VOLUME 3 JANUARY 1994

THE *SEM* INAR

The newsletter that addresses the *probing* concerns of Forensic Science

Welcome to 1994! We hope this year will be a great one for all of you.

One of the goals of THE *SEM*INAR is to provide a literature survey, of both Forensic science publications as well as others in relevant areas. We will accomplish this with the help of the Forensic Science Information Resources System (FSIRS) of the FBI, which receives most of the major journals of interest to our community. Publications will be reviewed by THE *SEM*INAR for articles of interest. If significant, an article will be reprinted in its entirety when permission from the publisher is granted. An important article that cannot be reprinted will at least be reviewed. All articles that appear to be worth reading will be mentioned. Additionally, please contact us if you uncover any article, paper, etc. of interest to the community. If you would be interested in occasionally reviewing an article, please contact us. We would appreciate your help!

Thanks, Frank and Mary-Jacque for the SCANNING '93 review. A review like this gets us all to the meetings even when we can't attend. Please think of THE *SEM*INAR when you attend a meeting (particularly the regional meetings).

Frank Platek is putting together the agenda for the Forensic Session at SCANNING '94. If you would like to contribute, call Frank at 513/684-3501. Hope to see you there!

P.S. Don't forget to include your commitment to contribute to THE *SEM*INAR in your list of New Years Resolutions.

Editors:
Robert O'Brien, Connecticut Forensic Science Laboratory, Meriden, CT
Dennis Ward, FBI, Washington, DC

SCANNING '93

Overview of the First Annual Session of "Applications of Scanning Microscopy in Forensic Science" held at SCANNING'93

S. Frank Platek[1] and Mary-Jacque Mann[2]

1 - USFDA - National Forensic Chemistry Center, Cincinnati, Ohio
2 - USDI - National Fish and Wildlife Forensics Laboratory, Ashland, Oregon

Scanning'93 convened at the Twin Towers Hotel and Convention Center in Orlando, Florida from April 20-23, 1993 and included the first annual session in forensics "Applications of Scanning Microscopy in Forensic Science". The focus of the sessions was to discuss new and unique applications of scanning microscopy in forensic science and highlight interesting case/sample analyses. The following is a brief overview of speaker top-

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ics. Speaker abstracts were published in ***SCANNING***, Vol.15, Suppl III (1993).

S. Frank Platek [USFDA - National Forensic Chemistry Center (NFCC)] served as session chairperson and presented a paper discussing the use of scanning electron microscopy at the recently formed NFCC. The samples discussed included food and drug tampering, drug counterfeiting and fraud. Tampering cases included the analysis of a drain cleaner added to powdered coffee creamer and replacement of a diabetic's insulin with household cleaners. Energy dispersive x-ray analysis (EDXA) in the SEM was used to determine the presence of sodium in a headache medication replaced (tampering) with sodium cyanide which resulted in a homicide. Image analysis on both the SEM and polarizing light microscopes was utilized to differentiate counterfeit from authentic bulk pharmaceuticals and perform tablet "ballistic" analyses.

Blair W. Schultz (Illinois State Police) presented an overview of the application of SEM/EDXA instrumentation in a large police crime laboratory. Three cases involving firearm ballistic analysis were discussed including resolution of each case by SEM/EDXA analysis.

Mary-Jacque Mann (USDI - National Fish and Wildlife Forensic Laboratory) discussed SEM/EDXA applications in the identification of biological specimens, taxonomy and firearms analysis. Unique applications include the direct analysis of light and electron micrographs of cartridge casing firing pin impressions as well as overlaying the impressions in the SEM using image comparison hardware. Studies for the presence of gunshot residue on bow hunters were described and showed transfer of gunshot residue on bow hunters in Oregon and Washington was minimal. (NOTE: It is illegal to use a gun during bow season in the State of Washington). The determination of the fate of an illegally shot bear and snow geese was also performed by SEM/EDXA.

Roxie Laybourne (Smithsonian Institution - Department of Ornithology) and "The First Lady of Feathers" (per chairperson's citation) discussed the analysis of bird feathers. Both light and scanning electron microscopy were discussed in their role to determine the identity of birds responsible for downing civilian and military aircraft (bird strikes) and feathers found at crime scenes. Ms. Laybourne also showed the procedure of preparing, analyzing and identifying the origin of a feather from no more than a single barbule.

Robin Keeley (Metropolitan Police Forensic Science Laboratory, London, UK) discussed a unique method for analyzing latent fingerprints using a silver salts substitution process and subsequent analysis in the SEM. Tracking the source of a piece of circuit board plastic after a fatal terrorist bomb explosion to a watch with a timer using SEM analysis was also detailed.

Peter F. Schmidt (Institute for Medicine and Biophysics, Munster, Germany) presented a most interesting application of laser microprobe mass spectrometry (LAMMS) in combination with the SEM/EDXA instrumentation to analyze materials on fabrics including fabric softeners and dyes. Particular applications detailed the analysis of various indigo dyes on cotton jean fabrics. Jeans and other items of clothing are often related to crime scene investigations and play a major role in subsequent investigations.

Timothy F. Watson (Department of Conservative Dental Surgery, Guy's Hospital, London, UK) discussed the use of scanning confocal microscopy to analyze for the presence of hard tissues including bone and dentition fragments in food products. The microstructure of the bone-like objects was imaged to perform positive identification of contamination.

Richard S. Brown (MVA, Inc., Norcross, GA) detailed the identification of inorganic paint pigments, fillers and extenders. The use of SEM/EDXA along with polarizing light microscopy was demonstrated to accurately identify most of the paint constituents making the application to forensic analyses invaluable.

"Applications of Scanning Microscopy in Forensic Science" was held in two sessions during the *Scanning'93* meeting. Both sessions were very well attended and provided an excellent forum for the exchange of new applications and techniques between attending forensic scientists. The *Scan-*

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ning'94 meeting (May 17-20, 1994 in Charleston, SC) will once again offer a dedicated forensics session with both invited and contributed papers presented. It appears that the attraction and enthusiastic reception of scanning microscopy (especially scanning electron microscopy) in forensic science is to become a featured annual session. The authors look forward to meeting our fellow *SEM*INAR readers at future meetings.

EMPLOYMENT

JOBS WANTED

Retiring Microscopist, (from NSA with TS-SI clearance), seeking part time employment. Three years experience with a Scanning Electron Microscope (AMRAY 1840), XRF/Imaging System (TN 8500) and an E-Beam probe analyzer (ICT 8420). Available after January 31, 1994. (Robert E. Lamothe--Home Telephone: (401) 849-5032).

WHATS NEW?

Minitool carries a line of carbide tipped micro cutting tools, in needle, knife, chisel shapes each in several sizes. These are useful in sample preparation situations where a scalpel blade *just doesn't cut it.* Available from **Minitool, Inc**, Los Gatos, CA 408/339-1585.

BOOKSHELF

The Role of Microscopy in Semiconductor Failure Analysis, by B.P. Richards and P.K. Foorner.

