EVANESCENT-WAVE MICROSCOPY: PEEKING JUST UNDER THE SURFACE OF THE CELL

Stephen W. Carmichael,[1] Mayo Clinic

There are many techniques available that enable us to know what is happening at the surface of a living cell. These techniques have allowed us to characterize many aspects of the process of exocytosis, the final cellular event in secretion. Patch clamping has given us quantitative measurements of the capacitance changes as the membrane of the secretory vesicle is added to the surface of the cell during exocytosis, and the change in the opposite direction as membrane is retrieved back into the cell during endocytosis. Tiny probes have measured electrochemical changes just outside the surface of the cell as reactive molecules are released into their surroundings. Differential interference contrast microscopy has given us high resolution pictures of the cell surface during exocytosis; real-time images are suggestive of bubbles breaking the surface of a boiling pot of water. However, exocytosis is known to be proceeded by an ordered series of events that occur below the surface of the cell. The secretory vesicles are products of the endoplasmic reticulum and Golgi apparatus. After they are formed, there is a maturation process, and then the vesicles apparently enter a "reserve" pool of mature vesicles. Unknown mechanisms take vesicles from the reserve pool and transfer them to the relatively small "readily-releasable" pool where they are primed for exocytosis. The readily-releasable pool can be quickly depleted, then the cell waits for vesicles to be moved up from the reserve pool.

These important last steps in secretion have been difficult to observe for a number of reasons. What we have needed is an optical tool that would allow us to peek just below the surface of a secretory cell. This much-needed tool appears to be evanescent-wave microscopy. As described by Martin Oheim, Dinah Loerke, Robert Chow, and Walter Stühmer, this clever utilization of optics promises to provide more information on exocytosis from neuroendocrine cells and neurons.[2]

The word evanescent means "rapidly fading," and it is the rapidly fading light at an interface between two media of higher and lower refractive indices. When light travels through a medium with a high refractive to an interface with a medium of lower refractive index, the light is refracted at the interface as the incident angle is moved from the perpendicular. When the incident angle is increased beyond the "critical angle," all of the light is reflected back into the medium of higher refractive index, a phenomenon called total internal reflection. This critical angle may be on the order of 60^{O} and 65^{O} (with 90^{O} being perpendicular to the surface). Evanescent waves are formed that penetrate only a fraction of the wavelength of light into the other medium. The optical properties are such that the intensity of the evanescent light at the surface can be 3 to 4 times the intensity of the incident light, but decays exponentially so that typically only a shallow volume (80-300 nm from the surface) is illuminated.

In experiments described by Oheim *et al.*, neuroendocrine cells from the adrenal medulla were grown on coverslips. The "footprint" where the cells were in contact with the glass was the interface surface. The volume just inside this surface was available for evanescent-wave microscopy. The chromaffin vesicles could be labeled with an acidophilic fluorescent dye (acridine orange) because the vesicle interior is at pH 5.5 and selectively takes up the dye. By modifying a standard upright microscope, Oheim *et al.*, could direct a laser beam to the glass/water interface, with the cells in the aqueous medium. With a hemicylindrical prism coupled to the glass (there is a "prismless" method, also, but it doesn't allow variations in the incident angle), a laser beam was directed at the specimen about 62^{O} from the perpendicular. They were able to determine that the evanescent wave penetrated about 260 nm into the cell.

Oheim *et al.*, were able to visualize distinct fluorescing points that appeared to be chromaffin vesicles approaching the cell surface at relatively rapid rates, then seemingly stopped in a "docked" position. With stimulation, the fluorescence disappeared in a puff at the cell surface, evidence of exocytosis. It is clear that evanescent-wave microscopy has opened a window into how secretion progresses just below the surface of the cell. This technique allows for vesicles to be visualized and tracked quantitatively in three dimensions. Different biophysical properties can be described for vesicles in various functional states just beneath the surface of the cell. We are now equipped to answer important questions about the terminal events in vesicle trafficking. This new method of microscopy will be the wave of the future!

