

Application Note

Leica EM AMW – Epoxy resin embedding of *Drosophila* embryos

Courtesy of:

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Living up to Life

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Epoxy resin embedding of *Drosophila* embryos

Ultrastructural analysis

Epoxy resin embedding of *Drosophila* embryos

(Y. Schwab, IGBMC, Illkirch, France and K. Tiklova, Univ of Stockholm, Sweden)

i. Materials and reagents

Cellulose capillary tubes (from Leica microsystemes)

10 µl pipetman and tips

halocarbon oil

Heptane

4% Paraformaldehyde in PBS with 0,01% Glutaraldehyde

PBS

Osmium tetroxyde 1% in PB

Uranyl acetate 1% in d-water

Epoxy resin, for infiltration and embedding (18% araldite M; 23% epon812; 55% DDSA; 4% DMP30)

Acetone

ii. Fixation protocol

Fixation

1. Pick *Drosophila* embryos with a needle from the egg laying plate to a microscope slide, on which you have double sticky tape.
2. Dechorinate the embryo by pulling them on the tape until the chorion breaks
3. Transfer the "naked" embryos to halocarbon oil (to prevent drying)
4. Pick the embryos of your interest (stage)
5. Transfer the embryos from the oil to a vial containing 3ml of Heptane. Try to get rid of oil as much as possible before submerging the embryos in the Heptane.
6. Add the same volume of fix solution: 4% Paraformaldehyde in PBS with 0,01% Glutaraldehyde (concentration of GA depends on later use)
7. Shake the vial on a shaker for 30-40 min (maximum speed can be used).
8. Remove as much of the Heptane (upper phase) as possible.
The embryos should lie in the interphase and float on the fix phase after the Heptane is removed.
9. Put the embryos on microscope slide to a drop of 1xPBS with the glass Pasteur pipette. Use two thin tungsten needles to rip apart the vitelline membrane.
! Important. Do not let the embryos dry.
10. Transfer the embryos to 4% PFA until you use them.

Preparation of the capillary tubes:

A length of 5cm of the capillary tube is mounted on 10 µl pipette tips with nail polish and filled with buffer by pipetting.

Embryos are introduced in the capillary tube by pipetting. Pieces of 0.5 to 1 cm of tube, containing the embryos are produced by crunching with a pair of curved forceps. Ensure that the extremities of the tube are sealed. The sample containing tubes are then transferred into the baskets of the automate and processed for embedding.

Reagent List

MaxTemp (°C)	MaxPower (watt)	Type	Name	DrainPause
50	30	Fixative	Buffer + OsO4 1%	0
90	30	Rinse	dH2O	0
50	30	Fixative	U-acetate 1%	0
46	30	Solvent	Acetone 30%	0
46	30	Solvent	Acetone 50%	0
46	30	Solvent	Acetone 75%	0
46	30	Solvent	Acetone 95%	0
46	30	Solvent	Acetone abs.	0
50	30	Resin	Resin 3:1	30
50	30	Resin	Resin 1:1	10
50	30	Resin	Resin 1:3	20
95	30	Resin	Epon	50

