Endless Possibilities...
EMS Microscopy Academy
Soya Bean Leaf, colorized

Phenacetin sublimate, crossed polars with lambda waveplate. Shows utility of thermal microscopy in solid-state analysis.

Fusion of acetyl salicylic acid with phenactin. Acetylsalicylic acid is on the right and phenactin on the left. The eutectic forms in the middle mixing zone.
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**SEM image of primary drug particle.** Particle morphology can help understand pharmaceutical performance in some dosage forms.

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Electron Microscopy Sciences

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Giving Back To The Scientific Community

With deep appreciation for all we have accomplished in partnership with the scientific community, we are excited to announce the opening of our newest facility, the **EMS Microscopy Academy**.

We firmly believe in the endless possibilities of learning and working together. And so we have built a dedicated space where we can offer an ongoing series of workshops and training courses covering all fields of microscopy, including materials and biological science.

Here you can take advantage of state-of-the-art equipment and hands-on training led by our certified faculty. Our spacious labs have been set up to provide you with what we sincerely hope will be a valuable learning experience, both practically and theoretically.

This Course Catalog provides an introduction to our current offerings, including full workshops as well as shorter personal courses. We also give equipment demonstrations.

Most importantly, we want this to be about you! This Course Catalog is just a starting point. We welcome your ideas and suggestions. Whether it’s a full workshop, short course, or equipment demonstration, let us know what you want.

We hope to see you soon.

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The EMS Microscopy Academy is located next to our main facility in Hatfield, PA, just minutes from Philadelphia.
Faculty

**Al Coritz, Electron Microscopy Sciences, Hatfield, Pennsylvania, USA**
Al has been doing Electron Microscopy for 38 years, beginning at the Yale School of Medicine and ending up on the commercial side with several key EM companies. His specialty is Cryo-techniques and Thin Film Technology: i.e. Freeze Fracture/ Rotary Shadowing, High Pressure Freezing, and more. He is currently with Electron Microscopy Sciences where he has been the Technical Director for over 20 years.

**Helmet Gnaegi, Diatome, Ltd., Biel, Switzerland**
Helmet’s background is in engineering and he is one of the founders of Diatome, the leading supplier of diamond knives and related accessories, such as ionizers and manipulators, etc. He is also one of the leading instructors for ultramicrotomy courses (Biological and Materials) around the world.

**Michael Kostna, Electron Microscopy Sciences, Hatfield, Pennsylvania, USA**
Michael was the program director of the Electron Microscopy Technician program at Madison Area Technical College and has more than 35 years in EM technical education and research experience. He has been training EM students for 29 years and has developed curricula and lab exercises for TEM, SEM, OLM, lab safety, introductory and advanced biological EM, EM, maintenance, and x-Ray microanalysis. He has worked with companies such as SC Johnson Polymer, Dow Chemicals, Io Genetics, Virent Technologies, ABS Global, NanoOnocology, and Microscopy Innovations, and in the process gained insight to the various applications of EM.

**Peter van de Plas, Aurion, Wageningen, The Netherlands**
Peter has a background in histology and immunocytochemistry and joined Aurion in 1991. During the pioneering phase of Aurion in the early nineties, he worked closely with Dr. Leunissen in founding a firm basis for Aurion. He contributed not only to the development of product applications, but also in designing the Aurion Immuno Gold Silver Staining workshop. He has been invited to many international microscopy conferences and workshops, and is especially experienced in providing hands-on training. Former workshop attendees and customers appreciate Peter for his technical support on, and his thorough knowledge of, the Immuno Gold Silver Staining techniques. The Aurion Immuno Gold workshops in Europe, Asia, Australia, and the US have all been fully attended and very well received.

**Robert Carlton**
Robert worked for nearly 40 years in the research and development of fiberglass insulation, orthopedics, and pharmaceuticals. His specialty is solid-state analysis with a particular interest in microscopy. Robert retired from full-time employment in early 2016. He is now teaching microscopy and consulting on solid-state analysis in pharmaceutical development. Robert’s education is in chemistry, with a Ph.D. from Lehigh University. He has taken numerous courses at McCrone Research Institute on microscopy from Skip Palenik and Walter McCrone. Robert worked for pharmaceutical companies Rhone-Poulenc Rorer (Aventis, Sanofi), Elan (Nanocrystal), and GlaxoSmithKline in microscopy and solid-state analysis for 24 years. He published a book on Pharmaceutical Microscopy in 2011 with Springer.
Scope of Class

The objective of the course is to provide researchers with the opportunity to learn the theory and practice of Immuno Gold labeling. Participants will process their own samples under the expert guidance of our tutors, who are experts in Immuno Gold Silver Staining techniques.

During the workshop attendees will receive theory, including but not limited to immune detection, in situ hybridization, silver enhancement, as well as background issues. There will be time for practice as well. Attendees will be able to work with their own specimens, as well as ones we will have prepared. A full review of incubation methods, testing of antigenicity and reactivity, complete principles of Immuno Gold labeling, and preparation of conjugates for EM and LM will be covered.

