OMS Atlas OCT Spatial Mapping V.2

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1 Works for me
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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.

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MATERIALS TEXT

MATERIALS

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

Citation: Brett Johnson, Danielle Galipeau, Todd Camp, George Thomas (06/17/2021). OMS Atlas OCT Spatial Mapping. https://dx.doi.org/10.17504/protocols.io.bvw7n7hn

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	Tanner slide	5µm	H&E	OHSU, HSR
3	Tanner slide	5µm	Cyclic Immunofluorescence (Tumor Panel)	HMS, Alyce Chen
4	Tanner 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 Tanner slide according to OCT spatial map (step #3 above).

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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.*

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