Dil Stain Protocol

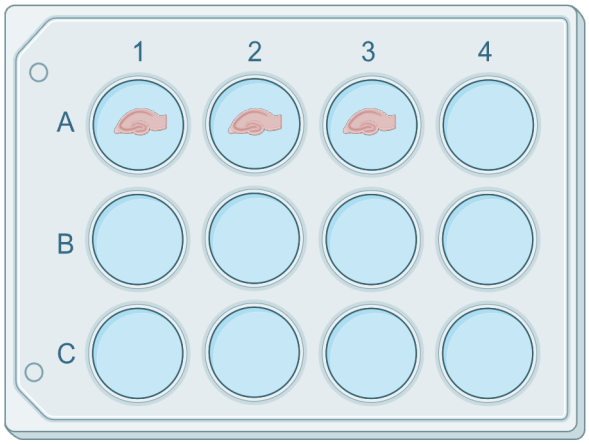
Mia Till, adapted from Hannah Reid's Protocol
July 2025

What is Dil?

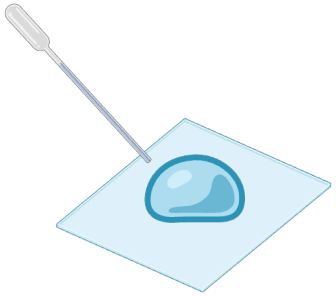
Dil (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) is a fluorescent dye that inserts into the lipid bilayer of the cell membrane. It is commonly used to label the dendrites of a neuron, allowing for quantification of dendritic spines and their morphologies.

Day 1

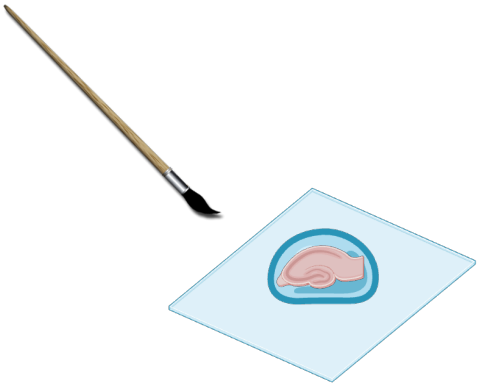
1. Extract the brain from the animal and generate 200µm thick transverse sections using a compresstome. Typically 2-3 slices per brain are collected to account for error.



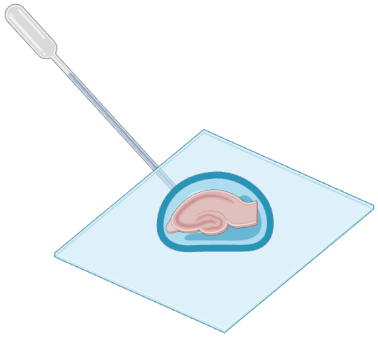
2. Transfer the slices to 1% PFA in a 12 well plate immediately after they are cut. Place the plate on a shaker at RT for 1hr.



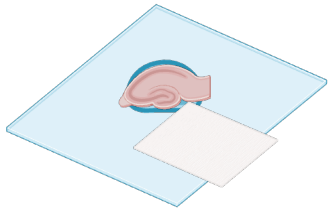
3. Place a large drop of 1x PBS on a square of parafilm.



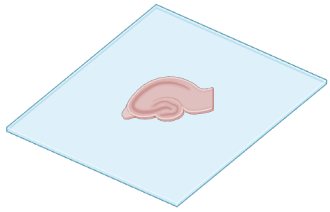
4. Use a paintbrush to transfer the slice from the 12 well plate to into the drop of PBS. Ensure the slice is not folded.



