TEM Flat Embedding Technique

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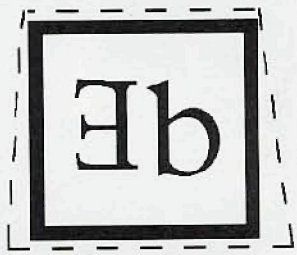
Individual BHK-21 (Baby Hamster Kidney-21) cells growing on 25 mm photo-etched alpha-numeric glass coverslips (Belico Co.) are identified by phase contrast microscopy and photographed. (Saredi, Howard and Compton, 1996). This method should work for other cell lines, but likely will require some empirical adjustment of the times, concentrations, and pH.

1) The coverslips are fixed in 2% glutaraldehyde in 0.1 M Na Cacodylate buffer pH 7.4 for one hour at room temperature.
2) Rinse three times in 0.1 M Na Cacodylate buffer pH 7.4.
3) Post-fix in 1% 0s04 in 0.1 M Na Cacodylate at pH 7.4 for 1 hour at room temperature.
4) Rinse in 0.1 M Na Cacodylate at pH 7.4.
5) Rinse in distilled H_20.
6) En-Bloc stain in 2% Uranyl acetate in distilled H_20 for 30 minutes in the dark at room temperature.
7) Rinse in distilled H_20.
6) Dehydrate through EtOH series: 30%, 50%, 70%, 85%, 95%; 3-5 minutes each.
9) Three 5 minute changes in 100% EtOH.
10) Four 5 minute changes in Propylene oxide in 60 mm glass petri-dishes. Make sure coverslips stay covered with fluids at all times.
11) Four changes of Epon/Araldite:Propylene oxide 1:1 mixture. Make sure coverslips stay covered with fluids at all times.
12) The flat-embedded samples are left overnight in a dessicator (NO vacuum) to remove Propylene oxide.
13) Fresh resin from the same batch is removed from freezer and thawed for about 1 hour. Coverslips are carefully removed from glass petri-dishes. Excess Epon/Araidite is removed from bottom surfaces by wiping with cotton swabs. Be sure to keep track of which side is the CELL SIDE! Coverslips are placed in clean, flat-embedding slide mold and a thin layer of fresh epon/araidite is added, by drops, to the surface of the coverslip. Leave for one hour.
14) Coverslips are placed in a 60° C oven and Epon/Araldite is fully polymerized for 24 hours.
15) Remove coverslip from heat. Check bottom surface of coverslip. GENTLY remove any epoxy layer from the bottom surface with fine sandpaper (120-240 grit). This will expose the glass surface. Glass is removed by etching in cold concentrated hydrofluoric acid* (store HF at 4°C for 24 hour before use) (Moore, 1975; Rieder and Bowser; 1987). Once the glass is etched away, the plastic water is rinsed thoroughly with distilled water and then dried. Etching times: Belico etched glass coverslips - 2.5 to 6 minutes, depending on temperature of HF (4°C - 15°C). Be careful not to scratch surface during water rinse. Works 100%, with a nice, clean surface.
16) Mark area of interest, using a dissecting scope. Place epoxy square inside slotted, plastic petri-dish. Insert a single edge razor through the slot:: tap GENTLY with hammer, in order to cut out section of interest. The slotted petri-dish will keep cut pieces from flying across the lab bench. Attach these pieces to blank epoxy blocks with crazy glue. Mark each block with the two letter code for reference. After glue has set, check block for strong attachment, then CAREFULLY trim away excess epoxy around chosen cell or cells. In this case, each etched square is about 1 mm^2.



Cell side up, after embedding

References:

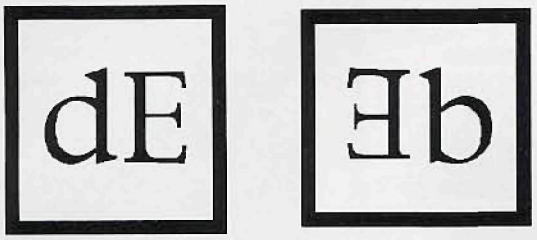
Moore, M.J., 1975, Removal of glass coverslips from cultures flat embedded in epoxy resins using HF. Microscopy 104, 205.

Rieder, Conly and Bowser, Samuel, 1987, "Correlative LM and EM on the same epoxy section", in Correlative Microscopy in Biology, Chapter 11 (ed. M.A Hayet), pp. 249-277, Academic Press

Saredi, A., Howard, L. and Compton, D., 1996 "NuMa assembles into an extensive filamentous structure when expressed in the cell cytoplasm". J. Cell Sci. 109: 619-630.

Coverslip Source:
Belico Glass co.
P0 Box B
340 Edruduo Road.
Vineland, N.J. 08360
1-800-257-7043

Each etched square is about 1 mm with 2 letters or 1 letter/1 number marking center area of each square. For example:



Cell side up, before embedding | **Cell side up, after embedding**

EPON / ARALDITE:

10 mL	LX112 (POLYBED812, etc.)
10 mL	Araldite 502
24 mL	DDSA
0.9 mL	DMP 30 (added just before use)

Mix together first three by shaking vigorously. Warm in 50°C oven for 5-10 minutes to improve mixing. Add DMP-30 and mix vigorously. Resin mix may turn orange depending on pH of DMP-3O. Remove air bubbles with vacuum for about 30 minutes. Store aliquots of resin mix in 5 or 10 mL disposable syringes in -20° C freezer; will keep for 1 week. Be sure to use same resin mix for all steps of infiltration.

* The hydrofouric acid is used straight from the bottle. It comes as 48%, with a well designed pour spout to avoid spilling, splashes and drips down the side of bottle. Use under fume hood! It is a strong, dangerous acid, as are all concentrated acids. Bottle comes with all the caveats and recommended precautions. Since only about 5 mL is used in a closed plastic petri dish, lab coat, neoprene gloves and eye protection are sufficient precautions. ■