Sequential Imaging with AP/MALDI for Enhanced Tissue Characterization

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In Situ Imaging of N-Acetylglucosamine

In collaboration with Edwin Escobar and Jennifer Brodbelt – UT Austin

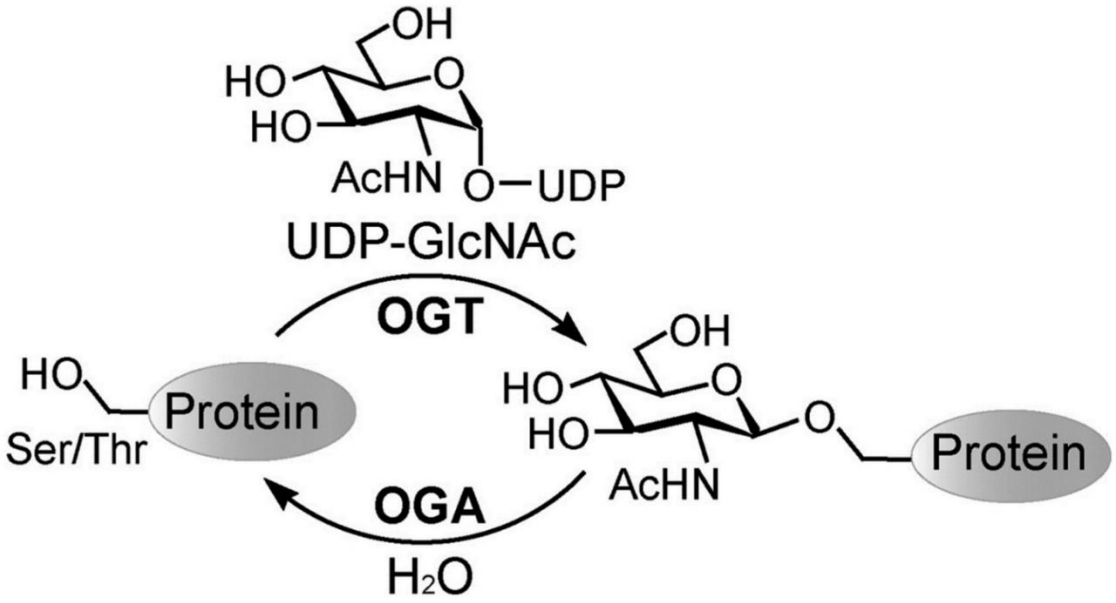
Escobar EE, Seeley EH, Serrano-Negrón JE, Vocadlo DJ, Brodbelt JS. Cancers (Basel). **2023**, *15(4)*, 1224. doi:10.3390/cancers15041224

Sequential Imaging Rationale

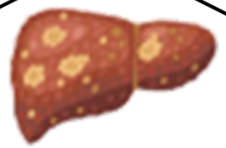
- Diagnostic biopsies are often quite small, 1-2 mm^2
- Histological diagnosis may be challenging and only a few molecules can be detected with each specialized test
- Limited material is available for research
- The cells may be different between sections and alignment of serial sections may be challenging
- MSI allows for detection of hundreds to thousands of analytes from a single tissue section
- Careful experimental planning allows for multiple mass spectrometry images to be collected from the same section

O-GlcNAc Protein Modification

- Single sugar co- and post-translational modification that occurs on serine and threonine
- Reversible modification implicated in stress response, epigenetic regulation, and proteostasis
- Critical metabolic marker in tumorogenesis
- Controlled by two enzymes, O-GlcNAc transferase (OGT) that installs sugars on proteins and O-GlcNAc hydrolase (OGA) that removes them
- We sought to develop methods to monitor O-GlcNAcylation of proteins by MSI



Methods



- Rabbit inoculated VX2 tumor tissue (tumors and normal) grown for 9-11 days
- Snap frozen and sent for MSI

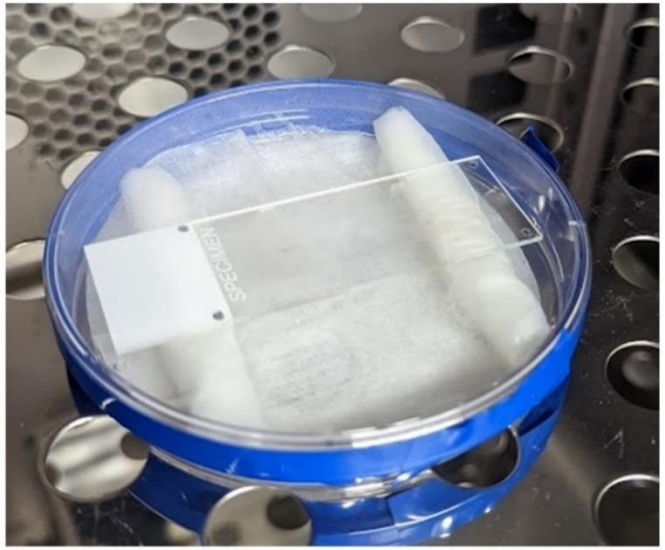
- Thermo NX50 Cryostat
- Sectioned at 12 µm thickness
- Serial sections for H&E staining

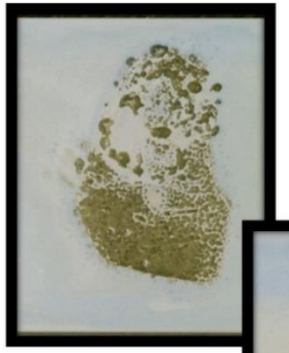
- Images combined for normalization and visualization in SCiLS Lab Pro

- Hamamatsu NanoZoomerSQ
- Digital microscopy images at 20X magnification

- MassTech AP/MALDI source
- Thermo Fusion Lumos Orbitrap
- Positive ion mode
- 100 µm imaging resolution

- HTX M5 Sprayer
- Sections Carnoy's fluid washed
- wtOGA reaction in 100 mM ABC
- 10 mg/mL CHCA in 70% ACN, 0.1% TFA



