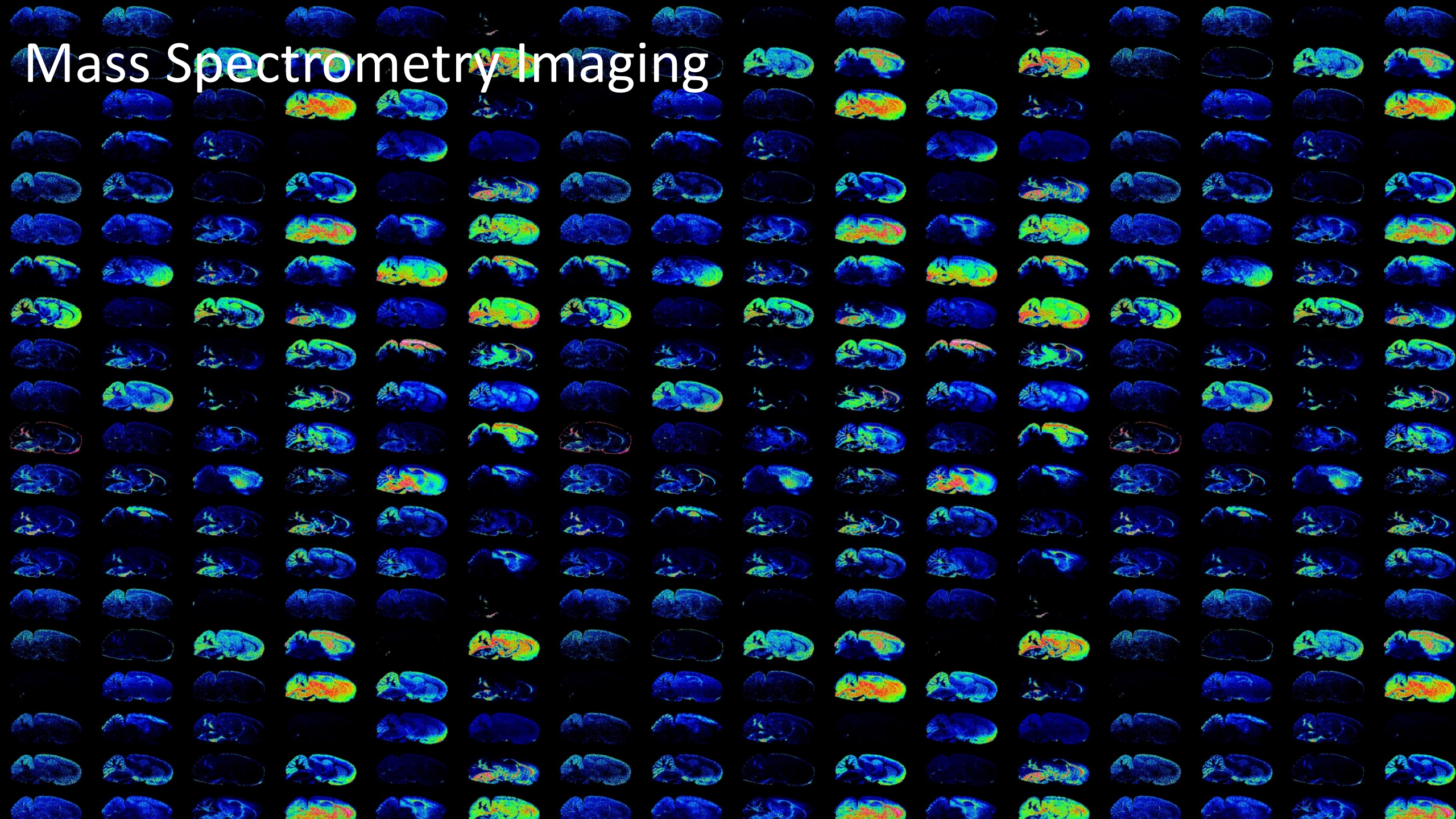
