MALDISTAR

Quality Assessment and Cross-normalization for MALDI MSI

Improving inter-lab comparability and multi-center studies

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CoI disclosure: TB is part time employee at SCiLS / Bruker Daltonik (Bremen, Germany)

Universität Bremen*

Zentrum für Technomathematik

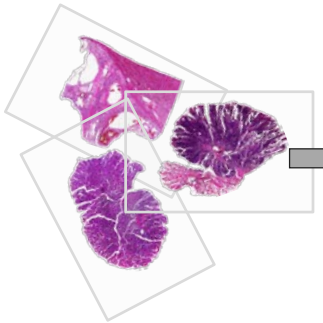
Klaus Tschira Stiftung gemeinnützige GmbH

KTS

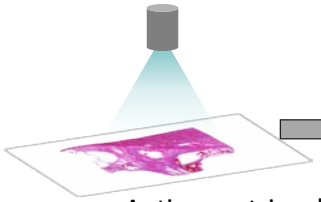
SCiLS

The Role of Reproducibility

FFPE tissue samples



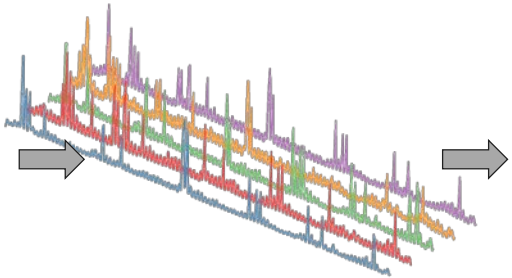
Sample preparation



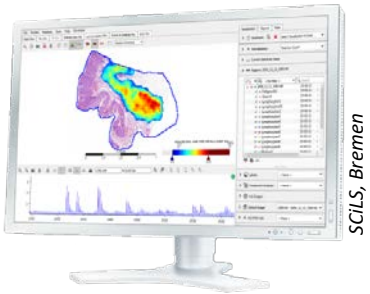
Antigen retrieval
Tryptic digestion

Bruker Daltonik, Bremen

MALDI data acquisition



Data analysis



SCiLS, Bremen

Process complexity

Operator dependence

Technical variability

Reproducibility and comparability …

- *across measurements*
- *over time*
- *across instruments*
- *across laboratories*

Large cohort studies

Longitudinal studies

Multi-center studies

Biomarker discovery

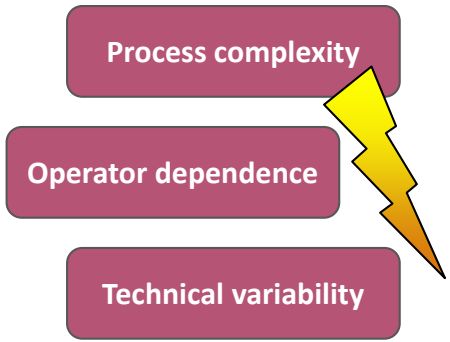
Pathway analysis

Prognostic factors

Drug efficacy

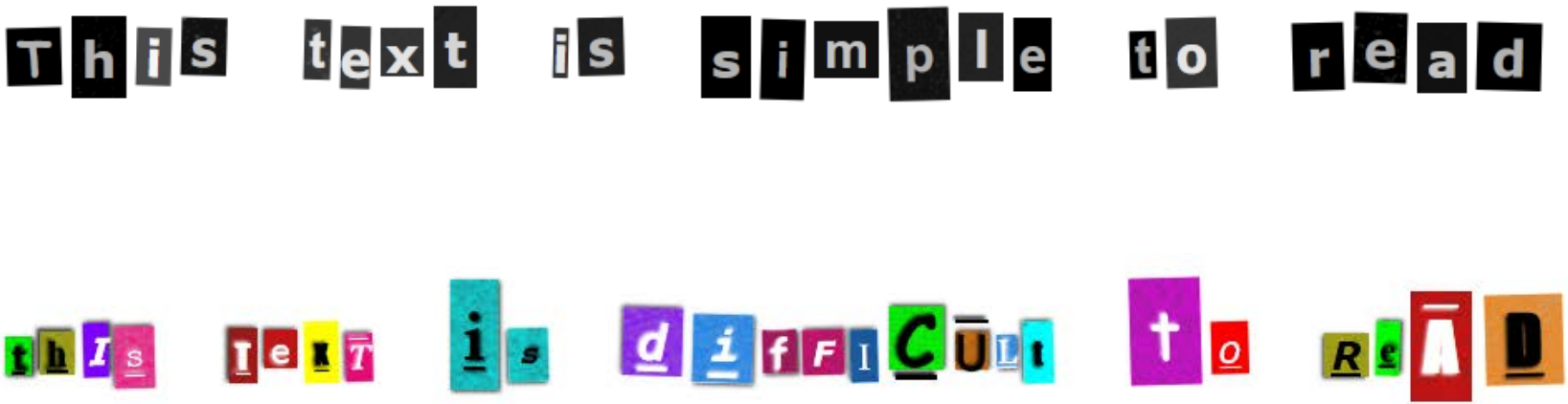
Technical Variability in MALDI MSI

- High sensitivity to process variations
- Differences between replicates often larger than between phenotypes



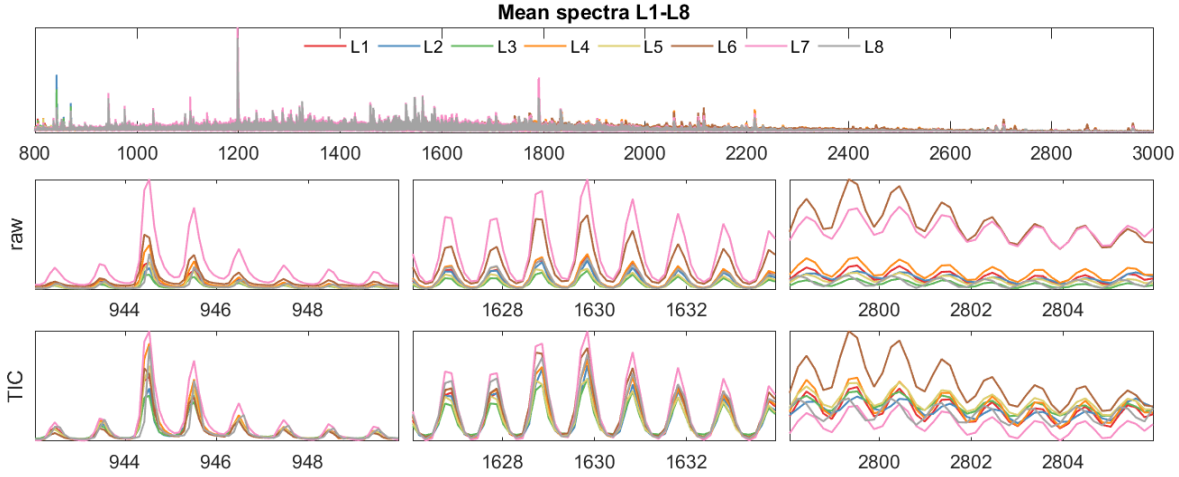
- Variability effects include
  - ***Ionization / ion suppression***
  - ***Delocalization***
  - ***Noise***
  - ***Intensity / sensitivity variations***
  - ***Mass distortions***
  - …

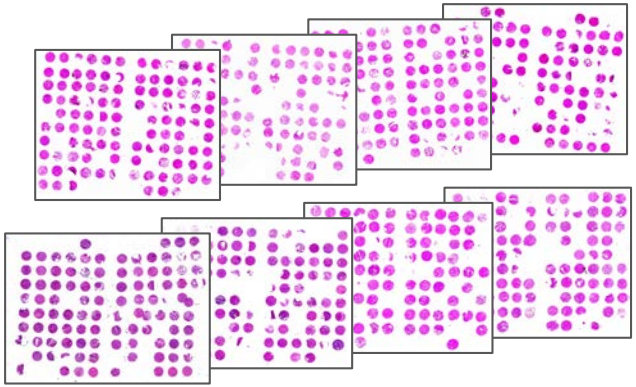
Technical Variability



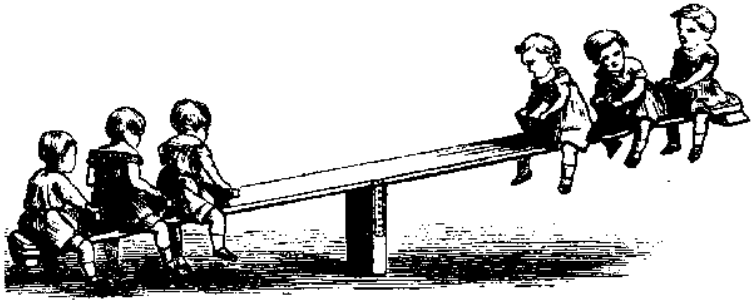
http://www.ransomizer.com

Example: Intensity Variations



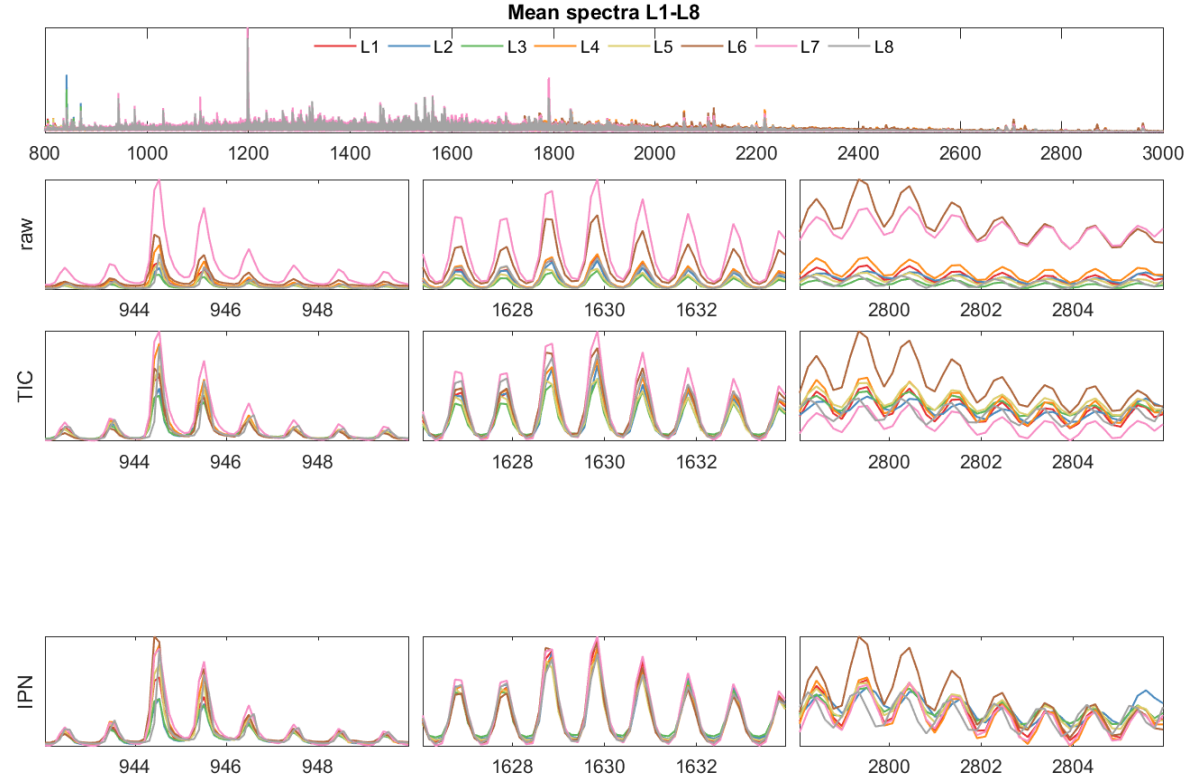


MSI Data: *Kriegsmann et.al. Mol Cell Proteomics. 2016 Oct;15(10):3081-3089. Epub 2016 Jul 29.*

