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We use this protocol and it's working

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Sectioning Mouse Brain with Sliding Microtome

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ABSTRACT

This is a protocol that details sectioning a mouse brain fixed in 4% PFA and 30% sucrose on a sliding microtome. This protocol details the microtome set up, the mounting of the brain on the microtome stage in OCT or 30% sucrose, slicing the brain with the microtome blade, microtome take down and clean up, and tissue section storage.

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Keywords: Histology, Microtome, Sectioning, Immunohistochemistry

MATERIALS

Sample

Dry Ice **Contributed by users**

Sucrose **MP Biomedicals Catalog #**04821721

Tissue-Tek® O.C.T. Compound, Sakura® Finetek **VWR International Catalog #**25608-930

10xPBS **Ambion Catalog #**AM9624

Sodium Azide 5% **Ricca Chemical Company Catalog #**71448-16

Milli-Q Water **Contributed by users**

Ethyl alcohol **Grainger Catalog #**12352104

500ml Mineral Oil **G-Biosciences Catalog #**RC-118

	Materials	Product number
	Sliding microtome	Leica, SM2010 R
	48 well clear TC-treated multiple well plates	Costar 3548
	Fine tip paintbrush	Amazon, PBR-01
	Rubber mallet	Uline, H-2215
	Petri dish	Thermofisher Scientific, 263991
	Single edge razor blade	Uline, H-595B
	Stir plate	MilliporeSigma, Z693510
	Magnetic stirring bar	MilliporeSigma, Z328839
	Kimwipes	MilliporeSigma, Z188956-60PAK
	Scale	Mettler Toledo, 30317530
	Weighing paper	MilliporeSigma, WHA1034767
	Disposable spatulas	VWR, 80081-194
	Parafilm	Uline, S-25928

Recipes:

1L 1xPBS:

Combine the following reagents into a container with a stir bar. Mix well on a stir plate at high speed (300 RPM or higher) for 00:02:00 or until solution is mixed. Store at Room temperature for 1 month.

	Reagent	Volume
	10xPBS	100ml
	Milli-Q water	900ml

1L 1xPBS & Sodium Azide 0.01% :

Combine the following reagents into a container with a stir bar. Mix well on a stir plate at high speed (300 RPM or higher) for 00:02:00 or until solution is mixed. Store at Room temperature or 4 °C for up to 1 year.

	Reagent	Volume
	Sodium Azide 5%	2mL
	1xPBS	998mL

50ml 1xPBS & Sucrose 30%:

Measure sucrose using a scale and place into a container, then add 1xPBS with a serological pipette. Mix well on a stir plate at high speed (300 RPM or higher) for 00:05:00 or until sucrose crystals are dissolved in 1xPBS. Store at 4 °C for 1 month.

	Reagent	Volume
	Sucrose	15g
	1xPBS	50mL

1L 70% Ethyl Alcohol:

Measure Milli-Q water and Ethanol into a container. Stir for 00:10:00 .

	Reagent	Volume
	Milli-Q	300mL
	Ethyl alcohol	700mL

SAFETY WARNINGS

Paraformaldehyde is carcinogenic. Wear gloves at all times when handling specimen fixed in paraformaldehyde, as the tissue may contain trace amounts of the chemical.

Take care when handling microtome knives. The cutting edge is extremely sharp and may cause severe injury.

Sodium Azide is toxic and carcinogenic. It should be handled and prepared with care. Do not breathe dust, do not use metal utensils. Wear gloves when handling this chemical.

Personal Protective Equipment (PPE) should be used at all times while operating this protocol. If you are unsure what PPE you should be using, see your immediate supervisor.

BEFORE START INSTRUCTIONS

The mouse brain must be fixed in 30% sucrose for 1-2 days prior to sectioning on microtome, or until the brain sinks to the bottom of the container of 30% sucrose.

Setup

1m

1

Work station setup:

1.1 Set up a well plate next to the microtome. This will be used to store the brain sections that are sliced during this protocol. Wells should be filled with 1xPBS & Sodium Azide 0.01% for tissue

preservation. Label the well plate lid with relevant information: brain ID number, section thickness, date, etc.