1. The author gratefully acknowledges Dr. Martin Oheim for reviewing this article.

2. Oheim, M., D. Loeke, R.H. Chow, and W. Stühmer, Evanest-wave microscopy: A new tool to gain insight into the control of transmitter release, *Phil. Trans. R. Soc. Lond. B* 354-307-318, 1999. See also: Steyer, J.A., H. Horstmann, and W. Almers, Transport, docking and exocytosis of single secretory granules in live chromaffin cells, *Nature* 388:474-478, 1997. These two laboratories have led in the development of applying evanexcent-wave microscopy to studies of secretion.

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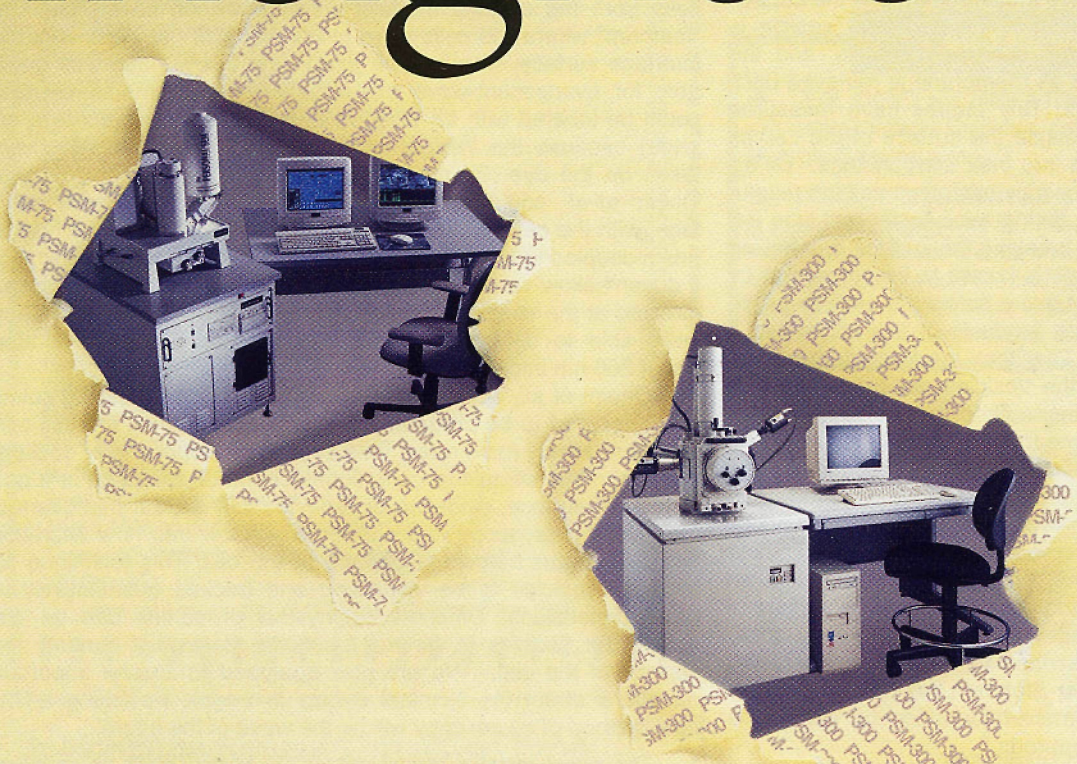

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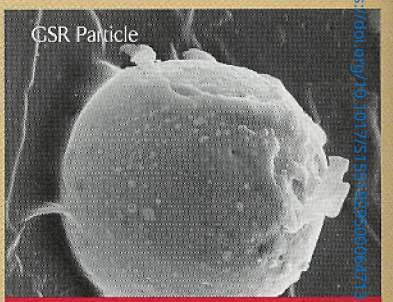

