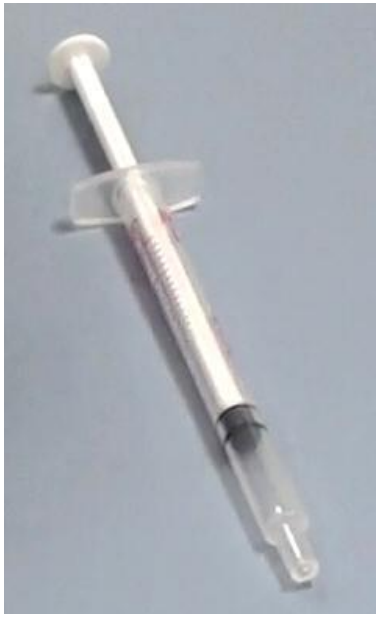
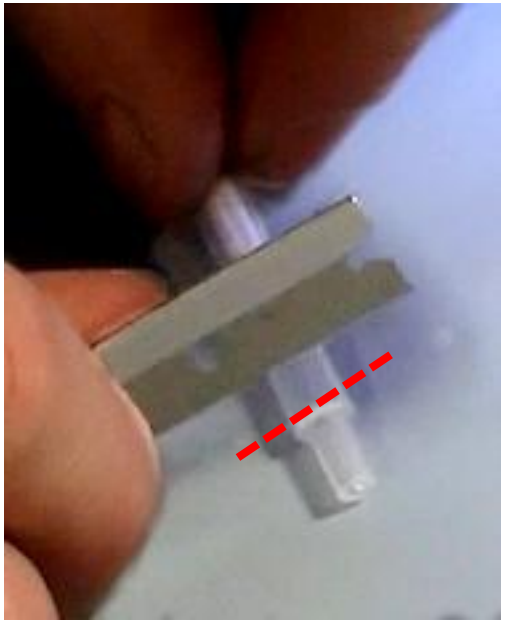
Lightsheet Z.1
Sample Preparation Slides

Annette Bergter
Product Management

Embedding of larger samples
Syringe – Cutting





Use 1 ml syringe with the following characteristics:

- Soft plastic material for easier cutting
- Without needle
- Not too prominent printing, which might block the view onto the sample during embedding

Use a one sided razor blade or a scalpel for cutting.

Cut straight just behind the orifice opening (red dotted line).
This might take some force .

Embedding of larger samples
Syringe – Embedding

Fine Tweezers Cut Syringe Sample
(here green plastiline)

Liquid agarose
- Concentration: 1-1.5%
- Appropriate medium
- Fluorescent beads

Embedding of larger samples
Syringe – Embedding

Fill syringe with agarose.

- Before entering agarose, the plunger pokes out of the syringe (red star) to prevent air bubbles
- Enter liquid agarose and pull the plunger to fill about 0.5 to 1 cm

Use the tweezers to introduce the sample into the agarose

For alignment:
- Rotate syringe slowly horizontally
-Use a dissecting needle
- The agarose will harden within minutes

Embedding of larger samples
Syringe – Embedding

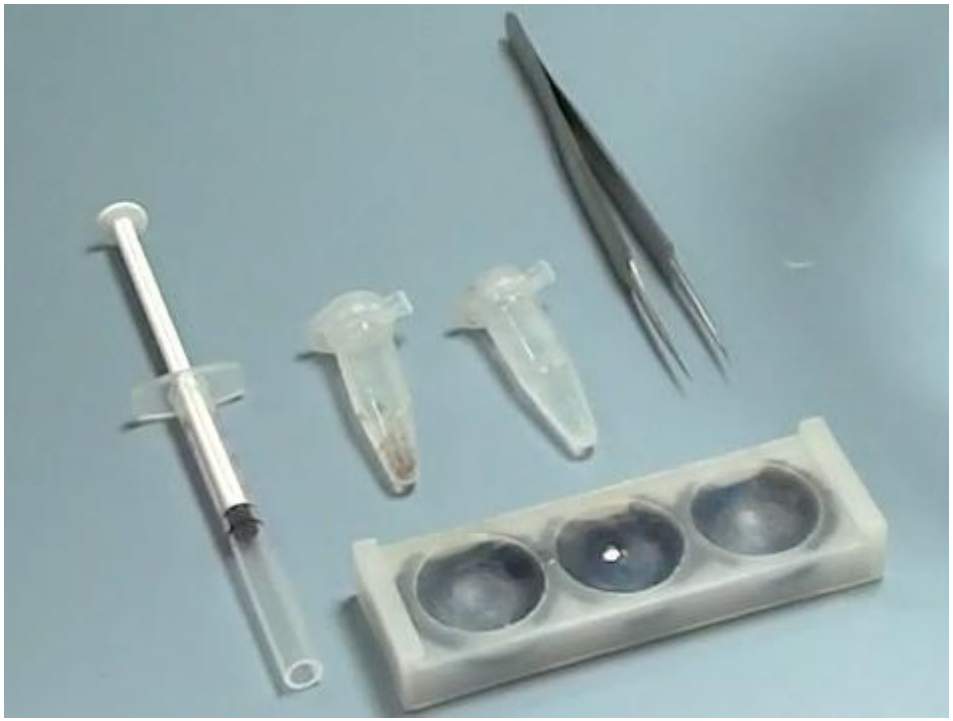
Extra agarose at the end will destabilize the sample during imaging

