Modern Microscopy on the Light Side: TEM in the Trenches

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Great strides have been made in the last decade in high resolution transmission electron microscopes (TEMs) which can also provide elemental information via energy dispersive X-ray analysis (EDX) or energy loss spectroscopy (EELS), and proponents of various TEM techniques make bold claims. Convergent beam electron diffraction and microdiffraction shine as techniques for defect structure analysis and means for solving crystal structures. The spectroscopies can now be used to map chemical state information at a level which until recently might be encountered in science fiction. As a pure imaging device, electron holography holds great promise for providing the ultimate (would you believe 0.1Å?) imaging resolution. Although conventional TEMs will never approach this, it appears that we are learning more and more about less and less, until we will soon know everything there is to know about nothing. What about the person in the trenches, battling not on the frontiers of technique development, but with the samples of industry?

Certainly there are impressive capabilities available, but for most materials science applications the real power of the technique resides in the complementary nature of transmission electron microscopy with respect to light microscopy and scanning electron microscopy, in its ability to determine structure and composition on a scale surpassing the other two. What about the efforts to apply the techniques to samples which are neither routine nor ideally suited to test the limits of the characterization front? What about the microscopist who, although appreciating the improvements in resolution and ease of use of today's TEMs, finds little practical advantage? For instance, there may be minimal benefit to a 1.8 Å point-to-point resolution if the specimen requiring characterization is a scraping of corrosion debris or a collection of untidy minerals. Here, the specimen itself is more often the limiting factor in obtaining satisfactory results, rather than the microscope. For this, the proper preparation is paramount. We look at such real samples all the time, and constantly struggle with the limitations of the specimen. When the sample of interest is a wipe from a crime scene, it is unlikely that large-angle convergent beam electron diffraction or extended energy loss fine structure analysis will be techniques of first choice.

For microscopy, just as in any other line of research, we normally do not have the luxury to pursue answers until the instrumental limitations are reached. More mundane constraints such as time and money loom large, and often make the decision for us. So when do we stop gathering information, as with a biomedical client whose materials are not straightforward, as is often the case with real world materials such as corrosion products? Apparently non-crystalline according to diffraction observations, but of variable composition within fairly well-defined limits according to EDX, his samples posed an interesting problem. At what scale does heterogeneity end and homogeneity begin? The practical problem became: When should he stop upping the analytical ante and give up? We had hoped to arrive at the happy conclusion of discovering end-members to account for the range of compositions observed, but unfortunately this did not happen. The solution for him became clear after a morning's work on the 4000FX, and he was comfortable stopping at about a hundred Å spatial resolution of analysis, although we did not succeed in determining the precise scale on which the compositional variation was operative.

Another interesting question addressable with TEM concerns where we can draw a line between crystalline and non-crystalline. We are currently dealing with an interesting set of mineralogical specimens which have large amounts of disorder. Some may be non-crystalline altogether, and all of them suffer to a great extent from beam damage. Powder X-ray diffraction provides important clues to the structure of the phases, but is for these materials mostly a fingerprint, and the disorder and small particle sizes seem to require high resolution TEM for elucidation. This is a case where iimaging resolution

Sample Preparation: Observations in Light Microscopy

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This is the first in a series of articles relating to sample preparation. Following articles, by knowledgeable individuals in the field, will cover all aspects of microscopy. We understand that some readers may find the articles somewhat basic and expect that others may take exception to some approaches. We hope, however, that the series will stimulate interest and the exchange of ideas in this critical aspect of microscopy.

Have you noticed that some biologists first want to look at their samples at high magnification - and find the fine details quite fascinating? The problem with this approach, of course, is that the understanding of the structure should begin by simply looking at it in its' natural form, as a whole, and not just at a small part. Aspects such as color, density, size, environment, mobility, etc. play a large part in understanding the overall structure and are critical in fully understanding a delicate structure. Have you ever looked at an electron micrograph even at 2,000 times magnified and said to yourself "what is this"? Yes, that's an experience we've all had. So let us consider what one may do to first examine the specimen at low magnification.