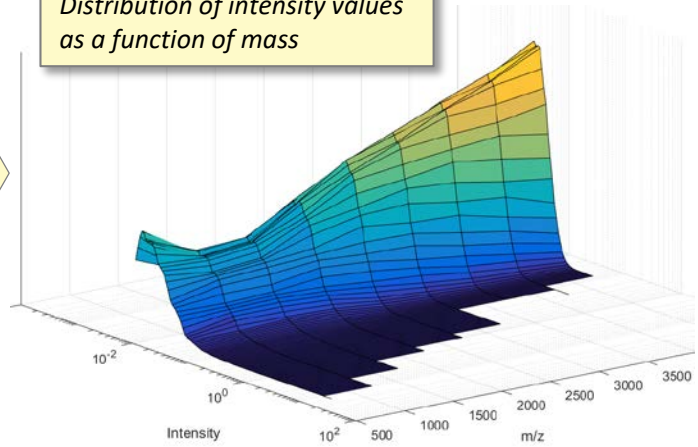


- MALDI MSI of 8 FFPE lung cancer biopsy TMAs (L1 – L8), identical protocol (incl. trypsin digestion), constant acquisition conditions
- Strong intensity shifts between L1 – L8 mean spectra, varying across m/z range
- Standard normalization (TIC, median, RMS, …) cannot avoid ***"seesaw effect"***

Intensity Profile Cross-Normalization



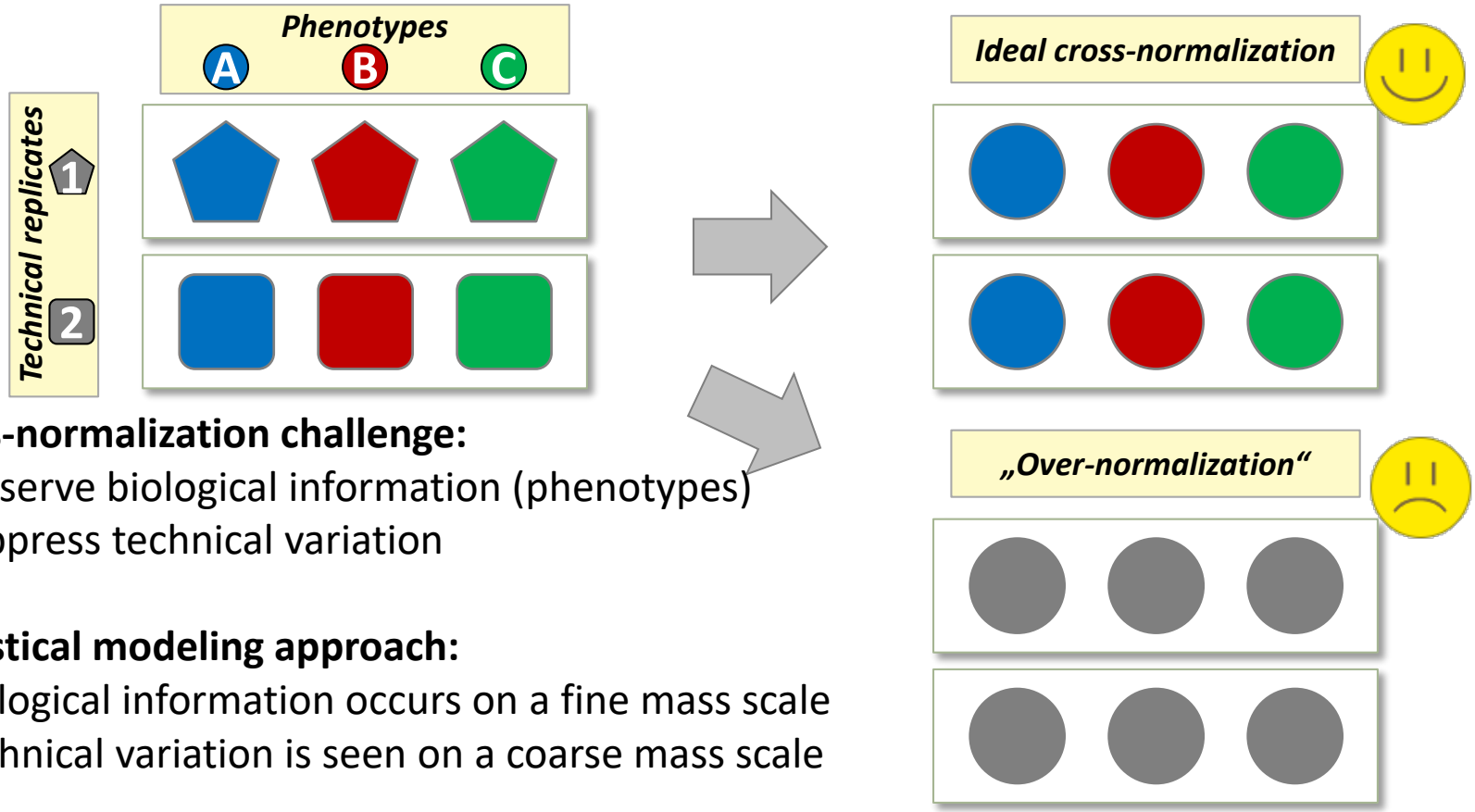
Spectral intensity profile:
Distribution of intensity values as a function of mass



Intensity profile normalization (IPN):
Mass-range dependent intensity variations reduced

Boskamp et.al., Ourcon 2017, ASMS 2018

Cross-Normalization



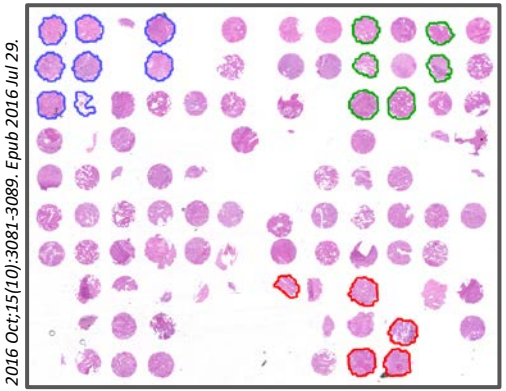
Cross-normalization challenge:

- Preserve biological information (phenotypes)
- Suppress technical variation

Statistical modeling approach:

- Biological information occurs on a fine mass scale
- Technical variation is seen on a coarse mass scale

Example: Mass Shift Variation



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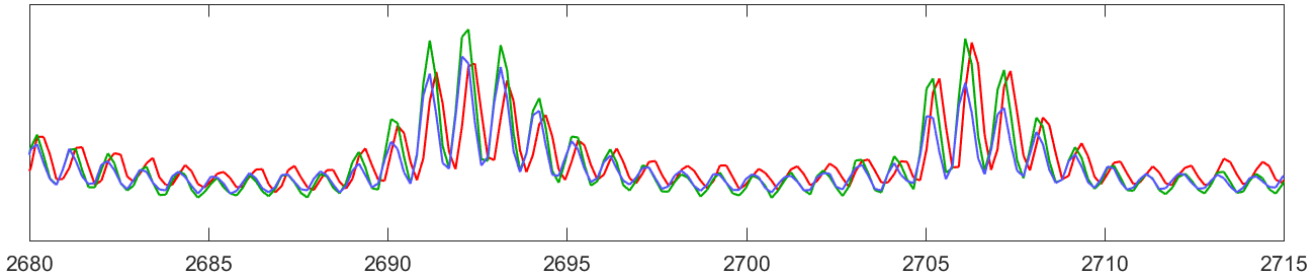
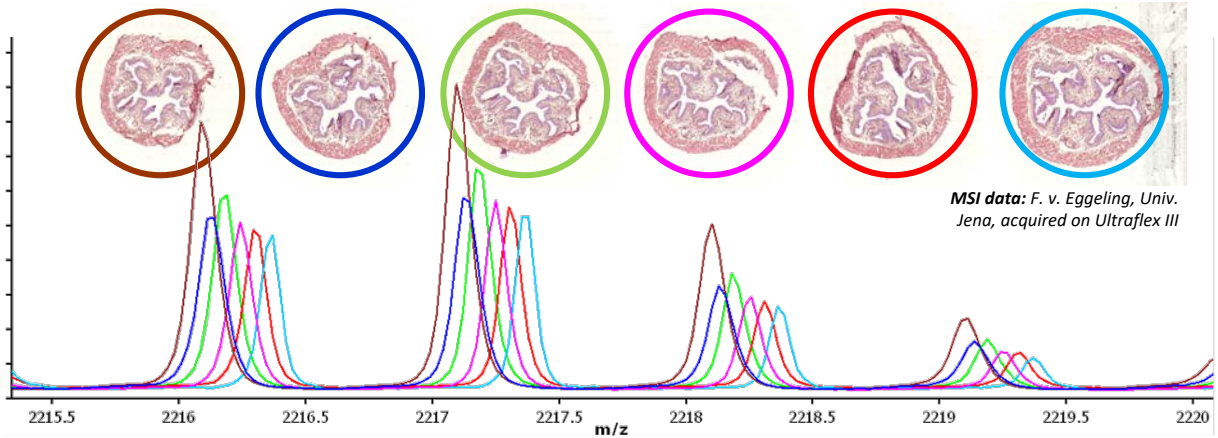


