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Seeing more with less

A noise-reduction algorithm decreases the amount of light needed for live-cell fluorescence microscopy by orders of magnitude.

Researchers who want to watch cells over time face an inherent conflict: the light necessary to see their cells interferes with the behavior they wish to observe. But a new technique can reduce the amount of light necessary in fluorescence microscopy by several orders of magnitude (Carlton *et al.*, 2010). John Sedat of the University of California, San Francisco and colleagues combined a denoising algorithm with a fast image-acquisition program to observe entire cell divisions without the photoxicity or bleaching that typically limit such experiments.

Sedat had previously noticed that yeast cells stopped dividing after being exposed to light during imaging experiments and decided to analyze this effect. If the amount of light was reduced tenfold, the cells paused in the midst of the cell cycle for a few hours, but eventually moved on. At one-hundredth the light typically used in a fluorescent microscopy experiment, the cells divided normally. That level of excitation light, however, produced extremely noisy images.

Separately, Jerome Boulanger at Institute Curie and Charles Kervrann, now at the University of Rennes, had developed a method to denoise images in fluorescence microscopy (Boulanger *et al.*, 2010). Their strategy was to focus on patches of consecutive images, use statistics to analyze noise and then average appropriate patches together to reduce it. In effect, they used redundancy across images to eliminate noise.

Sedat, together with the scientists in France and other colleagues, found that these algorithms could be used to study the three-dimensional structures of living, dividing cells at light levels one-hundredth to one-ten-thousandth of that normally used in fluorescence microscopy, levels at which yeast cells are unperturbed. The researchers found that this technique could be used not only to detect green and red fluorescent proteins at extremely low levels, but also to glimpse overall cell structure, such as nuclei and cell boundaries.

These low levels of light are good for the fluorescent proteins as well as for the cells. Usually, such proteins become less bright
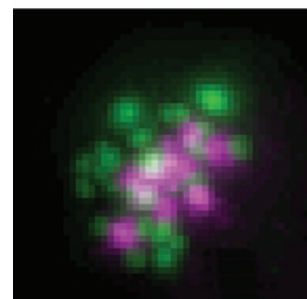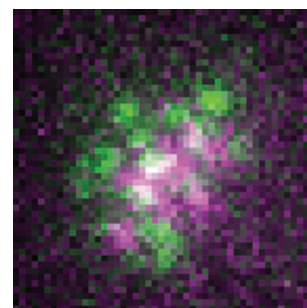
Imaging yeast cells at low light levels. Raw (top) and denoised (bottom) images of yeast cells.

as they are imaged over time, but Sedat found that very low light levels ablate photobleaching.

Many long-term live-cell imaging experiments using fluorescence limit images to around one every 10 minutes to prevent phototoxicity, so these algorithms could allow images to be taken more frequently or over a longer period of time. "You can do this forever," says Sedat, as there is no fading and the biology is the same as in control cells kept under normal light conditions.

For Sedat's experiments, he incorporated the algorithms into data collected with an 'optical microscopy experimental' high-resolution instrument he helped design, but in principle these algorithms should help any fluorescence microscope attenuate light, he says. The software can be installed on Mac, Windows and Linux platforms, and Kervrann is currently making it freely available through the University of Rennes.
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RESEARCH PAPERS
Boulanger, J. *et al.* Patch-based nonlocal functional for denoising fluorescence microscopy image sequences. *IEEE T. Med. Imaging* **29**, 442–454 (2010).
Carlton, P.M. *et al.* Fast live simultaneous multiwavelength four-dimensional optical microscopy. *Proc. Natl. Acad. Sci. USA* advance online publication (12 August 2010).