Many low power observations, looking at the sample in it's own environment, can be done with simple lenses. In these cases one may use some very simple techniques: a drop of methyl cellulose placed on a slide

improvements are beneficial, but nonetheless, the sample itself is the main stumbling block in obtaining desired information.

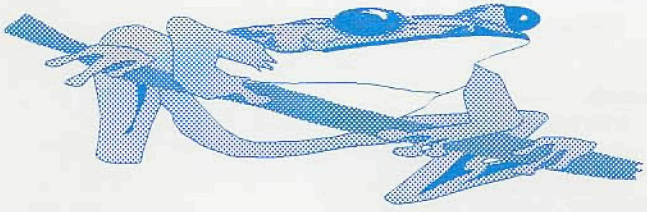
Admittedly, with TEMs we can do wonderful things:

* like with X-ray diffraction, we can obtain structural information about the crystalline structure of materials, but at smaller scales.

* like with X-ray photoelectron spectroscopy, we can obtain spectroscopic information from EELS which reveals much about the chemical environment of the elements of interest, and

* like with other chemical analytical methods, we can determine the chemical composition of materials, but at higher spatial resolution.

However, it would be well for the analytical microscopist who works at lower resolution to keep in mind that although we can seemingly answer almost any analytical question with the modern TEMs, provided we are able to put the time and effort and dollars into it, the specimen will often determine the extent of progress. Usually the sample is in that vast middle ground where it requires a great amount of effort to characterize, cannot itself be modified to suit the analysis because it is strictly constrained by a process or synthesis or accident, but does not merit a research project. The key is not always in extracting one more nanometer-worth of information, but in picking the right aspect of a problem to attack, so that when the specimen, which is never quite thin enough, or pure enough, or stable enough under the beam, or of sufficient crystal size, or sufficient contrast, or....perhaps you can add some more here. And if the sample does not decide for us, the accountants will. ■

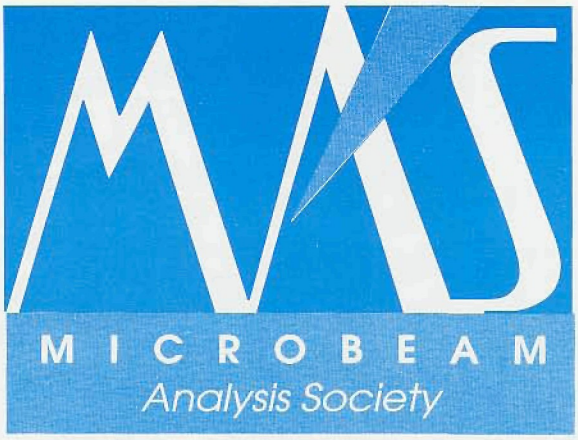


with a protozoa solution in order to slow movement and make observation easier, a thin layer of a transparent glue when spread on a slide or dish can hold an ant still for viewing, a vital stain ingested by an amoeba can be seen easily at low power, a blood smear stained with Wright's stain for better viewing of the various types of cells, or perhaps a few of one's own cheek cells could be spread on a slide, then a coverslip added with a moderate amount of pressure applied and one can see single cells spread across the field of view. These cheek cells may not be readily visible but they are an example of looking at a living cell with a minimum of preparation. One can see movement within the cytoplasm if you look closely. To improve the image, a drop of vital stain (non-toxic) can be added to the slide and, as the cells absorb the dye, they can be seen quite readily. Other ways of improving the knowledge of a sample can come from using various forms of illumination with varied combinations of light through the microscope. I will mention only two of these excellent lighting methods. The first is called phase contrast microscopy. Here the light rays are diffracted by the components of the specimen and those that pass through are given an extra path difference. The result is that the details of the specimen stand out quite clearly. One can see the sample without staining and therefore living cells can be observed without any destructive processing. The second outstanding method is a differential contrast method named after it's originator, G. Nomarski. In this case, the subject is illuminated by polarized light which is separated into two beams by a Wollaston prism. The beams are then combined by a second prism and a three dimensional image can be seen. The effect is outstanding! All of the methods and techniques mentioned above are reliable and efficient when used on living cells or tissues. They permit us to see the specimen in it's natural form - without any chemical processing.