Lung cancer TMA L8: Regional mass and intensity shifts within individual measurements



MSI data: *F. v. Eggeling, Univ. Jena, acquired on Ultraflex III*

Mouse bladder sections: Heavy mass shifts due to non-optimal acquisition conditions

Characteristic Peptide Background

Chemical noise in MALDI peptide imaging

- Chemical noise largely dominated by peptides
- Characteristic wavelength = 1+δ Da
- Kendrick factor δ determined by peptide mass rule

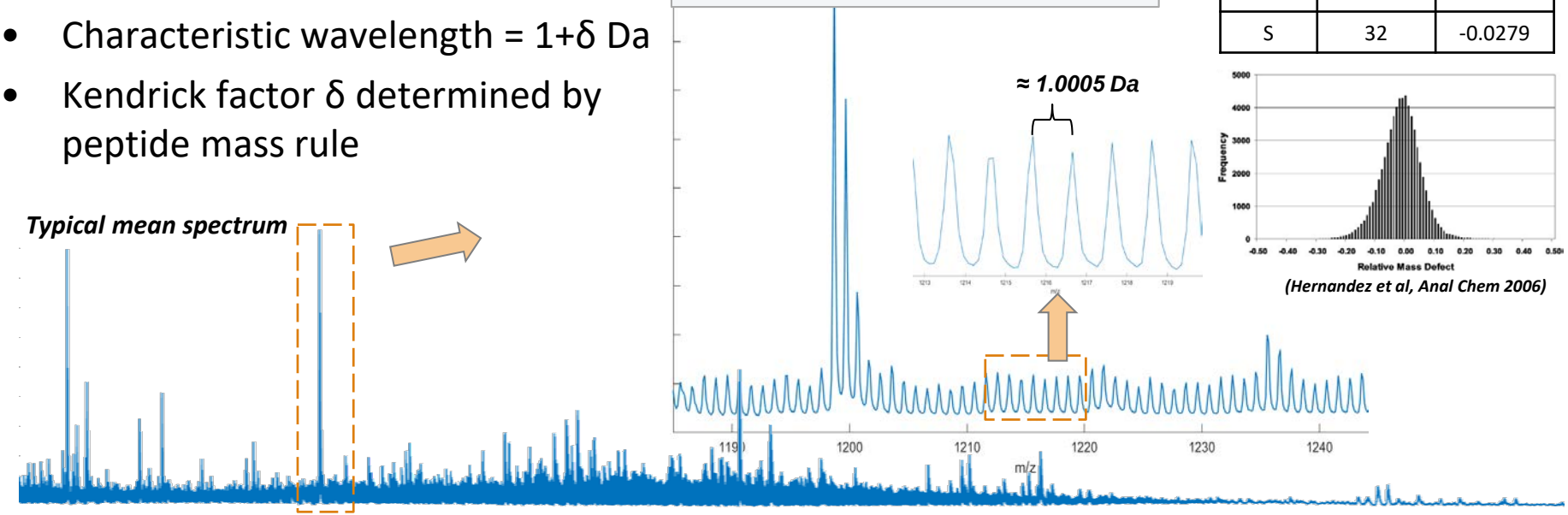
Peptide mass rule:

$$\overline{m} = (1+\delta)m_N, \delta \approx 4.95 \times 10^{-4}$$

Peptide scale Kendrick shift:

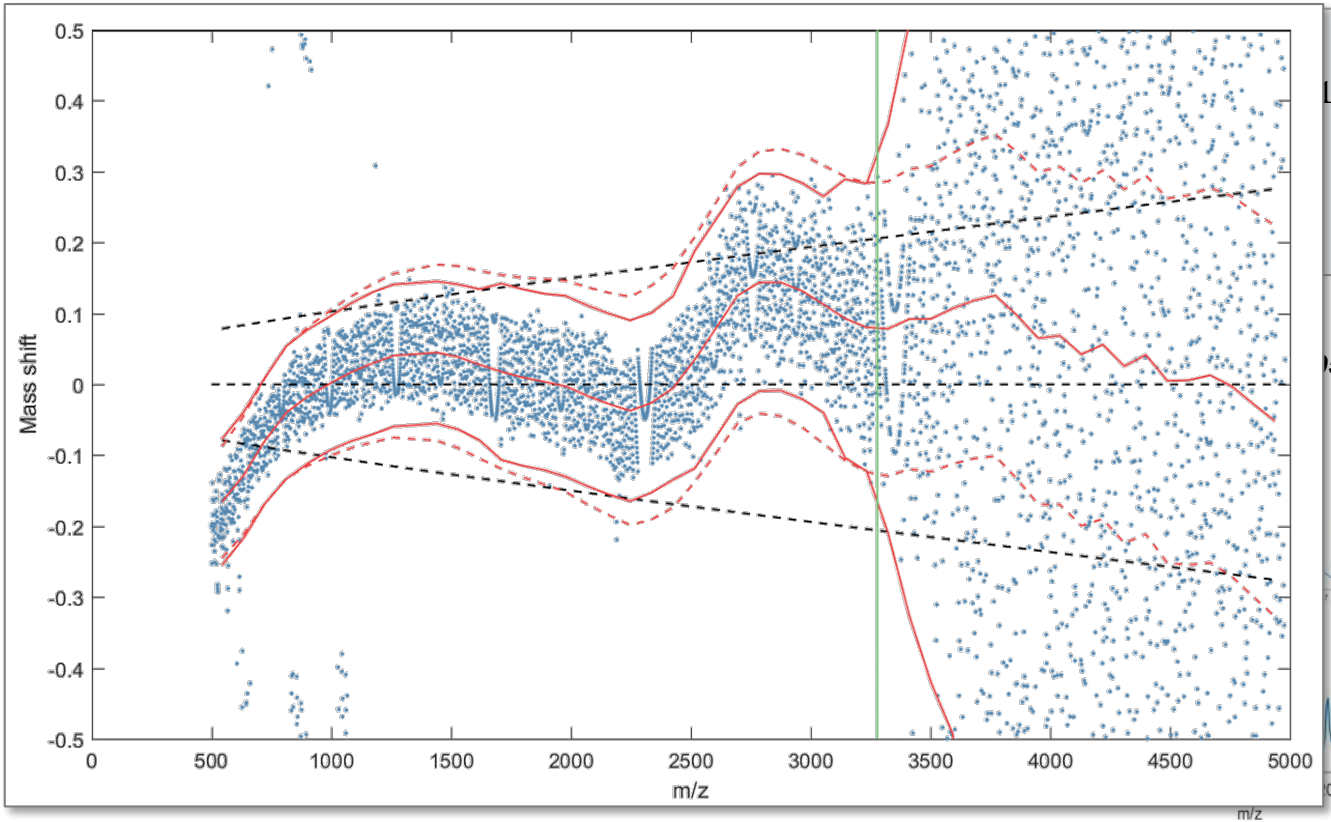
$$\Delta = \mathrm{frac}\left(\frac{m}{1+\delta} + 0.5\right) - 0.5$$

Element	Nominal mass m_N	Mass defect
H	1	0.0078
C	12	0.0000
N	14	0.0031
O	16	-0.0051
S	32	-0.0279



(Hernandez et al, Anal Chem 2006)

Kendrick Profile / Mass Shift Profile



Boskamp et.al., Ourcon 2016

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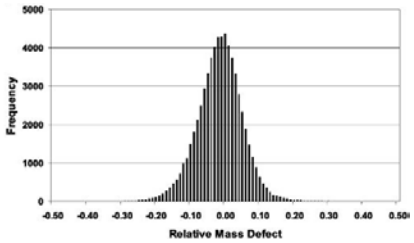
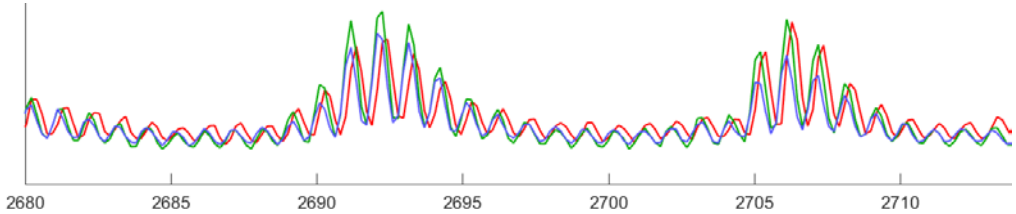
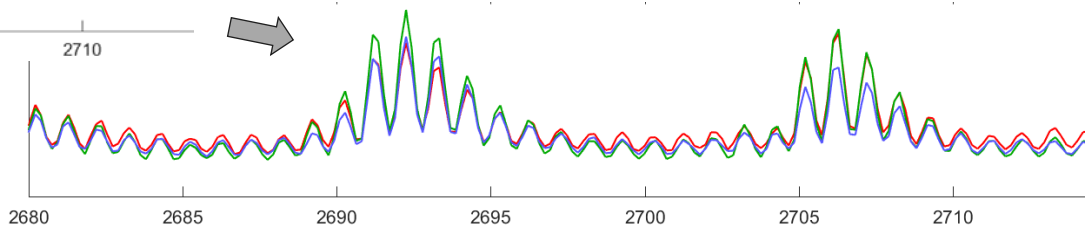
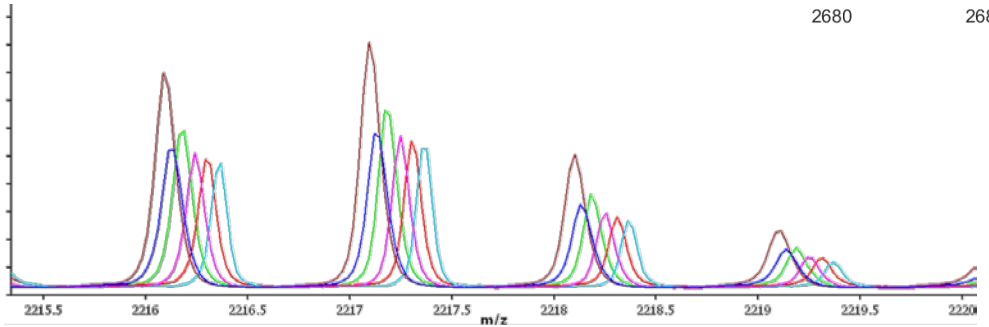


