

Batch effects in mass spectrometry imaging



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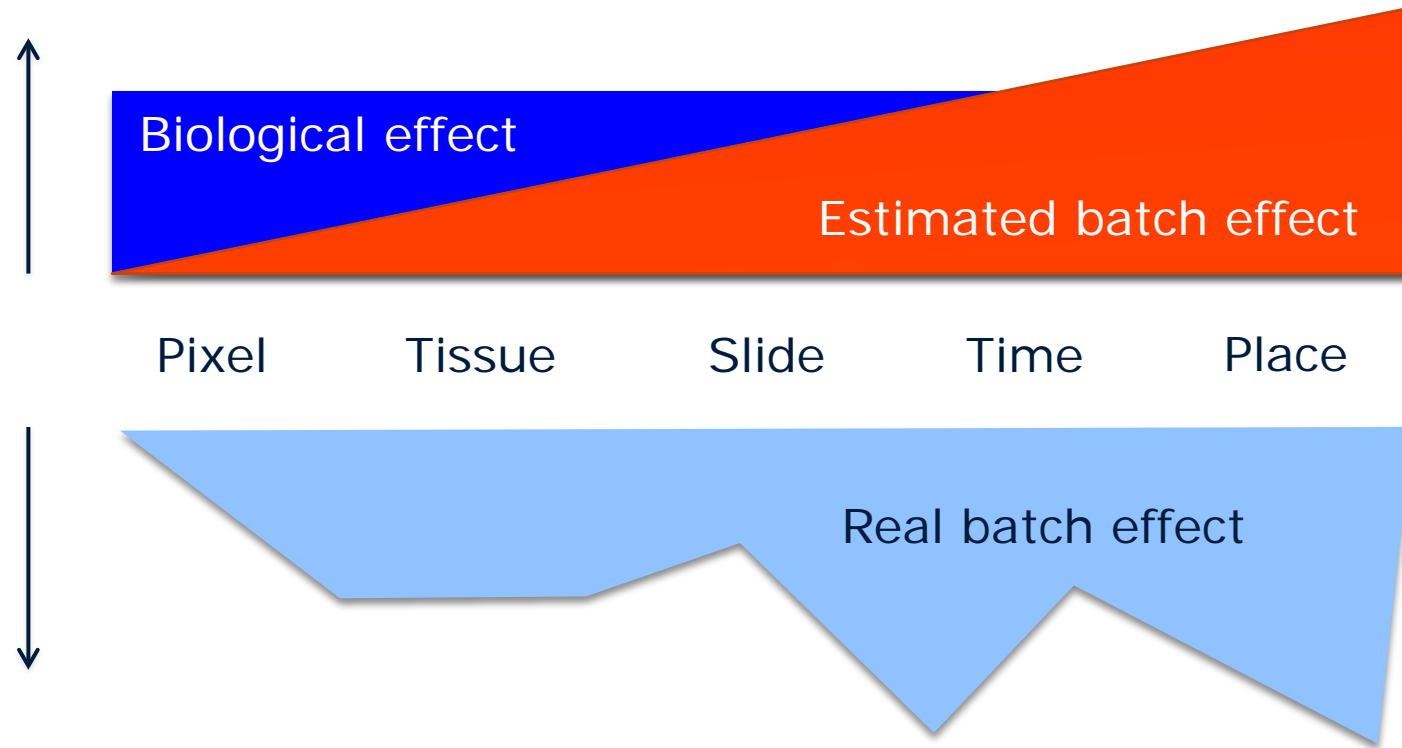
Ourcon VII, Workshop 2:
A MALDISTRAR is born!

October 28, 2019



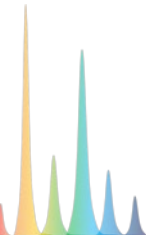
Maastricht University

Batch effects can mask biological effect

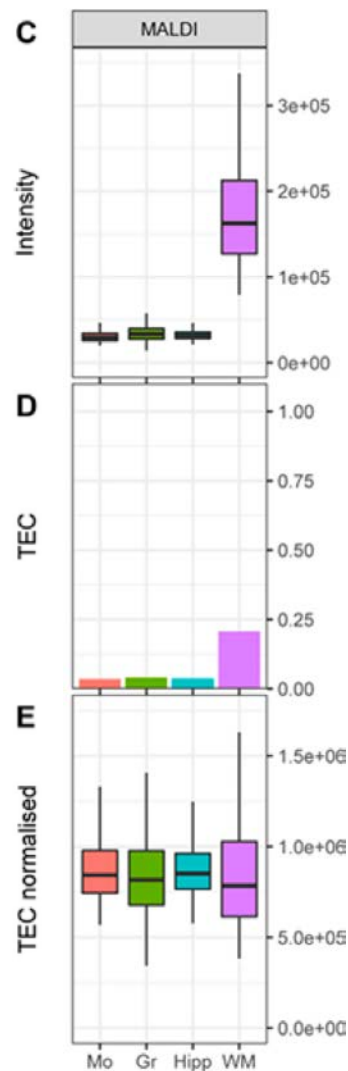
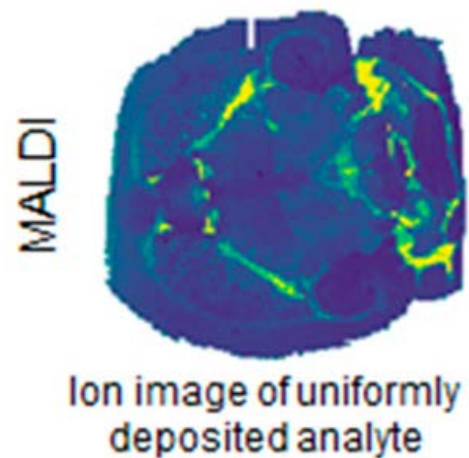


Given constants:

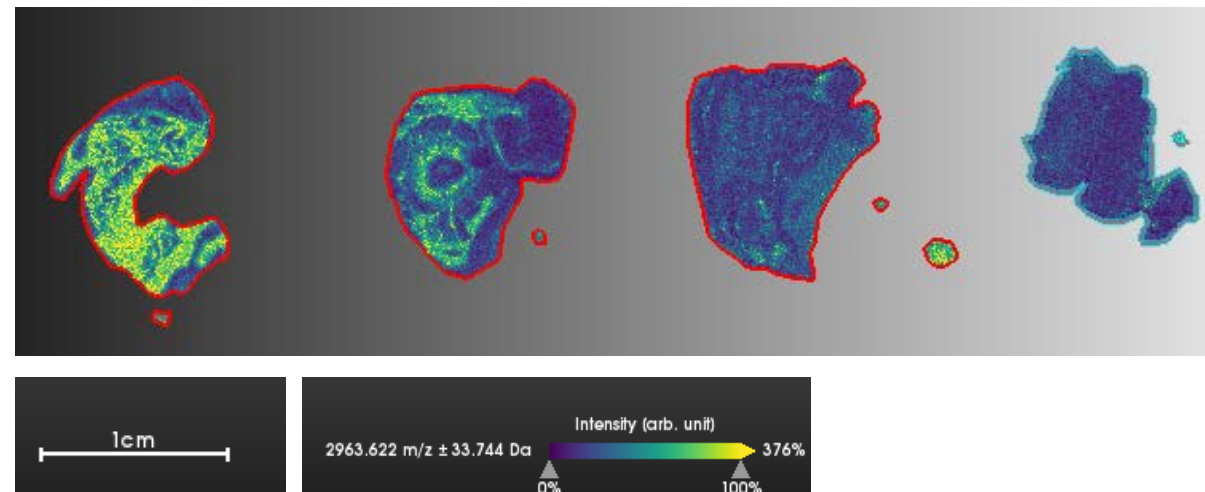
- Experimenter, instrumentation, ...