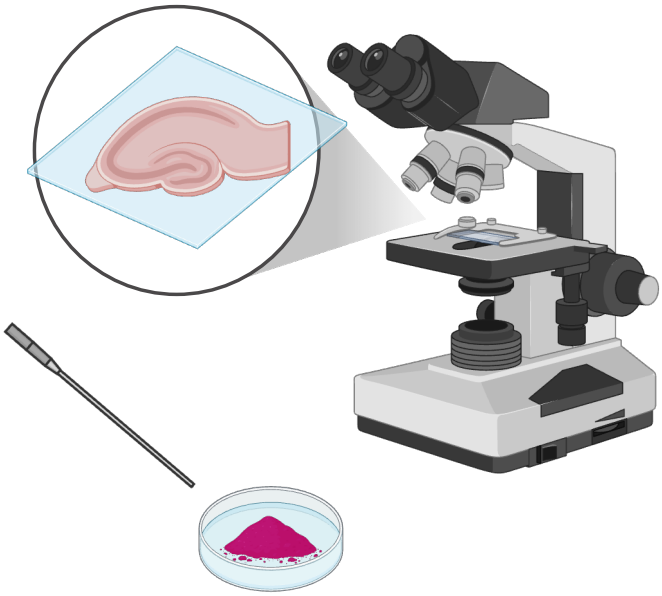
5. Use an eye drop to carefully remove the PBS from the slice. Place the eyedrop around the edges of the slice, do not pipette from the top. Ensure the slice lies flat.



6. Use the corner of a kimwipe to carefully remove the rest of the PBS by gently dabbing around the slice.



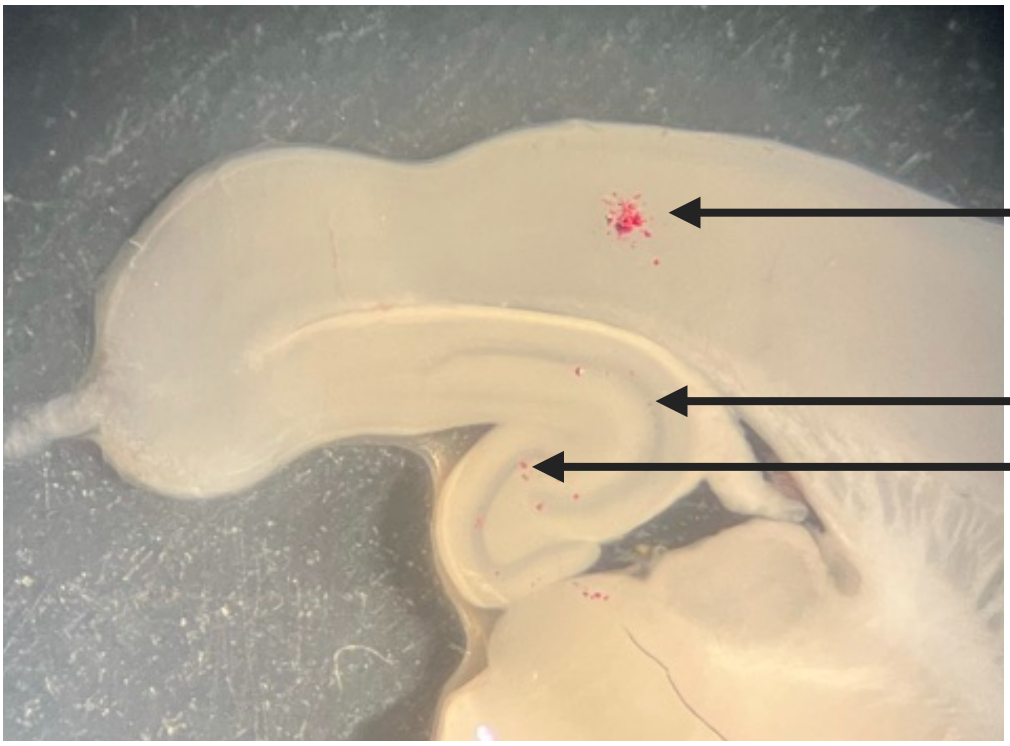
7. Let the slice dry on the parafilm for approximately 5min, or until the slice no longer appears shiny.