This concise handbook describes the applications of microscopy to semiconductor failure analysis. Generously illustrated, it covers each type of microscopy. (Royal Microscopical Society Microscopy Handbooks 24). ISBN 0-19-856432-5, 1992, Paperback.

Available from Ted Pella, Inc, Redding CA. 1-800-237-3526 $24.95.

LEAD-FREE AMMO

Gary Lawrence, Arkansas State Crime Laboratory, presented the paper "LEAD-FREE or CLEAN-FIRE" at the Southwestern Association of Forensic Scientists (SWAFS) spring 1993 meeting in South Padre Island, Texas.

Realizing that ammunition without barium, antimony, lead or other heavy metals has the "potential of creating a major problem of identification and analysis", he reports the results of his studies on "Clean-Fire" and "Lead-Free" primers, including organic (mass spectrometer) analysis of the primer, inorganic analysis (XRF and SEM/EDXA) of residues, and cloth target residues and patterns. He also details distribution of the product.

Particles containing Sr and Ti are demonstrated by SEM.

For a copy of this paper, contact Gary Lawrence, Arkansas State Crime Laboratory, PO Box 5274, #3 Natural Resources Drive, Little Rock, AR 72215-5274.

FROM THE READERS

I applaud the efforts in producing **THE *SEM*INAR**. This is a sorely needed communications tool for our field, since many well meaning SEM/EDS experts outside of Forensic Science just don't have the necessary life experience to appreciate our particular needs and requirements. JK

I would like to congratulate **THE *SEM*INAR** on the Meetings and Schools column. Excellent source of information. RL

Congratulations on **THE *SEM*INAR**, it's a good publication and I hope it continues. GL

MICROALIENS

If you're looking for a Christmas gift that is fun and also tells young and old something about what you do, consider a new publication. The book is called "Microaliens, Dazzling Journeys with an Electron Microscope". In its 80 pages with virtually all of them displaying EM illustrations, the book tells a little about how an electron microscope works, and then illustrates the images that many groups of materials produce. Categories include pollen, butterfly wings, bird feathers, mosquitoes, water, fish, the backyard, the home, (watch out for those dust mites, they look grim) and fibers. Written by Howard Tomb, the book is based on work and illustrations done by Dennis Kunkel, Senior Scientist at the University of Hawaii. This is a great way to help people comprehend the world of microscopic images and can remind those of us who use microscopes every day of the beauty and mystery that is all around us. It is something worthwhile to consider every now and again. Published by Farrar, Straus, and Giroux, 19 Union Square West, NY, NY 10003, Cost $16.00. A best buy.

..reprinted with the permission of Microscope Technology & News, Vol 5, No 12, Dec 1993.

FEDERAL LAB DIRECTORY

A Special Edition of Microscope Technology & News (Volume 5, Number 10, October 1993), includes a Directory of Federal Laboratory and Technology Resources. Listed are 27 facilities for Materials Science, and 15 for life science applications, that are available for technology development. Included is a description of the laboratory services and equipment available, applications specialties, and contacts.

Those interested in obtaining subscription information for **Microscope Technology & News**, and a complementary copy of **The Microscope Book,** a catalog of microscope and related equipment, call MT&N at 800/440-0311.

WORTH READING

Paint

"Effects of Acidic Deposition of Paint: A Chamber Study", J W Spence, *Journal of Coatings Technology*, Vol 65, No 823, Aug 1993.

Describes study of artificial exposure of latex and alkyd films to smog, light and dew and resulting changes in surface structure and loss of Ca.

XRF

"High-sensitivity energy-dispersive XRF technology, Part 1: overview of XRF technique", *American Laboratory*, July, 1993, 24C-H, and "Part 2: Advances in Instrumentation", September, 1993, 36H-O, Boris Yokhin, Jordan Vailey Applied Radiation, Migdal Haemek, Israel, and Robert Tisdale, Baird Corp, Bedford MA

Reviews general concepts of XRF, and describes preliminary energy selection (PES) which lowers detection limit of metals in aqueous and organic materials.

"Sampling and Sample Preparation in EDXRS", Barbara Holynska, *X-Ray Spectrometry*, Vol 22, 192-198 (1993).

Discusses principles of sampling, sample storage and sample preparation of soil, biological and geological specimens prior to EDXRF, including contamination concerns, digestion/preconcentration, and use of reference materials.

SEM

"Combining computer technology with SEM", Michael P. McCarthy, Vice President, Topcon Technologies, Inc, *American Laboratory*, November, 1993. 510/462-2212.

Discusses the extent to which SEM functions should be automated to obtain maximum benefit from computerization without losing the "art" of microscope operation.

"10 Timely Applications for Environmental SEMs", Robert Cassidy, *R&D Magazine,* Dec, 1993.

The title says it all. Includes corrosion, particles in hydraulic fluid, release coatings, analyzed with the ESEM of Electroscan Corp.

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MATERIALS

"Quantitative Analysis of Surface Topography using Scanning Electron Microscopy", N. K. Myshkin, et al, *Wear*, 153, (1), 119-33 (1992).

A detailed survey is made of the methods available for studying the textural and morphological characteristics of surfaces. A method of evaluating topography using SEM and a personal computer is described.

for additional information regarding any of the above articles, write or fax ***THE SEMINAR*** *(see last page)*

DOWNTIME

We have all experienced the frustration of down time when maybe the scan generator didn't scan, or the pumps didn't pump, but consider:

- The lab above **Nick Madary's** EM suite at AFIP (Washington, DC) had a fire, and where did the water used to extinguish it go? Shorting this and corroding that, the SEM was "totaled", and Nick still resorts to borrowing SEM time from facilities in the area until the new SEM arrives.

- and **Jim Wallace** and staff (Northern Ireland Forensic Science Lab) has had to temporarily relocate the entire EM Lab because of bomb damage caused by terrorists.

So next time you *just* have to replace a filament to get back in operation, consider yourself very, very lucky.

MEETINGS, SCHOOLS

February 1994

PITTCON '94, Feb 27-Mar 4, 1994, Chicago, Il. Alma Johnson, 412/825-3220. Conference/exhibition for laboratory equipment and chemical analysis.

American Academy of Forensic Sciences, February 14-19, 1994, San Antonio, TX.

March 1994

Using Ultramicrotomy in Materials Science, Mar 8-11, 1994, Univ of Arizona/Research and Manufacturing Co Short Course, Tucson, AZ. Emphasis on diamond knife sectioning. Bob Chiovetti, 602/889-7900.

Practical Aspects of Scanning Electron Microscopy, Session I: Mar 14-18,1994, Session II: March 21-25,1994, Univer. of Maryland, College Park, MD. Tim Maugel 301/405-6898.

April 1994

Advanced Materials. Apr 11-14 (Lake Bluff, IL), Nov 7-10 (Tucson, AZ). Institute for Microstructural Analysis, Buehler Ltd, Lake Bluff, IL. Preparation and characterization techniques for microscopic analysis, including SEM. 708/295-4659.