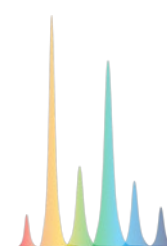
Batch effects between pixels and tissue sections



Peptide MSI of tissue sections from same patient on same slide after tryptic digest:

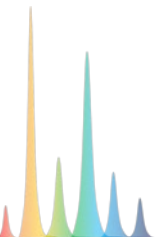
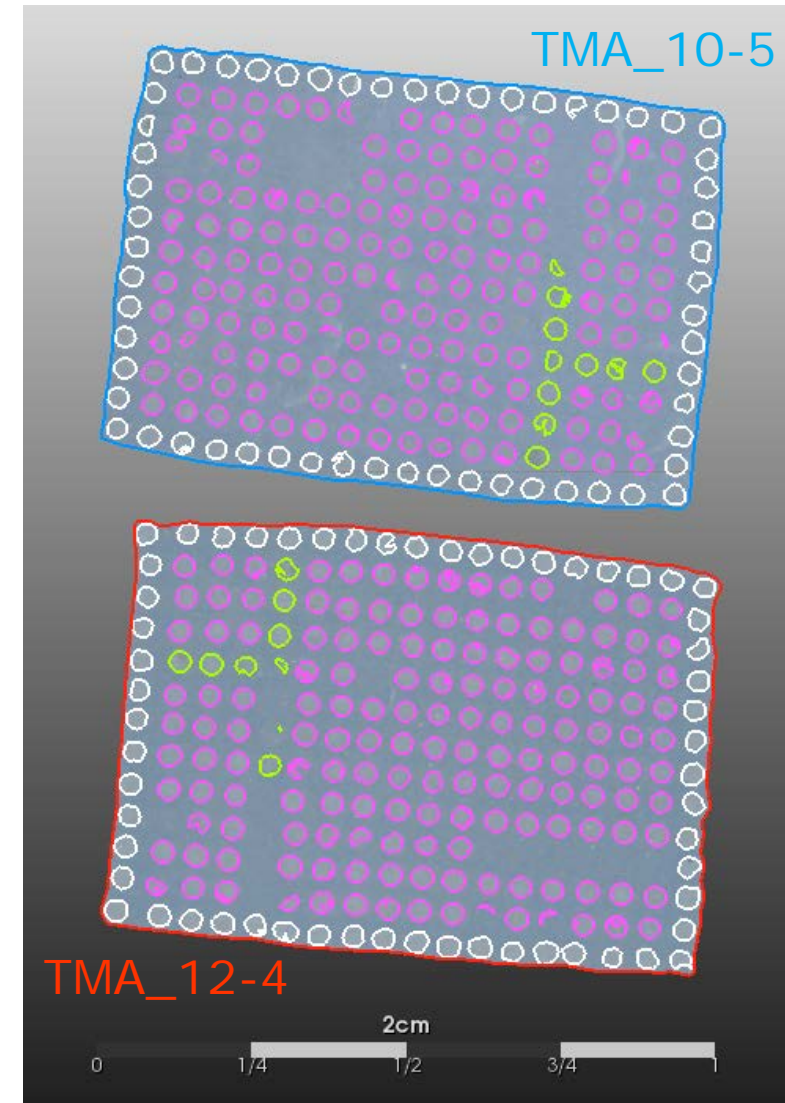


Solution: randomization of sections on slide



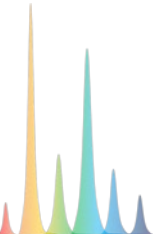
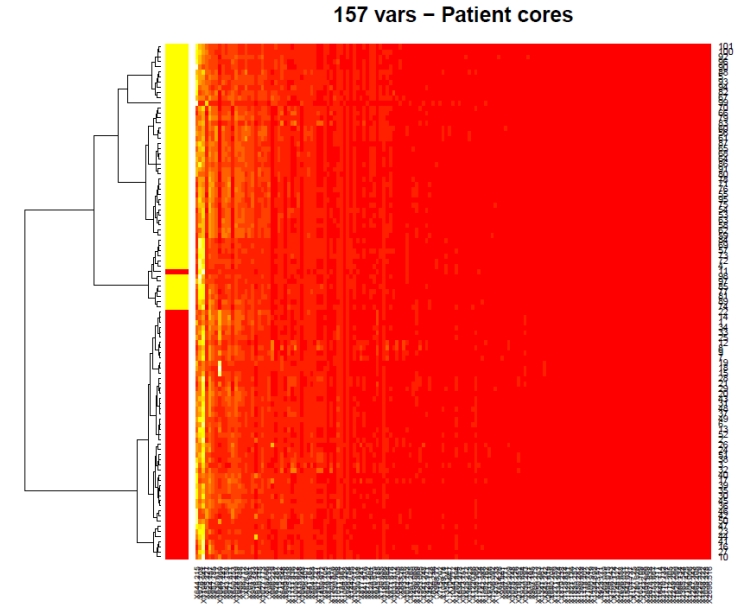
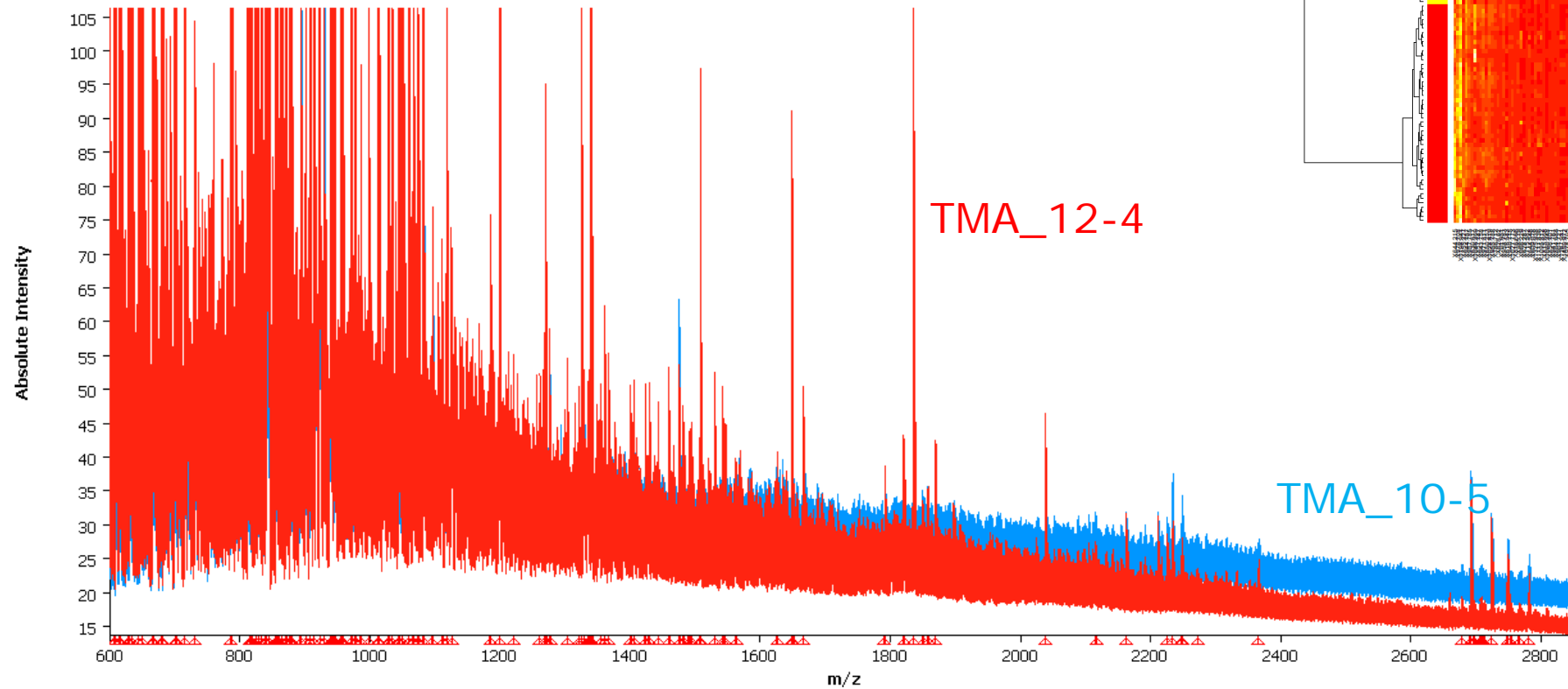
Batch effects between slides

