Antibody staining on *Drosophila* post-emergence wings

(Nicolas Gompel, Eva Ayla Schröder, 2015)

This protocol is for the preparation of Drosophila wings, immediately after the emergence of the fly from the pupa. Upon dissection of the wings, separation of the dorsal and ventral surfaces and fixation, these wings can be processed for antibody staining. This protocol can be adapted for RNA *in situ* hybridization.

Material

Heptan glue

Unroll 4 full rolls of tesa® fotostrip (15 mm x 10 m) double-sided tape. Remove the protective liner and immerse the tape into 200 ml n-heptane in a closed bottle. Make sure that the tape is covered by heptane. The glue dissolves in 2 to 3 days at room temperature, resulting in a glue solution. Transfer this glue solution (but not the remaining tape ribbon) to a new bottle (figure 1).

After a long storage, the glue can become viscous, which makes its handling more complicated. Add heptane and mix well to make it more fluid; alternatively, prepare new glue.

Figure 1. Heptane glue. Left, Tesa film soaking in heptane. Right, dissolved tape glue transfered to clean bottle. (Photos: ©Ayla Schröder)

Dissection slide

The dissected wings will be collected and temporarily arranged on a dissection slide, before their transfer to a final mounting slide. The dissection slide is a microscope slide coated with a 3.5 cm piece of double-sided tape (with its protective liner on the other side). The protective liner provides the smooth surface, necessary to not damage the dissected wings. For an illustration, see the last picture of figure 4 or the first picture of figure 5.

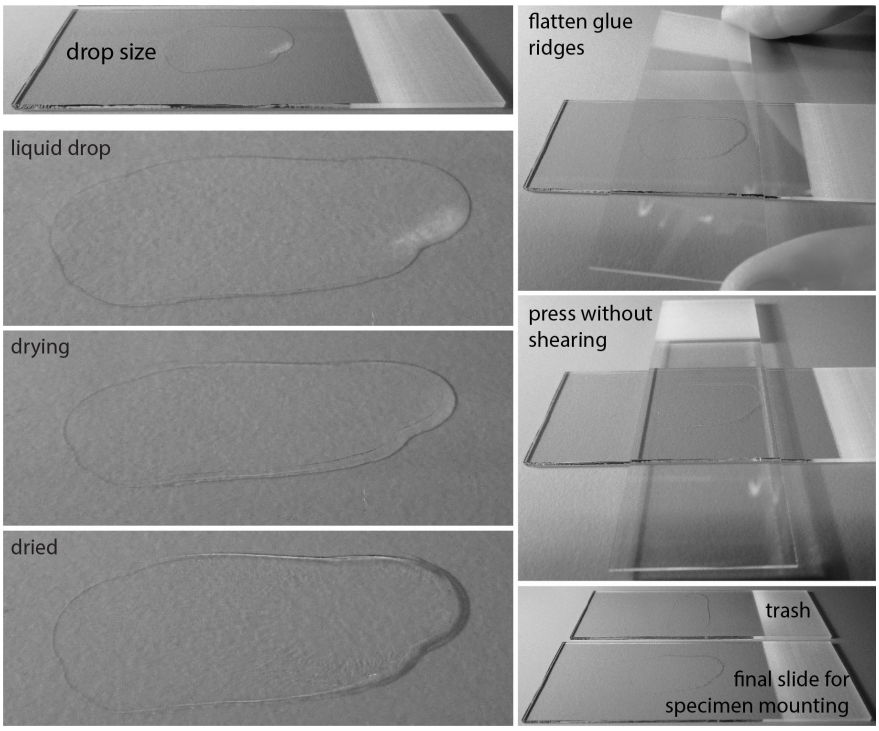
Slides for final wing mounting

The slides, onto which the dissected wings will be mounted, must be coated with a thin layer of heptane glue in their central part.

- Wipe any dust or glass debris off the slide with a clean tissue (Kimwipes®).
- Add a drop of heptane glue with a Pasteur pipette and directly spread it evenly with the pipette tip to an oval shape of approximately 1 x 3 cm (see figure 2). Spread the glue quickly to prevent the edges of the liquid from drying as you are spreading. This would result in unwanted ridges.
- Let the glue dry while the slide is placed on a flat surface. Do not move the slide until the heptane is fully gone.

- To flatten the elevated edges of the dried drop, stamp another clean slide onto the glue, ideally turned at a 90° angle. Avoid shearing movements as these will impair the smoothness of the surface.

