Limnological Research Center Core Facility Draft v.1.2
SOP series 1/26/05
thinsections.pdf A. Myrbo
(technique and initial writeup kindly provided by Jessica Black and Pierre Francus)

Epoxy impregnation of lake sediment slabs for thin sections

Note: this procedure covers only embedding of freeze-dried slabs with Spurr's-type epoxy. Other techniques, such as acetone-exchange with Spurr's epoxy, and PEG-80 embedding of wet sediments, are not routinely used in the Core Facility.

Purpose
Sediment thin-sections allow study of virtually undisturbed sedimentary structures, fabrics, and particles. This technique is used primarily in high-resolution studies of laminated sediments where individual sets of years can be identified. Sediment components studied include charcoal, precipitated components, diatoms, pollen, insects, zooplankton, and in-washed materials.

Carefully prepared blocks of sediment core are embedded in epoxy resin. The blocks are then hardened in a drying oven. The hardened blocks can then be treated as "rocks" for thin-sectioning. This includes cutting, mounting to a slide, making a second cut, and finally grinding and polishing the slide to the desired thickness (usually 30 to 50 µm).

Some of the specialized materials and equipment listed below are pictured at the end of this procedure.

Materials:
Slabbing boxes, ends snipped and folded in
Aluminum trays
Styrofoam cooler
Liquid nitrogen
Pencil
Plastic craft mesh
Aquarium gravel (brightly colored so you can tell it from natural materials if it gets in a slide)
Disposable glass pipets and bulb
Disposable beakers (400 mL or larger for full batch of resin)
Stir sticks

Equipment:
Slab cutter and/or fishing line, guitar string, wire, etc
Engraver
Long tongs
Cryo gloves
Freeze dryer
Scoopula
Neoprene gloves
Lab coat, goggles
Drying oven in hood
Desiccator with port attached to (house) vacuum
Balance weighing to 0.1g (in hood), covered with plastic wrap or foil

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Safety:
All chemical components of Spurr's type resin are hazardous – carcinogenic, blistering, respiratory hazards, etc. Every step involving resin (mixing, pouring, degassing, etc.) must be done in a fume hood while wearing neoprene gloves, lab coat, and goggles. Keep the workplace clean and wipe up all spills. Towels, wipes and other materials should be haz-wasted in a baggie at the end of the day. Leftover prepared resin can be cured in its beaker in the oven along with slabs and discarded in the trash when hard.

Notes:
- Humidity can cause problems because one of the chemicals (NSA) is anhydrous, so a climate-controlled environment (such as 6 floors underground) is preferable.
- Slab samples must be completely dry when you begin the impregnation. For clay-rich samples, this can take three days in the freeze dryer, depending on the number of slabs and whether the freeze dryer is defrosted daily.
- The embedding process cannot be interrupted once it is started.
- Impregnation is done by introducing resin from beneath the slabs: resin is poured in beside the slabs and then drawn up into the sample by capillary forces. If the resin is poured on top of the samples, it can destroy the fragile sediments and there is a higher likelihood of air bubbles. Rely on capillary forces.
- Resin has a pot life of 1-4 days (depending on the recipe) if kept cool and airtight.

1) Label everything::
   a. Engrave the sample depth and an UP arrow on each slabbing box.
   b. Engrave the name of each sample on the outside bottom of the aluminum trays in approximately the position where the samples will be placed. (3-4 samples can fit in each tray, depending on width of sample boxes used.)
   c. On ~2x10 cm pieces of paper, write a label for each sample with the sample name and an UP arrow in PENCIL (pen will be dissolved by solvents in the resin chemicals). Place label face down inside the aluminum tray in the position where the sample will go.

2) Cut plastic mesh to fit in the bottom of the aluminum tray and place on top of paper labels. Mesh elevates the sample boxes in the aluminum trays while allowing the resin to flow underneath it.

3) Insert a labeled slabbing box into the core and cut under it using the slabbing cutter or fishing line. Lift out, clean exterior of sampling box and set aside. Repeat for additional samples, overlapping 1-2 cm to provide continuity between slabs.

4) Pour liquid nitrogen into the styrofoam cooler to a depth of ~10 cm. Lower the slab into the LN using tongs. If your sediments are extra watery, you may wish to use a slush of dry ice plus alcohol or acetone for a potentially less violent freeze. (Freezing in a regular freezer will allow ice crystals to grow slowly and get large, potentially disrupting the sedimentary structures.) Once the samples are fully frozen (bubbling will slow or stop) move them to a

freezer temporarily (more than a few hours and the ice may recrystalize) or directly to the freeze dryer.

5) Freeze dry overnight (≤4 slabs) or for a couple days; drying will progress more quickly if freeze dryer is defrosted and restarted each day, but the process of bringing the chamber to room pressure and moving slabs out and in may disturb samples. Samples that are completely or partially dry, especially if they started out with a high water content, are very fragile and should be handled as little as possible. Check temperature of the slabs (by hand) for completeness of drying: a cold slabbing box indicates remnant ice in the sample.

