MALDI imaging analysis of the canine heartworm, *Dirofilaria immitis*, using a timsTOF fleX

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Objectives

- Demonstrate SpatialOMx capabilities of the timsTOF fleX on a complex tissue sample
- Correlate molecular phenotypes across glycomics. lipidomics, and metabolomics imaging experiments

Background

Parasitic nematodes afflict much of the world population and limit agricultural production by infecting livestock and cash crops. The intensity, distribution, and geographic reach of parasitic nematodes is projected to be influenced by global climate change. These diverse species have the potential to further impact crop production rates and expand to new hosts, with impacts on plant and animal life across the planet. A comprehensive understanding of such organisms is critical to best devise strategies to reduce these impacts.

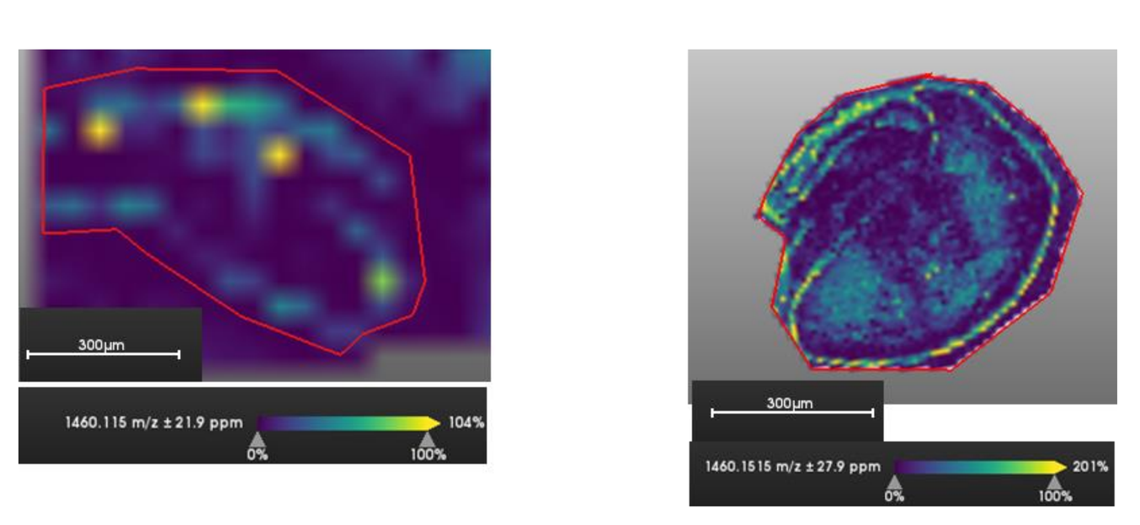
Experimental Methods

- MALDI Imaging experiments performed on fresh frozen canine heartworms, *Dirofilaria immitis*
- Glycomic analysis: tissue embedded in OCT, sectioned at 10 μm thickness and thaw mounted onto conductive IntelliSlides at -19°C. Samples were delipidated using standard protocols and digested with PNGaseF, followed by spraying with CHCA matrix
- Lipidomic analysis: tissue was embedded in CMC, sectioned at 10 μm thickness, thaw mounted onto IntelliSlides, then sprayed with CHCA.
- Metabolomic analysis: tissue was embedded in CMC , sectioned at 10 μm thickness, thaw mounted onto IntelliSlides, then sprayed with DHAP or norharmane.
- All samples were imaged using a timsTOF fleX at 10 micron lateral spatial resolution in positive or negative ion mode (Bruker Scientific, Billerica, MA). Data analysis was performed using SCiLS lab and MetaboScape (Bruker Scientific, Billerica, MA).

