Fully automated mass alignment and recalibration of MALDI TOF imaging data from N-linked glycans

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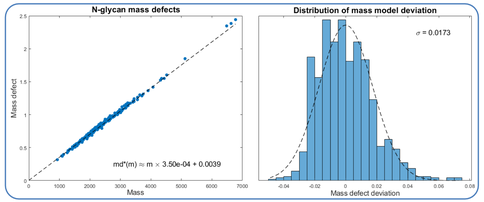
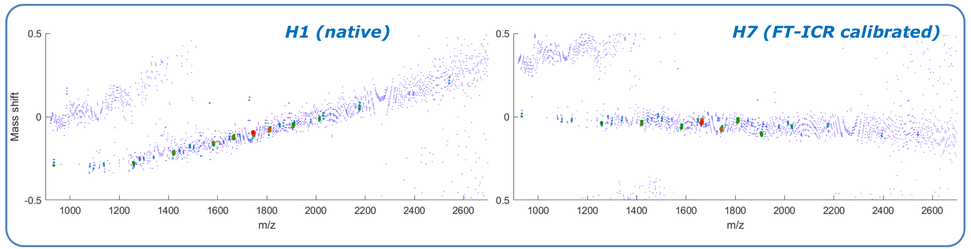
Introduction

- MALDI imaging of N-linked glycans from FFPE tissue is a valuable tool for tissue typing and biomarker discovery.
- Mass misalignment in MALDI TOF data represents serious issue for clinical research and assay development.
- An automated mass alignment and calibration method specifically tailored to N-glycan data is proposed.
- Method is evaluated on four human HCC tissue samples analysed using MALDI TOF (reflector mode, 50 µm raster), reference obtained from prior FT-ICR measurement of same section.



▲ Fig. 1: Mass defects of N-linked glycans are accurately predicted by linear regression model (computation based on 981 human N-glycans listed in GlyTouCan database).



▲ Fig. 2: Mass defect plots show deviation of mean spectrum peaks (local maxima) from mass defect as predicted by N-glycan mass model. Peak intensity is represented by dot size and color. Left: Diagram for Sample H1 as acquired showing a strong mass shift that varies with m/z. Right: Diagram for Sample H7 after cross-calibration to FT-ICR reference data, resulting in good correspondence to N-glycan mass model.

Method

Molecular masses of N-linked glycans show a strong linear correlation between exact mass and mass defect (Fig. 1). This phenomenon allows to investigate the overall mass shift of a dataset using mass defect plots (Fig. 2).

Local mass misalignment is analyzed by computing individual mass shift profiles for each spectrum (Fig. 3). Per-spot calibration curves yield reduced misalignment (Fig. 4, Table 1).

Absolute mass accuracy is evaluated by comparing model based calibration to FT-ICR reference measurements (Fig. 5).

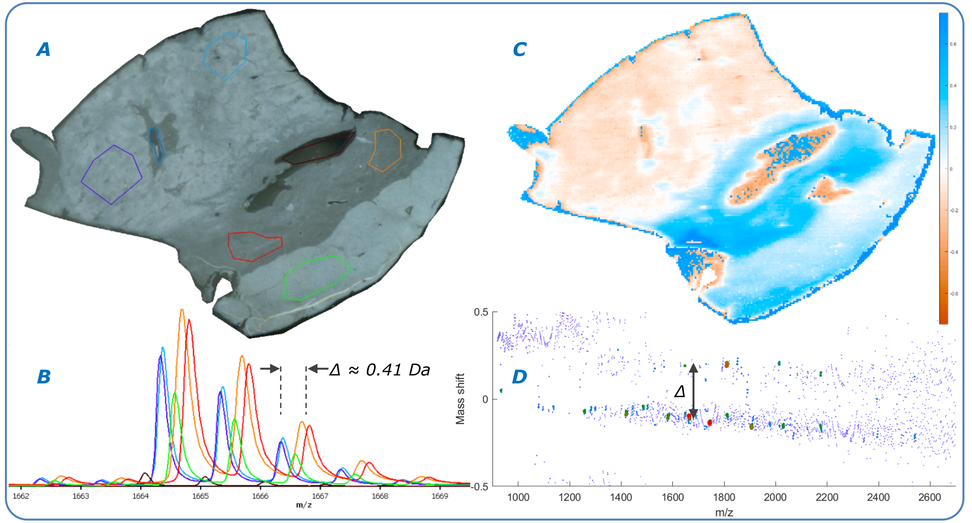
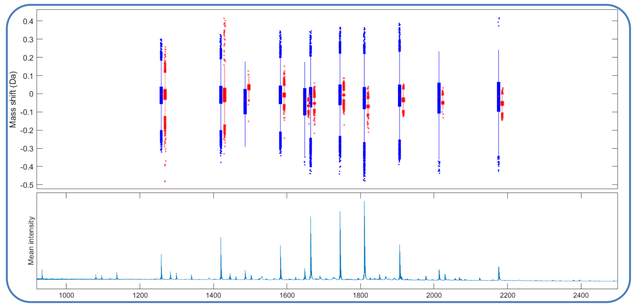
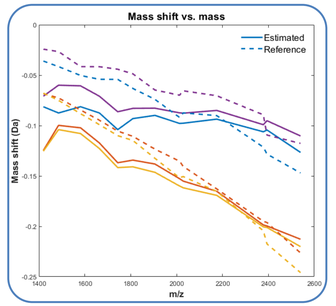


Fig. 3: (A) Darker tissue region in Sample H4 corresponds to tumor. (B) Regional mean spectra show mass shifts depending on tissue type. (C) Local mass shift analysis yields spatial misalignment map with correlation to tissue anatomy. (D) Misalignment is also reflected by overall mass defect plot. ▶



▲ Fig. 4: Mass misalignment of major peaks measured by Gaussian matching pursuit on Sample H2 before (blue) and after (red) applying the model based alignment method.



▲ Fig. 5: Model based (solid) and FT-ICR based (dashed) calibration curves.

Sample	original		aligned	
	std	iqr .95	std	iqr .95
H1	0.095	0.376	0.005	0.022
H2	0.101	0.408	0.006	0.026
H4	0.149	0.494	0.007	0.028
H7	0.073	0.268	0.005	0.020

▲ Table 1: Median m/z dispersion, given as standard deviation and 95% confidence range.

Conclusion

- **Mass misalignment significantly reduced** by recalibration based on N-glycan mass model
- **Absolute mass accuracy within 25 ppm** compared to FT-ICR reference data
- **Spatial mass shift distribution** reveals dependency on tissue anatomy

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