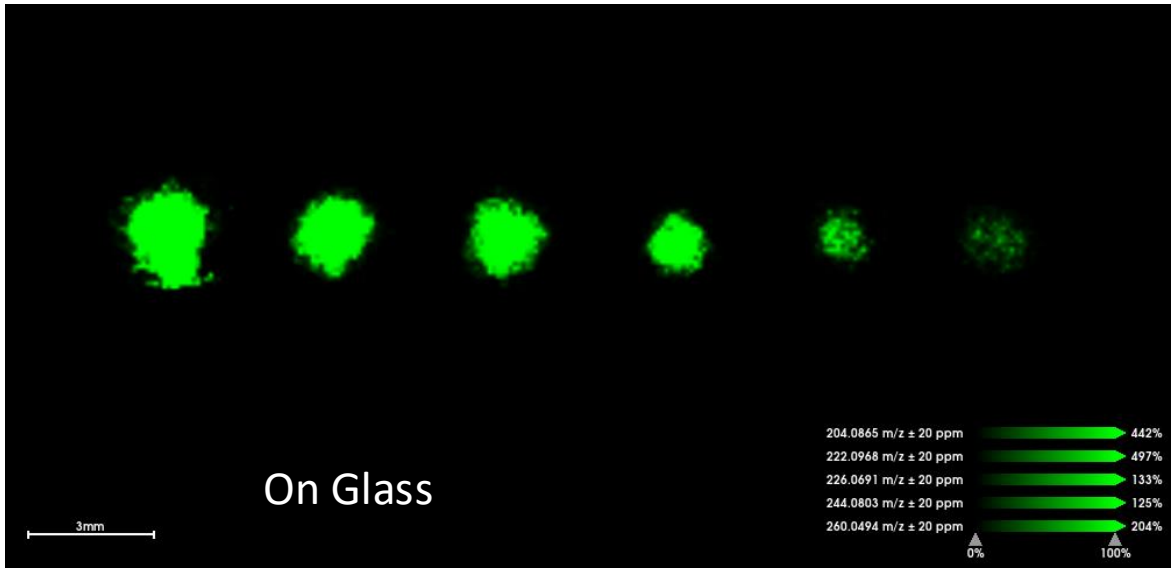


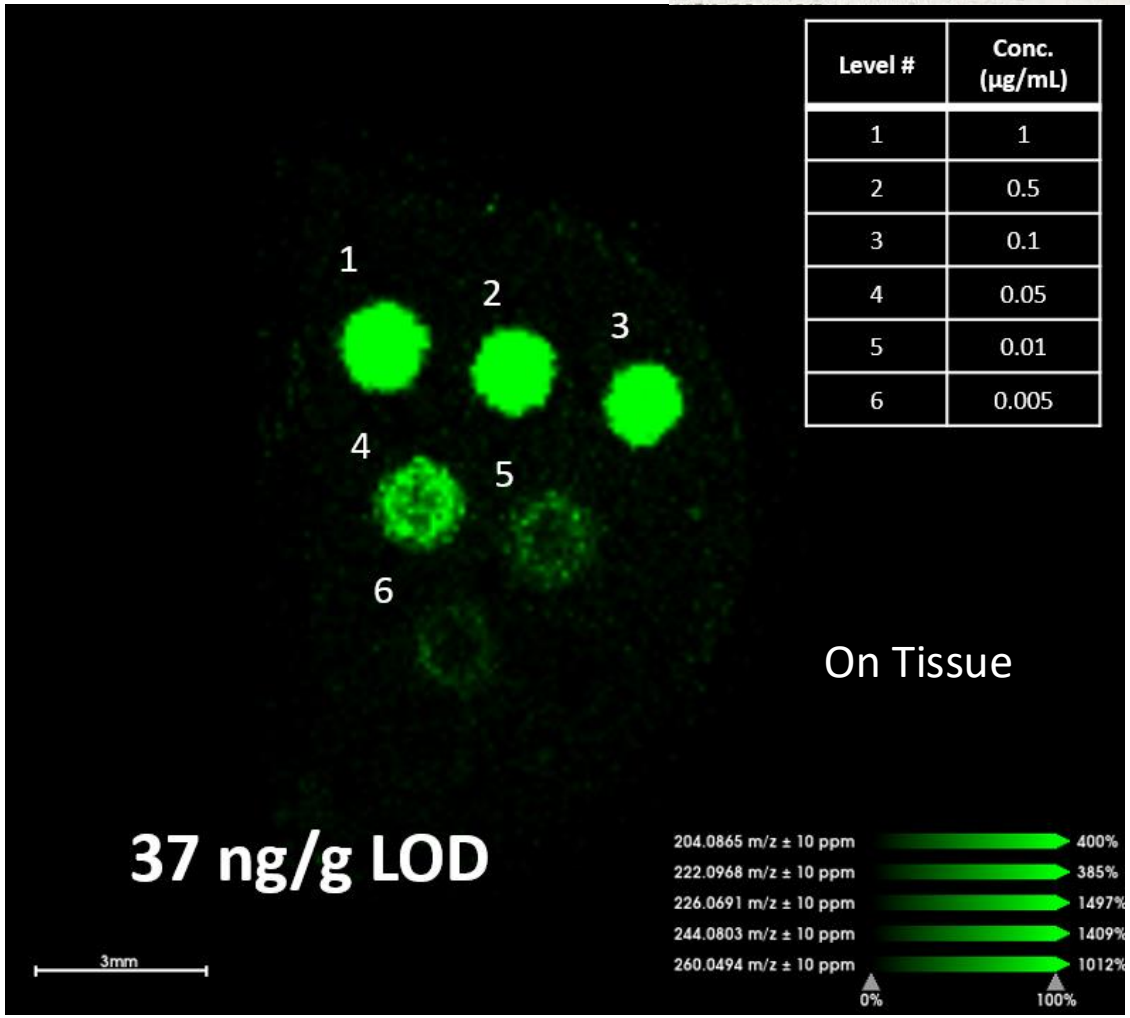
Overnight incubation 10 mL H_2O @ 37°C oven

Heated lid @ 40°C Incubation using 10 mL H_2O @ 37°C

GlcNAc Limit of Detection Determination

- Dilutions of GlcNAc standard spotted onto glass and tissue
- Slide coated with CHCA matrix
- Imaged on a Thermo Fusion Lumos
- LOD calculated based on mass of GlcNAc applied, mass of tissue over which spot spread, and % of tissue ablated