FDLE

Florida Department of
Law Enforcement

James T. "Tim" Moore
Commissioner

**Division of Local Law
Enforcement Assistance
Orlando Regional
Crime Laboratory**

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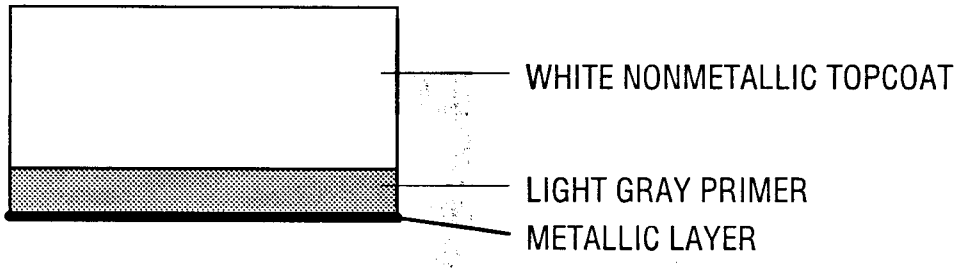
November 28, 1993

WHAT AND WHY IS IT?

In the not too distant past I came across a multiple layer automotive paint sample in a homicide investigation which had an unusual thin metallic coating on the bottom surface of the layer structure. I'm looking for anyone who has also encountered this type of specimen and might know the purpose of the layer and how it is applied. What and why is it?

The paint sample originated from a rear panel (unsure if it was the hatch back or the panel underneath it) of a white 1991 GEO Metro. The paint layer structure is as follows:



The topcoat binder is a polyester-melamine enamel, indicative of an original (OEM) finish system. The thin metallic coating on the bottom is visually dissimilar to the somewhat crystalline appearance of a typical zinc phosphate conversion coating found between the base primer and the sheet metal substrate on most vehicles. It has a metallic luster and is in the form of a continuous layer with no apparent binder resin. When viewing the layer from the bottom of the paint chip, its surface topology resembles the low magnification orange peel topology noted on the bottom surface of the base primer of most OEM automotive paint fragments. (See Figure 1, an SEM image obtained using a negative bias on the secondary electron detector to enhance topographical characteristics rather than a stereomicroscope) Increasing magnification of the uncoated metallic surface reveals the presence of numerous cracks or fissures "spider webbing" their way across the entire surface. (See Figure 2) Imaging with a quad backscatter detector revealed that the resulting mosaic pattern is somewhat consistent in dimension with no apparent chemical inhomogeneity. (See Figure 3) The layer is magnetic and consequently the paint chip can readily be picked up with a magnet. Elemental analysis using a 20 KV beam and

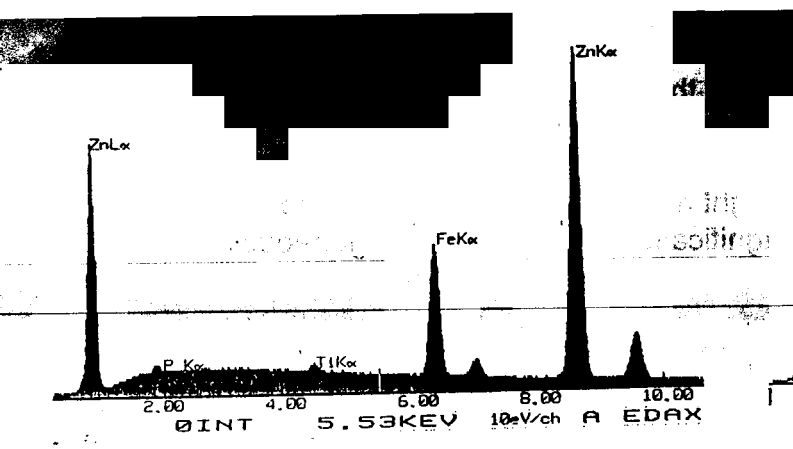


Figure 4: EDX spectrum of the metallic layer of the 1991 Chevy GEO sample - 20 KV beam potential, 32 degree take off angle.

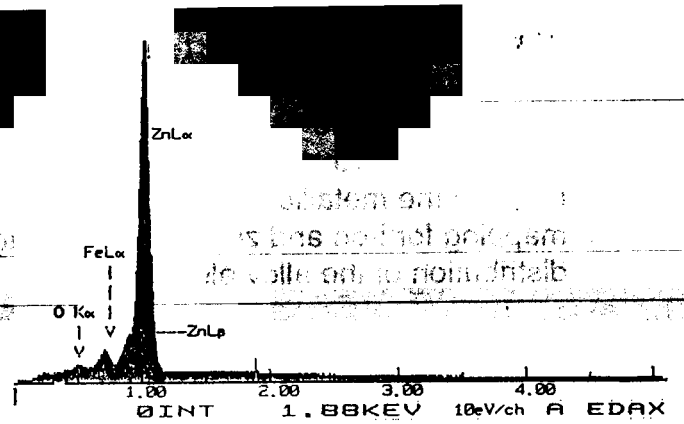


Figure 5: Windowless EDX spectrum of the metallic layer of the 1991 Chevy GEO sample - 5 KV beam potential, 32 degree take off angle.

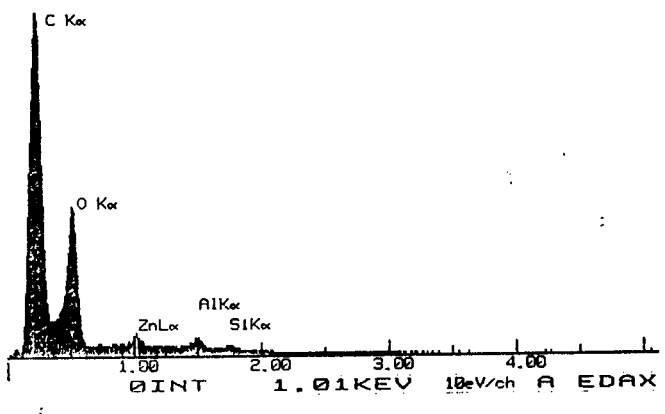


Figure 6: Windowless EDX spectrum of the light gray layer 2 primer of the 1991 Chevy GEO sample - 5 KV beam potential, 32 degree take of angle (major extender pigments are clay and titanium dioxide)

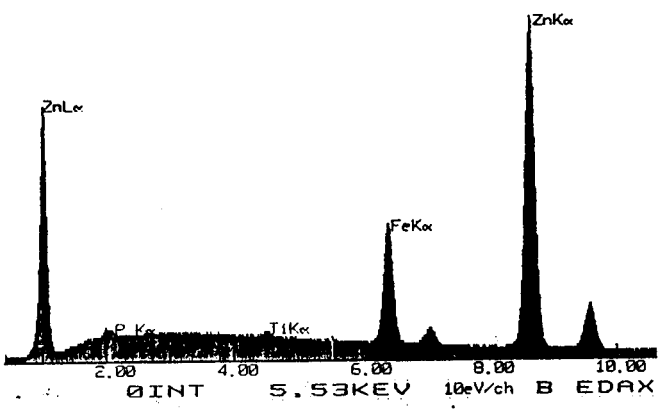


Figure 8: EDX spectrum of the metallic layer of the Broward County sample - 20 KV beam potential, 32 degree take off angle.