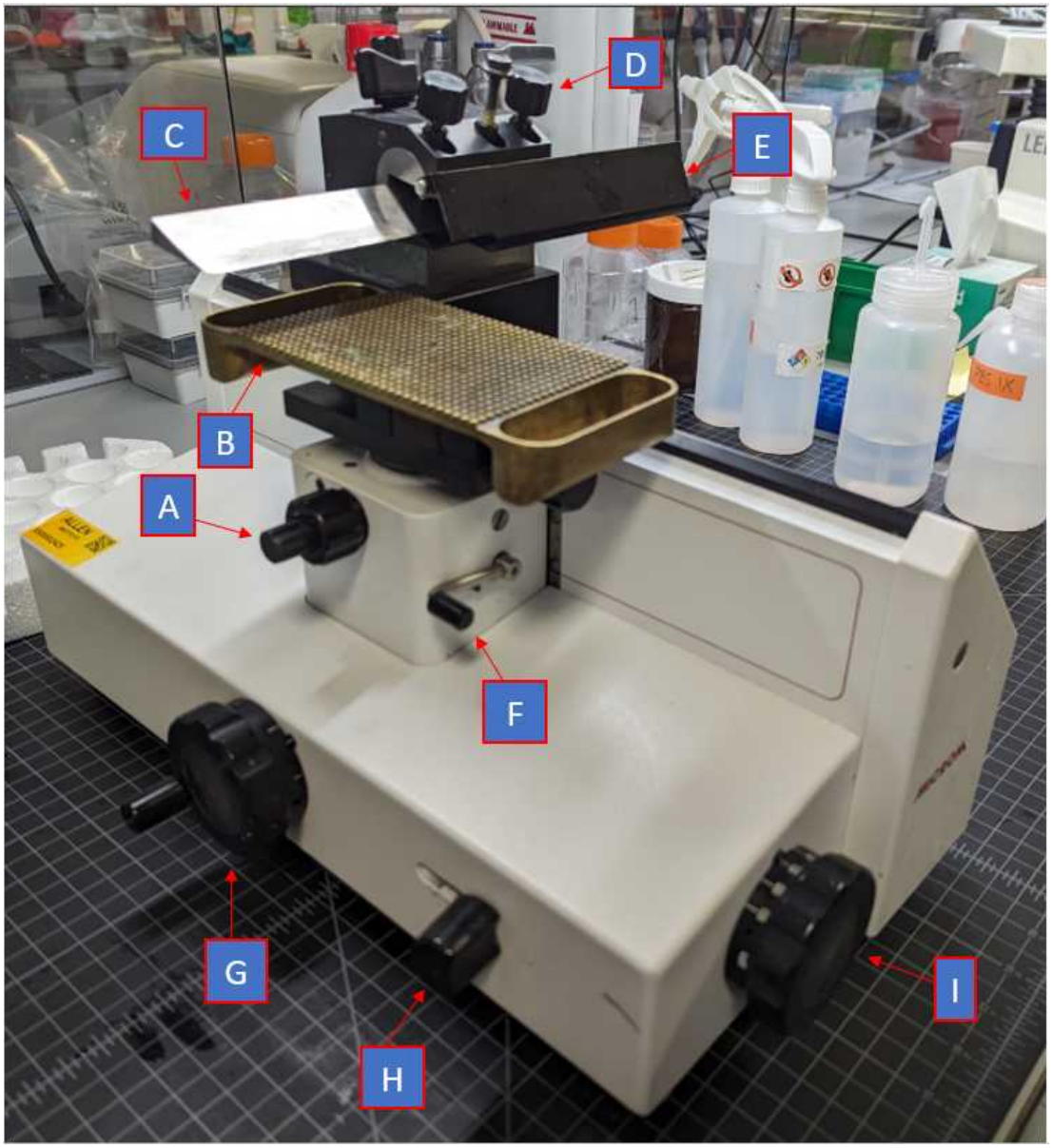
Safety information

Sodium Azide is toxic and carcinogenic. It should be handled and prepared with care. Do not breathe dust, do not use metal utensils. Wear gloves when handling this chemical.

1.2 Set up a dish filled with 1xPBS next to the microtome to collect any miscellaneous sliced tissue that will be discarded and not saved for storage.

2 Microtome setup:

2.1 Microtome diagrams with labeled parts:



Left side of microtome
A - knobs adjust angle of stage
B - stage
C - knife
D - knife clamps
E - knife guard - slides left to cover knife
F - lever locks stage in place
G - coarse feed wheel - raises stage up and down
H - manual feed lever - raises stage up by amount of microns set with dial "I"
I - section thickness adjustment dial (up to 60 microns)