- 2 gastric cancer tissue microarrays
- $50+51 = 101$ patients ■
- 2-3 cores per patient
- Clinical data:
 - TNM, G
 - Hist. type
 - Survival
- 58 wall cores of cow liver □
- 9 matched human control samples ■

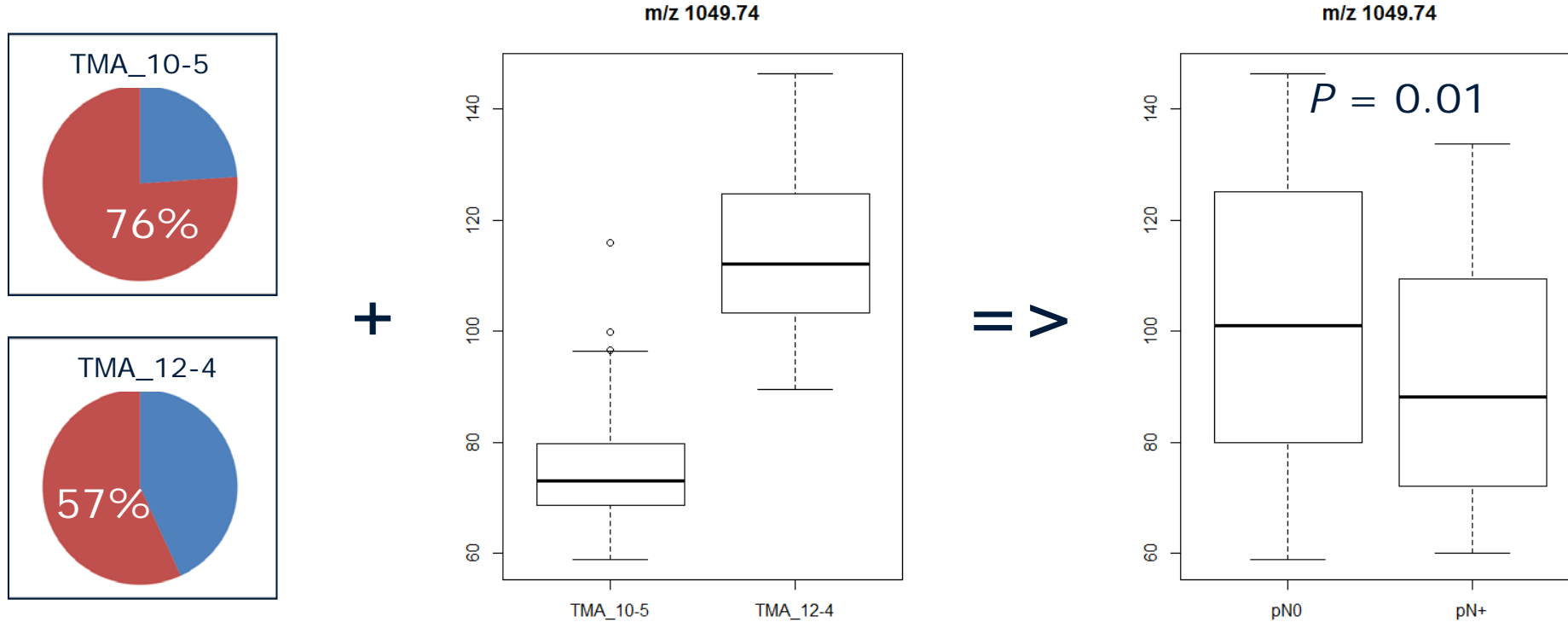


TMA = batch effect

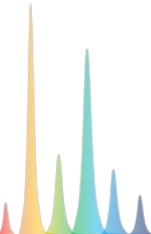
- Average peptide MSI spectra
- After TopHat baseline subtraction:



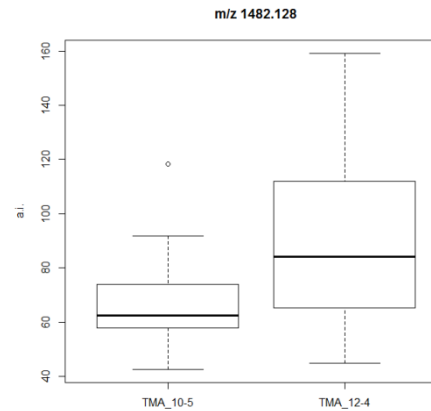
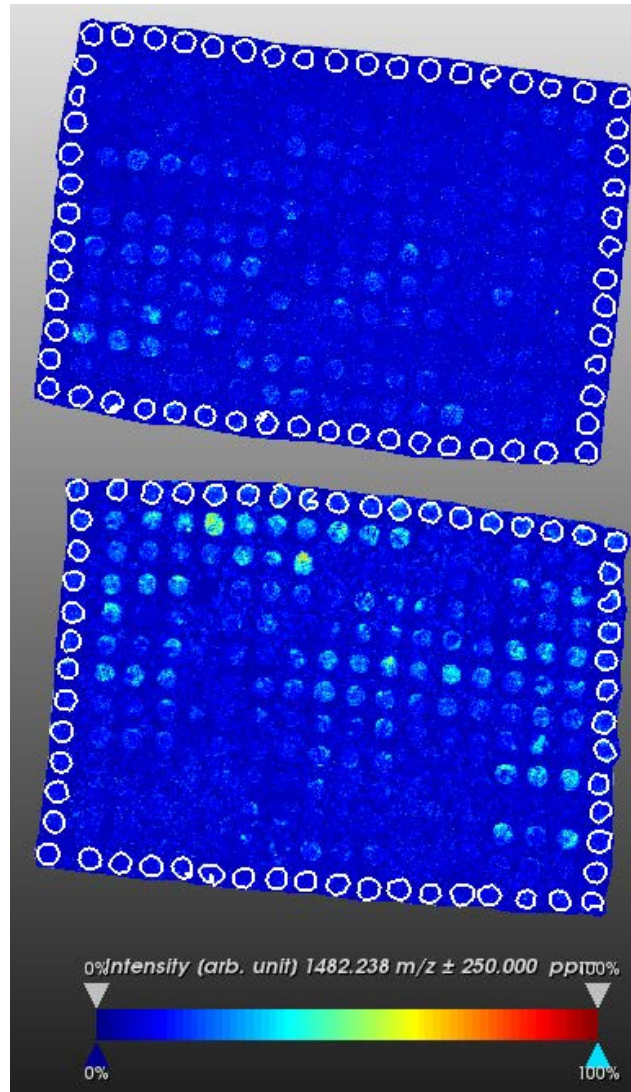
Implications: how to distinguish biological from technical effects?



