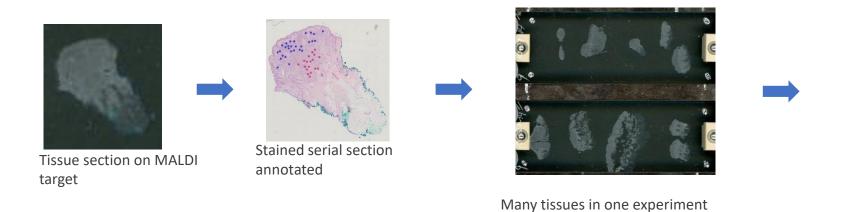
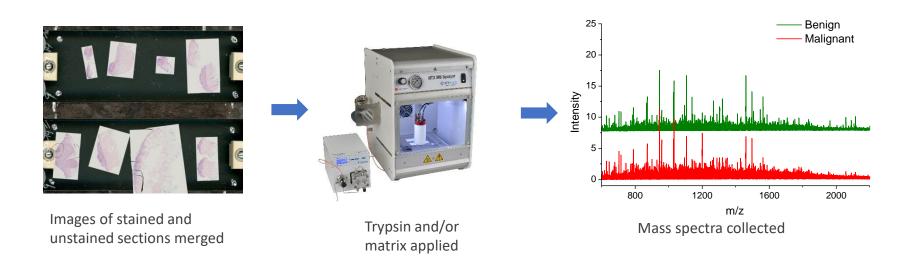
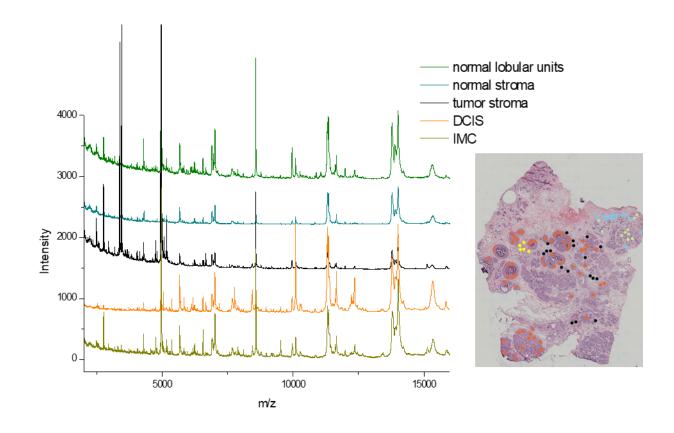
Histology-Guided MS Profiling Workflow





Histology-Guided Mass Spectrometry Profiling

Histology-Guided Mass Spectrometry (HGMS) Profiling is a targeted approach in which only discrete areas within a tissue section are analyzed. Histological staining is used to guide the acquisition of spectra from the tissue section allowing each analyzed spot to be enriched for a single cell type. Due to the reduced data volume, this approach is highly conducive to statistical analysis and classification algorithm generation. HGMS can provide biological insight not attainable by standard histology; such as predicting disease outcome, improving diagnosis, predicting treatment response, etc.



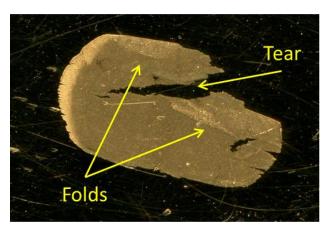
Tissue Sectioning - Patience, Persistence, and Perfection

Practice is necessary to achieve quality sections for the HGMS approach. This is especially the case when working with frozen sections that are not in an embedding medium. Frozen sections are more prone to tearing and folding than are formalin fixed, paraffin embedded sections.

It is extremely important that the sections used for histology-guided mass spectrometry be serial to each other. Structures can change quickly with distance through a tissue specimen. The sections need to be superimposable to ensure accurate alignment for targeting of features of interest. It is essential that the sections be complete and free from defects like folds and tears that will limit the ability to find target areas in serial sections.



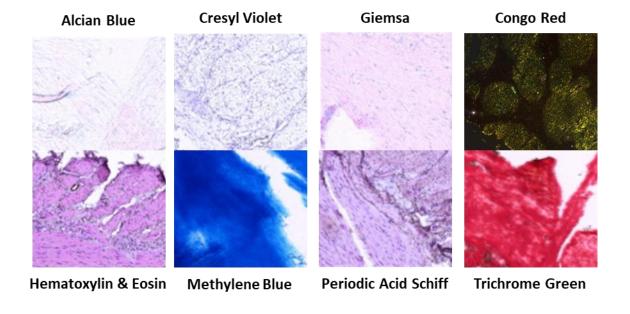
Serial Sections?





Histological Staining

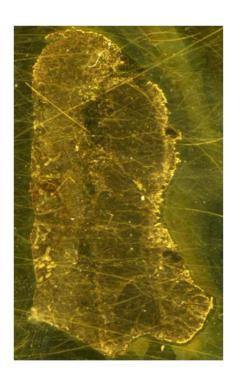
Histological staining is used to allow for visualization of features and cell types of interest in a tissue section. Staining is typically carried out on a section immediately serial to the one to be analyzed by HGMS, but there are cases where staining can be used on the same section, if it will not interfere with data collection*. A variety of different stains can be used depending on the disease or types of cells to be targeted. These include: Hematoxylin and Eosin, Cresyl Violet, Congo Red, and Giemsa, among others, as well as immunohistochemical staining.



Digital Imaging

Digital images are acquired of both the section for mass spectrometry and the stained serial section. Images of the mass spectrometry section are typically acquired with a flatbed document scanner with a resolution of 2400 dpi or higher.