Format

Lecture, demonstration and hands-on practice, as well as round table discussion. Participants may bring their own samples to work on during lab time.

Note: Specimens need to be “ready to use”, e.g., sections of chemically fixed cells on coverslips. For each combination of primary antibody and gold conjugate that you want to test during the workshop, you need at least 3, preferably 4, specimens. This enables you to test different dilutions of the primary antibody and to check the Immuno Gold/Silver reagents (negative control).

For pre-embedment immuno labeling, also include specimens that will be immuno labeled for evaluation on the light microscope. These specimens are used to see if the experiment is successful. During the workshop there is no time/no options to further process pre-embedment specimens for EM evaluation. Plastic embedment and sectioning needs to be done at your own facility.

Main Curriculum

- The properties of gold particles and their protein conjugates
- Theory underlying Immuno Gold labeling protocols
- Silver enhancement of gold particles
- Immuno Gold labeling on a variety of sample preparations of OLM
- Immuno Gold labeling for EM
- Pre-embedment Immuno Gold labeling using ultra small gold conjugates and silver enhancement
- Post-embedment Immuno Gold labeling on plastic and Tokuyasu cryo sections using conventional colloidal conjugates and ultra small gold conjugates
- Manual and automated Immuno Gold labeling
- Pre/Post-embedment double Immuno Gold labeling
- Background minimization in Immuno Gold labeling
- Troubleshooting

Facility

The EMS Microscopy Academy: Located in Hatfield, Pennsylvania, the Academy provides electron microscopy classes, workshops and training sessions for all fields of microscopy, including materials science and biological science.
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Aurion Immuno Gold Silver Staining

**Left:** Immuno Gold Silver Staining of E-cadherin on a paraffin section of human skin.

Courtesy of R. Moella, Dept. of Exp. Path., EUR, The Netherlands
- Mouse monoclonal anti E-cadherin,
- GAM IgG Ultra-Small,
- Aurion R-Gent SE-LM

Immuno Gold Silver Staining of alpha-amylase on Lowicryl HM20 section of rat pancreas.
- Goat-anti-Rabbit, 15nm
- Ultra-small R-Gent (Dansher Method)

Pre-embedding Immuno Gold Labeling of Huntington Interacting Protein 3 in Mouse Brain using Aurion GAR Fab-US and Aurion SEEM.

Courtesy of Ms. Hong Yi, Emory University, Atlanta, GA

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IGSS of tubulin on coverslip culture of PtK2 cells. Courtesy of Peter van de Plas, Aurion Costerweg 5, The Netherlands.

2 views of a model of a COPII-labeled ER-exit site, resolved from 400nm thick cryo-sections of Hepg2 cells, labeled like described for the ultrathin section before.

Note that the labeling for COPII is assessable throughout the section.

Courtesy of: Dagmar Zeuschner, Judith Klumperman (Department of Cell Biology, UMC Utrecht, The Netherlands) and Willie Geerts, Abraham Koster (Molecular Cell Biology, Utrecht University, The Netherlands)

ER = light blue, Free membrane carriers of vesicular and tubular shape, partially labeled for COPII = yellow, COPII = silver enhanced-red
Targeted Participants
Lab managers or technicians who are responsible for the processing of samples for TEM analysis and currently do not incorporate either a microwave or automated processor in their lab.

Scope of Class
The preparation of samples for EM requires many steps, with extended wait times in between, requiring an entire day of a technician’s time. This process consists of several fixative, wash, dehydration, and resin infiltration steps, which are tedious and prone to temporal variability between runs.

With the use of an automated tissue processor or microwave, a technician’s time is reduced and better continuity between processing runs is obtained.

This class will introduce participants to the use of a dedicated lab microwave, EMS 9000, and 2 models of automated tissue processors Lynx II, and Microscopy Innovation’s ASP 1000 for processing tissue samples for TEM.

Format
Lecture, demonstration, and hands on operation of systems used. Discussions about advantages and disadvantages, along with participant questions, are a significant component of this workshop.

Main Curriculum
- EM sample preparation requirements / time table
- Microwave theory and processing
- Lynx II setup and operation
- Microscopy Innovations ASP 1000 setup and operation

Facility
The EMS Microscopy Academy: Located in Hatfield, Pennsylvania, the Academy provides electron microscopy classes, workshops and training sessions for all fields of microscopy, including materials science and biological science.

Left: Glutaraldehyde-fixed kidney specimen
Above and Below: Eight mPrep/s capsules containing glutaraldehyde-fixed kidney specimens were mounted on the ASP-1000 before initiating the 45-minute processing program. Rapid processing, achieved by fluid exchanges occurring every half second, constantly delivered fresh reagent to the specimens.