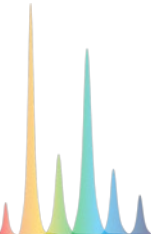
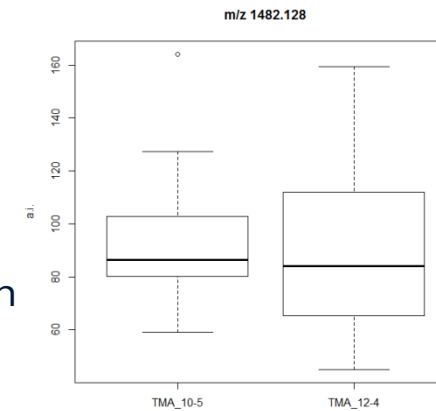
- Metastasized (pN+)
- Metastasis-free (pN0)



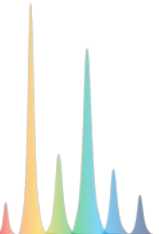
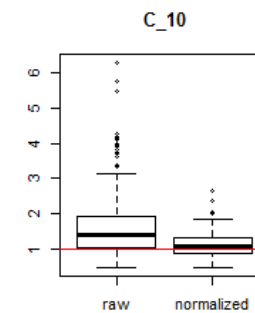
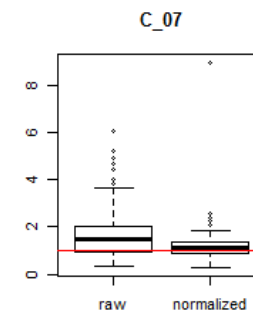
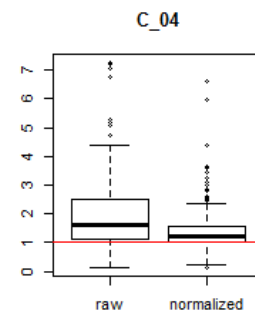
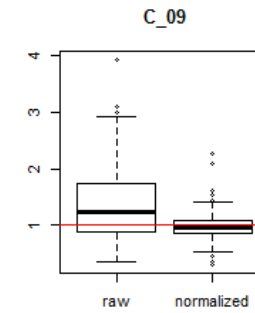
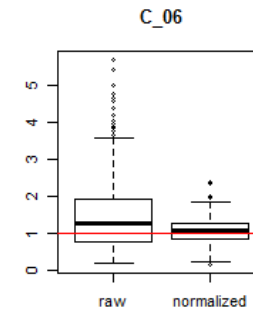
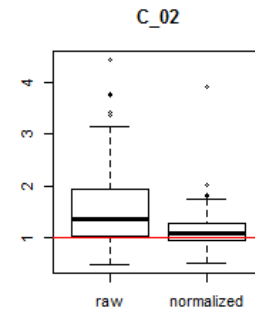
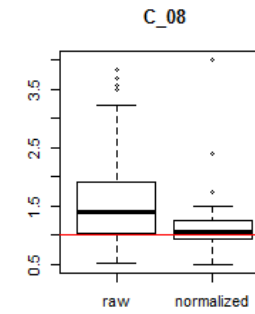
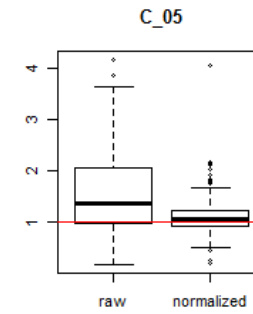
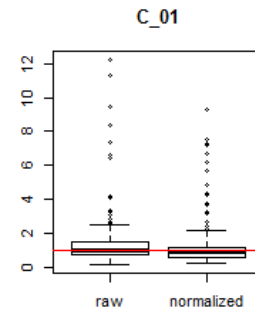
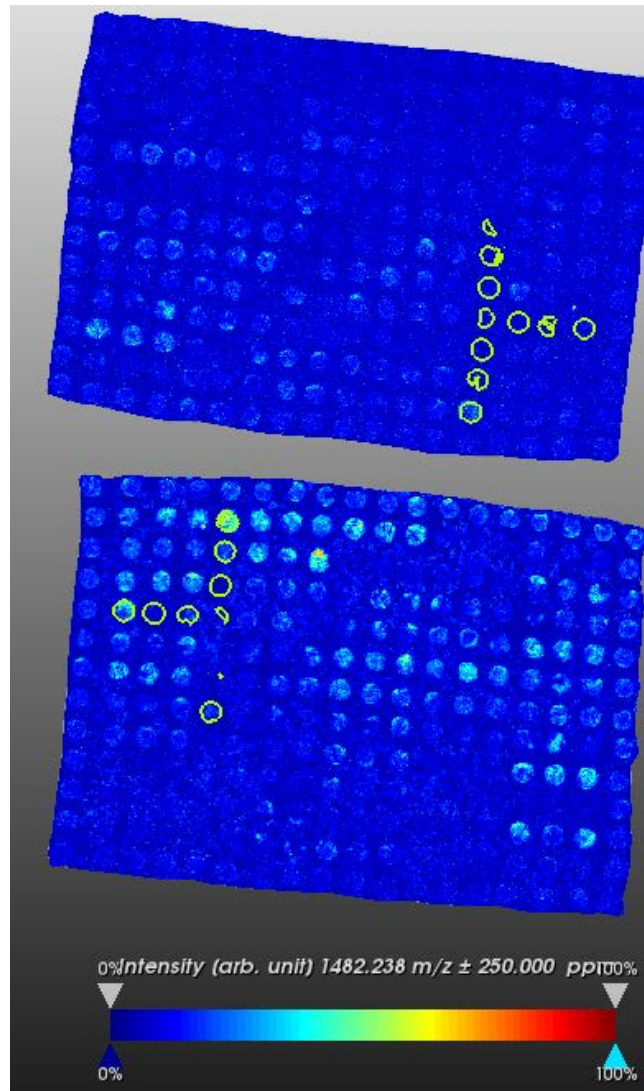
Can we use wall cores for normalization?



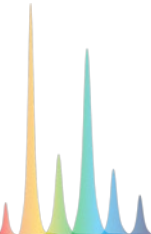
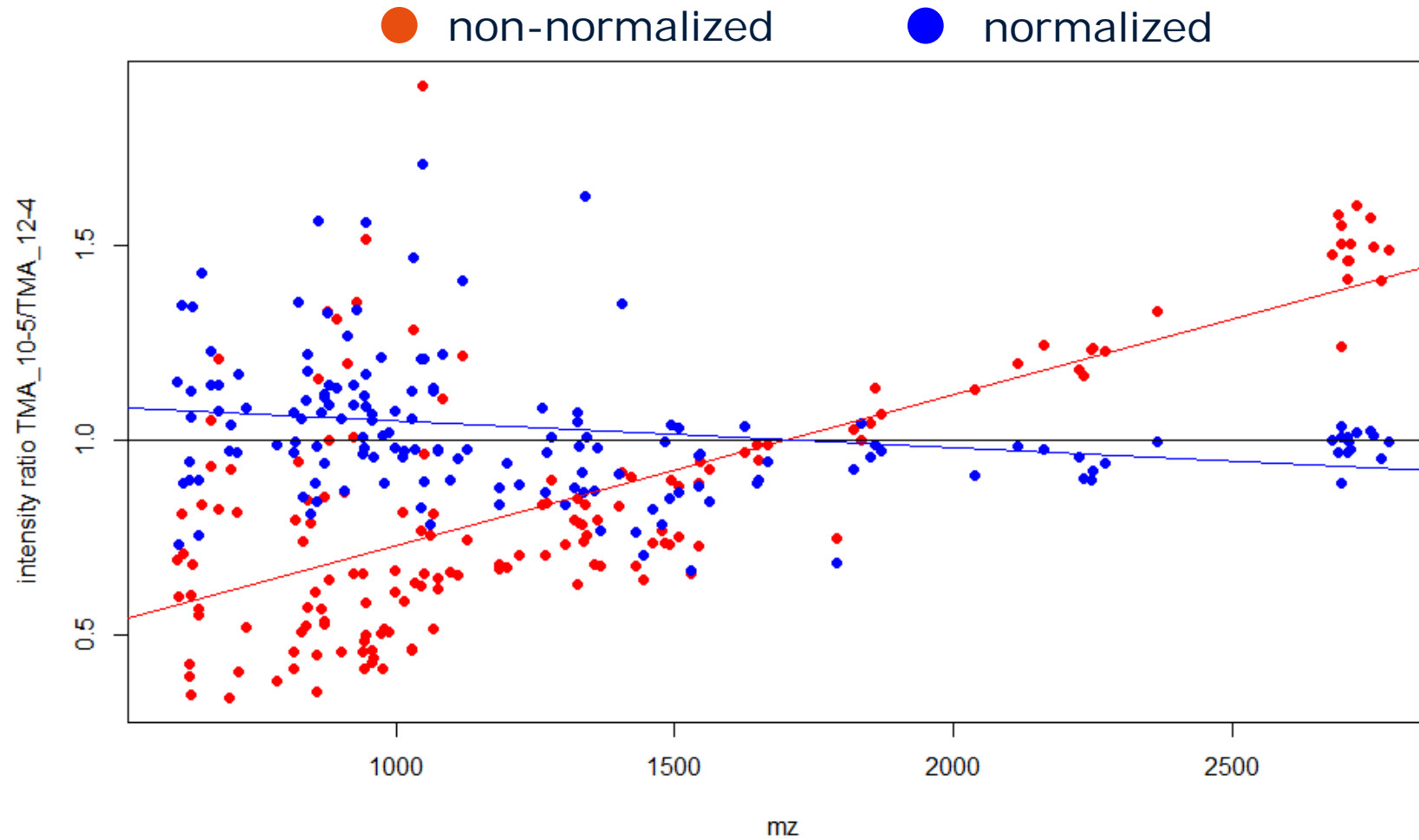
Correction factor x