To remove the agarose:

- Pull the sample back, the extra agarose sticking out
- Cut along the syringe opening

The sample is ready for imaging

Embedding of larger samples
Syringe – Embedding



Depression Slide or watch glass

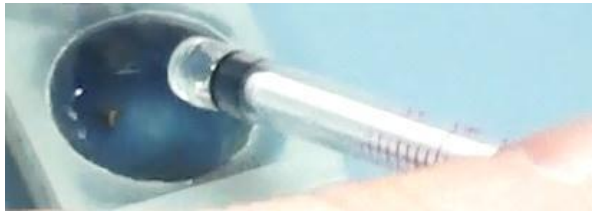
Cut syringe

Adult Drosophila in 70% EtOH or 50% glycerol

1 – 1.5% agarose

Tweezers or Preparation needle

Embedding of larger samples
Syringe – Embedding



Place the fly with the tweezers in the depression slide removing all extra liquid with a pipette.

Use the syringe to fill the depression with liquid agarose and immediately suck the agarose with the fly back into the syringe.

Use tweezers or preparation needle to align the fly within the agarose.

Cut off extra agarose. The fly is ready for imaging.

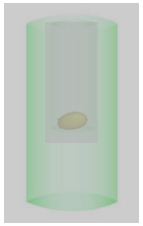
Embedding of larger samples
Mounting Chamber

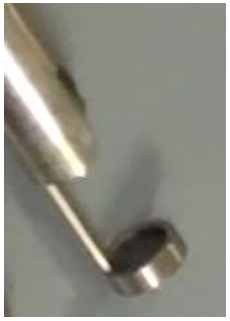
The syringe is modified* so a metal rod of the wanted diameter is at the front of the plunger (here an O-ring, red arrow).

Agarose concentration: 1.5%. If too low, the walls won't be stable.
Phytagel: stable and good optical characteristics

The agarose can be poured into the syringe or suck into the syringe using the plunger.

The mounting chamber is removed by pressing the rod out and cutting along the edge of the syringe with a tweezers.





Sample Holder

The chamber can be used for incubation in specific medium, or to grow plants.

For imaging the mounting chamber is held by a syringe or the same diameter.

* Flood P.M., Kelly R., Gutiérrez-Heredia L. and E.G. Reynaud
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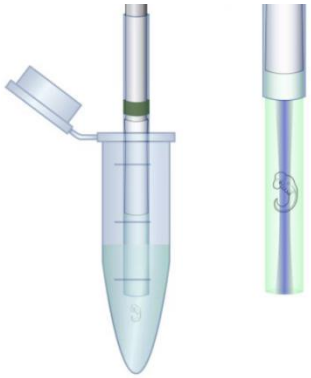
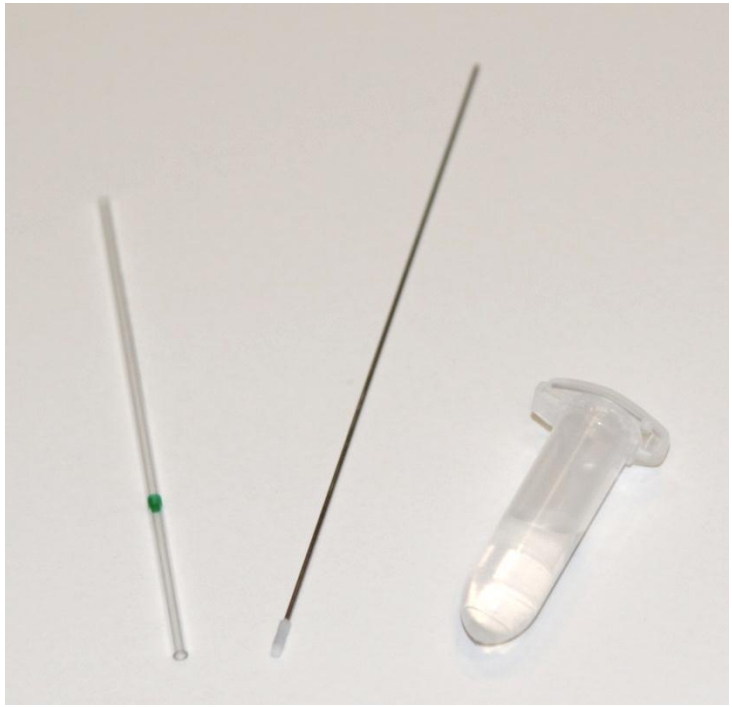
Embedding in Capillaries
Medium sized samples



Capillary
Sample size should be no more than 2/3 and no less than 1/3 of the capillary diameter