Results

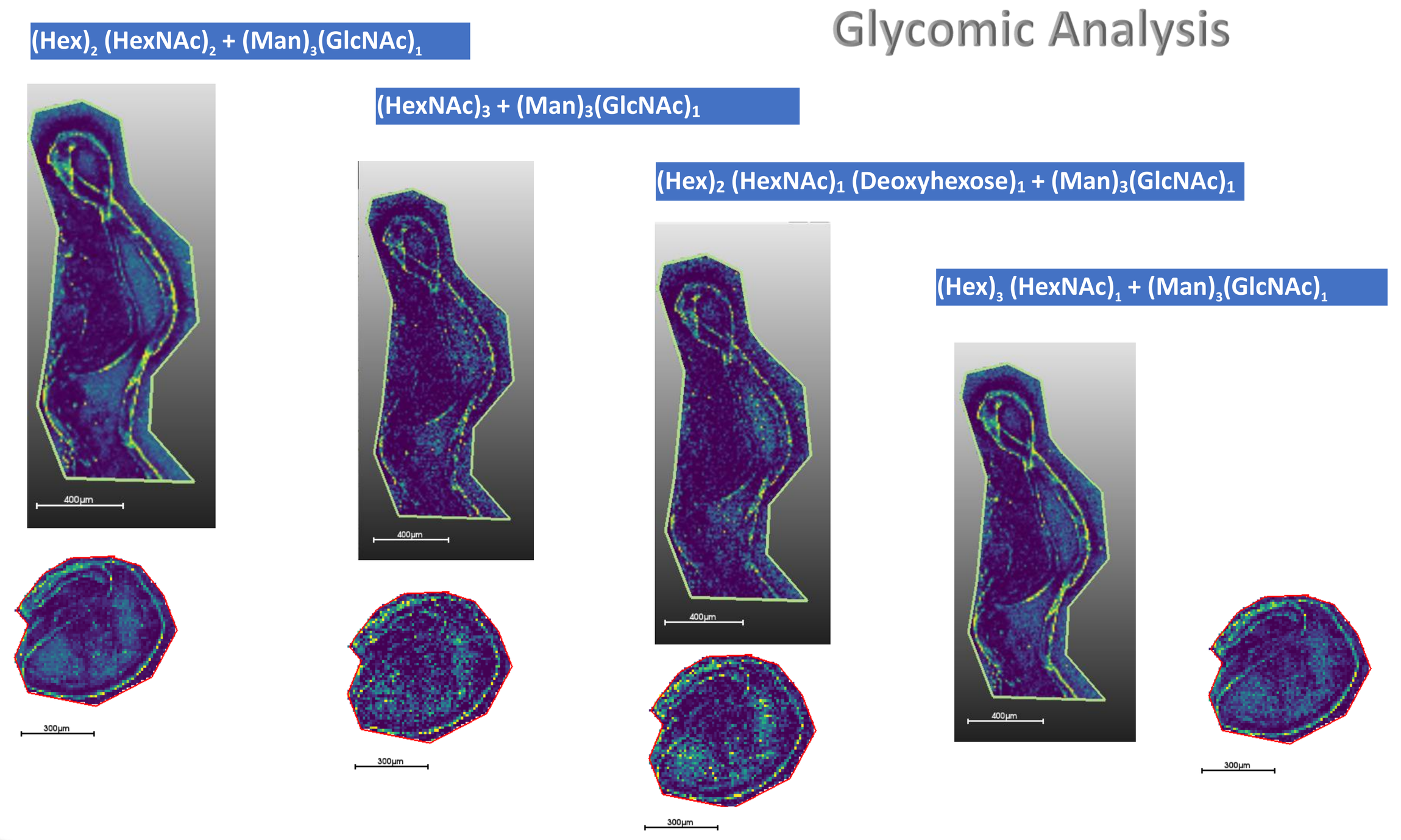
The importance of lateral spatial resolution

50 μm 10 μm