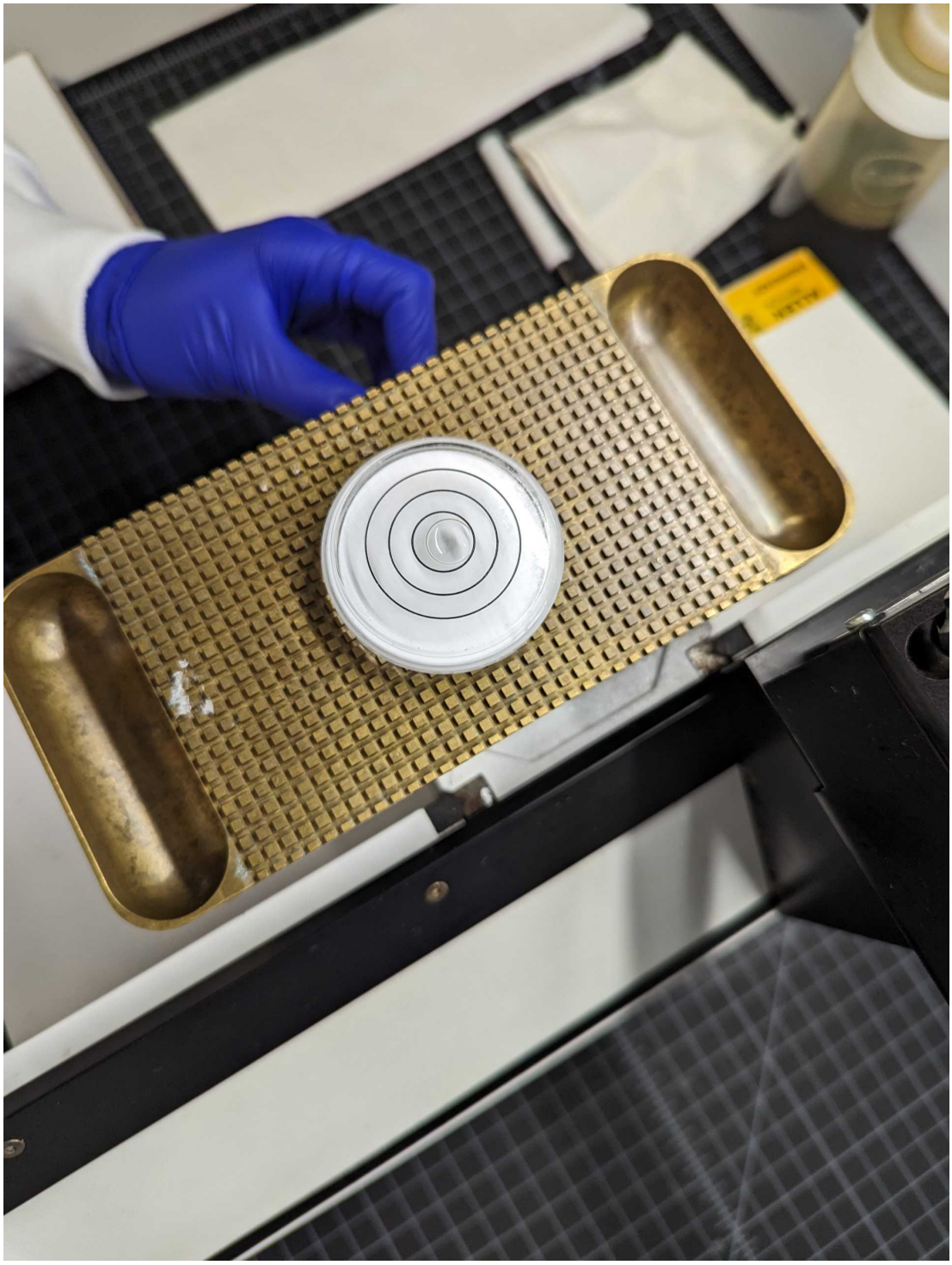
Right side of microtome
J - knife sledge movement grip
K - lock handle for knife (up = locked, down = unlocked)
L - number indicate angle of knife
M - knife angle adjustment knob

2.2 Ensure lock handle for knife is in the closed position before making any adjustments to, or mounting tissue onto, the microtome.

Knife guard is locked when the handle is up. See "K" in diagram in step 2.1.

Knife guard is unlocked when the handle is down. See "K" in diagram in step 2.1.

2.3 Attach the stage to the microtome and ensure that it is level.



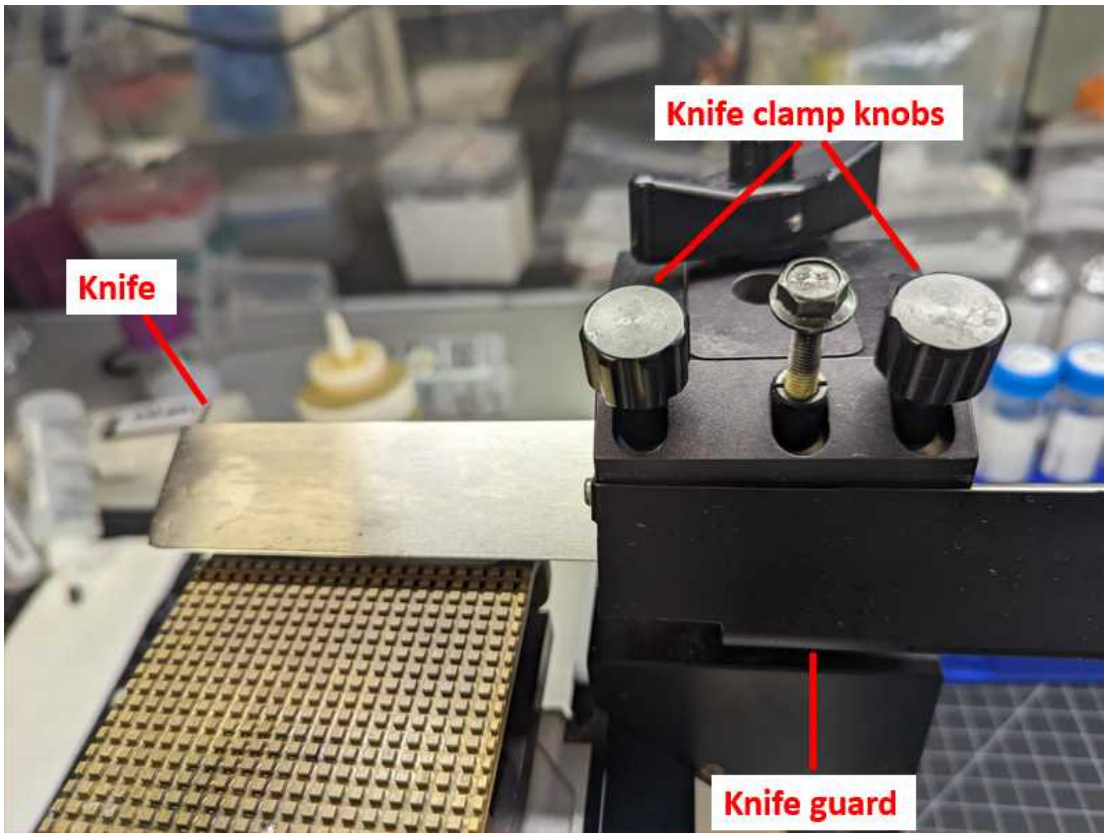
Pictured is a level tool sitting on top of the metallic microtome stage. The angle of the stage can be adjusted from a side knob (see "A" in step 2.1) until the level tool confirms that the stage is level when the bubble is in the center/bulls-eye of the circles.

