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Embedding Four Brains For Serial Section Imaging V.2

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We use this protocol and it's working

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Abstract

Describes how to embed four perfused mouse brains for serial section microscopy using the SWC's embedding molds.

Guidelines

It helps to have a stock of the 4% agar. Do not boil this stock: aliquot the required amount and work with that.

Materials

- 4% agar *suspended* in 50 mM PB
- ~50 ml beaker
- Embedding mold with steel wires (guitar strings work) to support brain
- Forceps

Safety warnings

This protocol uses no dangerous substances.

Ethics statement

This protocol does not describe perfusion or tissue collection. Please follow all relevant government and ethical guidelines when sourcing tissue for this protocol.

Before start

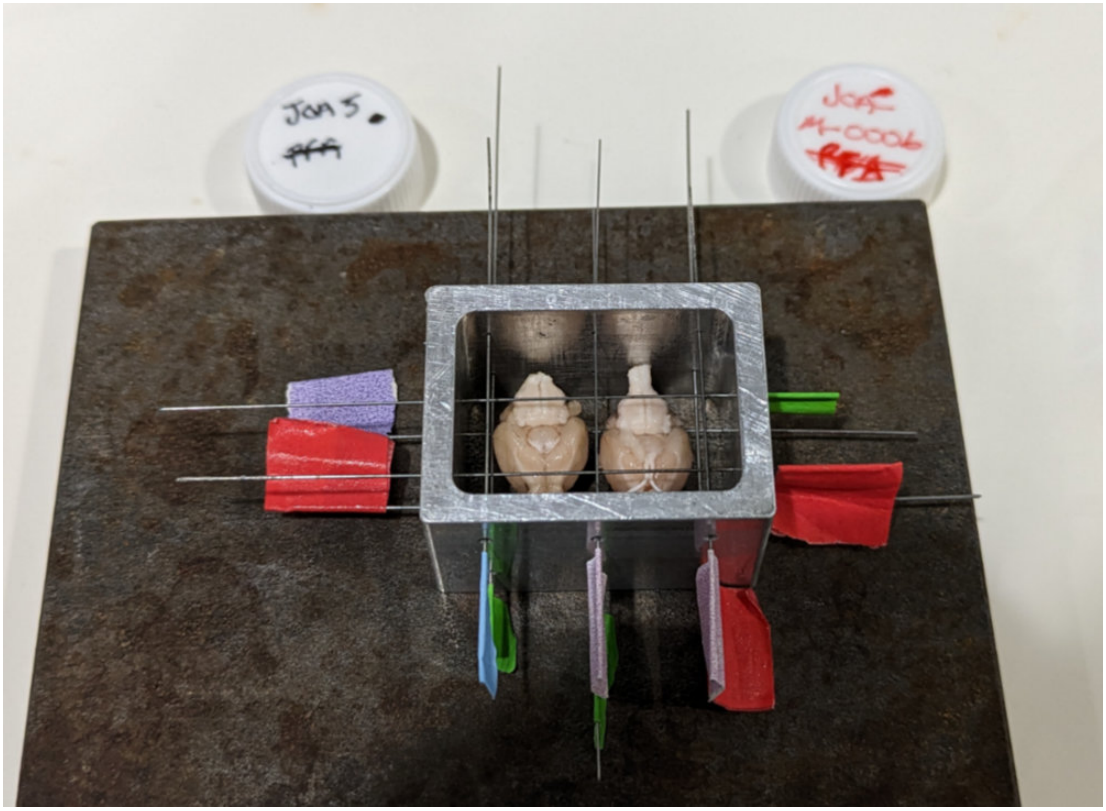
A major advantage of serial sectioning is that it generates a seamless 3D data set that can be registered to a standard atlas. For this whole process to work well the sample should be well perfused (no blood) and be undamaged. Badly perfused samples can cut poorly, producing artefacts. Damaged brains tend to register badly to the atlas.

- Post-fix overnight in 4% PFA at 4 degrees C. This is more important for rat brains: these may need two days.
- Store in regular PBS or 50 mM PB at 4 degrees C for at least 12 hours before imaging. There is usually PB down in the AMF prep area in 2L bottles. Avoid going straight from PFA to 50 mM PB then imaging the same day: these brains tend to swell whilst they are being imaged. (Sucrose is not involved: we aren't using a cryostat.)
- Longer term storage (months) is not encouraged because brains deform over time. If necessary, however, use 0.01% azide **and label as such**. Do not use higher azide concentration: that stuff is really dangerous. **Thoroughly wash all samples stored in azide before bringing them down for sectioning**.

