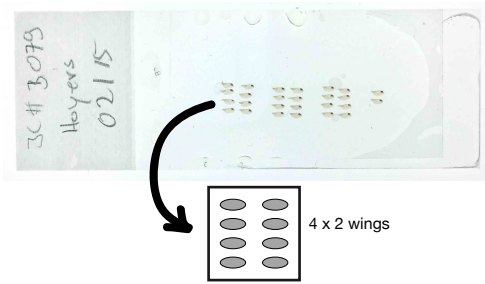


# Protocol for standard mounting of *Drosophila* adult wings (Mechthild Kredler/Nicolas Gompel, April 2015)

## Final result



## Material

- fine forceps
- disposable plastic pipettes
- watch glass dishes
- microscope slides and 20 x 46 mm cover slips
- a matrix for wing positioning, taped under a glass stage (50 x 50 x 2 mm)
- a Sylgard coated 6 cm Ø Petri dish
- mounted needles, (our favorites are cactus spines)
- a small (15 x 15 cm) metal plate to be heated at 70-80°C
- 100% ethanol, non denaturated
- Hoyer's medium

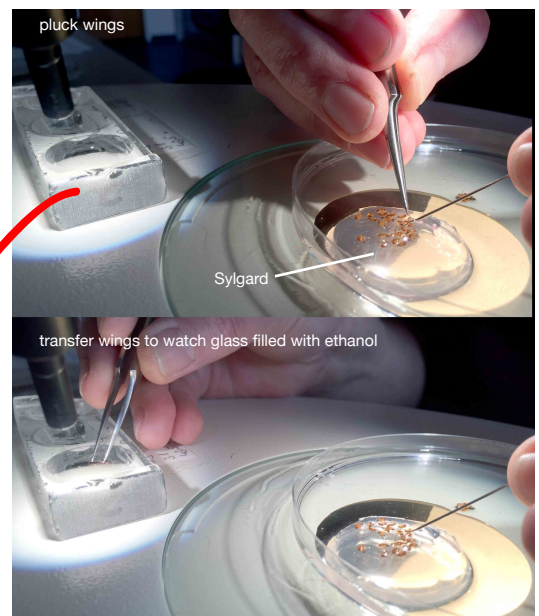
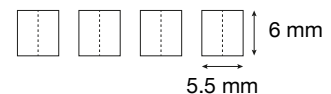
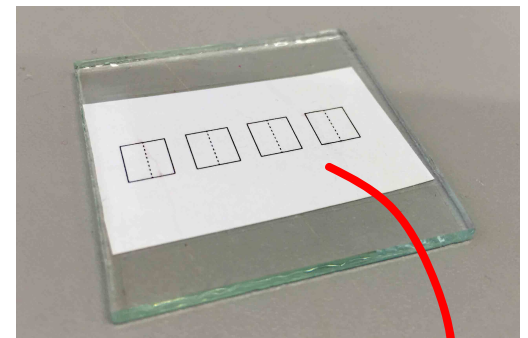
25 ml water  
15 g gum Arabic  
10 ml glycerine  
100 g chloral hydrate

Mix the gum Arabic, the water, and a crystal of chloral hydrate the size of a pea (to prevent fungal growth).  
Soak for 24 hrs  
Add 100 g of chloral hydrate and wait until dissolved (this may take several days)  
Once everything is dissolved add glycerine.