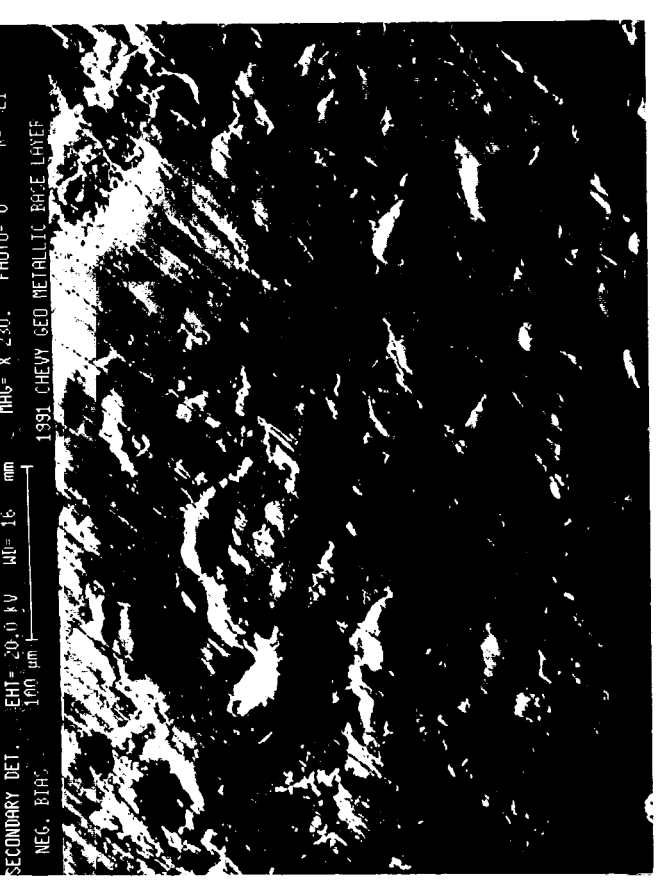


Figure 1: Topology of the metallic layer surface on the 1991 Chevy GEO sample imaged using a negative bias on the secondary detector.

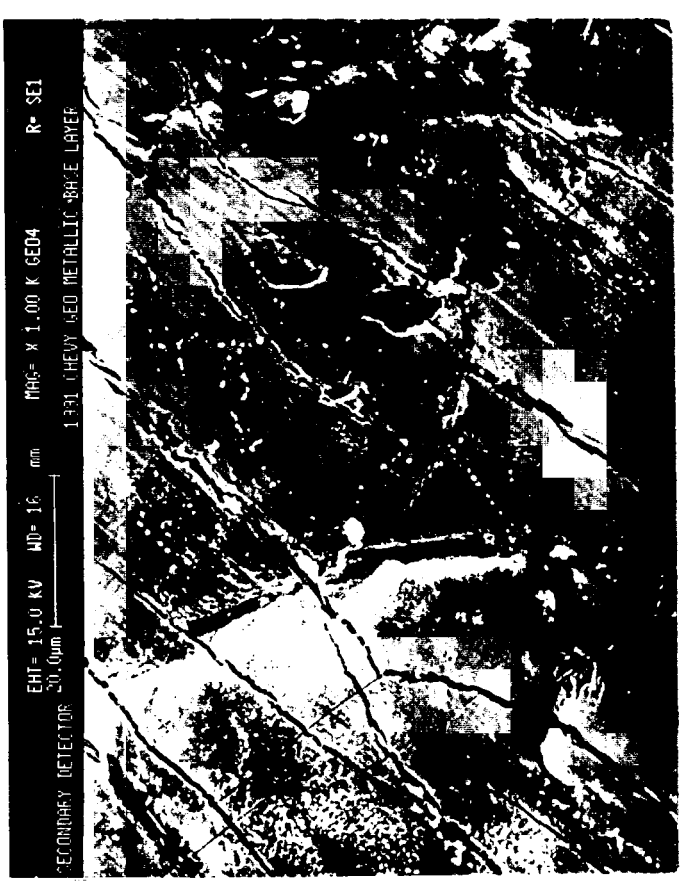
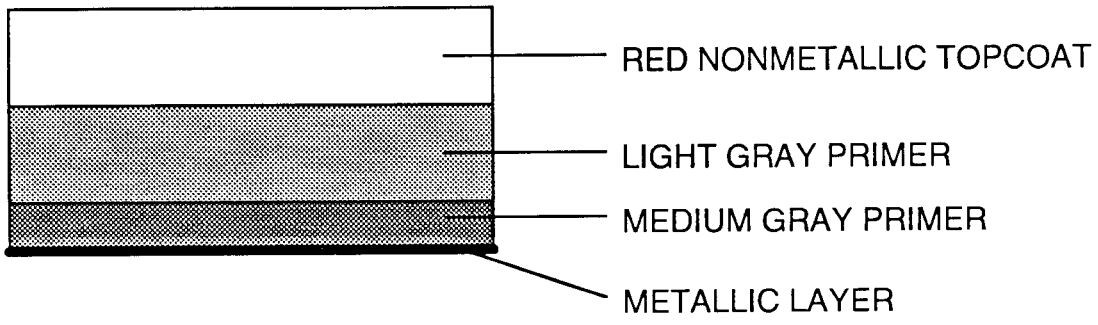


Figure 2: Higher magnification image of the metallic layer surface of the 1991 Chevy GEO sample acquired using a positive bias on the secondary detector.

a beryllium window EDX detector disclosed the layer to consist of an iron - zinc alloy. (See Figure 4) Windowless EDX analysis revealed little indication of organic constituents, i.e., binder. (See Figures 5 and 6, contrasting the C, O, N ratios of the metallic layer 3 versus the light gray layer 2 primer) X-ray dot mapping for iron and zinc at 4000 x magnification revealed a homogeneous distribution of the alloy elements.

Recently, I received a call from the Broward County, Florida, Sheriff's Laboratory (Bruce Ayala) inquiring about an apparently similar type of specimen they had encountered. The sample was acquired from a "chop shop" operation and the make/model origin was not known. Visual examination of the thin metallic layer on the bottom of the paint fragment under the stereomicroscope disclosed a similar appearance to the previously reported sample, only differing by slightly less metallic luster. The topology of the surface is slightly different than that of the 1991 GEO sample. (See Figure 7) The paint fragment has the following layer structure:



The metallic coating (or layer) was subjected to SEM/EDX analysis and found to be elementally indistinguishable from the 1991 GEO sample. (See Figure 8) A difference was noted in the two sample's fissure patterns, as can be seen in Figure 9. X-ray dot mapping for iron and zinc at 4000 x magnification also revealed a homogeneous distribution of the alloy elements in the Broward County sample.