2.4 Take microtome knife out of its case and use 70% ethyl alcohol and a Kimwipe to remove the protective oil coating on the knife.

Insert knife into the holder and tighten both black-knobbed clamps to hold knife in place. Black knife guard located below knobs can be used to cover blade when microtome blade is inserted but not in use.

Safety information

Microtome knives are extremely sharp and may cause severe injury, take special care when handling.



Metal microtome blade installed into microtome ("C", step 2.1). Two knife clamps with black knobs located to right of blade ("D", step 2.1), and black plastic knife guard located below clamps slides left to cover knife ("E", step 2.1).

2.5 Set the knife angle to desired setting with knife angle adjustment knob. For example, 0 degrees.

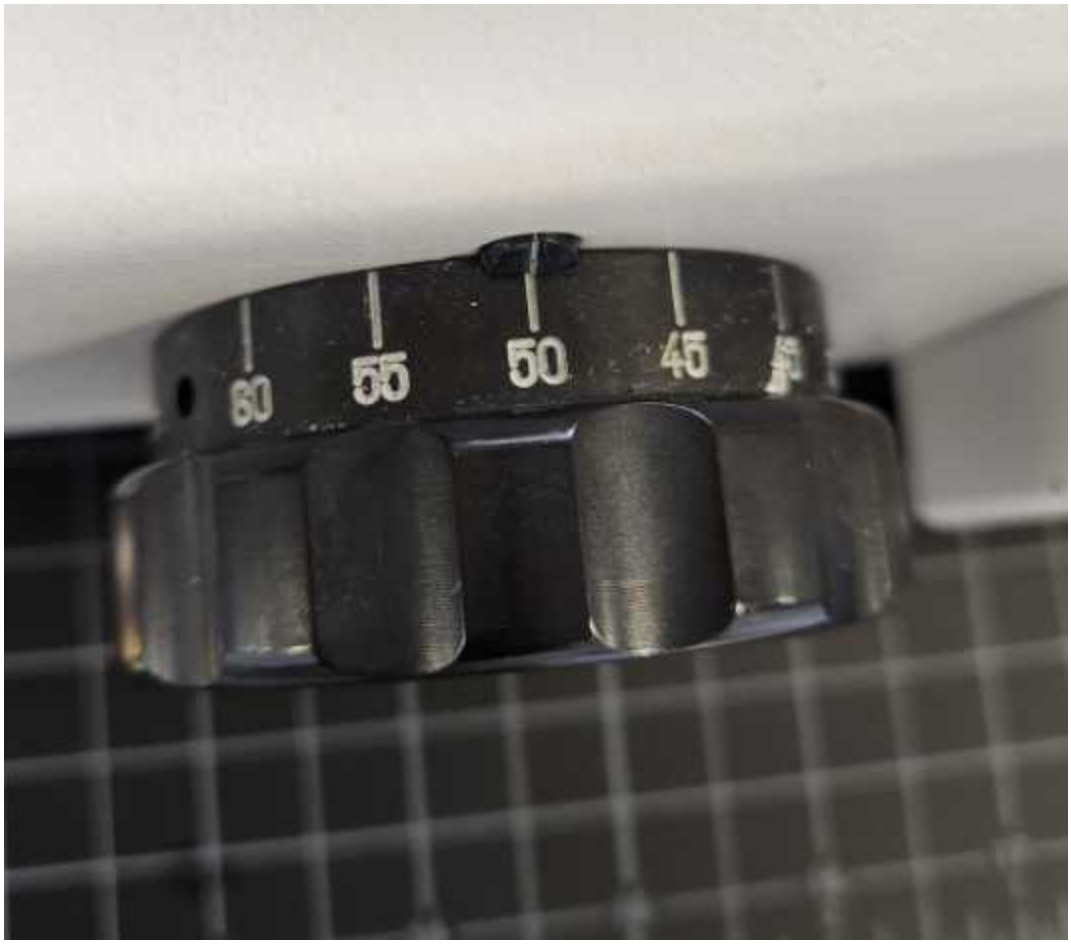
Knife angle adjustment knob located on top of microtome (see "M", step 2.1 diagram) and numbers indicating knife angle located on right side of microtome (see "L", step 2.1 diagram).

2.6 Adjust the section thickness in microns by turning the section thickness adjustment dial.

Note

The dial only adjusts up to 60 microns. If thicker sections than 60 microns are desired, adjust the dial to a multiple of the section thickness needed and press the manual feed lever ("H" in step 2.1 diagram) multiple times.