-Microscopy Innovations Application Note #601
Targeted Participants

Individuals who currently perform or will be responsible for the preparation of samples and/or operation of the SEM in a research, academic, or industrial setting.

Scope of Class

The preparation of samples will start with determination of ROI and subsequent selection of gross cut orientation and determination of which dehydration technique to use: freeze drying, HMDS, Hitachi’s ionic liquid or critical point drying (CPD). The chemical processing required before CPD will be stressed. Special attention will be paid to orientation, stability and grounding of the sample when mounting to facilitate ease of imaging. The advantages, disadvantages and instrument requirements for the various coating materials: Au, Pt, Pt/Pd will be discussed.

Selection of accelerating voltage (kV) and spot size are critical for surface detail, resolution, and charging and will be covered in detail. Parameters such as working distance for depth of field (Df) and resolution, plus tilt and raster rotate will be examined for proper image collection.

Format

Lecture, demonstration and hands-on practice, as well as round table discussion. Participants are encouraged to bring their own samples, if possible.

Main Curriculum

- Identification and isolation of ROI
- Theory and hands-on chemical processing
- Theory and hands-on CPD, HMDS, and Hitachi’s ionic liquid
- Theory and hands-on freeze drying
- Mounting
- Theory and hands-on Sputter coating
- SEM theory and discussion of parameters affecting image quality
- Demonstration and hands-on practice of SEM operation and effects of parameters
- Theory and demonstration and hands-on practice of variable pressure and Hitachi’s ionic liquid

Facility

The EMS Microscopy Academy: Located in Hatfield, Pennsylvania, the Academy provides electron microscopy classes, workshops and training sessions for all fields of microscopy, including materials science and biological science.

Faculty

Michael Kostrna
Al Coritz

Equipment

Hitachi S3500 SEM
EMS 850 Critical Point Dryer
PP3010T Cryo Preparation System
TES 150T ES Sputter Coater

This course will introduce participants to methods of sample preparation and SEM parameters and operation needed for accurate analysis.
Endless Possibilities...

**Biological SEM**

- **Knot of human hair.**
  
  Courtesy of Frans Holthuysen

- **Aphid on white poplar.**
  
  Courtesy of Riccardo Antonelli

- **Scanning Electron Microscope image of sunflower lower leaf surface.**

  Louisa Howard, Dartmouth College

- **Scanning Electron Microscope image of Nicotiana alata stem cross section.** Image shows outer epidermal layer, followed by the cortex and then large vascular bundles. The vascular bundles contain the phloem (nearest the cortex) and xylem.

  Louisa Howard, Dartmouth College

- **Human Blood Cells: Red blood cells/Platelets.**

  Louisa Howard, Dartmouth College
Targeted Participants
Individuals who are, or will be, responsible for chemical processing and sectioning of biological samples for TEM examination.

Scope of Class
The proper chemical processing of biological samples for TEM observation is essential to maintain representative morphology and ultrastructural detail. This course will cover the buffers, fixatives, dehydrants, and embedment resins most often used for EM, with their individual advantages and disadvantages discussed. The preparation of these various solutions, when necessary, will be calculated and preformed. The microwave will be utilized for all steps except polymerization. The need for specialized protocols when using specific tissues, such as myelinated nerve which requires extended infiltration, will be discussed. Epoxy (Embed 812) and acrylic (LR White) resins will be available. The TEM's ability to provide sub-nanometer resolution is dependent ultimately on sample thickness, typically 60 nm or less. To obtain sections of this dimension requires specialized equipment, ultramicrotomes, high quality diamond knives, and a skilled technician. The process of trimming, thick sectioning for OLM observation, thin sectioning, section retrieval, and section assessment will be the major focus of this workshop. Basic operation of the TEM will include specimen insertion, condenser and objective astigmatism correction, and critical focusing.

Format
Lecture, demonstration and hands-on practice, as well as round table discussion. Participants are encouraged to bring their own samples, if possible.

Main Curriculum
- Theory and practical preparation of buffers, fixatives, and other solutions required for chemical processing of biological samples for TEM
- Fixation theory and application of primary and secondary fixatives
- Dehydration, choosing the correct one, ETOH, Acetone, or propylene oxide
- Infiltration and choice of embedment resin, epoxy, methacrylate, or acrylic
- Embedment and maintaining orientation if applicable
- Block evaluation
- Trimming for thick survey and thin sections
- Practical aspects of microtomy and instrument parameters affecting section quality
- Adjustment of clearance angle and block face:knife edge alignment
- Thick (0.5 µm) sectioning and chromatic staining for OLM assessment
- Thin (≤ 60 nm) sectioning and section retrieval
- Post staining Pb and UA
- Section assessment and troubleshooting potential artifacts such as chatter, knife marks, and tears

Facility
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Biological TEM

Bone Marrow: Transmission electron microscope image of a thin section cut through an area of bone marrow near the cartilage/bone interface in a mouse kneecap. Image shows small opening in the thin endothelium of the vascular sinus wall, where a blood cell is crossing the thin vascular sinus wall and into the sinus lumen.