Our current hypothesis as to the purpose of the coating hinges on the samples originating from plastic body panels (a fact which is not known with the samples described). The thin metallic coating placed over the surface of nonconductive plastic body panels may permit them to be painted with the same electro-deposition primers as metallic body panels, thus reducing manufacturing costs and primer/ topcoat deviations between different types of panels. If anyone has more information or samples to share, please contact the author at 407-423-6800 (extension 124) and write a short article for the *SEMINAR*. Thanks for the help!

Scott Ryland
Senior Microanalyst

Figure 3: Higher magnification image of the metallic layer surface of the 1991 Chevy GEO sample acquired using a quad backscatter detector.

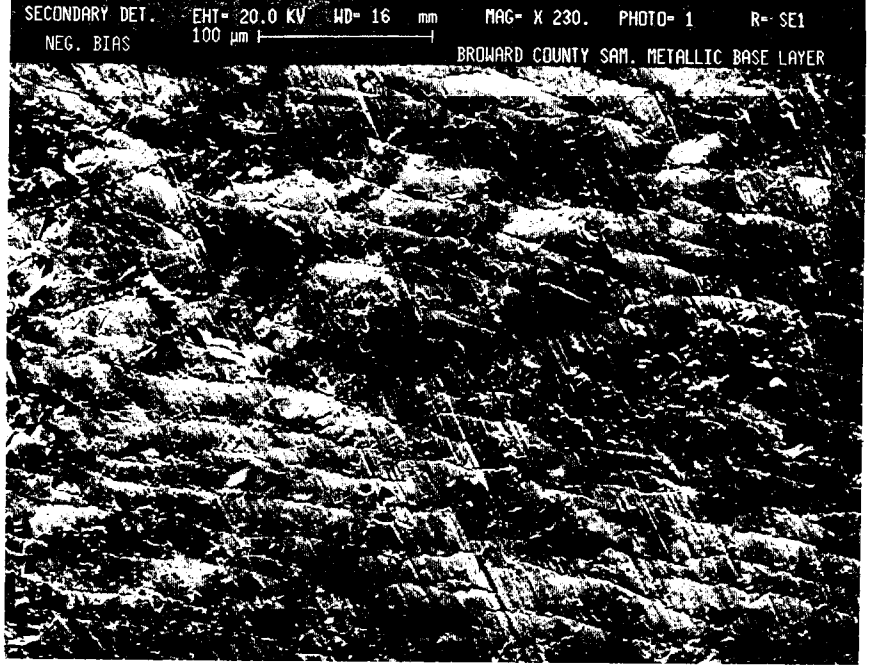


Figure 7: Topology of the metallic layer on the Broward County sample imaged using negative bias on the secondary detector.

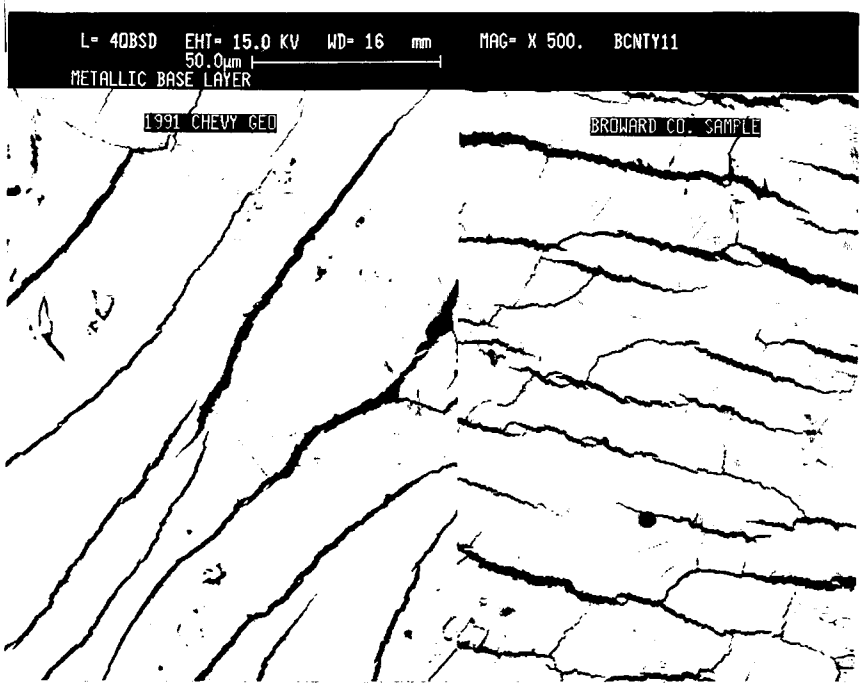
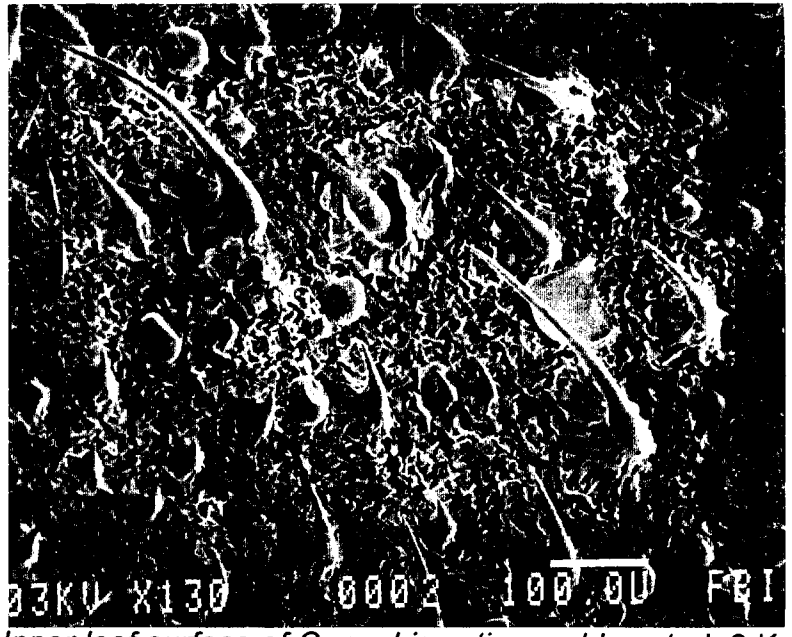


Figure 9: Comparison of the metallic layer surfaces of the two specimens acquired at 500 X magnification using a quad backscatter detector.

PICTURE THIS!



Upper leaf surface of Cannabis sativa, gold coated, 3 Kv, 130X

Southern Association of Forensic Scientists (SAFS) and Southwestern Association of Forensic Scientists (SWAFS), April 12-16, 1994, Little Rock, AR. Ken Michau or Gary Dallas, 501/2275747.

May 1994

Mid-Atlantic Association of Forensic Scientists (MAAFS), May 4-6, l994, Virginia Beach, VA. Harry Finley or Marc Jaskolka, NCIS, 9079 Hampton Blvd, Norfolk, VA 23505.

Scanning Microscopy 1994, May 7-12,1994, Toronto (Downtown, City Hall) Canada. Contact Dr. Om Johari, Scanning Microscopy International, 708/529-6677.

