

# WORKSHOP SUMMARY

Workshop: Sample preparation I/II

Topic: Fresh frozen tissue: washing and histology staining

Session: 1C & 2C

Time: Tuesday 10:30 am & 1:30 pm

## Background

Tissue washing and histological staining are essential for targeting molecules of interest and correlating signals to tissue features. Tissue washing removes molecular species that contribute to ion suppression or tissue degradation. For endogenous peptides and intact protein imaging, series of organic solvent washes are used to fix proteins, limit degradation, and remove lipids and endogenous salts that may suppress protein signal. For lipid imaging, the tissue is washed in aqueous buffer to remove endogenous salts that enhance matrix cluster formation and suppress lipid signal.

Histological staining may be performed on serial sections or on the same sections used for imaging after the imaging experiment is complete. The hematoxylin and eosin (H&E) stain is a classical histology stain that provides a comprehensive view of cellular features within the tissue and is widely used by pathologists to diagnose disease states. Hematoxylin stains nuclear components, while eosin stains cytoplasmic components. A high quality H&E stain allows one to correlate signal arising from unique tissue structures to a cellular level. This workshop will present practical aspects of sample preparation prior to matrix application and staining for image correlation after imaging mass spectrometry experiments.

## Highlights

- Strategies for washing fresh frozen tissue sections for lipid imaging
- Discussion on how tissue washing prior to lipid imaging simplifies data analyses
- Protocols for washing fresh frozen tissue sections for endogenous peptide or intact protein imaging
- Discussion on why washing is important prior to storage
- Discussion on storing tissue prior to protein or lipid imaging experiments
- Staining fresh frozen tissue with hematoxylin and eosin
- Discussion on the importance of histological staining for imaging experiments
- Mention image fusion or automatic correlation of ion images with histology staining images

## Summary

In this workshop, attendees will learn how to prepare fresh frozen thin sections for MALDI IMS studies. Thin tissue sections from adult mouse brain will be used for hands-on washing experiments for protein and lipid imaging experiments and for hematoxylin and eosin staining. Discussion will include aspects of tissue storage and how preparative processes contribute to the imaging data results.