EVOLUTION AND REVOLUTION IN MICROSCOPY - II

Jean-Paul Revel, CALTECH

While today a picture of a serious looking person peering into a microscope may suggest "science" to the general public, we noted in our last column that this was not always so. In the early 19th century, for example, the microscope was seen by many as a hobby, interesting yes, but not as worthy of respect as an amateur's telescope.

Microscopes improved and soon, through the insight of the likes of Ernst Abbe, reached a state of perfection which was not greatly improved upon until recently. Once aberrations were well corrected for and glasses of high refractive index commonly utilized, further improvements in numerical aperture became increasingly difficult to receive. The only way to improve resolution seemed to be to decrease the wavelength of light used to illuminate the sample.

One such step was to use UV illumination as proposed by Koehler in 1900. The light source was an electric discharge between two cadmium electrodes and a uranium glass was used to focus the image. The optics were made of quartz.

Even in the 1940's, UV microscopy was still contributing heavily to biological research because it was found that there was UV absorbing "stuff" inside cells. This turned out to be due to the presence of ribonucleic acid (RNA) abundant in cells which make lots of protein and deoxyribonucleic acid (DNA) found in the cell nucleus, where it serves as the repository of all the information the cell needs. The famous names are those of Casperson in Denmark and the Belgian Brachet who worked with him. Of course there were multiple problems which hindered the wide use of this machinery. One problem was that to use UV of reasonably short wavelength meant that one had to use quartz optics (still today quartz optics are expensive, not that glass optics are cheap). Another difficulty was that the images could not be seen by eye, since we do not register UV. The data had to be recorded by photography. That too presented real problems not only because focusing blind is difficult but also because photomicrography had shaky beginnings, starting off on the wrong foot when overenthusiastic workers decided that one could just keep on magnifying the image.

Photomicrography began a popular approach to gathering data before most workers clearly distinguished between magnification and resolution. Objectives were claimed to work at 4000X and pictures were magnified 10-20000X by faithful if uncritical believers in the idea that seeing was proof of reality. As a result of such excesses many doubted that microphotography produced results accurate enough to ever replace the drawing of specimen. That in fact reminds me of my Biology 1 laboratory, where the lab instructor used to come over and peer in my microscope demanding to see the cell I had just drawn... The pioneer pathologist Koch remained convinced, in spite of such problems, that photography would eventually contribute mightily to microscopy. In 1934 the engineer V. K. Zworykin built a massive instrument he called an electric microscope, by which he meant a UV microscope using an iconoscope TV camera to produce a visible image[1]. Zworykin soon got involved with the newfangled electron microscope that was being designed by Knoll and Ruska and by Marton and Prebus and Hillier. In the electron microscope, resolution was not limited by the wavelength of the radiation used in imaging, but by the shortcomings of electron lenses. X-ray microscopy also is on the same evolutionary path. Again here the resolution is helped by having radiation of short wavelength but at first there were no lenses which could be used to make an X-ray image. So the first X-ray micrographs were contact micrographs. Soon Scanning Electron Microscopy was used to examine the patterns left by the specimen in photoresist. Today, with the advent of bright X-ray sources (synchrotron radiation) and zone plates of appropriate spacings, real X-ray projection microscopes (scanning) can be built and are having their first successes.

The revolution, as distinguished from the evolution, in microscopy is of course represented by the scanned probe approaches. With these techniques even photonic microscopes have managed to get around the problem of the diffraction limit (i.e. the near field scanning optical microscope). There is actually yet another microscope design, which has been shown to achieve high resolution with photons, a microscope which confounds the distinction between lensed (i.e. diffraction limited) and lens-less instruments. This is the newly introduced "Proton Tunneling Microscope" described earlier on these pages[3], a descendent of the internal reflection and surface contact microscopes. In the photon tunneling microscope, a thin sheet of material ("transducer") is placed over the specimen. If there is no object close to the transducer, the beam is reflected back. If there is an object present close to the transducer some photons tunnel into this medium, and total reflection does not take place. The intensity of the light reflected by the transducer is thus modulated by the microtopography of the sample. Guerra[4] showed that such an apparatus is capable of a vertical resolution of .65 nm, and that quantitative height information is easily obtainable. The lateral resolution is of the order of .16 μ, significantly better than the Abbe limit. The maximum feature depth which can be studied is of the order of 300 nm, giving images similar to those obtainable by SEM, but with less depth of field and resolution.

It thus seems that the Abbe limit can be breached (revolution!) in a number of ways, and this even in systems using lenses as in the photon tunneling microscope. Poor Abbe, one can wonder how he feels now as he watches us through a chink in the pearly gates. Is he really happy to find that his conclusions are now so routinely circumvented?

References:

1 Zworykin K. V. (1934) Electric Microscope. 1 Congresso Internazionale di Electroradio-biologia 1934 (1) pp 672-686.

2 McMullen D. (1990) The prehistory of scanned image microscopy Part 1 Scanned optical microscopes. Proc. Roy. Mic. Soc. 25, 127-131

3 Guerra J. M. (1992) Photon Tunneling Microscopy. Micr. Today #6.

4 Guerra J. M. (1990) Photon Tunneling Microscopy. Appl Opt. 29, 3741-3752

COMING EVENTS

(***): **Contact Microscopy Today for further information.**

✓ Feb 2/5 '93: **Digital Microscopy School** (Gatan, Inc.) Pleasanton, CA. Hans de Ruljter: (510)463-0200.