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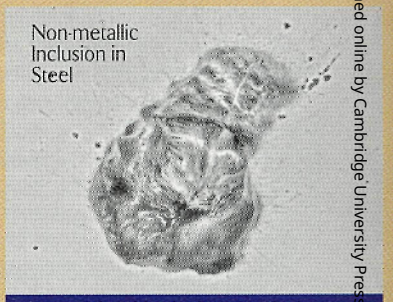

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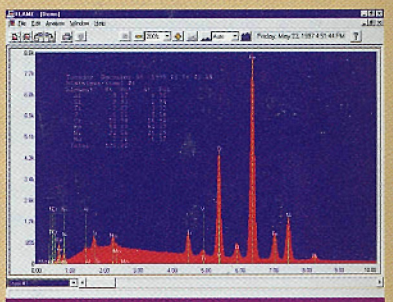

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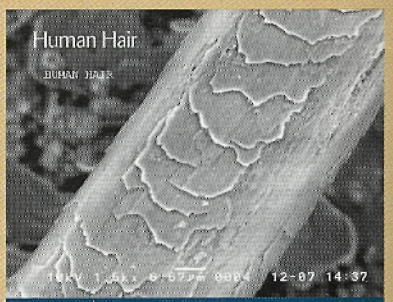

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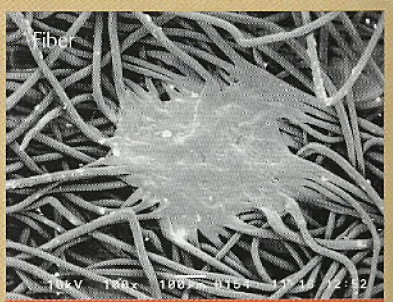

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MICROSCOPY & MICROANALYSIS '99

An Editorial Comment

This happened to be the nineteenth EMSA/MSA/MAS conference that I have attended—and it far outshined all others, in all categories!

There were a total of 2,901 participants, 1,866 being attendees and 1,035 being exhibitors. There were over 710 scientific papers presented. The attendance was some 25% over the previous year, and may well have been the largest in conference history.

Exhibitors advised that they were very pleased with the attendance—both in quantity and quality (meaning that many were purchasing!).

The Local Arrangements Committee did a truly outstanding job in organizing the affair. The highlight (at least for me) was the Sunday Evening Reception—held at the Portland Zoo. There were three separate serving areas, each with a different food type! No long waiting in lines to be served cafeteria style food! There were plenty of tables and chairs and the room to mill around and renew old acquaintances.

And at the conference even the coffee served was very, very good.

Credit for an outstanding meeting should also go to M&M Meeting Management—Annamarie Dowling and her associates.

---Don Grimes, Editor

2000 MICROSCOPY SOCIETY OF AMERICA AWARDS

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All MSA Members are encouraged to nominate candidates for these Awards—to recognize our eminent Scientific and Society leaders. Deadline for the receipt of completed nominations is December 30, 1999.

Details available from the MSA Business Office:

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NEW AND/OR INTERESTING IN MICROSCOPY

➔ Dr. Robert Wick has joined NORAN Instruments as Director of Sales and will be responsible of their worldwide sales effort. Dr. Wick has over 15 years of experience in marketing and sales and has held senior positions at Carl Zeiss and Hamamatsu.

➔ **THE EIGHTEENTH ANNUAL SYMPOSIUM OF ADVANCES IN MICROSCOPY** will be held on October 29—30, 1999 at the Coastline Convention Center, Wilmington, NC.

The Symposium, sponsored by the North Carolina Society for Microscopy and Microbeam Analysis, has been planned with the theme of *"Nanoscopy" for the New Century?"* Continuing with the tradition of the symposium, the guest lecturers are composed of both nationally and internationally distinguished scientists. The meeting has several purposes, not the least of which is to draw attention of the scientific community to emerging developments in the practical and basic research aspects of exciting new fields, and to bring people together from diverse disciplines to discuss how innovative techniques will be relevant to the future direction of microscopy and microprobe analysis. Special emphasis will be placed on how recent advances in nanoscience and nanoengineering have resulted in new knowledge that has benefited microscopy in general and are having a significant impact in the biological and physical sciences.

