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VU Biomolecular Multimodal Imaging Center (BIOMIC) kidney characterization pipeline for tissues collected through the Cooperative Human Tissue Network (CHTN) V.4

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

VU Biomolecular Multimodal Imaging Center

Human BioMolecular Atlas Program (HuBMAP) Method Development Community



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ABSTRACT

We aim to develop high resolution, chemically informative imaging methodologies for building an atlas of human organs, such as the kidney.

Scope:

Provide an overview of the methods used by the Vanderbilt Tissue Mapping Center as part of the Human Biomolecular Atlas Program (HuBMAP, NIH Common Fund) and contextualize individual protocols within our larger workflow.

- 1 Collection of post-surgical tissue.
Collection: dx.doi.org/10.17504/protocols.io.7gehjte
- 2 Stabilize and freeze tissues.
Freezing Tissue: dx.doi.org/10.17504/protocols.io.6wghfbw
- 3 Initial Rapid Pathology Assessment of Kidney Tissue
Staining: dx.doi.org/10.17504/protocols.io.4qngvve
Assessment: dx.doi.org/10.17504/protocols.io.9dph25n
- 4 Cryosection tissues into micrometer thick sections, alternating between thaw mounting onto indium tin-oxide and positively charged glass slides (proceed to step 4), or collecting several tissue sections within a microcentrifuge tube for proteomics analysis.
Cryosectioning: dx.doi.org/10.17504/protocols.io.7ethjen
- 5 Perform LC based Proteomics on adjacent tissue sections and/or continue to Step 6.
Sample Preparation: dx.doi.org/10.17504/protocols.io.67nhhme
Data Acquisition: dx.doi.org/10.17504/protocols.io.bfsjincn
Data Analysis: dx.doi.org/10.17504/protocols.io.bfshjnb6
- 6 Perform autofluorescence microscopy on all tissue sections before IMS (step 5) or MxIF analysis (step 9)
Autofluorescence: dx.doi.org/10.17504/protocols.io.7e3hjgn

- 7 Coat tissue sections with MALDI matrix for IMS analysis.
Matrix Application: dx.doi.org/10.17504/protocols.io.4srgwd6
- 8 Perform high resolution IMS analysis of matrix coated tissue sections.
IMS: dx.doi.org/10.17504/protocols.io.7gdhjs6
- 9 Perform fluorescence microscopy to visualize laser ablation spots.
Post IMS AF: dx.doi.org/10.17504/protocols.io.879hxr6
- 10 Annotation of Lipids from IMS Data
Cal & Annotate: dx.doi.org/10.17504/protocols.io.864hzgw
- 11 Remove MALDI matrix and perform PAS staining.
PAS Staining: dx.doi.org/10.17504/protocols.io.4qngvve
- 12 Alternatively, MxIF can be performed after step 4.
Antibody labeling: dx.doi.org/10.17504/protocols.io.667hnhn
MxIF: dx.doi.org/10.17504/protocols.io.665hhg6
- 13 Registration of autofluorescence images from both IMS and MxIF sections allow for the direct correlation of the two orthogonal approaches.
Registration: <https://dx.doi.org/10.17504/protocols.io.bed2ja8e>
Publication: <https://doi.org/10.1021/acs.analchem.8b02884>
- 14 MALDI imaging mass spectrometry data processing.
Data Processing: <https://dx.doi.org/10.17504/protocols.io.bed3ja8n>
- 15 RNA Assessment from tissue.
RNA extraction: dx.doi.org/10.17504/protocols.io.86nhzde