

Imaging spatial omics: Single cells in context.



Ron M.A. Heeren

The Maastricht MultiModal Molecular Imaging Institute (M4i)

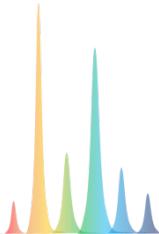
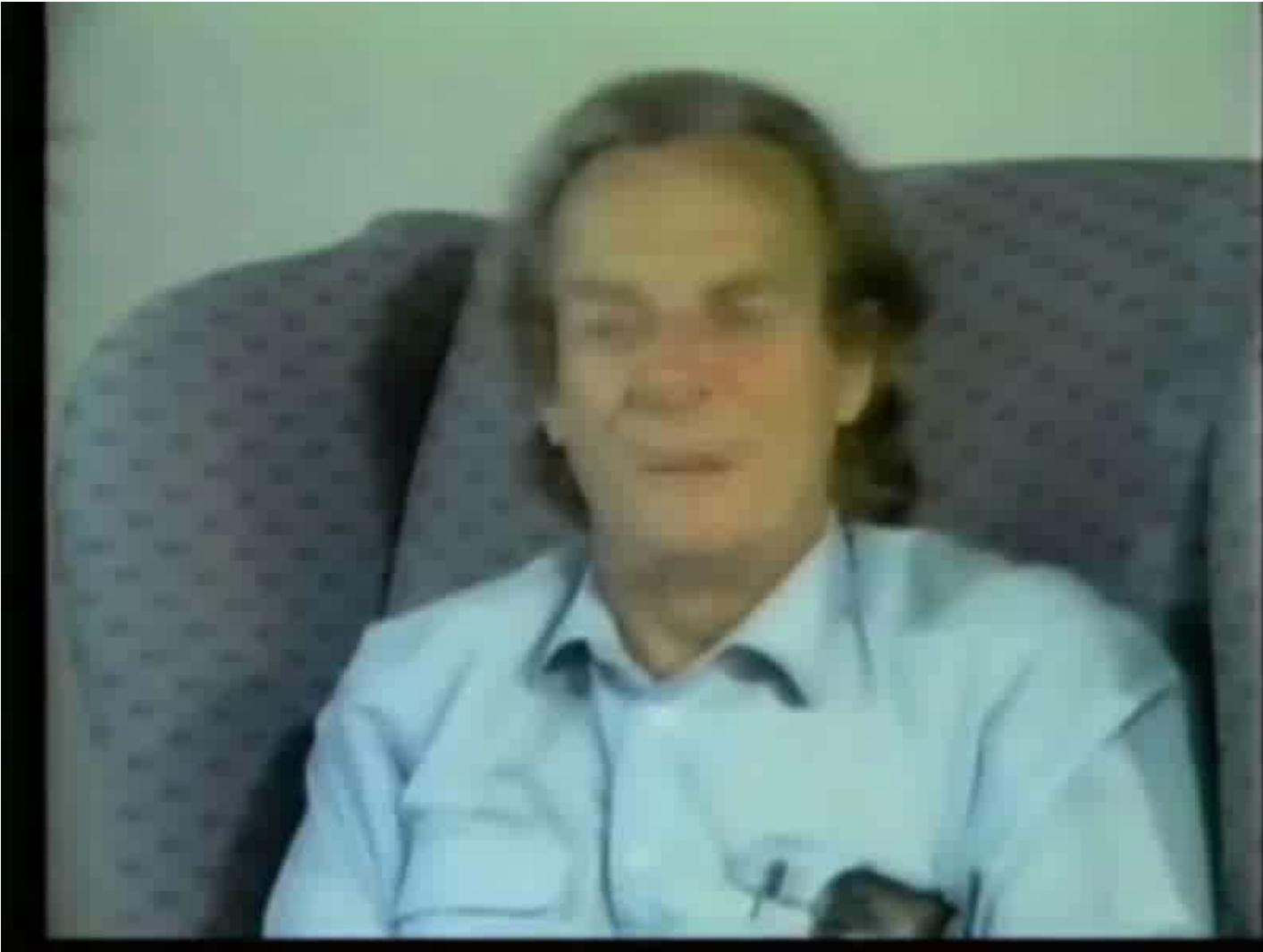
**X-omics festival,
Nijmegen, April 11th, 2022**