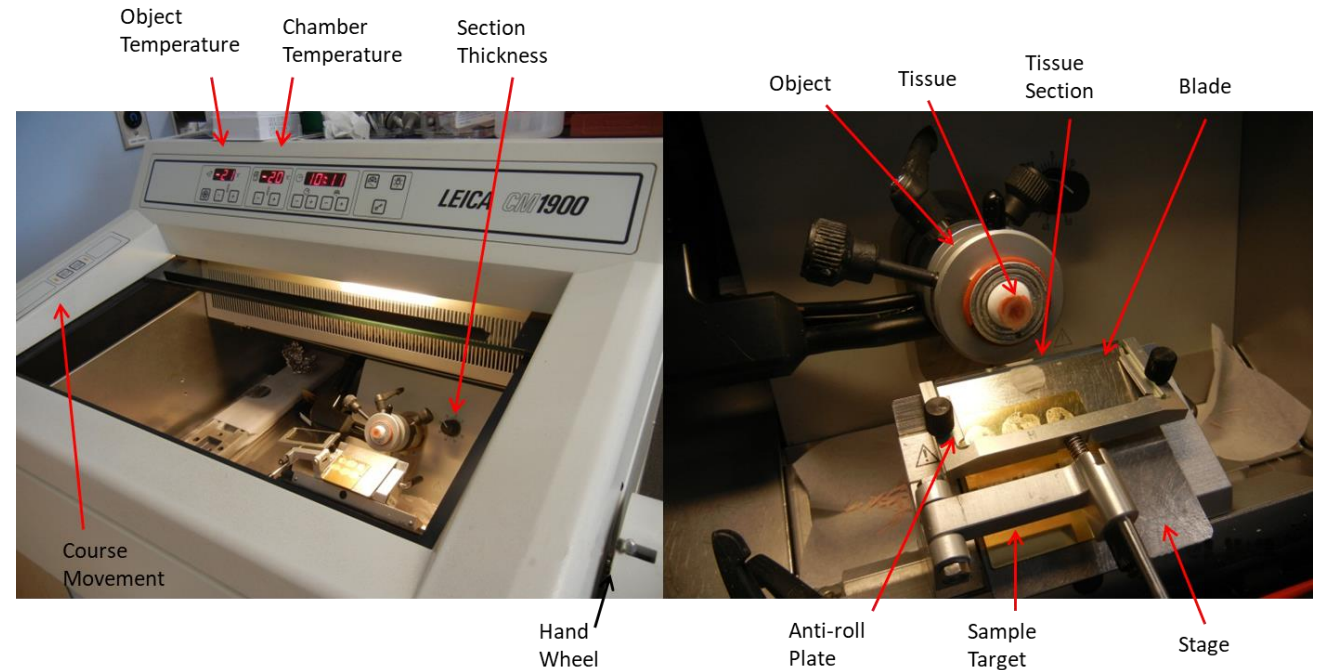


