Hints On Removing Epon Specimen Blocks From Glass Slides

Wolfgang Muss
Landeskrankenanstalten, Salzburg, Austria

Depending on the quality of the glass slide (not all brands seem to be the same quality and therefore display variable physical properties), post-polymerized specimen blocks can be separated from object slides. These can be of varying area size, and can include a selected area of your former semi-thin section or the whole area (as used in re-embedding techniques).

If the concern is:

Re-embedding of (immuno-) histochemical reacted light microscopy sections from, for example, thick paraffin sections (10 to 40 µm), then after several steps of processing such as incubations, washing, osmication, dehydration, infiltration and embedding:

1) Flush and infiltrate the specimen area repeatedly with fresh resin (complete with hardener/accelerator), after an intermediate/solvent (*e.g.*, propylene oxide-PO, PO:resin, etc...) step to get rid of any trace of solvent.

This repeated exchange of resin should be done only by dropping fresh resin into the middle of the section area to be re-embedded. The former resin would be transferred to the outer margins of the area to be re-embedded.

2) Place a polymerized resin block over the selected specimen area (*e.g.*, also re-embeddings of bigger light microscopy or histological sections).

3) Polymerize the resin block (with a clean and plane-smooth block-face) on your section to be re-embedded for correlative LM/TEM for at least 1.5 hours at only 37° C, then for, say, one hour at 80 to 90° C.

Let the "combo" of resin block-intercalated section-glass surface cool down to room temperature.

Prescore the four sides of the resin block with a scalpel blade. Be careful! Try to cut through resin remnant "walls" down to the glass slide surface. Next, there are two choices:

1) (faster) A flat container made from styrene foam is filled about 1 cm high with LN_2 (liquid nitrogen).

- Dip ONLY the object slide in the LN_2, until evaporation and bubbling of N_2 gas has stopped. If the surface of the object slide gets a little bit of LN_2, it won't matter.
- Pull the "combo" out of the LN_2 and either let warm up (resin block in between your fingers) until the blocks gives a "popping click", or try to apply a "shearing force" to the block to separate the resin block from the object slide.

2) Needs longer for warming up your "combo in an oven - warm up the "combo" to *e.g.*, 80° C for about 10 to 15 minutes, then proceed as above.

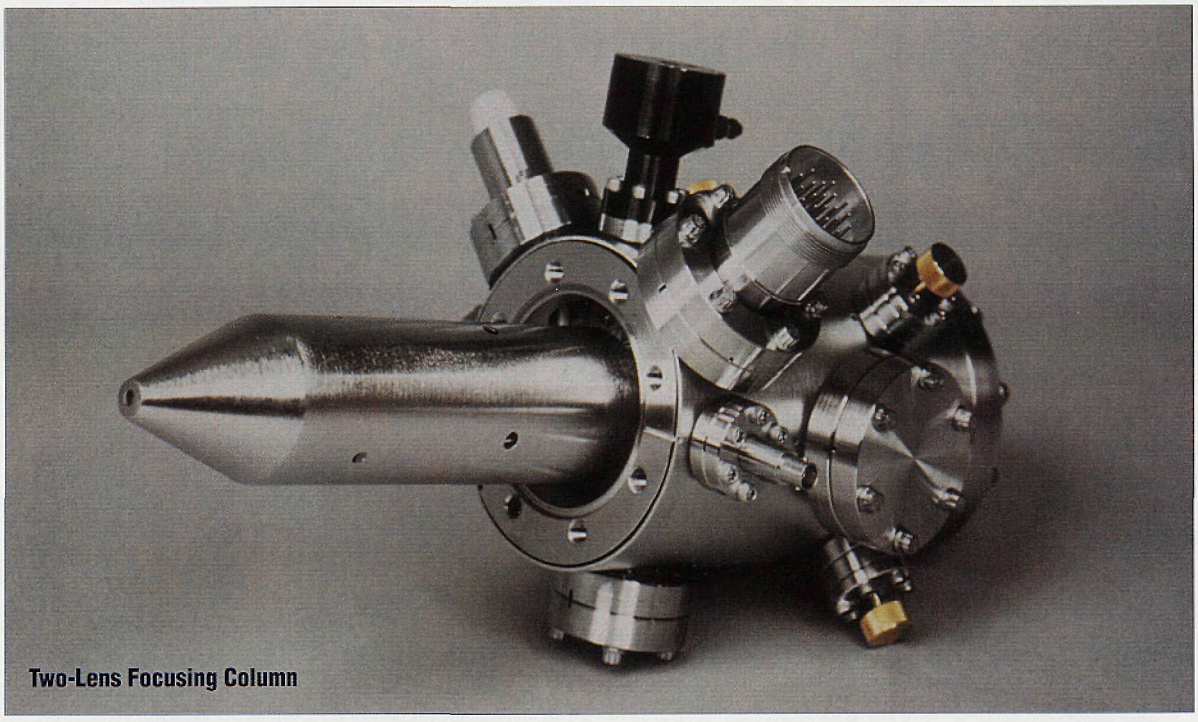
Note: Depending on the quality of glass slides (physical properties) and (individual) technique used, the separation process may not be not uniform, *i.e.*, sometimes some glass particles are left on the block surface after separation, or there is no separation at all. Elegant separation normally takes a few tests or trials. So don't use important or valuable tissue/section preparations for your first or second attempt.