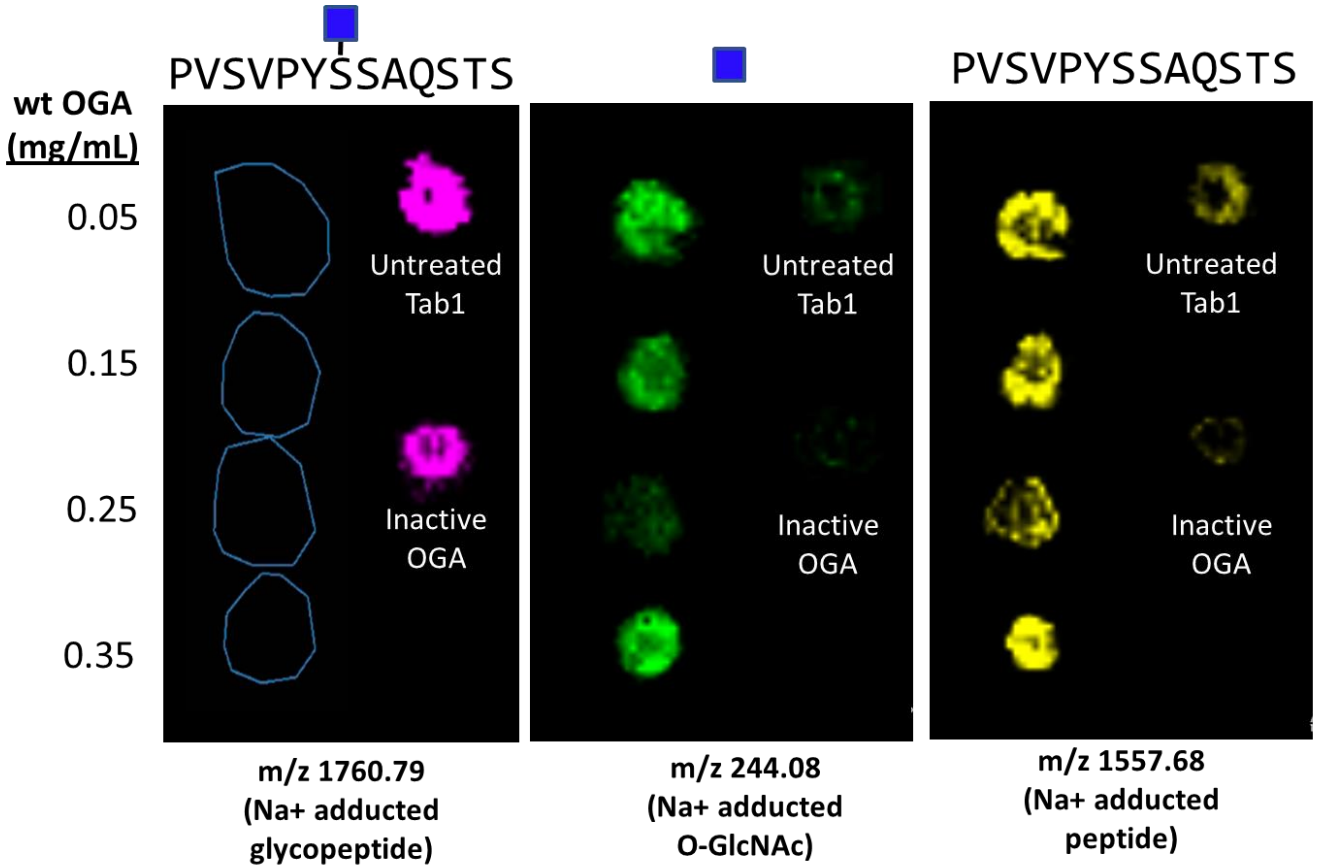




Level #	Conc. (µg/mL)
1	1
2	0.5
3	0.1
4	0.05
5	0.01
6	0.005

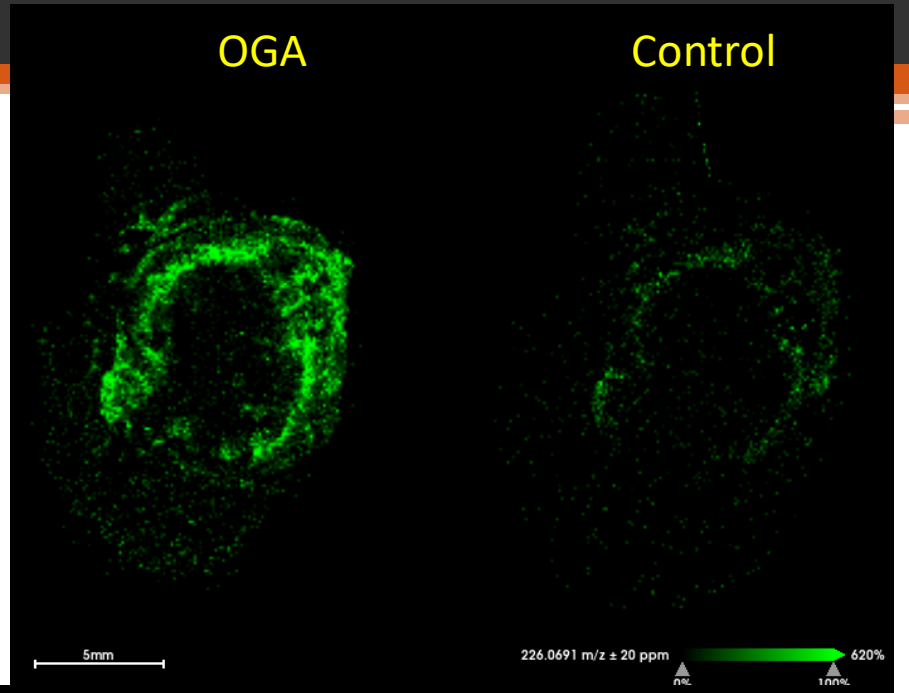
Peptide Standards

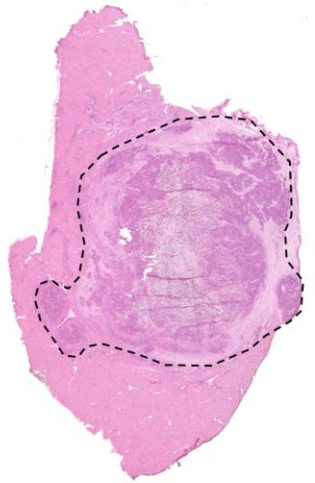
- O-Glycopeptide standards were used to evaluate the efficacy of OGA in an imaging environment
- OGA concentration in the spray solution was varied and optimized
- Optimized methods were then applied to tissue sections

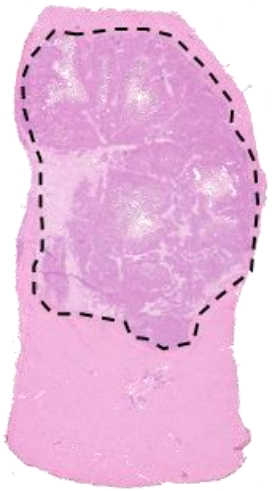


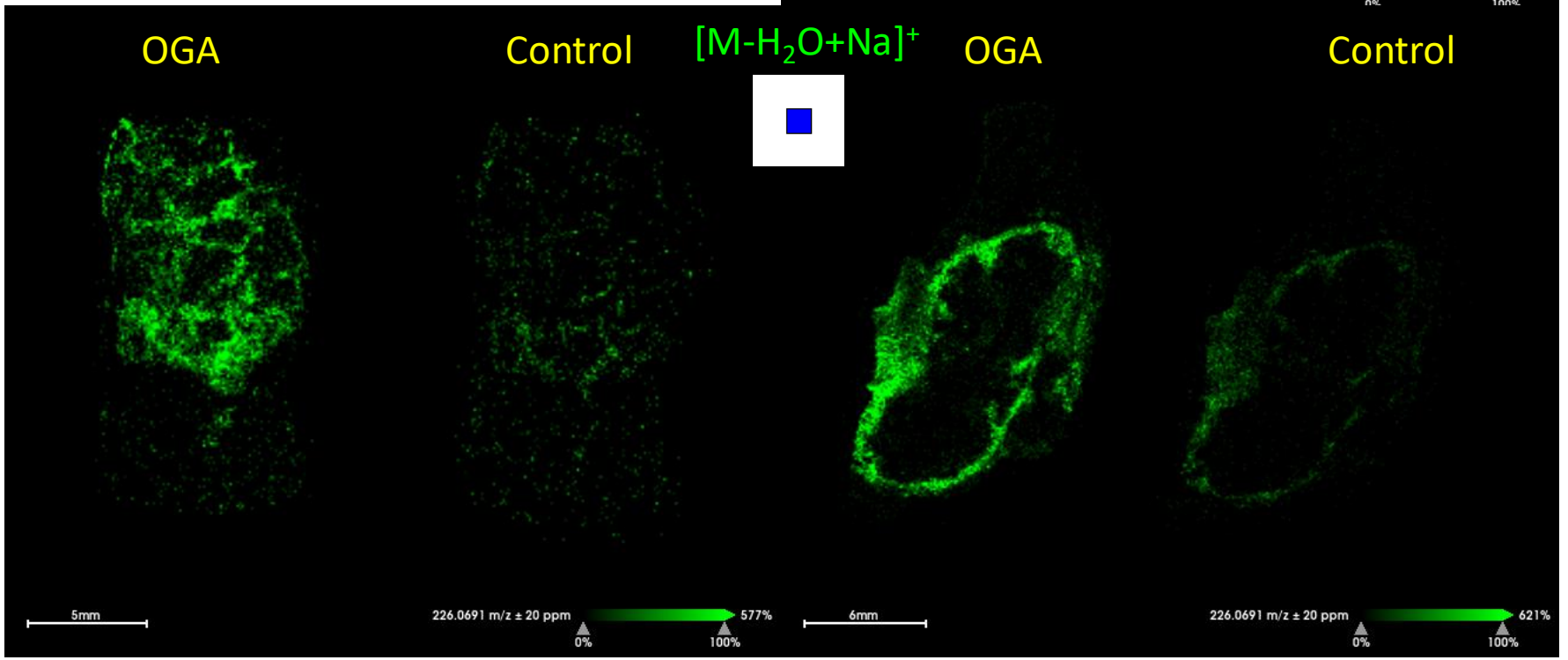
O-GlcNAc Imaging

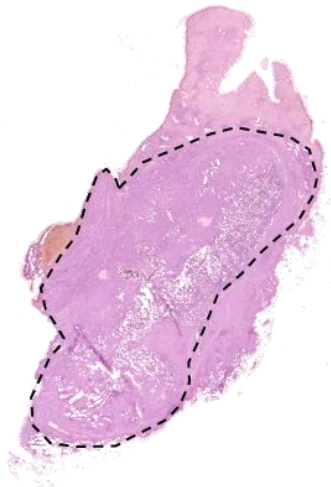
- Most abundant in viable tumor areas
- Slight signal detected in untreated tissue, likely due to laser induced fragmentation