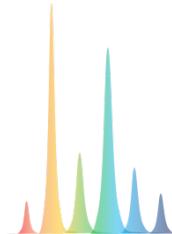
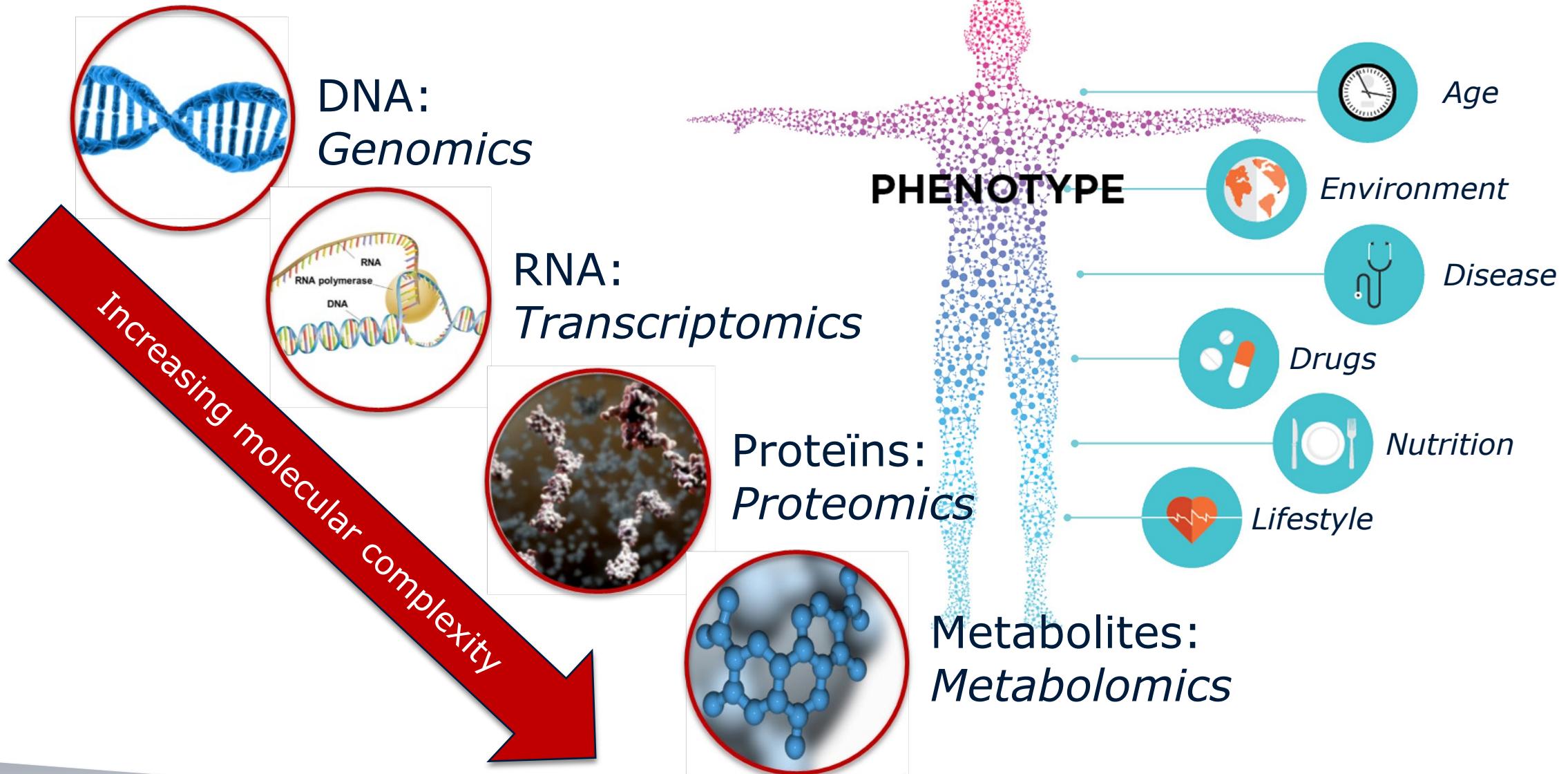
Maastricht University

Complexity of “Omics”

Richard Feynmann 1918-1988

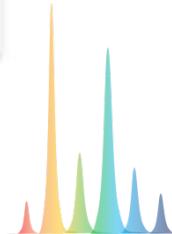
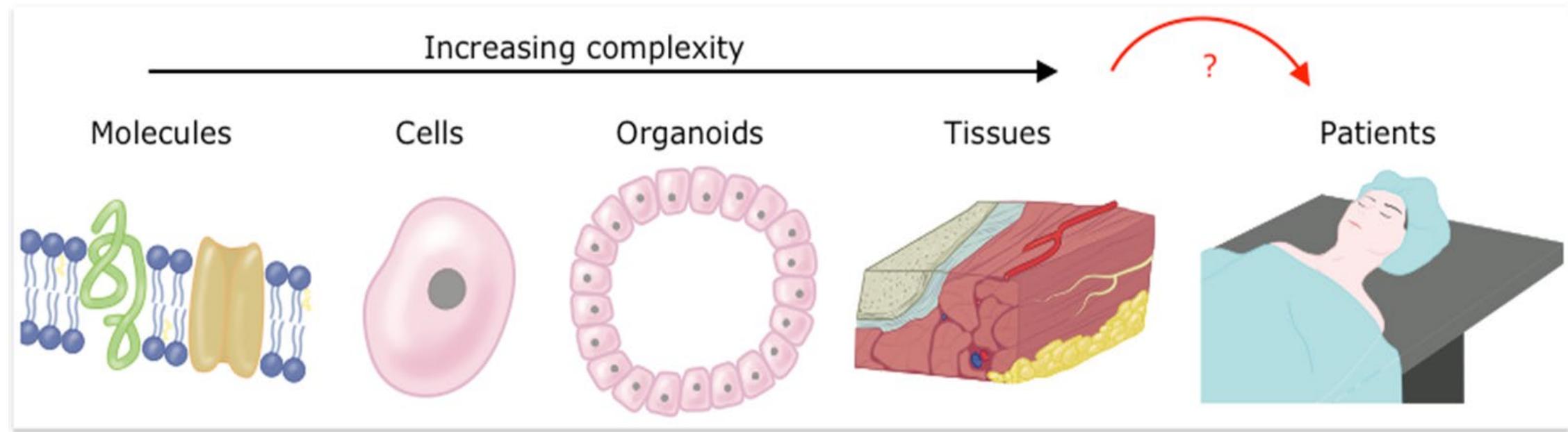


Who are we (at the molecular level)?