Verification on internal controls

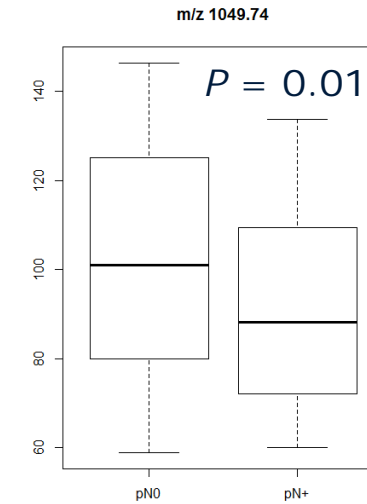
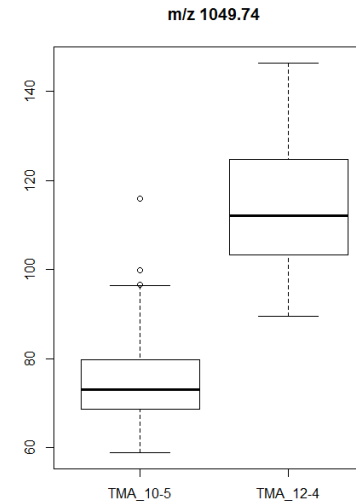
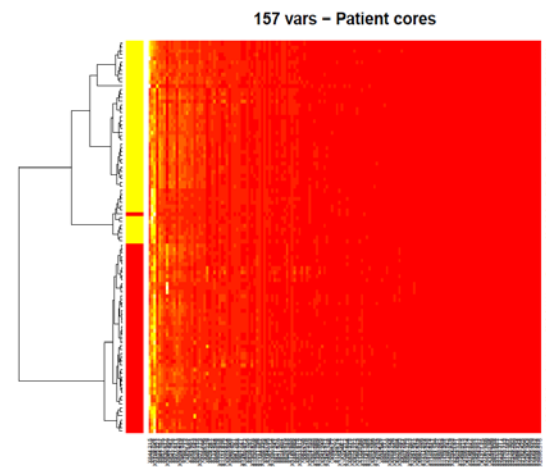


Normalization of patient cores

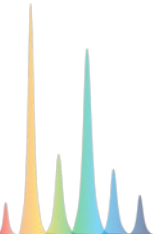
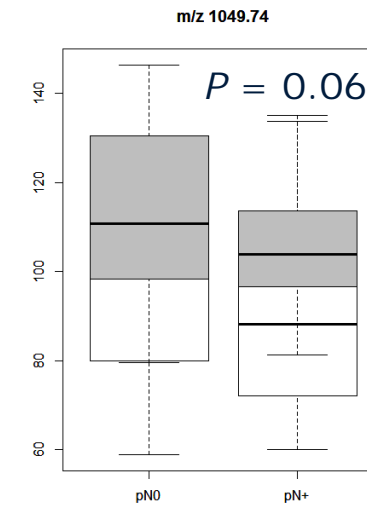
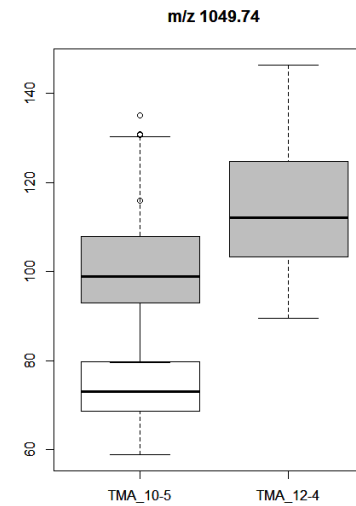
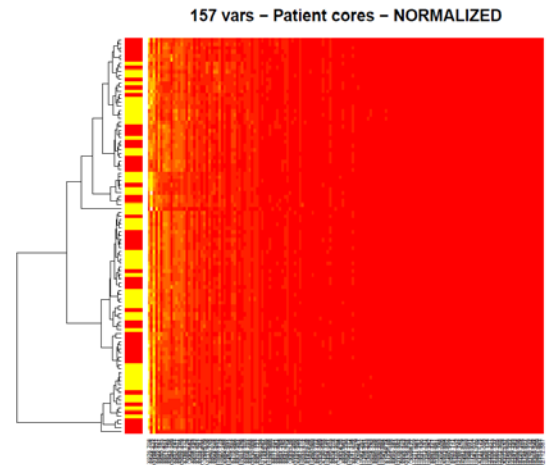


Statistics on normalized patient cores

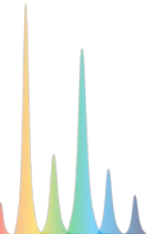
Raw:



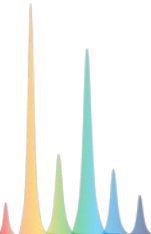
Normalized:



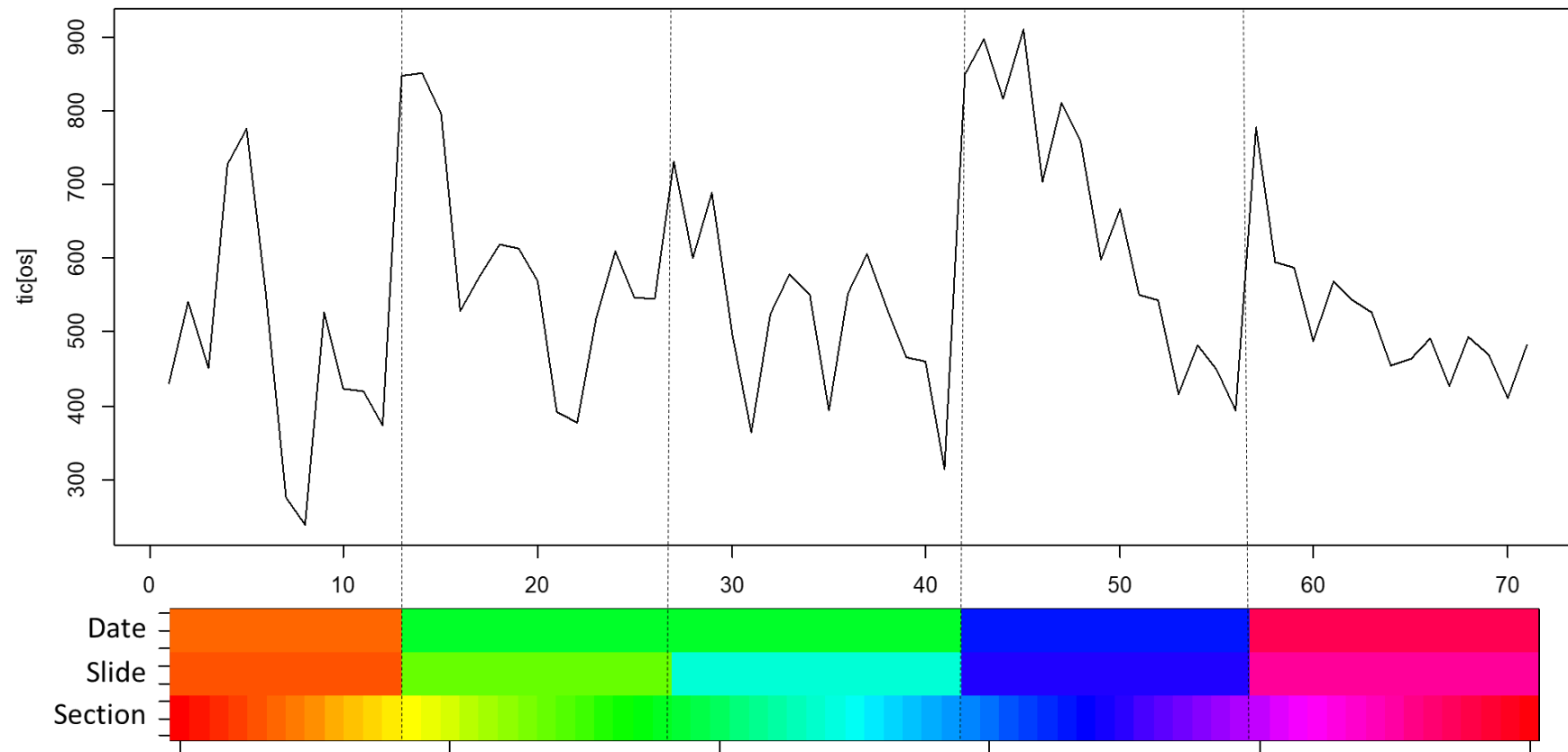
Batch effects in 3D MSI



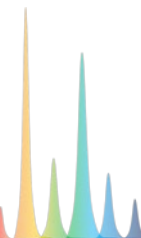
Batch effects in 3D MSI



Time and slide effect in 3D data

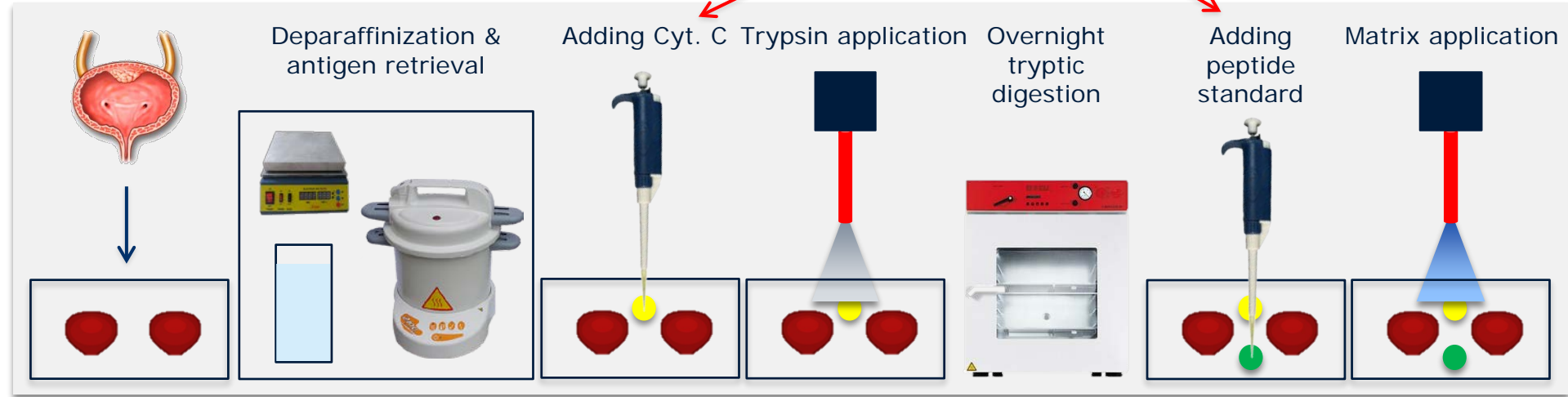


Solution: randomization of slides and tissues on slide

