

STANDARD OPERATING PROCEDURE

For Users Providing Samples for Clearing using SmartBatch+ and Imaging on Miltenyi Blaze Ultramicroscope

Samples need to be prepared as described below before submission for clearing and imaging. *The current protocol has been tailored to mouse brains.* A basic pricing list is included below. Once imaging is complete, users may pick up samples and imaging data. See Figure 1 below for a visual description of the steps from sample preparation to clearing and imaging to data analysis.

Please contact Dr. Kelly Summers (kelly.summers@uwo.ca) ASAP to discuss the following:

- Number and type of samples
- Project timeline
- How sample preparation needs to be adjusted for samples other than mouse brains
- Antibodies and labelling
- Price estimates and potentially available discounts

Sample Preparation for Clearing using SmartBatch+

1. Transcardial perfusion of the animal is to be completed according to the following two step procedure:
 - i) Begin the perfusion with ice-cold 1X PBS. We recommend using heparinized PBS to remove as much blood as possible and prevent clotting (1 uL heparin: 1 mL PBS). Make sure the fluid is running completely clear before proceeding with 4% PFA.
 - a. For mice, use about 20 mL of 1X PBS and a 5 mL/min flow rate.
 - b. For rats, use 200 mL of 1X PBS and a 60 mL/min flow rate.
 - ii) Once the required volume of PBS has been administered, continue the perfusion with ice-cold 4% PFA in PBS. Use the same volume(s) and flow rate(s) described above. Note, when switching between the PBS and PFA solutions, be careful not to introduce air bubbles inside the tubing. Moreover, if fluid comes out of the mouth or a lung swells, adjust the position of the needle in the heart.
2. Dissect out the brain or other organ of interest. Careful dissection is essential to preserve the sample's structural integrity.
3. Incubate the sample in 4% PFA in PBS for 24 hours at 4°C with shaking.
4. The sample can then be stored in 1X PBS with 0.02% sodium azide at 4°C until you are ready to continue with clearing (up to ~2 months). If storing for longer periods of time (> 2 months up to a year), please contact Kelly to arrange for the samples to be stored in epoxy. Storage beyond a year is not recommended.

Antibody Quantities Required for SmartBatch+ Staining

Users are required to purchase their own antibodies. This expense is not included in the clearing and imaging package price. A list of validated primary and secondary antibodies can be found at <https://lifecanvastech.com/products/smartbatch/> and is updated frequently. The amount of each antibody required varies with the antibody of choice and the number of samples to be stained. Please contact Kelly for assistance in determining how much of each antibody will be required.

Images from the Miltenyi Blaze Ultramicroscope

Two images per sample are included in the clearing and imaging package price. Typically, this will include a coarse scan at 1x with 100 µm z-steps, and a finer scan at 4x with 4 µm z-steps, but a 12x image may be possible (depending on the sample area). **Additional imaging is possible for an additional fee.**

Users will also require a plan for data storage and analysis. Light sheet imaging data sets are large and data transfer takes a substantial amount of time. For example, images collected at the lowest resolution with the 1× objective and 100 µm step size generate ~10 GB of data per channel. Images collected with the 4× objective and 4 µm step size generate ~300 GB of data per channel. The highest resolution imaging with the 12× objective may generate TBs of data. Users are asked to bring a **NEW, UNUSED** external hard drive with sufficient space to accommodate their data. Alternatively, users may borrow an SSD from the facility to transfer data from the light sheet computer to the Imaris workstation **ONLY**. Users may also upload data onto their lab’s network drive (Kelly may need to be provided with access). Imaris is currently the data analysis software available on the Imaris workstation within the Western Advanced Microscopy Core. Contact Claudia Seah (cseah@uwo.ca) for access. Additional information on data analysis using Imaris, including video tutorials, can be found at <https://imaris.oxinst.com/learning/>. Additionally, Dr. Ali Khan (ali.khan@uwo.ca) is developing a data analysis workflow that allows registration of light sheet images with MRI and available brain atlases. Please contact Ali for more information.

Package Type	Number of Samples	Price
Full clearing, labelling, and imaging	First 6	\$1000/mouse brain
	Each additional sample	\$900/mouse brain
Clearing and imaging, no labelling (for samples with endogenous fluorescence)	First 6	\$800/mouse brain
	Each additional sample	\$720/mouse brain
Additional light sheet images	Any	\$60/hour



Figure 1. Workflow for clearing and imaging samples using the light sheet microscope. Steps that are the responsibility of the user are shown in blue and steps that are included in the clearing and light sheet package are shown in purple.