MSI in clinical diagnostics

- Fast diagnostics & prognostics: markers, therapy
- Tissue typing with (fast) digital pathology
- Personalized medicine: surgery guidance & in situ diagnosis



Untargeted versus Targeted

Targeted

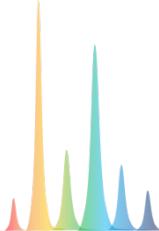


Visualizing specific molecules

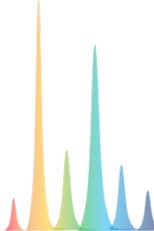
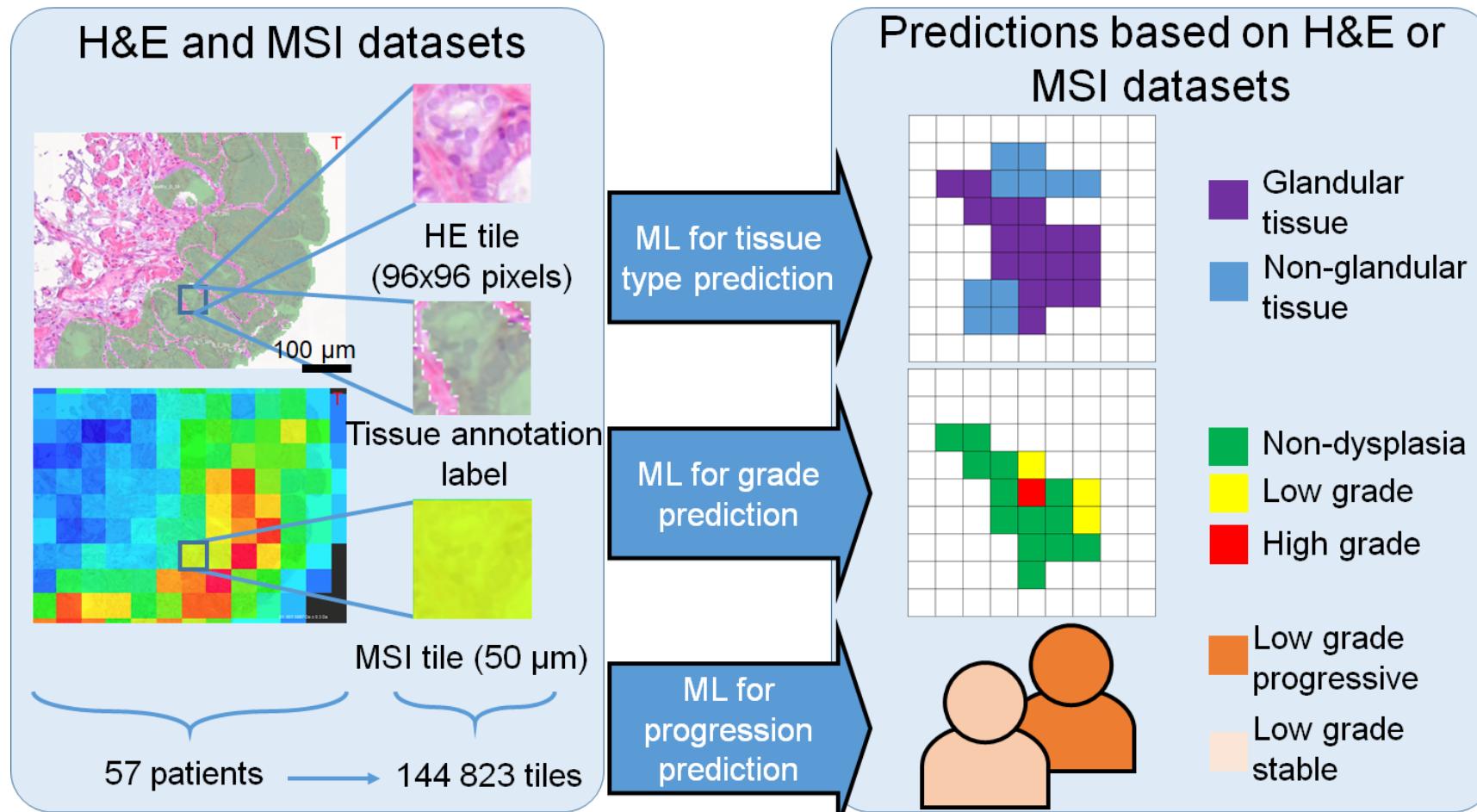
Untargeted



Visualizing as many molecules possible

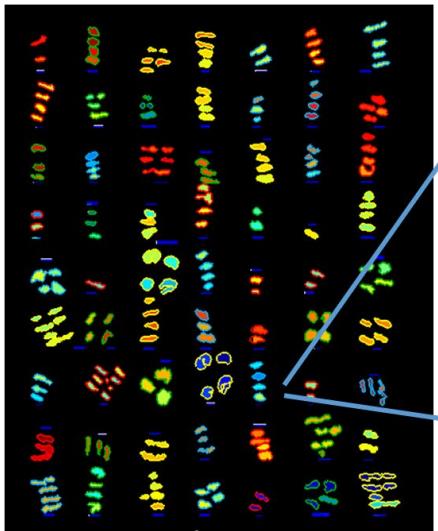


MSI, Pathology and Machine learning

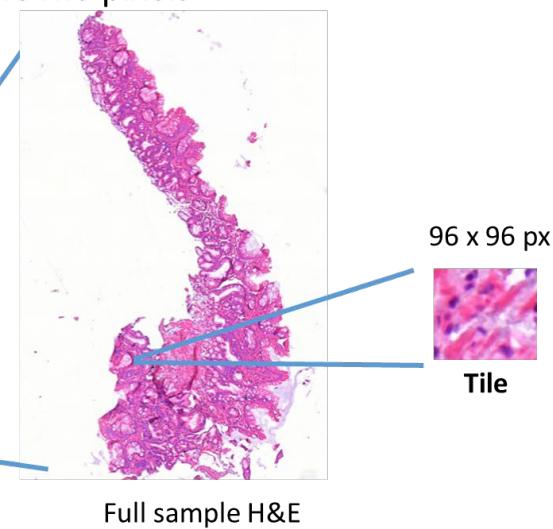


MSI grades tumors better than H&E-path

Dataset crops in tiles matching the MS pixels



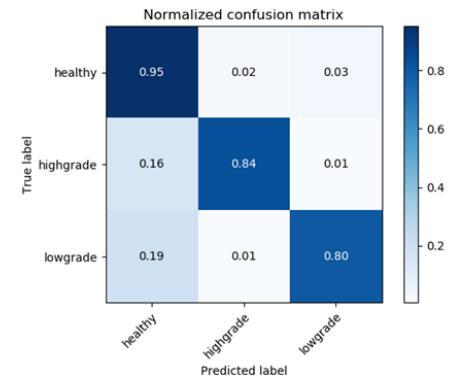
Representation of all the MSI acquisition



