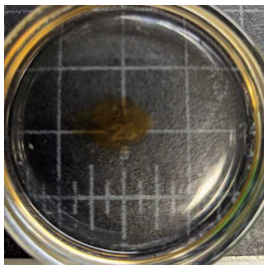
Feb 06, 2025

Refractive Index Matching - EasyIndex



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Holly Myers[1], Daphne Toglia[2]

[1]Allen Institute for Neural Dynamics; [2]Boston University

Allen Institute for Neural D…

Holly Myers
Allen Institute

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Protocol status: Working
We use this protocol and it's working

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Protocol Integer ID: 81480

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Abstract

This protocol is modified from the Index Matching protocol found in the LifeCanvas Technologies Full Active Pipeline Protocol (see references). At the end of this process, the specimen should have the same refractive index as LifeCanvas EasyIndex, allowing for lightsheet imaging on the SmartSPIM. Refer to the following protocols to prepare for imaging: **Embedding Protocol - SmartSPIM. EasyIndex Agarose Embedding Protocol**, **SmartSPIM setup and alignment**, and **Imaging cleared mouse brains on SmartSPIM**.

Materials

Reagents:

LifeCanvas EasyIndex **Catalog #** EI-Z1001

Nuclease-free water (e.g. MilliQ or HPLC grade water) **Contributed by users**

	Materials	Product number
	Serological pipets, 25mL	Fisher Scientific, 13-675-30
	50mL high clarity conical centrifuge tube	Fisher Scientific, 14-959-49A
	Serological pipet filler	ThemoFisher, 9501
	Oven	ThermoFisher, 51028063
	Aluminum foil	Amazon, B074NB5CDZ
	Shaker	OHAUS, 404P87
	Metal spatula	Cole Parmer, ux-06287-07
	Petri dish	ThermoFisher, 150460

Recipes:

50% EasyIndex for 1 Mouse Brain:

Using serological pipet to measure 10mL Nuclease free water into 50mL conical tube. Add 10mL LifeCanvas EasyIndex to container. Place upright on shaker at low speed for 00:05:00 to mix.

	Reagents	Volumes
	LifeCanvas EasyIndex	10mL
	Nuclease free water	10mL

Safety warnings

Wear PPE while performing the steps of this protocol: gloves, eye protection, lab coat.

Before start

This protocol is intended for a brain that has already been delipidated. See protocol **Whole Mouse Brain Delipidation - LifeCanvas Active** for preceding steps.

To allow time for troubleshooting (step 5), it may be beneficial to begin this protocol at the start of day.

LifeCanvas Index Matching

2d 0h 30m

1 Shake bottle of LifeCanvas EasyIndex well to homogenize the solution. Let the bottle sit for 00:30:00 to allow any bubbles to settle. 30m

2 Using a serological pipet, create 20mL of 50% EasyIndex by measuring 10mL of nuclease free water and 10mL of LifeCanvas EasyIndex into a 50mL falcon tube. Place upright on shaker at low speed for 00:05:00 to mix. 1d 0h 5m

Add one delipidated whole mouse brain specimen into the tube and make sure specimen is completely submerged in solution. A long-handled spatula may be used to transfer the mouse brain into the tube. Seal tube tightly to prevent evaporation and cover in aluminum foil to protect from light. Incubate for 24:00:00 at 45 °C on shaker at slow speed.

> Note
>
> The 50% EasyIndex solution may be stored at Room temperature if needed.

3 Remove the 50% EasyIndex solution from the tube containing the specimen by using a long-handled spatula to scoop the brain out of the tube and pouring out the 50% EasyIndex solution from the tube. Replace the brain into the tube. Using a serological pipet, measure 20mL LifeCanvas EasyIndex into the tube and incubate for 24:00:00 in oven at 45 °C on shaker at slow speed. 1d