Three workshops will be offered: *(a) Cryo-preparation Techniques, (b) Atomic Force Microscopy and (c) Digital Imaging Methods.*

For further information, contact Ms. Betty Gooch: (919) 286-0411, email: b.gooch@cellbio.duke.edu

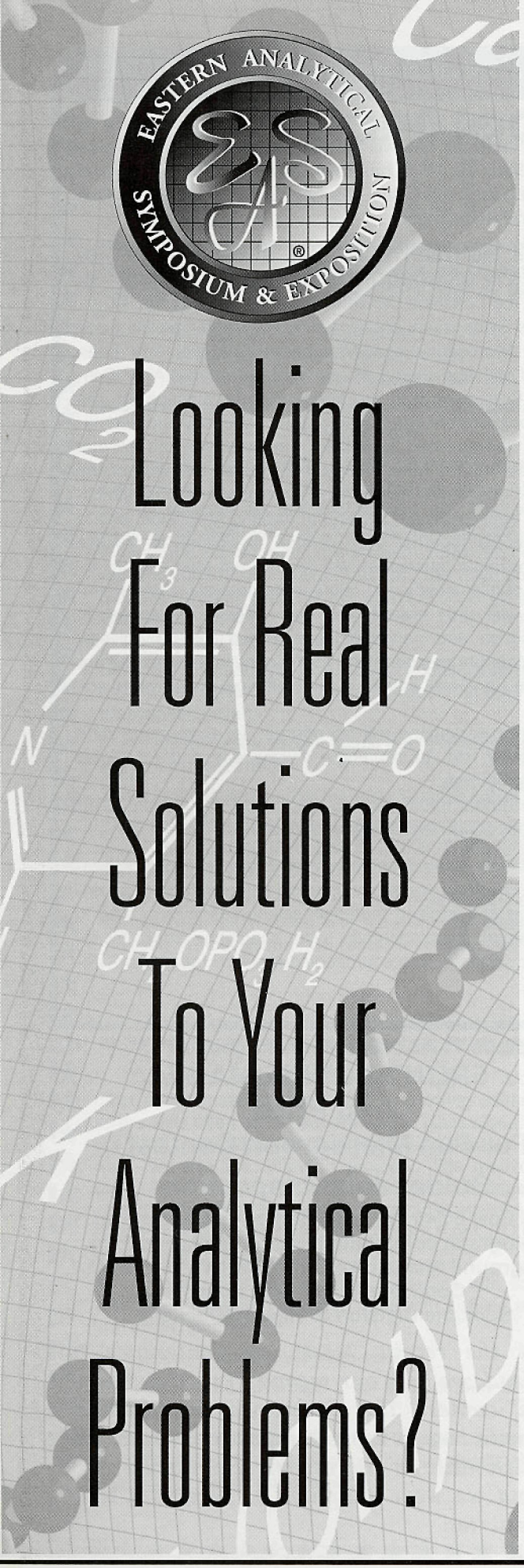
➔ A new Nanoindentation Web Site is intended to provide an overview of nanoindentation along with practical engineering and exciting materials science examples. Visit to learn about nanoindentatin with scanning probe microscopes: spm.aif.ncsu.edu/nanoindentation (no "www" required).

FRONT COVER IMAGE
First Prize
For Fun Micrograph Contest
Solder Joint Inspection

**Cold flow 60/40 Tin Lead Solder on Copper Pad.
Photograph combination using PaintShop Pro.
Background image taken with AMRAY 1600 SEM.
Contributed by: Jerry Long,
Spokane Division of Hewlett-Packard**

At the recent M&M '99 Conference in Portland, we held our second "For Fun Micrograph" contest. The contest was based on entries with two or more composite images, one of which must be microscopical in nature.

And we were delighted to receive a total of 27 entries!