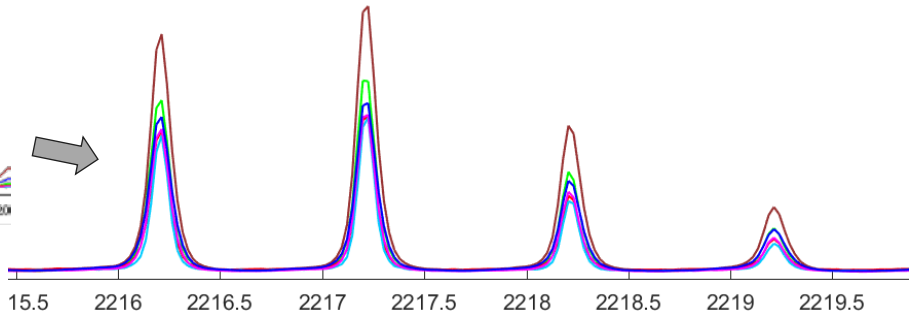
(Hernandez et al, Anal Chem 2006)

Mass Shift Alignment / Calibration







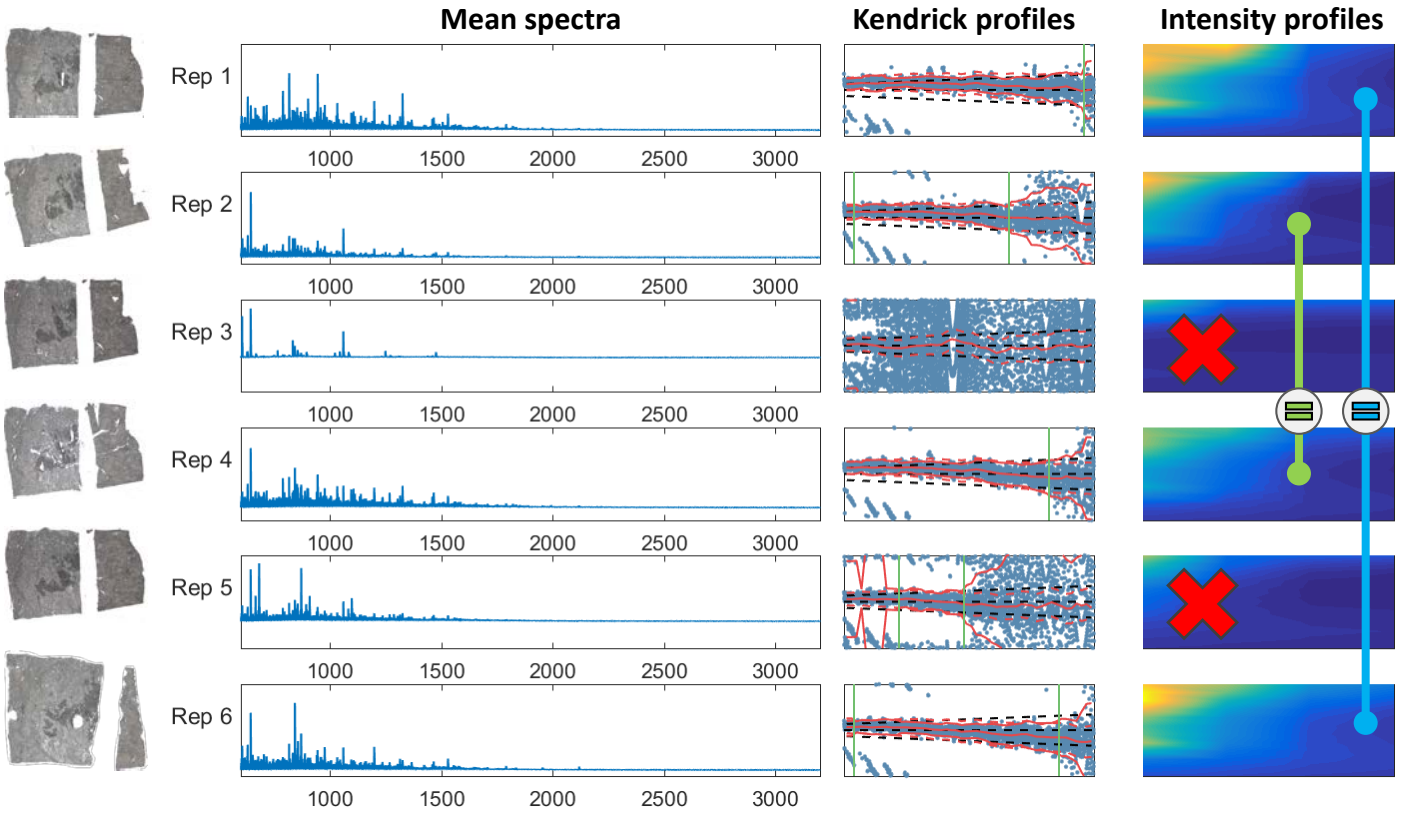


Boskamp et.al., Ourcon 2017, ASMS 2018

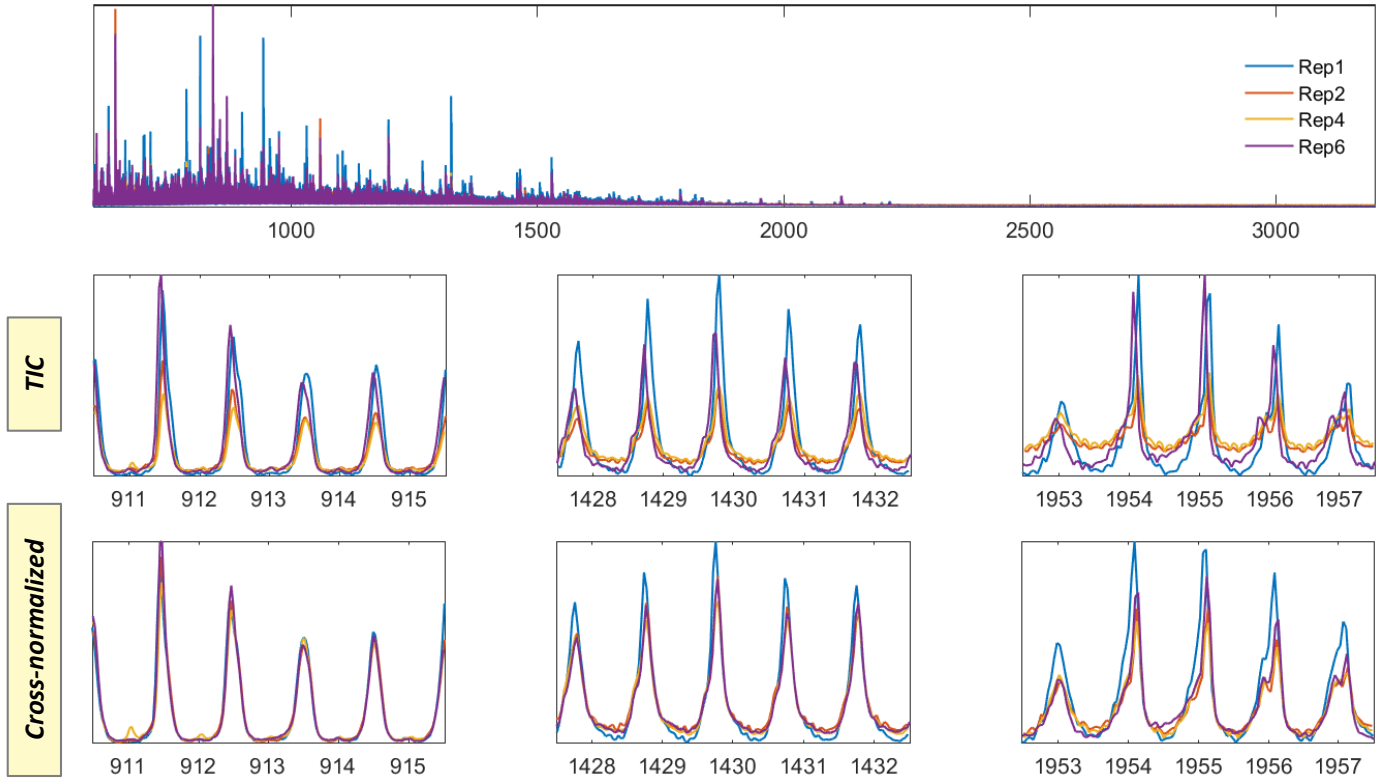
MALDI TOF Reproducibility Study

- Homogenous liver tissue
- Six serial sections, mounted on separate slides
- Measured on six days within two weeks
- Identical preparation and acquisition conditions (TM Sprayer, Rapiflex)
- ***Two out of six experiments failed – which?***

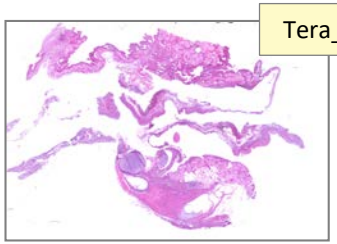
MSI data: *J. Kriegsmann, R. Casadonte, Proteopath, Trier*