# Mass Spectrometry Imaging



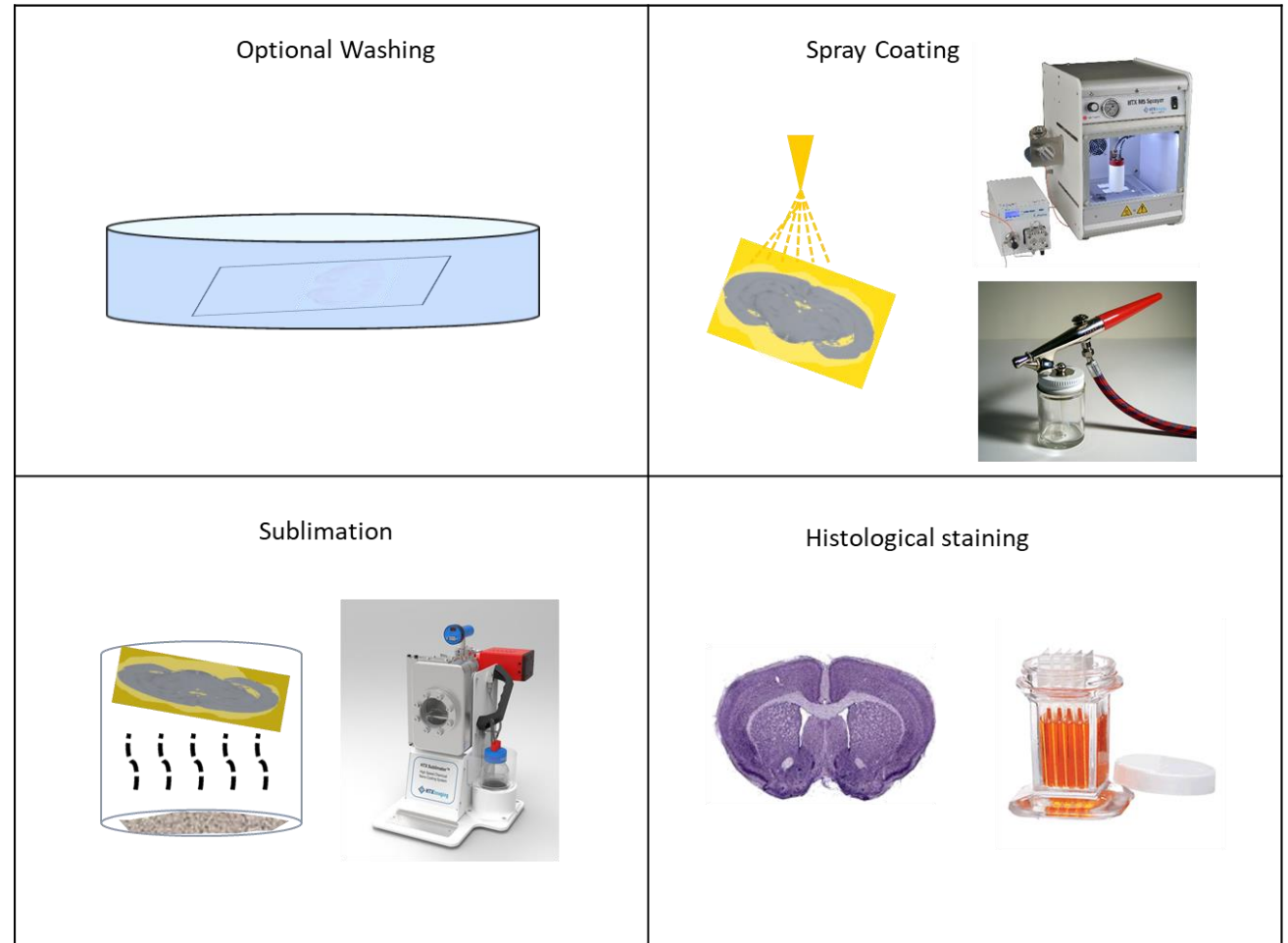
# Mass Spectrometry Imaging (MSI)

MSI uses thin tissue sections, similar to those a pathologist uses to evaluate a biopsy, except that the MSI tissue section is collected onto a specialized target that can be used in a mass spectrometer. In the case of MALDI-TOF MSI, the slides have an indium-tin oxide coating to make them conductive. Other instruments may or may not have this conductivity requirement. MSI can be performed on both fresh frozen and formalin fixed, paraffin embedded (FFPE) tissue specimens, although there are limitations on the types of molecules that can be detected from FFPE tissue.