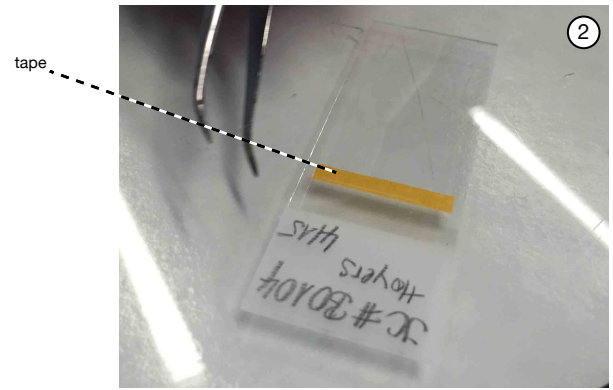
- a 1:1 mix of Hoyer's solution and ethanol (prepare fresh, keep no more than 3-4 days, vortex before use as it Hoyer's precipitates into ethanol)
- a hot plate for heating the metal stage (70-80°C)
- a heating table to pre-cook the mounted wings (temperature ~60°C)
- a large steel plate fitting the heating table and small magnets ([www.supermagnete.de](http://www.supermagnete.de), ref. S-02-02-N, disc magnet Ø 2 mm, height 2 mm, neodymium, N48, nickel-plated) to cook the Hoyer's mounted wings
- a 70°C oven
- adult flies preserved in 70% EtOH

## Preparation

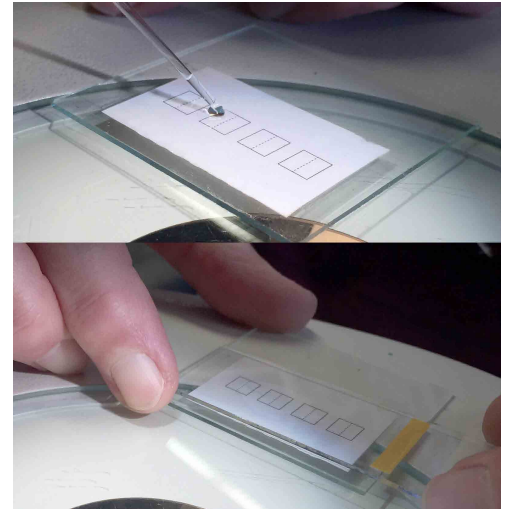
- transfer the flies into the Sylgard-coated Petri dish, filled with 50% ethanol
- under the stereoscope, pluck the wings of each fly and transfer them to a watch glass dish filled with 50% ethanol
- When sampling male pigmentation, discard males with asymmetric wings, such as different pigmentation patterns on each wing and mount a single wing per male



-prepare a microscope slide for mounting the wings: label it and fit a strip of tape close to the label to prevent the ethanol to spill on the label during mounting



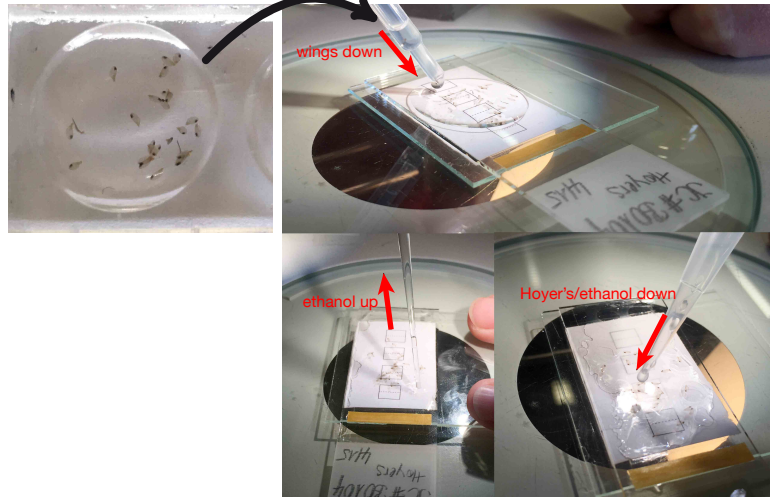
-position the slide onto the matrix fitted to the glass stage, fix it by capilarity with a drop of ethanol



-transfer ~30 wings onto a microscope slide in a droplet of ethanol, using a disposable pipette

