USING ANTIBODIES TO MAKE IMAGES

Stephen W. Carmichael,[1] Mayo Clinic

The atomic force microscope (AFM), the workhorse of scanning probe microscopes, has become even more versatile. Anneliese Raab, Wenhai Han, Dirk Badt, Sandra Smith-Gill, Stuart Lindsay, Hansgeorg Schindler, and Peter Hinterdorfer have demonstrated that the AFM, in the dynamic force mode, can use antibodies as a probe.[2] Dynamic force microscopy uses a magnetized tip that is oscillated in an alternating magnetic field as the tip scans the surface. This provides a very gentile interaction that can be recorded as a high resolution topographic image. Raab *et al.*, showed that more information can be obtained from the specimen.

Their "trick" was to attach a portion of an antibody molecule to an AFM tip. The antibody was tethered to the tip with a polyethylene glycol derivative. The specimen was some molecules of lysozyme adsorbed to a mica substrate. The tip was oscillated at an amplitude that was less than the length of the polymer tether (the length of the tether was 6 nanometers and the amplitude used as 2.5 nanometers) and at a rate that provided adequate time for the antibody to recognize and bind with its antigen (calculated to be 1 millisecond so a "dwell time" of about 12 milliseconds was used). As the oscillating tip with the antibody attached approached a lysozyme molecule, the tethered antibody attached to the lysozyme molecule before the tip arrived. The antibody-antigen attraction acted as a non-linear spring, decreasing the amplitude of the tip. This reduction in amplitude was maximum when the tip was directly over the antigen molecule. As the tip moved past the antigen, the effect on the amplitude decreases until the antibody dissociates from the antigen and the attractive force went to zero. These force changes could be mapped out with a spatial resolution of about three nanometers. Control experiments confirmed that the interactions were specific for the antigen and antibody used. It appeared as though this method could only recognize molecules with their antigenic sites exposed, and molecules "facing down" were not imaged.

As an analogy, let's imagine a blind man exploring a surface by tapping a cane. As the surface is sampled, he builds an image of the surface in his brain. For our purpose, we will say the surface is patches of carpet scattered on a hard floor. Now let's attach a Velcro-covered ball on a short tether to the tip of his cane. The Velcro sticks to pieces of carpet that are facing up, but does not stick to pieces of carpet facing down, or to the hard floor. Only with this modified cane can the blind man picture where the pieces of carpet facing up are located.

Not only is spatial resolution on the nanometer scale possible, but some temporal resolution was also demonstrated. As a tip-link-antibody was scanning the specimen, excess antigen was added to the solution, binding to the antibody, thereby neutralizing the probe and converting to the tapping mode alone. This event was resolved within a few seconds. Raab *et al.*, point out that virtually any functional ligand can be tethered to an AFM tip. Not only could one image antigens distributed on a biologic specimen, but the location of receptors or other molecules that have a specific interaction with another molecule could be imaged. And not only with a spatial resolution of a few nanometers, but temporal changes on the scale of seconds to hours could be detected as well! ■

1. The author gratefully acknowledges Dr. Peter Hinterdorfer for reviewing this article.

2. Raab, A., W. Han, D. Badt, S.J. Smith-Gill, S.W. Lindsay, H. Schindler, and P. Hinterdorfer, Antibody recognition imaging by force microscopy, *Nature Biotechnology* 17:902-905,

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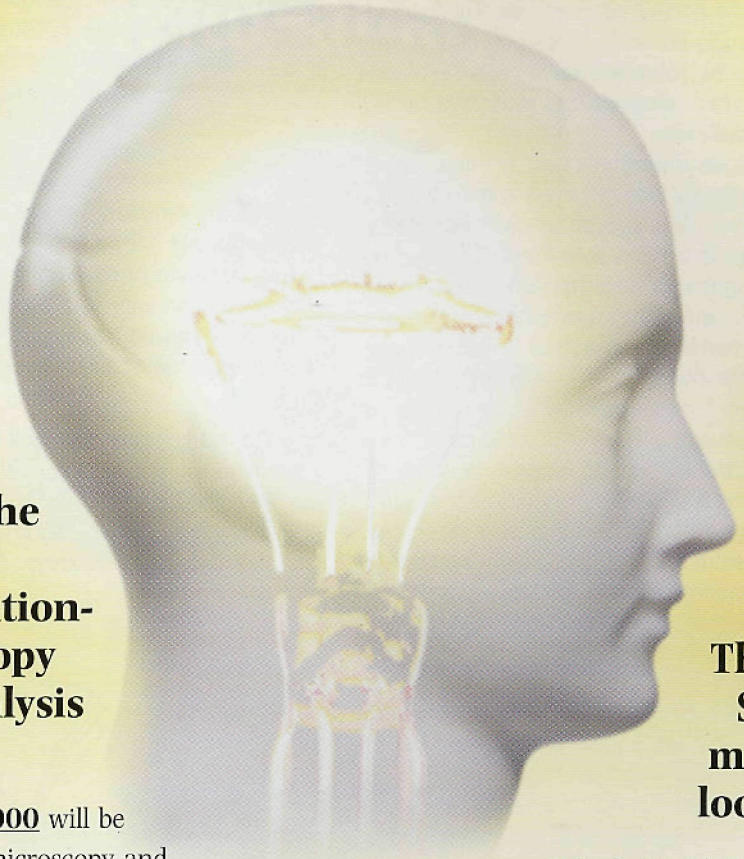
Smart



