ONBI Practical 1: Preparing samples for microscopy

RM Parton revised 2014

Aims of the practical:

In this practical the aim is to understand the issues and constraints of sample preparation for microscopy.

Onion epidermis prep

This is a simple-to-prepare live sample that can be used to demonstrate bright field contrast techniques. Cells should show clear subcellular structures and dynamic movements of cytoplasmic streaming and organelle transport.

Reagents and materials provided

Large onion.
Lens tissue.

Mounting solution.
Distilled water.

Plastic Pasteur pipette.
4 × Glass slides and coverslips.

Forceps.

Scalpel or blades.

4 × Plastic mounting frames with double-sided sticky tape.

Humidified chamber (square petri dish with wet filter paper on the lid).

Small plastic dishes (for storing samples).

Onion Peel Prep:
http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artoct11/wd-onion9.html

Experimental Protocol

1. Peel away the papery outer layer of the onion then cut a square into the fleshy layers about 3×3 cm using a scalpel. Using the scalpel again, lever off a layer, there will be a thin epidermal layer attached to the concave side.

2. This epidermal layer can be conveniently isolated for viewing by sticking it to a **"plastic frame" with double sided sticky tape on one side**. Using the pre-prepared plastic frame you will isolate a section of epidermal peel: first, remove the covering of the double-sided sticky tape (do not touch the sticky surface); next press the sticky side down firmly but carefully against the epidermal layer then trim away the excess outside the frame with the blade and peel away. You can produce several peels and store them floating on drops of medium in a Petri dish.

3. Mount the isolated peel in a drop of medium on a glass slide and place on the microscope.

4. Select a low mag objective (**10x or 20x**), focus and view the specimen.

5. Remove the sample from the microscope, add an extra drop of water on top and add a coverslip on top, don't add too much water and avoid air bubbles.

6. Change to a 40× lens. Adjust the condenser iris to generate contrast.

Macrophage prep

Drosophila macrophages, or haemocytes are the blood cells of *Drosophila*. Some of them circulate whereas others are immobilised under the larval (or adult) cuticle. They form part of the innate immune system of the fly. Macrophages can easily be isolated from the haemolymph of third instar *Drosophila* larvae and cultured on simple media.

Reagents and materials provided

* Kim wipes
* Pair of fine forceps / person (with good tips)
* Dissecting needle
* Access to a dissecting microscope
* Glass slides
* Humidified chamber (9cm square plastic dish, dampened filter paper, supports for glass slides)
* Schneider's medium or 1x PBS (eppendorf with 500 µl of medium)
* 200 µl Pipette, tips (to dispense 50 µl medium)
* Coverslips 25x25 mm (one for each glass slide)
* 3x 5cm plastic Petri dishes (for larvae)
* Third instar larvae

Experimental Protocol

1. Collect 4 larvae to a 5cm plastic Petri dish and wash thoroughly with tap water.

2. Take a glass slide to the dissecting microscope and add a single 50 µl drop of Schneider's medium or PBS to the centre with a 200 µl pipette.

3. Take the first larva with the first pair of forceps and transfer to the drop of medium.

4. Take a second pair of forceps in the other hand. While looking under the dissecting microscope, hold the larva gently with both sets of forceps by pinching the cuticle.

5. Tear the cuticle by pulling the tweezers away from each other. Try to see if anything leaks from the wound. Holding the pierced larva with one set of forceps, move it around in the medium in a circular manner to disperse the haemolymph. The whole procedure should take less than a minute. Repeat for all four larvae and discard the remains on tissue paper.

