. The natural environment of many cells, including blood cells, is in suspension. By using optical tweezers, researchers can constrain a floating cell in order to measure its Raman spectrum.[1] This technique—called optical tweezers Raman spectroscopy (OTRS)—is non-destructive, non-invasive (and therefore sterile), and requires little or no sample preparation. Some successful biological applications of OTRS include the detection of glutamate in a single nerve terminal[2] and the study of the effect of alcohol solution on single human red blood cells.[3]

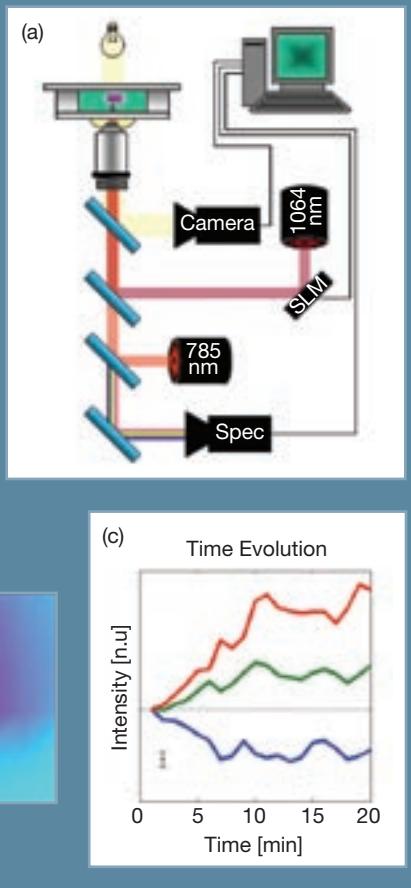
Our group succeeded in keeping a single yeast cell alive in the trap for up to three hours while studying its living processes, one of which was a hyperosmotic stress response.[4] We are now using this time-resolved information to investigate the cell cycle of a single yeast cell.

In many cases, determining where certain biochemical changes take place inside a cell may be just as desirable as learning when these changes occur. Raman images show the distribution of chemicals within a cell. Biomedical researchers who are familiar with imaging techniques can interpret these images more easily than spectra alone. In addition, such images contain more information than those obtained using conventional microscopy.

In a single beam trap, a cell continues to move and rotate to a certain degree due to Brownian motion and organelle motility. During the acquisition time, scientists measure time-averaged Raman signals, but Raman imaging cannot be performed. To facilitate imaging, we propose using multiple trapping beams around the periphery of the cell in order to immobilize it. This allows asymmetric cells to be immobilized and distributes the optical trapping power more evenly throughout the volume, thereby limiting photo damage.

Holographic optical tweezers, which are produced by a spatial light modulator, are used to generate such multiple tweezing sites and to scan a floating cell back and forth across the focus of a stationary Raman excitation beam. In this way, an image of the entire cell is built up. We demonstrated the first example of a Raman image of a cancer cell in suspension, with movement completely controlled by holographic tweezers.[5]

This new technique will make it possible to acquire previously inaccessible time- and space-resolved information about cells in suspension. It will allow researchers to learn more about cell-cell interactions and cell signalling and may have biomedical applications in areas such as drug delivery systems.



(a) Experimental setup for optical tweezers Raman spectroscopy: The 785-nm laser excites the Raman spectra and the 1064-nm laser generates a number of trapping points by means of a spatial light modulator. (b) Raman image of the protein content within a floating Jurkat cancer cell (1005 cm^{-1} band imaged). (c) Time evolution of different components of a single *S. cerevisiae* cell (RNA, lipids, proteins).

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