

# 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

$[(\text{Hex})_1, (\text{HexNAc})_2 + (\text{Man})_1(\text{GlcNAc})_1]$

$[(\text{Hex})_2, (\text{HexNAc})_1, (\text{Deoxyhexose})_1 + (\text{Man})_1(\text{GlcNAc})_1]$

- 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

$m/z$  672.03

$m/z$  721.48

$m/z$  808.58

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

### Metabolomic Analysis

Norharmane negative ion images

CHCA positive ion images

Norharmane positive ion images

DHAP positive ion images

- 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

- Segmentation analysis reveals molecular phenotype layers within the cuticle

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

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

- CCS-Aware SpatialOMx using MetaboScape

Sample annotations from Norharmane

m/z	m/glyc	Abund (ppm)	Size	CCS (Å <sup>2</sup> )	Name	Molecular Form.	Annotations	AG
278.1622	158.3	5.811	1*	164.4	the the gly	C <sub>12</sub> H <sub>16</sub> O <sub>5</sub>	new	C23
773.6264	318.6	4.471	1*	303.1	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
789.6599	315.3	2.680	1*	307.9	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
235.5049	294.8	-3.382	1*	281.3	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
308.5779	303.8	2.533	1*	293.2	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
488.4191	216.0	6.743	1*	208.5	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
502.2142	168.3	-3.289	1*	237.3	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
616.6614	303.8	-6.405	1*	288.0	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
636.6225	289.7	-6.782	1*	258.9	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
684.6671	289.8	6.691	1*	259.1	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
847.1855	133.2	0.531	1*	111.9	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
982.2010	133.2	4.645	1*	233.0	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
808.5899	196.0	-6.409	1*	227.7	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23

Sample annotations from DHAP

m/z	m/glyc	Abund (ppm)	Size	CCS (Å <sup>2</sup> )	Name	Molecular Form.	Annotations	AG
187.5094	13.7	6120	1*	202.1	SM-1815-0318	C <sub>12</sub> H <sub>16</sub> O <sub>5</sub>	new	C23
312.2310	24.4	3.206	1*	289.0	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
364.1391	35.2	6.980	1*	272.2	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
384.2194	27.2	6.762	1*	180.2	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
215.8845	119.4	-5.976	1*	142.8	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23

Sample annotations from CHCA

m/z	m/glyc	Abund (ppm)	Size	CCS (Å <sup>2</sup> )	Name	Molecular Form.	Annotations	AG
105.4799	81.1	2.246	1*	201.1	SM-1815-0318	C <sub>12</sub> H <sub>16</sub> O <sub>5</sub>	new	C23
163.4310	15.3	6.890	1*	204.4	SM-1815-0318	C <sub>12</sub> H <sub>16</sub> O <sub>5</sub>	new	C23
794.9994	290.0	3.771	1*	309.0	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
801.9788	277.6	6.524	1*	284.4	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
868.8194	254.5	5.540	1*	240.0	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
874.8102	42.9	6.664	1*	284.7	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
884.8219	193.3	5.539	1*	237.7	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
884.8219	184.4	4.827	1*	234.4	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
784.8824	293.5	2.746	1*	292.0	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
804.9451	277.0	-6.270	1*	284.4	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
784.8824	182.7	7.475	1*	254.4	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
774.8439	183.1	-6.290	1*	263.7	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
782.7942	183.0	3.140	1*	263.0	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
782.8050	181.1	-4.471	1*	253.0	SM-1815-0318	C <sub>24</sub> H <sub>32</sub> O <sub>10</sub>	new	C23
804.9451	31.0	3.361	1*	193.0	SM-1815-0318	C <sub>12</sub> H <sub>16</sub> O <sub>5</sub>	new	C23

## 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