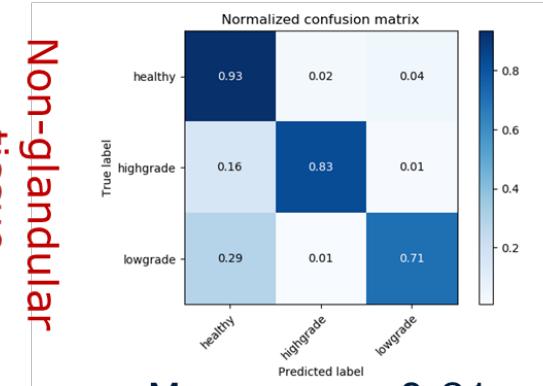
Full sample H&E

Glandular tissue
Non-tissue

MSI

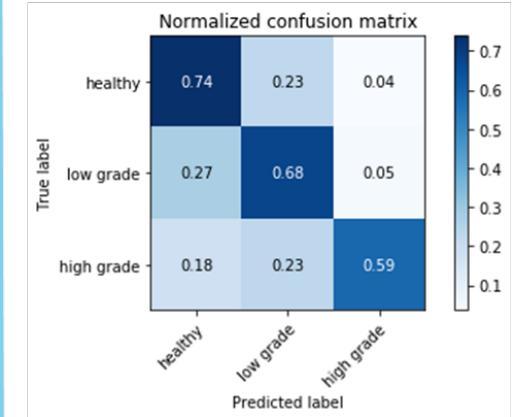


Mean acc = 0.86

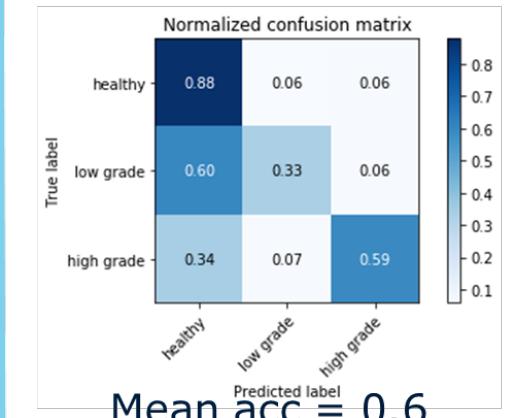


Mean acc = 0.81

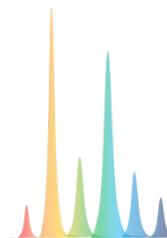
HE



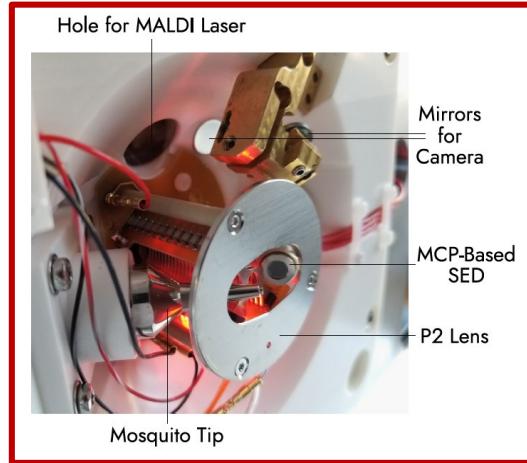
Mean acc = 0.67



Mean acc = 0.6



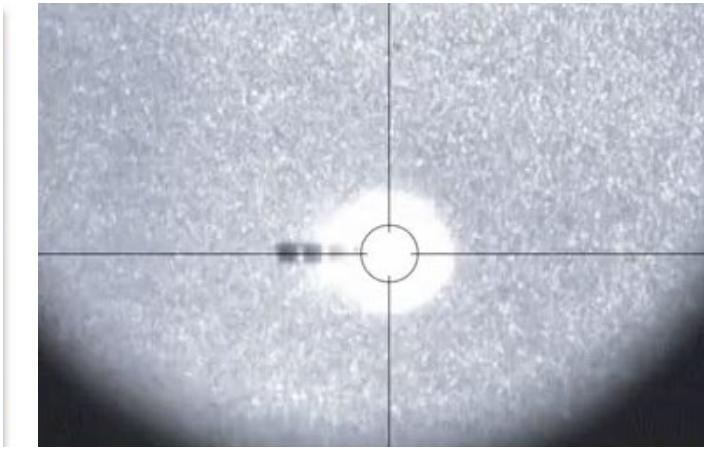
Adding value in molecular imaging



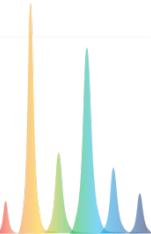
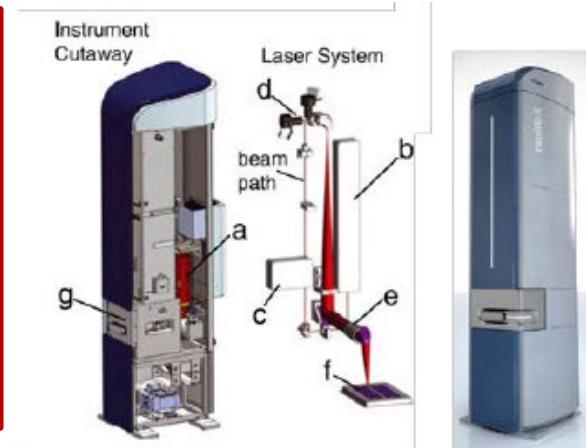
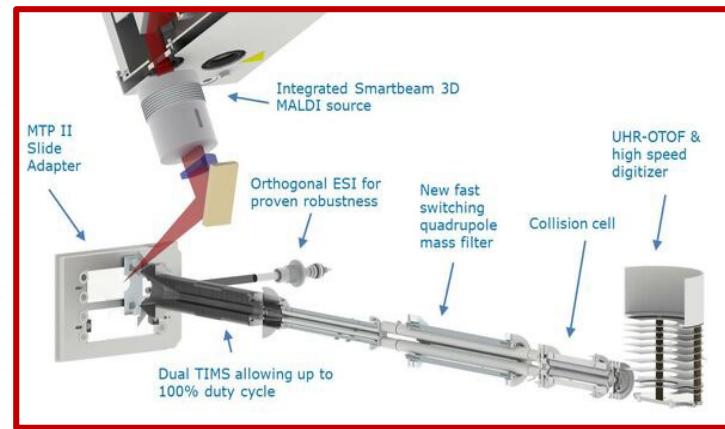
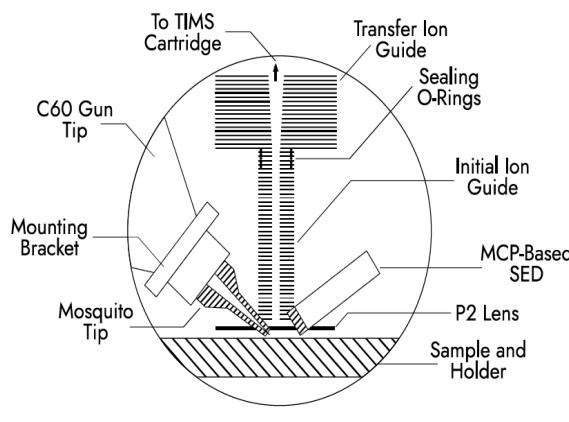
Spatial detail



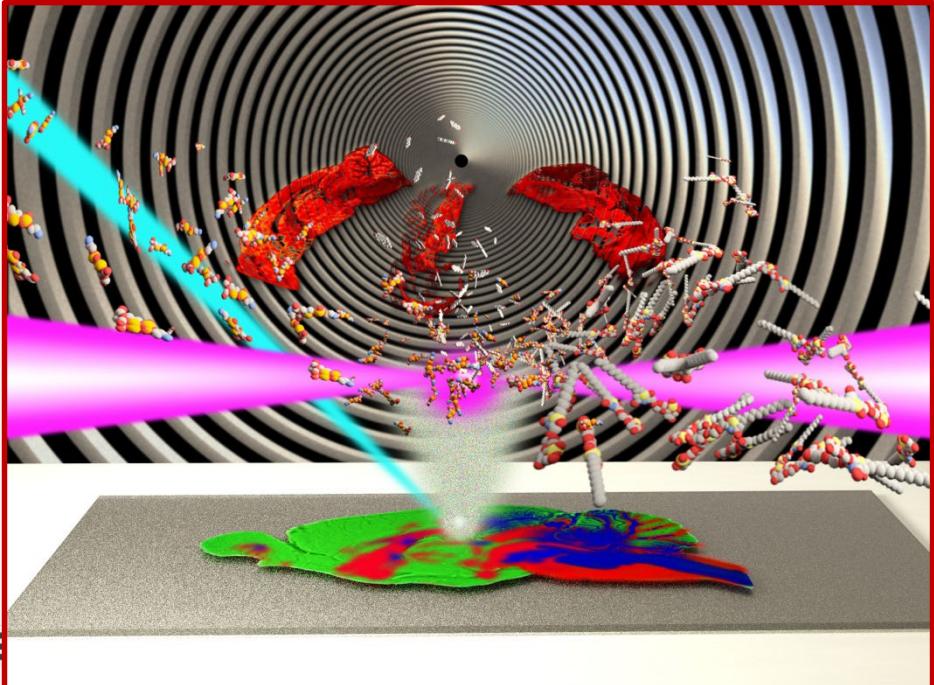
Molecular detail



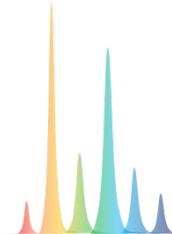
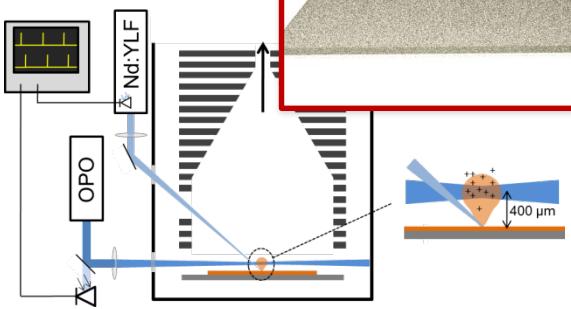
High throughput