Example: If 100 micron sections are desired, set the dial to 50 microns and press the manual feed lever twice.



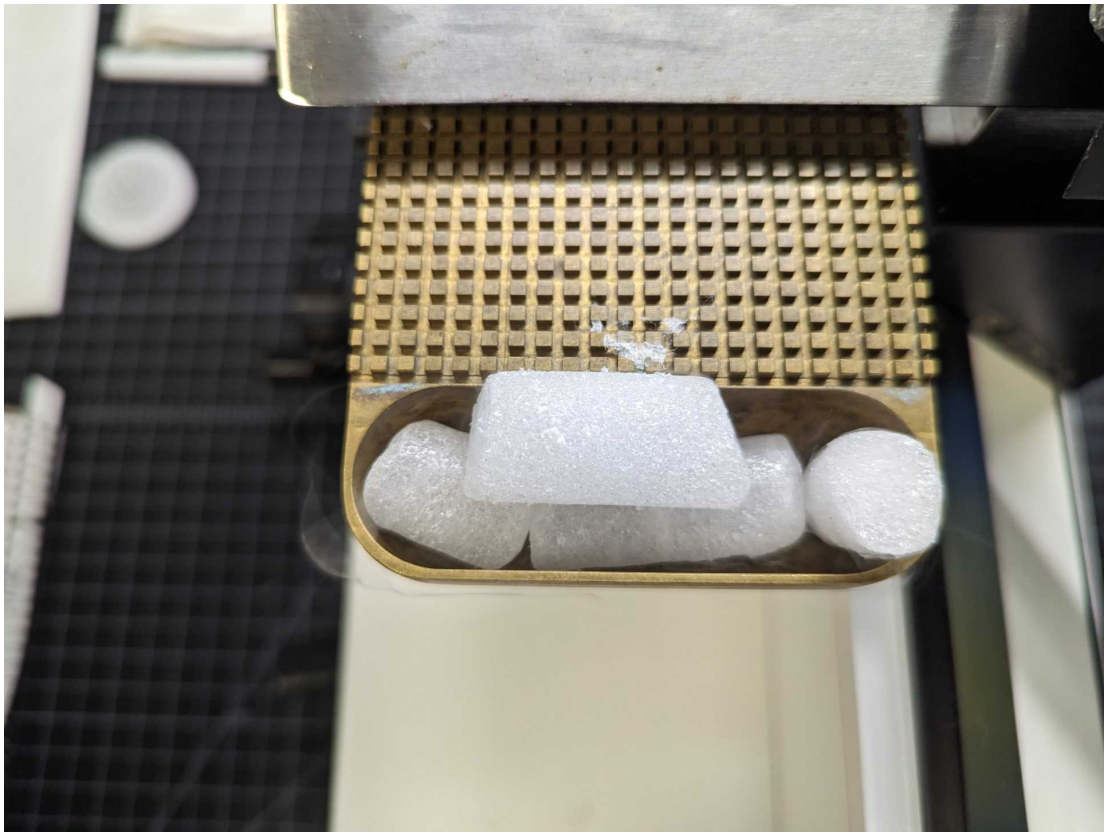
Dial located on the front of the microtome measures section thickness in microns. ("I" in step 2.1 diagram) Dial is set to 50 microns in this image.

3 Create a base of 1xPBS, OCT, or 30% sucrose on the microtome stage:

Note

See image in step 3.3 for example of 1xPBS base.