California Association of Criminalists (CAC), Spring Seminar, May 11-14, 1994, Oakland, CA. Contact Mary Gibbons, Oakland PD Lab, 510/238-3386.

SCANNING 1994, May 17-20,1994, Charleston, SC. Contact Mary Sullivan, 201/818-1010.

June 1994

Lehigh Microscopy Short Courses, June 13-23, 1994, Bethlehem, PA. Contact David Williams, 215/758-5133. SEM & X-ray microanalysis.

July 1994

INTER/MICRO-94, July 18-21, 1994, McCrone Research Institute, Chicage, IL, 312/842-1078.

MAS/MSA 1994, July 31-August 5, 1994, New Orleans, LA. Contact: MSA Meeting Office, 800/538-3672.

August 1994

Denver X-ray Conference, August 1-5, 1994, Steamboat Springs, CO. Lynne Bonno, Dept of Engineering, U of Denver, Denver, CO 80208.

October 1994

Midwestern Association of Forensic Scientists (MAFS), Oct 11-16, 1994, Cleveland, OH. Mary Wenderoth or Cathy Denissoff, 216/623-5646.

Northeastern Association of Forensic Science (NEAFS) Oct 13-15, 1994, Manhattan, NY, NY, Jeff Luber, 516/853-5585.

California Association of Criminalists (CAC), Fall Seminar, Oct 19-22, 1994, Pasadena, CA. Contact Manuel Munoz or Dan Anderson, Los Angeles County Coroner, 213/343-0530. (This will be the first joint CAC/Forensic Science Society meeting!)

Canadian Society of Forensic Science (CSFS) and Northwest Association of Forensic Scientists (NWAFS), October 31-November 5, 1994, Vancouver, British Columbia, Canada. Jeffrey Coughlin, 604/264-3507.

November 1994

Advanced Materials, Nov 7-10, 1994, Buehler Ltd. Tuscon AZ. 708/295-4659. (See listing under April).

Australia and New Zealand Forensic Science Society, November 21-25, 1994, Auckland, New Zealand. Douglas Elliot, Auckland, New Zealand, 09-815-3670.

MAS 1995, Denver, CO
MSA 1995 ?
MAS/MSA 1996, Minneapolis

G. W. Bailey and C. L. Rieder, Eds., Proc. 51st Annual Meeting of The Microscopy Society of America
 Published by San Francisco Press. Inc.. Box 426800. San Francisco, CA 94142-6800. USA

STANDARD FORMATS FOR THE EXCHANGE AND STORAGE OF IMAGE DATA

R.F. Egerton (1), D.S. Bright (2), S.D. Davilla (3), P. Ingram (4), E.J. Kirkland (5), M. Kundmann (6), C.E. Lyman (7), P. Rez (8), E. Steele (2) and N.J. Zaluzec (9)

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(2) National Institutes for Science and Technology, Gaithersburg, MD 20899, USA;
(3) 4pi Systems, 117 West Lynch St., Durham, NC 27701-1929, USA;
(4) Departments of Cell Biology and Physiology, Duke University, NC 27710, USA;
(5) Department of Applied Physics, Cornell University, NY 14853, USA;
(6) Gatan Inc., 6040 Washington Street, Downers Grove, IL 60516, USA;
(7) Department of Metallurgy, Lehigh University, Bethlehem, PA 18015, USA;
(8) Physics Department, Arizona State University, Tempe, AZ 85281, USA;
(9) Electron Microscopy Center, Argonne National Laboratory, Illinois 60439; USA

In microscopy, there is an increasing need for images to be recorded electronically and stored digitally on disk or tape. This image data can be shared by mailing these magnetic media or by electronic transmission along telephone lines (e.g. modem transfer) or special networks, such as Bitnet and Internet. In each case, the format in which the image is stored or transmitted must be known to the recipient in order to correctly recover all the information. Because there are many image formats to choose from, it would undoubtedly save misunderstanding and frustration if a group of individuals with similar interests and needs could agree upon a common format. The MSA Standards Committee has surveyed several formats which could be of particular interest to microscopists, with a view to making a recommendation to our community.1

Our chief concern has been compatibility with existing software, combined with an adequate representation of the data, compactness of data storage (on disk) and reasonable rate of data transfer. In some forms of microscopy, the image intensity covers a wide dynamic range, demanding a large number of bits per pixel or representation by floating-point numbers. Although data transfer rates can be increased through various forms of data compression, compactness of storage demands that the image be represented in binary code rather than in more immediately readable ASCII numbers. The formats which we have considered include raw binary files, PICT, GIF, FITS, TIFF and HDF. A raw binary file is highly compact but not self-describing: the dimensions of the data array have to be specified external to the dataset. Such a format is too rigid for general use.

PICT is the main graphics format used by Macintosh computers and is recognized by many software programs. In its most recent manifestation (PICT2), grey-scale and color images are supported. Data can be represented by 8-, 16- or 32-bit integers and the file contains other information describing the image. But because this format is not commonly supported by computers which use the DOS and Unix operating systems, we do not recommend PICT as a general standard.

GIF (Graphics Interchange Format) was originally developed for the interchange of files over the Compuserve network. A compression scheme can be used to compact the data and thereby shorten the transmission time. Support for color tables is included, but not floating-point numbers, so we feel that this format is not flexible enough for general microscopy.

>>

(Flexible Image Transport System) is a standard format used by optical and radio astronomers. It is capable of handling large, multidimensional arrays and can support several data types, including ASCII characters, 8-, 16- and 24-bit integers and floating-point numbers. Each file contains a header (written in ASCII text) to describe the data, which is usually in binary form.

TIFF (Tagged Image File Format) was developed jointly by the Microsoft and Aldus companies in order to store images from scanners and other desktop-publishing equipment in a machine-independent form. It has become the mostly widely used format for Macintosh and IBM-type personal computers. The data itself can be stored as 8-,16-, or 32-bit unsigned integers, and (in version 6.0) as signed integers and floating-point numbers. Besides the picture data, a TIFF file may contain supplementary information concerning the ownership, acquisition conditions etc., and even extensive documentation about the image. Each particular element of information is identified by a tag - a label which the computer needs to interpret the information.

HDF (Hierarchical Data Format) was developed by the National Centre for Super computing Applications (NCSA) at the University of Illinois (Urbana). It uses a machine-independent binary-file standard for recording matrix arrays and other types of scientific data, which can be quite extensive. This format permits flexibility; for example, subarrays corresponding to part of an image can be accessed for read/write purposes. Data are stored as 1- or 3-byte integers, or as floating-point numbers, in a binary-format continuous-byte file (not subdivided into blocks).