Assessing for Successful Index Matching

22h

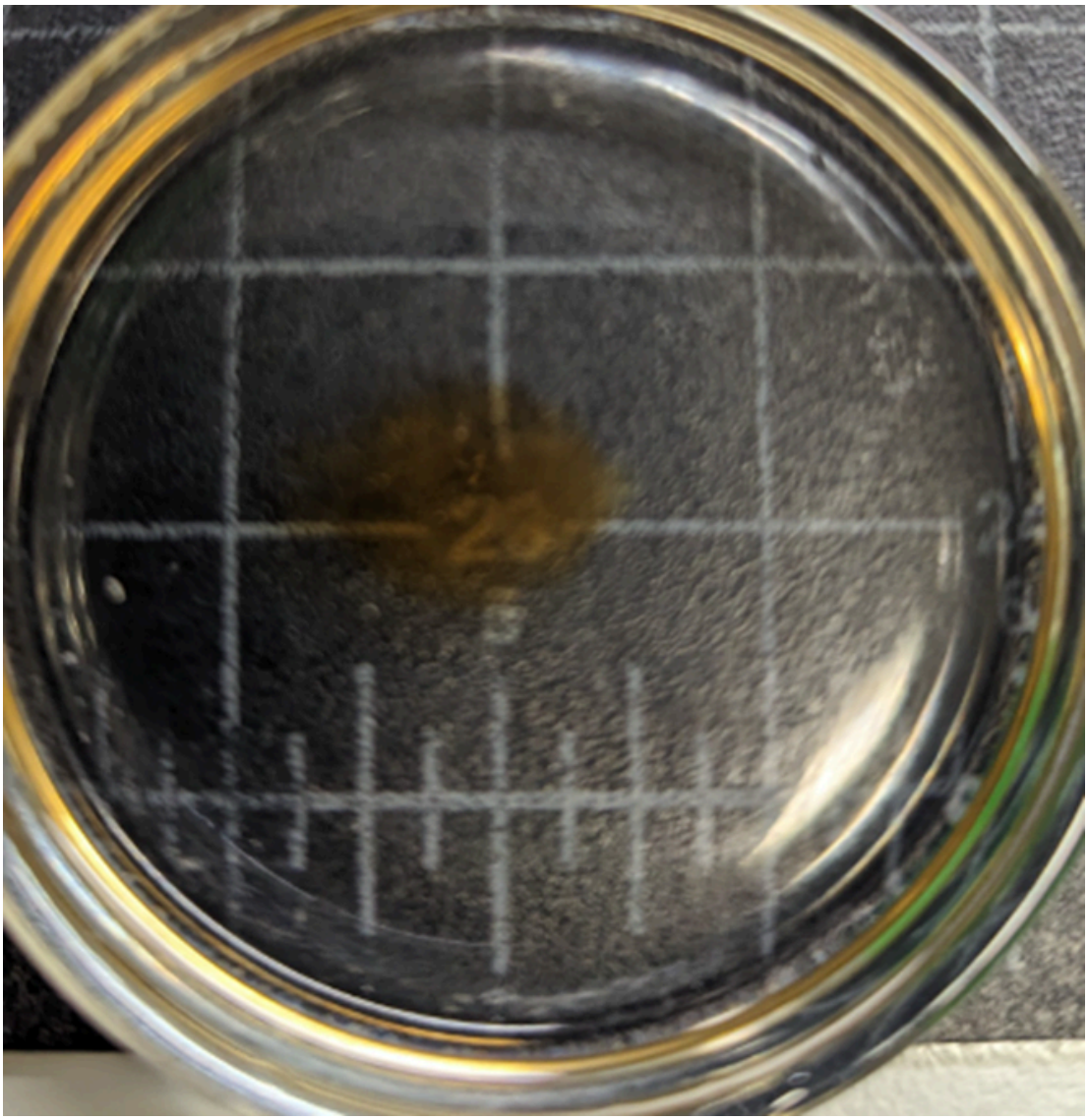
4 Remove tube with brain specimen and assess brain for successful index matching. The brain may be temporarily immersed in EasyIndex in a clear petri dish for evaluation.

> Note
>
> If processing a batch of multiple brains at once, assess each brain individually, as there may be variance in clearing between brains in the same batch.

Successful index matching:
When fully immersed in EasyIndex solution, the brain should be completely transparent. It should be possible to view numbers, letters, or fine lines on a grid through the brain without

warping.