Reproducibility Study – Cross-Normalization

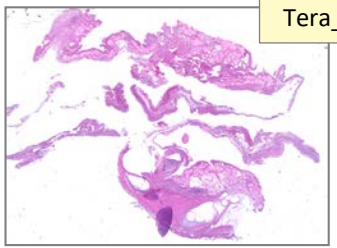


Normalization Across Instruments



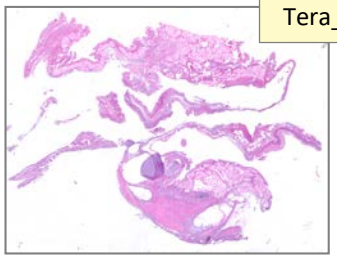
Tera_12

ImagePrep + Autoflex



Tera_13

TM Sprayer + Autoflex

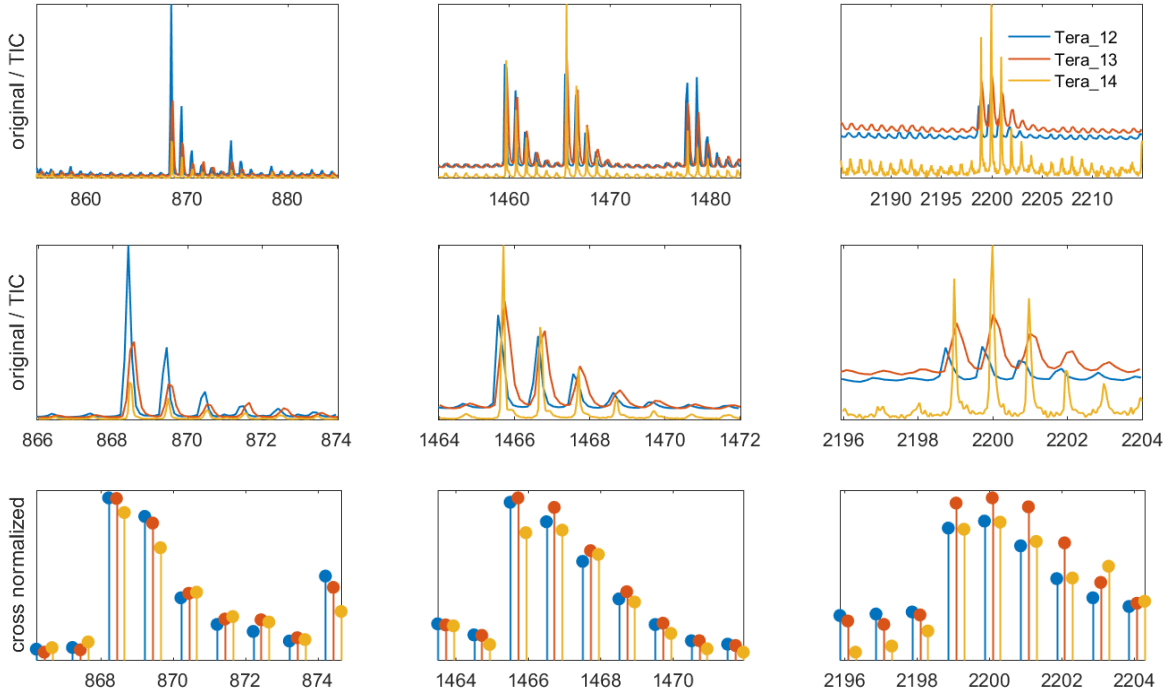


Tera_14

TM Sprayer + Rapiflex

MSI data: *J. Kriegsmann, R. Casadonte, Proteopath, Trier*

Three serial sections of human teratoma prepared and measured using different instrumentation and protocols



Cross-normalization allows combined analysis of all three datasets

Optimized data analysis pipeline for MALDI MSI based tumor typing from FFPE tissue samples evaluated on six benchmark classification tasks

D. Lachmund[1], J. von Schröder[1], T. Boskamp[1,2], L. Hauberg-Lotte[1], J.H. Kobarg[2], S.O. Deininger[3], K. Kriegsmann[4], M. Kriegsmann[4], R. Casadonte[5], J. Kriegsmann[5], P. Maaß[1,2]

University of Bremen

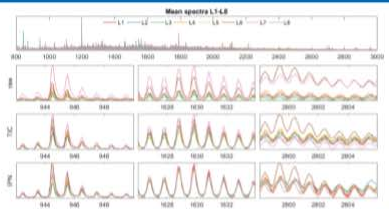
BRUKER

ASMS 2019, MP 340

[1]University of Bremen, Bremen, Germany [2]SCiLS, Bremen, Germany [3]Bruker Daltonik, Bremen, Germany [4]University of Heidelberg, Heidelberg, Germany [5]Proteopath, Trier, Germany

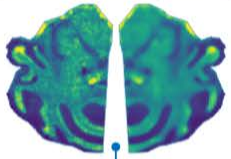
Goals

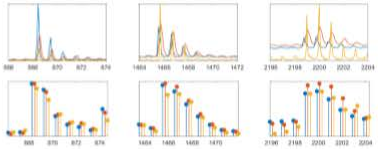
- Develop optimized pre-processing pipeline for MALDI MSI based tumor typing
- Consider different clinical tumor typing and subtyping tasks
- Consider intra- and inter-lab scenarios and different instrument types



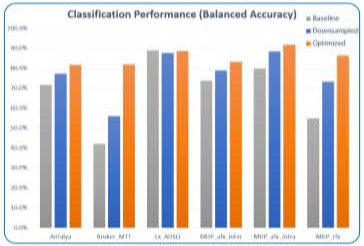
Non-linear **intensity profile normalization** (IPN, bottom row) improves comparability across different acquisitions *(Boskamp et al, ASMS 2018)*

Mild Gaussian kernel **spatial denoising** (right) increases signal-to-noise ratio as compared to original data (left)





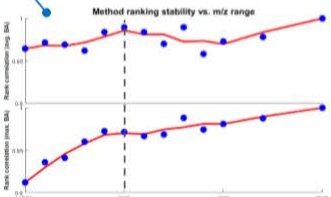
Dimensionality reduction by downsampling to peak areas over 0.4 Da intervals *(Boskamp et al, ASMS 2018)*



Data ▶ **m/z range** ▶ **Normalization** ▶ **Alignment** ▶ **Spatial denoising** ▶ **Downsampling** ▶ **Normalization** ▶ **Transformation** ▶ **Classification**

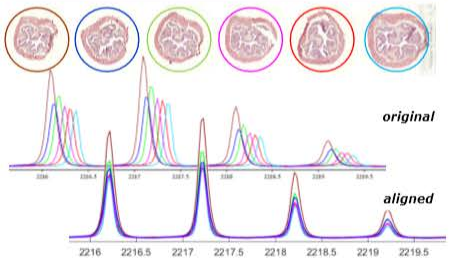
Benchmark panel acquired from **25 TMAs**, **2031 cores** and **1410 patients** total

Task	Instrument	Description
Antalya	autoflex	• Four tumor entities, 8 TMAs • Lung, pancreas, colon, breast
Bruker MTT	rapiflex	• Six tumor entities on one TMA • Five measurements in four labs • Training and test data from different SOP's
Lx ADSQ	autoflex	• Eight TMAs with mix of adeno- and squamous cell carcinoma, afx
MDP afx inter	autoflex	• Breast, ovary tumors, 5 TMAs • Measured in two labs • Inter-lab cross-validation
MDP afx intra	autoflex	• Same as above, but intra-lab cross-validation
MDP rfx	rapiflex	• Breast, ovary tumors, 5 TMAs • Single lab

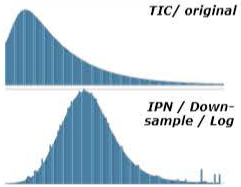


Reducing mass range for feature selection down to 700 … 2000 m/z increases robustness and speed without affecting accuracy

Mass calibration based on statistical peptide mass model reduces misalignment *(Boskamp et al, ASMS 2018)*



Logarithmic transform with appropriate scaling results in more symmetric intensity distributions – beneficial for subsequent LDA classification



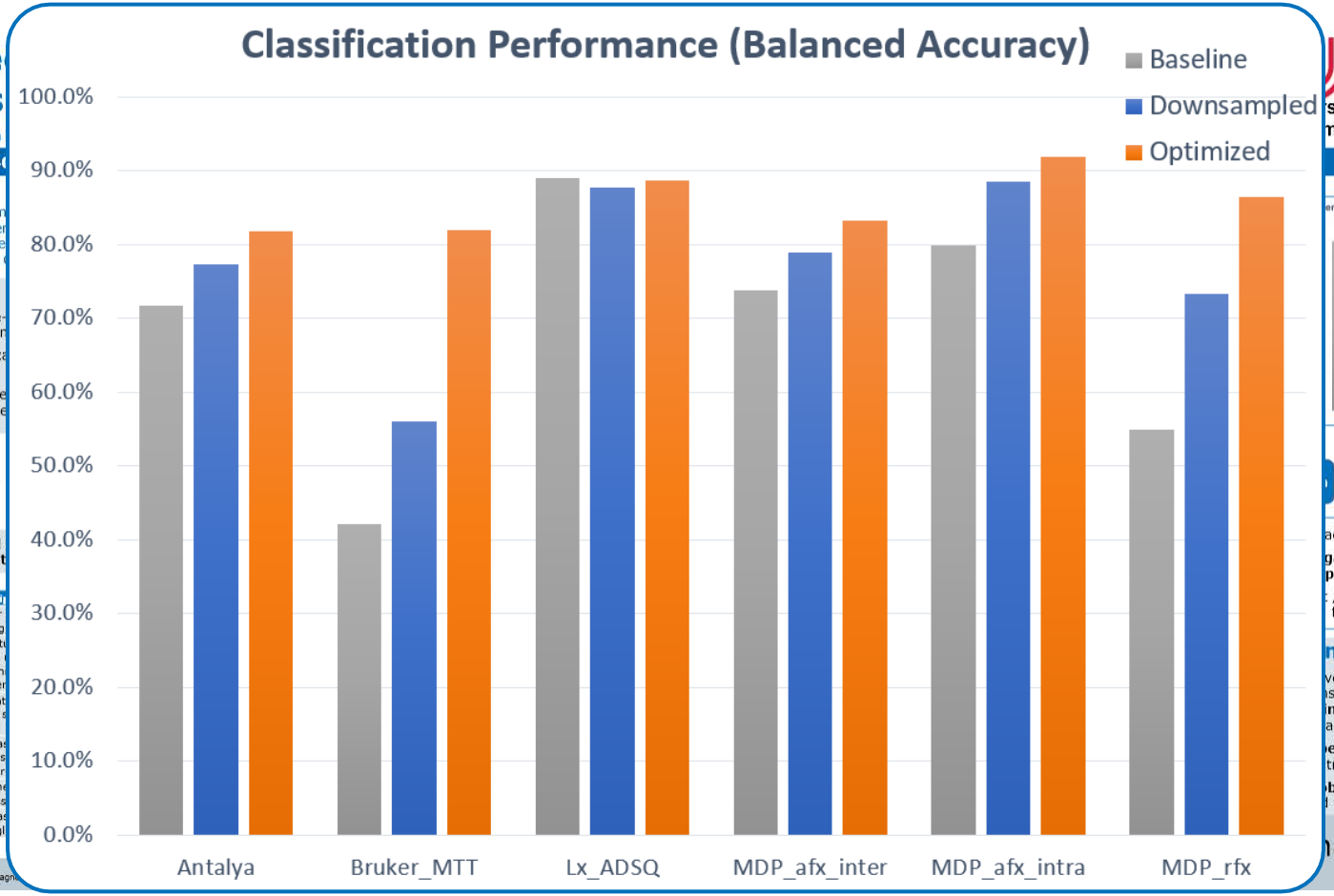
- Balanced accuracy 82% and 92%
- **Performance gain** over baseline (TIC only) **9.5 … 39.8% pts.** for five of six tasks
- Mass alignment / downsampling alone yields 5 … 18.5% pts. for five of six tasks

Conclusion

- Systematic investigation of six benchmark problems yields an **optimized pre-processing pipeline** for MALDI MSI tumor typing applications
- **Significant performance gains** achieved in intra- and inter-lab scenarios
- **Improved robustness** towards SOP variations and technical variability