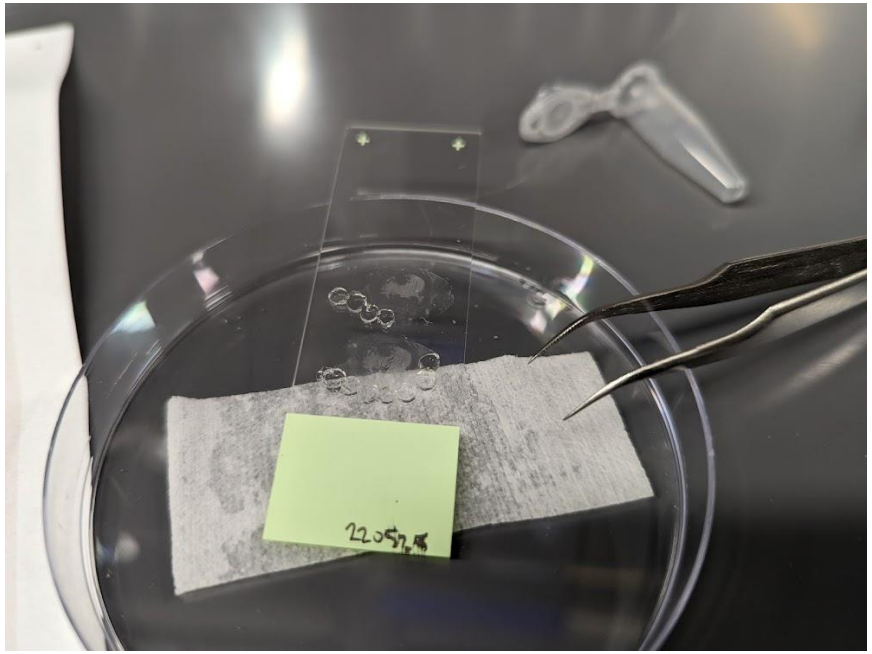






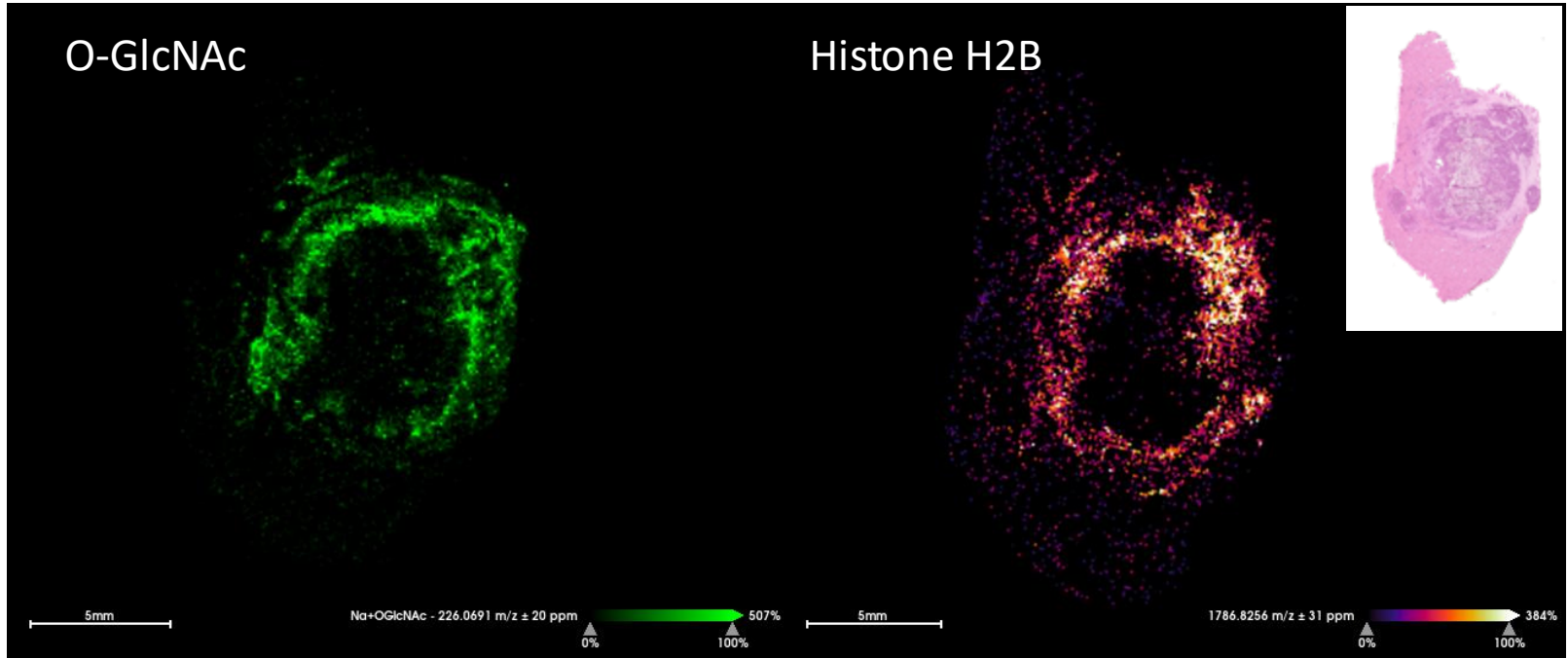


On Tissue Digestion and LC-MS/MS Peptide Identification



- Trypsin-loaded SDS-PAGE gel pieces placed on tumor region and incubated for 4 hr
- Peptides extracted and nanoLC-MS/MS performed (HCD and UVPD)
- Analyzed with Protein Metric Byonic software
  - 1916 proteins, 14727 unique peptides, 23 O-GlcNAc modified peptides identified

Identified Protein	Protein Best Score	Identified Peptide + O-GlcNAc site	Peptide Byonic Score
Actin, cytoplasmic 1	1428	HQGVMVGMGQKDS[+203.07937]YVGDEAQSKR	531
Calnexin	917	SKPDTSTPPPS[+203.07937]PK	639
Histone H2B	803	acetyl-GIMNS[+203.07937]FVNDIFER	455
PDZ and LIM domain 5	746	EVVKPVPITSAVS[+203.07937]K	636

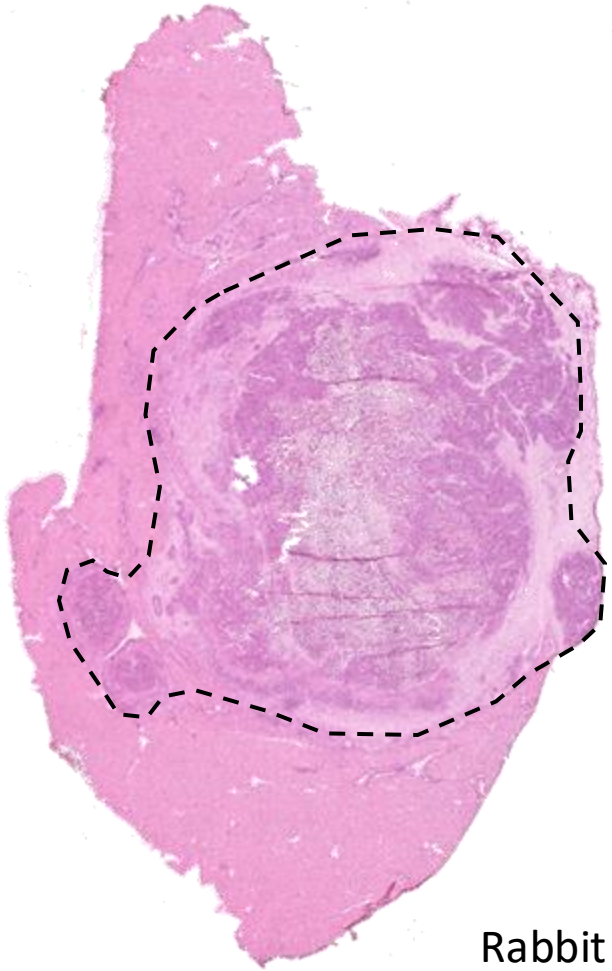


Sequential Enzymatic Treatment

N-Glycan Imaging
- Carnoy's fluid wash
- Treatment with PNGaseF
- Application of CHCA matrix
- MALDI Imaging

O-GlcNAc Imaging
- Matrix removal and Carnoy's fluid wash
- Treatment with OGA
- Application of CHCA matrix
- MALDI Imaging