We see both TIFF and HDF as suitable formats for microscopy. In terms of immediate use, TIFF has the largest amount of support; it is used by all IBM-PC and Macintosh page-scanner programs, by page-layout applications such as Ventura Publisher, QuarkXPress and Aldus PageMaker, and by image-editing programs such as Image, Digital Darkroom, Photoshop, Image Studio, Image Edit and Snapshot. Because these applications typically read several formats, they can be used to translate TIFF files into other forms. TIFF is flexible and still evolving; it currently allows multiple images per file, several types of data compression, and various types of color image. Because the image data is organized into ‘strips’, an application program can call into memory only those parts of an image which require processing . TIFF documentation is available over the Internet network from several FTP sources,including zippy.nimh.nih.gov (see the /pub directory).

Microscopists should remain aware of the HDF standard, which might grow in popularity within the scientific community. NCSA’s program Import2HDF can read several ‘foreign’ formats including TIFF, FITS, PICT, GIF and ASCII Text Files. HDF documentation, source code and Macintosh applications are available free of charge (via anonymous FTP) over Internet (ftp.ncsa.uiuc.edu).

1- MSA Standards Committee report, MSA Bulletin, vol.23, No.2, 1993.

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TACKY DOT ARRAYS FOR ORDERED PARTICLE MOUNTING

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We describe for the first time a rapid way to mount particles on regularly spaced centers for microscopic examination. This innovative method mounts particles in ordered arrays rather than the random chaos of usual methods and therefore simplifies particle examination, identification and analysis. Advantages include the ability to easily mount single particles in controlled patterns and known locations, to mount tight clusters of 2 or more particles per center (Fig. 3); to repeatedly mount the same pattern/number of particles; to mount particles in unusual orientations (Fig. 5, 6) and to easily get the average weight of single particles. Examples of applications include mounting and examination of ores (Fig. 1), pollens (Fig. 2, 3), milled products, seeds (Fig. 4) and crystalline products (Fig. 5, 6).

The mounting medium consists of a pattern of fine adhesive centers typically on 1 by 3 inch glass slides or on clear plastic film that is easily cut to any size or shape. Many other substrates are possible and the size and shape of the adhesive centers and their spacing are adjustable. Dots as small as 13 um have been produced. To facilitate mounting, holders have been designed that hold the slides and contain the particle sample as it is applied to the medium. The process is as simple as dusting the particles on the medium, rocking the particles back and forth briefly, and tapping off the excess.

The method is well suited for 25 to 1000 um particles. Mounting thousands of nontouching particles one-to-a-center in a regular array on a slide greatly facilitates particle counting, automated image analysis, preparation of standards and analysis for quality assurance and control. For singular attachment the ratio of the largest particle to smallest should be three or less and the particles should be larger than the dot diameter. For round particles, ratios of particle to dot diameters of 1/1 to 4/1 give greater than 90% singular attachment. For crushed, rough particles the ratio should be 3.0 or greater for singular attachment. For mixtures of similar particles the mounting process is not critical. For mixtures of particles with different properties, the sample should be split to obtain representative samples and attached under kinetic control where all particles attach upon initial contact and no exchange occurs. Low attachment velocity and increasing tackiness of the centers favor kinetic control.

By mounting in an ordered pattern, each particle can be identified by coordinates and examined by multiple techniques. By weighing slides before and after mounting particles and easily counting the number of particles in the regular array an average weight per particle is determined. By turning the tacky dot slide and the mounting holder upside down and shaking up and down so the particles hit the inverted slide vertically, a high number of edge and corner mounts are obtained (Fig 5, 6). Tacky dot patterns can be prepared by a variety of methods. Du Pont has several technologies and products for making tacky patterns by photo imaging that we find convenient. 1,2

References:

1. U. S. Patents no. 3,649,268; 4,174,216; 4,282,308.
2. F. A. Raymond and W. R. Hertler, J. of Imaging Science and Technology, 36 (1992) 243.