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✱ **HORROR STORIES, ANYONE?**

Recently, on the Microscopy Listserver, participants presented a series of short "stories" relating to events in their microscopy labs which bordered on disaster. Many events were caused by equipment/material misuse by unqualified operators (and some, interestingly, by qualified operators).

I am accumulating these "horror" stories and will publish an edited summary in the next issue of this publication. I also expect to publish the summary on our web site where they may be downloaded by any interested party.

Should you not be active on this listserver, and have experienced or know of such events, kindly advise me and I will include your experience in the summary—by email (microtoday@mindspring.com) or fax (608-836-1960).

BTW—the microscopy listserver is actively used by several thousand microscopists around the world to present questions, answers or otherwise cover topics of interests to microscopists. Typically there are around a dozen messages each day.

If you are not already a reader, you may subscribe by sending a message to:

ListServer@MSA.microscopy.com
Then, as the subject type: *subscribe*
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Then, after this last subscribe, space and type your email address

And to unscribe, simply follow the above but substitute "unsubscribe" for "subscribe" in the two places.

✱ The National Association of Graduate-Professional Students (NAGPS) has recently received a grant from the Alfred P. Sloan Foundation to conduct a survey of doctoral students on their graduate school experiences. The survey will be conducted on the Web <http://survey.nagps.org/> by current and recent doctoral students from January-May 2000, and the results made publicly available on the Web on a departmental specific basis in September.

The effort is a follow-up to a more limited survey which occurred this past spring, which was aimed at science and engineering doctoral students. The aggregate results from that survey are available at <http://www.phds.org/survey/results/>.

The survey we are conducting is unique in at least two important ways: it collects information on a department-specific basis, not only averaged over entire institutions or disciplines (though discipline-level results will also be available). So it will be possible to look at, for instance, responses from individual biology programs, or to rank history departments based on faculty mentoring.

The survey is based on best practices and covers issues in a number of areas, including information for prospective students, curriculum breadth and flexibility, career guidance and placement services, faculty mentoring, time to degree, department climate, teaching, professionalism and overall satisfaction.

For this survey to be useful, it is vital that we reach as many current and recent doctoral students (anyone who has been enrolled for at least one semester in the past five years) as possible.

✱ **A reminder** that Kona, Hawaii is the site of the International Union of Microbeam Analysis Societies (IUMAS 2000) meeting this summer. There will be SPM and nanomechanics sessions. The scheduled presentations for the meeting may be found on the following website:

http://www.microanalysis.org/iumas2000/

✱ **FLUORESCENCE 2000**—Advanced Courses of Fluorescence Microscopy and Confocal Microscopy will be held on 16-20 October 2000 in palazzo Feltrinelli Gargnano (BS), Lake of Garda, Italy, University of Milan-Italy.

Courses will be held with an eminently practical approach concerning the use of all light microscopy, including confocal microscopy. For details, contact: Dr. Annalisa Imberti, Institute of Human Anatomy, University of Milan, V8ia Mangiagalli 31, 20133 Milan (Italy) or vist the web site: http://users.unimi.it/~fl2000

✱ ***Workshop on Quantitative Image Analysis*** *will be held* on May 11-17 and May 15-17 2000 at North Carolina State University and on June 15-17 2000 at the Danish Technological Institute, Taastrup, Denmark.

This highly regarded hands-on course taught by Dr. John Russ and other expert faculty has been presented annually for more than 15 years. It deals with all phases of quantitative and computer-assisted imaging from acquisition and processing through measurement and stereo logical interpretation. Attendees receive The Image Processing Handbook plus a CD-ROM containing images, algorithms (Photoshop-compatible for Mac and Windows) and an extensive on-line tutorial. The course is appropriate for professional scientists, technicians and administrators using these techniques for research. Attendees typically come from material science, geology, biological and medical sciences, pharmaceuticals, food science, industrial quality control, remote sensing, and other disciplines.