Preparing for agar embedding

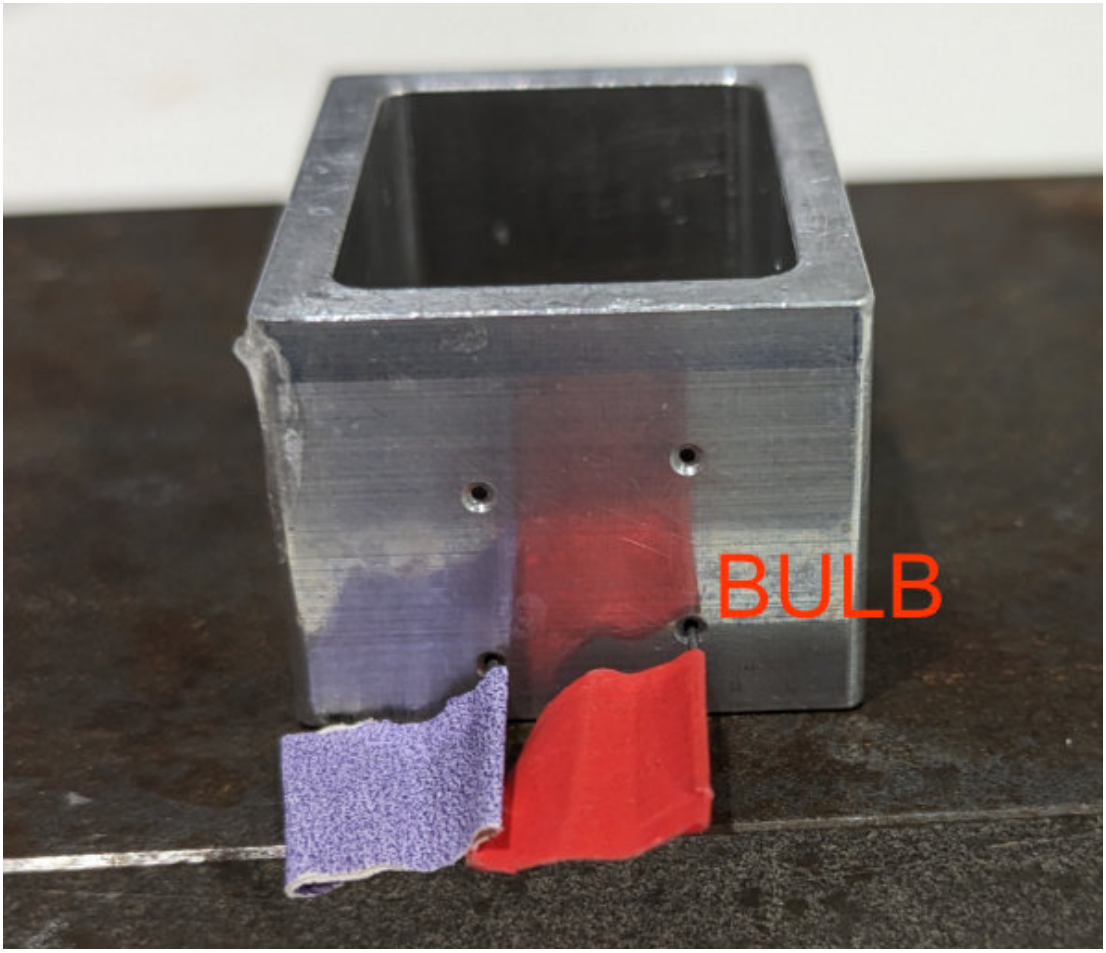
1 Before starting, set out all the brains on absorbent paper. Place the lid or tube next to each brain to ensure you know which is which. **Do not mix up the brains!**

2 Brains are to be suspended in a metal enclosure through which thin wires are inserted. We will mount the lower brains first. Insert 5 steel wires into mold as shown (colors on the tape are not meaningful).



3 Place two brains into the mold then put in the remaining 5 wires. Note that the bulb sits on the higher (here red) wire.