3.1 Fill the trough surrounding the stage with dry ice chunks.



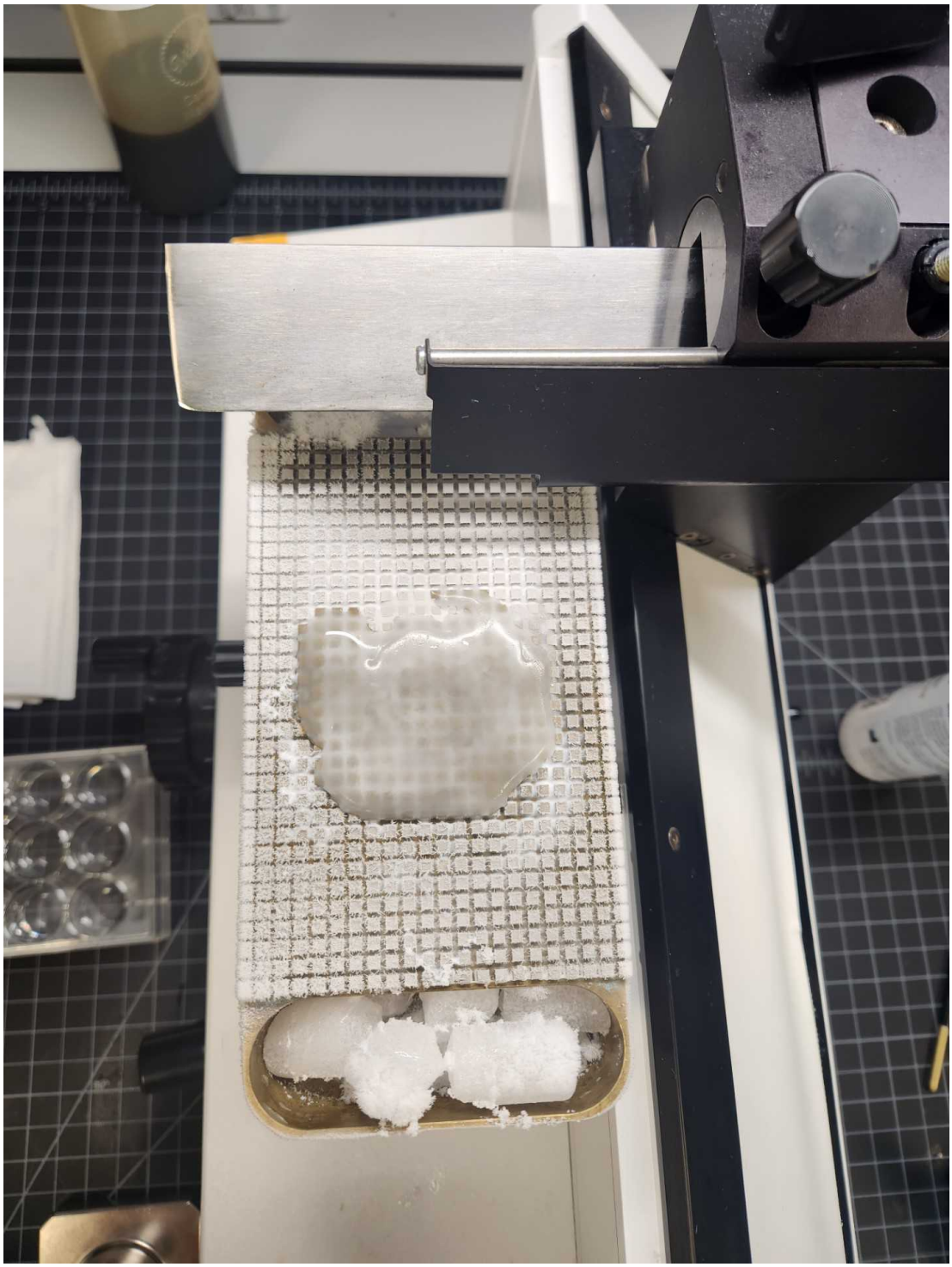
Dry ice placed in microtome stage trough.

3.2 Wait until stage has cooled enough so that the 1xPBS, OCT, or sucrose will freeze soon after it is applied. The metallic stage should look slightly white and frosty, and might take 00:05:00 to reach this point. 5m

3.3 Apply a 4cm square layer of 1xPBS on the stage to section one brain.

Note

If sectioning multiple brains (up to 4), apply a layer that spans the width of the stage and is 12cm long.



1xPBS frozen on microtome stage, appropriate for sectioning one brain

Example of 4 brains mounted in OCT in a staggered formation, with a larger 1xPBS layer that spans the entire stage.

3.4 Wait until the 1xPBS is completely frozen before proceeding.

3.5 Determine if you will mount the specimen in OCT or sucrose. If mounting in OCT, proceed to step 4. If mounting in sucrose, proceed to step 5.

	Mounting solution	Notes
	OCT	-Quicker process to mount the specimen.

	Mounting solution	Notes
	30% sucrose	-Easier to cut through with a knife. - Good for larger specimens like macaque brain. -Easier to replace remaining specimen back in 30% sucrose for storage for later use.

Mounting specimen using OCT

1m

4 Mount the specimen on microtome stage using OCT:

Safety information

Use gloves at all time while handling specimen, as tissue may have trace amounts of paraformaldehyde and azide, which are carcinogenic.