Louisa Howard, Dartmouth College.

Pancreas: Transmission electron microscope image of a thin section cut through part of a nerve cell and part of a capillary within the pancreatic tissue. The capillary lining consists of long, thin endothelial cells, connected by tight junctions. The image shows a fenestration in this endothelial cell. Basal lamina is present at the edges of the acinar cells.

Louisa Howard, Dartmouth College.

Transmission electron micrograph of Zebra Fish muscle.
Specimen courtesy of Dr J Leslie.

Transmission Electron micrograph of Zika Virus.
Virus particles are 40 nm in diameter, with an outer envelope and an inner dense core.
Courtesy of Cynthia Goldsmith, CD.

Transmission electron microscope image of a region in the Drosophila germarium. Specific cells in the germarium contain synaptonemal complex in their nuclei.
Targeted Participants

Individuals who are new to the field of cryo SEM or desire a technical refresh to maintain current skills or just those that want to see and learn all of the possibilities of the technology.

Scope of Class

Many types of samples such as ice cream, pastes, paints, and gels do not lend themselves to routine SEM sample preparation methods such as critical point drying or freeze drying due to the morphologic changes caused by desiccation. To avoid these artifacts it is necessary to image the hydrated or natural state. This requires that the sample be rapidly frozen, to reduce ice crystal damage, and fractured to reveal their physical / functional sub-surface morphology. These samples are applied to the stub, immersed in liquid nitrogen slush loaded into the Cryo fracturing and coating chamber, and finally the SEM. This final preparation takes place in a high vacuum environment thus minimizing the possibility of frost contamination. Within the SEM, while viewing, the sample temperature can be maintained at -130° or warmed slightly to facilitate sublimation of the surface. The selection of accelerating voltage (kV), for surface detail, and spot size for resolution and charging are critical and will be covered in detail.

Parameters such as working distance which affects depth of field (Df), and resolution, plus tilt and raster rotate will be examined for proper image collection.

Format

Lecture, demonstration and hands-on practice, as well as round table discussion. Participants are encouraged to bring their own samples, if possible.

Main Curriculum

- Theory and overview of cryo SEM
- Mounting and adhering
- Freezing, loading and fracturing
- In situ coating
- Operation of SEM
- Cryo face-off Leica UC 7 Crion
- Specific techniques of Cryo SEM imaging

Facility

The EMS Microscopy Academy: Located in Hatfield, Pennsylvania, the Academy provides electron microscopy classes, workshops and training sessions for all fields of microscopy, including materials science and biological science.

Left: Cryo-SEM prepared myosin (muscle protein)
Endless Possibilities...

Cryo SEM

**Foodstuffs**

Mayonnaise – fractured

Image courtesy of FEI Company

Soya Bean Leaf, colorized

Fungi

Fungal spore sac

Spore sac of a fungus. The sacs tend to burst open as soon as they are exposed to moisture, so the only way to visualize them is to use cryo-SEM. Image courtesy of Miranda Waldron at the Electron Microscope Unit at the University of Cape Town, South Africa

Carnivorous Plants

Sundew leaf and insect

A small fly trapped on a carnivorous South African sundew plant. The whole plant is about 3 cm across and has sticky hairs on its leaves to capture small insects. Image courtesy of Miranda Waldron at the Electron Microscope Unit at the University of Cape Town, South Africa

Vertebrates – Tissues

Frozen hydrated mouse lung

Low-temperature SEM retains all of the cellular and extra-cellular fluids in the lung tissue. Consequently, the micrograph clearly illustrates that the bronchioles are covered with a thin layer of mucus (marked by arrows). None of the alveoli show any collapse. Bar: 5μm

Fractures through the myocardium

Small pieces of heart have been mixed, washed in buffer then cryo-protected with 30% glycerol. Contracted myofilbrils can be seen in longitudinal aspect. The fracture in places has occurred along the sarcolemma and the positions of the Z-lines (marked by ‘z’) and the T-tubules (marked by arrows) are clearly visible in places. The intimate relationship between the capillaries and the myofilbrils can be appreciated from this preparation. The contents of the capillary have a smooth appearance due to the presence of glycerol. Bar: 5μm

Whipped Double Cream

Whipped double cream is another example of a fat-stabilized foamed emulsion. The first image shows that, at low magnification, milk fat droplets in the continuous phase are visible as 2-10 μm diameter particles (marked by arrows). Bubbles of a variety of sizes between 10-100 μm are also obvious. Bar: 100 μm (inset 50 μm). At higher magnification, the second image shows that fat can be seen associated at the air/liquid interface with a continuous layer of liquid fat at the surface (marked by small arrows). In places (marked by larger arrows), fat droplets can be seen to be coalescing. Over-beating would result in the emulsion in the continuous phase inverting.
Scope of Class

