MALDI Mass Spectrometry Imaging

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) is an emerging tool to assess the spatial distribution of analytes (proteins, peptides, drugs, lipids, metabolites) in thin tissue sections. General information regarding MALDI mass spectrometry imaging is available from the MALDI MSI Interest Group. Additionally, a number of reviews discussing MALDI MSI have recently appeared.[1-4]

Sample preparation for MALDI mass spectrometry imaging: The tissue of interest is ideally snap-frozen in liquid nitrogen or dry ice in isopentane immediately after excision to minimize protein degradation. Tissue orientation and morphology are best preserved when tissues are loosely wrapped in aluminum foil prior to freezing, and tight plastic tubes or cassettes that deform the sample should be avoided. Tissues should be stored at -80 °C, free from embedding media such as OCT (optimum cutting temperature compound) to prevent diffusion of analytes into hydrophobic OCT polymers. Ideally, the tissue is sectioned using a minimum amount of OCT to mount the tissue in the cryostat, and thin tissue sections (usually 10-12 um thick) are mounted on glass MALDI slides with conductive surfaces (coated with gold or indium tin oxide). Washing protocols depend on the analytes of interest, but usually involve dipping the slide into water to remove OCT and then graded ethanol or isopropanol washes to dehydrate the tissue and eliminate physiological salts. When the tissue is completely dry, MALDI matrix is applied. The choice of matrix depends on the analytes of interest, and commonly used matrices include sinapinic acid (SA) for intact proteins and α-cyano-4-hydroxycinnamic acid (CHCA) for peptides. The matrix can either be deposited as individual droplets using a spotter (e.g. with Labcyte Portrait 630 Spotter) or as a homogeneous layer using a sprayer (e.g. with SunCollect MALDI sprayer-spotter), and both of these systems are available in the proteomics core facility. This overall process is illustrated below (Figure 1, workflow 1).

***In Situ* tryptic digestion for protein identification:** Proteins present in the tissue section may be digested with trypsin and the spatial distribution of the resulting peptides determined.[5-7] To accomplish this, trypsin solution is preferably applied in discrete droplets on the tissue to minimize lateral diffusion (using the Labcyte Portrait 630 spotter), and the tissue is incubated in a humidity chamber at 37 °C for several hours to facilitate digestion. Following *in situ* trypsin digestion, MALDI matrix is applied as discrete droplets to the same coordinates and the tissue is imaged on the mass spectrometer. *In situ* tryptic digestion enables the imaging of tissue preserved as formalin fixed paraffin embedded (FFPE) blocks.[5,6]

Imaging data acquisition and analysis: In conjunction with mass spectrometry imaging, ideally the mass spectrometer is tuned using both external and internal standards (added to matrix prior to deposition) and mass spectra are acquired from the surface of the tissue at a predefined special resolution while moving the mounted tissue section under a fixed laser position in the inlet of the instrument. In the proteomics core facility, the AB SCIEX 5800 TOF/TOF system is used for MALDI mass spectrometry imaging. To facilitate the acquisition of the data, AB SCIEX recently released imaging acquisition software developed in collaboration with the proteomics core facility. MALDI MSI data can be analyzed using any one of three free dedicated software packages that include CARDINAL (open-source R package) developed by Olga Vitek and Kyle Bemis at Purdue University in collaboration with the proteomics core facility; MSiReader (open-source Matlab package);[8] and Biomap (from Novartis).

As illustrated below (Figure 1, workflow 2), to identify proteins after *in situ* digestion one can either perform MS/MS directly on the tissue, or preferably compare the m/z values observed on the tissue with a list of m/z values (and the corresponding peptide IDs) generated by digestion of one or more adjacent tissue sections that are subsequently processed by LC-MALDI on the same mass spectrometer. Additionally, a stained adjacent tissue section can be used to determine regions of interest (ROIs) within the MALDI imaging data for further analysis (Figure 1, workflow 3). The software allows the user to view single ion (m/z) images or to perform segmentation analyses over the entire

mass spectrum. To compare mass spectra in different ROIs within the tissue, the software can generate mass lists corresponding to one or more ROIs, and use statistical segmentation algorithms to generate clusters based on similarities and differences in the mass spectra. Further, mass lists corresponding to ROIs can be used for supervised classification or alternatively an unsupervised classification can be done by principal component analysis.