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COMING EVENTS

✓ September 21/24 '99: **Using Ultramicrotomy in Materials Science** (Ventana/RMC) Tucson, AZ, Steve Miller: (520)903-9366

✓ October 2 '99: **5th California Microscopy Colloquium.** (CSU & NCMS) San Francisco, CA. Http://online.sfsu.edu/~camicro/

✓ October 6/14 '99: **Optical Microscopy & Imaging in the Biomedical Sciences** (Marine Biological Lab) Wood Hole, MA. Carol Hamel: (508)289-7401, eMail: admissions@mbl.edu

✓ October 20/22 '99: **Asbestos Analysis by Transmission Electron Microscopy** (McCrone Research Institute), Chicago, IL Nancy Daerr: (312)842-7100, eMail: ndaerr@mcri.org

✓ October 22 '99: **5th California Microscopy Colloquium** (CA State Universities & Northern CA Microscopy Society). San Farncisco State University. Http://online.sfsu.edu/~camicro/

✓ October 25/29 '99: **American Vacuum Society's 46th International Symposium** Seattle WA, Della Miller: (408)246-3600, fax: (408)246-7700

✓ Nov 14/19 '99: **1999 Eastern Analytical Symposium.** Somerset, NJ.(302) 738-6218, eMail: easinfo@aol.com, http://www.eas.org/

✓ Nov 15/18 '99: **EuroFE '99.** Toledo, Spain. www.cmp-cientifica.com/EuroFE/schedule.htm

✓ March 12/16 '00: **High Resolution Electron Microscopy in Materials Science Symposium** (TMS Physical Metallurgy Committee) Nashville, TN, Diane Albert, Los Almos Natl Lab: (505)665-2266, Fax: (505)667-5268

✓ April 3/4 '00: **Microscopy of Composite Materials V** (RMS & Oxford Centre for Advanced Materials and Composites) St. John's College, Oxford, U.K.+44-1865-248768, Fax: +44-1865-791237

✓ April 11/13 '00: **MICRO 2000** (Royal Microscopical Society) London www.rms.org.uk

✓ May 9/12 '00: **SCANNING 2000** San Antonio, TX., Mary K. Sullivan: (201) 818-1010, Fax: (201)818-0086, scanning@fams.org

✓ May 22/June 2 '00: **PASEM 2000** (Univ. of Maryland) College Park, Md., Tim Maugel: (301)405-6898, tm11@umail.umd.edu

LEHIGH MICROSCOPY SCHOOLS

✓ June 12/16 '00: SEM and X-ray Microanalysis
✓ June 11 '00: Introduction to SEM and EDS
✓ June 19/23 '00: Advanced Scanning Electron Microscopy
✓ June 19/23 '00: Quantitative X-ray Microanalysis
✓ June 19/22 '00: Analytical Electron Microscopy
✓ June 20/23 '00: Atomic Force Microscopy
✓ June 19/23 '00: Microdiffraction
✓ June 21/23 '00: Cryo SEM

For further information, contact Ms. Sharon Coe at Tel.: (610)758-5133 or by eMail at sharon.coe@lehigh.edu

✓ June 26/30 '00: **7th Asia-Pacific Conference on Electron Microscopy** Singapore. eMail: micngml@nus.edu.sg or medlab2@nus.edu.sg http://www.med.nus.edu.sg/micsoc/7apem

✓ July 8/13 '00: **2nd Meeting of the International Union of Microbeam Analysis Societies.** Kailua-Kona, Hawaii. www.microanalysis.org/iumas2000/

✓ July 9/14 '00. **12th European Congress on Electron Microscopy.** Bruno, Czech Republic. http://www.eurem2000.isibrno.cz/regform.html

✓ August 13/17 '00: **Microscopy & Microanalysis '00:** (MSA) Philadelphia, PA. Maryanne Rebedeau: (708)361-6166, mas@tradeshownet.com

✓ September 3/8 '00: **11th International Congress of Histochemistry** York, U. K., www.med.ic.ac.uk/external/ichc_2000

✓ November 19/23 '00: **First International Conference on Advanced Materials Processing** Rotorua, New Zealand. Prof. Nigel Sammes: n.sammes@walkato.ac.nz