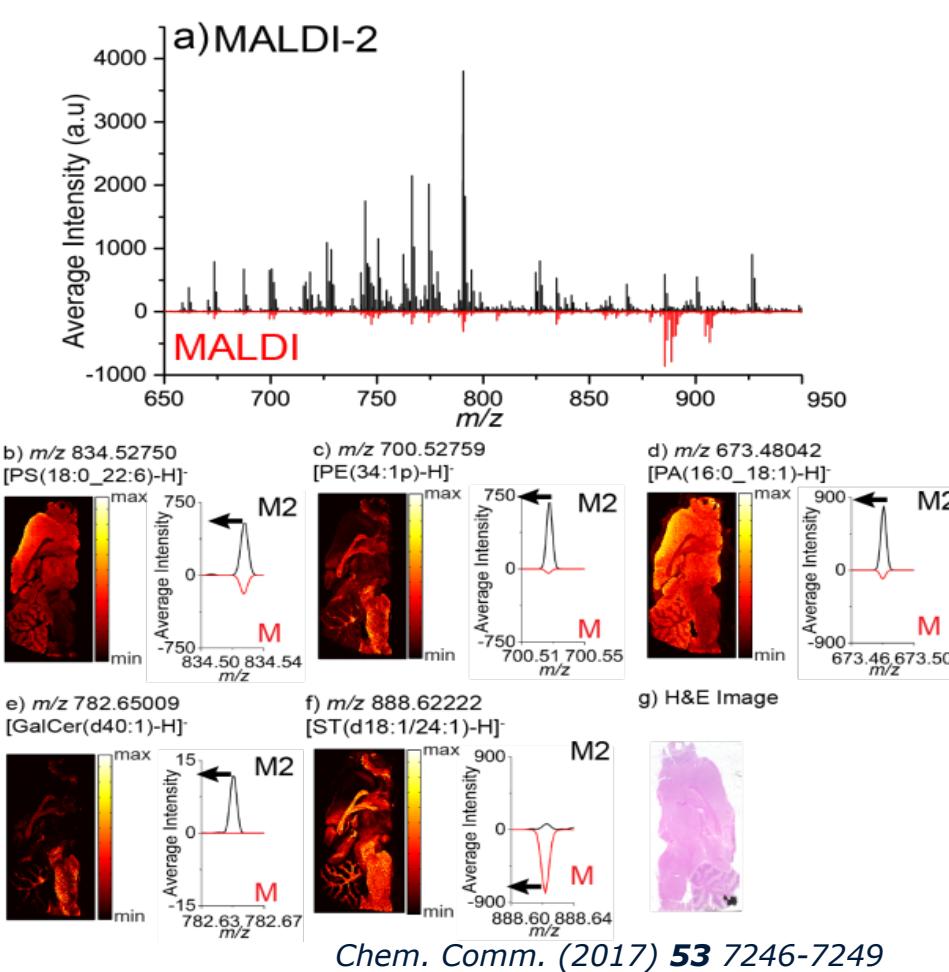
Improving Ionisation Efficiency: MALDI-2



