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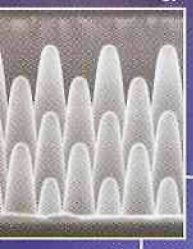

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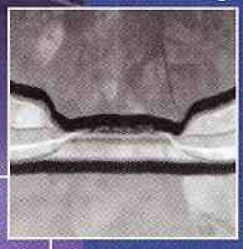

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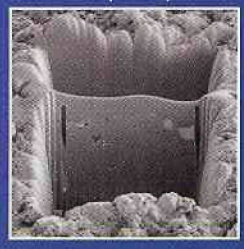

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Correlating Fluorescence Microscopy with Electron Microscopy

Stephen W. Carmichael and Jon Charlesworth,[1]

Mayo Clinic carmichael.stephen@mayo.edu

The use of fluorescent probes is becoming more and more common in cell biology. It would be useful if we were able to correlate a fluorescent structure with an electron microscopic image. The ability to definitively identify a fluorescent organelle would be very valuable. Recently, Ying Ren, Michael Kruhlak, and David Bazett-Jones devised a clever technique to correlate a structure visualized in the light microscope, even a fluorescing cell, with transmission electron microscopy (TEM).[2]

Two keys to the technique of Ren *et al.* are the use of grids (as used in the TEM) with widely spaced grid bars and the use of Quetol as the embedding resin. The grids allow for cells to be identified between the grid bars, and in turn the bars are used to keep the cell of interest in register throughout the processing for TEM. Quetol resin was used for embedding because of its low autofluorescence and sectioning properties. The resin also becomes soft and can be cut and easily peeled from glass coverslips when heated to 70°C.

For demonstrating the technique, Ren *et al.* grew neuroblastoma cells on coverslips. After fixing and permeabilizing the cells, they were exposed to monoclonal antibodies for promyelocytic leukemia (PML) protein. The specimen was then treated with a second antibody that was tagged with a fluorophore. The coverslips were mounted on slides using a glycerol-based mounting media and the fluorescing cells (or cell) of interest were identified at high magnification then imaged at low magnification with a light microscope (LM), with or without fluorescence. After an image record of the cells had been made, a 50 mesh copper grid was positioned over the cells of interest and attached to the coverslip with transparent tape. The slide was then returned to the LM and the exact location of the cells with respect to the 16 grid openings was noted. The coverslip was separated from the slide, placed in a 40-mm petri dish, washed with phosphate buffered saline to remove the mounting media and processed for TEM.

For the final embedding step the coverslip (cells up) was covered with a thin layer (0.5-2 mm) of Quetol 651 mix and polymerized. Once polymerized, a second grid was glued to the resin in register to the grid on the coverslip. The petri dish was placed on a hot plate at 70°C. After a few minutes the resin softened and was cut within the perimeter of the coverslip with a scalpel. The cut resin was then carefully peeled off the coverslip. A third grid was placed on the cell side of the Quetol block in perfect register with the second grid. The region of interest was marked with a sharp scalpel and the grid opening was also marked with a felt-tip pen. A large selected area was cut with a razor blade and glued onto a blank bullet and mounted onto an ultramicrotome for sectioning.

When the block surface was lightly wiped with 95% ethanol, the cells could be seen and compared with images captured earlier with the fluorescence microscope. The exact cell(s) was identified, the block further trimmed, and thin sectioned. The grid with the sections was placed on a glass slide, covered with a coverslip, and imaged with a fluorescence microscope at low (to determine the position of the cell of interest with respect to the grid bars) and high (to obtain the highest resolution of fluorescence labeling present) magnification. The grid was then transferred to the electron microscope where conventional TEM and electron spectroscopic imaging were employed. Correlative fluorescence and ultrastructural images from the region(s) of interest were compared and contrasted. With this method detail at the suborganelle level was obtained. We followed the methods of Ren *et al.* in our lab, and it worked on the first try.

It will be exciting to see the interesting uses for this advance in correlative microscopy! ■

[1] The authors gratefully acknowledge Dr. David Brazett-Jones for reviewing this article. Jon Charlesworth is the Coordinator of the Electron Microscopy Core Facility at Mayo Clinic.

[2] Ren, Y., M.J. Kruhlak, and D.P. Bazett-Jones, Same serial section correlative light and energy-filtered transmission electron microscopy, *J. Histochem. Cytochem.* 51:605-612, 2003.

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ABOUT THE COVER

Aberration Corrected, High Resolution Image
P.E. Batson, IBM Research

Annular Dark Field STEM image of a Au island on a carbon resolution test specimen using a 1 Angstrom probe. The data have been Fourier filtered to reduce noise. Single Au atoms, and monolayer "rafts" of Au atoms are visible nearby. The large Au island is about 50 Angstroms in diameter.