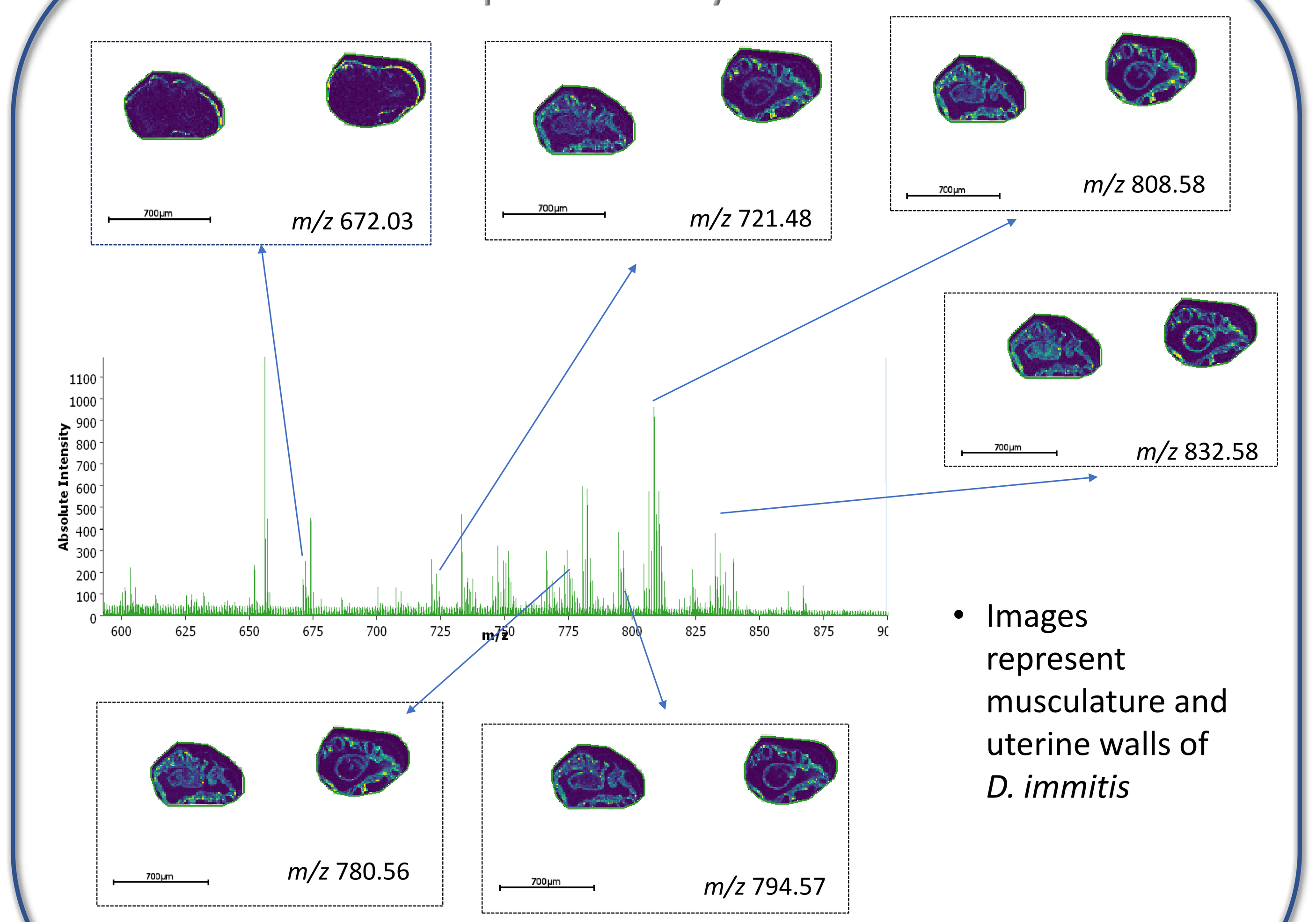
- Fine features in small organisms require high spatial resolution to gain meaningful information