Imaging MS

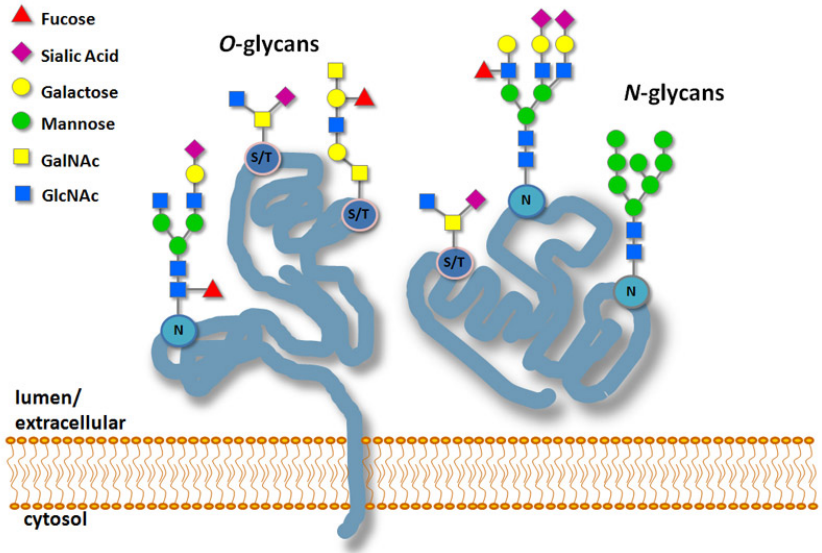
Lachmund et.al., ASMS 2019



Lachmund et.al., ASMS 2019

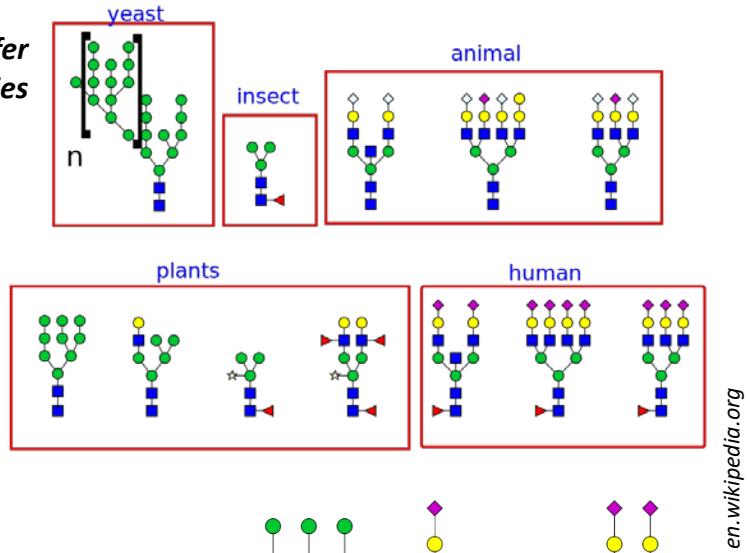
N-Linked Glycans

N-glycans: *Sugar molecules bound to asparagine („N“) amino acids outside of cell membranes*



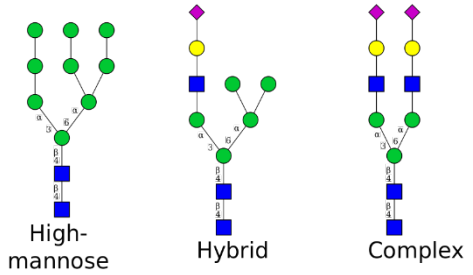
www.neb.com/applications/glycobiology-and-proteomics/glycobiology

N-glycans differ between species



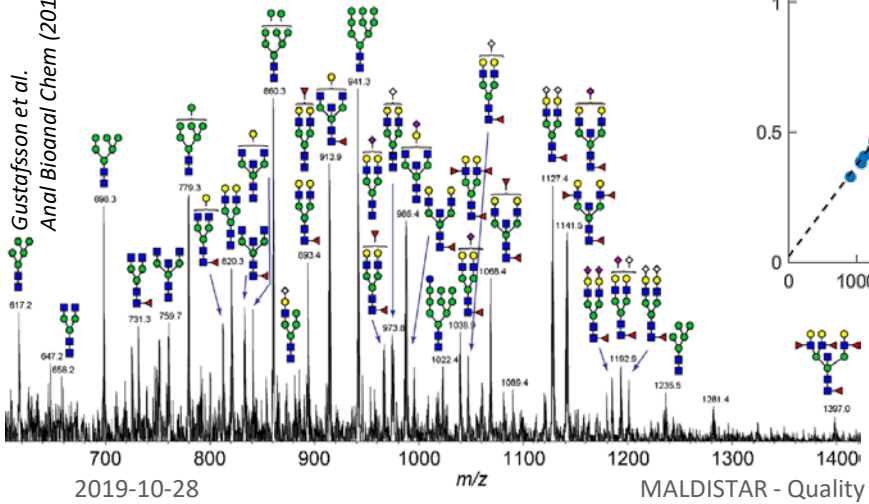
en.wikipedia.org

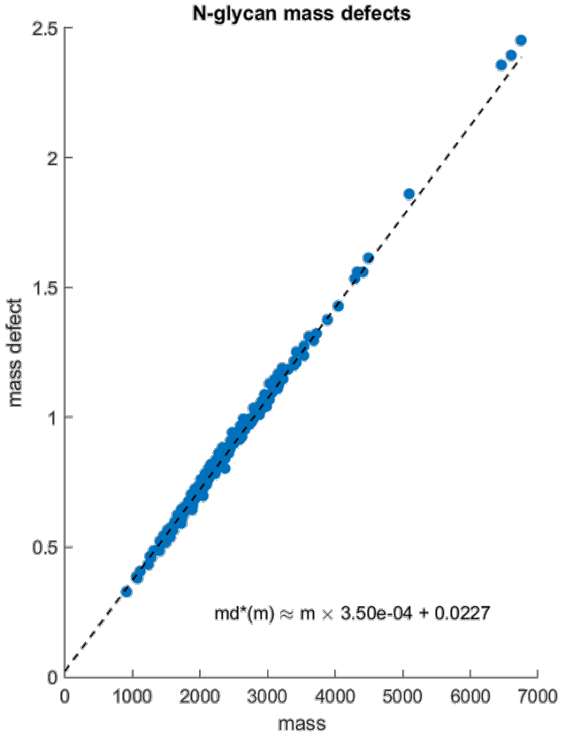
Three major types of N-glycans

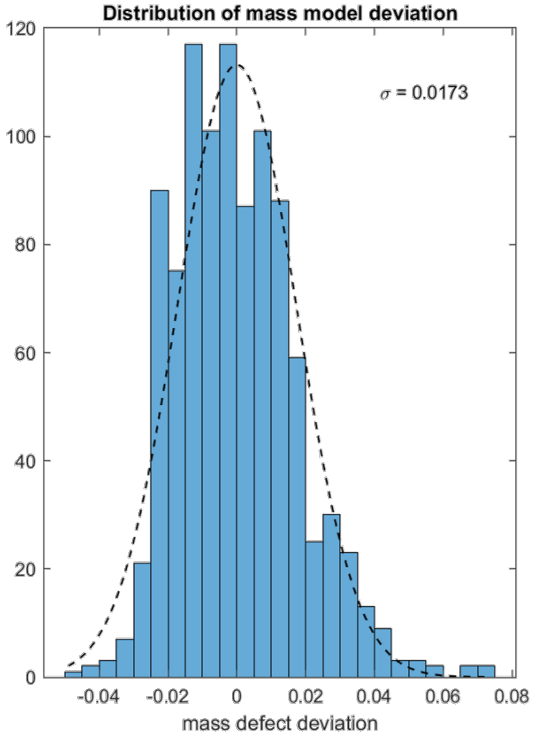


N-Glycan Mass Model

- Regular molecular composition
  - Six classes of monosaccharides
  - Elements C, H, N, O
- GlyTouCan database:
  - ~ 900 N-linked glycans identified in humans
  - Most in mass range 1000 … 4000 Da
- Strong linear correlation between mass and mass defect







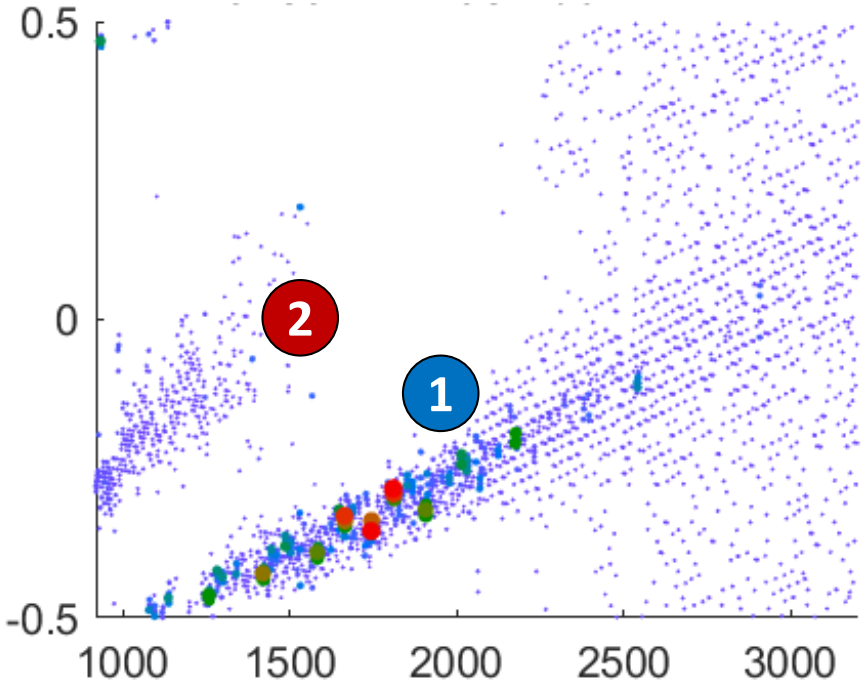
N-Glycan Chemical Background Pattern

- **Glycan scale Kendrick plot:**
  Visualize accordance of background signal with glycan mass model
  Color dots represent spectral peaks

1 **N-glycans** concentrated in dominant cluster

- **Original signal:**
  Strong mass shift reflected in mass defect plot

2 **Additional cluster** observed below 1500 Da
  Possibly peptides?