Techniques used in the research laboratory are more complex and require a knowledge of chemistry, safety procedures, and specific information about the particular samples that are to be processed. In fact, these processes are fundamentally destructive to the sample. We would like to think that they preserve the sample in it's natural form, but we know that these chemicals must have a dilatory effect on some components. The only thing that can be said in their defense is that, over years of use, results have been obtained that verify structure by various techniques, in vivo and by processing - even with electron microscopy. I realize that this subject tends to be controversial and will leave future review to others.

For light microscopy specimen preparation, one should obtain fresh tissue and process the tissue as soon as possible. The most common fixative for light microscopy is Formalin. It can be used alone but is often used in combination with other ingredients including calcium, picric acid, and mercuric chloride. Such additives can act as fixatives themselves by preserving components other than those effected by Formalin. These can make a tissue easier to work with, be less brittle or perhaps even allow better contrast. The sample used for light microscopy remains in the fixative for fairly long periods of time depending upon it's size and density. If the tissue pieces are of conventional size, 3-5 mm, it may take three hours to days to fix. Larger samples may take days to months.

Pieces are washed after fixation in water or buffer overnight. Dehydration follows by immersing the sample in ascending alcohol baths from 50-100 %. Xylene or Toluene soaking followed by infiltration with paraffin takes place next. The transition into paraffin is a slow process which starts in a dilute combination of Xylene and paraffin chips. Finally the sample is placed in pure paraffin in an embedding oven and soaked for several hours. The sample is changed through several baths of fresh molten paraffin and then left to harden. The hardened blocks are timed to the tissue area and then sliced on the microtome at 7-14 µm thickness. Sections are recovered as they come from the microtome blade, are mounted on slides with mounting media and a coverslip. They are stored at this point or processed through staining. Many stains have been developed and are selected by the biologist or physician according to particular needs. For example, if the carbohydrate content of the tissue is of interest, a Periodic-Acid-Schiff stain might be used. The most common stains used for general morphology are a combination of Hematoxylin, which stains the nucleus, and Eosin which stains the cytoplasm. One should refer to texts on Histology or Histochemistry for further information. ■



Dear Colleagues:

Our Microbeam Analysis Society is pleased to announce and invite you and your family to attend this years meeting to be held at Loyola Marymount University in Los Angeles.

Our local arrangement committee has been hard at work putting this national meeting together for the past three years.

Loyola Marymount is an ideal setting for a MAS meeting as it is located very close to the beach, Marina Del Rey, and will be completely dedicated to our meting. The week will include several socials, beginning with a Fiesta welcoming mixer and our main event planned for Knott's Berry Farm amusement park.

Our program chairs are offerring what they refer to as a "diverse program" - which I am sure is an understatement.

There will be tutorial workshops, the AEM joint session on X-ray spectrometry, and the Chuck Fiori Memorial Symposium, not to mention the Presidential Symposium - Microbeams: here, there, and everywhere.

Our L.A.C. Committee has done and is continuing to do an outstanding job to provide us with a very memorable scientific and social event. Just add water and heat.

All we need now is you, our members.

Sincerely,

Thomas G. Huber, President
Microbeam Analysis Society

Preliminary Program

SUNDAY MORNING, JULY 11
- Short Courses:
  1. Image and Spectra Processing for Small Computers.
  2. Quantitative Electron Microprobe Analysis.
  3. Material Failure Analysis.
  4. Semiconductor Sample Preparation.
  5. Micro FT-IR.
  6. Specimen Preparation for AEM.

MONDAY MORNING, JULY 12
- MAS Presidential Symposium; Here, There and Everywhere.
- MAS Awards Ceremony.

MONDAY AFTERNOON, JULY 13
- MAS Plenary Lecture.
- Joint MAS-AEM Symposium - Chuck Fiori Memorial Symposium: X-ray Spectrometry in Electron Beam Instruments I. EDS.
- MAS: Microbeam Mass Spectrometry
- AEM Workshop: Quantitative EELS - I.