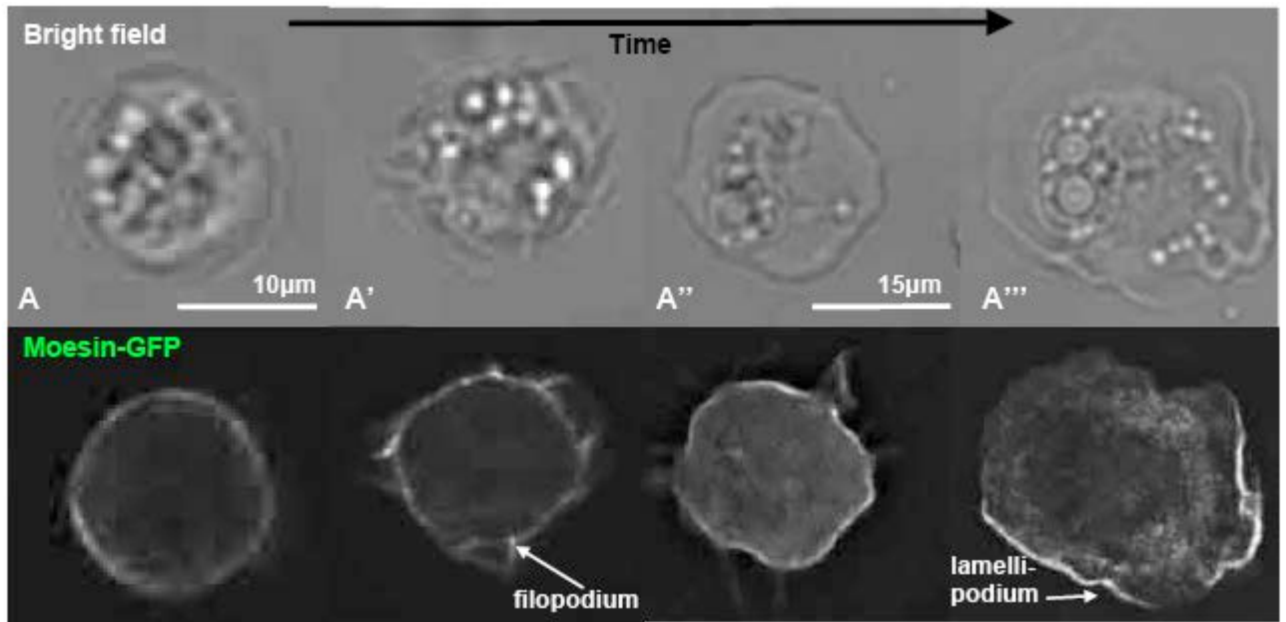
6. Place this slide in the pre-prepared humid chamber provided (square plastic dish). Ensure the lid is on the humid chamber and carefully set to one side for about 20 min to allow the macrophages to settle and adhere.

8. After 20 min, cover the 50 µl medium carefully with a 25x25 mm coverslip.

9. View under the microscope.

Macrophage:

Sequence of macrophage spreading

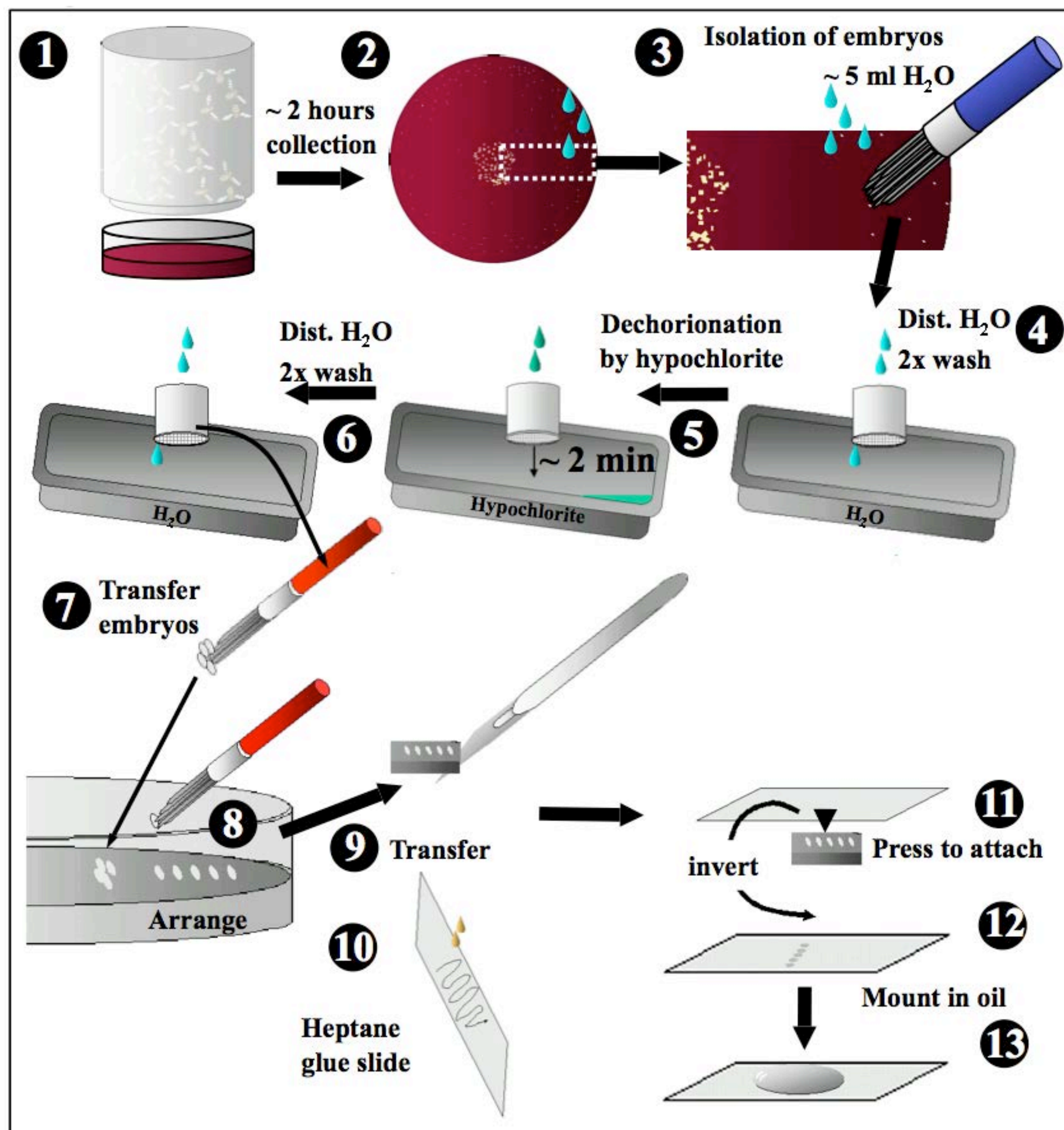


Embryo prep

Drosophila embryos are collected by allowing flies to lay eggs on a yeasted apple juice agar plate for between 30 minutes and 2 hrs. The eggs are washed from the plate and collected in a sieve. The egg shells (chorion) are removed by bleach treatment and dechorionated embryos can be stored on agar plates. Egg development up to emergence as a larva can be followed by mounting live embryos and examining them under the microssope.

Reagents and materials provided

* Pre-isolated, dechorionated Drosophila embryos on an agar plate.
* Coverslips and slides.
* Apple juice plates.
* Razor blades.
* Heptane glue.
* Halocarbon oil and membranes.
* Humid chambers.



Experimental Protocol

1. View embryos under a dissecting microscope, space them at least 1 embryo-width apart to avoid problems of anoxia. Arrangement in rows facilitates imaging. (This is step 7 in the diagram above.)

2. Cut out an agar block under the aligned embryos and use it to convey them to a slide.

3. Coat coverslips with "heptane glue". Application of ~20 μl can be made over a 1.5 x 3 cm area by applying at an angle and "streaking" left to right. The dried glue should only just be visible on the slide. The glue stock solution is prepared from Scotch double-sided sellotape (3 meters of "scrunched up" tape to 200 ml Heptane, shaken for 3 hours and then decanted). The glue should be clear and very thin.

4. Press the coverslip firmly against the agar block to transfer the embryos. The agar block prevents crushing and dessication.

5. Cover the embryos with halocarbon oil (Halocarbon Products Corporation, Series 700). If access to the embryos is not required a teflon membrane (such as may be obtained from YSI incorporated; standard membrane kit, 5793) helps to maintain viability for prolonged observations and improves optical clarity.

6. View under the microscope.

Leaf epidermis peels

Some plant leaves (especially from monocots) can easily be "peeled" to isolate strips of epidermis. This can be used to isolate live guard cells, which make up the stoma of plant leaves and regulate gas exchange.

Reagents and materials provided

* Leaf samples.
* Coverslips and slides.
* Tweezers.
* Razor blades.
* Tap water
* Plastic frames.
* Humid chambers.

Experimental Protocol

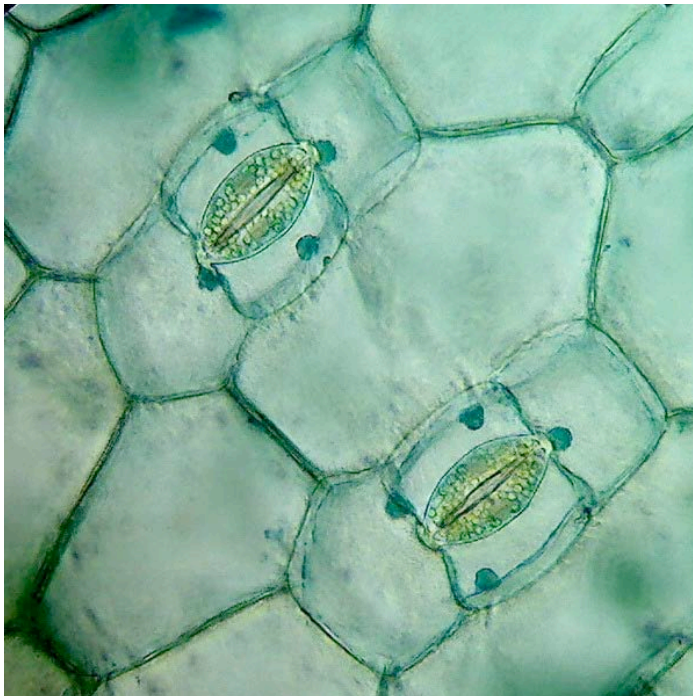
1. Try to isolate epidermal strips by peeling or tearing leaves.

2. Large sections may be anchored flat by attaching to a plastic frame.

3. Mount in tap water avoiding air bubbles and place a coverslip on top.

4. View under the microscope.

Guard cells in leaf epidermis:



http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artmay11/wd-Brilliant-Blue-1.html