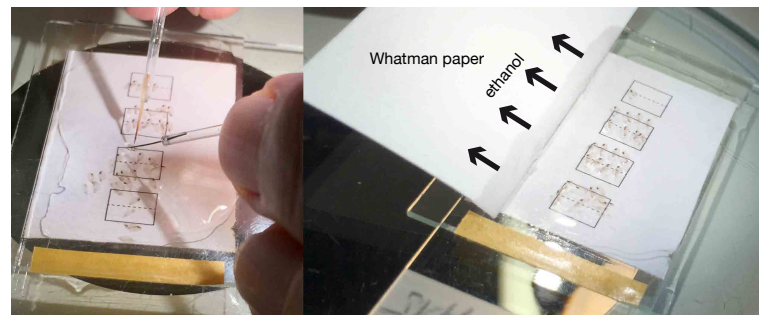
-carefully remove most of the ethanol with a pasteur pipette, do not let all the ethanol evaporate

-add 140  $\mu$ l of Hoyer's-ethanol mix (vortex briefly) **before** the ethanol dries out completely (to avoid bubbles)

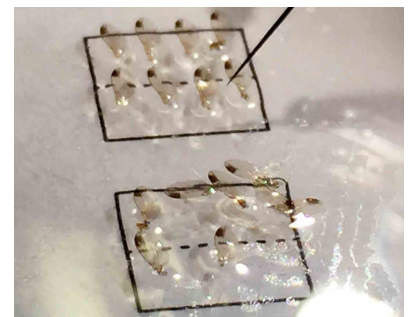


-using the mounted needles, flip the wings until they are all in the same orientation, position them roughly onto the matrices (up to 8 wings per template)

-remove most of the liquid with Whatman paper until the wings can no longer swim



-refine wing arrangements onto the matrices with a mounted needle on the stereoscope stage

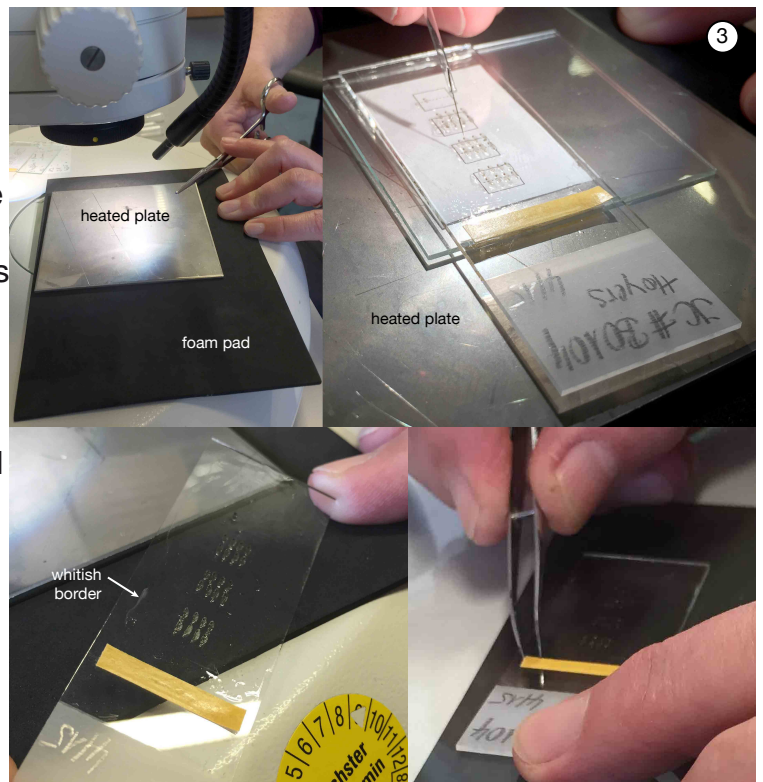


-Install a 70-80°C-heated metal plate on the microscope stage (use forceps to not get burned); use a slim foam pad to prevent the plate from slipping