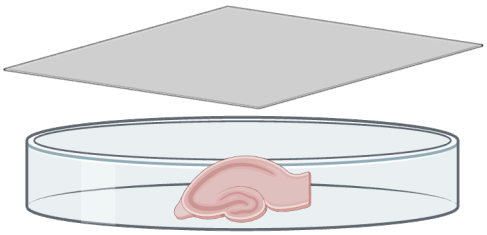


8. Transfer the slice on the parafilm to the microscope. Pick up DiI crystals with a metal dissecting probe. Very few crystals are needed.

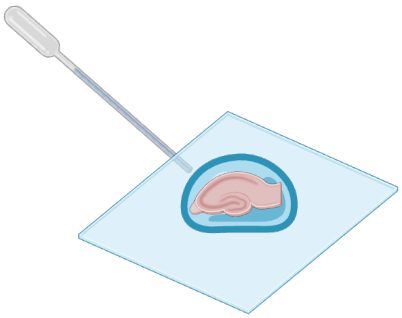
Be careful with the DiI crystals as they are very light sensitive. They should be temporarily stored covered in tinfoil. Long term storage is at -20°C.

9. First remove the excess DiI crystals on the probe by tapping on a part of the slice which you won't be imaging. Then place the crystals on your region of interest. You should barely be able to see the crystals placed on the slice. If you can see multiple, there are too many crystals in that spot. Be careful to place them on top of the slice without poking it.

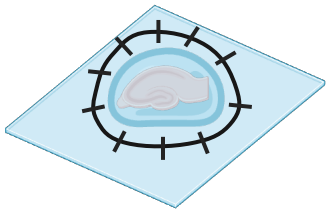




10. After crystal placement, allow the slice to dry on parafilm under a piece of tinfoil for 5min.

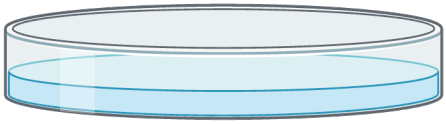


11. Place another drop of PBS covering the slice using an eyedropper. Do not pipette directly on the slice, but allow the drop to wash over the tissue from the side.

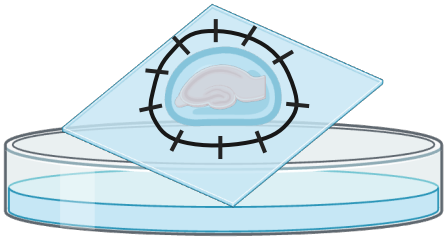


12. Place a second square of parafilm on top of the slice. Use pen to draw a circle around the slice, accounting for the liquid to expand outwards. Draw stitch marks to seal the parafilm pieces together. Place in a petri dish wrapped in tinfoil for 24hr at 4°C.

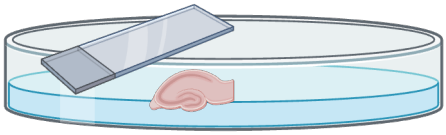
Day 2



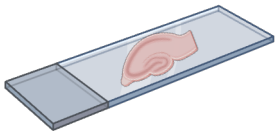
13. Fill a petri dish with 1x PBS. This will be used to wet mount the tissue.



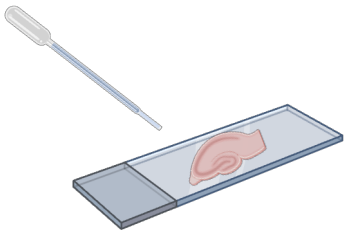
14. Separate the pieces of parafilm and transfer the slice from the parafilm into the petri dish.



15. Activate a slide by rubbing it with PBS using a paintbrush. Float the tissue onto the slide in the petri dish.



16. Allow the tissue to begin drying on the slide. This will take a couple minutes, but depends on the thickness of the tissue.



17. Place fluoromount on the tissue. Use a needle to poke any bubbles. Lay the cover slip down flat and allow the slide to dry under tinfoil.

Day 3

View the slide under the confocal microscope and obtain Z-stacks for image analysis. Note that you must be trained prior to use of the confocal microscope.