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VU Biomolecular Multimodal Imaging Center (BIOMIC) Eye characterization pipeline

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VU Biomolecular Multimodal Imaging Center / Spraggins Research Group | Human BioMolecular Atlas Program (HuBMAP) Method Developmen

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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 eye.

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

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We use this protocol and it's working

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Keywords: HuBMAP, BIOMIC, Vanderbilt, Multimodal Imaging, IMS, MxIF, Proteomics, Image Registration, Eye

Organ Procurement and Processing

1 Collaborating with the Advancing Sight Network (500 Robert Jemison Rd Birmingham, AL 35209), our team identifies and preserves human donor ocular tissue using the following protocols.

Donor Tissue Acceptance Criteria:
1. Caucasian or African American (AA)
2. **≥18-70** years of age
3. Acceptable: sepsis
4. Acceptable: long or short optic nerve stump

Exclusion Criteria:
1. Diabetes
2. Ventilator ≥5 days
3. Cataract surgery; glaucoma shunt; intravitreal injections; cornea donor, cornea with infiltrates
4. Conditions discovered through the Interview that can affect retina or choroid (i.e., AMD, macular edema, macular hole, retinitis pigmentosa; neurologic disease Parkinson, any dementia)
5. Surgeries affecting retina, discovered by Interview: vitrectomy, pan-retinal coagulation, scleral buckle
6. Known major head trauma (falls, motor vehicle accident, gunshot wounds, etc)

To facilitate downstream analysis, the anterior and posterior segment are dissected apart during tissue processing.

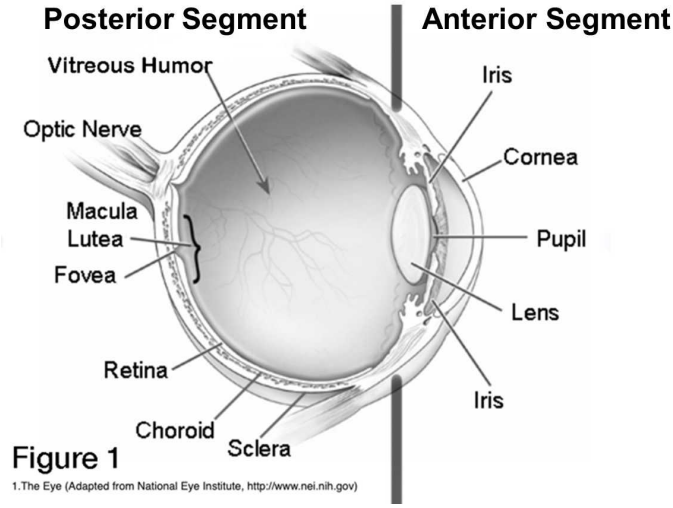

Figure 1. Division of the anterior and posterior segment during tissue processing. The anterior segment contains the cornea, iris, ciliary body, and lens. The posterior segment contains the retina, fovea, choroid, sclera, and optic nerve head.

Collection of post-surgical tissue and fixation.
Collection-Fixation: Fixation of Eye Tissue at UAB
Collection-Freezing: Freezing and Embedding Eye Tissue

Tissue Preparation and Screening

2 **Initial Rapid Pathology Assessment of Eye Tissue**

Eyes are assessed by ex vivo imaging using optical coherence tomography (OCT), color fundus photography, and near infrared reflectance scanning laser ophthalmoscopy. Normal retinas are those with even and uniform band thicknesses and reflectivity on OCT, with smooth changes in layer thicknesses between retinal regions. In the case of non-uniform band thicknesses and reflectivity levels, eyes that do not exhibit features beyond what can be explained by known post-mortem artifact are considered normal. Known artifacts include detachments of retina from retinal pigment epithelium (RPE), of RPE from choroid, of neurosensory retina from bacillary layer, RPE undulation, cystic change).

Optical Coherence Tomography (OCT)and Scanning Color Fundus Photography: (steps 5-6 of Fixation of Eye Tissue at UAB)

Ocular tissue is further evaluated using histological staining:
PASH Staining

3 **Cryosectioning** of tissues into micrometer thick sections, alternating between sections for MALDI, histopathology, MXIF and proteomics.
Sectioning: steps 12-14 of Freezing and Embedding Eye Tissue

Sample Assays

4 **LC-based Proteomics**
Sample Preparation
Data Acquisition
Data Analysis

5 **Autofluorescence microscopy (performed on all slide-mounted tissue sections)**
Pre-IMS Autofluorescence 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

6 **Imaging Mass Spectrometry (IMS)**
Matrix Application:

HTX M5 TM Sprayer
Sublimation

High resolution IMS data acquisition

7 **Post-IMS Autofluorescence Microscopy and Image Registration**
Post IMS AF
Matrix removal and staining
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

Registration of autofluorescence images from both IMS and microscopy sections allows for the direct correlation of two or more orthogonal approaches.

8 **IMS Data Analysis**
Data pre-processing
Lipid annotation

9 **LC-MS/MS Lipidomics Analysis**
LC-MS/MS from tissue cryosections

10 **Spatial transcriptomics via the Nanostring GeoMx Digital Spatial Profiler**
Manual slide preparation
NGS library preparation
Next-generation sequencing