6) Place sediment slabs in their appropriate order (to match labels) in the aluminum trays, on top of the mesh.

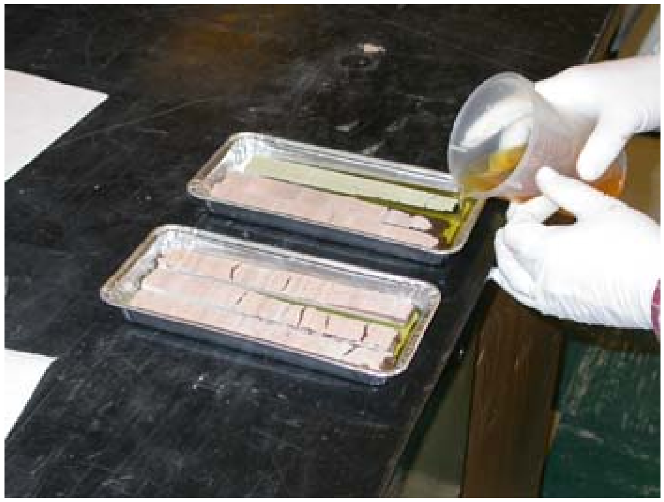
7) If there is less than a full tray of sediment slabs being impregnated, fill up the empty space between and around the slabs with aquarium gravel. Fashion a paper funnel to deliver gravel into the tray, or use the tip of a scoopula. Scattering gravel on dried slabs may damage samples.

8) Mixing of Spurr resin:
   - Tables at the end give proportions of different chemicals for various recipes and for a full aluminum tray of samples, as well as costs per tray.
   - Place a plastic beaker in an aluminum tray or other small containment vessel to protect the balance from spills (resin chemicals can eat or seep through plastic wrap). Add appropriate amount of one chemical at a time, taring balance after each addition. Most of the chemicals can be poured directly, but if this is awkward, or for small quantities (e.g., DMAE), use a disposable glass pipet.
   - The order in which chemicals are added doesn't matter; the amounts should be as precise as possible. (This can be difficult when airflow in the hood makes the balance reading unstable, but usually the variations are only a few tenths of a gram, which is within tolerances for the size batches we're making.)
   - Stir thoroughly with disposable stirring rod.

9) Once mixed, place the plastic beaker with the resin in the desiccator under vacuum for two minutes to degas.

10) Place aluminum tray in desiccator on a stiff piece of cardboard slightly larger than the tray itself. Check to be sure tray is horizontal and slabs are lying flat on plastic mesh, with no gravel fragments underneath. If resin beaker is more than about half full, transfer some resin to another container to allow better pouring control. Pour the resin into the aluminum trays, to the side of the samples, NOT ON TOP. Begin with a small amount of resin (~0.5 cm depth in tray).

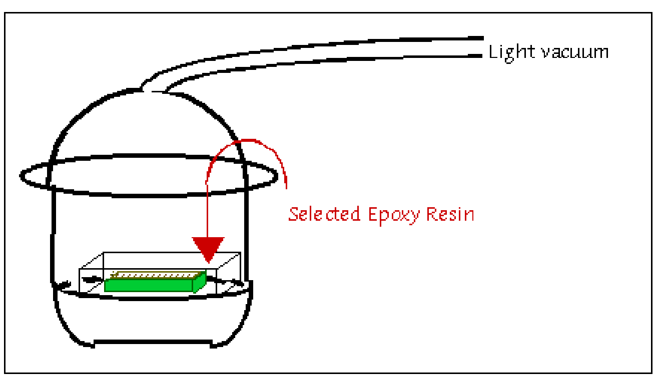
As the resin rises through the sediment and displaces the air in void spaces, we want the air to be able to exit easily out the top. Resin on top of the slabs leads to constriction of escape passages and to bubbles, which can move whole chunks of sediment and disrupt structures.



Adding resin. This can also be done while trays are in desiccator, and should be done under a hood. (figure courtesy of Pierre Francus)

11) Close dessicator and put under vacuum for 10 minutes to one hour to aid sediment in wicking up the resin. More clay-rich samples (with smaller void spaces) will take longer to take up the resin. If there is bubbling, reduce the vacuum.

12) When first round of resin has been sucked up by samples, add a second and third round of resin alongside the samples (making sure not to cover samples with resin), placing trays under vacuum after each addition. The process is complete when resin has visibly saturated the upper surface of slabs throughout.