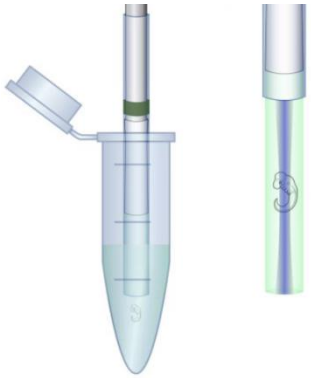


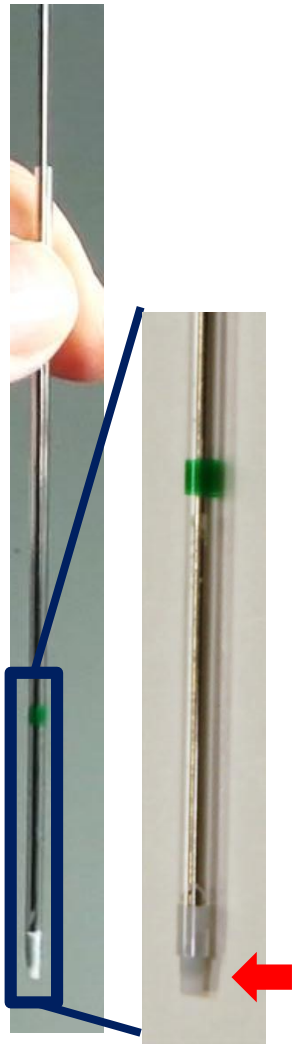
Plunger

0.5-1% liquid agarose
- Appropriate medium
- Fluorescent beads

The following show a zebrafish embryo, similar procedure as well for e.g. *Drosophila* embryos.
For smaller samples a steromicroscope during embedding can be useful.

Embedding in Capillaries
Medium sized samples

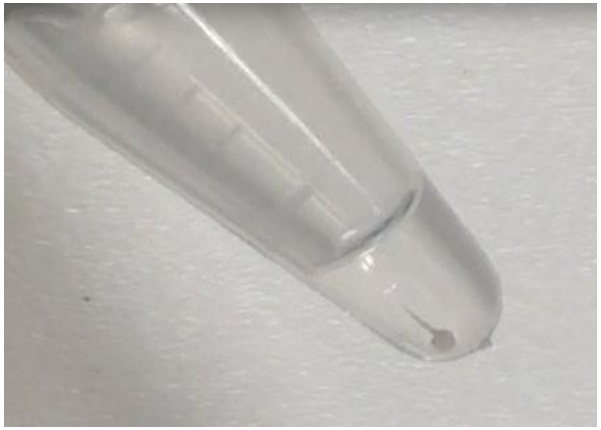




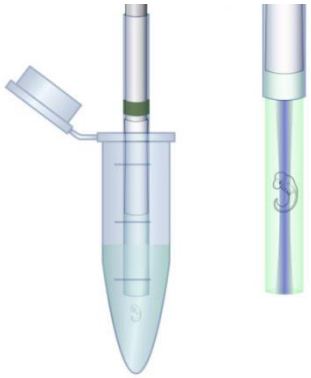
Place the sample in an eppendorf tube, remove all medium.

Add liquid agarose (ca. 200µl) into the eppendorf tube to the zebrafish.

Before dipping the capillary with plunger into the agarose, make sure the plunger sticks out a bit (red arrow) to prevent air bubbles.



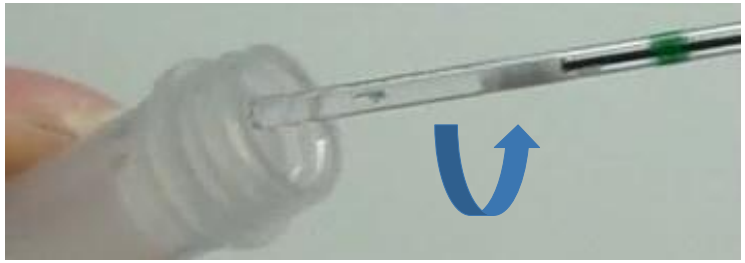
Embedding in Capillaries
Medium sized samples



Bring the capillary close to the sample.

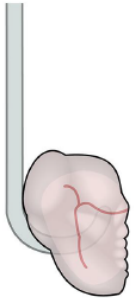
Move the plunger upwards to suck in some agarose, before sucking in the sample.

Proceed gently to not damage the sample at the edge of the capillary while it enters. Hold the capillary horizontally if necessary.



Take the capillary out of the eppendorf tube when the sample is embedded.
Start rotating the capillary, while holding it horizontally until agarose is solid.
Cut off extra agarose .

Hanging Samples
Hook or surgery clamp



Samples can be placed on a hook*, that is fastened to a capillary.
The Lightsheet Z.1 sample holder can be used.

Be aware:
The rotation of the sample is not centered and it might therefore collide with the detection optics.
The hook or clamp will damage the sample and might cast shadows or block the light during imaging.

Samples can be attached by a surgery clamp*, that is fastened to a syringe.
The Lightsheet Z.1 sample holder can be used.

* Flood P.M., Kelly R., Gutiérrez-Heredia L. and E.G. Reynaud
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