Chem. Comm. (2017) 53 7246-7249

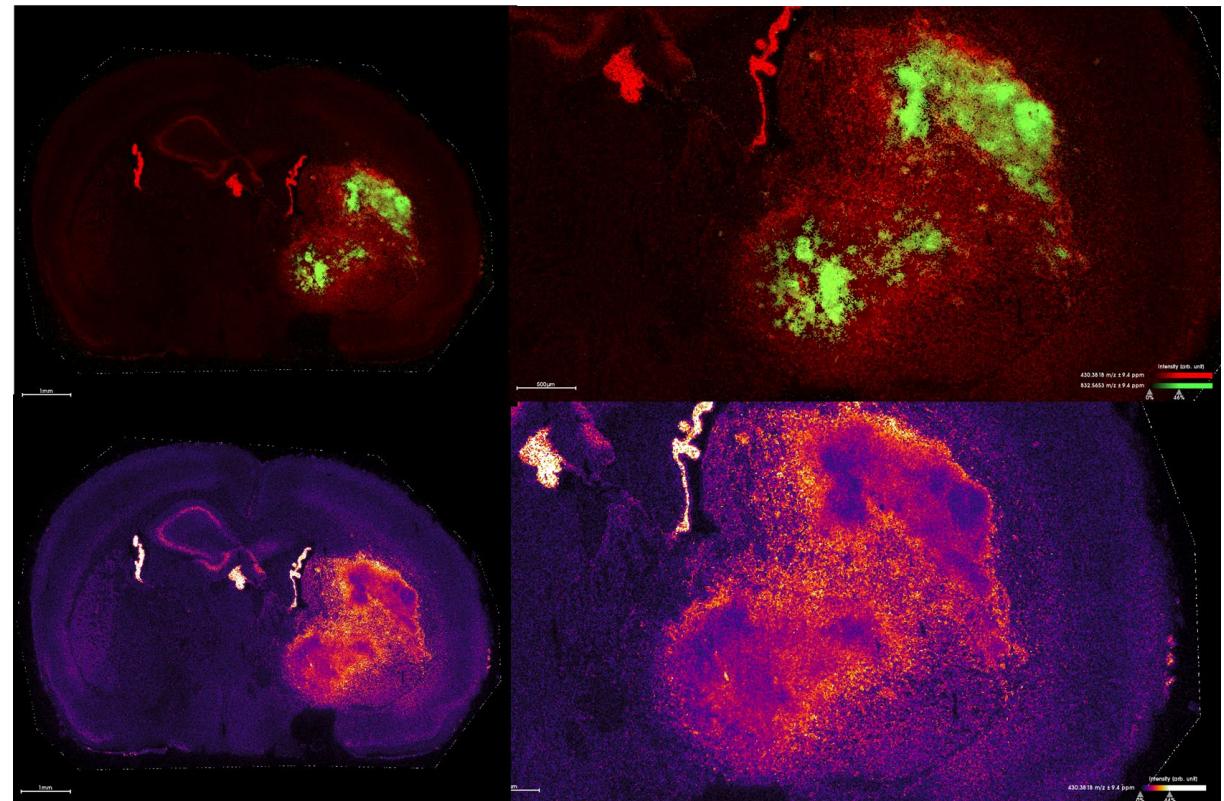


Lipidomics imaging with MALDI-2 MSI

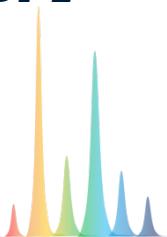