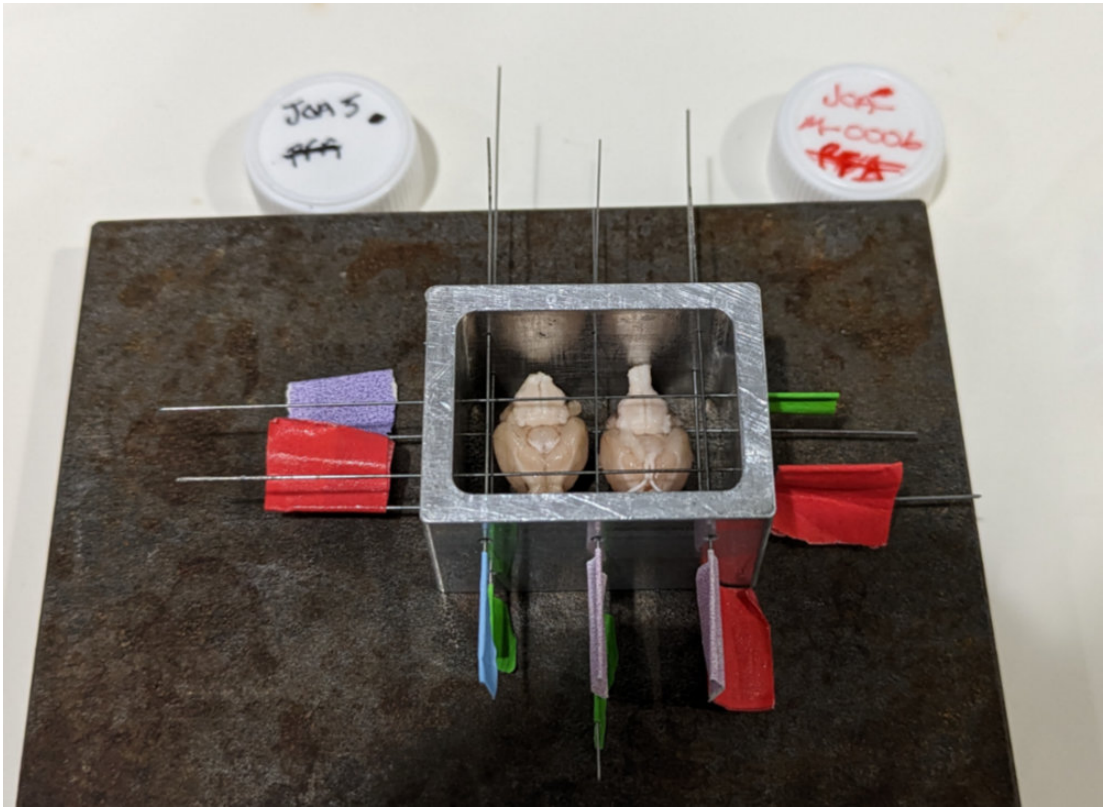
Two brains inserted side by side.



The bulb is supported by the higher (red) string. This ensures that the ventral surface of the brain is roughly parallel with the table.

4 It is **critical** to ensure that you know which brain is which. In the image below note that the lids from the tubes holding the brains are placed such that their locations indicate which brain is which.

The imaging proceeds from cerebellum to bulb, so the bulb is the last thing to be imaged. When we set the agar block onto the slide for imaging (step below), the surface closest to the camera in the image below will be the surface that is glued to the slide. Therefore the two brains shown here will the top row in the imaged FoV. This will make more sense later, but keep this thought in mind for now.



The side of the agar closest to the bulbs will be that which we glue to the slide.