To separate the dorsal and ventral cell layers of the wings, two slides are needed. The schematic in figure 2 refers to the positions and shapes of the glue layers on each slide.



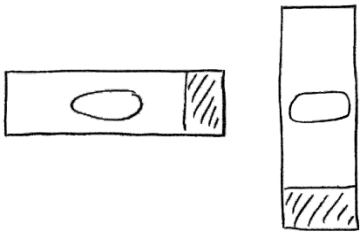


Figure 2. Preparing a slide for final wing mounting. Pictures on the left: processing of a single slide. Scematic on the right: shapes of the glue layers that need to be produced.
(Photos: ©Ayla Schröder)

Fixation solution

5% Formaldehyde/PFA in 1x PBT (Phosphate Buffer Saline, 0,1% Triton X-100).

Example: 75 ml 2x PBT + 75 ml 10% Roti®-Histofix (phosphate-buffered Formaldehyde solution; Roth)

Wash buffer

1x PBT (Phosphate Buffer Saline, 0,1% Triton X-100).

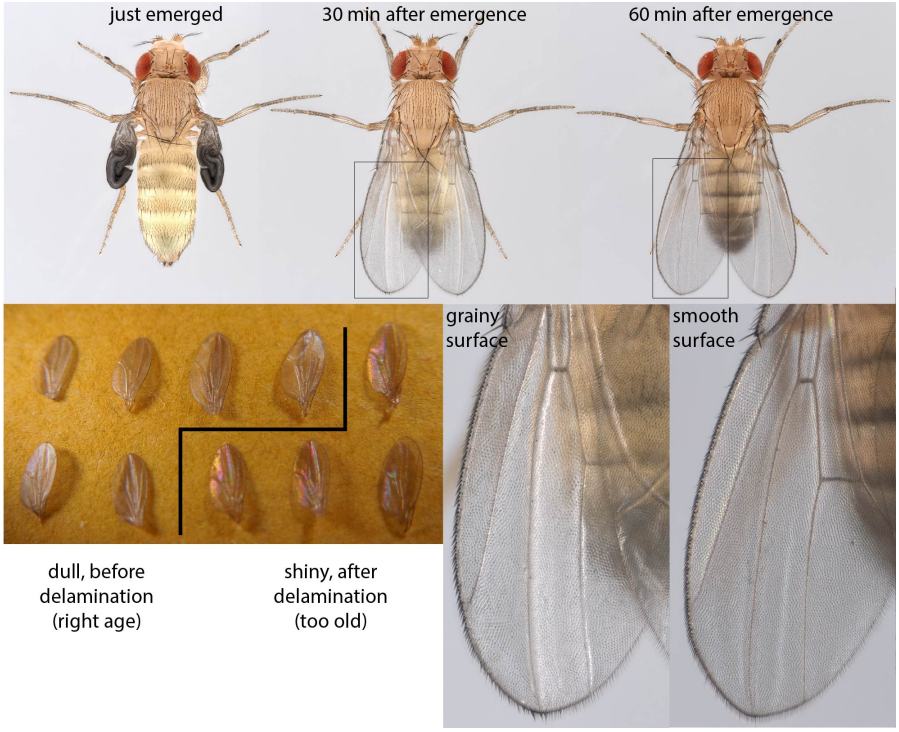


Figure 3. Staging wings. (Photos: ©Nicolas Gompel & Ayla Schröder)

Methods

Stage selection for wing dissection

Within 30 min of emergence from the pupal case, adult flies unfold their wings (figure 3). This process is generally blocked when the flies are anaesthetized with CO_2 and does not resume. Therefore, do not anaesthetize flies that have just hatched with CO_2.

Upon unfolding, the wing surfaces look dull, likely because the cells of the wing blade are still present. These cells start to delaminate as the wings unfold. Within two hours after emergence, the dorsal and ventral epithelia dissociate and the wing blade cells migrate to the thorax. The ventral and dorsal cuticle surfaces become tightly bound, looking shiny and iridescent (figure 3; Kiger et al., 2006).

For antibody staining, or *in situ* hybridization on unfolded post-emergence wings, before cell delamination, the flies must be staged as shown in figure 3 (30' after emergence). Additionally to the dull wings, flies of the right age remain overall lightly pigmented, in particular on the abdomen. Dorsally, the dark stripes at the posterior end of each segment are not yet distinct. Ventrally, the dark spot on the left side of the abdomen is still well marked.

Collection of flies with unfolded wings

An alternative staging method is to harvest flies that have just emerged from the pupal case, without CO_2 anesthesia, and to place them in a clean vial until they have unfolded their wings. They can then be anaesthetized if needed.

