Correlating Fluorescence Microscopy with Electron Microscopy

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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).

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 microscopy. The grid was then transferred to the electron 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.

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cleobert@tms.org
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March 22-26, 2004, University of California, Santa Barbara
www.lifesci.ucsb.edu/mcdb/events/imaging_workshop/index.php
✓ Focus on Microscopy 2004
April 4-7, 2004, Philadelphia, PA
www.focusonmicroscopy.org
✓ Materials Research Society
April 12-16, 2004, San Francisco, CA
info@mrs.org
✓ SCANNING 2004
April 27-29, 2004, Washington, DC
www.scanning.org
✓ Asia-Pacific Congress on Electron Microscopy
June 7-11, 2004, Kanazawa, Japan
keih@kanazawa-med.ac.jp
✓ 12th Int'l. Congress of Histochemistry and Cytochemistry
July 24-29, 2004, LaJolla, CA
www.fishc.org/index2004.html/
✓ Cryo-HRSEM/STEM/TEM
July 28-30, 2004, Atlanta, GA
rapkari@emory.edu
✓ Microscopy and Microanalysis 2004
August 1-5, 2004, Savannah, GA
www.msa.microscopy.com
✓ EMC 2004 (former EUREM)
August 22-27, 2004, Antwerp, Belgium
www.emc2004.be
✓ Society for Neuroscience
October 23-28, 2004
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2005
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April 12-16, 2005, San Francisco, CA
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✓ Materials Research Society
November 29- December 3, 2004, Boston, MA
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✓ American Society for Cell Biology 2003
December 4-8, 2004, Washington, DC
www.ascb.org

2006
✓ Microscopy and Microanalysis 2006
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