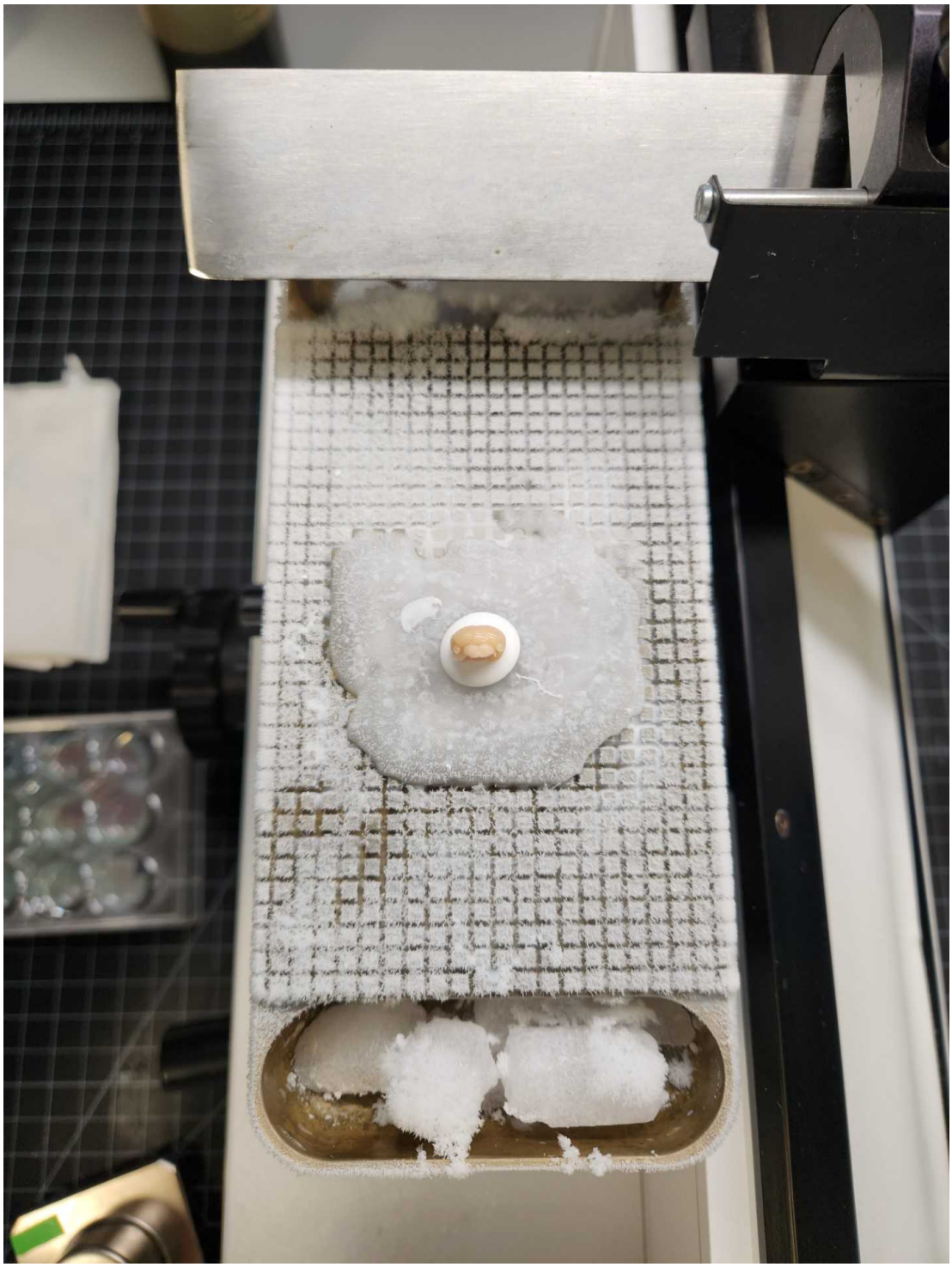
4.1 With a razor blade, trim tissue as needed so it will sit flat and level on the stage in the correct orientation (coronal, sagittal) when sectioning.

4.2 Blot specimen briefly on a Kimwipe to remove excess liquid.

4.3 Add a large drop of OCT onto the frozen, flat OCT base.

4.4 Quickly set the specimen into the large drop of OCT before it freezes.

4.5 Gently hold the tissue in place until the OCT has solidified enough to hold the tissue securely.



Mouse brain mounted in OCT, oriented with olfactory bulb facing up for coronal sectioning.

4.6 Crush dry ice with a rubber mallet and apply ice dust to the top of the mounted brain.

4.7 Wait until tissue freezes passively, about 00:01:00 , and gently brush away crushed ice to reveal brain specimen. The tissue should be hard and white after freezing. 1m

4.8 Proceed to step 6 for sectioning.

Mounting specimen using 30% sucrose

1m

5 Mount the specimen on microtome stage using 30% sucrose:

Safety information

Use gloves at all time while handling specimen, as tissue may have trace amounts of paraformaldehyde and azide, which are carcinogenic.

5.1 With a razor blade, trim tissue as needed so it will sit flat and level on the stage in the correct orientation (coronal, sagittal) when sectioning.