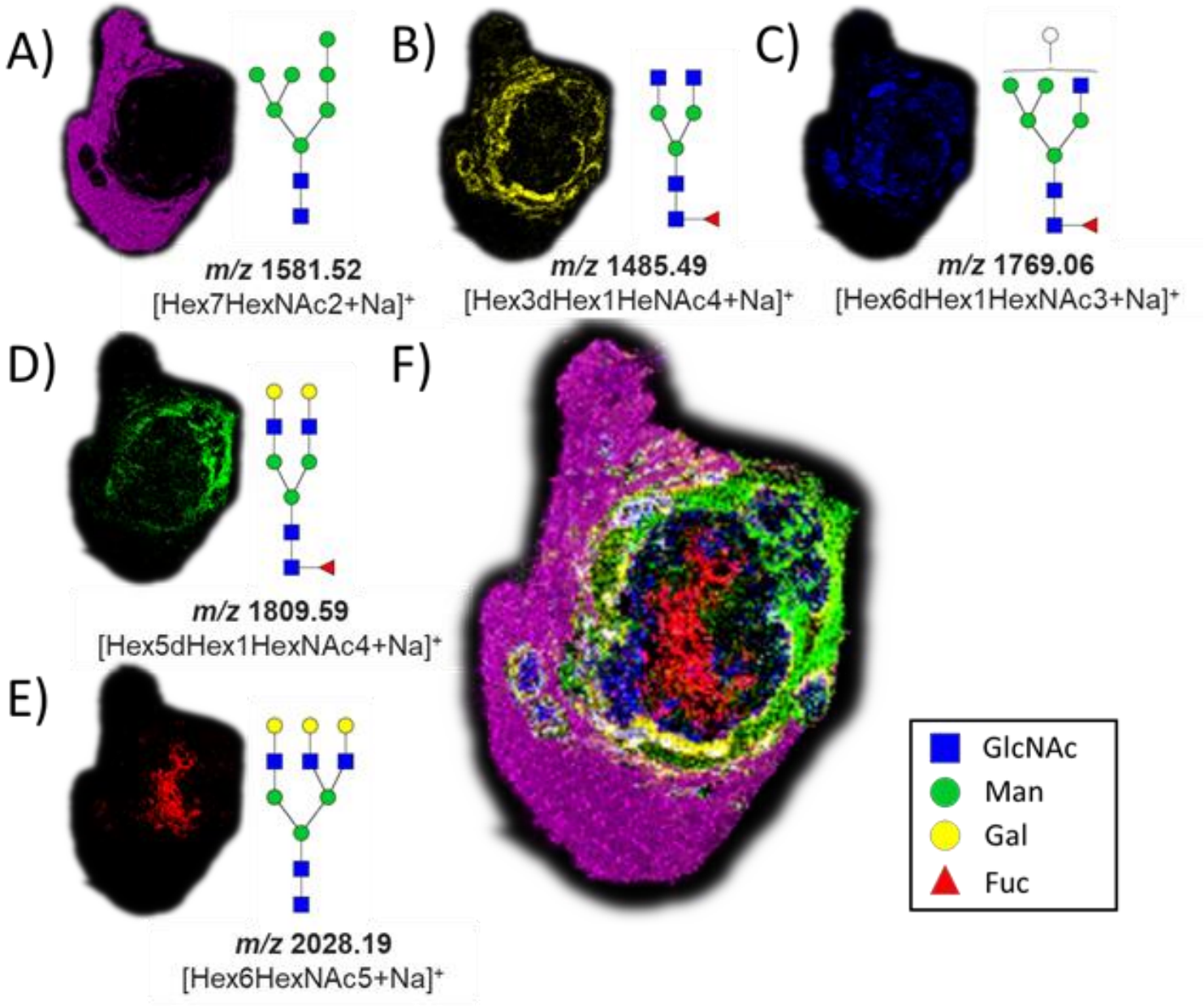
Peptide Imaging
- Matrix removal and Carnoy's fluid wash
- Treatment with Trypsin
- Application of CHCA matrix
- MALDI Imaging



Rabbit VX2 Liver Tumor

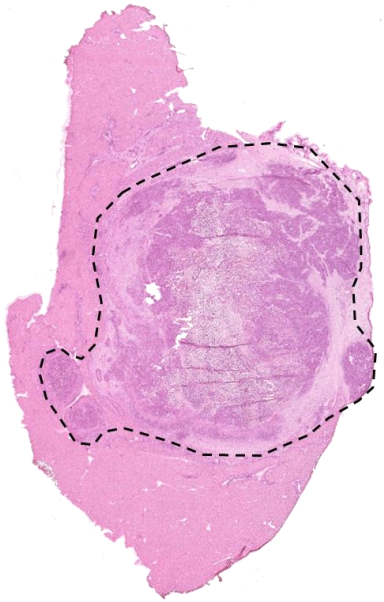
N-Glycan Imaging

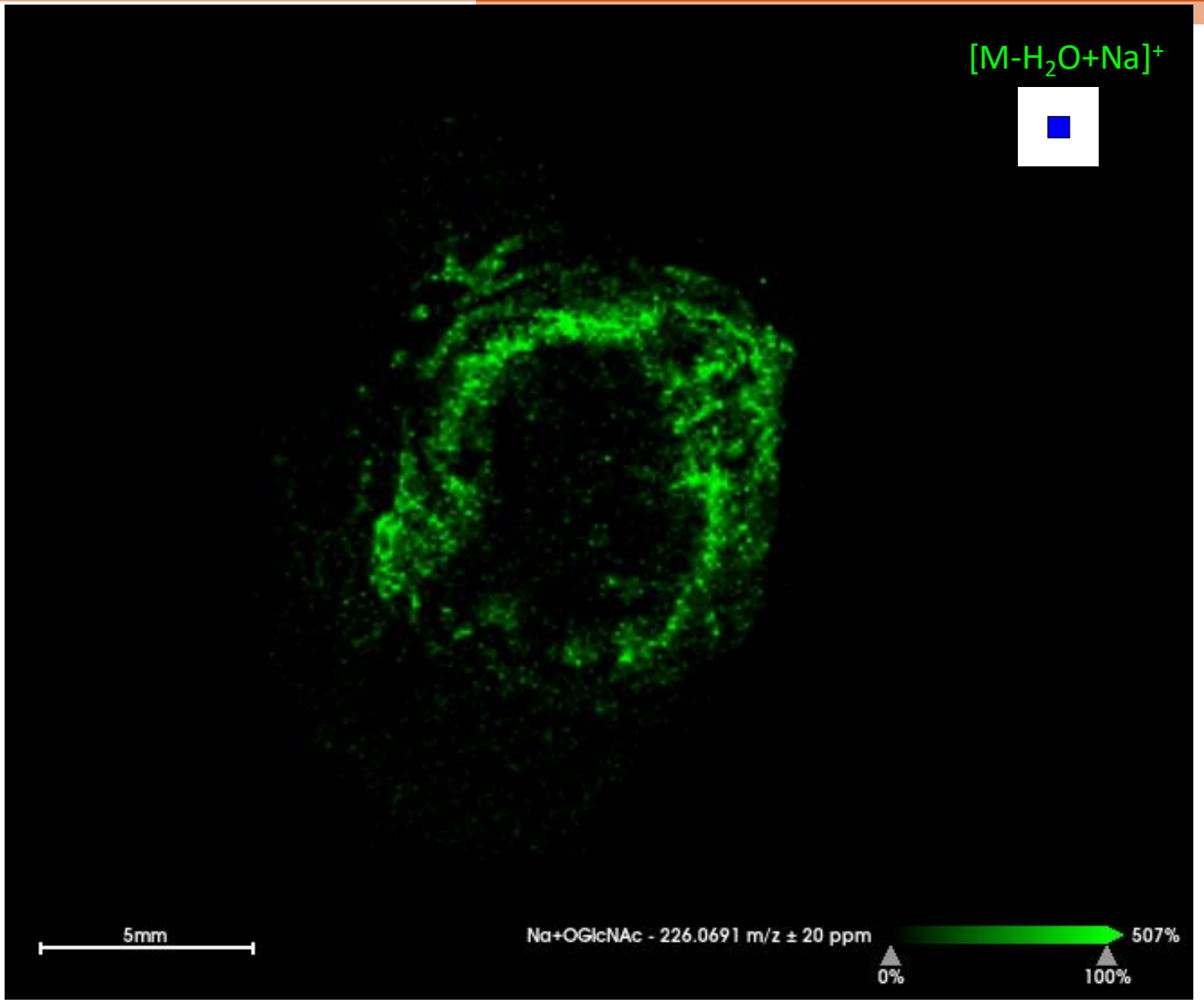
- Numerous glycans detected in different parts of the tissue
- Glycogen abundantly detected in normal liver tissue
- Removal of N-linked glycans reduces steric hindrance for OGA