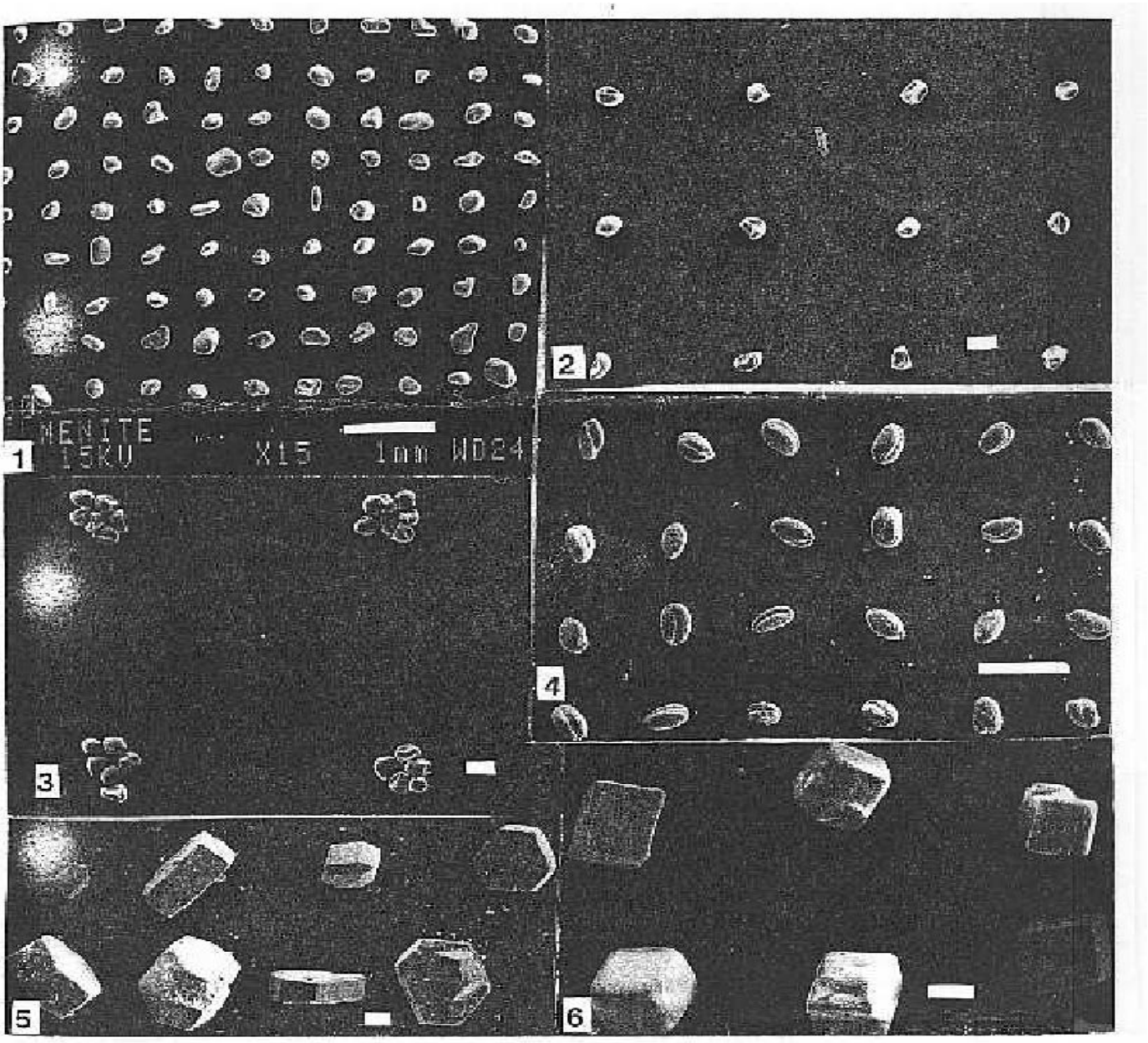

FIG. I . —SEM of ilmenite ore on 75 um tacky dots/ 500 um spacing. Bar = 1 mm
FIG. 2. —SEM of 90 um corn pollen on 40 um tacky dots/ 500 um spacing. Bar= 1 mm.
FIG. 3. —SEM of clusters of 90 um corn pollen on 150 um tacky dots/ 1000 um spacing. Bar = 100 um.
FIG 4 —SEM of arabidopsis seeds on 150 um tacky dots/ 1000 um spacing. Bar = 1 mm.
FIG 5 —SEM of sodium sulfite on 75 um tacky dots/ 500 um spacing, corner, edge. face mounting. Bar = 100 um.
FIG 6. —SEM of sodium chloride on 75 um tacky dots/ 500 um spacing, corner and face mounted cubes. Bar= 100 um.

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Scanning Electron Microscope Magnification Calibration Interlaboratory Study

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The National Institute of Standards and Technology (NIST) is in the process of developing a new scanning electron microscope (SEM) magnification calibration reference standard useful at both high and low accelerating voltages. This standard will be useful for all applications to which the SEM is currently being used, but it has been specifically tailored to meet many of the particular needs of the semiconductor industry. (4,5) A small number of test samples with the pattern were prepared on silicon substrates using electron beam lithography at the National Nanofabrication Facility at Cornell University. The structures were patterned in titanium/palladium with maximum nominal pitch of approximately 3000 um scaling down to structures with minimum nominal pitch of 0.4 um. Several of these samples were sent out to a number of university, research, semiconductor and other industrial laboratories in an inter-laboratory study. The purpose of the study was to test the SEM instrumentation and to review the suitability of the sample design. The laboratories were asked to take a series of micrographs at specific magnifications and accelerating voltages designed to test several of the aspects of instrument performance related to general SEM operation and metrology. This study represents data from a pool of 35 participating laboratories representing a total of 49 instruments.

A number of studies were done on the data submitted. (6) These studies included: analysis of the micrometer marker relative to the image, measurement of the image magnification measurement of the X and Y squareness, and analysis of the accelerating voltage compensation. The analysis of the micrometer marker length to the measured image of the prototype sample was made from the submitted micrographs. Overall, most of the SEMs involved in this study demonstrated some error in the adjustment of the micrometer bar. This is a very difficult adjustment to make since it is generally made directly from the micrograph, often from a relatively short fiducial line. Box plots of the percent error demonstrated by all the instruments of this study relative to the magnification range (for all accelerating voltages reported) are shown in Fig 1a. The mean of the error of these measurements was 2.23% with a standard deviation of +13.01%.

The actual magnification of the micrographs was also determined and compared to NIST measurements of the same sample in another portion of the study. The graphical representation of the magnification error is shown in Figure 1b. This figure represents box plots of the magnification error data obtained from all the instruments. In this figure, the mean of the error of these measurements was 1.77% with a standard deviation of +12.03%.

It can be concluded from this study that the NIST calibration sample could be issued without further modification, but several excellent suggestions made by the participants of the study will be incorporated in the final issued version. However, even with the issuance of an accurate standard capable of meeting the needs of modern SEM use, the coarseness of the calibration system of the instrument will become the limiting factor to the accuracy of the magnification calibration procedure.

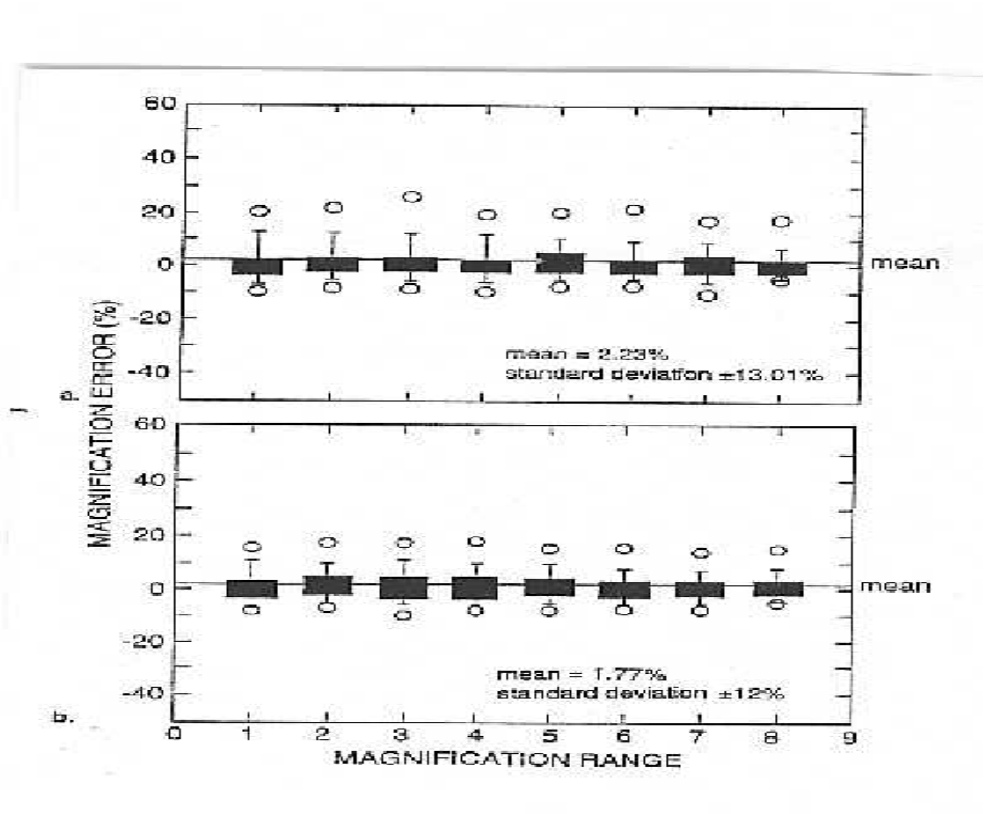

FIG 1.— SEM magnification calibration error. (a) Measurement of the error of the image magnification to the micrometer marker (b) Measurement of the error of the magnification to the NIST measurements of the same sample.

References

1. Guest Researcher from the Research Institute for Technical Physics of the Hungarian Academy of Sciences
2. Contribution of the National Institute of Standards and Technology. Not subject to copyright.
3. M.T. Postek and R.C. Tiberio, Proc. Ann. EMSA Meeting, 46(1988)198-199.
4. M.T. Postek, Scanning Microscopy 3(4),(1989) 1087-1099.
5. M.T. Postek et al., NIST J. Research, in press.

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