Chem. Comm. (2017) 53 7246-7249

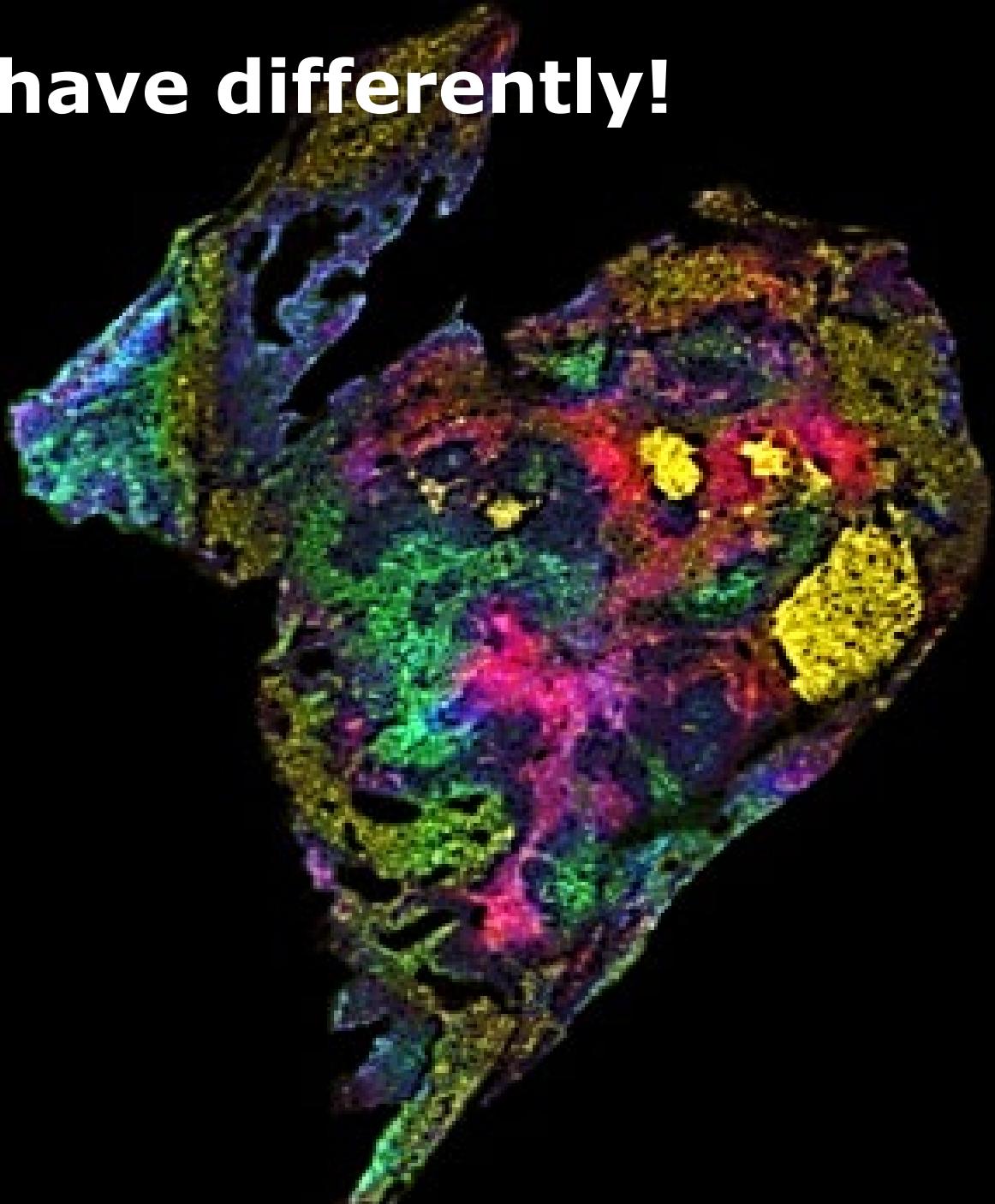
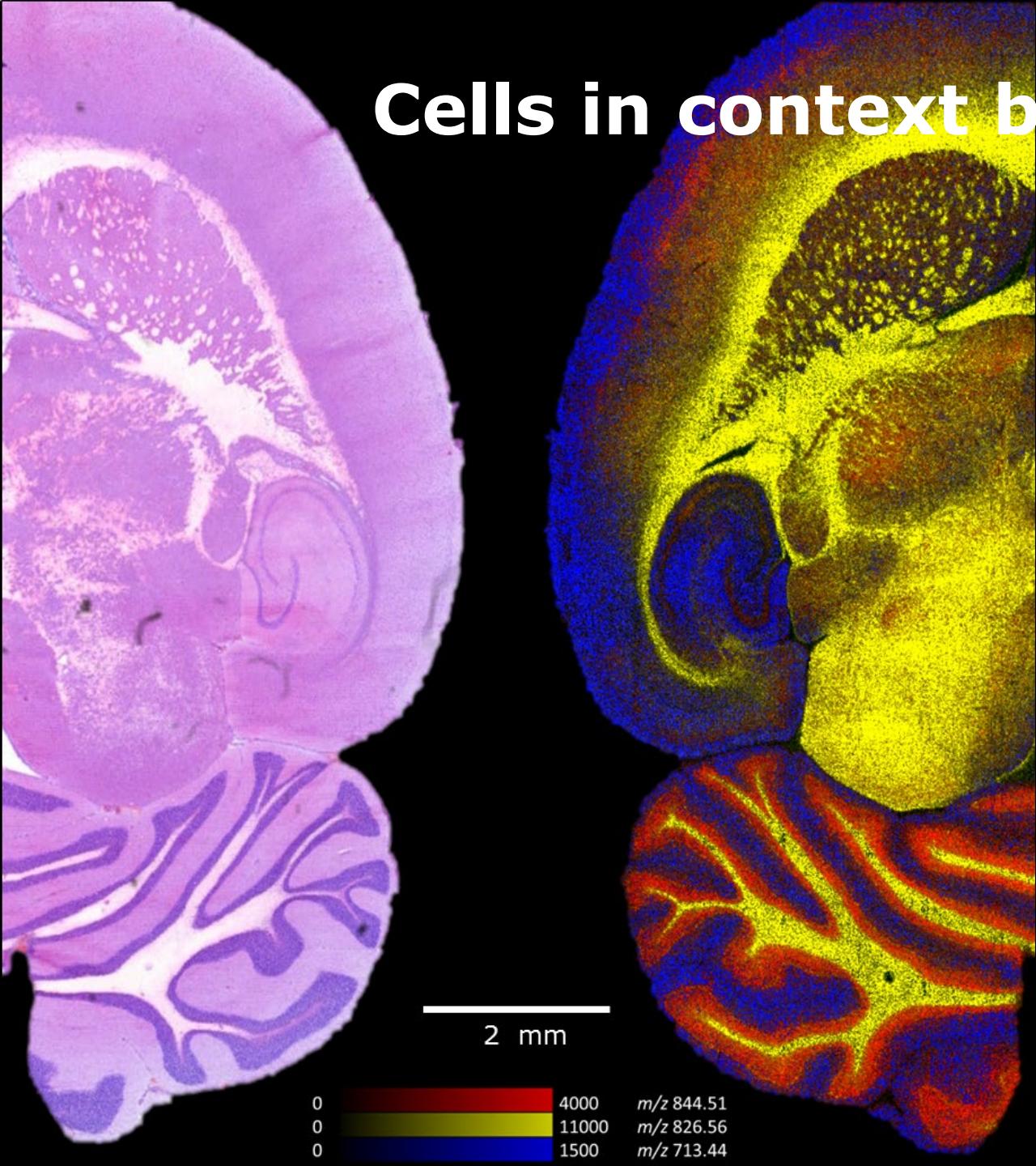
Human Glioblastoma