- Flush flies from a culture with lots of emerging flies.
- 30 minutes later, empty newly emerged flies in a clean dish. Most should have folded wings.
- Gently push the newly hatched flies into a clean vial with a brush.
- 30 minutes later, the same flies should have unfolded their wings, and have reached the appropriate stage for dissection. These flies can be frozen at -20°C in a Petri dish coated with Whatman paper for later dissection.
- Continue to collect every 30 minutes.

Dissection

The goal of the dissection is to carefully cut or pluck the wings at the stage described above. We use 2 pairs of forceps (5-Dumoxel-H, Dumont), but other tools (*e.g.*, mounted needles) may work too.

Figure 4. Dissecting wings. (Photos: ©Carolin Bleese)

- Put 1-10 flies of the correct stage on a CO_2 pad. They usually lie on their side, facilitating wing dissection. [Or use flies accumulated at -20°C].
- Hold a fly with one pair of forceps by gently pressing its thorax (figure 4).
- Pinch one wing at its hinge with the other pair of forceps. It is important to only manipulate the wing by its hinge, to not damage the fragile wing blade.
- In a gentle but firm motion, detached the wing from the thorax.
- Transfer plucked wings to the dissection slide; arrange them in the same orientation and in rows onto the liner of the double-sided tape, as illustrated above. This arrangement facilitates later imaging (figure 5).
- Avoid pressing onto the wing surfaces, to limit lesions.
- The wings are loose, but will be firmly attached to the glue of the mounting slide after transfer. Protect the loose wings from air movements such as slamming doors.

Wing transfer to the final slide

- Apply a slide for final wing mounting, glue-coated side down, onto the wings, aligned on the dissection slide. Gently press for a short time. Avoid sliding movements that would shear the wings.
- The wings should now be attached to the final slide (figure 5).

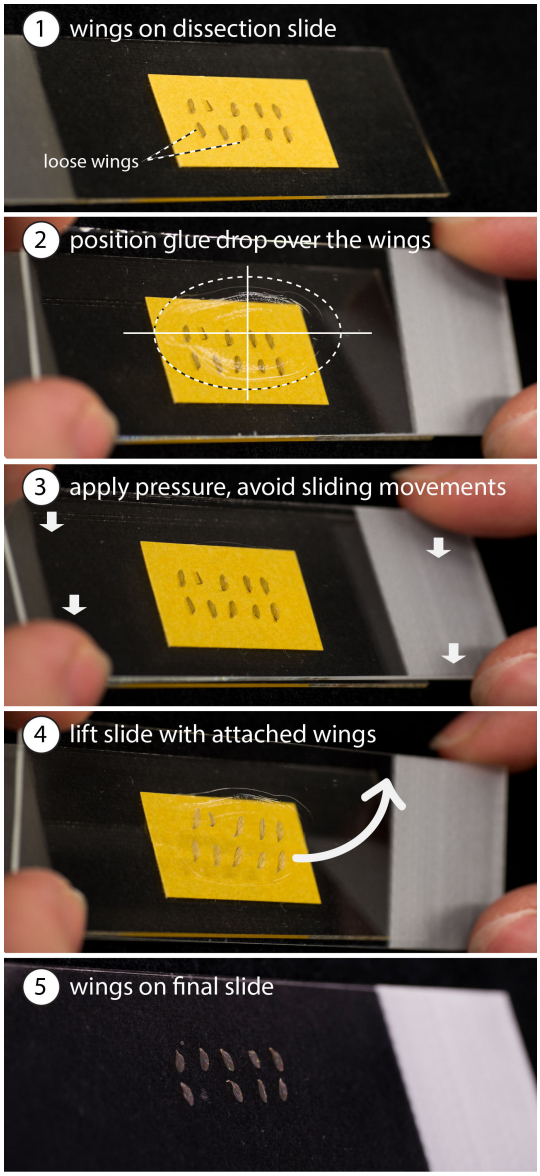


Figure 5. Transfering wings. (Photos: ©Carolin Bleese)

Wing surface separation

- Place the slide with the transferred wings on a soft pad (*e.g.*, a computer mouse pad)
- Apply a second glue-coated slide onto the wings, at a 90° angle (figure 6). The two glue drops must be positioned above each other.
- Shortly press on the top slide with your thumbs or a light object (*e.g.*, pack of microscope slides). The wings become visibly attached to the upper slide. It may be necessary to apply pressure directly in the region of the slide overlap.
- Separate the slides in a steady movement, without shearing. This is best done by holding the bottom slide with one hand at both ends and lifting the upper slide at one end with the other hand. The wings should open in halves along the wing margin, like a wallet.

