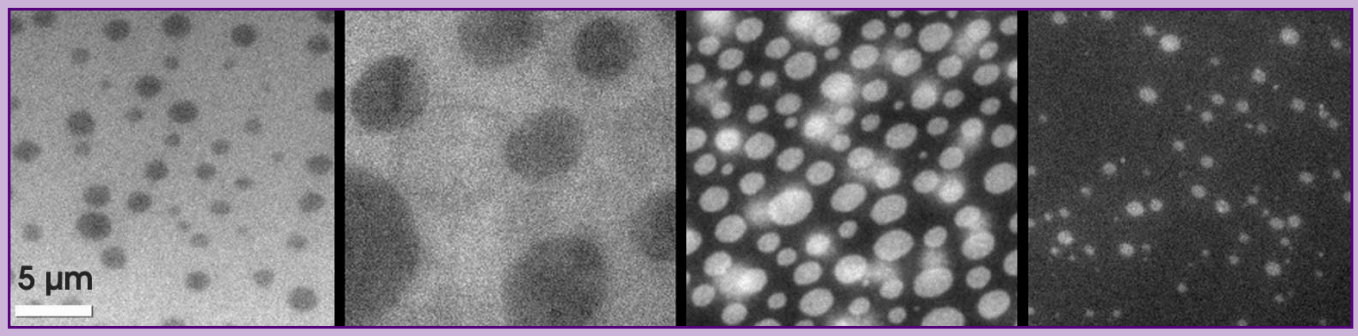
Red enables cell membrane study

In biological microscopy, simply being able to view a sample is half the battle. It is also crucial to capture and archive images for future reference. CCD cameras seem to be the technology of choice for this task, but not all of these devices are the same. Various microscopy applications demand cameras with appropriate technological features, such as adequate color sensitivity, speed, resolution or even physical specifications.



Researchers at the University of Virginia used a digital CCD camera to capture these images of an increasingly ordered lipid bilayer membrane (left to right) as signified by changes in the ratios of light to dark areas.

Researchers at the University of Virginia in Charlottesville recently attempted to find a CCD camera to facilitate their study of the varying states of phospholipid bilayers that mimic cell membranes. They wanted to create and image in vitro membrane models tagged with the fluorescent dye rhodamine. To capture the images, they needed a camera that not only has a high sensitivity to rhodamine's red light, but also offers the standard advantages of a CCD device.

Cell membranes from different species and even different cell membranes from the same species vary in their cholesterol fractions. The phospholipids that compose these membranes tend to naturally aggregate in the more fluid state, and the greater the number of cholesterol molecules present, the more ordered the lipid bilayer. Understanding why may be paramount to understanding several membrane-based cellular processes, such as protein segregation within membranes.

The researchers are using fluorescence microscopy to create visual guides of these membrane states from artificial planar membranes formed by layering phospholipid molecules on a glass microscope slide. Approximately 0.5 percent of the phospholipids are labeled with rhodamine — enough to provide a detectable fluorescence signal, but not so much that the dye interferes with the fluidity of the membrane. Team member Jonathan M. Crane said that they use rhodamine because it is bright, doesn't photobleach easily and can be covalently linked to phospholipid molecules.

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Imaging the membranes

They alter the fluidity of the membranes by adjusting their cholesterol and phospholipid content and imaging them so they have future visual correlations of the membranes' states. Various distributions in the fluorescence correspond to varying concentrations of cholesterol in the membranes and, therefore, their relative fluidity. To do this, they use an inverted epifluorescence microscope manufactured by Carl Zeiss Inc. of Thornwood, N.Y., with a 63× objective for a total magnification of 630× with the eyepiece. The resultant fluorescence is detected by a SensiCam QE digital CCD camera from Cooke Corp. of Auburn Hills, Mich., and transformed into an image.

The camera uses an enhanced progressive-scan interline transfer chip manufactured by Sony Electronics Inc.

of Park Ridge, N.J. Previous versions of these chips have commonly been used in high-resolution biological imaging because they can image a wide variety of intensities with high signal-to-noise ratio. Although they tend to be more sensitive to shorter wavelengths, they also often sacrifice performance in the red region. The enhanced chip, however, has increased quantum efficiency because a higher amount of light is being directed to the active sensor area instead of to the transfer area. Furthermore, it features a thinner light isolation area and a special internal lens. All of these increase the ability to image red and near-infrared light.

"We use a lot of rhodamine red in our experiments," Crane said. "We chose this camera because it has a higher sensitivity at the longer red wavelengths."

So far, they have achieved images of enough quality to allow an easy distinction between the membrane states simply by assessing them with the naked eye. Crane said the ability to quickly and accurately obtain visual evidence of membrane condition should provide verification in future experiments. For instance, they plan to label various proteins with a different fluorescent tag, such as GFP, to see if the proteins preferentially associate with any of the rhodamine-labeled membrane. Alternatively, he said they might forgo lipid fluorescence and use fluorescent-tagged proteins exclusively, then use the images they are acquiring as a reference of what the various phases look like.

Another advantage of using a camera with an enhanced progressive-scan interline transfer chip is that, while offering enhanced efficiency in the red and near-IR, it retains its effectiveness in the green region for double-label experiments. "The camera has good signal-to-noise ratio for both red and green," Crane said. "This allows us to get good images of both tags on the same bilayer."

Image quality is not compromised by the camera's ability to image red fluorescence, and the rest of its features are at least comparable to those of others on the market. Crane said the higher-quality chip does not require the extreme cooling that older CCD cameras did to obtain similar noise characteristics. Like other cameras featuring this type of chip, the SensiCam needs to be cooled only to approximately −12 °C. It also uses 12-bit digitization to create images with 1376 × 1040-pixel resolution, and comes with software that facilitates camera control and image archiving. □

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