Glycomic Analysis



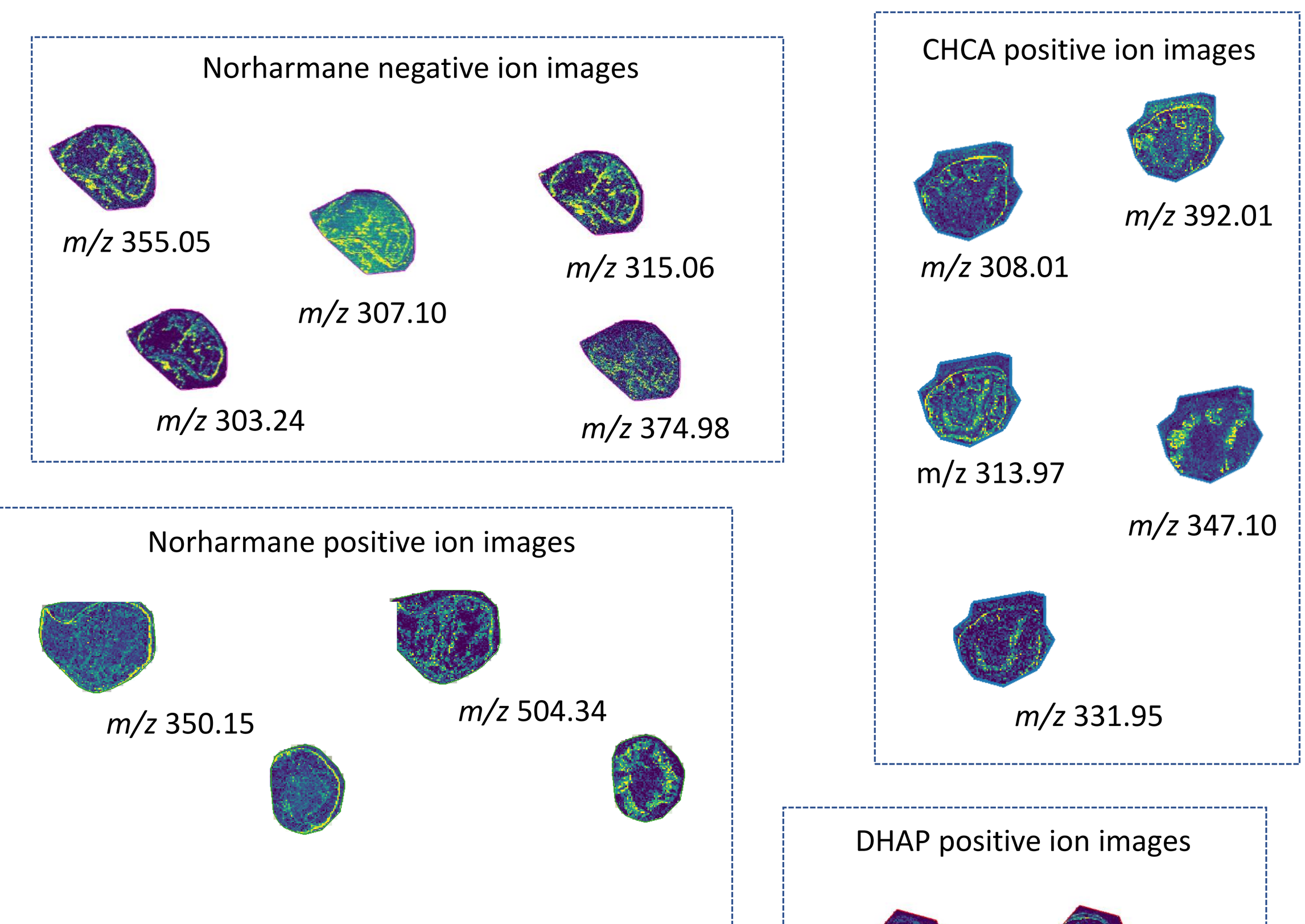
- Glycans expected to be predominately expressed in outer cuticle layers.
- Longitudinal and cross section views analyzed
- All putative compositions previously reported in *D. immitis (Martini et al., Nature Comm.* **2019**, *10.)*