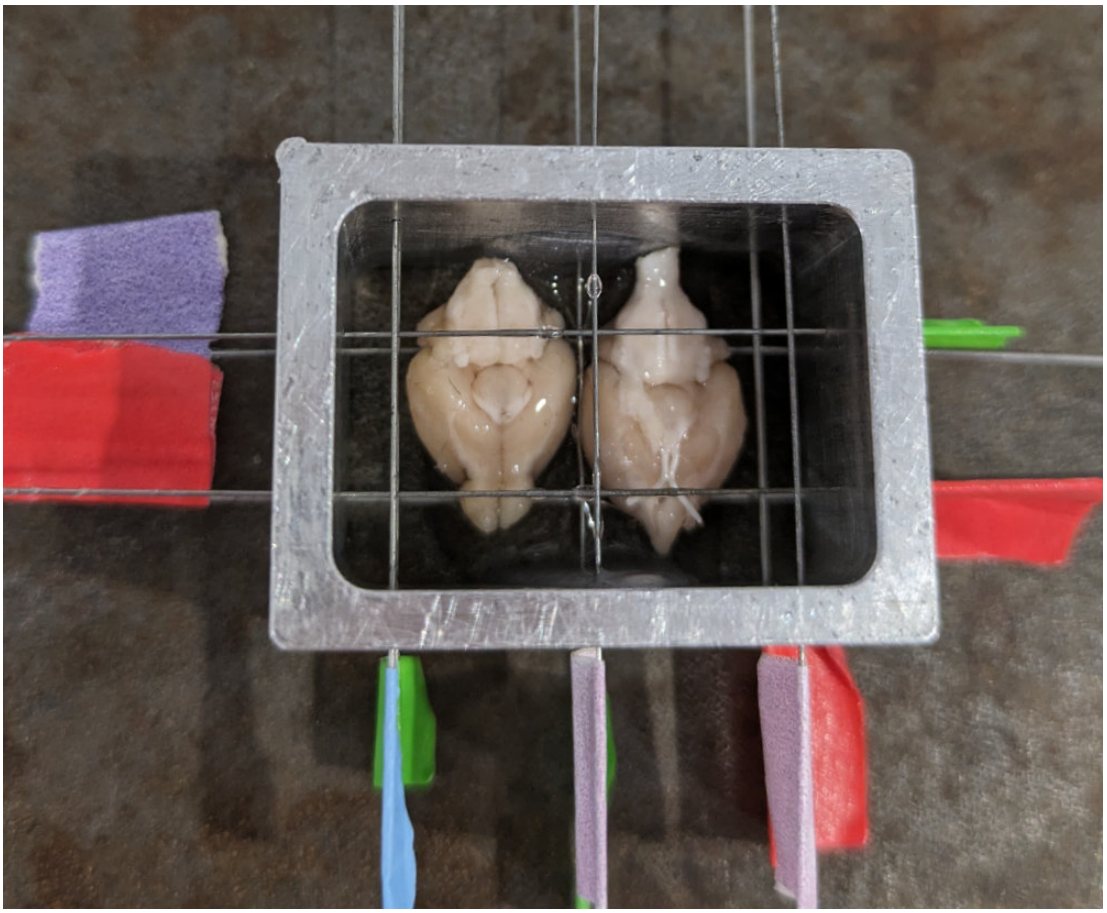
Pouring the initial agar

5 Briefly shake the agar suspension then pour about 20 mL of it into a small beaker.

6 Set microwave to low or medium power and heat carefully. Watch carefully so it does not over-boil. It is sufficient if the agar has only just melted. It does not have to boil thoroughly. Don't worry about air bubbles.

The agar is ready to use once it is about about 50 °C to 60 °C You can either use a thermometer or simply judge it according to when the underside of the beaker is no longer painful when pressed against your wrist.

