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Getting The Most Out Of Light

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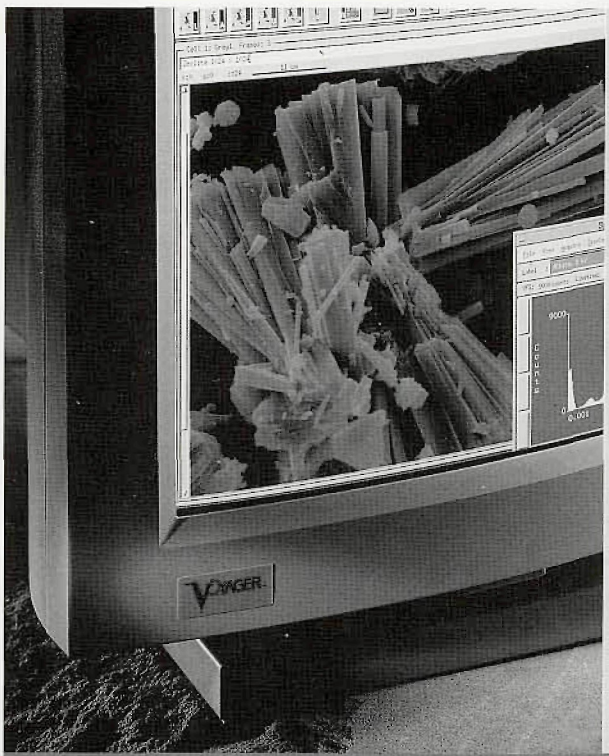
Advances in the development of light microscopy have been very impressive over the last decade. However, even with modern confocal microscopes, may of us yearn for even better resolution, both spatially and temporally. The trick would be to maximize the information from each meaningful photon that emanated from your specimen. This is essentially what has recently been accomplished by Walter Carrington, Kevin Fogarty, and Fredric Fay from the University of Massachusetts Medical School (Worcester), Ronald Lynch from the University of Arizona (Tucson), Edwin Moore from the University of British Columbia (Vancouver), and Gerrit Isenberg from the Julius-Bernstein-Institut für Physiologie (Halle, Germany)[2].

Using conventional methods, one encounters a trade-off when trying to image fluorescent molecules that have been introduced into a cell: light sufficient to make an image either damages the cell directly, or bleaches out the fluorescence. At low light levels, the signal to noise ratio becomes unfavorable, plus light from parts of the cell that are out of focus obscures the image. The solution to the problem was largely a mathematical one. Carrington *et al.* developed an algorithmic framework to produce images of high resolution from fewer image planes, thereby optimizing resolution with minimal light. Whereas many microscope/camera systems make a one-to-one correlation between a voxel (a pixel with three dimensions) in a cell and a voxel in the acquired data, they rightly pointed out that a cell is a continuous object, rather than an accumulation of voxels. While the cell of interest was modeled as a continuous object, the acquired data was considered as a discrete-data model, meaning that it consists of a finite amount of data. This allowed them to construct an algorithm with flexible data sampling requirements. The cell did not need to be divided by rigid grids or sections. Each acquired pixel represents light from a considerable volume of the cell, and the sensitivity of the pixel to a point source depends on the location of the source. They then use mathematical techniques to reverse this modelling process and calculate an accurate estimate of the dye density in the cell of interest. Carrington *et al.* dealt with edge effects mathematically. An additional advantage to their approach was that they could restore the data on a finer grid than the sampling of the data. With camera pixels of 100 nm, they restored the data to a grid of 25 nm. This allowed the resolution of two point sources 100 nm apart in the same plane. Resolution of 400 nm could be obtained in the vertical axis; to improve on this would require more light, thus getting back to the disadvantages they wanted to avoid in the first place. The bottom line is that Carrington *et al.* were able to obtain images better than the theoretical limit of resolution that can be obtained with light. This phenomenon is termed superresolution. Furthermore, they did it with minimal light, avoiding the damage that stronger light causes with fluorescent molecules.

Using images from a conventional wide-field microscope restored with their algorithmic framework, Carrington *et al.* examined some biologic specimens. They resolved individual microtubules only 112 nm apart, whereas an ideal confocal microscope with the same optics would require a separation of 420 nm. The light from each stained microtubule was restored to 84 nm, only slightly larger than the diameter of a microtubule covered with primary and secondary antibodies. They also imaged a fixed smooth muscle cell in which two different types of receptors were tagged. The resulting observations suggested specific interactions between calcium ions and the receptors. Finally, they demonstrated that the algorithm can work to image living cells by visualizing the movement of hexokinase in a cultured smooth muscle cell. The hexokinase was tagged with a fluorescent molecule, microinjected into the cell, and could be clearly visualized to be localized within mitochondria. Predictably, the hexokinase dissociated from the mitochondria when the cell was poisoned and it could be displaced by 2-deoxyglucose.

Carrington *et al.* pointed out that there are limitations in their method. The computational time does not allow for viewing restored images in real time, but this problem may be solved by faster processing. The wide-field optics that they used limited the specimen thickness to about 350 μm, but using their image



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restoration algorithm with confocal optics or two-photon excitation would be expected to overcome this limitation. It will be interesting to see this advance in image reconstruction being put to use, advancing our knowledge of what happens inside cells. ■

1 The author gratefully acknowledges Walter A. Carrington for reviewing this article.

2 Carrington, W.A., R.M. Lynch, E.D.W. Moore, G. Isenberg, K.E. Fogarty, and F.S. Fay, Superresolution three-dimensional images of fluorescence in cells with minimal light exposure, *Science* 268:1483-1487, 1995

My Microscope, Snoopy

I have a friend able to see
Objects much too small for me
And through his eyes, he lets me peek
At tiny wonders which I seek
To see as if I could go
Into that tiny world below

He is my own light microscope
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And spend the pleasant hours of time
To see the wonders I have seen
Of pond and flower and insect wing

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