# MALDI Sample Preparation

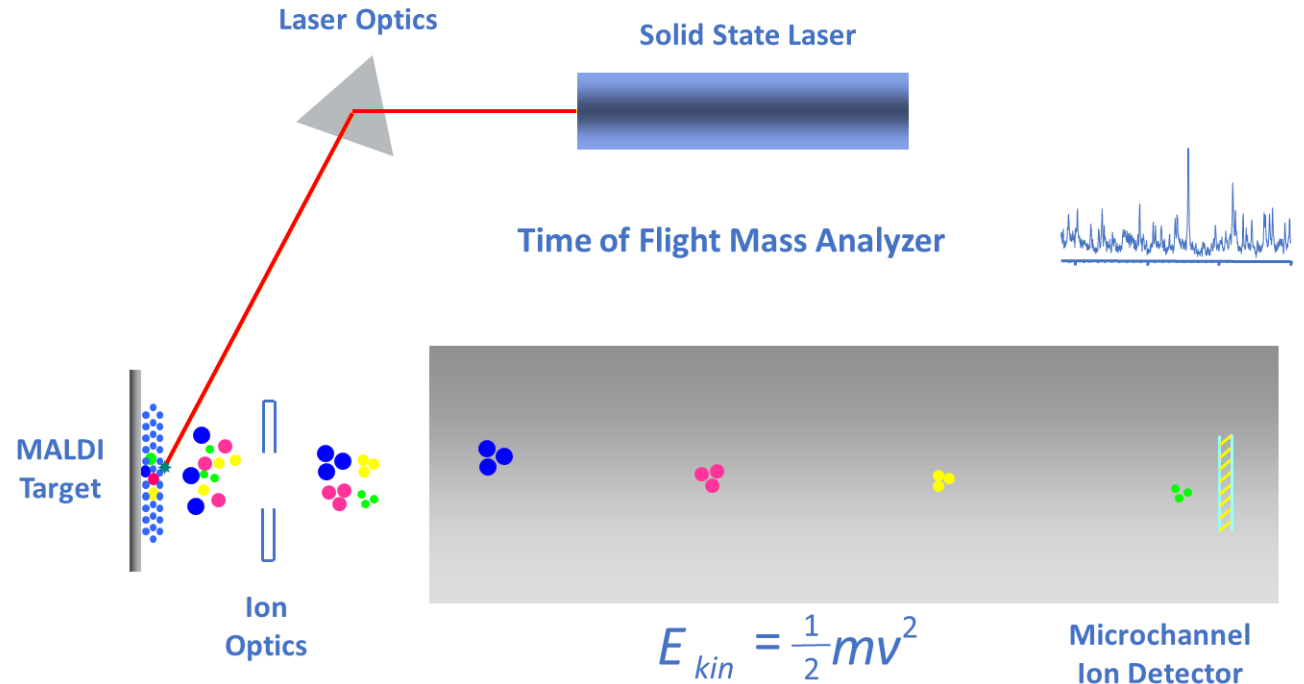
Appropriate sample preparation is carried out for the target molecular class (*e.g.* protein, peptide, lipid, or drug/metabolite) of interest. The section may be washed to remove interfering molecules and/or to enhance the signal of the analytes of interest. A matrix, typically a small organic acid, is applied to the surface of the specimen, usually in a solvent that helps to extract and co-crystallize the analytes of interest with the matrix. The matrix can be applied using sublimation, robotic sprayers, or manually using an airbrush or thin layer chromatography sprayer. Enzymatic digestion performed with a robotic sprayer may be used as part of sample preparation. A serial section is typically stained for histological review.



