

DiI Stain Protocol

M. Till, Adapted from Hannah Reid's protocol | July 2025



What is DiI?

DiI (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 typically used to label the dendrites of a neuron, allowing for quantification of dendritic spines and their morphologies. DiI is commonly performed in tandem with electrophysiology in order to use brain slices efficiently.

The first day of this protocol consists of obtaining the slices and placing the crystals. On the second day of the protocol the slides will be cover slipped, and then imaged on the third day.

Day 1

Materials Needed

- 1% PFA solution
- 1x PBS
- 12 well plate
- Tinfoil
- Kimwipes
- Eyedrop
- DiI crystals
- Paint brush
- Compresstome
- Dissection materials
- Parafilm
- Dissecting microscope
- Pen
- 200 μ m thick transverse brain slices

Protocol

1. Extract the brain from the animal on ice.
2. Use a compresstome to slice the brain into 200 μ m thick transverse slices.
3. Immediately place the slices in 1% PFA in a 12 well plate for 1hr at room temperature on a shaker. Prepare the PFA in the fume hood.
4. Cut 2x parafilm squares per brain.
5. Place a large drop of 1x PBS on the parafilm.
6. Use a large paintbrush to transfer the slice from the 12 well plate to the PBS drop.
7. Hold the slice with a paintbrush to help it lie flat while removing the PBS surrounding the slice with the eyedrop.

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8. Once majority of the PBS is removed with the eyedrop, use a kimwipe to dab around the edges of the slice and remove the last of the PBS. Extra PBS left on the slice will cause the DiI crystals to wash away.
9. Allow the brain to dry on parafilm for approximately 5 minutes, or until no longer shiny. The exact timing will depend on how quickly you remove the excess PBS.
10. Move the slice to the dissecting microscope and focus the microscope on the slice.
11. Pick up DiI crystals using a metal pin by gently tapping the crystals in their storage petri dish. DiI crystals are extremely light-sensitive, so ensure the lid is replaced on the petri dish afterwards.
12. Use the dissecting microscope to first tap the metal pin on a piece of the slice that you will not be imaging in order to remove extra crystals.
13. Place one crystal on each spot you want to image. You will barely be able to see the crystal after placement. Ensure you place the crystal on top of the tissue and do not stab it in.
14. Let the slice dry for another 5 minutes under tinfoil to allow the crystals to settle into the tissue.
15. Cover the slice with another drop of PBS using the eyedrop. Do not put the PBS directly on the slice or the crystals may wash away. Angle the eyedrop to the side of the slice.
16. Place the second square of parafilm on top of the slice and gently press down on the edges.
17. Draw a circle using pen on the parafilm around the slice to join the pieces together. Account for how the PBS will spread when you draw the circle.
18. Draw stitch marks on the circle to further join the parafilm pieces, making the circle resemble a sun.
19. Place the parafilm squares in a small petri dish and cover it in tinfoil. Label the tinfoil and put it in the fridge for 24 hours.

Day 2

Materials Needed

- Large petri dish
- Microscope slides (labelled)
- Microscope cover slips
- Paintbrushes
- Fluoromount
- Needle
- Eyedrop
- 1x PBS

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Protocol

It is best practice to have everything set up ahead of time to limit the amount of photobleaching on your slices.

1. Gather all necessary materials.
2. Pour 1x PBS into a large petri dish.
3. Remove the petri dish from tinfoil and separate the pieces of parafilm. Place the piece of parafilm with the brain slice stuck to it face down in the petri dish to transfer the slice.
4. Activate the slide by rubbing it with PBS using a paintbrush.
5. Float the slice onto the slide in the petri dish using a paintbrush.
6. Place the slide flat suspended off the bench using tube racks to prop them up. Cover the slides with tinfoil until they begin to dry. The tissue should be dry enough so it lies flat and is not obviously wet, or the staining will be wavy. You can use a kimwipe to wick away excess PBS if needed.
7. Once dry, cover the slice in fluoromount using an eyedrop. Use a needle to pop any bubbles. Pick up the cover slip and gently lay it down on the slide using the needle to lower it.
8. Leave the slide suspended flat off the bench for 24 hours, covered with tinfoil. Once dried they are ready to be imaged using the confocal microscope. It is best to image the DiI on the third day of the protocol, as within a few days after the dye will have dissociated. Note that you must complete training prior to use of the confocal.