MSI data: *R. Drake, A. Black, MUSC Charleston, USA*

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

Universität Bremen · MUSC · BRUKER

ASMS 2019, MP 340

Tobias Boskamp[1,2], Alyson Black[3], Anand Mehta[3], Richard Drake[3], Yujin Hoshida[4], Dennis Trede[1], Peter Maaß[1,2]

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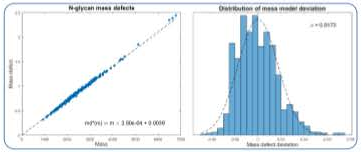
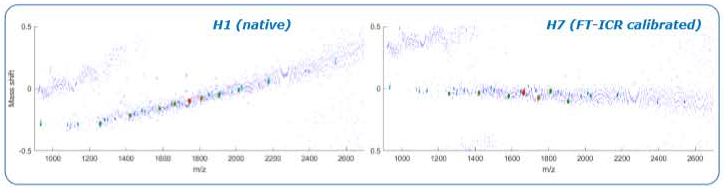
Introduction

- 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.

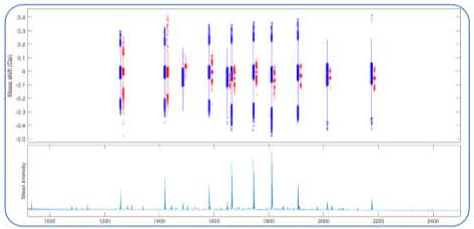
Method

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).

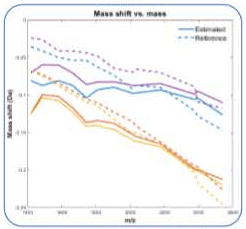
Local mass misalignment is analyzed by computing individual mass shift profiles for each spectrum (Fig. 3). Per-spot calibration curves yield reduced misalignment (Fig. 4, Table 1).

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

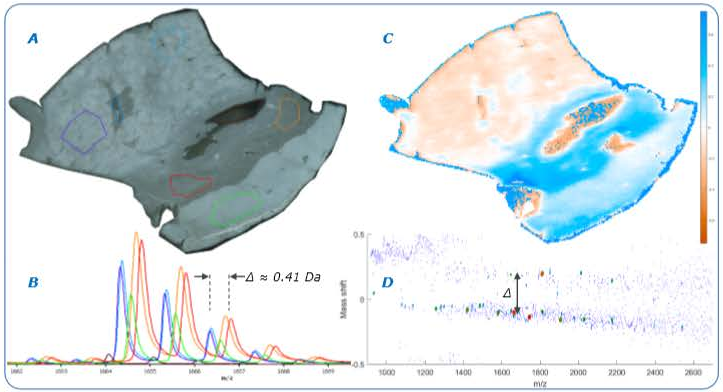
Fig. 3: (A) Darker tissue region in Sample H4 corresponds to tumor. (B) Regional mean spectra show mass shifts depending on tissue type. (C) Local mass shift analysis yields spatial misalignment map with correlation to tissue anatomy. (D) Misalignment is also reflected by overall mass defect plot. ▶



▲ 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

Imaging MS

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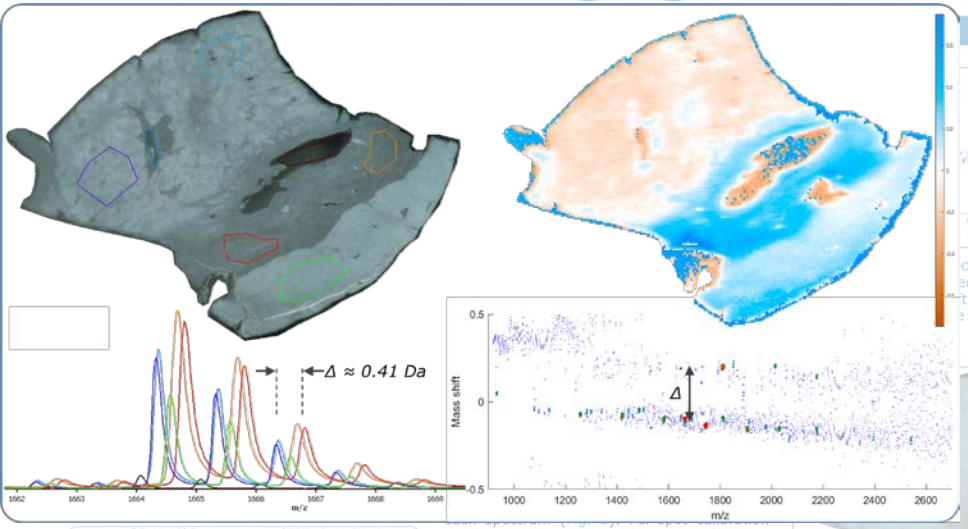
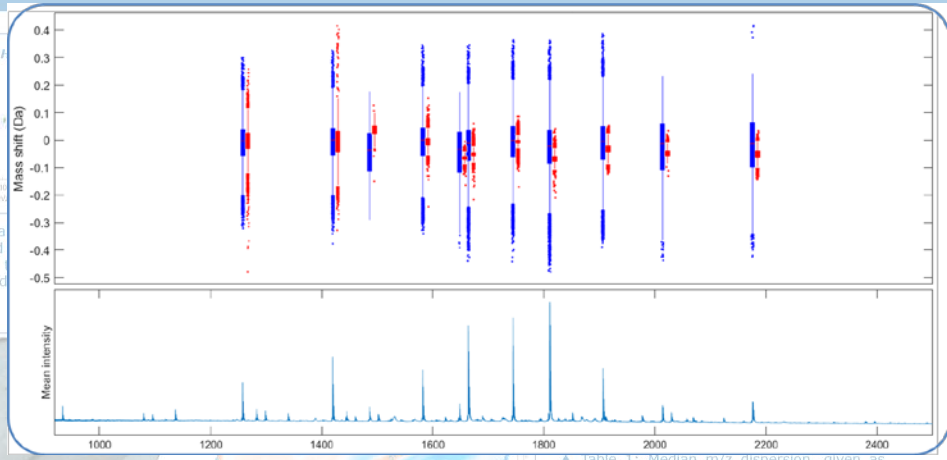
Fully automated mass alignment and recalibration of MALDI TOF imaging data from N-linked glycans

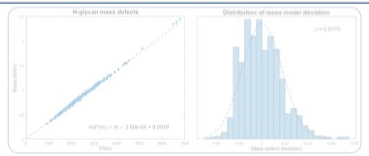
Universität Bremen

MUSC

BRUKER







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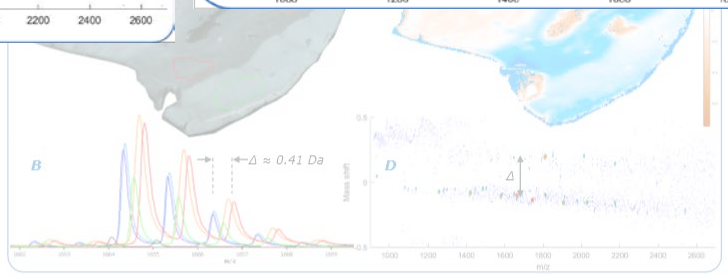

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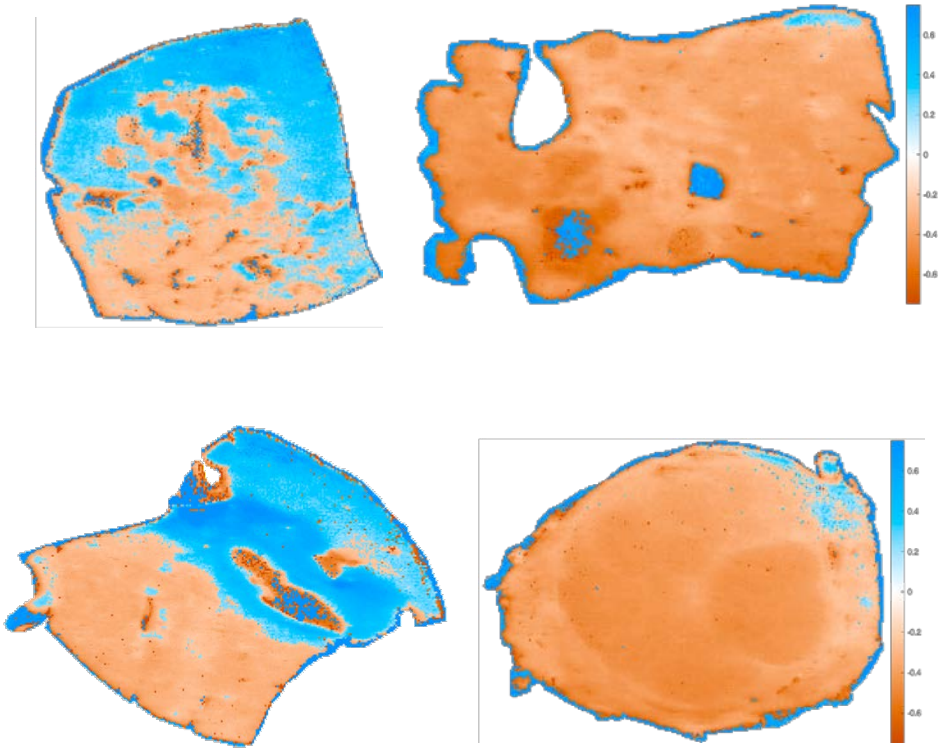
Mass Shift Mapping – Rapiflex vs Solarix