# MALDI-TOF Data Collection

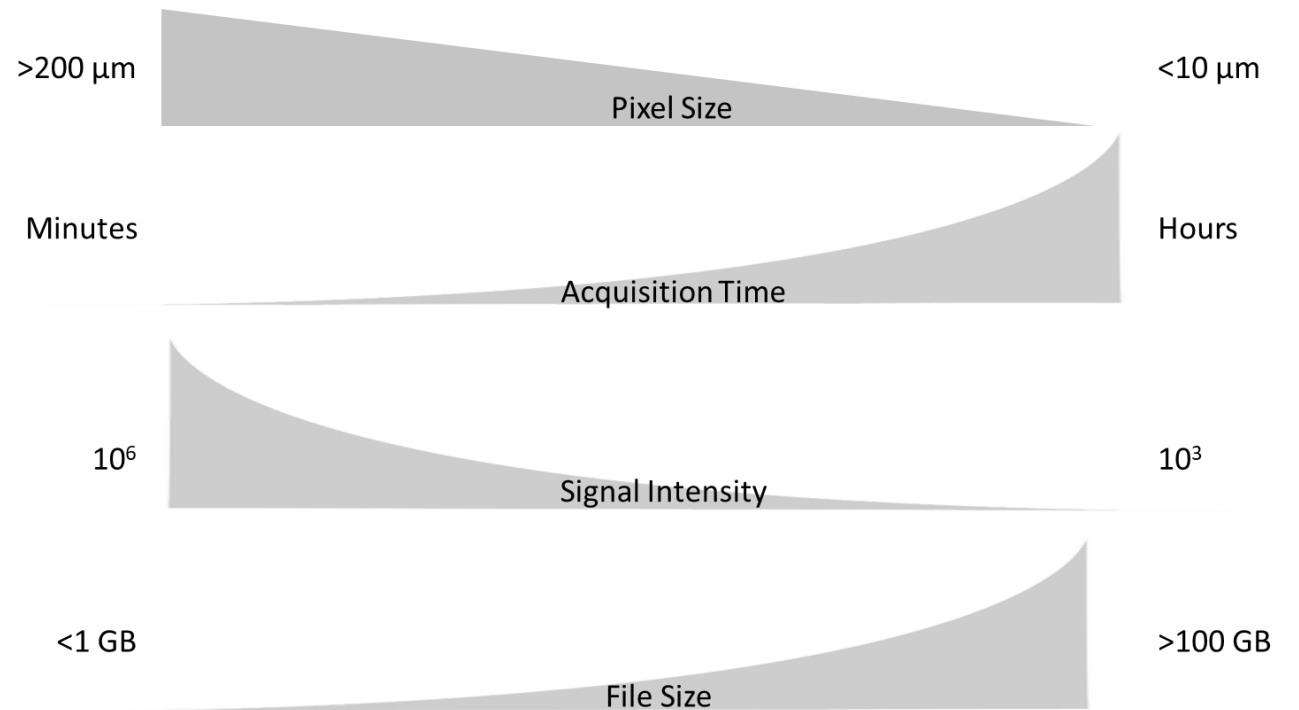
The specimen is interrogated in the mass spectrometer using a UV laser that is fired at the tissue in an ordered array of regular spacing. The matrix absorbs the laser light and acts to desorb and ionize the molecules from the tissue surface. In time-of-flight analysis, ionized molecules are accelerated with high voltage and travel through a flight tube under high vacuum until the ionized molecules arrive at a detector at the end of the flight tube. Since all ions are accelerated with the same energy, those that are smaller will fly faster, while those that are larger will fly slower, across the fixed distance. By measuring the time it takes for the ions to reach the detector, and through the use of calibration standards of known molecular weight, the mass-to-charge ratio of the ions can be determined.

Time-of-flight mass analyzers have been most commonly used for MSI, but MALDI sources have also been coupled with QTOF, Ion Trap, FT-ICR, and Orbitrap mass analyzers