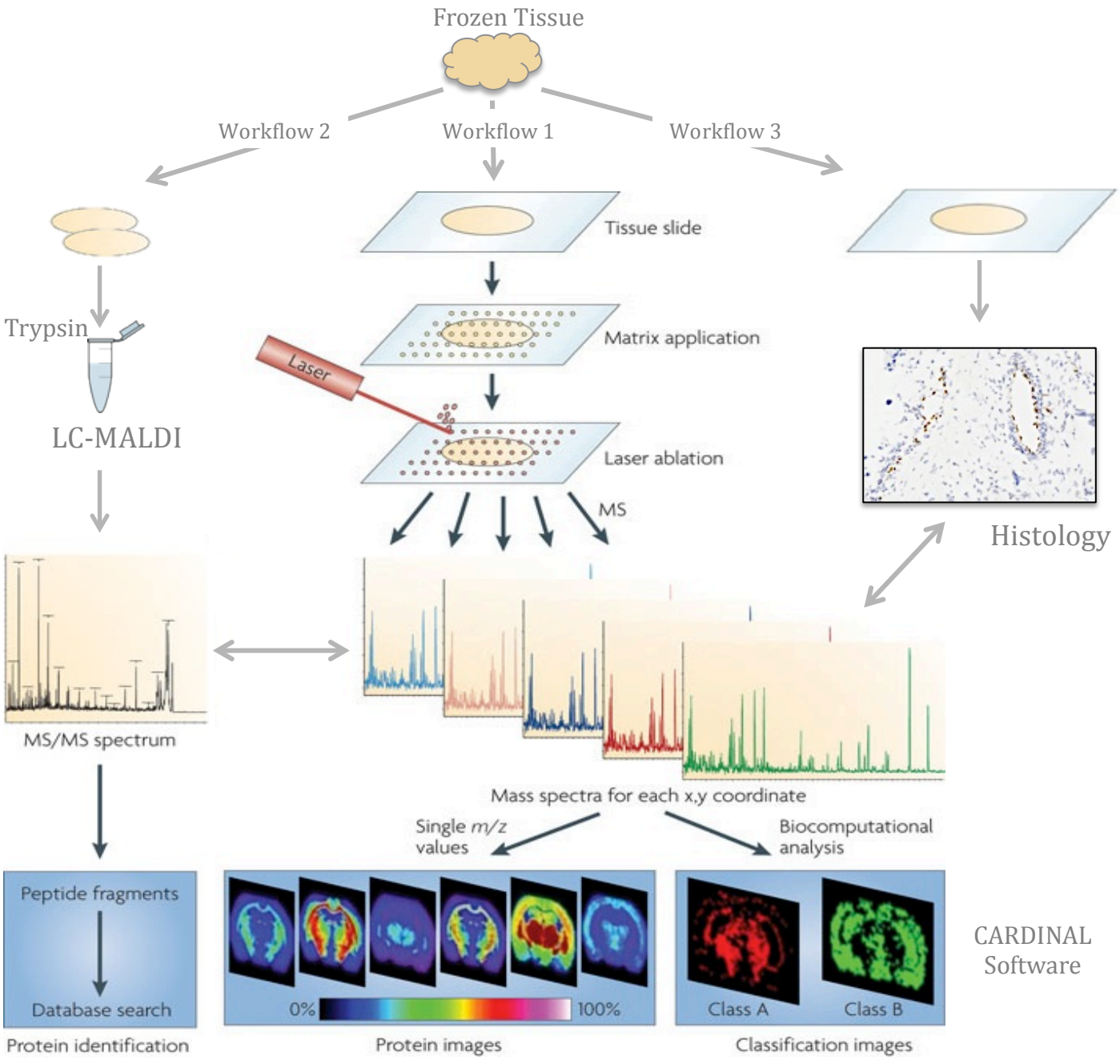

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Figure 1. Workflow 1, MALDI mass spectrometry imaging with matrix deposition as discrete droplets; Workflow 2, Analysis of adjacent tissue section(s) by tryptic digestion and LC-MALDI for protein identification; and Workflow 3, Analysis of adjacent tissue section by immunohistochemistry.

References

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