If nothing changes and separation is not performed, try using a separation media such as Teflon spray or silicone pasting before mounting your sections to be reembedded.

Another *important* point is that if the whole "combo" (resin block - at least at its end connected to the glass slide) is being dipped into the LN_2 to cool it down, there might be problems due to cracking of the resin block. This may interfere with cutting the whole areas selected to be sectioned.

The effect of separation ("pop-off-technique") is explained by the difference of the coefficients of expansion of glass versus resin. Since glass has a different coefficient compared to resin formulations, glass contracts on low temperature and expands on warming differently than the resin used.

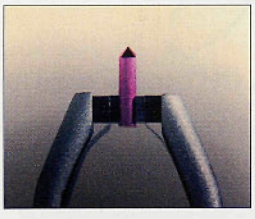
I have done "re-embeddings for TEM" of 2 µm to 40 µm thick immunohistochemically reacted tissue cryosections pre-embedding labeling techniques up to section areas of 5 X 5 mm, and most of my attempts worked. It is, however, very, very tricky and good results seem to depend on the time of day, or at least on how you got up in the morning (you know, one foot at a time). ■



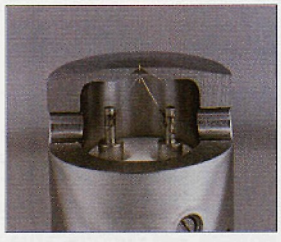
Two-Lens Focusing Column

What does fei® components stand for?

Our compact, UHV, *field* emission columns are used by researchers worldwide. Innovative *electrostatic* optics and dedicated electronics allow you to *integrate* a high current density electron or ion column into most vacuum systems. FEI also supplies researchers with other specialized products.



LaB_6 and CeB_6 Cathodes
FEI's Mini Vogel Mount, the *first* universally compatible long-life, high stability LaB_6 cathode, provides *excellent* performance and the best cost-per-use value for *installation* into your EM systems.



Schottky Field Emission Cathodes
FEI supplies Schottky *field* emitters to EM manufacturers worldwide. Schottky *emission's* high current *intensity* has established it as the preferred electron source for high resolution SEM,TEM, Auger, ESCA, EDX, and lithography.

New Components Facilities
Dedicated FEI Components Group *facilities enabling* new technology development through key *investments* in R&D and manufacturing.

fei® components

FEI Company
7425 NW Evergreen Parkway
Hillsboro, Oregon 97124-5845
(503) 844-2520 Fax (503) 640-7509
E-mail <components@feico.com>
Subject of e-mail: "MTfei"

Now, when you think of FEI Components, you'll know we are the Specialists in *Field Electron* and *Ion* Technology.

Circle Reader Inquiry #6

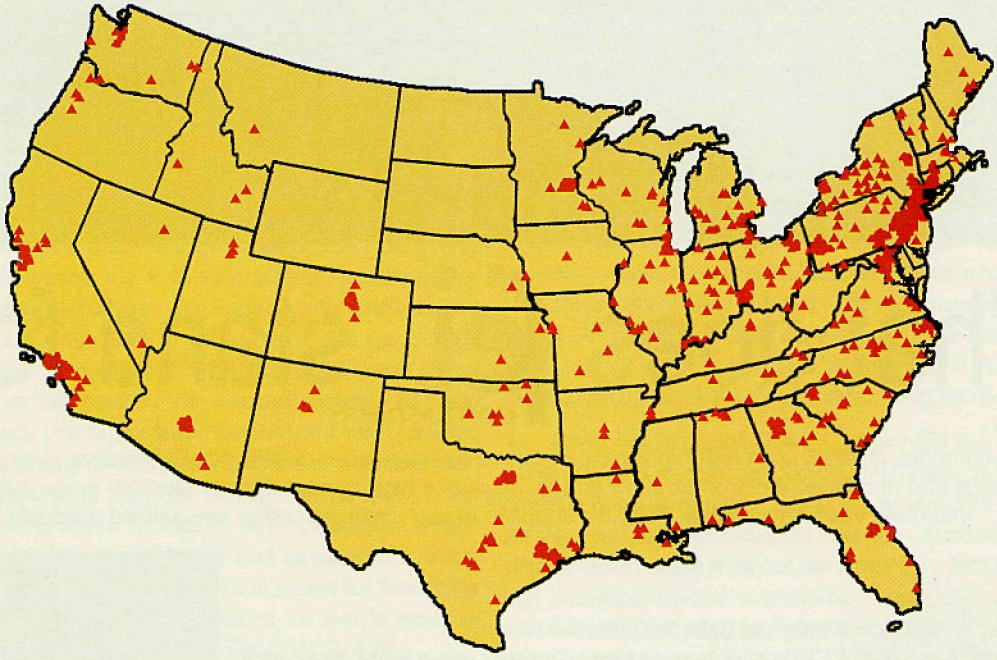
Lehigh Microscopy School

SEM, X-ray Analysis, AEM, AFM

for Materials Engineers, Geologists, Biologists, Polymer Scientists

The World's Best SEM Courses

Participants come from "everywhere"