From now on, it is crucial that the wings do not dry out. Transfer the slides directly after opening the wings into the staining jar with fixation solution (figure 7).

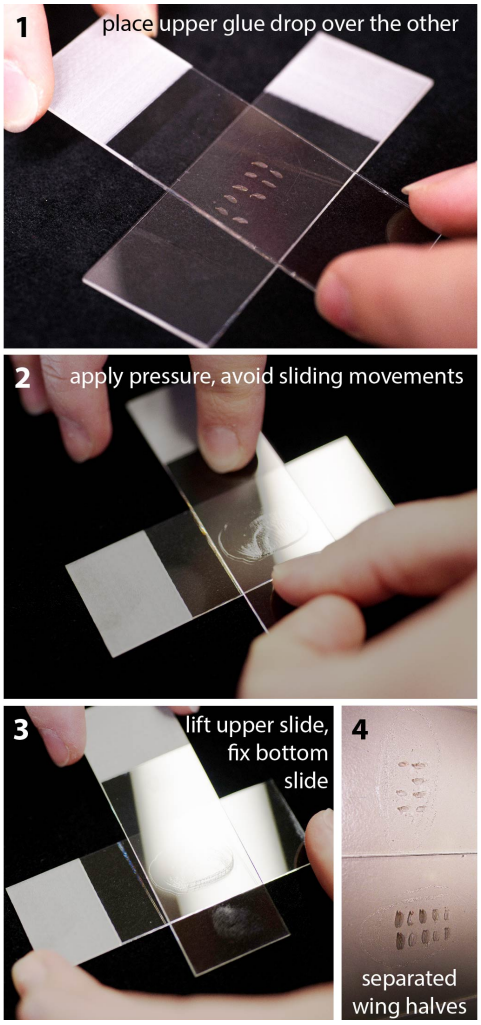


Figure 6. Separating wing surfaces.
(Photos: ©Carolin Bleese)

Fixation

- Transfer the slides into a vertical staining jar with fixation solution (under the fume hood).
- Incubate for 30 minutes at room temperature.
- Wash the slides 3x 15 minutes with fresh 1x PBT in another staining jar.

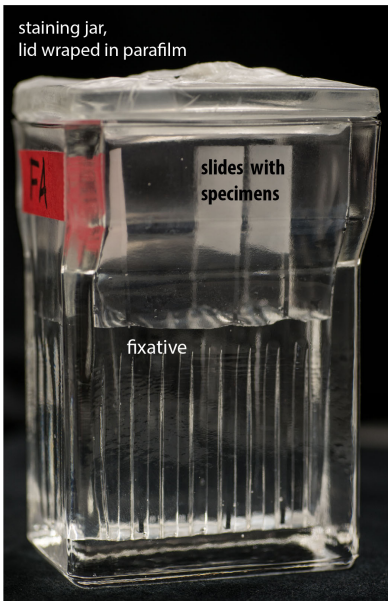


Figure 7. Jar for wing fixation and washes.
(Photos: ©Carolin Bleese)

Antibody staining

Prepare a humid chamber by placing wet tissues at the bottom of a slide box (or a similar kind of box) (figure 8). Allow enough space between the tissues and the upper end of the spacers to prevent contact between the edge of the slide and the tissue. This would result liquid drain from the slide.

Figure 8. Antibody incubation.
(Photos: ©Carolin Bleese)

- Take the slide with specimens out of the staining jar and place it over two spacer of the humid chamber (figure 8). Do not let the wings dry out, proceed quickly to the next step.
- Dispense 200 µl of primary antibody solution onto the wings. Fully cover the slide to prevent the liquid from escaping the hydrophobic glue surface.
- Incubate over night at 4°C.
- Wash with PBT: 3x briefly, then 3x 10’
- Apply secondary antibody, incubate 1 hour at RT in the humid chamber.
- Wash with PBT: 3x briefly, then 3x 10’

Mounting

- Cut thin strips of double-sided tape (approximately 3 cm x 2 mm) that will later be used as spacers between the slide and the coverslip.
- Remove the liquid from the long edges of the slide with the fixed, open wings, using a Kimwipes® tissue. This allows the spacer to stick to the slide.
- Place a spacer strip on each side of the slide; use forceps or a dissecting needle to position the strips; remove the protective liner.
- Immediately apply a drop of Vectashield® on the wings (they must not dry out).
- Slowly lower a coverslip onto the wings at an angle to avoid air bubbles (figure 9).