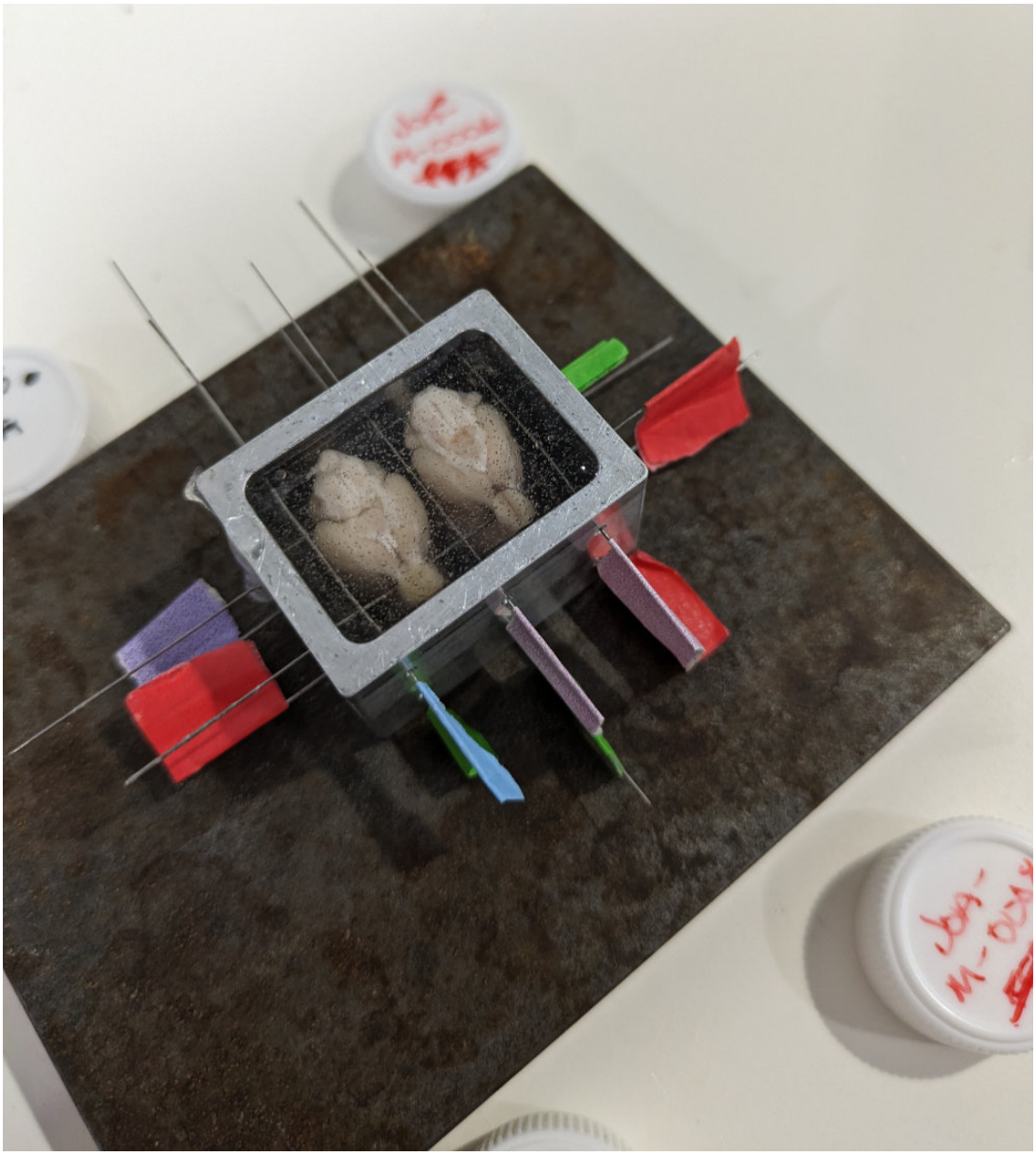
7 Pour a small quantity of agar between the two brains to keep them in place.



A small quantity of agar has been poured between the brains

Placing the next two brains

8 Place the next two brains into the mold then fill with agar. Use forceps to stop brains from floating. It helps to use your dominant hand to hold the forceps whilst pouring with the other hand. The image below shows the mold with all four brains. Note the tube cap with a brain ID name written on it refers to the right brain of the top row.



