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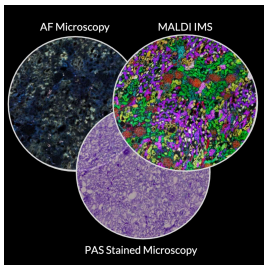
VU TIS Multimodal Molecular Imaging Pipeline for KPMP Biopsy Interrogation V.2



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VU Biomolecular Multimo… KPMP

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol provides an overview of the Multimodal Molecular Imaging pipeline used by the Vanderbilt University Tissue Interrogation Site (VU TIS) as part of the Kidney Precision Medicine Project (KPMP). Individual protocols are contextualized within our larger workflow.

Materials

Optimal cutting temperature (OCT) compound

Sample Shipment, Storage, and Handling

1 **KPMP Biopsy Shipment to VU TIS**

KPMP biopsies are shipped on dry ice to VU TIS. Temperature is monitored using MadgeTech data loggers. Specimens and relevant information are recorded in Redcap, and samples are store at -80^{O}C until sectioning. Prior to sectioning, samples are assessed according to **Table 1**, described below.

Tissue Screening and Assessment

2 **Asses Kidney Tissue Sections**

To accept biopsies for multimodal molecular imaging analysis by VU TIS, the samples must meet the following Go/No-Go conditions (**Table 1**).

Table 1. Sample Processing Go/No-Go Criteria

	A	B
	Go/No-Go Criteria	**Reasoning**
	Biopsy must be LN2 core	Other storage conditions can induce analyte suppression effect in MALDI IMS analysis
	Biopsy size must be > 3mm	Smaller tissue sections are difficult to handle, section, and mount
	Time to preservation must be < 10 min	Prolonged exposure prior to preservation can cause metabolite/lipid degradation

Sectioning

Accepted biopsies are sectioned and visually assessed following the QC metrics described in **Table 2**. Biopsies are cryo-sectioned to 10 µm thickness on indium-tin-oxide (ITO)-coated (for imaging mass spectrometry) or glass (for stained microscopy) slides. For workflow QC, a normal human kidney reference tissue section (termed BlockQC) is sectioned along with KPMP samples. For cryo-sectioning, samples are mounted to a chuck using a small amount of optimal cutting temperature (OCT) compound. Photos are taken before, during, and after sectioning (with and without mounting media) to document the process and ensure proper handling. After sections are obtained, the OCT is removed from the tissue block, and the samples are stored at -80oC. Tissue sections are visually assessed following the QC metrics described in **Table 2** before data collection.

Table 2. On-Site Tissue Integrity Considerations

	A	B
	Tissue QC	**Reasoning / Data Flags**

	A	B
	Are there any glomeruli in the biopsy section?	Ensures proper coverage of biological features
	Visible cracks/folds on the tissue surface	This can lower FTU segmentation performance, due to non-segmentable features
	Visible freezing artifacts on the tissue surface	Freezing artifacts can induce analyte delocalization, jeopardizing MALDI IMS spatial integrity

Sample Interrogation Assays

3 **Autofluorescence Microscopy (AF)** is collected on all KPMP tissue sections prior to IMS. AF is also collected on an external reference tissue termed "*BlockQC*".
AF QC Data Collection Protocol: **AFQC Protocol**
AF protocol: **AF Microscopy**

CITATION

Patterson NH, Tuck M, Van de Plas R, Caprioli RM (2018). Advanced Registration and Analysis of MALDI Imaging Mass Spectrometry Measurements through Autofluorescence Microscopy..
LINK
https://doi.org/10.1021/acs.analchem.8b02884

4 **MALDI Imaging Mass Spectrometry (IMS)** is collected on all KPMP tissue sections and on a *BlockQC* tissue section.
Matrix Deposition Protocol (Sublimation): **Tissue Washing and Matrix deposition Protocol**
High Spatial Resolution MALDI IMS Data Acquisition: **MALDI IMS Protocol**

5 **LC-MS/MS Lipidomics** is performed on a single serial KPMP biopsy and a *BlockQC* tissue section.
Protocol: **LC-MS/MS Protocol**

6 **Post-IMS Autofluorescence Microscopy** is collected on all KPMP tissue sections and a single *BlockQC* tissue section to aid in multimodal data registration.
Protocol: **Post-IMS AF**

7 **PAS Staining** is performed on all tissue sections including *BlockQC*
Matrix removal and Staining: **PAS Staining Protocol**

Data Pre-Processing and Analysis

8 **Data Pre-Processing i**s performed on all MALDI IMS data.
Protocol: **MALDI IMS Data Pre-Processing**

9 **Image registration - AF, PAS and IMS** is performed on all tissue sections including *BlockQC*
Protocol: **Image Registration**

CITATION

Patterson NH, Tuck M, Van de Plas R, Caprioli RM (2018). Advanced Registration and Analysis of MALDI Imaging Mass Spectrometry Measurements through Autofluorescence Microscopy..
LINK
https://doi.org/10.1021/acs.analchem.8b02884

10 **Functional Tissue Unit (FTU) Segmentation** is performed on all KPMP biopsies and *BlockQC* tissues.
Protocol: **FTU Segmentation**

CITATION

Patterson NH, Neumann EK, Sharman K, Allen J, Harris R, Fogo AB, De Caestecker M, Caprioli RM, Van de Plas R, Spraggins JM (2021). Autofluorescence microscopy as a label-free tool for renal histology and glomerular segmentation. bioRxiv.
LINK
https://doi.org/10.1101/2021.07.16.452703

11 **Analyte Annotation**
Tentative lipid annotations are curated from the data
Protocol: **Tentative Lipid Annotation**

QC Data Collection and Recording

12 QC data collected during this work will be compiled in Levy-Jennings plots to longitudinally track the performance of the assays. QC data for both the KPMP samples and the reference

tissue are summarized and reported. QC/QA protocols for the multimodal molecular imaging pipeline are summarized in the protocol **QC Metrics for KPMP Data Collection by VU TIS**.

Citations

Step 10

Patterson NH, Neumann EK, Sharman K, Allen J, Harris R, Fogo AB, De Caestecker M, Caprioli RM, Van de Plas R, Spraggins JM. Autofluorescence microscopy as a label-free tool for renal histology and glomerular segmentation
https://doi.org/10.1101/2021.07.16.452703

Step 3

Patterson NH, Tuck M, Van de Plas R, Caprioli RM. Advanced Registration and Analysis of MALDI Imaging Mass Spectrometry Measurements through Autofluorescence Microscopy.
https://doi.org/10.1021/acs.analchem.8b02884

Step 9

Patterson NH, Tuck M, Van de Plas R, Caprioli RM. Advanced Registration and Analysis of MALDI Imaging Mass Spectrometry Measurements through Autofluorescence Microscopy.
https://doi.org/10.1021/acs.analchem.8b02884