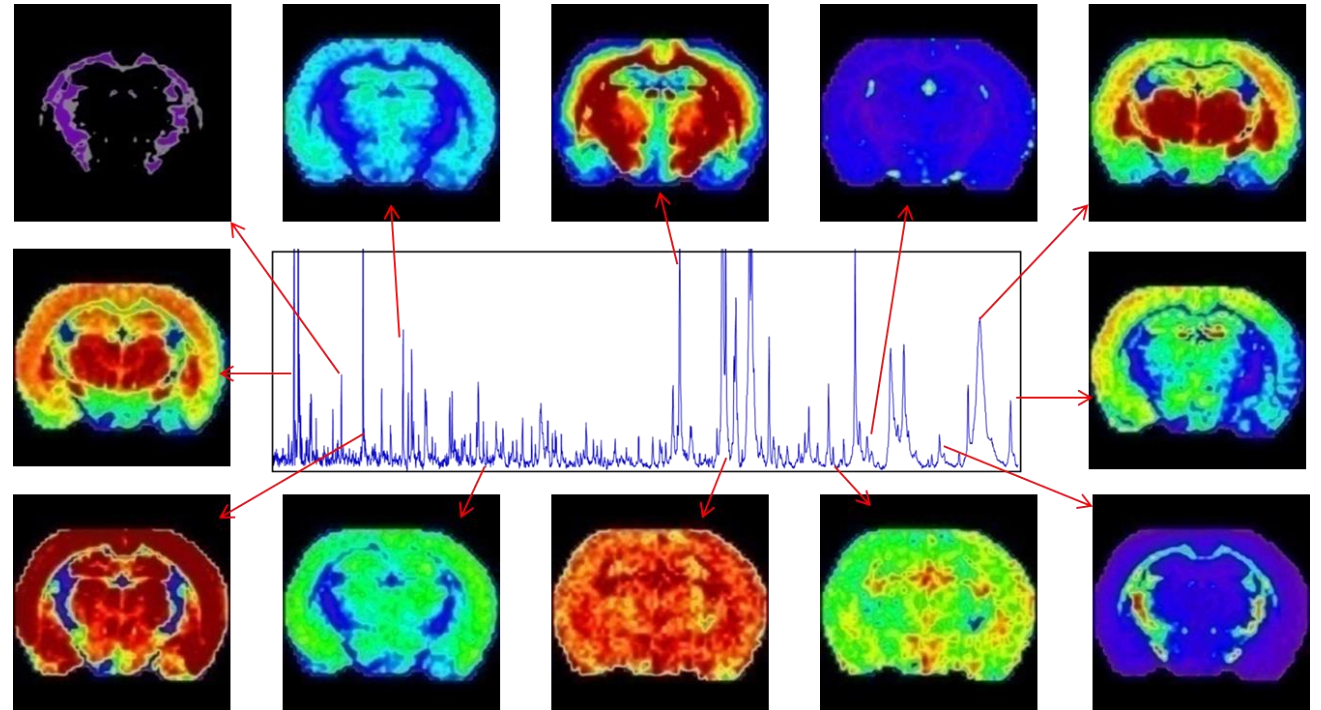
# Imaging Trade-Offs

There is a trade-off between the spatial resolution, or pixel size, used for an image and other parameters such as the time for acquisition, sensitivity, and file size. The number of pixels increases exponentially with an increase in spatial resolution, leading to exponential increases in acquisition time and file size, while leading to a decrease in signal intensity. When designing an imaging experiment, the spatial resolution should be limited to what is necessary to answer the biological question being asked.



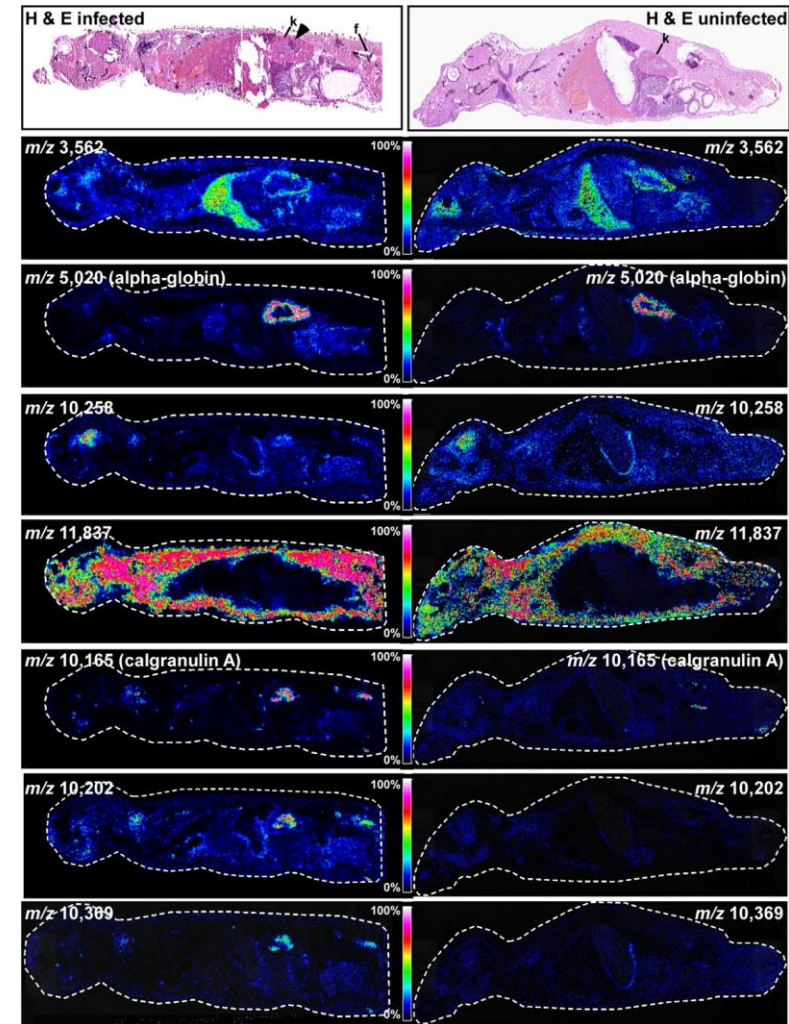
# Data Visualization

A full spectrum is collected at each targeted location in the tissue. This can be likened to the pixels in a picture or on your television, except that instead of having three color channels (red, green, and blue), hundreds to thousands of biomolecules are detected. Any one of these biomolecules can be displayed as a false color image indicating its spatial localization and relative intensity across the surface of the tissue section. Ion images can be directly compared to histological staining to gain biological insights.