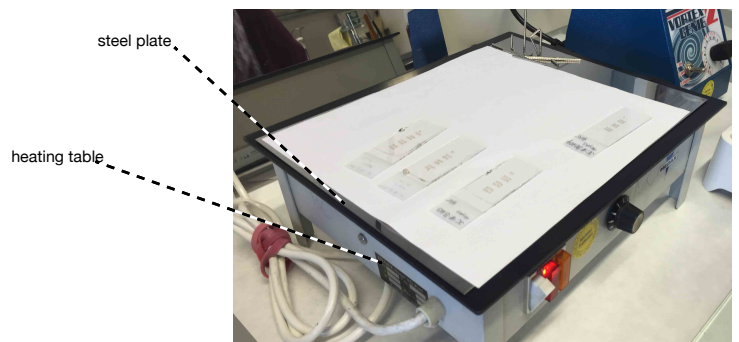
-immediately put the slide and the template on the hot metal plate. Finalize the arrangement of the wings in the next few minutes and until the Hoyer's medium is dry enough to let the wings protrude slightly from the medium

-When the Hoyer's medium is dry enough, a whitish border becomes visible at the edge of the initial drop

-remove the slide from the template and remove

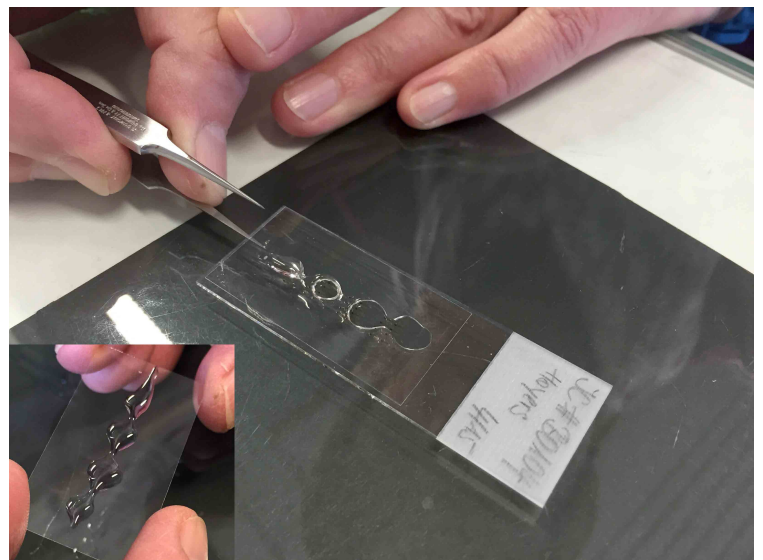


-let the Hoyer's dry for another few minutes on the heating table (60°C)

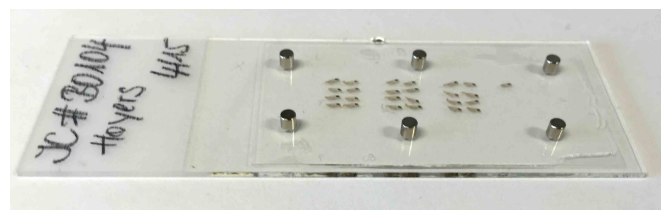


-transfer 4 drops of pure Hoyer's medium on a cover slip with the tip of a needle

-carefully apply the cover slip, Hoyer's down, onto the mounted wings, let the Hoyer's medium spread between the slide and the cover slip



-sandwich the slide between the steel plate and small magnets. Allow the preparation to dry on the heating table for a few hours. Place the entire plate in an oven at 70°C for 2 days





# Protocol for standard imaging of *Drosophila* adult wings (Mechthild Kredler/Nicolas Gompel, April 2015)

④

Microscope: Leica Wild M420

Camera: Manta G-609B/C (GigE camera with Sony ICX694)

Driver: custom driver, developed by Christian Schmid

Lens: 1x

Zoom: 12.5

Aperture: 1 graduation before completely open

Light stage: Durchlichtbeleuchtungen DBL-2020-WT (200x200 mm LED Back Lighting white, MBJ, Hamburg)

Setting file: *2015-04-07 wing1x12,5 settings.mat*



1x lens

light stage



Aperture

Zoom

All flies kept in the same folder, which is named as follows: *YYYY-MM-DD experiment name*