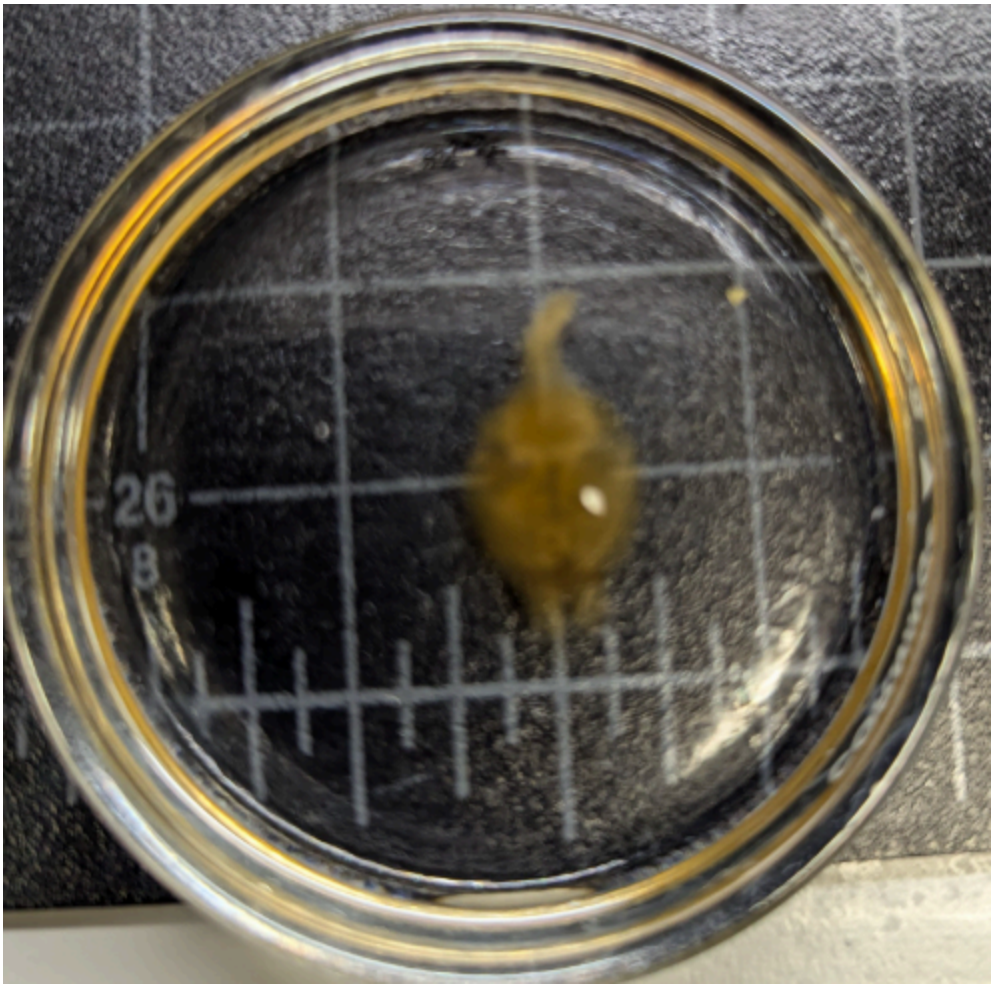
If the brain successfully index matched, proceed to step 6.



Example of a successfully index matched brain. Note that you can see the lines of the grid and read the number "23" through the brain when it is immersed in a dish of EasyIndex.

Not successfully index matched yet:
When fully immersed in EasyIndex solution, some opaque, white, or partially transparent structures may be visible inside the brain. It may be difficult to view images or lines through the brain without warping.

If the brain does not look successfully index matched yet, proceed to step 5.



Example of an unsuccessfully index matched brain. Note that you cannot clearly read the number "27" through the brain when it is immersed in a dish of EasyIndex. This brain could use the additional index matching step.

5 Place tube with the EasyIndex solution and brain specimen back into the 45 °C oven on shaker at low speed for an additional 06:00:00 and assess brain specimen for transparency again (step 4). 22h

If brain specimen is not yet completely transparent, remove the EasyIndex solution from the tube and replace with 20mL fresh EasyIndex. Replace specimen in 45 °C oven for at least 16:00:00 on shaker at low speed.

This additional step should maximize index matching between the brain and the EasyIndex solution as much as possible. Proceed to step 6.

6 The brain specimen is now ready for embedding, if desired. See protocol **Whole Brain Embedding - EasyIndex with 2% Agarose** for more details.

Sample may be stored in EasyIndex for several months at Room temperature .

Protocol references

Full Active Pipeline Protocol SmartBa…