The objective of the course is to provide researchers with the opportunity to learn the theory and practice of the use of cryosectioning with diamond knives, and immunogold labeling. Participants will process their own samples under the expert guidance of our tutors, who are experts in cryosectioning and immunogold silver staining techniques. During the workshop, attendees will receive theory, including but not limited to, cryosectioning, immuno detection, in situ hybridization, silver enhancement, as well as background issues. There will be time for practice as well. Attendees will be able to work with their own specimens, as well as ones we will have prepared. A full review of sectioning, incubation methods, testing of antigenicity and reactivity, complete principles of immunogold labeling, as well as preparation of conjugates for EM, as well as LM, will be covered.

Format

Lecture, demonstration and hands-on practice, as well as round table discussion. Participants may bring their own samples to work on during lab time.

Note: Specimens need to be “ready to use”, e.g., sections of chemically fixed cells on coverslips. For each combination of primary antibody and gold conjugate that you want to test during the workshop, you need at least 3, preferably 4, specimens. This enables you to test different dilutions of the primary antibody and to check the immunogold/silver reagents (negative control).

For pre-embedment immuno labeling also include specimens that will be immuno labeled for evaluation on the light microscope. These specimens are used to check if the experiment is successful. During the workshop there is no time; there no options to further process pre-embedment specimens for EM evaluation. Plastic embedment and sectioning needs to be done at your own facility.

Main Curriculum

- Introduction in cryosectioning
- Theory and full review of Diamond knives
- Sectioning and practice with section pick-up, etc.
- The properties of gold particles and their protein conjugates
- Theory underlying immunogold labeling protocols
- Silver enhancement of gold particles
- Immunogold labeling on a variety of sample preparations of OLM
- Pre/Post-embedment double immunogold labeling
- Background minimization in immunogold labeling

Immunogold labeling for EM:

- Pre-embedment immunogold labeling using ultra small gold conjugates and silver enhancement
- Post-embedment immunogold labeling on plastic and ToKuyasu cryo sections using conventional colloidal conjugates and ultra small gold conjugates
- Manual and automated immunogold labeling
- Troubleshooting

Facility

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Endless Possibilities...
Cryosectioning/ Immunogold

Left: Immuno Gold Silver Staining of E-cadherin on a paraffin section of human skin.
Courtesy of R. Moella, Dept. of Exp. Path., EUR, The Netherlands
- Mouse monoclonal anti E-cadherin,
- GAM IgG Ultra-Small,
- Aurion R-Gent SE-LM

Immuno Gold Silver Staining of alpha-amylase on Lowicryl HM20 section of rat pancreas.
- Goat-anti-Rabbit, 15nm
- Ultra-small R-Gent (Dansher Method)

2 views of a model of a COPII-labeled ER-exit site, resolved from 400nm thick cryo-sections of Hepg2 cells, labeled like described for the ultrathin section before.
Note that the labeling for COPII is assessable throughout the section.
Courtesy of: Dagmar Zeuschner, Judith Klumperman (Department of Cell Biology, UMC Utrecht, The Netherlands) and Willie Geerts, Abraham Koster (Molecular Cell Biology, Utrecht University, The Netherlands)

Pre-embedding Immuno Gold Labeling of Huntington Interacting Protein 3 in Mouse Brain using Aurion GAR Fab-US and Aurion SEEM.
Courtesy of Ms Hong Yi, Emory University, Atlanta, GA

IGSS of tubulin on coverslip culture of PtK2 cells. Courtesy of Peter van de Plas, Aurion Costerweg 5, The Netherlands.

ER = light blue, Free membrane carriers of vesicular and tubular shape, partially labeled for COPII = yellow, COPII = silver enhanced-red
Scope of Class
Introduce individuals to the unique application of ultramicrotomy to materials, which provides several advantages over other common techniques such as ion milling, FIB, and tripod polishing for TEM analysis. The thin (≤ 30 nm) sectioning of metals, embedded powders, and polymers is a technique that provides samples with a uniform thickness, retention of elemental distribution, lack of ion implantation contamination, and proves to be much faster than other preparation methods such as ion milling, tripod polishing and FIB milling.

Format
Lecture, demonstration and hands-on practice, as well as round table discussion. Participants may bring their own samples to work on during lab time.

Main Curriculum
- Sample discussion/evaluation to determine method of support
- Embedment in Epofix or Cyanoacrylate glue, if necessary
- Trimming using razor blades, diamond trim blades, or the TXP for precision trimming
- Sectioning of brittle materials and brittle water sensitive materials at room temperature
- Sectioning of polymers at room temperature using an ultra sonic knife
- Cryo trimming and sectioning of softer polymers
- OsO₄ and RuO₄ staining of sectioned polymers

Facility
The EMS Microscopy Academy: Located in Hatfield, Pennsylvania, the Academy provides electron microscopy classes, workshops and training sessions for all fields of microscopy, including materials science and biological science.