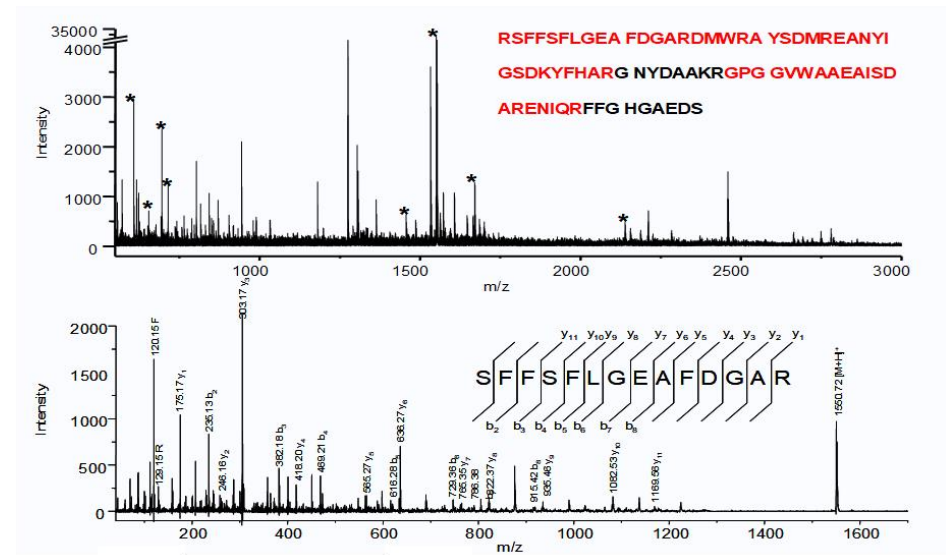
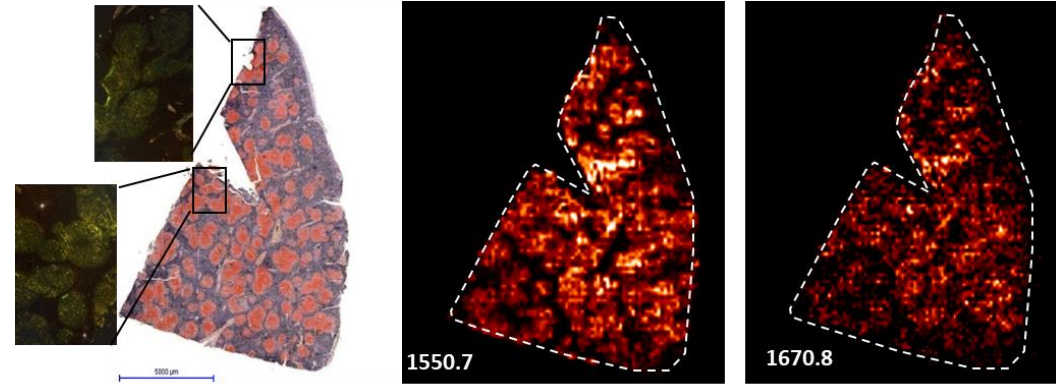
# Intact Protein/Peptide Imaging

Intact protein/peptide analysis must be carried out on fresh frozen tissue specimens. Sections are usually fixed with graded alcohol to remove biological salts and lipids that can suppress signal. Sinapinic acid or super dihydroxybenzoic acid (super DHB) are the matrices of choice for protein analysis. While  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) is often used for peptides. Proteins and peptides analyzed are typically small, with molecular weights ranging from 1 kDa to 30 kDa, and detected in positive ion mode.



