

The Truth in Imaging

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Several well (and also several not so well) publicized incidents of unethical conduct have raised the concerns of scientists, politicians and common folk that the truth and only the truth may not always emerge from scientific investigation. While this is a problem which clearly needs attention, I believe that Science alone of all fields of human endeavor has built in mechanisms which ensure that truth will emerge sooner or later, usually sooner (although some frauds, for example the Piltdown hoax, survived for quite a long time). This self correcting feature arises from the fact that today's interpretations are repeatedly tested against nature, whenever new methods or hypotheses arise. Only correct beliefs survive this repeated scrutiny. The misguided workers who for whatever reasons "cheat" or get so personally involved that they fall in traps made of their own gullibility are eventually discovered and at the very least lose the recognition that they longed for.

Microscopy presents special problems in searching out the truth. This is because the outcome of microscope observations in the old days were drawings. Accurate rendition of the detail required careful observation. In fact good microscopists have always been keen observers. As far back as 1673 Loewenhoek drew frog red cells with a central dot, a detail now identifiable as the cell nucleus even by beginners, but which was not "discovered" till 1820 by Brown. If more evidence is needed of the powers of observation of early microscopists, one has but to look at the detail in the illustrations in Hooke's "Micrographia" to become convinced. Even though technology improved (for example by the introduction of drawing tubes) a drawing is an interpretation of what can be seen and therefore leaves open the possibility of error (traduttore, traditore). It is rarely possible to reproduce all of the detail by hand. Put in the best of lights this is an advantage because it allows the researcher to simplify, to eliminate unnecessary features and thus communicate to others only the most important things about the object under study. The price to pay for this is that observers have the opportunity (conscious or not) to inject their own biases to the rendition. The drudgery of producing accurate drawings and the fear of giving misleading information has certainly been a factor in the popularity of photographic approaches in recording observation made with a microscope. With photography, after all, the data is recorded as it is, is it not? In fact an intense debate about the value of photography in depicting microscopic objects raged at the end of the last century. The problem was that not everyone appreciated the distinction between resolution and magnification. Pictures were published at magnifications of 10 and even 20,000, in the belief that the higher the magnification, the more could be learned from them. Because of such excesses there arose much skepticism about the value of photography. An ardent supporter was Robert Koch, the renowned pathologist, whose aphorism "omnis cellula e cellula" put a final period to ideas about spontaneous generation, the discoverer of the bacilli responsible for tuberculosis and for cholera. The work of an American, Joseph Janvier Woodward, a physician first in Philadelphia and then Washington, was of major importance in establishing confidence in the new technology. Woodward took microphotographs of the very fine gratings made by the famous German optician Friedrich Nobert and thus established the limits of resolution of light microscopy as well as the usefulness of photography in recording images through a microscope. Even today however, photographs are seldom perfect representations of microscopic objects. It is common to find that the response of the eye and that of the film are different enough to prevent rendition in silver of detail that the retina can detect well. The film can often be much more demanding than the eye. Detail seen by looking into a microscope, is lacking or not as clearly delineated in a micrograph. As a result the researcher often compensates by manipulating the photographic print. Sometimes this is done by altering contrast in order to emphasize objects difficult to perceive clearly otherwise. It is also common to "dodge", exposing parts of the micrograph more than the rest in order to obtain a more uniform, esthetically pleasing result. Although these practices fall in the category of tampering with the evidence,

they are usually not deemed objectionable, because they affect the image as a whole not one detail versus another. What type of enhancement is acceptable and what type is not, however varies greatly. Is it OK or is it not to remove the blemishes left by motes of dust or other such defects? Those of us who do indulge in this practice see it as only as an esthetic enhancement. Those of us who object on the other hand, see it as a first step towards retouching to emphasize structures which, may be, can't even be seen in the photomicrograph, but which the observer nevertheless believes to be there. Obviously that would be fabricating data and therefore unethical. But then all of biological microscopy can be considered suspect because by and large very little can be seen when examining an unadulterated biological sample. Live cells and other biological materials are mostly transparent and largely colorless. To see them one has to use dyes to stain the sample. To stain one usually first has to permeabilize cells, using fixatives which cross link at best and at worst coagulate proteins. By then one is so far from a living sample that there can be genuine concern about artifacts. Such worries in fact cast a pall over biological microscopy which lasted for much of the early 20th Century. Only if the same structures can be detected by independent means can one have a modicum of confidence in its reality. With the rise of modern microscopic techniques such as phase and interference contrast which permit observation of living, transparent cells, confidence in the reality of what one can observe and record even after histological processing has in fact been restored. The question we will ask next time is whether such confidence is still justified when digital, rather than analog approaches are used. ■

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

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

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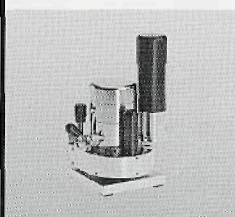
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- ✓ June 21/25 '93: **Advanced Courses**.
- ✓ June 21/24 '93: **Analytical EM..**
- ✓ June 24/25 '93: **Thin Specimen Prep**.
Info: Prof. Joe Goldstein: Tel.: (215)758-5133
- ✓ July 11/16 '93: **Microbeam Analysis Annual Meeting**. L.A., CA. Jack Worrall, MAS '93, PO Box 1014, Monrovia, CA 91017-1014.
- ✓ July 17/23 '93: **Immunocytochemistry and Cryosections '93**. A practical course. Yale School of Med. Paul Webster: (203)785-5072
- ✓ July 19/22 '93: **INTER/MICRO-93**. Chicago, Ill. Nancy B. Daerr, McCrone Research Institute: Tel.: (312)842-7100, Fax: (312)842-1078.
- ✓ July 19/23 '93: **Freeze Fracture Course**. Colorado State Univ., Ft. Collins, CO. Eileen Diepenbrock: (303)491-5847.
- ✓ 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: **51st Annual Microscopy Society of America (MSA/EMSA) Meeting**. Cincinnati, OH. MSA Business Office: Tel.: (800)538-3672; Fax: (508)548-9053.
- ✓ 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
- ✓ Sept 26 - Oct 2 '93: **Second International Congress on Electron Microscopy**. Cancun, Mexico. Mario Meki: Tel.: (525)622-50-33, Fax: (525)548-31-11.
- ✓ Oct 5/7 '93: **Third Annual Analytical Laboratory Exposition and Conference (ALEX '93)**. San Francisco, CA. (618)449-8938.
- ✓ Nov 17/21 '93: **Nat'l Assoc of Biology Teachers Convention**. Boston. (703)471-1134

- ✓ Nov 15/19 '93: **40th Annual Symposium of American Vacuum Society**. Orlando, FL. Marion Churchill: (212)661-9404.
- ✓ July 17/22 '94: **13th Annual Congress on Electron Microscopy**. Paris, France (**)

REGIONAL MSA/MAS EVENTS

- ✓ Oct 14/15 '93: **Louisiana SEM Meeting**, New Orleans, LA. Sherry Gibson: (504)346-3338.
- ✓ Oct 21/23 '93: **Great Lakes Electron Microscopy Affiliates Conference**. Airport Hilton Inn, Indianapolis, IN. Sandy L. White: (317)737-6423.

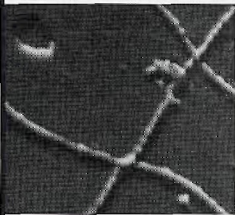
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AFM image (1112x1112nm) of the protein F-Actin showing 35nm helical twist

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