Images of the stained section are taken at microscopy resolution to allow for evaluation of histological features in the tissue. Histological images may be acquired with a stitching microscope using a 10X or higher objective or with a digital slide scanner.



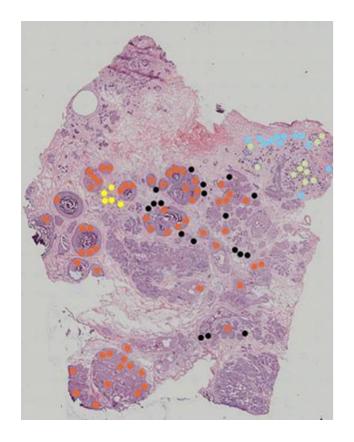
Section on an MS target - from a flatbed scanner



Serial H&E stained section - from a digital slide scanner

Image Annotation

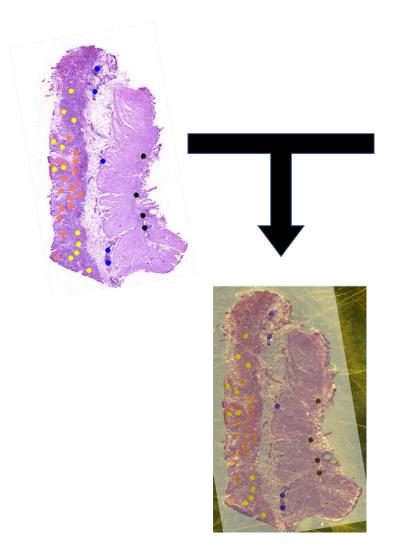
A pathologist or other clinician annotates the digital image of the stained section to indicate the areas from which to acquire mass spectral data. The annotations are of the same size as the area to be targeted for a single spectrum. The use of color coding to indicate different histologies is used for data tracking throughout the project. This approach allows for each spectrum to be enriched for a single cell type. While the size of the target area can be adjusted, it is important to remember that there are tradeoffs between the number of cells sampled (size) and sensitivity for detection of analytes from the tissue section, as well as possible misalignment between serial sections.



- Normal Lobular Units
- Normal Stroma
- Invasive Mammary Carcinoma
- Ductal Carcinoma in situ
- Tumor Stroma

Image Merging

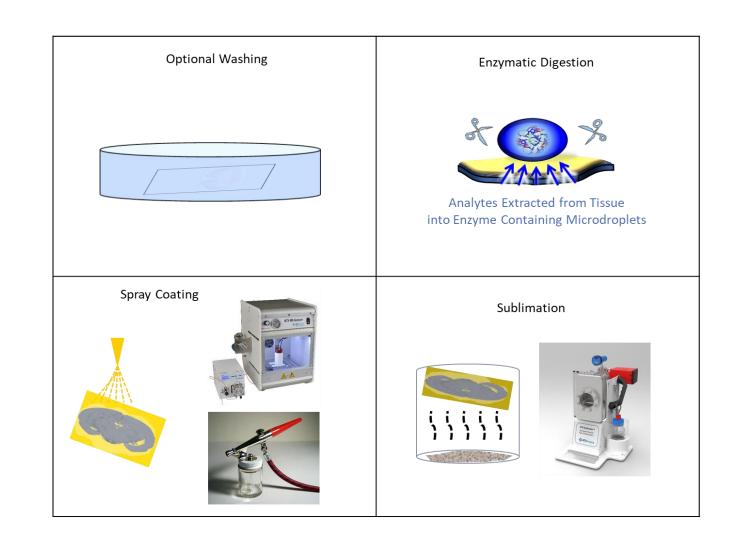
Because the annotations have been placed on a serial stained section to the one being analyzed, the coordinates of those annotations must be transferred to the unstained MSI section. In order to accomplish this task, the images of the two sections must be digitally merged. This is accomplished using Photoshop or other image processing software. When necessary, the annotated image may be broken up into multiple pieces to account for slight differences between the two sections, including bends, folds, or tears. The merged digital image is then used to guide the data acquisition in the mass spectrometer.





Sample Preparation

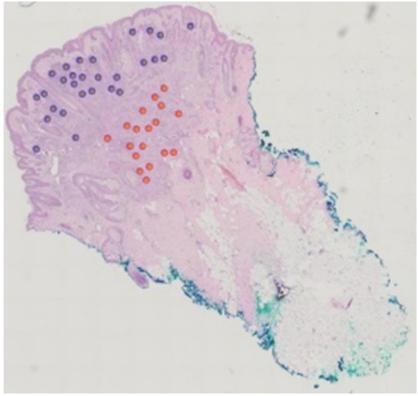
Just as in traditional mass spectrometry imaging, appropriate sample preparation must be carried out for the class of molecules to be analyzed from the tissue section. This may include washing to remove biological salts and enhance signal, enzymatic digestion, and/or matrix application.



Data Collection

The merged MSI and histology image is loaded into the data acquisition software. Fiducial points are used to align the digital image to the target via a 3-point registration. The locations from which data are to be collected are tagged as shown in the image on the right. Different samples and histologies can be saved as regions of interest for downstream data analysis. Data are collected only from the locations of the annotations as opposed to the entire tissue as is the case with traditional MSI. This allows for much higher throughput and more facile data analysis.





Data Analysis

Depending on the goals of the study, the data can be subjected to a variety of statistical analyses. These include hypothesis testing, discriminant analysis (receiver operating characteristic curves), and principal component analysis, as well as the generation of a variety of machine learning algorithms for diagnostic or prognostic classification of samples.