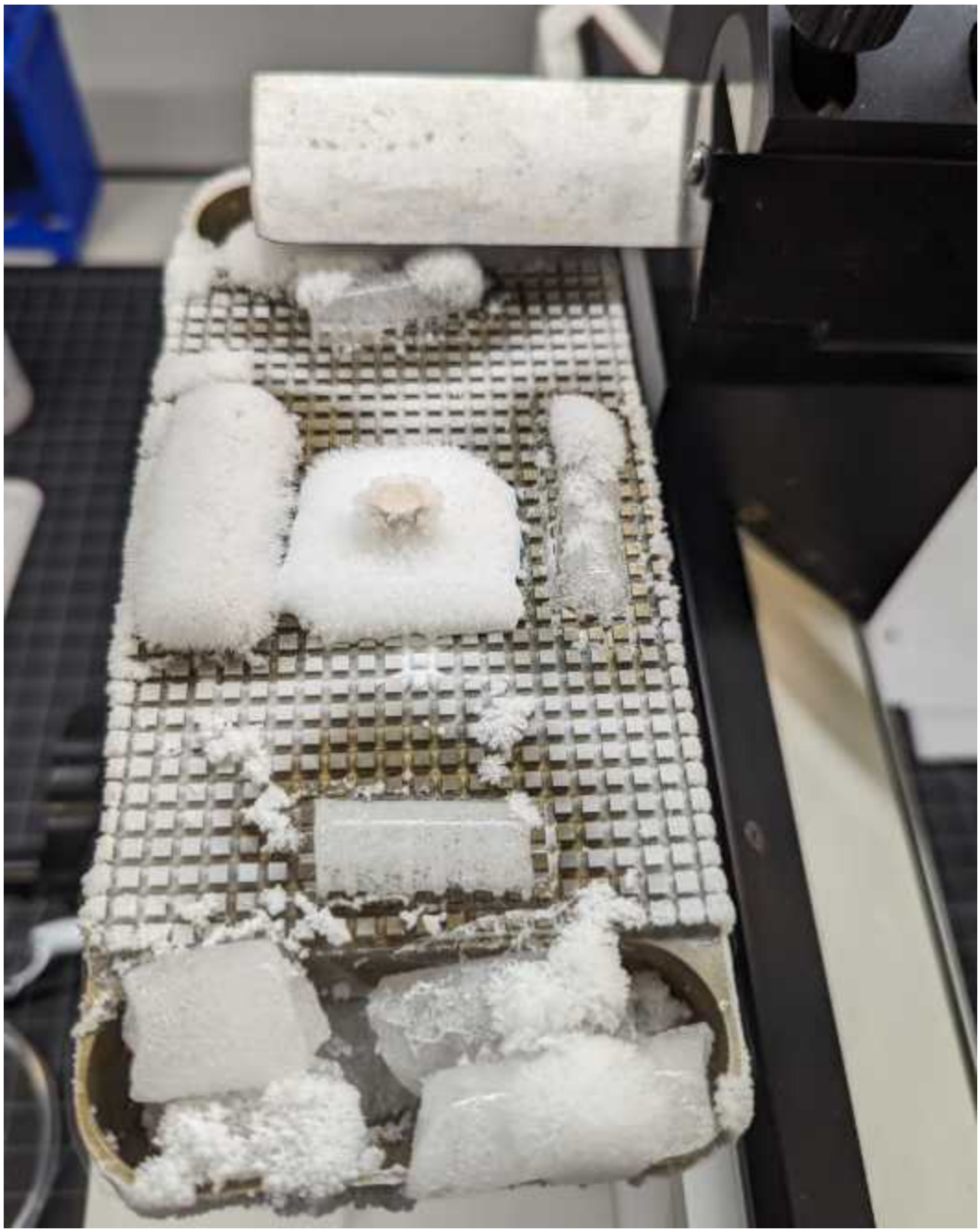
5.2 Blot specimen briefly on a Kimwipe to remove excess liquid.

5.3 Add a large drop of 30% sucrose onto the frozen, flat 30% sucrose base.

5.4 Quickly set the specimen into the large drop of sucrose before it freezes.

5.5 Surround base with more sucrose until you have a sturdy base that will hold the specimen securely. Add slowly drop by drop and avoid making hollow cavities.

5.6 Keep adding sucrose until specimen is securely held or if preferred, completely covered with sucrose.



Mouse brain mounted in sucrose, cut on sliding microtome.

Sectioning

1m

6 Section the tissue:

6.1 If desired by requestor, use a razor blade to cut a shallow notch on either the left or right side of the cortex of the brain that runs posterior to anterior. Make a note of which side is notched and refer to it for left/right orientation when mounting sections on slides.

6.2 Move the platform and specimen vertically towards the knife by turning the coarse feed wheel located on the left side of the microtome.

6.3 To position blade as close as possible to the specimen, pull the manual feed lever forward to raise the platform by microns.

6.4 Pull the knife sledge movement grip forward to bring the knife through the tissue.

6.5 The tissue section will remain on the top of the knife. Wrinkles and deformation of the tissue is normal.

6.6 Pick up the section with a fine-tipped paintbrush. Move each section to the well plate filled with 1xPBS & Sodium Azide 0.01% .

Safety information

Sodium Azide is toxic and carcinogenic. It should be handled and prepared with care. Do not breathe dust, do not use metal utensils. Wear gloves when handling this chemical.

6.7 If needed, wipe the blade with a Kimwipe to clear any excess moisture or debris.

6.8 Push the knife sledge movement grip back to bring the blade back to the starting point.

6.9 Pull the manual feed lever forward to raise the specimen for the next section.

6.10 Add more dry ice as needed to the troughs in the stage to prevent the OCT or sucrose mounting solution from softening or melting, as the dry ice added initially will melt over time.

6.11 Repeat steps 6.4 - 6.10 until desired sections are collected. Tissue may be double or triple stacked in each well in the well plate, but ideally sections in the same well should be separated by at least a few hundred microns (ex: every 6th section or more) so it is easier to see anatomical differences and determine section order when mounting.

6.12 Wrap well plate in parafilm and secure with tape to prevent spillage, then wrap in aluminum foil to protect from light, and store at 4 °C until ready to stain the tissue and mount the sections onto slides. Tissue may be stored for several months.

Clean up

3m

7 Take down microtome and clean up equipment:

7.1 Spray the knife and microtome with 70% ethyl alcohol and carefully wipe dry with Kimwipes.

7.2 Coat the knife with mineral oil and carefully store in its case.

7.3 Detach the specimen stage and run warm tap water over it to release 1xPBS, OCT, or 30% sucrose and any residual tissue.

7.4 Based on requestor preference, either retain tissue or dispose of tissue in biohazard waste bin.

7.5 Spray the stage with 70% ethyl alcohol. Soak for 00:03:00 before wiping with a Kimwipe. 3m

7.6 Dry the stage thoroughly to prevent frost buildup.