Materials Ultramicrotomy Workshop
Three days of hands-on training for technicians, researchers, and students who want to apply a faster and cleaner preparation method that provides samples with uniform thickness, no embedded contamination, and is cheaper than a FIB.

Faculty
Helmut Gnaege, Diatome Ltd., Switzerland
Michael Kostma
Al Coritz

Equipment
Leica UC7
Leica TXP
Diatome Ultrasonic
Boeckler Autotome
DM4
Diatome Diamond Knives

Left: ABS, stained with OsO₄, sectioned at room temperature with the DIATOME Ultrasonic Knife, section thickness 50nm. Note the almost perfect spherical shape of the large rubber particles and the preservation of the inclusions inside. Also the smaller dense rubber particles are well preserved. B. Vastenhout, Dow Benelux N.V. Terneuzen, The Netherlands.
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Materials Microtomy

High Impact Polystyrene. Claudia Maymofler, TU Graz

GaAs-AlGaAs nanowires grown epitaxially on Si Substrate. Resin embedded and sectioned with a ultra 35° diamond knife; HAADF-STEM. Hanne Kauko, Dept of Physics Trondheim

Polycarbonate modified with rubber. Jens Sicking, Bayer Technology Services, Leverkusen

Polypropylene with montmorillonite clay nanoparticles. Sectioning with the DIATOME Ultrasonic Knife.

Zeolite USY30 Crystal morphology STEM Analysis. The Mesopores (2-50 nm) (left) and the Crystalline Micropores (0.7 nm) (right) are clearly visualized. Tom Wilhammar, Sara Bals EMAT, Antwerp

Sectioning brittle samples

Sectioning Metals

Cerium inclusions in a zinc coating. Philippe Steyer and Emile Calvie, INSA, Lyon
Targeted Participants
This course is intended for the intermediate level analyst who is skilled at some of the microscopy techniques but needs training in other techniques and in interpretation.

Scope of Class
This workshop is designed to introduce the major applications of microscopy in pharmaceutical development: polymorphism, particle size analysis, contaminant identification, and glass corrosion.

The workshop is conducted over three days with a mixture of theory, demonstrations and hands-on work. The course is designed to prepare the student to apply microscopy to solve solid-state pharmaceutical issues. The emphasis is on the practical use of the microscope.

Format
Lecture, demonstration and hands-on practice, as well as round table tips and tricks discussion. Participants are encouraged to bring their own samples, if possible.

Main Curriculum
- Polymorphism: using Polarized light and thermal microscopy
- IR and Raman Microspectroscopy
- Size and shape analysis: using OLM, SEM/EDS, and fluorescence with image analysis
- Contamination identification and glass corrosion analysis: using polarized and scanning microscopes
- EDS and Microspectroscopy

Faculty
Robert Carlton
Michael Kostrna
Al Coritz

Equipment
Polarizing Light Microscope
Hitachi S3500 SEM
Image Analysis Software
Linkam Thermal Microscope
Bruker Esprit (SDD)
IR and Raman Microscopes

The EMS Microscopy Academy: Located in Hatfield, Pennsylvania, the Academy provides electron microscopy classes, workshops and training sessions for all fields of microscopy, including materials science and biological science.

Left: Differential interference contrast (DIC) image of liquid drops on interior of parenteral glass vial. DIC is used to examine parenteral glass vials.
Endless Possibilities...
Microscopy of Pharmaceuticals

PLM image of anthraquinone cooled after thermal microscopy. Crossed polars with lambda waveplate.

SEM image of primary drug particle. Particle morphology can help understand pharmaceutical performance in some dosage forms.

Fluorescence microscopy image of a sectioned multivitamin tablet. Different imaging techniques can be correlated to better understand drug performance.

Energy dispersive x-ray chemical map of a sectioned multivitamin tablet.

SEM image of Teflon tape. Teflon is used in pharmaceutical manufacturing and is a source of contamination.

SEM image of interior glass surface showing evidence of parenteral glass vial corrosion (delamination).

Left: Uniaxial interference pattern used to determine optical crystallographic properties of polymorphs.
Targeted Participants
This course covers the basic microscopy techniques used in pharmaceutical development and is designed for the novice or beginner.

Scope of Class
Microscopy has a place in nearly all solid-state studies and is often a critical component to solving drug development issues.
For the optimum use of microscopy, one needs both good instrumentation and a skilled microscopist. This course is designed to teach the basic techniques and operations of pharmaceutical microscopy. We assume no prior knowledge of microscopy but a basic knowledge of solid-state pharmaceutical analysis

Format
Lecture, demonstration and hands-on practice, as well as round table tips and tricks discussion. Participants are encouraged to bring their own samples, if possible.