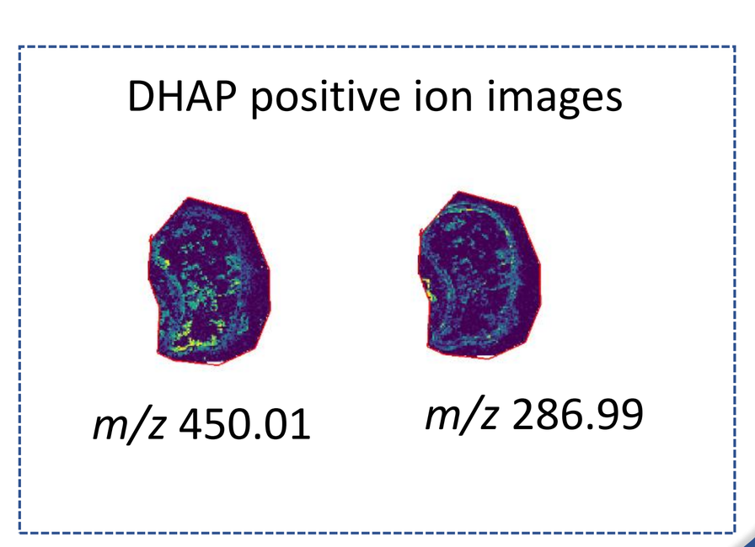
Lipidomic Analysis



- Images represent musculature and uterine walls of *D. immitis*

Metabolomic Analysis

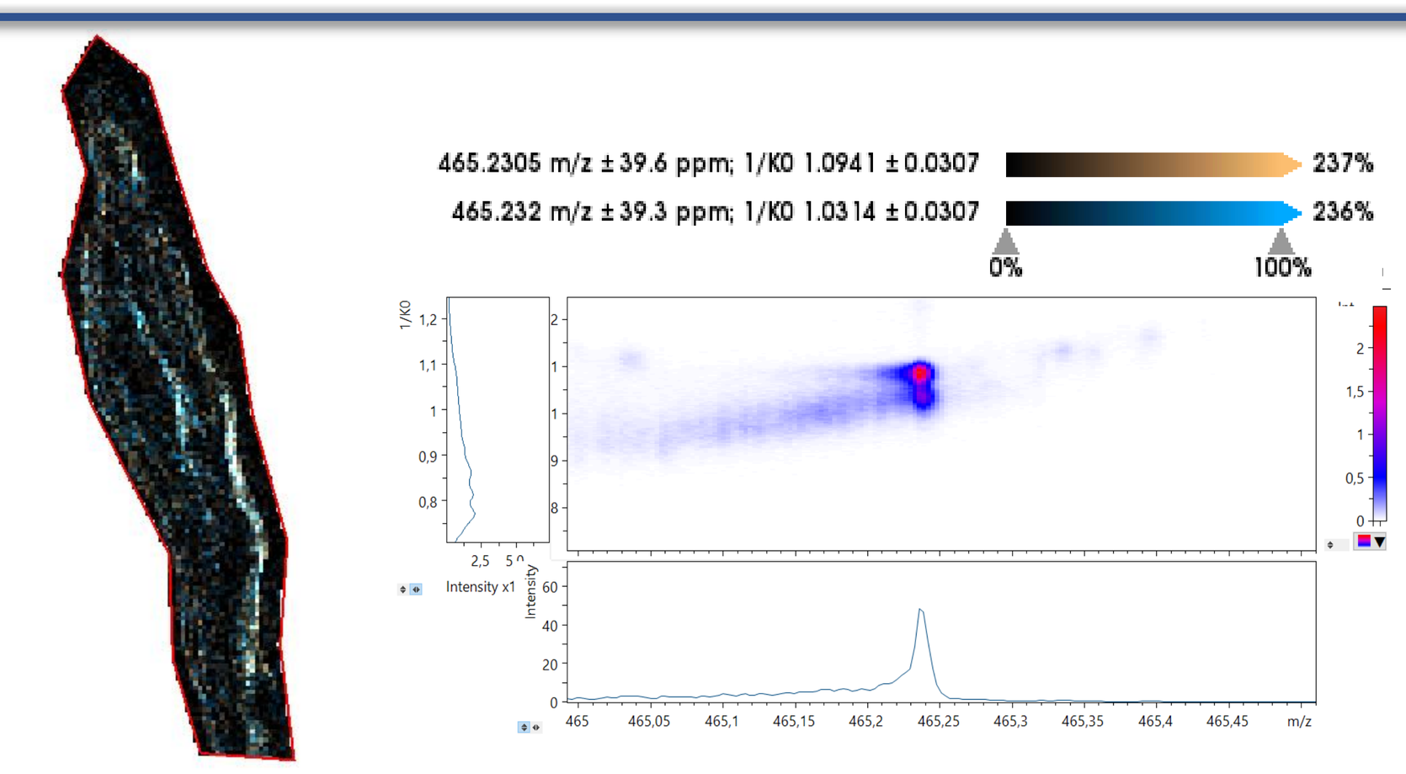




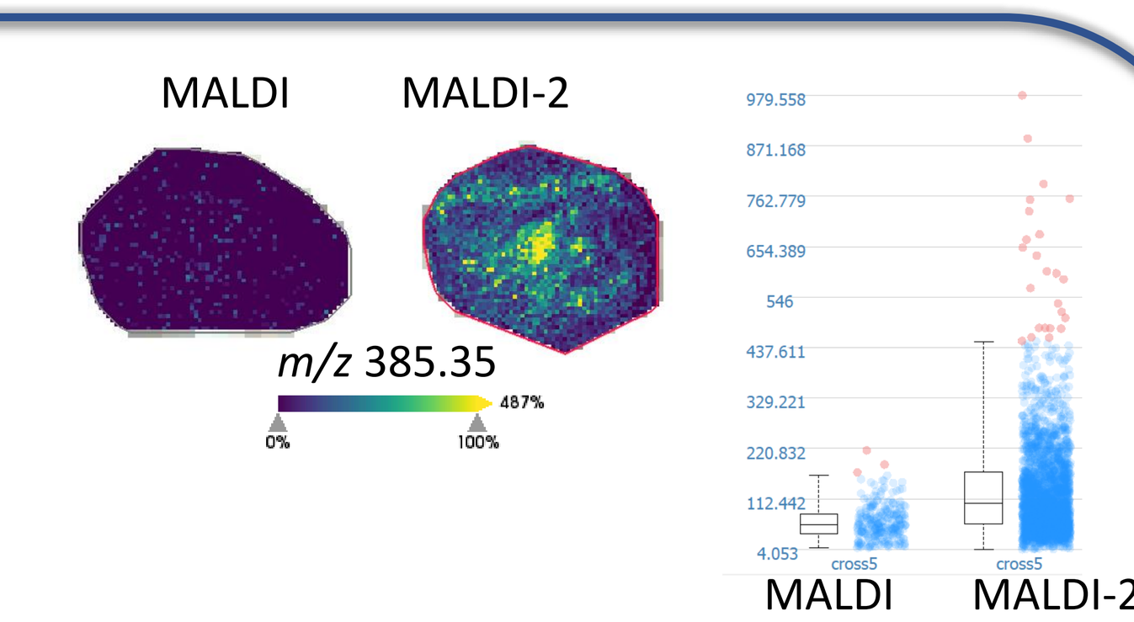
- Multiple matrices and both polarities make detection of more metabolites accessible

