Optical trapping and Raman spectroscopy examine single cells

Techniques contribute to understanding of cell response to stress

Understanding cell response to stresses such as increases in temperature, pH and saline concentration as well as the presence of toxins could contribute insight into the ways in which drugs affect living cells. But the methods currently used to study this response are limited as to what they can reveal.

For example, high-performance liquid and other chromatographical methods combined with molecular biology protocols can detect and monitor the changes, but they provide only statistical information about populations of cells and cannot track the cells' reactions to changes in situ. Fluorescence and confocal microscopy can follow single cells in real time but typically require the use of exogenous fluorophores or stains.

To address these limitations and to explore the stress response in a single cell, researchers with Institut de Ciències Fotòniques, Institut de Biologia Molecular de Barcelona and Institució Catalana de Recerca i Estudis Avançats, all in Barcelona, Spain, combined Raman microspectroscopy and optical tweezers to investigate the fermentation process in a single *Saccharomyces cerevisiae* yeast cell. Investigators often use this yeast as a model for fundamental studies of cellular processes because many of its processes occur in human cells, too.

Combining the techniques provided an effective means to achieve the scientists' goals. Recent developments in Raman microspectroscopy have made it possible to monitor kinetic processes in living cells in real time. An optical trap allowed them to manipulate cells in their natural environments, without having to fix them to substrates, for example. And because both techniques require a tight focus, combining them proved to be relatively straightforward.

As described in an *Analytical Chemistry* paper published in April, the researchers developed an experimental setup based on a 785-nm diode laser made by CrystaLaser LG of Reno, Nev., used for both excitation of Raman spectra and trapping. Cells were placed inside a custom-made holder with a 100-µm fused silica coverslip, which was then positioned on an inverted microscope made by Olympus of Melville, N.Y., and outfitted with a 100×, 1.25-NA oil-immersion objective. The same objective collected backscattered light and sent it to an Acton spectrometer incorporating a Princeton Instruments CCD camera. A CCD camera made by JAI Corp. of Yokohama, Japan, acquired optical images.

The researchers optimized this system so they could achieve stable trapping at

low powers without compromising the Raman signal-to-noise ratio. "This allowed us to study a living yeast cell in the optical trap for up to two hours without damaging it," explained Dmitri Petrov, the principal investigator of the study.

Petrov and colleagues then conducted experiments to demonstrate that they could perform real-time detection in a single cell. They looked for glycerol and ethanol, which are produced by glucose-induced hyperosmotic stress of a yeast cell. To this end, they trapped an *S. cerevisiae* cell and performed Raman microspectroscopy under hyperosmotic stress as well as under normal and heat-treated conditions. They found that, even with a number of other chemical changes occurring at the same time, they could easily detect and monitor these two substances.

This finding suggests a variety of applications of the combined approach for shedding light on biochemical processes at the level of the single cell and in real time. "I think the most exciting application is in the study of how drugs affect living or diseased cells," Petrov said.

Indeed, the investigators plan to develop the technique so that it can provide spatially and temporally resolved measurements using multiple tweezing points, such as those produced by holographic tweezers. This will allow them to discern where and when changes occur within the cell following the administration of drugs. It also will allow them to image cells — blood cells, for instance — that are typically found in suspension. In this area, they plan to collaborate with investigators in the Cell Signaling and Cancer Research group of Institut de Biologia Molecular to address specific questions relating to cell death.

Use of multiple points also opens up the possibility of cell tomography. By rotating a cell with the holographic tweezers, the researchers could produce a three-dimensional map of its chemical components.

Besides allowing investigators to explore the cell stress response, the combined technique offers the advantages of being noninvasive and fast. Perhaps most importantly, it acquires all spectra at once, enabling subsequent analysis of any chosen chemical. The primary disadvantage, Petrov said, is that, at this stage, it cannot distinguish between different proteins produced inside the cell. □

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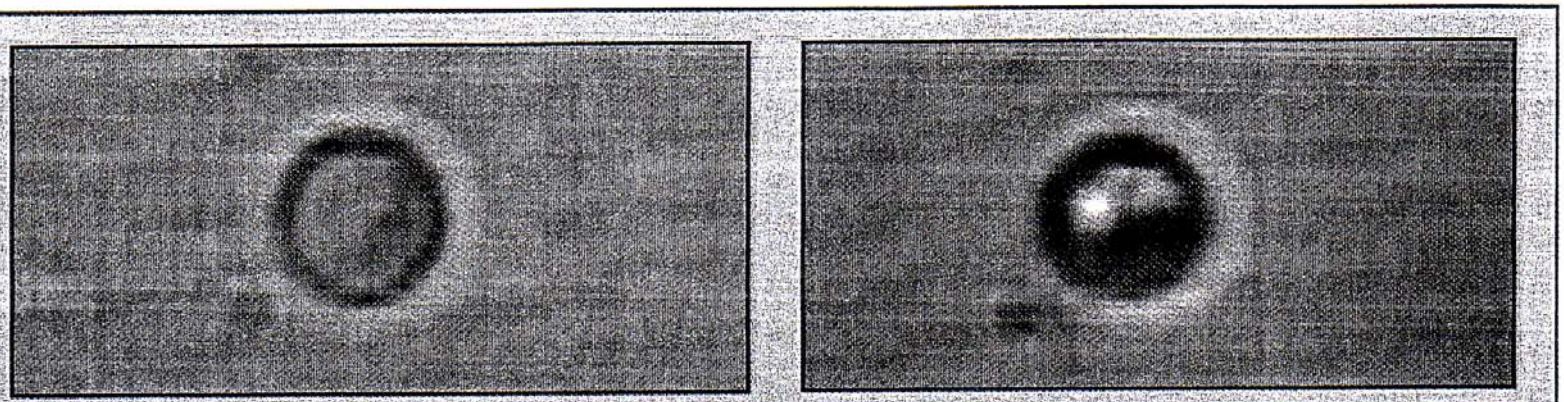

Using a combination of Raman microspectroscopy and optical trapping, researchers have monitored single cells' responses to hyperosmotic stress in real time. Shown here are images of an optically trapped yeast cell under normal (left) and hyperosmotic (right) conditions.