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MICROSCOPY TODAY

This Special Pre-MSA/MAS Conferences issue of our monthly newsletter is dedicated to the unique interests in microscopy of, and published at no cost to, 12,021 professionals in North America - 10,488 in the U.S., 1,164 in Canada and 359 in Mexico.

--- Don Grimes, Editor

NEW AND/OR INTERESTING IN MICROSCOPY

- ★ Polaroid has announced its 12th annual photomicrography competition. It will offer 35 prizes worth a total of \$14,450, including a grand prize of \$2,500. Two new categories have been added: 1) Polaroid instant micrographs produced with any of the new microscopies (acoustic, atomic force, confocal or scanning tunneling) and 2) photographs made with the new Polaroid MicroCam SLR microscope camera. Other categories include Electron: Life Science, Electron: Materials Science, Optical Black and White, and Optical Color. Up to three entries may be made by each contestant at, of course, no charge. The deadline is 1 October 1993. To receive entry forms and other information, call Polaroid at (800)225-1618.
- ★ DIATOME U.S. has introduced the STATIC-LINE II Anti-static Device for eliminating electrostatic charging in the cryo chamber and for preventing sections from sticking to knife edges, curling or bunching up one a top the other, or flying away during cryò sectioning. The STATIC-LINE II has been enhanced to include a control knob on the power pack to allow the varying of ion emission from the electrode. The electrode now remains stationary and, with a turn of a knob, the current can be either increased or decreased as desired. For more information on the DIATOME STATIC-LINE II, please call or write today. DIATOME US, PO Box 125, Fort Washington, PA 19034. Tel.: (215)646-1478 or FAX: (215)646-8931.
- ★ The proper contact for information regarding the Second Interamerican Congress on Electron Microscopy (Cancun, Mexico: Sept. 26-Oct. 2, '93) is:

Ms. Olga Leticia Perez Ramirez

Direccion Adjunta de Investigacion Cientifiica

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★ ELECTRON MICROSCOPY SCIENCES has introduced the EMS 820 Precision Pulsed Laboratory Microwave Oven for many steps in sample preparation prior to either light or electron microscopy — including but not limited to staining, fixation, decalcification, impregnation, dehydration and immunochistochemistry. For a complete brochure or for more information please call or write today. ELECTRON MICROSCOPY SCIENCES, PO Box 251, Fort Washington, PA 19034. Tel. (215)646-1566 or FAX: (215) 646-8931.

- ★ Microspec Corporation will introduce two new wavelength dispersive x-ray spectrometer systems at the 1993 MSA Conference in Cincinnati. The WDX-400 (four crystal) and the WDX-600 (six crystal) contain several new features, including a re-designed crystal change mechanism which allows crystals to be changed anywhere along the 2 θ range. Software control of both spectrometers under Microsoft Windows™ is specifically designed to seamlessly integrate with other computer controlled microanalysis products. For more information, please visit the Microspec booth (428-430) at the MSA Conference or contact Joseph Carr at Microspec Corporation, 45950 Hotchkiss Street, Fremont CA 94539. Tel: (510)656-8820, Fax: (510)656-8844
- ★ Material science is coming to TV! Scheduled to air nationwide on the Public Broadcasting Service (PBS) this fall, "The Stuff of Dreams" is a new three-part TV series that will explore how researchers are creating new materials that promise to revolutionize the way people live. The series is funded by Dow Corning, which will also underwrite a curriculum kit that will be sent to 25,000 high schools.
- ★ ELECTRON MICROSCOPY SCIENCES has now released an updated version of the EMS Oscillating Tissue Slicer. With many new enhancements, the EMS Oscillating Tissue Slicer is clearly the best solution for sectioning fresh or fixed tissue without the need for embedding or freezing. Sample preparation time is reduced dramatically and the risk of distortion and artifacts is eliminated. ELECTRON MICROSCOPY SCIENCES, PO Box 251, Fort Washington, PA 19034. Tel.: (215)646-1566 or FAX: (215)646-8931.
- ★ Mac ababy bye bye and bye! In our last issue we reported on the several heists of computers at the Univ. of Washington, as well as at UCLA and UC-Irvine. Yes, it happened again. In this case at the UW, not even clamp-on locks proved effective in protecting full computers. And CPUs and even units wired into central mainframes were removed. The only truly effective method of protection for computers may be to permanetly mark them to reduce or eliminate their resale value.
- ★ Sorvall MT-2/2B users can now eliminate costly repair bills with motor bracket, belt replacement and/or light replacement kits from Microtome Service: (315)451-1404.

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- ★ Leybold is introducing an improved stabilizer version of its highly successful TURBOVAC 340M Magnetic Bearing Turbomolecular Pump. This new version, which is designed to be impervious to shocks, is well-suited to the SEM market. Leybold Vacuum Products, 1-800-433-4021. MSA Conference: Booth 835.
- ★ A short course/workshop covering the principles of TEM specimen preparation will be offered at the Arizona State University on August 16/18, 1993. Parrticipants will gain a working knowledge and hands-on experience with preparation of specimens from semiconductors, ceramics, composites, etc. For information. contact Dr. Farhad Shaapur at (602)965-0399.
- ★ ASTM is publishing a new, free newsletter entitled "ASTM Standards International." Covered will be information on standards development from all 131 technical committees. Contact: Ellen McGlinchey: (215)299-5400.