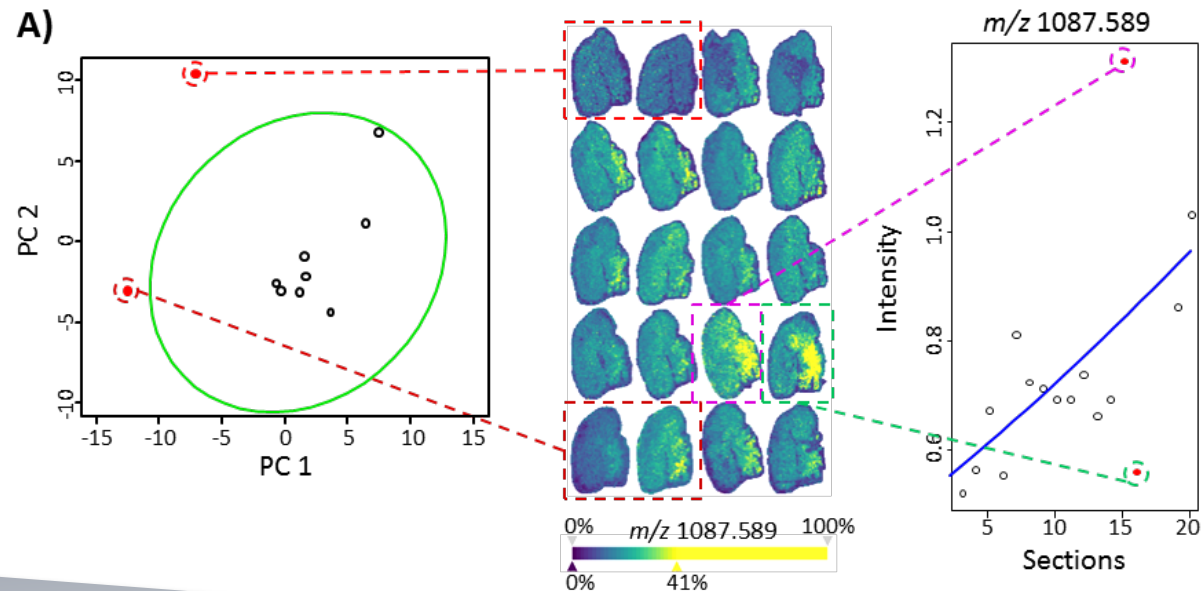


3D MSI: outlier detection

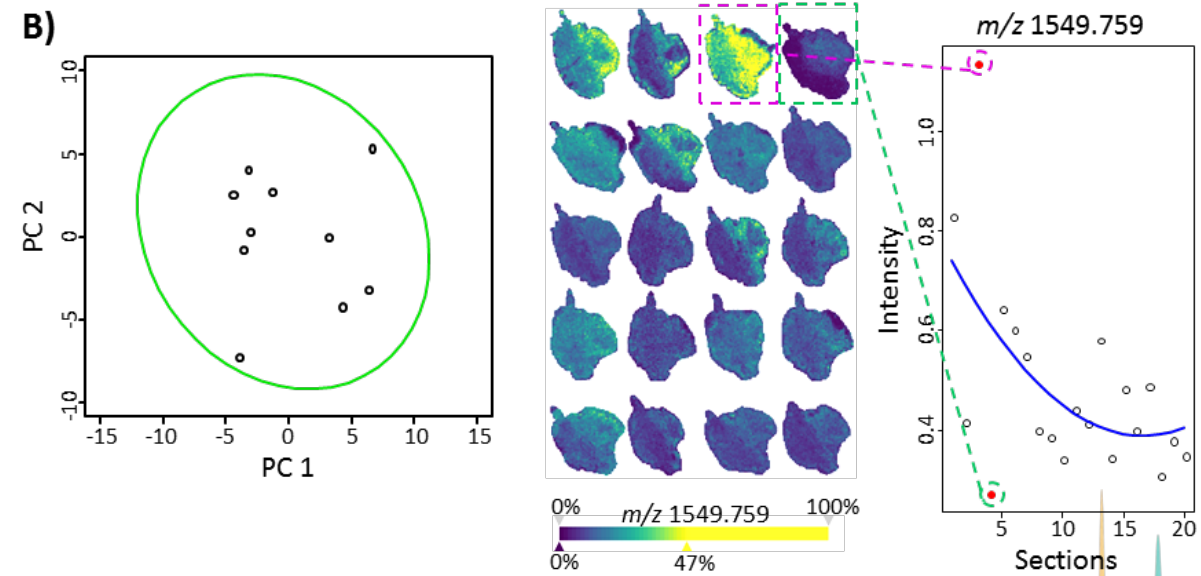
Quality control



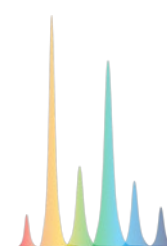
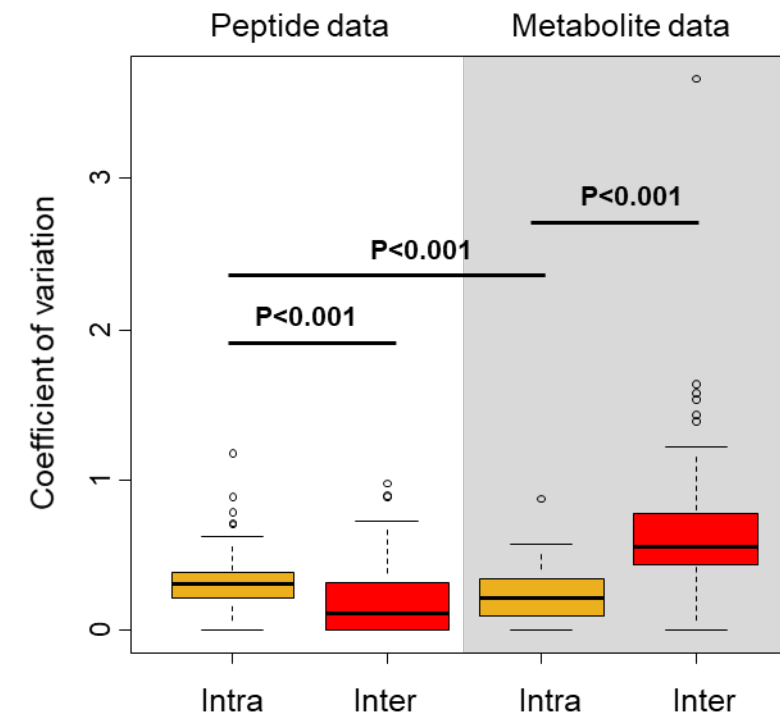
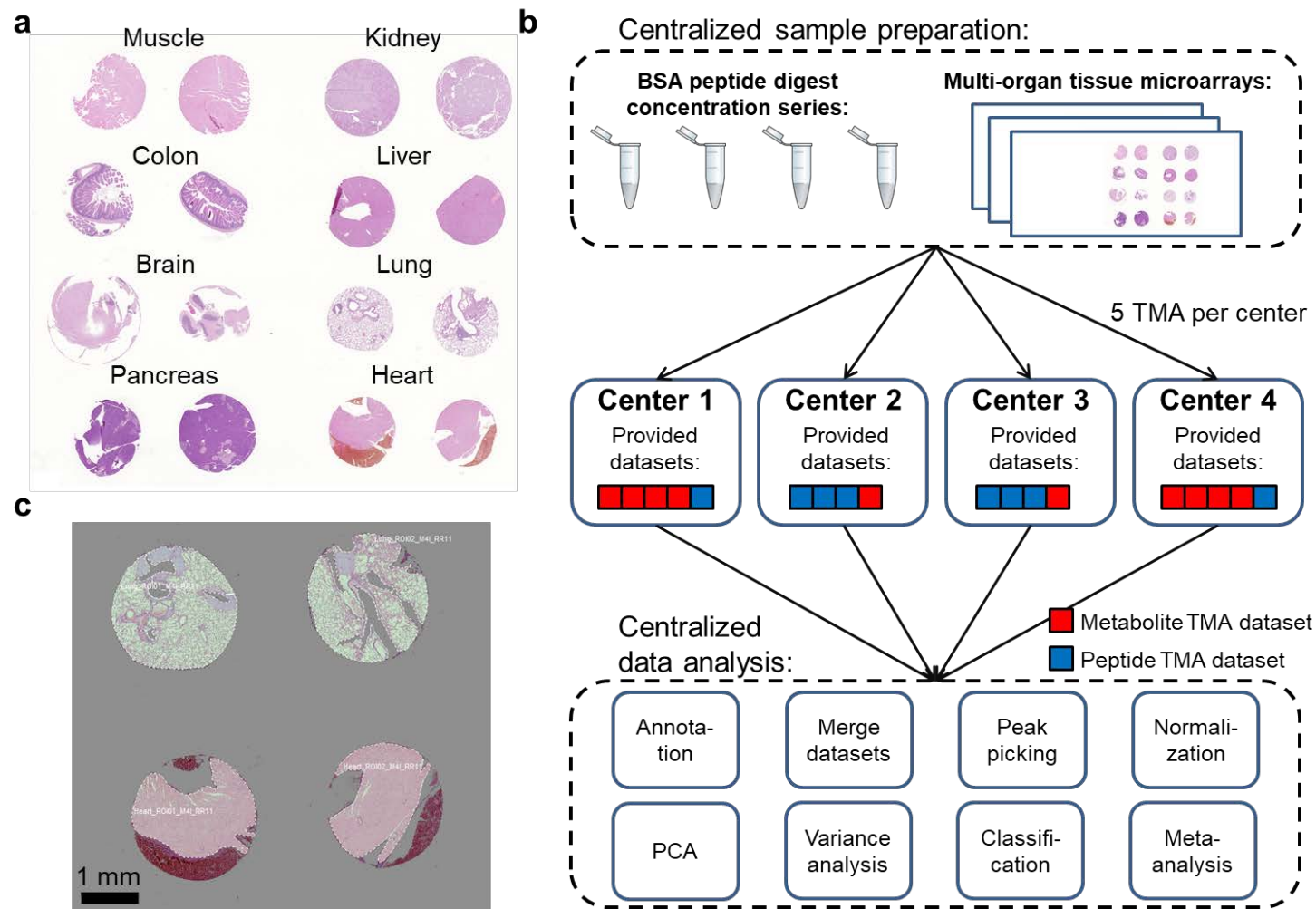
A)



