OMS Atlas OCT Spatial Mapping V.3

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ABSTRACT

This protocol describes the procedure by which the OMS Atlas serially sections an OCT block, prepares the resulting slides and samples, and then distributes the specimens for downstream analysis.

PROTOCOL CITATION

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MATERIALS TEXT

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Superfrost Plus Microscope Slides **Fischer Scientific Catalog #12-550-15**

Tanner Scientific 45° White Adhesive Slide with Beveled Edge **Mercedes Medical Catalog #TNR WHT45AD**

1.0mm PEN membrane covered slides ; 100pieces **Zeiss Catalog #415190-9041-000**

Additional equipment:

- UV lamp
- Cryostat
- Cryotubes

BEFORE STARTING

Transfer OCT blocks to OHSU Knight Histopathology Shared Resource (HSR) for sectioning and processing.

Preparation

1 Verify the identity of the OCT block to be cut against written request for sectioning.

2 Remove OCT block from **-80 °C** freezer and acclimate to cryostat (**-20 °C**) for minimum of **03:00:00** .

3 Label all slides and cryotubes with a unique BEMS ID and Part#, corresponding to the written request and OCT spatial map (below).

A	B	C	D	E
Part#	**Description**	**Thickness**	**Assay**	**Recipient**
1	Tanner slide	5µm	Cyclic Immunofluorescence	OHSU, Koei Chin
2	Superfrost Plus slide	5µm	H&E	OHSU, HSR
3	Superfrost Plus slide	5µm	Cyclic Immunofluorescence (Tumor Panel)	HMS, Alyce Chen
4	Superfrost Plus slide	5µm (Set Cryostat at 12µm)	Cyclic Immunofluorescence (Tumor Panel)	HMS, Alyce Chen
5	Cryotube	7µm	Single Cell DNA Sequencing	MD Anderson, Nick Navin
6	PEN membrane slide	12µm	Topographic Single Cell Sequencing	MD Anderson, Nick Navin
7	PEN membrane slide	12µm (Set Cryostat at 40µm)	Topographic Single Cell Sequencing	MD Anderson, Nick Navin
8	Cryotube	40µm (2 sections)	Single Cell DNA Sequencing	MD Anderson, Nick Navin
9	Remainder of OCT block	NA	Single Cell Indexing ATAC Sequencing	OHSU, Andrew Adey

4 Prepare PEN membrane slides by exposing close (~15-20cm) to a UV source for **00:15:00** .

Sectioning

5 Affix OCT block to cryostat chuck.

6 Orient and face block to get adequate amount of core.

Note: Avoid excessive facing to reduce tissue loss.

7 Set cryostat to 5 micron sections.
Note: All sections cut from here on should be sequential. The serial order, adjacency, and consistent orientation of the sections are all important factors. Please note any deviations from the protocol.

8 Cut first four sections at 5 microns (Part#1-4) and affix onto appropriately labeled slide according to OCT spatial map (step #3 above).

9 Change section thickness to 12 microns.

10 Cut one section (Part#5) and place in a cryotube.
Note: This is an intermediate section generated when the Cryostat is switching thicknesses. The actual thickness of this section should be about 7µm.

11 Cut two sections (Part#6, 7) and place on appropriate membrane slides.

12 Change section thickness to 40 microns.

13 Cut 2 sections (Part#8) and place both sections in a single cryotube.

14 Place all slides, both cryotubes, and remaining OCT block in **-80 °C** freezer.
Note: No slides are to be fixed under this protocol.

Processing

15 Perform hematoxylin and eosin (H&E) staining on slide labeled Part#2 (see OCT spatial map in step #3 above).

16 Deliver unstained slides (Part#1, 3, 4, 6, 7), cryotubes (Part#5, 8), and remainder OCT block (Part#9) to BioLibrary for distribution.
Note: Keep samples frozen at all times. Store at **-80 °C** *. Transfer/ship on dry ice.*