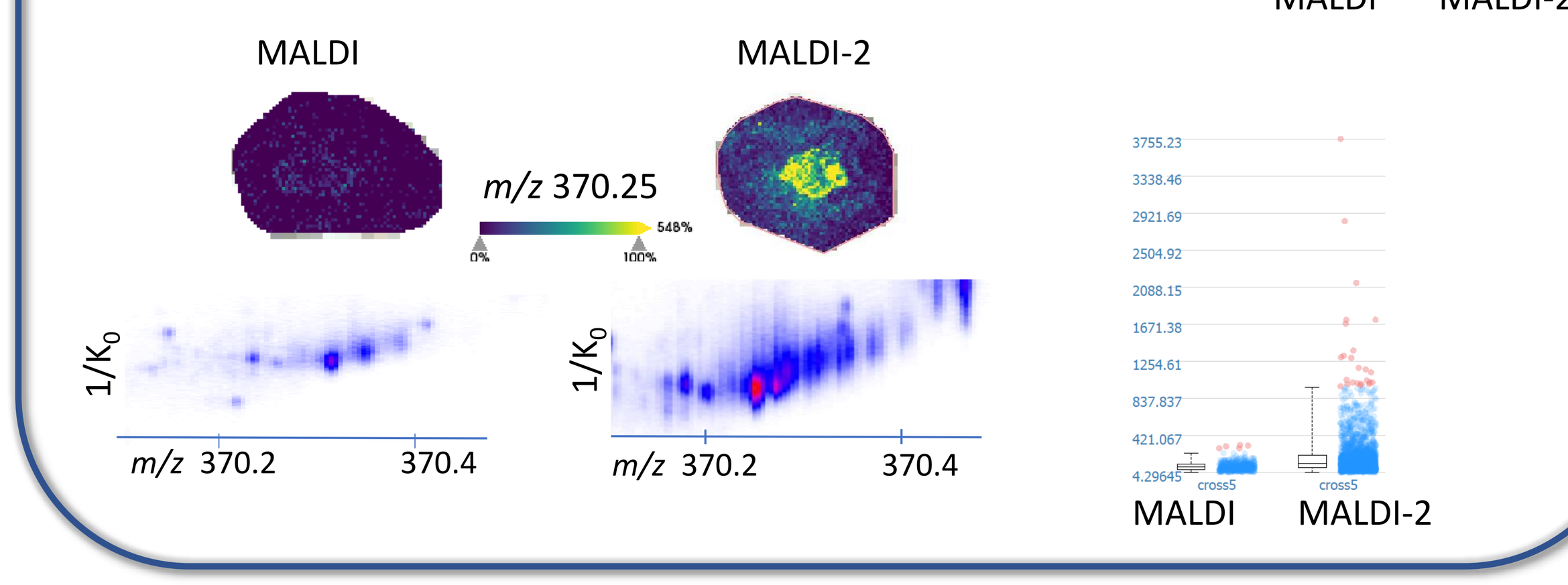
Results

- TIMS separation of small molecule isobaric species

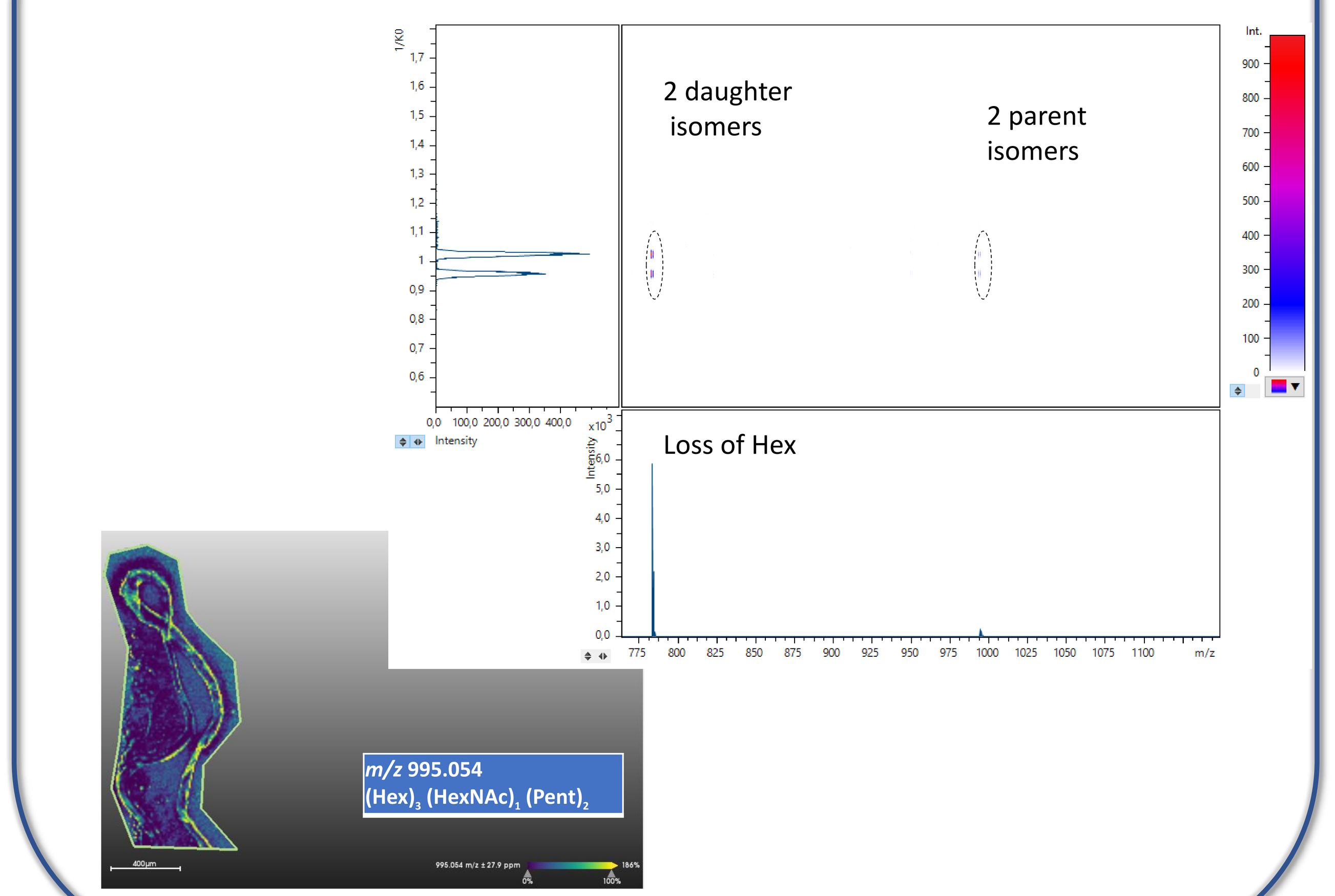


- MALDI-2 results in enhancement of small molecules
  - Higher intensity