✓ Feb 8/11 '93: **PEELS and Imaging Filter School** (Gatan, Inc) Mike Kundman: (510)463-0200.

✓ March 8/12 '93: **PITTCON '93.** Atlanta, GA. (412)825-3220.

✓ March 8/10 '93: **Microtomy for Material Science Application.** (Univ of Arizona & ARM Inc). Tucson, AZ. Bob Chiovetti: (602)889-7900.

✓ March 15/19 '93: **Microspec User Training Course.** Fremont, CA. (510)656-8820

✓ March 22/26, 29/April 2, '92: **Practical Aspects of Scanning Electron Microscopy** (Univ. of MD Short Course). College Park, MD. Tim Maugel: (301)405-6898.

✓ April 4/8 '93: **8th Oxford Conference on Microscopy of Senmiconducting Materials** (Royal Microscopy Society) Oxford, UK (***)

✓ April 18/23 '93: **EM Spring School** (Royal Microscopy Society) Manchester, UK (***)

✓ April 21/23 '93: **SCANNING '93 Conference** Orlando FL Mary Sullivan (201)818-1010

✓ April 23/23 '93: **2nd Annual Cal State Univ EM Colloquim.** Hayward, CA/San Leandro Marina Inn. Nancy Smith: (510) 881-3527.

✓ May 5/7 '93: **1st International Sym- posium on Computerized Data Standards: Databases, Data Interchange, and Information Systems.** Atlanta, GA. Dorothy Savini: (215)299-5413.

✓ May 8/13 '93: **Food Structure Annual Meeting.** Chicago, IL. Dr. Ohm Johari: (708) 529-6677.

✓ May 9/13 '93: **EMAS '93.** Rimini, Italy (***)

✓ May 11/13 '93: **Image Analysis and Measurement** (NC State Univ. Short Course) Raleigh, NC: (919)515-2261.

✓ June 2/4 '93: **Trends in Cell and Molecular Biology - 18th Annual Meeting.** George Washington Univ., Washington, DC. Fred Lightfoot: (202)994-2881.

✓ June 6/10 '93: **Molecular Microspectroscopy** (9th Annual short course & workshop). Miami Univ., Oxford, OH. (513)529-2873.

✓ June 9/11 '93: **15th Symposium on Applied Surface Analysis.** Case Western Reserve Univ, Clevelend OH. Jeffrey I Eldridge (216)433-6074.

★★★★★★★★★★★★★★★★★★★★

THE LEHIGH SEM/AEM/SPM COURSES '93

✓ June 14/18 '93: **Basic Course: Scanning Electron Microscopy & X-ray Analysis.**

✓ June 21/25 '93: **Advanced Courses:**
1) Microcharacterization of Electronic Materials, Devices and Packages.
2) Advanced Scanning Imaging.
3) Quantitative X-ray Microanalysis of Bulk Specimens and Particles.
4) AFM, STM and Other Scanned Probe Microscopies.

✓ June 21/24 '93: **Analytical Electron Microscopy.**

✓ June 24/25 '93: **Thin Specimen Preparation.**

For detailed information, contact Professor Joseph Goldstein: Tel.: (215)758-5133

★★★★★★★★★★★★★★★★★★★★

✓ July 11/16 '93: **Microbeam Analysis Annual Meeting.** Los Angeles, CA. Jack Worrall, MAS '93, PO Box 1014, Monrovia, CA 91017-1014.

✓ July 17/22 '93: **13th Annual Congress on Electron Microscopy.** Paris (***)

✓ July 31/Aug 1 '93: **A Practical Experience In Cryofixation and Freeze-Substitution.** (MSA Pre-Meeting Workshop) Miami Univ, Oxford, OH. A. Allenspach: (513)529-3100.

✓ August 1/6 '93: **MSA Meeting.** Cincinnati, OH. MSA Business Office: (508)540-7639.

✓ August 3/5 '93: **FT-IR Microscopy: A Hands On Sample Preparation Workshop.** Wesleyan Univ., Middletown, CT. Wallace Pringle: (203)347-9411, Ext: 2361/2791

✓ Nov 17/21 '93: **National Association of Biology Teachers Convention.** Boston, MA. NABT: (703)471-1134

REGIONAL MSA/MAS EVENTS

✓ March 11/12 '92: AZ SEM Meeting. AZ State Univ. Robert Robinson: (602)965-8618.

✓ March 16 '93: NYSEM Meeting. Columbia Univ. Joan W. Witkin: (212)305-3453.

✓ March 25/27 '93: TX SEM Meeting. Corpus Christi, TX.

✓ May 12/14 '93: SEEMS & AL SEM Joint Meeting. Birmingham, AL. Charles Humphrey: (404)639-3306

We are looking for scientists - with a sense of humor

- - - to help us develop a series of cartoons relating to the "Near Side" of science. Our objective will be to "throw fun" at all aspects of the industry - including technology, people, applications and whatsoever.

And to help in the fun, we will issue a $50 U.S. Savings Bond for each accepted cartoon contribution - and, should you not be gifted in drawing, a $25 bond for each accepted cartoon "thought". Many of you certainly can do at least as well as those in the biz!!!

- - - Ed.