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OMS Atlas OCT Spatial Mapping

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1 Works for me dx.doi.org/10.17504/protocols.io.4awgsfe

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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
- Cryotube

BEFORE STARTING

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

Preparation

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

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

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

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	6µm (Set Cryostat at 12µm -Step 9)	Cyclic Immunofluorescence (Tumor Panel)	HMS, Alyce Chen
5	PEN membrane slide	12µm	Topographic Single Cell Sequencing	MD Anderson, Nick Navin
6	Cryotube	12µm (6 sections)	Single Cell DNA Sequencing	MD Anderson, Nick Navin
7	PEN membrane slide	12µm	Topographic Single Cell Sequencing	MD Anderson, Nick Navin
8	Tanner slide	6µm (Set Cryostat at 5µm -Step 14)	Cyclic Immunofluorescence (Tumor Panel)	HMS, Alyce Chen
9	Tanner slide	5µm	Cyclic Immunofluorescence (Tumor Panel)	HMS, Alyce Chen
10	Tanner slide	5µm	H&E	OHSU, HSR
11	Remainder of OCT block	NA	Single Cell Indexing ATAC Sequencing	OHSU, Andrew Adey

Note: Part#6 is to be placed in a single cryotube and should be labeled "Part#6" along with a unique BEMS ID.

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.

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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 three section at 5 microns (Part#1-3) and affix onto appropriately labeled Tanner slide according to OCT spatial map (step #3 above).

9 Change section thickness to 12 microns.

10 Cut one section (Part#4) and place on appropriate Tanner slide.
Note: This is an intermediate slide generated when the Cryostat is switching thicknesses. The actual thickness of this slide should be about 6µm.

11 Cut one section (Part#5) and place on appropriate membrane slide.

12 Cut 6 sections (Part#6) and place all sections in a single cyrotube.

13 Cut one section (Part#7) and place on appropriate membrane slide.

14 Set cryostat to 5 micron sections.

15 Cut remaining 3 sections (Part#8-10) and affix onto appropriate Tanner slide.
Note: Part#8 is an intermediate slide generated when the Cryostat is switching thicknesses. The actual thickness of this slide should be about 6µm.

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

Processing

17 Perform hematoxylin and eosin (H&E) staining on slides labeled Part#2 and Part#10 (see OCT spatial map above).

18 Deliver unstained slides (Part#1, 3-5, 7-9), cryotube (Part#6), and remainder OCT block (Part#11) to BioLibrary for distribution.
Note: Keep samples frozen at all times. Store at **-80 °C** *. Transfer/ship on dry ice.*

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