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

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VU TIS Multimodal Molecular Imaging Pipeline for KPMP Biopsy Interrogation

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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.

Tissue Screening and Assessment

1 Asses Kidney Tissue Sections

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

On-Site Tissue Integrity Considerations

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

2 **Autofluorescence Microscopy (AF)** is collected on all tissue sections prior to IMS

AF QC 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

3 **MALDI Imaging Mass Spectrometry (IMS)**

Matrix Deposition Protocol (Sublimation): Tissue Washing and Matrix deposition Protocol

High Spatial Resolution MALDI IMS Data Acquisition: MALDI IMS Protocol

4 **LC-MS/MS Lipidomics**

LC-MS/MS is performed on a single serial biopsy section.

Protocol: LC-MS/MS Protocol

5 **Post-IMS Autofluorescence Microscopy**
Protocol: Post-IMS AF

6 **PAS Staining**
Matrix removal and Staining: PAS Staining Protocol

Data Pre-Processing and Analysis

7 **Image registration - AF, PAS and IMS**
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

8 **Data Pre-Processing**
Protocol: MALDI IMS Data Pre-Processing

9 **Functional Tissue Unit (FTU) Segmentation**
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

10 **Analyte Annotation**
Tentative lipid annotation from the MALDI IMS data
Protocol: Tentative Lipid Annotation