Trim agar block and mount on slide

5m

9 Wait for agar to cool. This is faster in the fridge. 5m

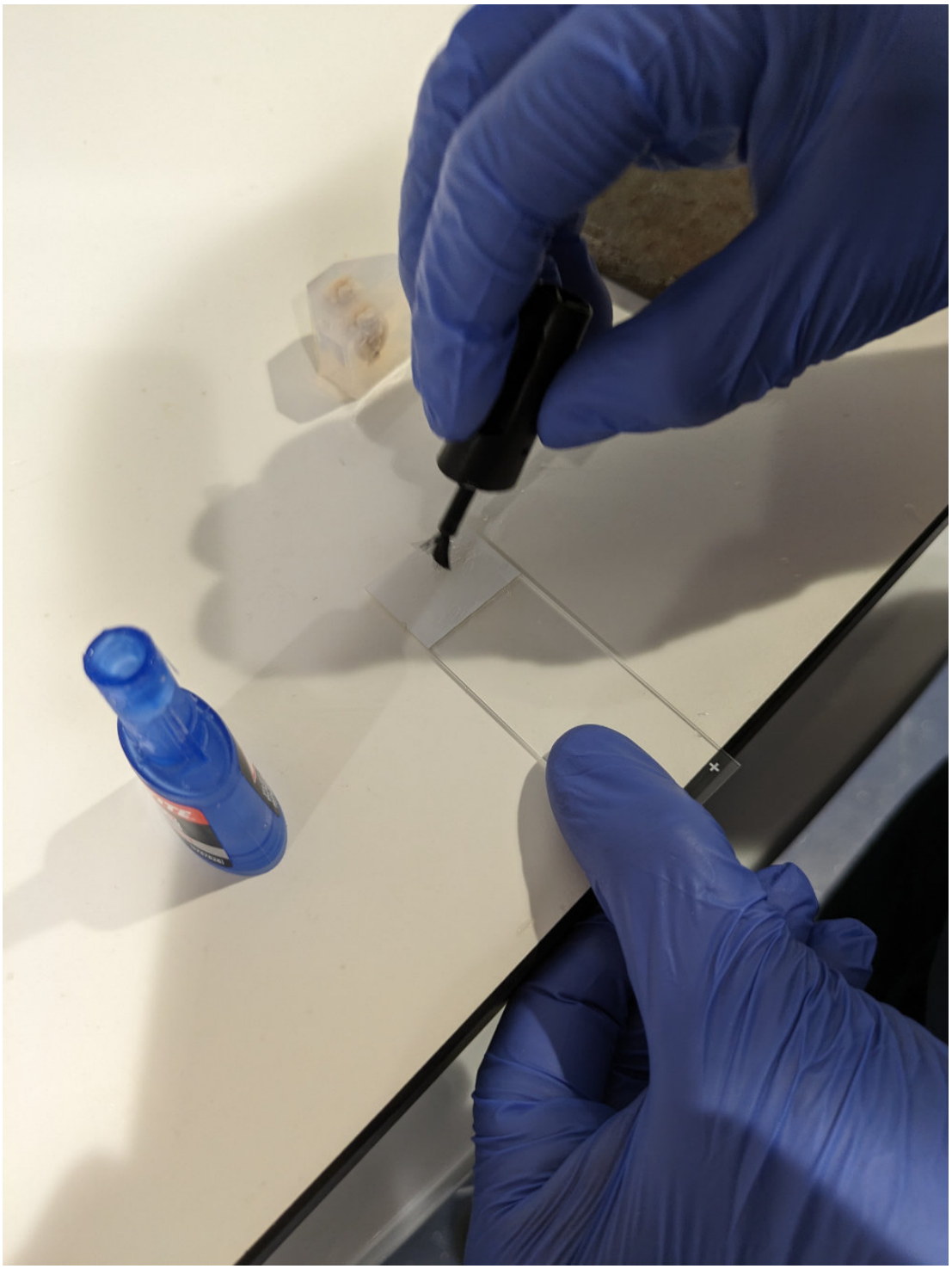
10 If the agar is projecting above the mold, trim off with a razor. Then pull out the strings and push out the mold.

11 Trim agar as shown. **Do not** trim the agar block tightly around the brains. The acquisition software automatically finds the brains for imaging, but at some wavelengths the agar exhibits