- Taken by over 4000 engineers, scientists and technicians
- Participants from 46 states and 23 countries
- Exclusive Lehigh software...free to every registrant
- At least one textbook included (see below)

Books Authored by Lecturers and Used in Courses

	US/Canada
J. I. Goldstein et al., *Scanning Electron Microscopy and X-ray Microanalysis*, 2nd Edition.....	$59.50
C. E. Lyman et al., *SEM, X-ray Microanalysis, and AEM: A Laboratory Workbook*.....	$39.50
D. E. Newbury, et al., *Advanced Scanning Electron Microscopy and X-ray Microanalysis*.....	$55.00
D. B. Williams and C. B. Carter, *Transmission Electron Microscopy: A Textbook for Materials Science*.....	$95.00 (HC) $55.00 (SC)

(Book prices are 20 percent higher outside the U.S. and Canada)

To order call: **1-800-221-9369** or

write: Order Dept., Plenum Publishing, 233 Spring Street, New York, NY 10013-1578

(Visit Plenum Publishing at http://www.infor.com:6800/)

Scanning Electron Microscopy and X-ray Microanalysis

The Lehigh Main Course

(June 8-12, 1998)

For scientists, technicians, engineers, and technical managers. **Core topics:** Image formation • EDS qualitative analysis • specimen preparation • basic maintenance of SEMs • strategies for practical microscopy and microanalysis. **Special Interest Options:** X-ray analysis, imaging analysis, organic materials, inorganic materials. **Textbooks provided:** Texts were written by the course lecturers: *Scanning Electron Microscopy and X-ray Microanalysis*, Plenum Press, 1992 and *SEM, X-ray Microanalysis, and AEM: A Laboratory Workbook*, Plenum Press, 1990

1

Introduction to SEM and EDS for the New SEM Operator

(June 7, 1998)

This course provides an introduction to the main SEM course for those who might otherwise find that course intimidating. It will introduce scanning electron microscopy and energy-dispersive x-ray analysis at a very practical "knob-twisting" level. Enrollment is limited to 30 participants who are also attending the main SEM course beginning on Monday, June 8.

1a

New for 1998!

Advanced Scanning Electron Microscopy

with Digital Image Processing

(June 15-18, 1998)

Advanced treatment of high resolution SEM • low voltage SEM • environmental SEM • digital image processing • Monte Carlo simulations of beam-specimen interactions • electron detectors • quantitative stereo microscopy • stereology • image processing with personal computers

2

Quantitative X-ray Microanalysis

of Bulk Specimens and Particles

(June 15-19, 1998)

Advanced topics include: ZAF and $\phi(\rho z)$ calculations using personal computers • WDS and EDS detectors • quantitative analysis of thin particles and rough specimens • light element analysis • trace element analysis • strategies for applying microanalysis techniques • specimen preparation

3

Analytical Electron Microscopy

and Quantitative Analysis of Thin Specimens

(June 15-18, 1998)

Advanced topics include: STEM optics • beam-specimen interactions • Z-contrast • x-ray microanalysis • electron energy-loss spectrometry • compositional imaging • convergent-beam electron diffraction • symmetry determination • microcomputer calculations • thin specimen preparation • digital imaging (**New textbook provided:** *Transmission Electron Microscopy: A Textbook for Materials Science*, by D. B. Williams and C. B. Carter, Plenum 1996)

4

Atomic Force Microscopy

and Other Scanned Probe Microscopies

(June 16-19, 1998)

Atomic force microscopy • scanning tunneling microscopy • scanning tunneling spectroscopy • feedback control • tip fabrication • tip-sample interactions • scan calibrations • in-situ imaging • UHV imaging • imaging in air and liquids • image processing • near-field optical probes • metrology • lateral force microscopy • electrochemical STM/AFM • other emerging scanned probe techniques

5

FOR DETAILED BROCHURE CONTACT:

Sharon Coe, Materials Science and Engineering Department,
Lehigh University, 5 East Packer Avenue, Bethlehem, PA 18015
Phone: 610-758-5133 **e-mail:** slc6@lehigh.edu **Fax:** 610-758-4244

http://www.lehigh.edu/~inmatsci/Microscourses.html

Free Brochure