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HuBMAP | GE/Vanderbilt MALDI IMS and Cell DIVE™ Modality Overview

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community



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ABSTRACT

This is an overview of all protocols currently in use for the GE/Vanderbilt University Cell DIVE collaboration for the Human BioMolecular Atlas Program (HuBMAP). It includes links to each of the individual protocols that make up this project workflow.

MALDI IMS

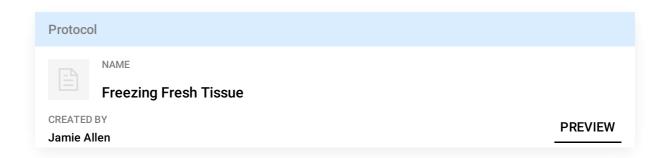
1

Collection of post-surgical tissue.

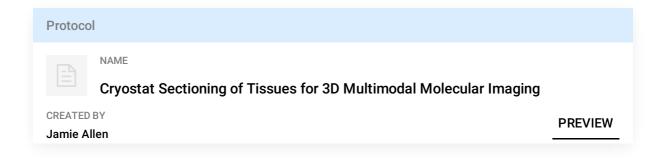
GE F



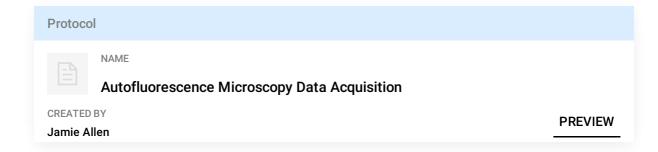
2 Stabilize and freeze tissues.



3 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 an microcentrifuge tube for proteomics analysis.



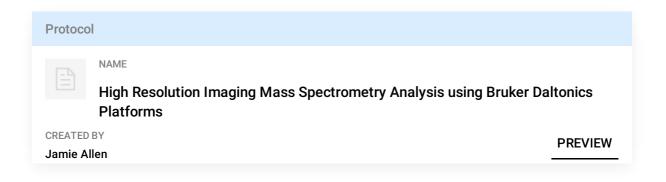
4 Perform autofluorescence microscopy on all tissue sections



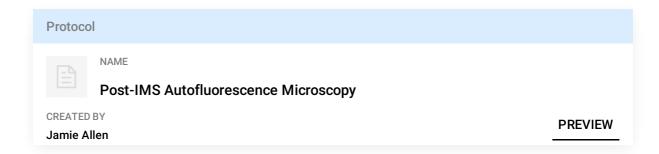
5 Perform Matrix Application



6 Perform high resolution IMS analysis of matrix coated tissue sections.

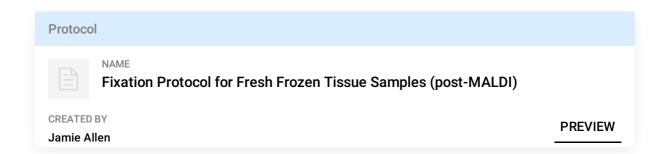


7 Obtain autofluorescence microscopy images of tissues after IMS analysis



Preparing Sample for MxIF

8 Perform Matrix Removal & Tissue Fixation



Cell DIVE

9 Characterize antibodies (primary/secondary, direct conjugates, and zenon labelled antibodies) and determine any antigen effects from the Cell DIVE dye inactivation process.

Cell DIVE™ Platform | Antibody Characterization for Multiplexing Cell DIVE™ Platform | Antibody Staining & Imaging

10 Prepare direct conjugates for study.

Cell DIVE™ Platform | Antibody Purification Chemistry

Cell DIVE™ Platform | Ab Conjugation: Initial Conjugation & Scale up Conjugation

11 Perform Cell DIVE™ multiplexed data acquisition on the final cohort.

Note

Staining is done manually using a humidity chamber and images are acquired on the Leica Cell DIVE imager utilizing a coverslipless imaging approach