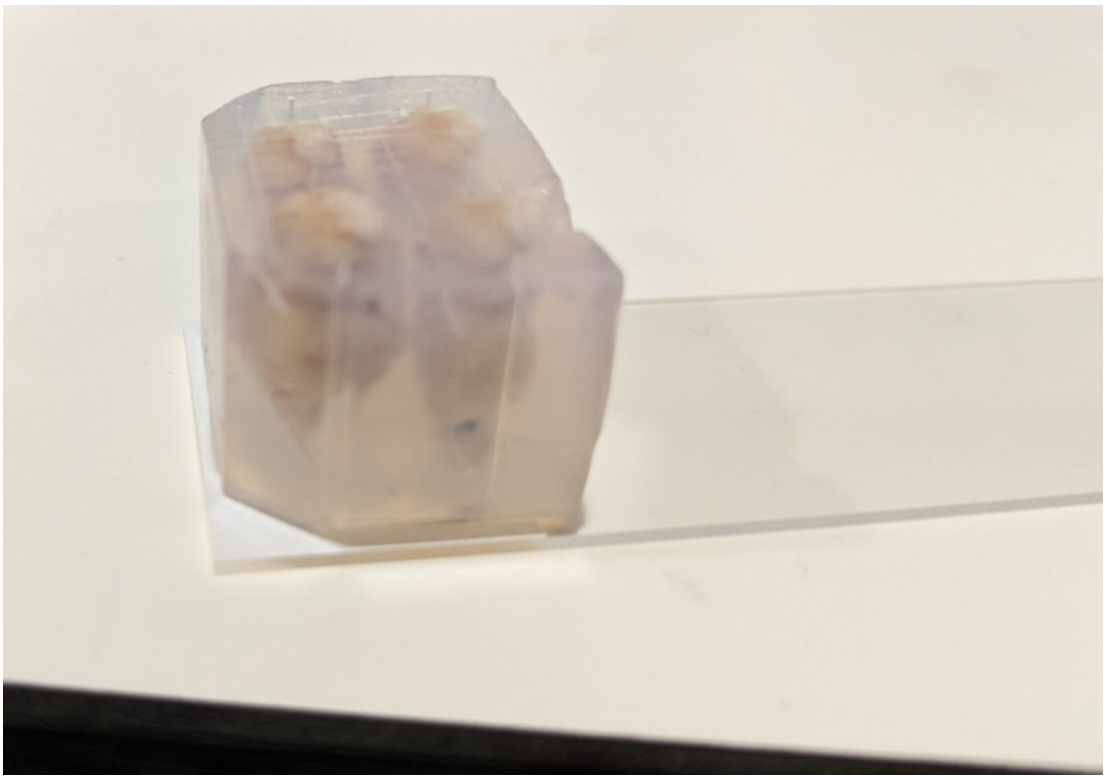
autofluorescence and the software might start to mistake the agar for your brain and image an excessively large area.

If there is a lot of agar above the cerebellum, this too can be trimmed off. Doing so will reduce setup time on the microscope, as trimming the agar block is relatively slow.

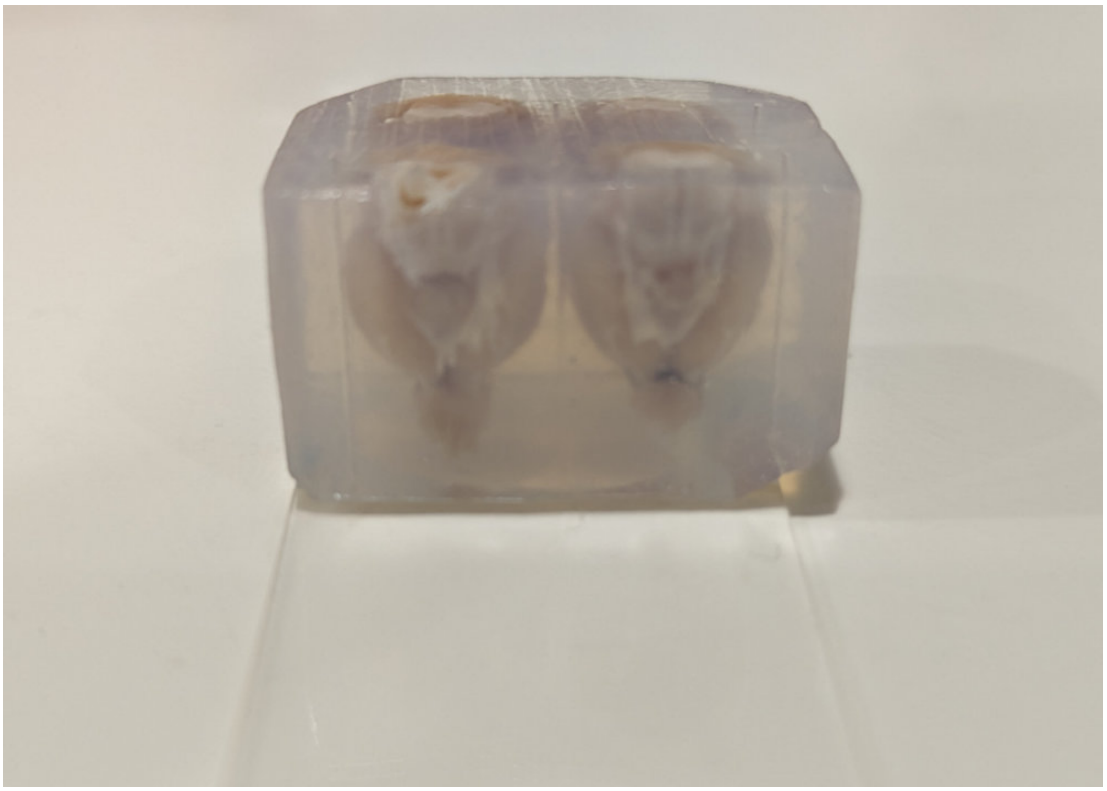
12 Apply superglue to the frosted portion of the slide