# Tryptic Digestion for Peptide Imaging

Peptide analysis, through use of on-tissue tryptic digestion, has mostly been demonstrated in FFPE tissue, although examples in fresh frozen tissue have been reported. Sections are subjected to deparaffinization and rehydration with xylene and graded alcohol followed by heat induced antigen retrieval. Subsequently, trypsin is applied via a robotic sprayer to allow for *in situ* enzymatic digestion of the proteins, a necessary step due to protein crosslinking that occurs during the formalin fixation process. CHCA matrix is used for MALDI, and peptides are generally detected in positive ion mode. Detected peptides may originate from proteins of any molecular weight. Peptide sequencing can be performed directly from the tissue sections.

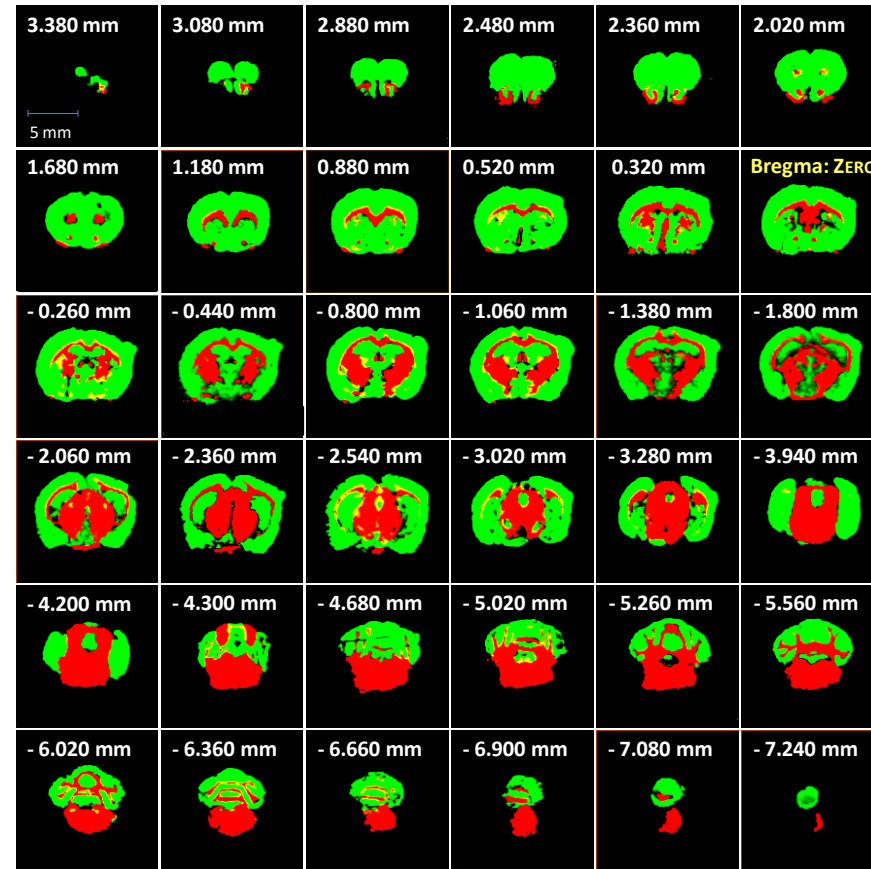




# Lipid Imaging

Lipid analysis are almost exclusively carried out on fresh frozen tissue specimens. Sections may be washed with cold ammonium formate to remove salt ions from the sections that can cause adducts to form on the lipid species, complicating the analysis. DHB or 1,5-diaminonaphthalene (DAN) are matrices typically used for positive and negative ion mode, respectively. Intact lipids, along with lysolipids and fatty acids, are readily detected, with different lipid classes being detected in positive and negative ion mode.

Thirty-six 2D overlaid ion images of the lipids PS 40:6 (green) and ST 24:1 (red)



# Drug/Metabolite Imaging

Drug/metabolite analysis has been demonstrated in both fresh frozen and FFPE tissue, although only polar metabolites have been detected in FFPE tissue. No washing is done on frozen sections while FFPE sections are subjected only to a xylene rinse to remove the paraffin wax from the sample. Matrix and/or solvent choices are highly variable depending on the target molecules of interest, often requiring extensive method optimization or the use of MS/MS to confirm identity. Quantitation of target molecules can be performed with the use of internal standards. Drugs and metabolites may be detected in either positive or negative ion mode.