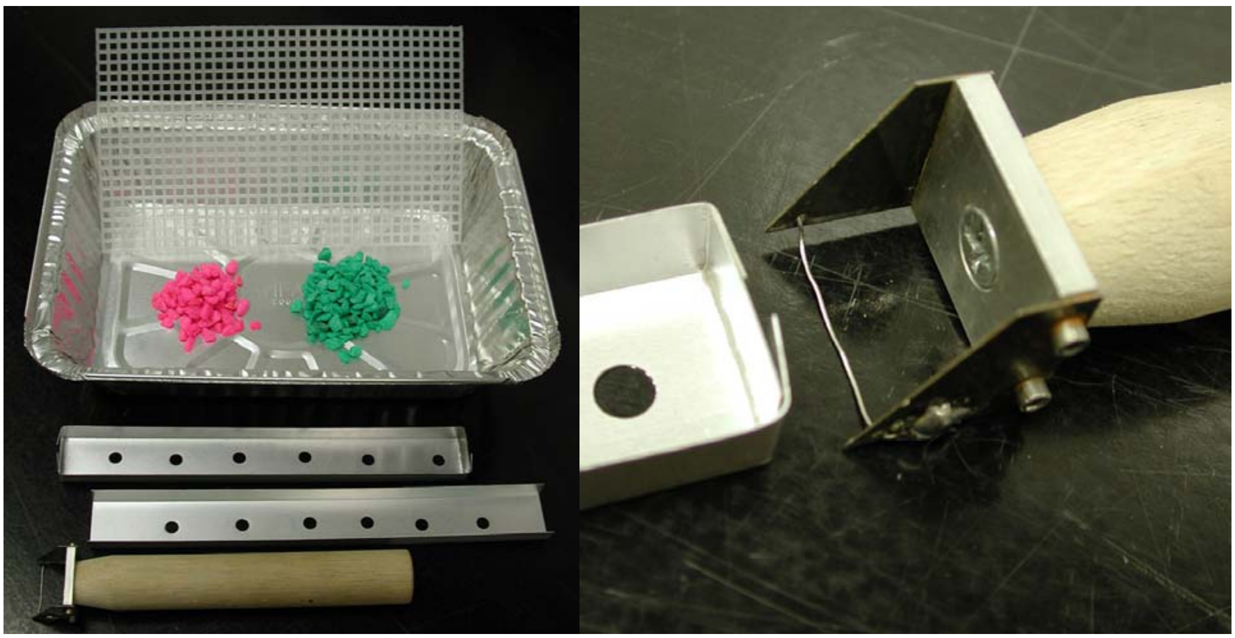


Schematic of desiccator with tray inside. (figure courtesy of Pierre Francus)

13) Once the sediments have finished taking up the resin, only then cover them with enough resin to make a flat surface on top of the slabs.

14) Leave in the desiccator under vacuum for two hours. After two hours, check to be sure no bubbles are evident on slab surfaces and that all slabs are still fully flooded with resin.

15) Remove tray CAREFULLY from the desiccator by lifting the cardboard piece it is sitting on. At this point the trays are heavy and unstable, and any bending can seriously compromise the samples. Slide trays carefully off of cardboard onto oven shelf. Leave trays in oven at 40°-50°C for 36-72 hours to cure; curing is complete when resin is hard and the surface is not sticky at all. Cure any leftover mixed resin at the same time.



Equipment: left, from top: craft mesh, aluminum tray, aquarium gravel, slabbing boxes (folded and unfolded), slab cutter. Right, detail of slab cutter head with folded slabbing box.

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Amounts used in epoxy preparation, per aluminum tray (about 4 slabs):

Standard recipe	amt. (g)	cost
4-vinylcyclohexene dioxide (VCD)	76.5	$ 24.40
DER 736	45.9	$ 2.63
nonenylsuccinic anhydride (NSA)	198.9	$ 13.70
2-diethylaminoethanol (DMAE)	3.1	$ 0.69
total	324.4	$ 41.42

Hard recipe	amt. (g)	cost
4-vinylcyclohexene dioxide (VCD)	76.5	$ 24.40
DER 736	30.6	$ 1.75
nonenylsuccinic anhydride (NSA)	198.9	$ 13.70
2-diethylaminoethanol (DMAE)	3.1	$ 0.69
total	309.1	$ 40.55

Long life/low viscosity recipe	amt. (g)	cost
4-vinylcyclohexene dioxide (VCD)	76.5	$ 24.40
DER 736	45.9	$ 2.63
nonenylsuccinic anhydride (NSA)	198.9	$ 13.70
2-diethylaminoethanol (DMAE)	1.5	$ 0.34
total	322.8	$ 41.08

Source for Spurr's type epoxy resin:

Polysciences, Inc. – polysciences.com – 800-523-2575

A search by "spurr" will bring up the epoxy kits offered; you can also buy each component individually:

Chemical name	Catalog number
VCD	01912
DER 736	02923
NSA	01542
DMAE	01458

MSDS for all chemicals are available as pdfs on the site as well.

Thin section preparation:

For slab cutting and slide preparation, we've used National Petrographic in Houston (nationalpetrographic.com; 713-661-1884) with fairly good success. They offer a student discount as well.