O-GlcNAc Imaging

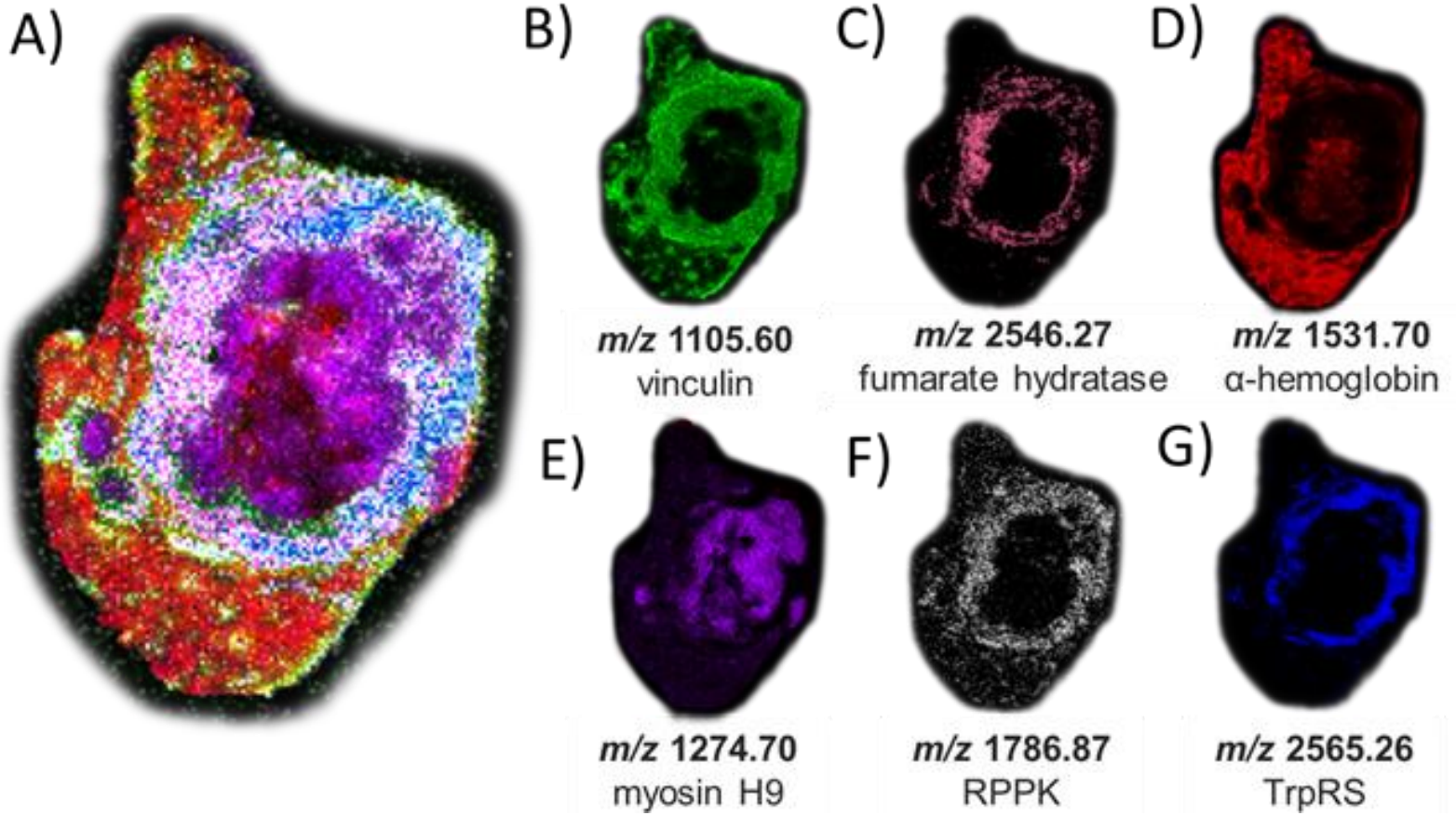
- Abundant in viable tumor region
- Signal enhanced after prior treatment with PNGaseF





Tryptic Peptide Imaging

- Robust signal, even after multiple enzymatic treatments
- Peptides detected with co- or differential-localization to O-GlcNAc