TUESDAY MORNING, JULY 13
- Joint MAS-AEM Symposium - Chuck Fiori Memorial Symosium: X-ray Spectrometry in Electron Beam Instruments II. EDS.
- MAS: Aerospace Materials.
- MAS: STM/AFM.
- MAS: New and Emerging Techniques.
- AEM Workshop: Quantitative EELS - II.

TUESDAY AFTERNOON, JULY 13
- MAS: Image Analysis and Compositional Mapping.
- MAS: Biological Microanalysis: I. X-ray Microanalysis.
- Joint MAS-AEM Symposium - Chuck Fiori Memorial Symposium: X-ray Spectrometry in Electron Beam Instruments III. EDS.
- MAS Symposium: Micro - X-ray Diffraction and Fluorescence, I.
- AEM Workshop: CBED Symposium.

WEDNESDAY MORNING, JULY 14
- MAS: Quantitative Microprobe Analysis, I.
- MAS: Biological Microanalysis: II. Imaging SIMS.
- MAS Symposium: Micro - X-ray Diffraction and Fluorescence.
- AEM Workshop: Quantitative STEM Imaging.

WEDNESDAY AFTERNOON, JULY 14
- Afternoon and evening at Knott's Berry Farm.

THURSDAY MORNING, JULY 15
- MAS: Quantitative Microprobe Analysis, II.
- MAS: Quantitation in the Environmental SEM.
- MAS: Materials Applications.
- MAS: Micro - X-ray Diffraction and Fluorescence, III.
- AEM Workshop: Biological Microanalysis - I.

THURSDAY AFTERNOON, JULY 15
- Joint MAS - AEM Symposium - Chuck Fiori Memorial Symposium, X-ray Spectrometry in Electron Beam Instruments IV. WDS.
- MAS: Particles, Geological and Environmental Applications.
- MAS Symposium: Strategies of Surface Analysis.
- AEM Workshop: Quantitative EDS - I.
- AEM Workshop: Biological Microanalysis - II.

FRIDAY MORNING, JULY 16
- Joint MAS - AEM Symposium - Chuck Fiori Memorial Symposium: X-ray Spectrometry in Electron Beam Instruments V. EDS.
- MAS Symposium: Micro-FT-IR Analysis.
- AEM Workshop: Quantitative EDS - I.

Should one wish more details about the program, meeting location, or registration, please contact Jack Worrall (213)740-1990. Don't forget the deadline for pre-registration is June 10. Registration after that date is $50 higher. Send in your forms soon and save $$$!

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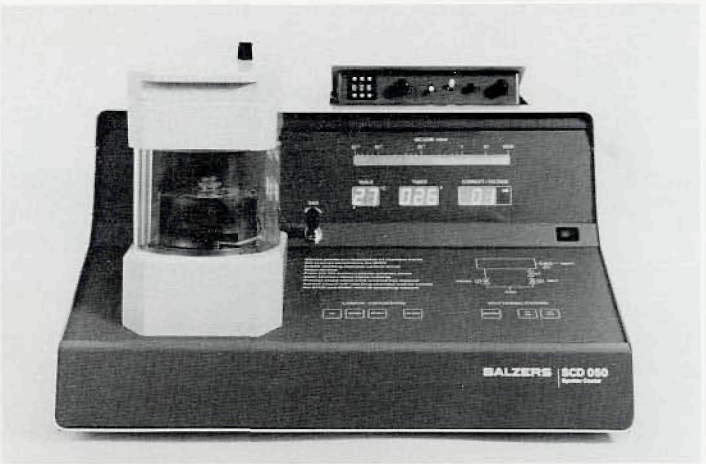