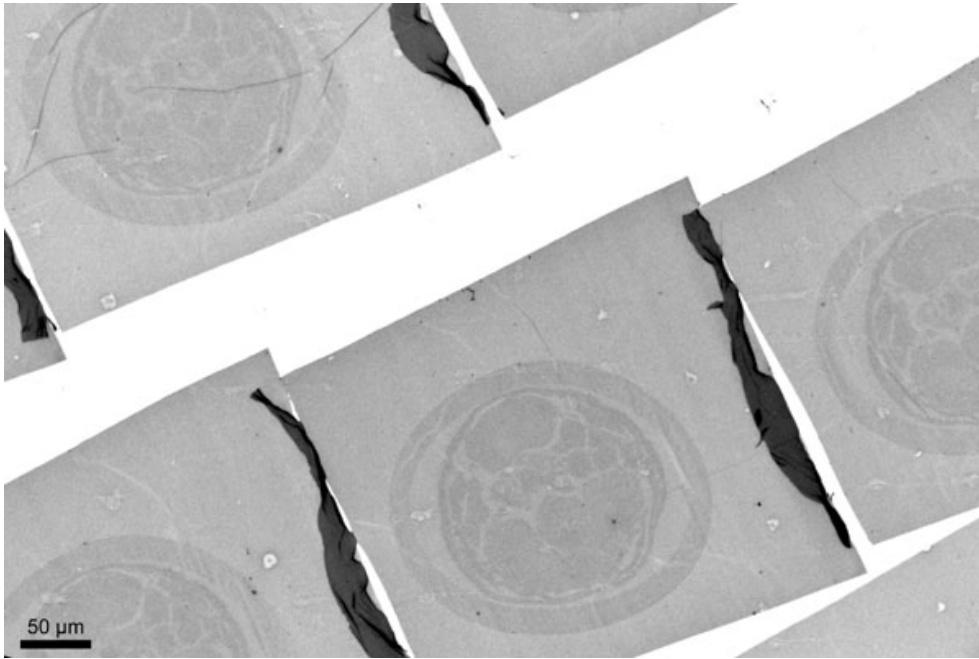
Epon embedding

Vial	Reagent	Power (watt)	Pause	Mode	Temp (°C)	Time (sec)
1	Buffer + OsO4 1%	15		Pulse	37	720
2	dH2O	15		Continuous	37	60
3	dH2O	15		Continuous	37	60
4	dH2O	15		Continuous	37	60
5	dH2O	15		Continuous	37	120
6	dH2O	15		Continuous	37	120
7	U-acetate 1%	15		Continuous	37	120
	U-acetate 1%	0		Continuous	20	120
	U-acetate 1%	15		Continuous	37	120
8	dH2O	15		Continuous	37	60
9	Acetone 30%	20		Slope	37	60
10	Acetone 50%	20		Slope	37	60
11	Acetone 75%	20		Slope	37	60
12	Acetone 95%	20		Slope	37	60
13	Acetone abs.	20		Slope	37	120
14	Acetone abs.	20		Slope	37	120
15	Resin 3:1	10		Continuous	37	180
16	Resin 1:1	10		Continuous	40	180
17	Resin 1:3	10		Continuous	45	180
18	Epon	12		Continuous	50	180
19	Epon	12		Continuous	50	180
20	Epon	12		Continuous	50	180

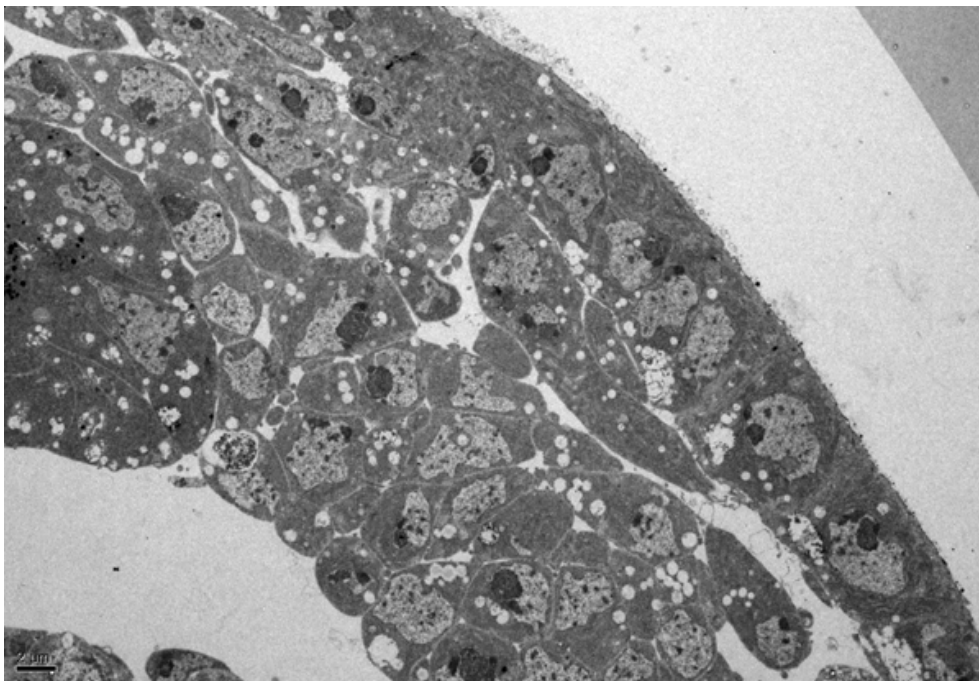
Poly Epon

Vial	Reagent	Power (watt)	Pause	Mode	Temp (°C)	Time (sec)
1	Epon	30		Slope	63	300
1	Epon	30		Slope	75	300
1	Epon	30		Slope	83	900
1	Epon	30		Continuous	83	6300

iv. Pictures



Serial cross sections of a drosophila embryo trapped in a cellulose capillary tube (arrows).



Magnification on the fly embryo lying on the inner side of the capillary tube (cp).