Sequential Imaging Results – 3 Separate Datasets

PNGaseF treated

OGA treated

Tryptic Digest

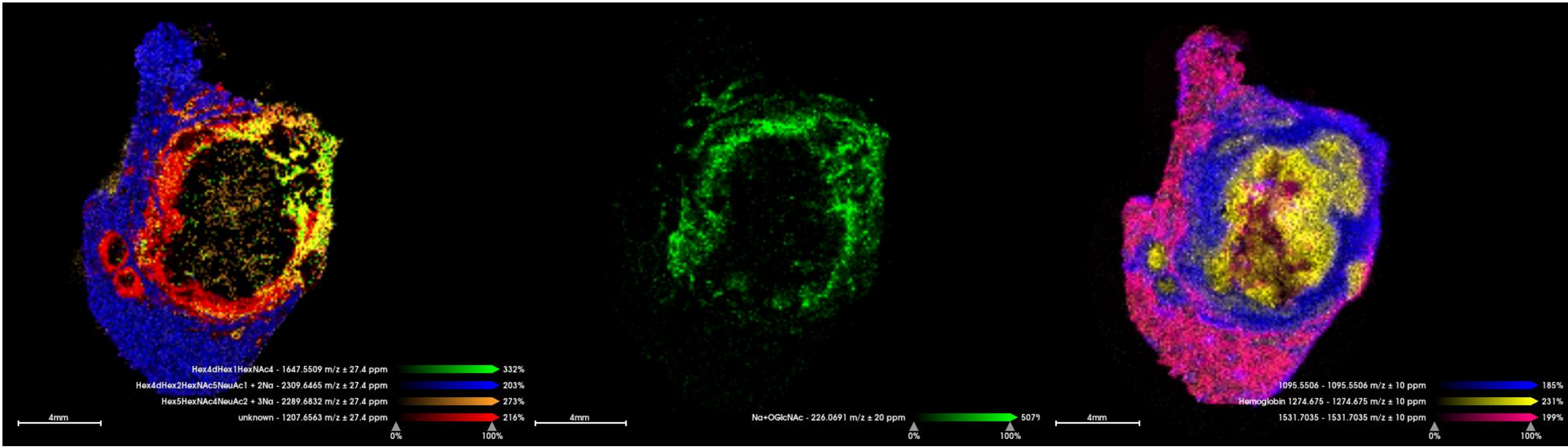
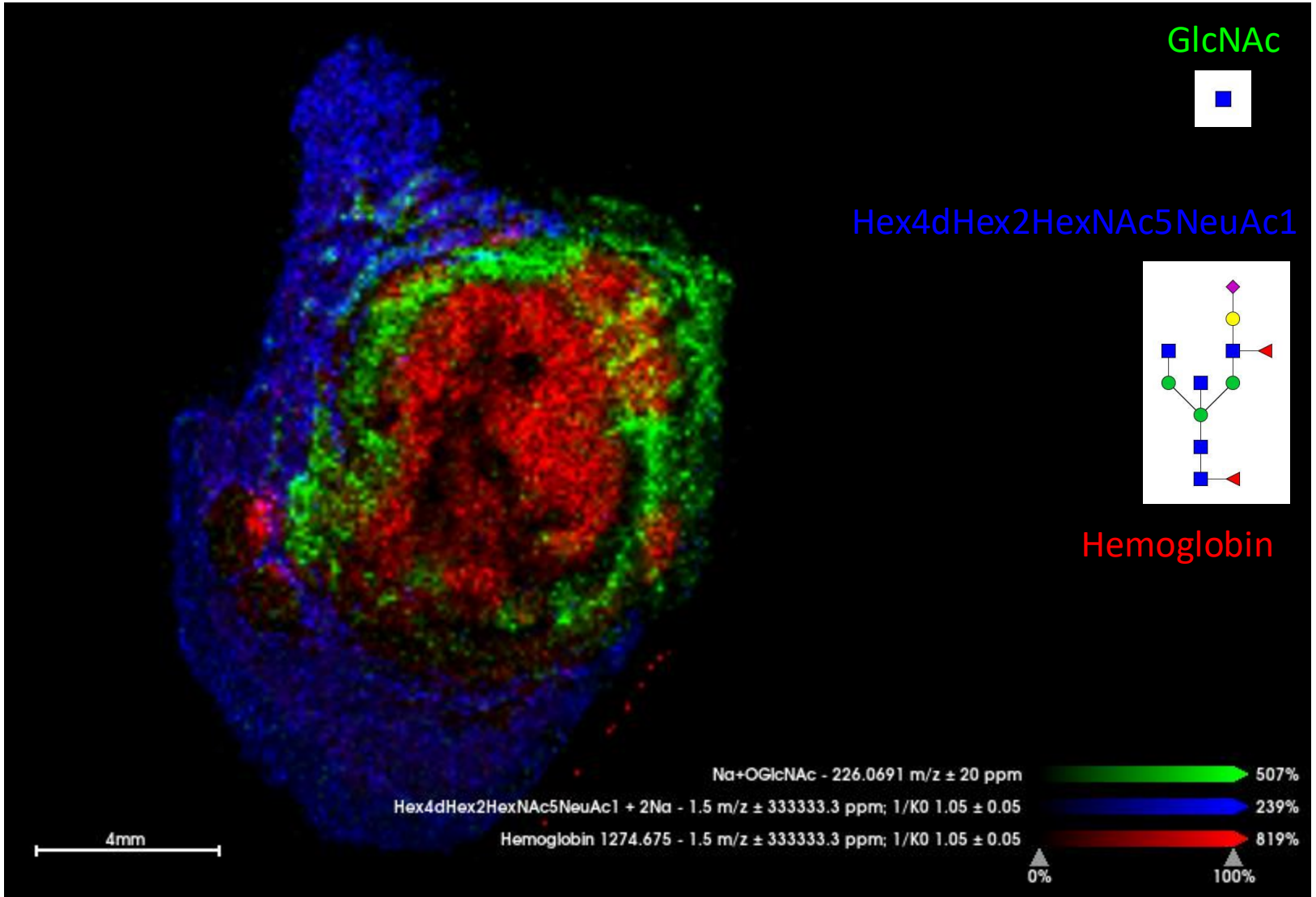
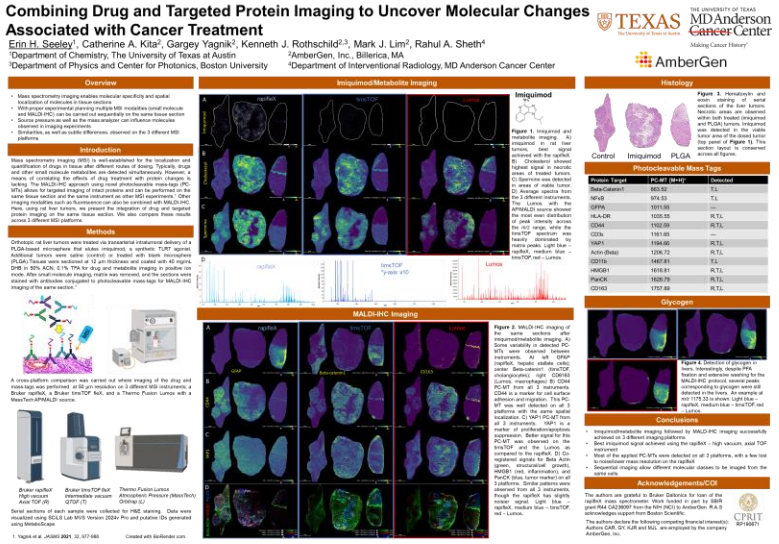


Image Co-registration



Sequential Metabolite and MALDI-IHC Imaging

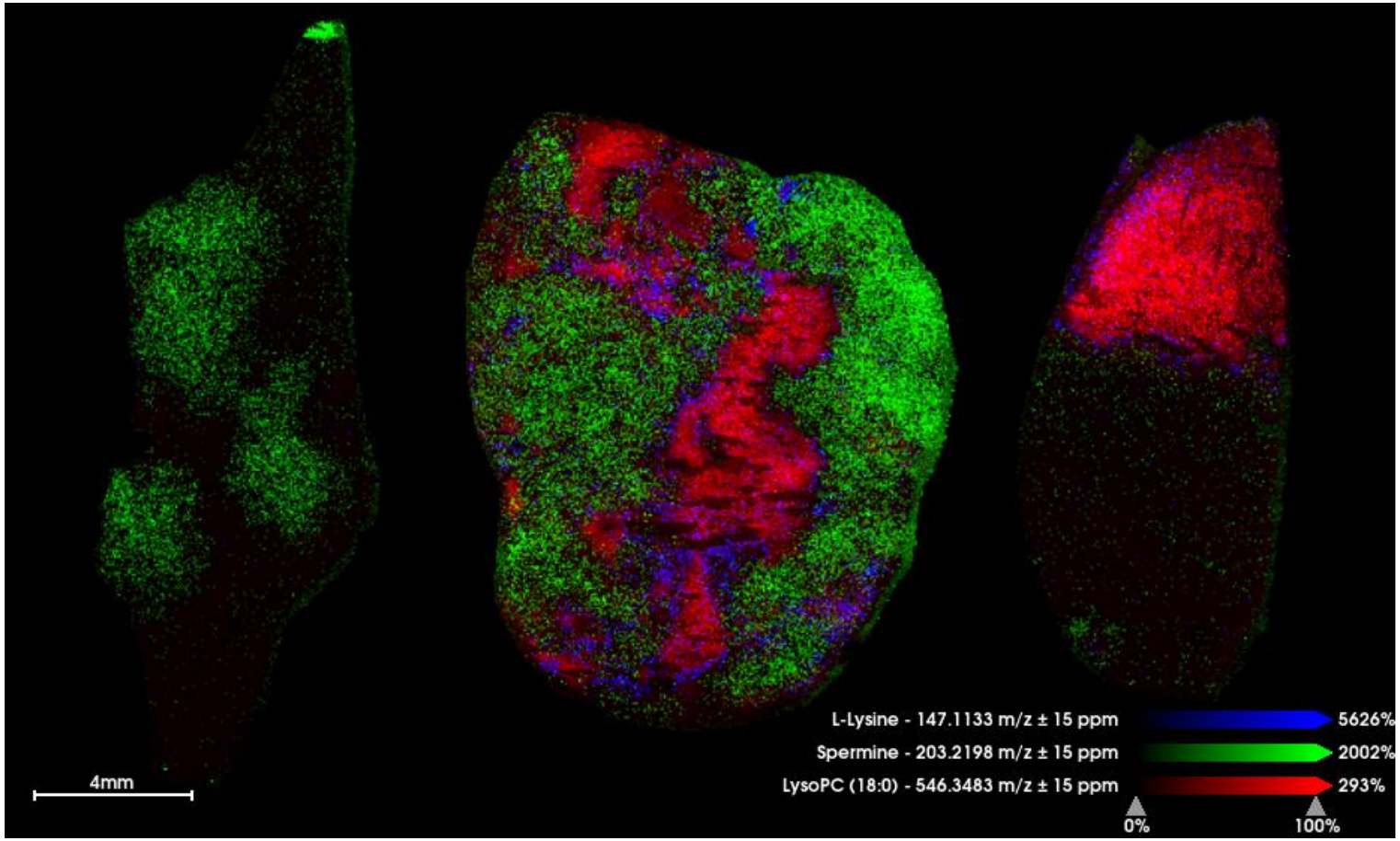
In collaboration with Rahul Sheth – MD Anderson, and AmberGen