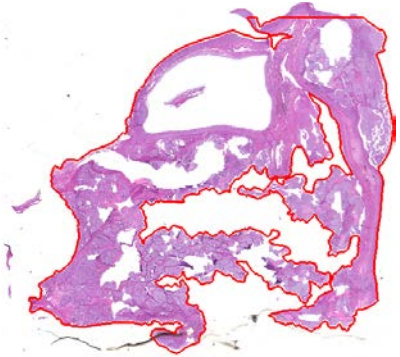
Rapiflex TOF

Solarix FT-ICR

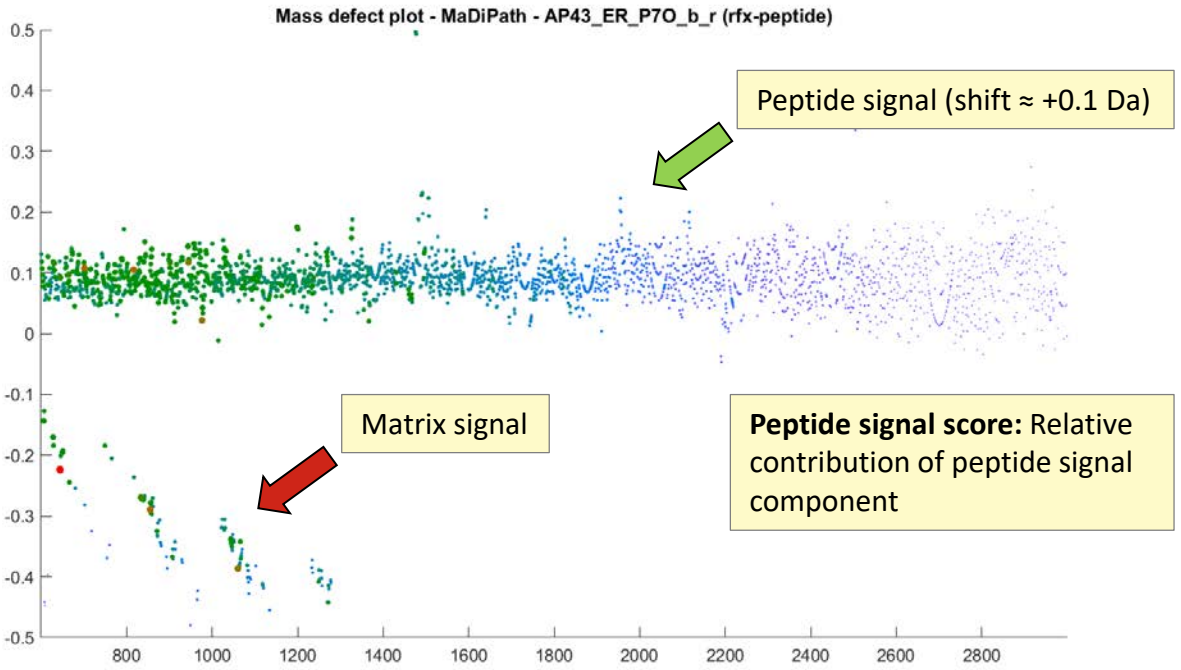


MSI data: *R. Drake, A. Black, MUSC Charleston, USA*

Peptide vs. Matrix Signal Components

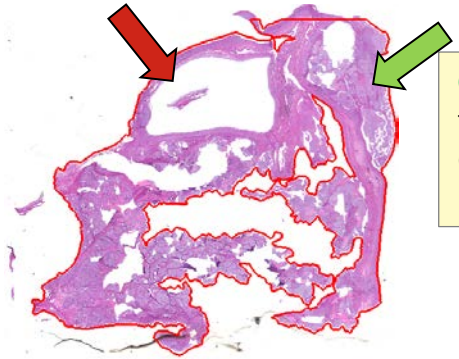


- FFPE tissue
- Tryptic digestion
- TM Sprayer & rapiflex

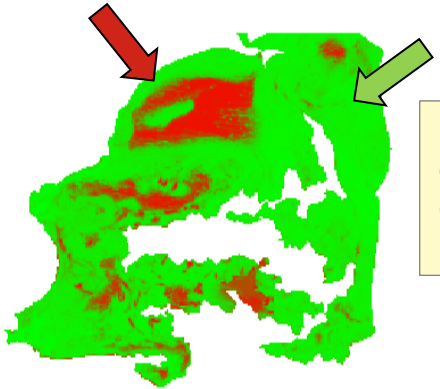


MSI data: *J. Kriegsmann, R. Casadonte, Proteopath, Trier*

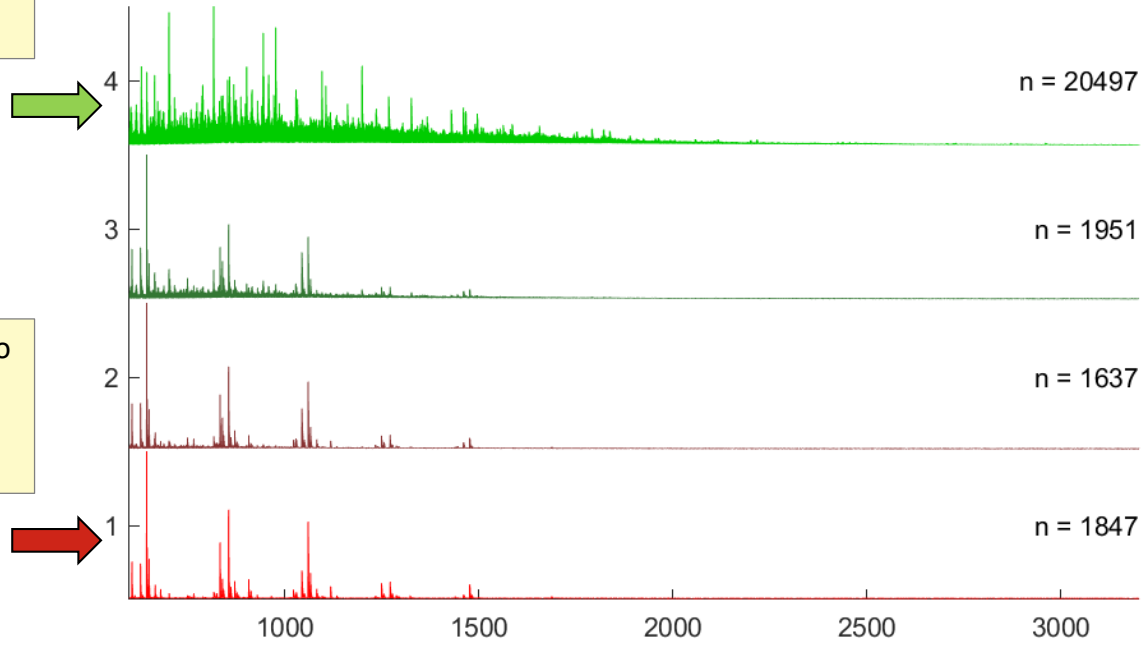
Peptide Signal Score – Off-tissue Regions



Green corresponds to tissue regions, dominated by peptide signal



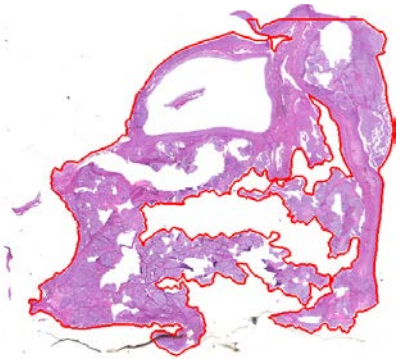
Red corresponds to off-tissue regions, dominated by matrix signal

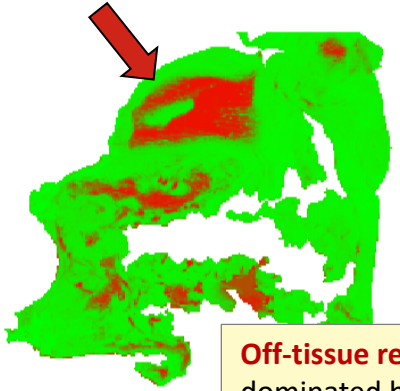


MSI data: *J. Kriegsmann, R. Casadonte, Proteopath, Trier*

Peptide Signal Score – Examples

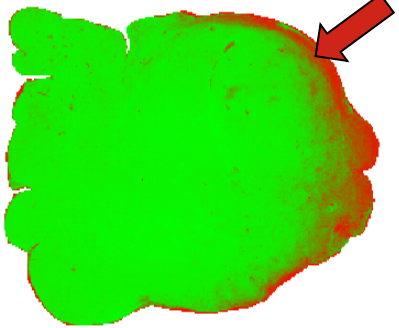
See talk on Wednesday 3:20 pm
Session 5: Bioinformatics

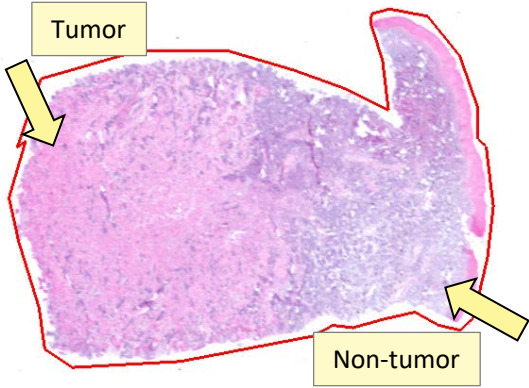


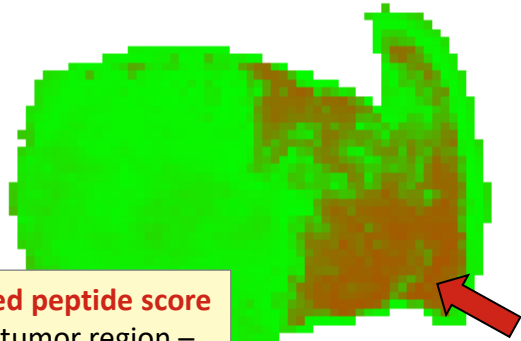


Off-tissue regions, dominated by matrix signal

Loss of peptide signal near tissue boundary – inhomogenous tissue preparation?







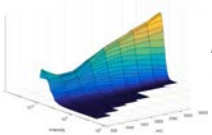
Reduced peptide score in non-tumor region – type of tissue?

MSI data: *J. Kriegsmann, R. Casadonte, Proteopath, Trier*

Conclusion

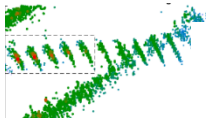
ALL DATA IS DIRTY!

Technical variation



Intensity variation ++

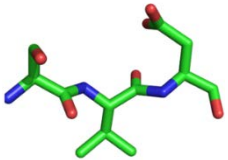
Mass shift +++



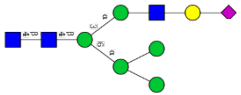
Matrix ++

Delocalization +

Molecule class



Peptides +++



N-Glycans ++

Lipids +

Metabolites -

Images: Wikimedia

Instrument class

Axial TOF +++

FT ICR +

O-TOF +

Orbitrap -

Images: Bruker Daltonik, Bremen

Conclusion

ALL DATA IS DIRTY!

1. Avoid dirt	• Clean experimental design • Proper statistics and data analysis	***Do it!*** … but it won‘t help
2. Dilute the dirt	• Collect more data • Open data repositories	***Do it!*** … will help only as part of 1. or 3.
3. Understand the dirt	• Investigate technical variation • Develop distortion models, quantitative metrics, (cross-)normalization	MALDISTAR

Thank You!

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- Dennis Trede

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- Janina Oetjen
- Alice Ly

Proteopath, Trier

- Rita Casadonte
- Petra Wandernoth
- Jörg Kriegsmann

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- Pierre Chaurand, Univ. Montréal

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