Main Curriculum
Use of the following techniques in solid-state analysis, particle shape and size studies, contaminant identification, and glass corrosion (delamination) assessments:
- Stereomicroscopy
- Polarized light microscopy
- Thermal microscopy
- Scanning electron microscope and EDS (energy dispersive x-ray spectroscopy)
- IR and Raman microspectroscopy
- Automated image analysis

Facility
The EMS Microscopy Academy: Located in Hatfield, Pennsylvania, the Academy provides electron microscopy classes, workshops and training sessions for all fields of microscopy, including materials science and biological science.

Left: Acetylsalicylic acid (aspirin) fusion preparation in crossed polars. Heated beyond melting point and then allowed to cool. Illustrates color progression of birefringent crystals.
Endless Possibilities...
Microscopy of Pharmaceuticals

Phenacetin sublimate, crossed polars with lambda waveplate. Shows utility of thermal microscopy in solid-state analysis.

Energy dispersive x-ray spectrum of a naproxen sodium tablet cross-section.

Carbamazepine Form 3 twinned crystals by optical microscopy.

Chemical Map (sodium and oxygen) of naproxen sodium tablet cross section overlaid on backscatter electron image.

Carbamazepine Form 3 twinned crystals by SEM. Optical microscopy and SEM allow for unique views of crystals.

Left: Polypropylene fibers under crossed polars on optical microscope. Such fibers are a common contaminant in pharmaceutical products.
Targeted Participants

This course is designed for the skilled analyst who is familiar with the basic pharmaceutical microscopy instruments. The emphasis of the workshop is on the practical application of microscopy in the analysis of polymorphism.

Scope of Class

The choice of the optimum solid-state form is critical for successful pharmaceutical development. Broadly defined, form includes polymorphs, hydrates, solvates, salts, co-crystals etc. Microscopy is well-suited to the study of solid-state form.

This workshop covers the use of the microscope in both early pharmaceutical development when the form is chosen and in later stages of development where the form is monitored for stability.

It covers the material in Chapter 8 Pharmaceutical Microscopy (Springer, 2011) along with selections from other chapters. The course emphasizes how microscopy is used in conjunction with other analytical techniques for form selection and analysis.

Format

Lecture, demonstration and hands-on practice, as well as round table tips and tricks discussion. Participants are encouraged to bring their own samples, if possible.

Main Curriculum

The material in this workshop is broadly separated by development phase into form discovery and form understanding. Form discovery usually occurs before first time in human (FTIH) into Phase 1. Form understanding generally occurs in Phase 1 and Phase 2, although some work extends into Phase 3.

This workshop emphasizes the role of microscopy in each aspect of form understanding. Although we will discuss solid-state theory and fundamentals, the workshop relies heavily on case studies, demonstrations, and instrument usage.

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Faculty

Robert Carlton
Michael Kostma
Al Coritz

Equipment

Polarizing Light Microscope
Hitachi S3500 SEM
Image Analysis Software
Linkam Thermal Microscope
Bruker Esprit (SDD)
IR and Raman Microscopes

Left: Acetylsalicylic acid (aspirin) fusion preparation in crossed polars. Heated beyond melting point and then allowed to cool. Illustrates color progression of birefringent crystals.
Caffeine Form 2 by polarized light microscopy with crossed polars and a full lambda waveplate. The large blue particle is cellulose.

Benzyl fusion preparation by PLM in crossed polars. Benzyl exhibits anomalous interference colors which can be used for form identification purposes.

Schematic energy vs. temperature diagram for three polymorphs. Forms I and II are enantiotropically related whereas forms I and III are monotropically related. Forms II and III are also monotropically related. Thermal microscopy can be used to generate such diagrams.

RG12525 Form 1 by SEM. The Form 1 habit is acicular and could not be altered using any of the attempted recrystallization techniques. Milling produced small fibers with poor flow properties.

Caffeine Hydrate by SEM. Caffeine hydrate readily converts to the metastable Form 1 which, in turn, converts readily to Form 2 - the stable form at ambient conditions.

RG12525 Form 2 by SEM. The Form 2 habit is twinned blocks. Milling produced small irregular particles with good flow properties.

Left: Fusion of acetyl salicylic acid with phenactin. Acetylsalicylic acid is on the right and phenactin on the left. The eutectic forms in the middle mixing zone.
Targeted Participants

Individuals who are, or soon will be, expected to operate an SEM, choose appropriate parameters for EDS, and perform qualitative and semi quantitative analysis on materials samples.

Scope of Class

The nondestructive elemental identification of a sample’s micro-composition is a powerful tool for the microscopist. This technique can detect elements from boron to uranium with a minimum concentration detectability of 1000 ppm in solid samples.

