Text by Mitch Leslie
mitchleslie@comcast.net

Two microscopes are better than one

Researchers reveal way to sharpen vision of double-microscope technique.

The cellular nuances revealed by electron microscopy (EM) and electron tomography (ET) would have thrilled microscope pioneer Anton van Leeuwenhoek. Today, researchers can see even more details by pairing up these techniques with fluorescence microscopy. Kukulski et al. (1) describe a new procedure that further boosts the sensitivity and precision of this combination of imaging techniques.

Certain structures and rare or ephemeral events are often difficult to discern with EM and ET. Even if researchers do manage to spot, say, an endocytic vesicle at the very moment it separates from the cell membrane, they need to repeat the feat again and again to collect enough data for statistical analysis. That's why scientists are working to mesh fluorescence microscopy with EM and ET. Although fluorescence microscopy can't match the resolution obtained with EM, it can be an excellent tool for tracking cellular events and differentiating similar structures. Fluorescence microscopy can help researchers narrow down where to look when using EM. "The cell is a big place by EM but a small place for fluorescence microscopy," says senior author John Briggs.

In the last few years, researchers have developed several procedures to integrate light and electron microscopy. One technique involves observing the sample first with fluorescence microscopy, then immediately freezing and fixing it for EM (2). However, this method cannot image rapidly changing processes, nor can it be used to accurately localize structures within cells. An alternative method entails preparing the specimen first, then using both types of imaging. With this approach, one group has pinpointed individual labeled cells in zebrafish embryos (3), and another team was able to take a close look at intact human mitochondria (4). But the method's precision remains around 0.5 microns, and researchers haven't been able to distinguish anything smaller than an organelle.

Focal Point

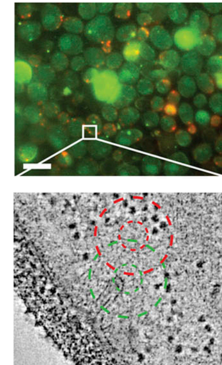

PHOTO COURTESY OF SONJA WELSCH

John Briggs (left), Wanda Kukulski (center), Marko Kaksonen (right), and colleagues (not shown) discovered how to increase the combined sensitivity of electron tomography and fluorescence microscopy. By using fluorescence microscopy to locate a protein that briefly adheres to endocytic sites (top right), the researchers pinpointed an endocytic vesicle (bottom right) as it pinches off from the cell membrane.

Kukulski et al. added a technical twist to increase the resolution of images obtained by correlating light and electron microscopy. They started with the standard steps of freezing the sample, encasing it in resin, and slicing off thin layers that they placed on an EM grid. Before making any observations, the researchers sprinkled the sample with tiny plastic spheres visible with both kinds of microscopy. The particles served as landmarks and enabled the rearchers to precisely match up images from fluorescence microscopy and EM or ET. Kukulski et al. determined that they could often narrow the location of an object to within 100 nm.

> "[We can] do EM on a bunch of problems that weren't previously practical."

The team then put the technique through its paces with three practical tests. The researchers first showed that they could pick out scarce HIV particles lurking among the filopodia protruding from the surface of a cell. Although the virus couldn't infect the dog kidney cells the team studied, the results suggest that the procedure could allow researchers to zero in on HIV particles as they enter human cells.

Another question Kukulski et al. addressed is whether growing microtubules flare at their tips like a pair of bellbottom jeans or adopt a sheet-like shape. Previous studies have supported both possibilities. By tagging microtubules with GFP-labeled mal3p, a protein that adheres to the tips, the team found that most of the tubules showed the flare. Kukulski et al. were also able to capture endocytic vesicles at the moment they break away from the plasma membrane. The protein Rvs167, which latches onto endocytic sites for only about 10 seconds, allowed the researchers to home in on vesicles that were about to separate. They discovered that a vesicle pinches off about halfway through Rvs167's 10-second attachment period.

The new procedure "allows us to do EM on a bunch of problems that weren't previously practical," says Briggs. The next step, he adds, is to determine whether other combination techniques are feasible. For example, researchers are working to merge super-resolution microscopy with EM and to integrate cryo-EM with fluorescence microscopy.

1. Kukulski, W., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201009037.
2. Verkade, P. 2008. *J. Microsc.* 230:317–328.
3. Nixon, S.J., et al. 2009. *Traffic.* 10:131–136.
4. van Driel, L.F., et al. 2009. *Eur. J. Cell Biol.* 88:669–684.

Downloaded from http://rupress.org/jcb/article-pdf/192/1/3/1259305/jcb_1921if.pdf by guest on 17 April 2021