  - More TIMS signal

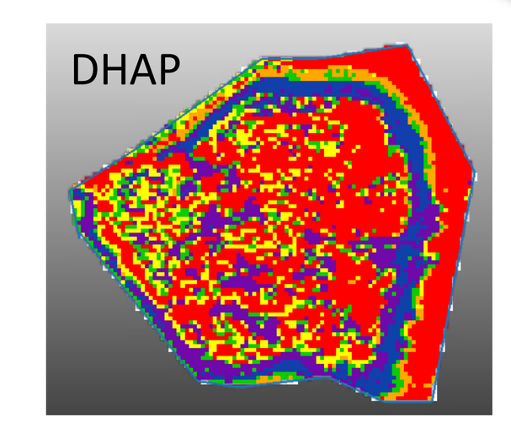




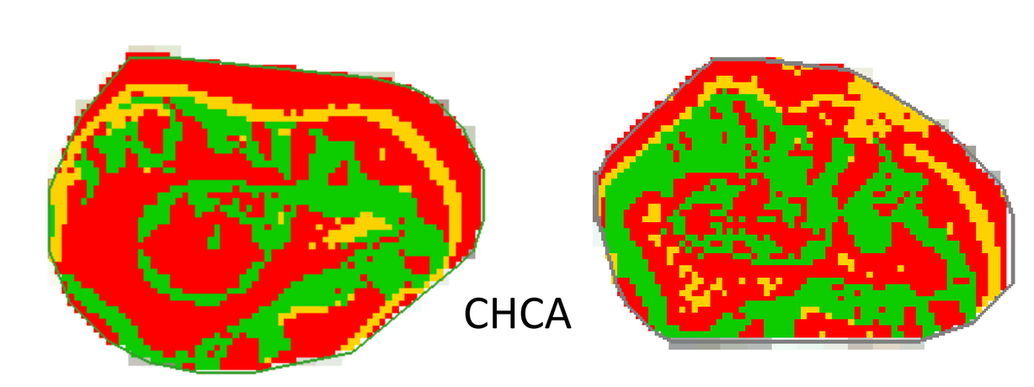
- MS/MS with CCS-aware detection enhances understanding of glycan parent and daughter species