Tuesday Poster 244

Metabolite Imaging

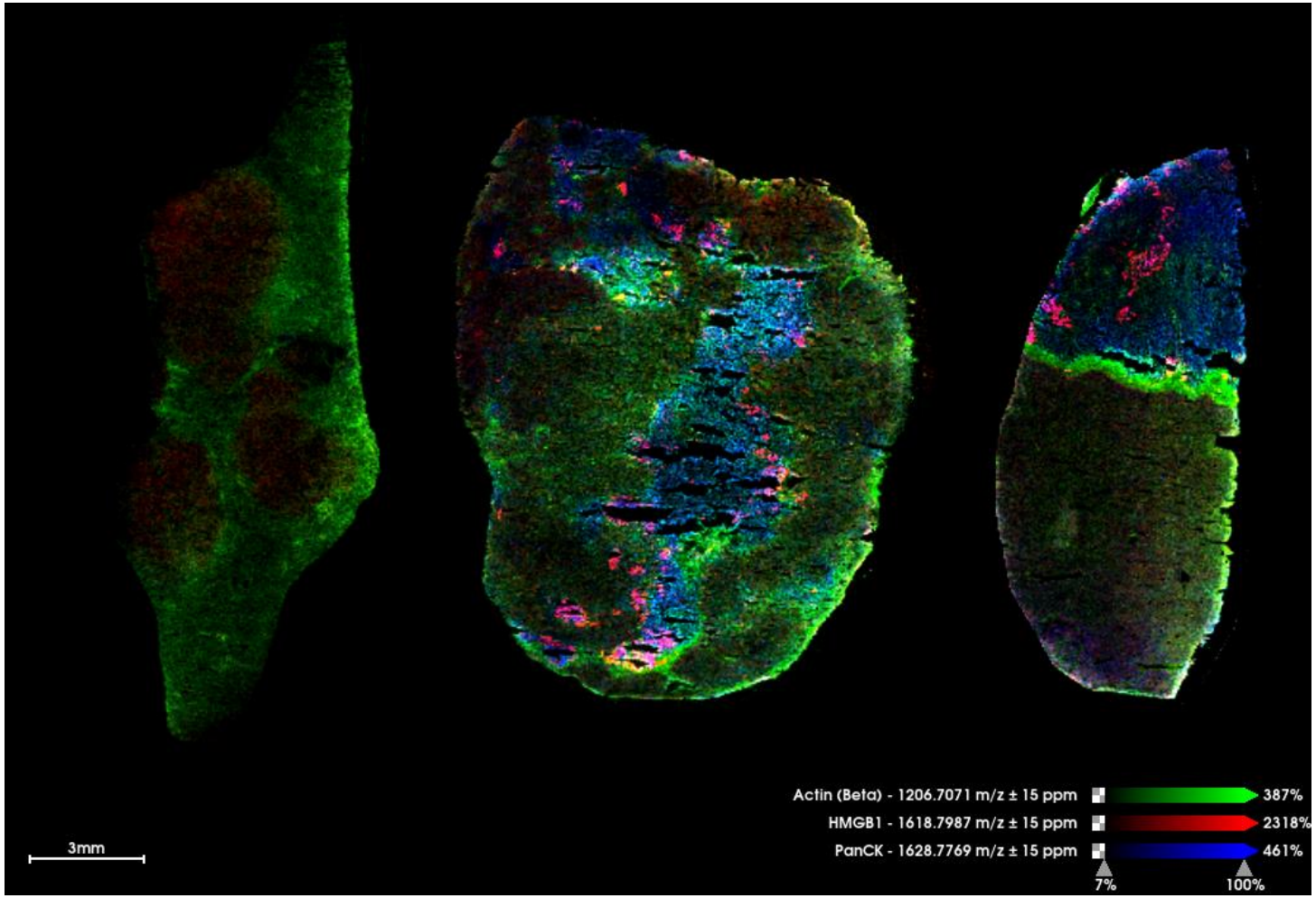
DHB Matrix, Positive Mode, 50 µm resolution



Control Imiquimod Vehicle

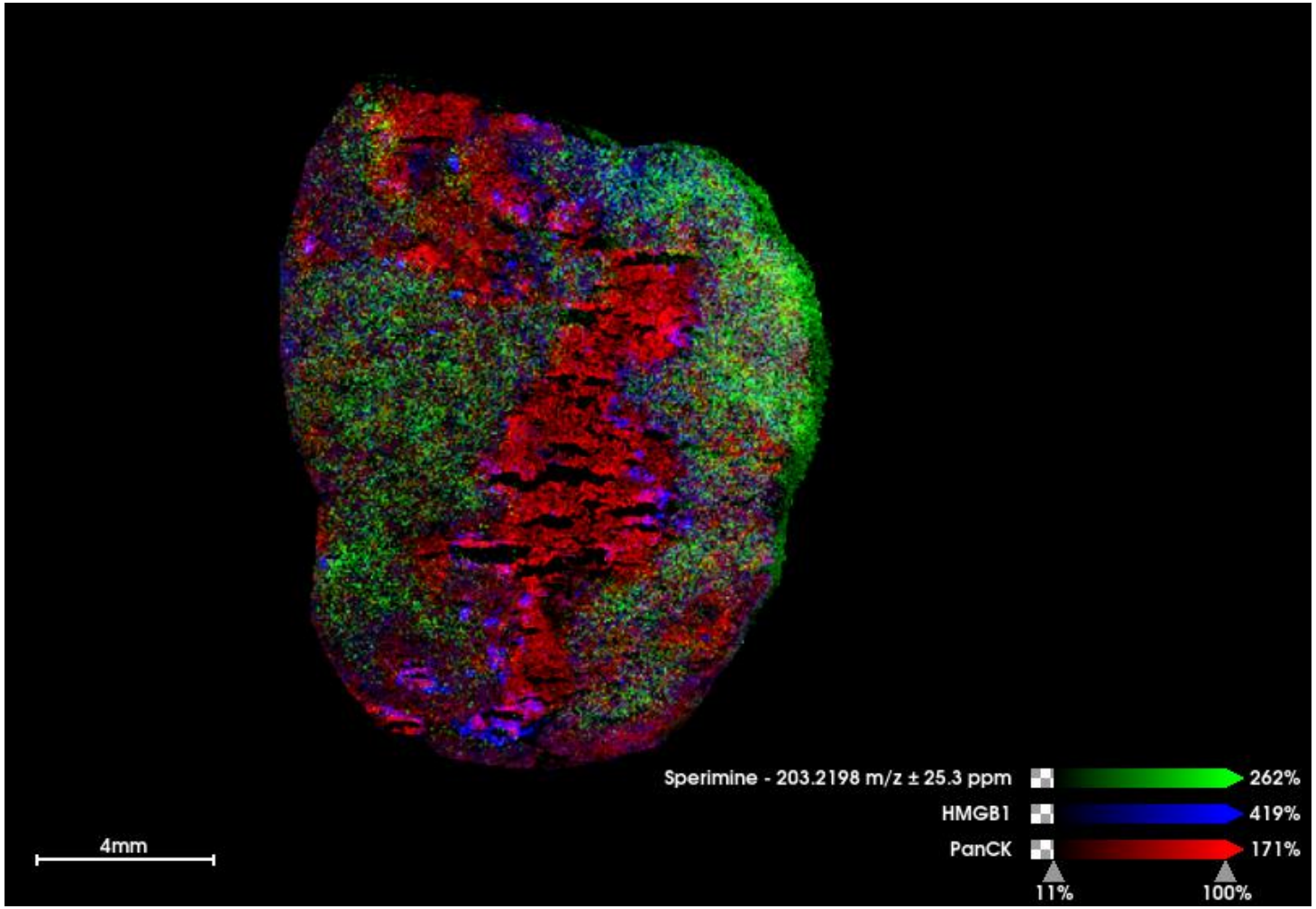
MALDI-IHC Imaging

Standard AmberGen Protocol, CHCA Matrix, 50 µm resolution



Control Imiquimod Vehicle

Image Registration



Conclusions

- New methodology for imaging of O-GlcNAc from tumor tissue
- Two demonstrations of sequential imaging workflows
  - N-linked Glycans → O-GlcNAc → Tryptic Peptides
  - Drug/Metabolites → MALDI-IHC
- Sequential analysis of the same tissue section enables deeper interrogation of tumor biology
- Co-registration allows for direct comparison of molecules imaged in separate datasets

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