ELECTRON TOMOGRAPHY

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The transmission electron microscope (TEM) was invented in the 1930's, and developments in specimen preparation in the 1950's led to its widespread use as a tool to study structure in biologic systems. Similar in principle to the light microscope, but utilizing a much shorter wavelength for better resolution, the TEM has the image-forming beam pass through the specimen. This results in a two-dimensional image which can be difficult to interpret because features from different depths of the three dimensional specimen are superimposed. Traditionally this was dealt with by cutting sections of plastic-embedded specimens so thin (in the 40 to 80 nanometer range) that they effectively had only two dimensions. To allow biologists to examine structures in three dimensions, serial sections are stacked and structures reconstructed. Even though computers have made reconstruction easier, the reality is that resolution in the depth dimension is limited by the section thickness. The technique of electron tomography is emerging as a way to overcome this limitation.

The development of electron tomography as an important method to examine the three-dimensional structure of organelles was impressively reviewed by Bruce McEwen and Michael Marko. As in the radiographic technique of computed axial tomography (the well known CAT scan), an intact specimen is examined from several angles and the accumulated data is computed as a three-dimensional structure. As currently used, plastic sections are cut thick enough (200 to 1000 nanometers) to include a significant portion of most cellular organelles. The section is tilted about an axis at about 1^{o} or 2^{o} increments over about $\pm 60^{o}$ to 70^{o}. At the extremes of this range the thickness that the beam penetrates is up to three times the thickness of already thick sections, so a high accelerating voltage is needed for optimal performance. To minimize certain problems from a limited tilt range (*i.e.,* less than $\pm 90^{o}$), the specimen can be rotated 90^{o} and tilted again. The physical aspects of electron tomography dictate that plastic sections up to about 2 microns in thickness can be visualized at a resolution of about 5 to 20 nanometers. This means that cell organelles are particularly well suited for examination by electron tomography.

McEwen and Marko give several examples of subcellular structures that have been examined using electron tomography. These include mitochondria, the Golgi apparatus, centrioles, membrane pores, muscle fibers, the nucleolar organizer region, and whole prokaryotic cells. As an example, it was controversial whether the folds of the inner mitochondrial membrane (cristae) enclosed a separate compartment within the mitochondria, or have unrestricted access to the intermembrane space and thereby to the cytosol. Using electron tomography, it was established that the space within the cristae communicate with the space between the inner and outer mitochondrial membranes through narrow (about 30 nanometers) tubular connections. This is a strong argument for what is called the "restricted access model" and has important consequences for the bioenergetics that occur in the mitochondrion. Another example is the Golgi apparatus where electron tomography revealed several previously unobserved features. Apparently, protein molecules do not have to go through the whole Golgi stack. Instead, vesicle transport can take place at any of the Golgi cisternae and travel through aligned fenestrations, or tubular extensions, or budding from the edges of any cistern.

The future of electron tomography looks bright. Improvements were predicted to be mainly in the area of specimen preparation, although advances in instrumentation and computing are being made. Rapid freezing and cryopreparation will more closely represent the native state and allow time-resolved studies, so improvements associated with these techniques are being developed. McEwen and Marko concluded that electron tomography has proven its worth as a method for three-dimensional reconstruction of ultrastructure. We can expect to better appreciate organelles with this technique. ■

1. The author gratefully acknowledges Dr. Bruce F. McEwen for reviewing this article.
2. McEwen, B.F., and M. Marko, The emergence of electron tomography as an important tool for investigating cellular utrastructure, J. Histochem. Cytochem. 49:563-563, 2001.

Index of Articles

NEW AND/OR INTERESTING IN MICROSCOPY

We note sadly the recent death of Ernest F. Fullam. Mr. Fullam was a pioneer in the applications of electron microscopy and electron optics. As early as 1940 he was involved in the purchase of an electron microscope to study printing inks and pigments. And, as one of the few electron microscopists in the country during World War II, he became involved in the Manhattan Project. In 1945 he collaborated in producing the first electron micrograph of a biological sample. When the war ended he moved to the General Electric R&D lab in Schenectady, NY.

Mr. Fullam founded Ernest F. Fullam, Inc., a scientific consulting company, in 1953 and was active in the business until his retirement in 1987. He was a charter member in EMSA (now MSA) and developed many instruments and sample preparation techniques now used by microscopists around the world. Mr. Fullam held memberships in a number of scientific societies and was a life member of the AAAS and Sigma Xi. His company is now managed by his son, Peter.

✖ Education Outreach Symposium at M&M 2002

This symposium will present contributions that emphasize creative, effective, and innovative educational approaches to microscopy, image acquisition and analysis, and the increased use of microscopy and images in a student curriculum. Pedagogical resources to be considered include: distance learning; online-tutorials, manuals, references and image databases plus a selection of shared utilities and discussion forums.

✖ American Society for Microbiology (ASM) MicrobeLibrary

Do you have:

- Stunning photographs of microorganisms?
- Time-lapse video photography of microbial processes?
- Innovative classroom activities?
- A laboratory that encourages students in their own learning?

If so, consider submitting materials to the ASMs MicroLibrary—a fully funded searchable database of more than 500 resources for microbiology education. Please contact Susan Musante, (202) 942-9282, smusante @asmusa.org for details.

✖ The Microbeam Analysis Society (MAS) has a new homepage. It is: http://www.microbeamanalysis.org

✖ M&M2001 Market Survey Available

A report recently released by Microscopy/Marketing & Education, Inc. highlights significant growth in key microscopy technologies such as confocal and digital imaging and the return of the quiescent transmission electron microscopy market. Contact Barbara Foster, MME President, at (413) 746-6931, or click on http://www.microscopymarket.com

M&M 2002—JUST FOR FUN MICROGRAPH CONTEST
YES, IT WILL BE HELD. DETAILS IN THE NEXT ISSUE OF THIS PUBLICATION.

News

For Readers And Advertisers Alike:

I am very pleased to announce that the Microscopy Society of America (MSA) is in the process of acquiring this publication. And that this will be my last issue as editor and publisher. I am equally pleased to advise that Ron Anderson, recently retired from the IBM Corporation, will be the new editor of Microscopy Today!

To respond to your possible questions:

- *U.S. readers will not have to join MSA to continue to receive this publication at no cost. Membership will likely be encouraged and is certainly recommended by me!*
- *Our current publication schedule and advertising rates will remain in effect this year.*
- *The objective of the publication, specifically to provide articles and other information of interest to the working microscopist will most certainly not be changed.*

Under Ron's leadership and the support of MSA, I fully expect a substantial improvement in both the quality and quanity of useful information in the publication. I also anticipate a major effort to increase content of interest in all microscopy techniques. To this end, I know that Ron will appreciate the help of all our readers. Ron may be contacted at:

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(Expect the address and phone numbers to change as Ron gets set up.)

I will be working with Ron for some time as needed to insure a smooth transition. See you in Quebec!

. . . Don Grimes

FRONT COVER IMAGE

Streptomyces — An Actinomyces Bacteria

Actinomyces are bacteria found in the soil. They are involved in the decomposition process. This particular species, Streptomyces, is responsible for the antibiotic, streptomycin.

Imaged on a LEO 440 SEM at 7 kV using the SEM wideband Multi-Detector Color Synthesizer (designed, built and patented by David Scharf). Then acquired digitally at 2,048 x 1,536 pixels directly into a Macintosh Power PC as a TIFF file, using Digital Micrograph software and Digiscan hardware. Approximately 16,000X.