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Lightsheet Microscope Acquisition Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.begajbse

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

ABSTRACT

The Zeiss Lightsheet 7 is used to quickly and efficiently scan large tissue volumes using laser lightsheet illumination. The focused laser sheets and imaging plane are static while the tissue is moved throughout the selected volume for three dimensional image acquisition. This protocol will cover the procedures used for the day of lightsheet image acquisition.

Setting the acquisition volume: Large scanning volumes are composed of multiple z-stack being stitched back together based on overlapping edges of the scanning window. This requires setting an overall z-stack, which must include the entirety of tissue as covered by adjoining z-stacks. This becomes tricky when considering irregularly shaped tissues and their orientation relative to the various laser and imaging objectives.

Dual-side lightsheet alignment: To obtain a strong and clear image from large tissues it is helpful to illuminate the sample with both the left and right laser. Each lasersheet can be angled slightly within the focal plane to account for differences in the R.I. of the tissue and R.I. media (TDE). This is a manual tuning process and is not intended for major R.I. differences.

Protocol Overview:
- Refractive index matching of cleared tissue samples.
- Mounting tissue onto sample holder.
- Image acquisition in Zen 3.1 LS (black edition), including:
  - Setting the acquisition volume
  - Dual-sided lightsheet alignment

MATERIALS

NAME	CATALOG #	VENDOR
Isopropanol		
Forceps (tweezers), 12.5cm, Blunt End	FC003.SIZE.1	Bio Basic Inc.
10 x PBS Solution (Phosphate Buffered Saline)	PD8117.SIZE.4L	Bio Basic Inc.
KimWipes		Fischer Scientific
Cyanoacrylate adhesive, optical grade	19806-00-1	Vwr
22'-Thiodiethanol	166782-500G	Sigma Aldrich

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Refractive Index (R.I.) Matching

1 Prepare 63% TDE, a refractive index matching solutions (RIMS).
Prepare a 63% 2,2'-thiodiethanol (TDE) solution, diluted with 1xPBS adjusted to pH 7.2. One can use a refractometer to confirm the target refractive index (R.I.) of n = 1.45. Prepare a volume of solution at least 10 times that of the tissue being incubated. Mix the solution gently to avoid creating bubbles.

2 Incubate tissue in RIMS.
Add a sufficient volume of 63% TDE to a clean tube then add the cleared tissue sample for incubation. Keep the sample in TDE at room temperature and provide gentle agitation (rocking, rotating) for at least 1 hour or until the tissue sinks to the bottom of the tube. Avoid creating bubbles during this stage as well, they will attach to the tissue and prevent the sample from sinking.

Tissue Mounting

3 Rinse tissue in PBS.
Transfer the tissue to a clean surface and rinse briefly with 1x PBS to remove excess TDE.

4 Apply glue to mounted capillary.
Set a glass capillary into the Sample Holder* and dip the capillary into cyanoacrylate and set aside momentarily.
*See page 18 pf 26 for a lightsheet system component guide.

5 Prepare and adhere tissue to capillary.
Set the R.I. matched tissue on the rim of a weigh-boat and wick away excess liquid with a Kim Wipe on the surface to be adhered to the glass capillary. Use a pair of forceps to stabilize the tissue while dab drying.

Take the Sample Holder with inserted glass capillary and join the glued end to the dried surface of the tissue. Take care to let the glue set to the sample before moving on. More glue may be needed surrounding the interface; keep an additional glass capillary nearby to apply extra if needed. Once adhered the tissue should hang straight down from the glass capillary.

6 Expunge the microbubbles.
The sample is next dipped briefly into 80% isopropanol/PBS to remove any microscopic bubbles. A 2-3 second dip while gently flicking of the sample holder should be sufficient. These bubbles will create massive shadow artifacts across the tissue otherwise.

7 Set mounted sample into microscope.
The sample is now ready to lower into the lightsheet microscope Sample Chamber*, which should be pre-filled with 63% TDE.

Image Acquisition - Setting the acquisition volume

8 Set tissue at 45 degree angle.
To set the acquisition volume first align the tissue to 45 degrees relative to illumination/objective plane. Zooming out fully helps to get a sense of the tissue shape and allows one to guage the tissue alignment.

9 Setting first and last z-stack slices.
Progress tissue to locate the furthest edge that is in focus along the z-axis, and select it as the first plane.

Repeat this procedure for the other side of the tissue, finding the furthest edge in focus and setting it as the last plane.

10 Determine bounds of x and y, all z considered.
A bounding region needs to be designated in which the multiple z-stacks will be sequentially acquired. Determine the upper and lower Y-axis values, recording them from the specimen navigator panel. Repeat this process for each X-axis value.

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11 Assign top-left and bottom-right bound for Multiview.
Use the specimen navigator panel to bring sample to the top-left most region, using the upper y-axis and lower x-axis values. Add this position to the multiview panel.

Repeat this procedure for the lower-right most region of the tissue.

Be sure to set these regions keeping in mind the entire z-stack scaning range of tissues mounted at various angles.

12 Load and generate Multiview z-stacks.
Save the Multiview positions and load into Zeiss TileStitcher, a supplemental script used to populate Multiview positions for every z-stack required to capture the entire tissue volume from top-left to bottom-right.

Within the Zeiss TileStitcher select the bounding box feature, set the scan to meander mode, and fill in imaging size parameters (Image Size, number of Slices, % overlap). The TileStitcher program will autopopulate the many z-stack Multiview coordinates which can be saved and loaded into the Multiview panel within the acquisition software. Also record the count of z-stacks along the x and y axes.

Image Acquisition - Dual-sided laser alignment

13 Adjust single side laser sheet angle.
It is likely that some R.I. mismatch exists within the cleared tissue and RIMS, therefore the angle of light entering the sample may not line up exactly with the objective focal plane. To account for small differences in R.I. the laser objectives can be tuned by adjusting the angle at which the light sheet enters and propogates through the tissue.

Select either the left or right side laser and adjust the corresponding laser objective angle in the Illumination Parameters panels. Visually inspect the live feed and adjust the laser angle until the clearest live image is observed.

Make note of the alignment angle and test other regions in the tissue, at multiple points in the z-stack range, to confirm that this angle adjustment is appropriate for the bulk of tissue.

14 Adjust the other laser sheet angle.
Perforom the same process as above to adjust the opposing laser sheet alignment.

15 Reconcile the dual-sided laser illumination.
To capture the clearest image illumination from each laser should have the same focal plane, so that the same features appear in the same location when illumination occurs from either side individually. This may require some fine tuning of the left and right laser alignment, and again should be checked within various regions of the tissue volume.

Image Acquisition - Other Settings

16 Laser Settings
Autofluorescence imaging is acquired using a 488 excitation laser at 90% power for 30ms exposure times.

17 Acquisition Settings
Dual-sided acquisition should be enabled along with online dual-sided fusion.
Pivot scan should be enabled as well.

18 Start Experiment
The experiment is ready to begin. Click on Start Experiment, designate a file name and directory location, then click Save.

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