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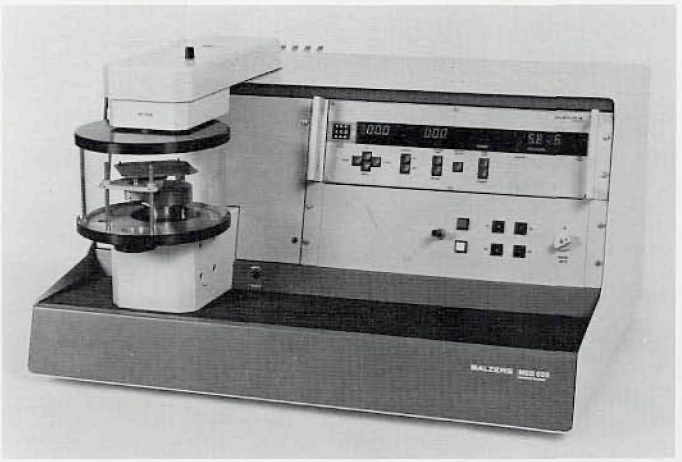

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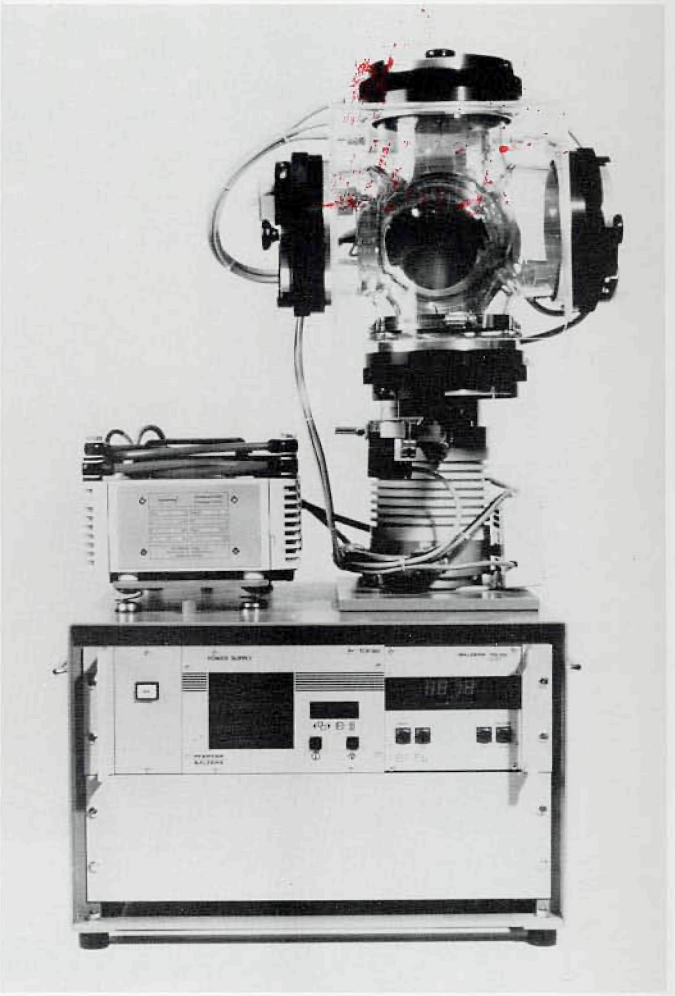

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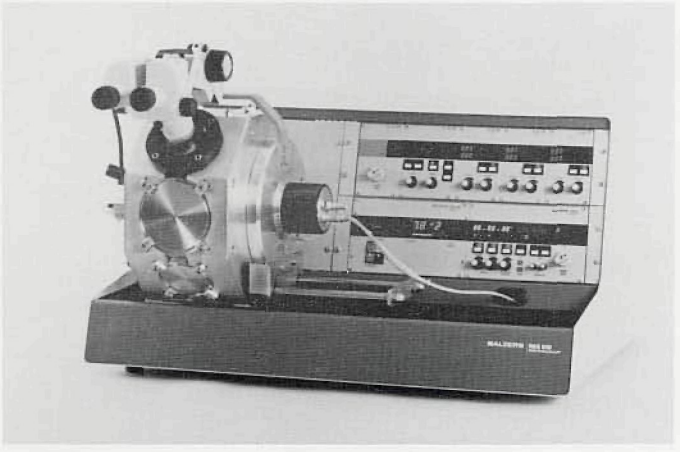

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