Collection parameter settings of both the EDS system and microscope, their effect on the spectrum and quality of the subsequent quantification are of primary importance. The non-variable parameters of working distance and tilt will be demonstrated as well as the effect of accelerating voltage on background shape, x-ray spatial resolution, over-voltage requirements, and accuracy of ZAF matrix corrections examined. With the advent of the silicon drift detector (SDD) the pulse processor time constant and beam current (spot size) settings to control % dead time are almost a moot point but will be introduced for those who work with a SiLi detector.

Identification of individual elemental lines as well as methods used for determining peak overlaps such as peak shape, peak family ratio anomalies, and the presence of a peak unassociated with known elements will be paid particular attention.

Quantitative analysis will be limited to the use of ZAF and PhiRhoZ routines but the collection of standards and their use in a full quant will be discussed. Backscattered (BSE) imaging will be correlated with x-ray maps and spectral imaging results. Energy calibrations will also be preformed.

Format

Lecture, demonstration and hands-on practice, as well as round table discussion. Participants are encouraged to bring their own samples, if possible.

Main Curriculum

- Generation and nomenclature of x-ray lines
- Spectral artifacts
- Deconvolution of peak overlaps
- Qualitative analysis
- Semi quantitative analysis
- Hardware settings/function
- Setup and operation of SEM for BSE imaging and spectral acquisitions
- Sample requirements for BSE / EDS

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Left: X-ray analysis of fluids: oxygen (green) and carbon (red), in chalk rock saturated with brine and oil.
Equipment Demos

All Available Equipment:

- LYNX II Tissue Processor
- EMS 9000 Microwave
- EMS 820 Precision Pulsed Microwave
- EMS Poly III
- LatticeAx™
- RMC/Boeckeler Power Tome PC
- DiATOME Diamond Knives
- EMS Family of Oscillating Tissue Slicers
- Vibrating Microtomes
- EMS-002 Rapid Immersion Freezer
- Edge® 3D Microscope
- NIGHTSEA™
- Leica EM UC 7
- Hitachi 7700
- Hitachi SU 3500
- Bruker Esprit
- X-Flash SDD
- EMS GloQube
- Evactron® Decontaminators
- Thermocirculator and Recirculating Heater/Chillers
- EMS Coolstage for SEM, LV or VP
- EMS 850 CPD
- EMS 750 FD
- EMS 150GB
- EMS PP 3010 T
- Specimen Transfer Systems
- Mini Coater and Carbon Attachment
- Rotary-Pumped Carbon and Sputter Coating System
- Turbo-Pumped Sputter and Carbon Coater
- Large Chamber Sputter Coaters
- EMS 300T D Dual Target Sequential Sputtering System
- EMS 1050 Plasma Archer
- Critical Point Dryers
- Freeze Dryer and Turbo Freeze Dryer
- Dessicators and Dessicants

If you would like an Equipment Demo of an item not listed here, you can submit a request online.
General Lab Skills [1 day]
- Solution preparation
- Molar, Normal, %, stock dilutions, and multi-component
- Glassware, pipettes, and mixing
- Weighing and pH adjustments

Microscopy OLM [1 day]
- Nature of light
- Optics and objective correction level
- Kohler alignment
- Focus and digital image collection

Sample Preparation [1 day]
Featuring the EMS 9000 Microwave
- Trimming
- Orientation determination
- Fixation for OLM and TEM
- Resin embedment

Microtomy [2 days]
Featuring the Leica EM UC 7 and RMC/Boeckeler Power Tome PC
- Thick sectioning (0.5 - 1.5 um) for OLM
- Chromatic staining for OLM
- Thin section (70 - 40 nm) for TEM — using DiATOME Diamond Knives
- Post staining for TEM contrast

Transmission Electron Microscope [2 days]
Featuring the Hitachi 7700
- Electron optics
- Aberrations and astigmatisms
- Beam specimen interaction/image formation
- Column alignment
- Focus and astigmatism correction
- Digital image collection
- Artifact identification

Scanning Electron Microscope (SEM) [2 days]
Featuring the Hitachi SU 3500
- Chemical specimen processing
- Critical Point Drying (CPD) and HMDS — using EMS 850 CPD
- Physical specimen processing
- Freeze Drying (FD) — using EMS 750 FD and EMS 150T sputter coater
- SEM Instrument
- Beam/specimen interaction theory
- Instrument parameters (kV, spot size/beam current, WD, tilt)
- Signal/Mode selection SE, BSE, VP, UVD
- Focus and astigmatism adjustment

Cryo SEM [1 day]
Featuring the EMS PP 3010 T
- Cryo sample prep
- Cryo transfer, fracture, coating
- SEM cryo stage and imaging

X-ray Microanalysis [1 day]
Featuring the Bruker Esprit and X-Flash SDD
- Generation and nomenclature
- Qualitative analysis
- Quantitative analysis (ZAF)

If you have an idea or request for a Personal Short Course not listed here, you can submit a request online.

Right: Cryo-SEM prepared soya bean leaf — fractured cross-section
Electron Microscopy Sciences

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