13 Place agar block on to the glue. This dries in a minute or so.

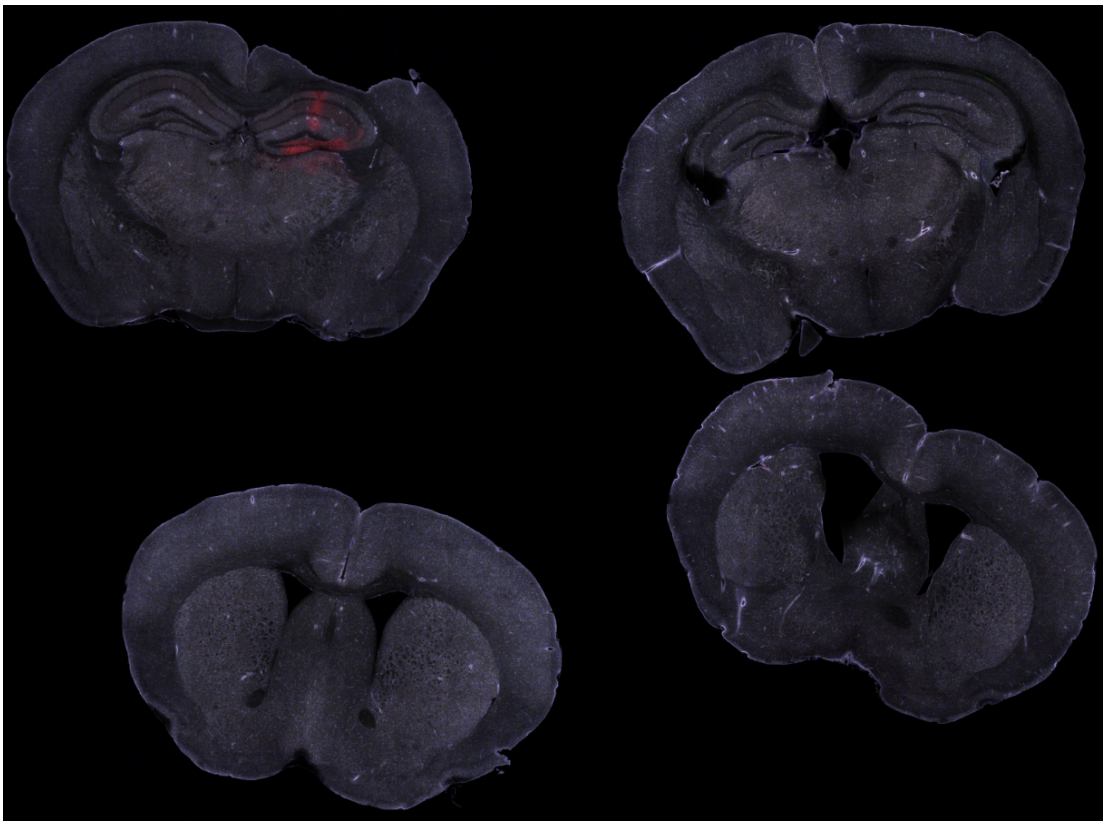


14 If the agar block is wider than slide, notch it out as shown in the bottom right of the block in the image below. This will allow the slide to be clamped into the water bath.



Outcome

15 Getting good results from automated whole brain imaging is far easier when brains are well perfused and undamaged. The image below illustrates problems that can occur if the above steps are not followed.



The above image is taken during acquisition of a poorly prepared sample. Damage, particularly in the top right brain, will impair registration to the Allen atlas template. These brains have not been perfused, as evidenced by the bright white vessels. This can cause cutting issues and interfere with automated analysis pipelines. The bottom right brain in particular is highly rotated. Rotation of this degree can cause automated registration to the Allen template to fail, and necessitate manual de-rotation of the raw data.