Nomenclature for files: *IC\_B0004\_slide1\_12.5x1\_015*

line name/  
experiment number

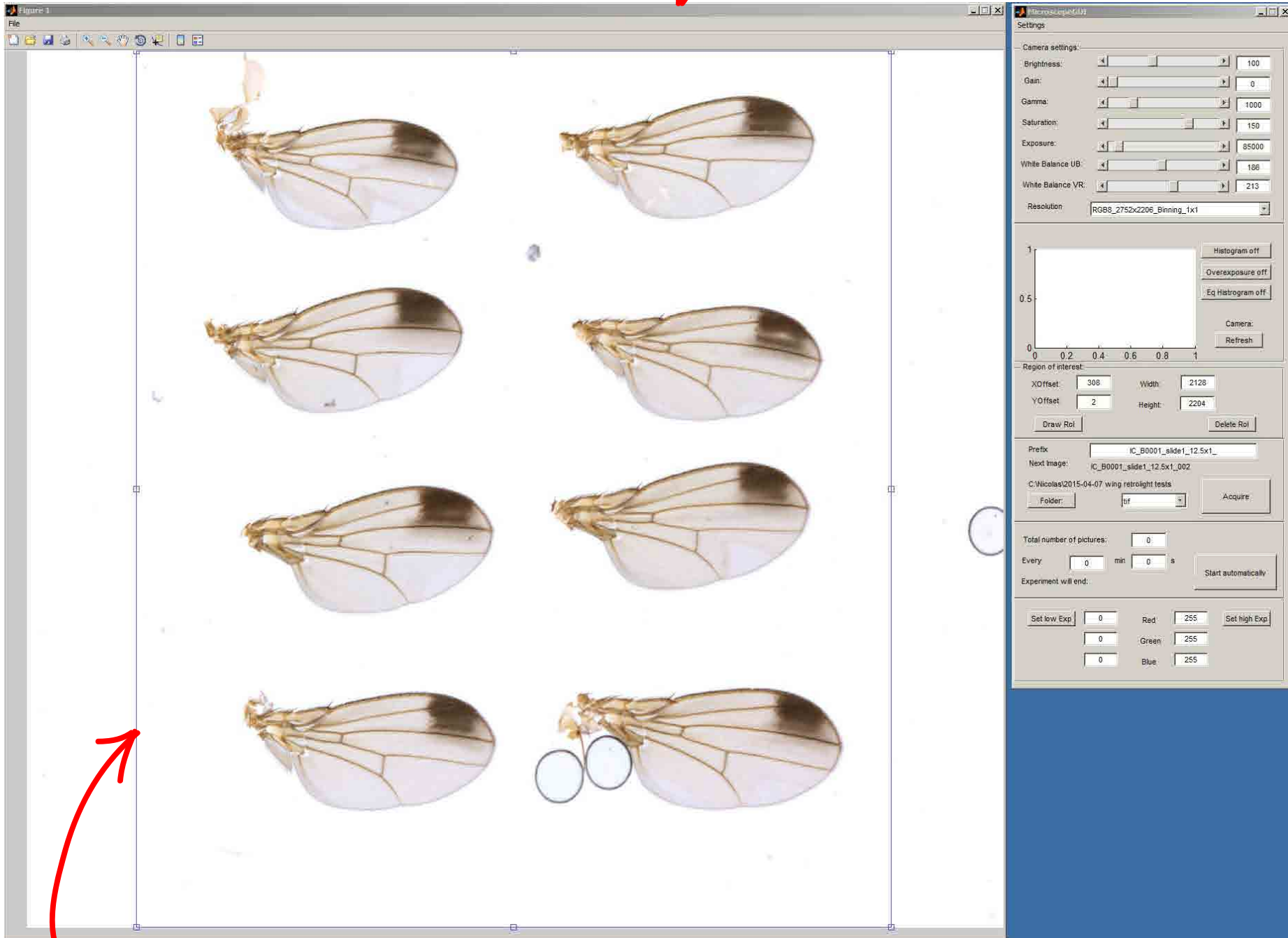
slide number

image number  
(added by the software)

magnification

5

Live display



camera  
settings

Histogram

ROI control

acquisition and  
saving options

time-lapse

saturation  
display

ROI (Region Of Interest)