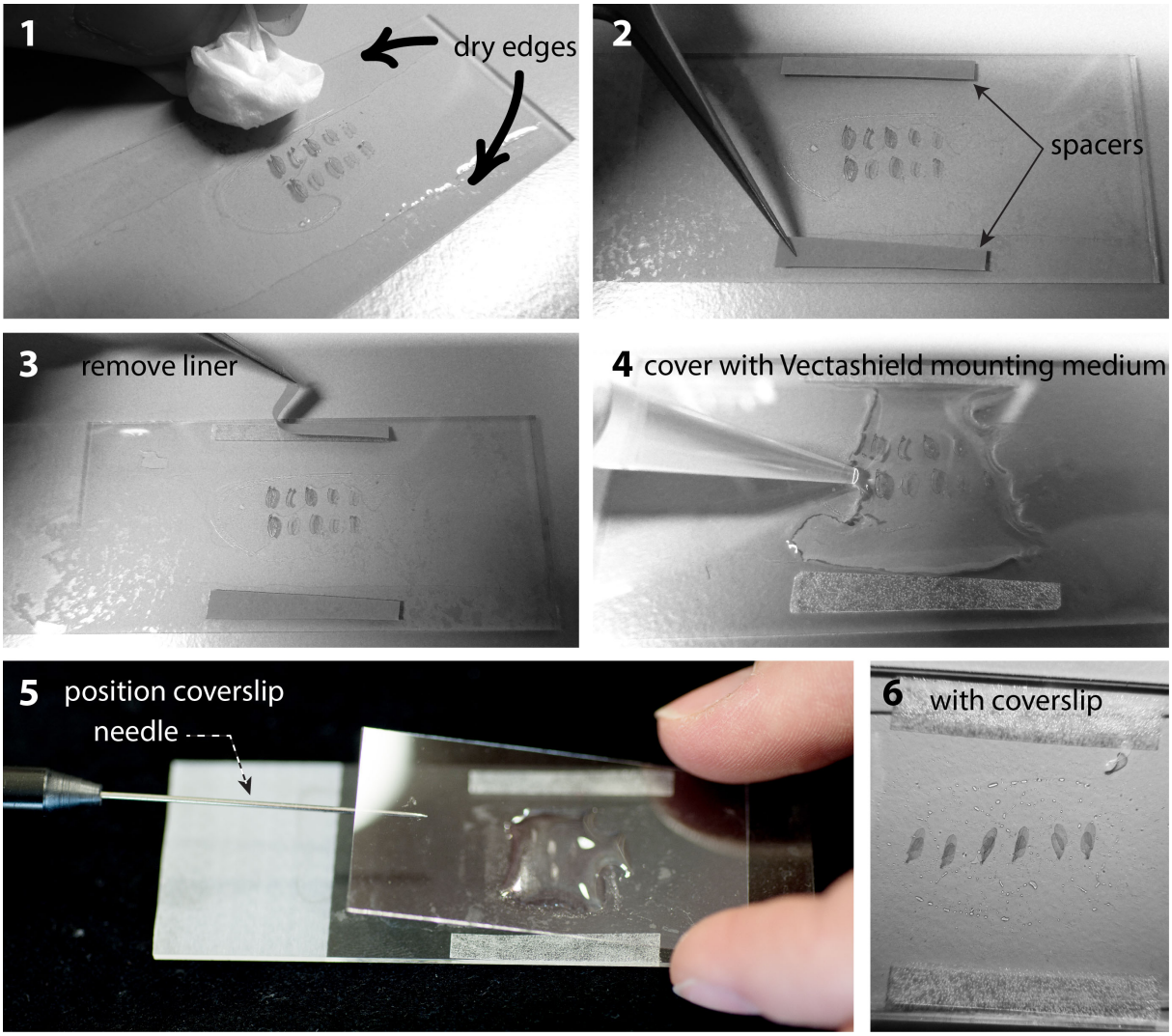


Figure 9. Final mounting of stained wings. (Photos: ©Ayla Schröder & Carolin Bleese)

Bibliography

Kiger, J. A., Natzle, J. E., Kimbrell, D. A., Paddy, M. R., Kleinhesselink, K., & Green, M. M. (2007). Tissue remodeling during maturation of the Drosophila wing. *Developmental Biology*, *301*(1), 178–191.