For detailed information and registration contact Department of Continuing and Professional Education, North Carolina State University at (919)515-2261 or visit the web site at: www2.ncsu.edu/cpe/

FRONT COVER IMAGE

Microcircuit metallization interconnects after removal of passivation layer. Imaged using AMRAY 1910 FESEM at 2,500X, 4 kV, tilt angle of 45 degrees, 50 µm final aperture, spot size –5, WD = 6 mm. Original b/w SE image was pseudo colored using Soft-Imaging's analySIS imaging processing software, Image Content's Lucis image analysis software and the Dindima Group's Spectrum image processing software.

Image© compliments of Dr. Gary Gaugler, Optical Reflections.

The Exam

The following story recently was presented on the web. The original author, unfortunately, is unknown

Sir Ernest Rutherford, President of the Royal Academy, and recipient of the Nobel Prize in Physics, is said to have told the following story:

Some time ago I received a call from a colleague. He was giving a student a zero for his answer to a physics question, and the student claimed a perfect score. The instructor and the student agreed to an impartial arbiter, and I was selected.

I read the examination question: "Show how it is possible to determine the height of a tall building with the aid of a barometer." The student had answered: "Take the barometer to the top of the building, attach a long rope to it, lower it to the street, and then bring it up, measuring the length of the rope. The length of the rope is the height of the building."

The student really had a strong case for full credit since he had really answered the question completely and correctly! On the other hand, if full credit were given, it could well contribute to a high grade in his physics course and certify competence in physics, but the answer did not confirm this.

I suggested that the student have another try. I gave the student six minutes to answer the question with the warning that the answer should show some knowledge of physics. At the end of five minutes, he hadn't written anything.

I asked if he wished to give up, but he said he had many answers to the problem; he was just thinking of the best one. I excused myself for interrupting him and asked him to go on.

In the next minute, he dashed off his answer, which read: "Take the barometer to the top of the building and lean over the edge of the roof. Drop the barometer, timing its fall with a stopwatch. Then, using a the formula $x = 0.5\ a\ t^2$, calculate the height of the building."

At this point, I suggested to my colleague that he would give up. He conceded, and gave the student almost full credit. While leaving my colleague's office, I recalled that the student had said that he had other answers to the problem, so I asked him what they were.

"Well," said the student, "there are many ways of getting the height of a tall building with the aid of a barometer. For example, you could take the barometer out on a sunny day and measure the height of the barometer, the length of its shadow, and the length of the shadow of the building, and by the use of simple proportion, determine the height of the building."

"Fine," I said, "and others?" "Yes," said the student, "there is a very basic measurement method you will like. In this method, you take the barometer and begin to walk up the stairs. As you climb the stairs, you mark off the length of the barometer along the wall. You then count the number of marks, and this will give you the height of the building in barometer units. A very direct method."

"Of course. If you want a more sophisticated method, you can tie the barometer to the end of a string, swing it as a pendulum, and determine the value of *g* [gravity] at the street level and at the top of the building. From the difference between the two values of *g*, the height of the building, in principle, can be calculated."

"On this same tack, you could take the barometer to the top of the building, attach a long rope to it, lower it to just above the street, and then swing it as a pendulum. You could then calculate the height of the building by the period of the precession."

"Finally," he concluded, "there are many other ways of solving the problem. Probably the best," he said, "is to take the barometer to the basement and knock on the superintendent's door. When the superintendent answers, you speak to him as follows: 'Mr. Superintendent, here is a fine barometer. If you will tell me the height of the building, I will give you this barometer."

At this point, I asked the student if he really did not know the conventional answer to this question. He admitted that he did, but said that he was fed up with high school and college instructors trying to teach him how to think.

The name of the student was Niels Bohr (1885-1962); Danish Physicist; Nobel Prize 1922; best known for proposing the first "model" of the atom with protons and neutrons, and various energy states of the surrounding electrons -- the familiar icon of the small nucleus circled by three elliptical orbits... but more significantly, an innovator in Quantum Theory. ■

COMING EVENTS

May 23/25 '00: **Fundamentals of Asbestos Analysis by Transmission Electron Microscopy**
October 17/19 '00: **Fundamentals of Asbestos Analysis by Transmission Electron Microscopy**
(MVA, Inc.) Norcross GA: (770)662-8509

✓ 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

✓ March 12/17 '00: **Pittsburgh Conference** New Orleans, LA, www.pittcon.org

✓ March 15/17 '00: **TEM Specimen Preparation Course.** (South Bay Technology & FEI Company) Univ. of Central Florida, Orlando FL. Lucille Giannuzzi: lag@mail.ucf.edu