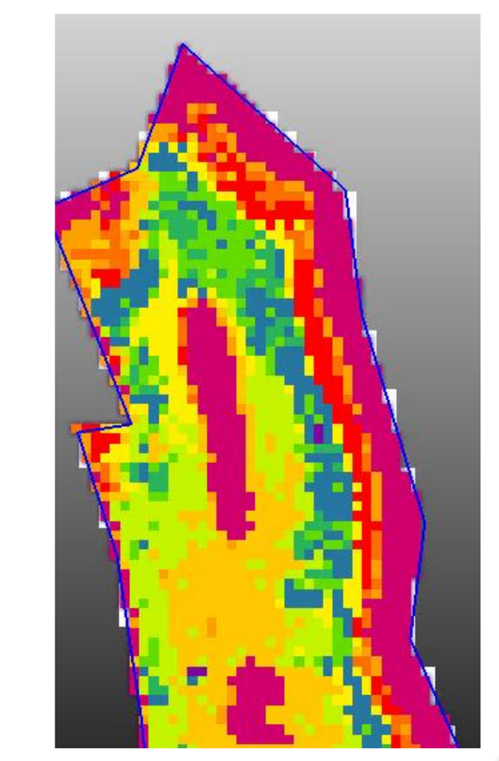


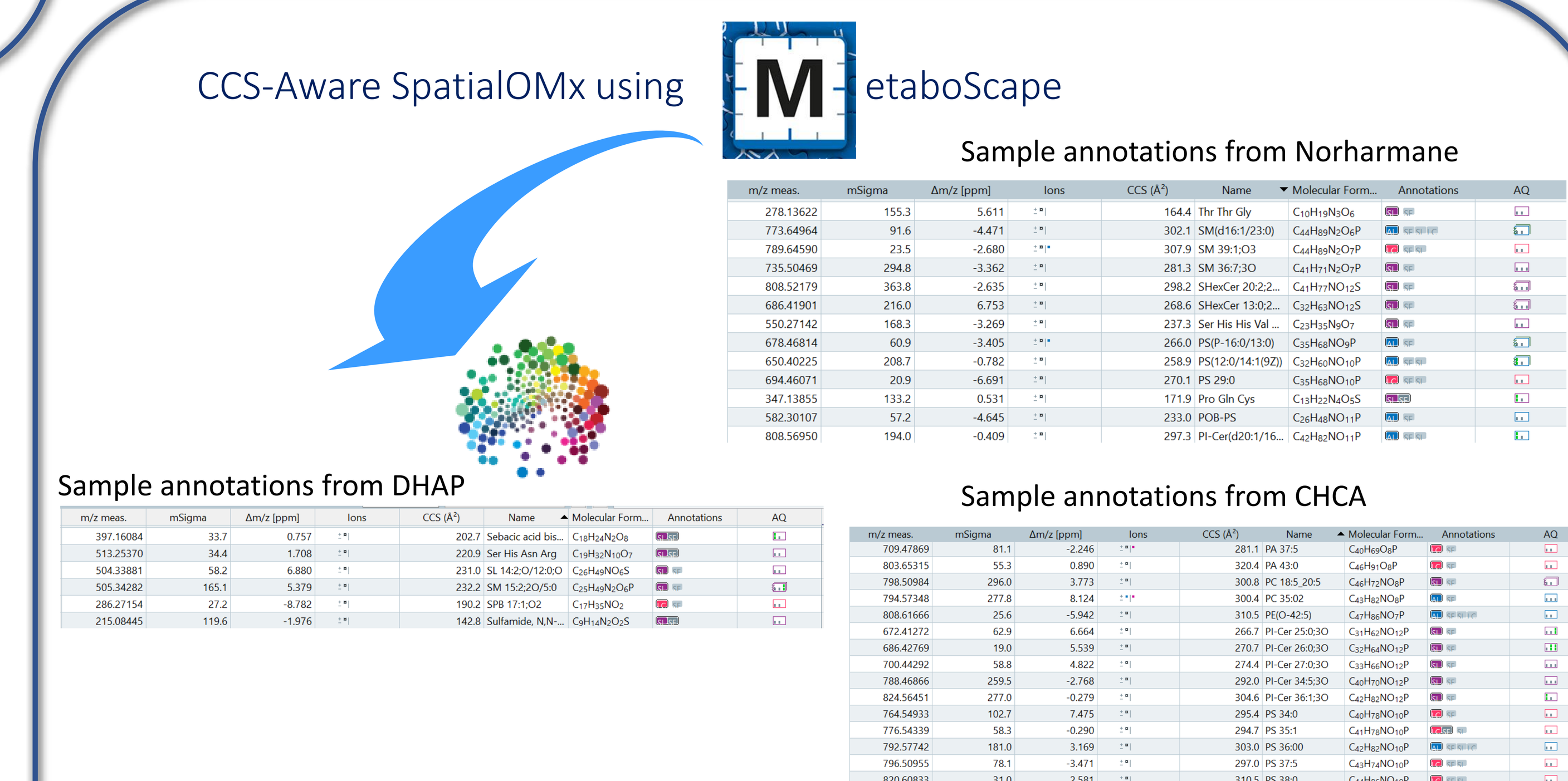
- Segmentation analysis reveals molecular phenotype layers within the cuticle



- Segmentation analysis for evaluation of consistency in detection across tissue sections







Matrix	Features	Annotations	Databases used
CHCA	358	312	LipidMapps, LipidClass, SmartFormula, MSDIAL
DHAP	1563	839	LipidMapps, LipidClass, SmartFormula, MSDIAL
Norharmane	556	235	LipidMapps, LipidClass, SmartFormula, MSDIAL

Summary Statements

- MALDI Imaging can be utilized to give multi-omics context to biological samples
- SCiLS Lab and MetaboScape provide comprehensive software solutions for SpatialOMx Imaging
- CCS-aware fragmentation allows for tracking of parent and daughter molecules and extraction of MS/MS information for each mobility separated species
- Key features that are only possible with the timsTOF fleX include: 10 micron lateral spatial resolution, trapped ion mobility separation of isobaric/isomeric species, and MALDI-2 enhancement