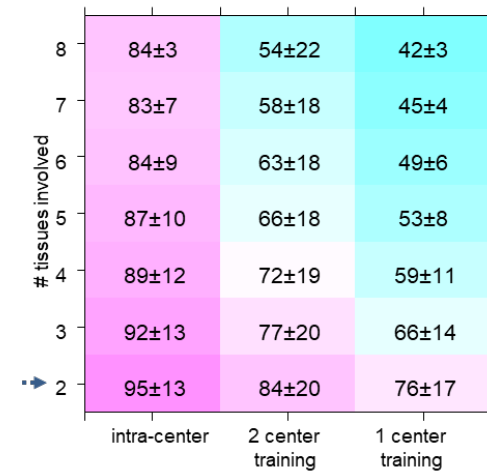
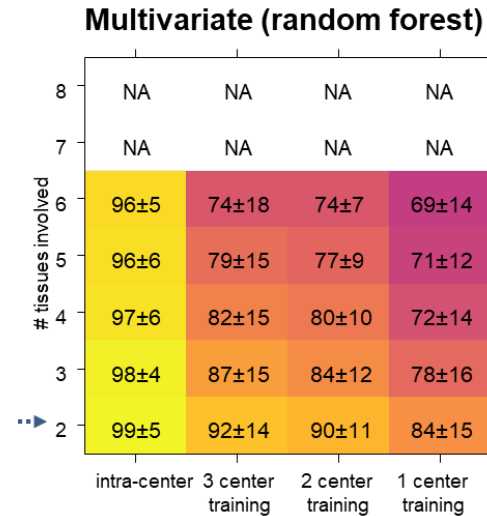
B)



Batch effects between centers



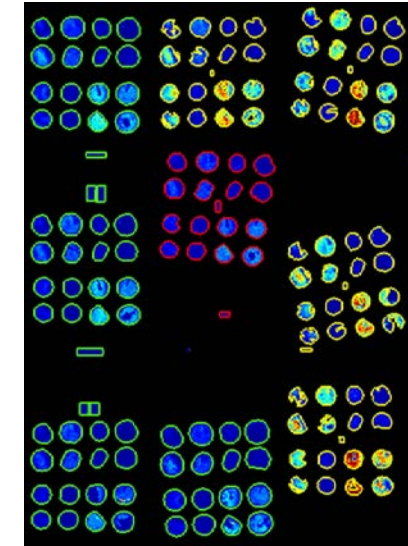
Absolute vs. relative effects



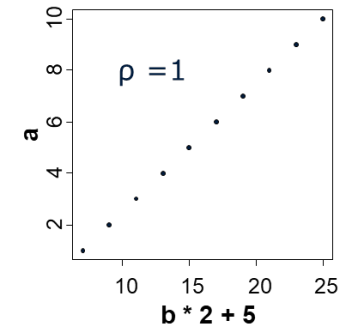
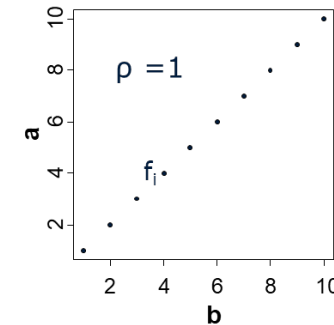
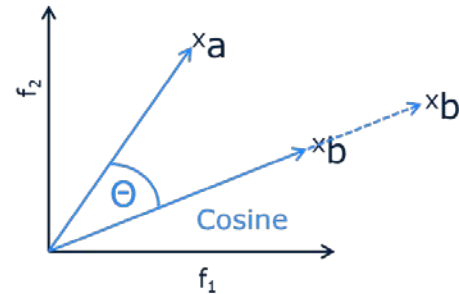
Correlation coefficients between centers:

Molecular class	Tissue	Intra-experiment	Intra-center	Inter-center
Peptides	Brain	0,96	0,88	0,72
Metabolites	Heart	0,98	0,93	0,66
Metabolites	Brain	0,96	0,91	0,65
Metabolites	Colon	0,99	0,92	0,58
Metabolites	Liver	0,94	0,86	0,58
Metabolites	Pancreas	0,97	0,95	0,57
Metabolites	Lung	0,98	0,87	0,56
Metabolites	Muscle	0,98	0,91	0,55
Peptides	Pancreas	0,91	0,75	0,53
Metabolites	Kidney	0,97	0,83	0,46
Peptides	Colon	0,97	0,94	0,40
Peptides	Heart	0,96	0,69	0,37
Peptides	Muscle	0,99	0,92	0,35
Peptides	Lung	0,97	0,86	0,31

m/z 283.3; $r = 0.964$



Solution: robust similarity metrics

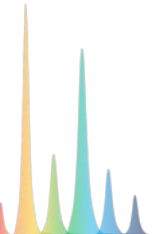


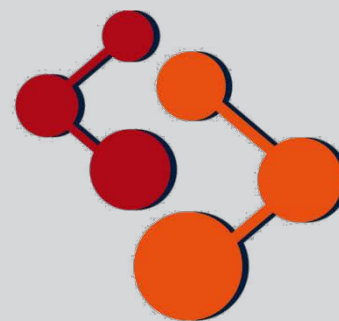
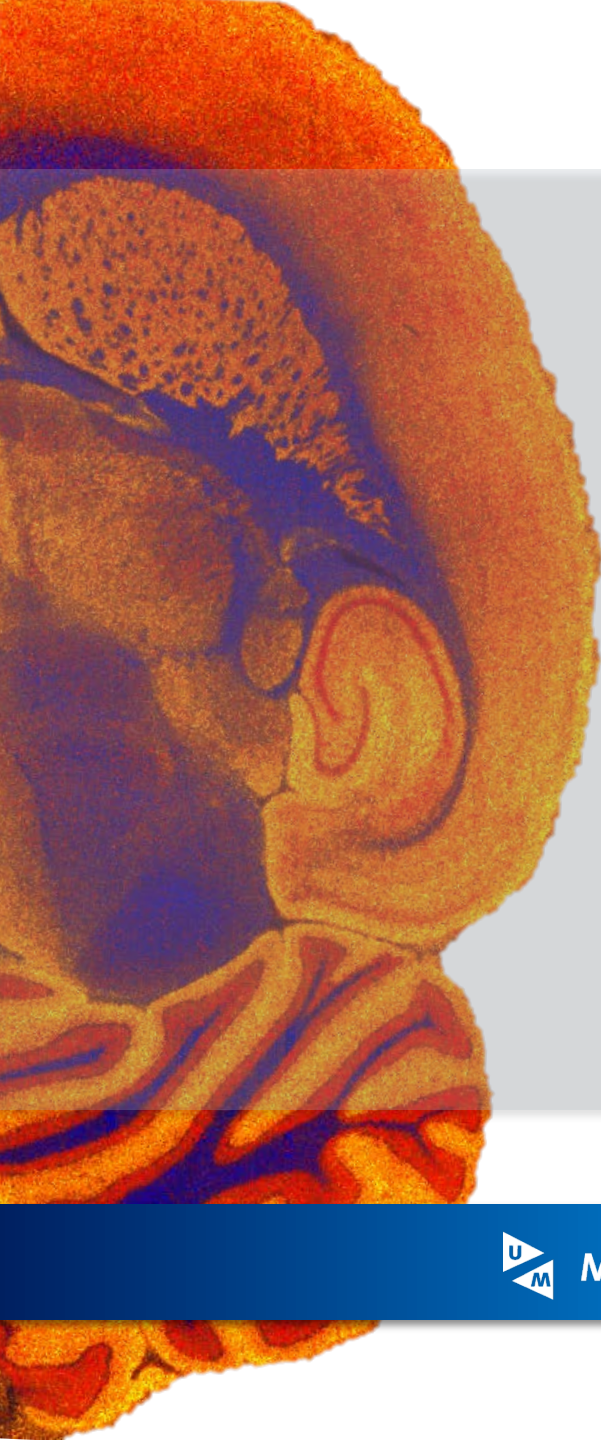
Conclusions

- (Block-) Randomize where possible!!
- Use robust similarity/distance metrics

There is still lot to do:

- Recipes to quantify (CV) batch effects in own workflow
- Establish internal quality controls for
 - instrument performance before and during measurements
 - sample preparation
- Establish new normalization methods
- More meta-studies on sample preparation protocols





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