✓ 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 9/14 '00: **Light Microscopy For The Biomedical Sciences (LMBS).** (University of North Carolina) Chapel Hill, NC. Dr. Wayne Litaker: (919)966-1730

✓ April 11/14: **Analytica 2000** Munich Germany, Kallman and Associates (201) 652-3938

✓ April 9/13 '00: **FOCUS ON MICROSCOPY 2000** (12th Annual Meeting of International Conference on Confocal Microscopy). Shjirahama, Japan. http://lasie.ap.eng.osaka-u.ac.jp/fom

✓ April 11/13 '00: **MICRO 2000** (Royal Microscopical Society) London www.rms.org.uk/mic2000.html

✓ April 30/May 4 '00: **2000 Annual Workshop on SIMS** Lake Tahoe, NE www.simsworkshop.org

✓ May 4/12 '00: **Analytical & Quantitative Light Microscopy** (Marine Biological Laboratory) Woods Hole, MA. Carol Hamel: (508)289-7401, admissions@mbl.edu

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

✓ May 11/13 & 15/17 '00: **Quantitative Image Analysis** (NC State University) Raleigh, NC. (919)515-2261, www2.ncsu.edu/cpe/

✓ May 16/23 '00: **Microinjection Techniques in Cell Biology** (Marine Biological Laboratory) Woods Hole, MA. Carol Hamel: (508)289-7401

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

✓ June 10/17 '00: **Optical Microscopy in the Biological Sciences.** (Univ. of Texas Health Science Ctr) San Antonio, TX. www.uthscsa.edu/gsbs/csbhome.html

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 22/24 '00: Thin Specimen Preparation
✓ 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

✓ **3D MICROSCOPY OF LIVING CELLS**
June 19/29 '00: 3D Microscopy of Living Cells
July 1/3,'00: 3D Image Processing
Univ. of British Columbia, Vancouver, BC,
www.cs,ubc.ca/spider/ladic/course/bulletin.html

✓ June 22 '00: **16th Annaul Short Course on Molecular Microspectroscopy** Miami University) Oxford, OH. http://www.muohio.edu/~sommeraj

✓ June 23 '00: **2nd International Conference on Scanning Probe Microscopy in Biomaterials Science.** Bristol, U.K., Dr. Klaus Jandt: K.jandt@bris.ac.uk

✓ 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

✓ June 26/30 '00: **Summer School on Computing in Electron Microscopy** (NCEM, Lawrence Berkeley National Lab) Berkeley, CA. (510)486-6036, http://ncem.lbl.gov/frames/workshops.htm#workshops

✓ July 2/5 '00: **International Kunming Symposium on Micdroscopy,** Kunming, China. http://www.iphy.ac.cn/microsc/IKSM.html

✓ July 3/8 '00: **Xith International Congress of Histochemistry and Cytochemistry.** (Royal Microscopical Society), York, U.K., www.med.ic.ac.uk/external/ichc_2000/

✓ July 9/14 '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/

✓ July 17/19 '00: **Electron Microprobe Analysis by Wavelength Dispersive Spectroscopy.** (MIT) Cambridge, MA. E-probe-www@mit.edu, (617)253-1995.

✓ July 27/29 '00: **International Kunming Symposium on Microscopy** (Chinese Electron Microscopy Society) Kunming, P.R. China. IKSM Office: IKSM@aphy.iphy.ac.cn

✓ August 13/17 '00: **Microscopy & Microanalysis '00:** (MSA) Philadelphia, PA. Annamarie Dowling / Mary Beth Rebedeau: (708)361-6045, rebgroup@earthlink.net

✓ August 22/26 '00: **Scanning Probe Microscopy of Polymers.** (American Chemical Society) Washington, D.C. Vladimir V. Tsukruk: ((515)294-6904

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

✓ September 10/15 '00: **CLEO/Europe-IQEC 2000**

✓ October 11/19 '00: **Optical Microscopy & Imaging in the Biomedical Sciences.** (Marine Biological Laboratory), Woods Hole, MA. Carol Hamel: (508)289-7401, admissions@mbl.edu

✓ October 16/20 '00: **FLUORESCENCE 2000-Advanced Courses of Fluorescence Microscopy and Confocal Microscopy.** Lake of Garda, Italy. Dr. Annalisa Imberti: +390270646234, http://users.unimi.it/~fl2000/

✓ November 12/16 '00: **International Symposium for Testing and Failure Analysis.** http://www.edfas.org/istfa

✓ November 13/17 '00, **8th Conference on Frontiers of Electron Microscopy in Materials Science** (National Research Instituite for Metals) Matsue, Japan. Dr. M. Takeguchi: 81-298-59-5055, www.nrim.go.jp/femm2000

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