- ★ The AVS is not publishing two volumes of *The Journal of Vacuum Science and Technology* on CD-ROM: 1) Vacuum, Surfaces, and Films and 2) Microelectronics and Nanometer Structures. Reasonable! Contact Angela Mulligan at (212)661-9404.
- ★ Philips Electronic Instruments has a list of used and surplus equipment for their TEM and SEM product lines. Contact Nathan Little: (201)529-6165.
- ★ Hessler Technical Services is now representing KORE Technology, Cambridge, England, a manufacturer of precision engineered, custom designed analytical systems. (203)358-0266.
- P.S. Should you find this newsletter of value, it would be appreciated if you would advise your salesmen. It is only through the advertising support of manufacturers and suppliers that we can continue to both improve its quality and send it to you at no charge.

Finger Painting or Digital Imaging

Jean-Paul Revel, CALTECH

In the good old days, when microscopists wanted to show others what they had found, they either drew by hand what they saw or hired someone to do same. Besides manual dexterity, and eye-hand coordination, drawing of course requires artistic interpretation, i.e. at least a smidgen of imagination. As a result one always had to be concerned with the objectively of rendering. Such concerns were more or less put to rest once the proper use of photography became established. The manual dexterity was reduced to twisting the focus knob, squeezing the shutter button without also shaking the camera and doing some handwaving in the dark room (artistic interpretation), surprisingly all non-trivial operations.

Today we have entered a different phase yet in the process of sharing morphological information, a phase characterized by digital recording and along with it, digital image processing. The digits now are not literally fingers anymore, but the numbers they stand for. Digital image recording and processing was originally devised to improve the quality of images returned by spacecraft and used to require highly sophisticated computing facilities. Today, however, digital image processing programs can be purchased for a few hundred dollars and can run well on desk top PCs with even 386 CPUs. Digital images are made of discrete picture elements (pixels) with which are associated numbers representing the brightness at a particular location in the image. The more points sampled in X and Y, the higher the spatial resolution, typically (but not always) limited to 256 X 256 (65,536 pixels) or 512 X 512 (262,144 pixels) so as not to be too demanding on memory. Brightness too is digitized, being represented by a binary number. Numbers consisting of 2 bits can represent 4 gray levels (00,01,10,11), numbers of six bits, 64 gray levels and so on, the more bits the better the gray level resolution. Digital processing consists of arithmetic manipulation of these parameters and is an incredibly powerful tool to modify the digital image.

Manipulation of contrast, the most commonly used adjustment to the quality of images, which used to be achieved by using different films, different grades of printing papers or filters (see "handwaving, above") can easily be implemented by digital processing. To adjust the contrast of digital images a histogram is constructed showing the number of pixels for each brightness level. A low contrast image would have a low dynamic range, with all the brightness values crowded close to each other. By first sliding all the values downward (by subtracting from each number a value corresponding to the lowest brightness level found in the image), and then stretching the histogram by multiplying all values by a constant number, one can obtain an image where the brightnesses cover the whole dynamic range, say, 0-225 if brightness is encoded as an 8 bit number. Voila!, contrast has been adjusted. No, you don't like what the picture looks like? Well, lets change the way we stretch the histogram, perhaps by multiplying with a logarithmic expression. It is easy to see that, using simple arithmetic

operations, images can be adjusted for the "best", the most revealing, result. Advertisements for a recently released program boast that one can even play photographer, "dodging" and "burning" digitally, I.e. apply the same manipulations to only part of the image. So dark room red lights are replaced by the computer screen's blues and grays and the wet and stained fingers of the old pros are replaced by unwettable binary digits.

But playing with contrast and brightness is only the beginning of what can be achieved. Much fancier things can be done easily using techniques which often have great sounding and mysterious names: Phong shading, morphological opening, statistical differencing. Edges can be enhanced, or instead the whole image can be softened. Lighting from any direction can be added where there was none, specular reflections placed. Images can be added to each other, distorted to compensate for defects in the recording system or just for the fun of it. They can be rotated, cropped, magnified or reduced. Colors can be added to code for brightness or other variables. Natural colors can be adjusted. Remember the first color picture of Mars returned by Viking? The surface of the planet was red and above it there was a beautiful clear blue sky like earth, beautiful! Only later did calibration show that the color had been wrong, the sky was really pink, even during the day. Processing to the rescue!

So where does this lead us? Of course it is to ask whether this is cricket, all this manipulation of the data. Does the result show something real? What is real? I don't wish to enter into a philosophical discussion, but the perception of reality does depend on the beholder. A bee sees the world differently from us and so does a dog whose world is monochrome and made as much of smells as of sights. The word of the very small is not visible to us directly, so in a narrow sense of "reality", the very small does not exist, at least not to the unaided eye. In order to circumvent this problem we take the data obtained with our various microscopes and let our imagination roam within the bounds of the observations they make for us. No matter that we use digits instead of fingers, they represent equivalent tools needed to allow us to see the invisible. In digital as in every other kind of microscopy the same rules apply. We make sure that what we observe is real by making multiple observations, preferably under different conditions. We must record not only how the original data was collected but also what manipulation of the data was carried out in order to achieve the image we present. The manipulations must be limited by common sense to doing what is needed to get the best image while not creating features where none exist. Whether encoded by digits or drawn by fingers holding a pen, the images collected by our microscopes are our windows to the invisible.

References:

Baxes, A.G.: Digital Image Processing. Prentice-Hall, NY 1984.
Dougherty, E.R.: An Introduction to Morphological Image Processing. Vol TT9, SPIE Optical Engineering Press, Bellingham WA 1992.
Hill, F.S.: Computer Graphics. Macmillan Pub. Co. New Yor, NY 1990.
Niblack, W.: Digital Image Processing. Prentice-Hall International, Englewood Cliffs, N.J 1986.
Washburn, M.: Mars, At Last! G.P. Putnam's Sons, NY 1977, page 236.