WORKSHOP SUMMARY

Workshop: Sample preparation I/II
Topic: Fresh frozen tissue: cryosectioning
Session: 1A & 2A
Time: Tuesday 10:30 am & 1:30 pm

Background

The majority of MALDI IMS studies utilize thin tissue sections from fresh frozen tissue. This allows a great flexibility in both selecting and developing imaging strategies and handling the unique aspects of each biological experiment. Fresh frozen tissue is the only tissue preparation used for intact protein imaging, as this tissue preparation does not fix or crosslink proteins like formalin fixation. Endogenous peptides, lipids, and small molecules may be better accessed through fresh frozen tissue as fixation processes typically remove these types of molecules. The requirements for analysis by imaging mass spectrometry are unique: the tissue is typically prepared for cryosectioning without embedding, as embedding media introduces contaminants incompatible with mass spectrometry analysis. This workshop will present practical aspects of cryosectioning specifically for imaging mass spectrometry experiments.

Highlights

- Mounting fresh frozen tissue without embedding media (whole organs or biopsy-size material)
- Basics of cryosectioning
- Thaw mounting onto different substrates
- Thaw mounting multiple sections on one substrate
- Special needs: handling optimal cutting temperature (OCT) embedded samples
- Special needs: fatty tissues (fat, breast)
- Discussion of optimal tissue thickness for experiment, considering matrix application method (precoating or postcoating) and laser geometry (reflection or transmission)
- Discussion on planning the experiment: number of sections needed and collection order of the tissue sections.
- Discussion on storing tissue prior to protein or lipid imaging experiments

Summary

In this workshop, attendees will learn how to prepare fresh frozen thin tissue sections for MALDI IMS studies. Intact mouse brain or portions of mouse brain will be used to demonstrate attachment of the brain to the cryostat chuck without contamination from embedding material. Brain tissue from mice will be cryosectioned and mounted on different substrates for imaging experiments and onto microscope slides for parallel histological studies. Thaw mounting for imaging mass spectrometry will be demonstrated. Discussions will include planning the experiment before cryosectioning and storage conditions for samples.