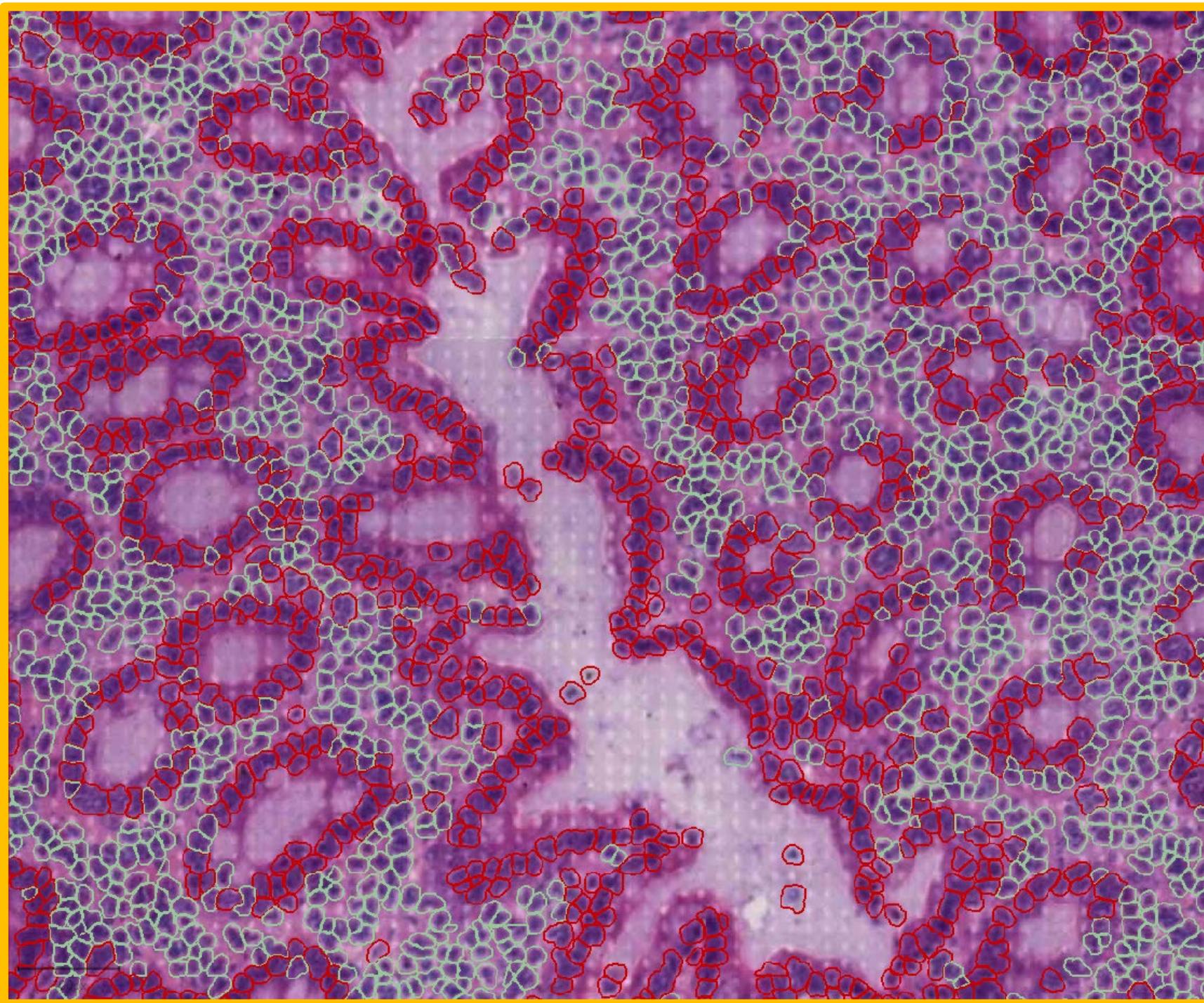


10 μ m, Norharmane MALDI-2



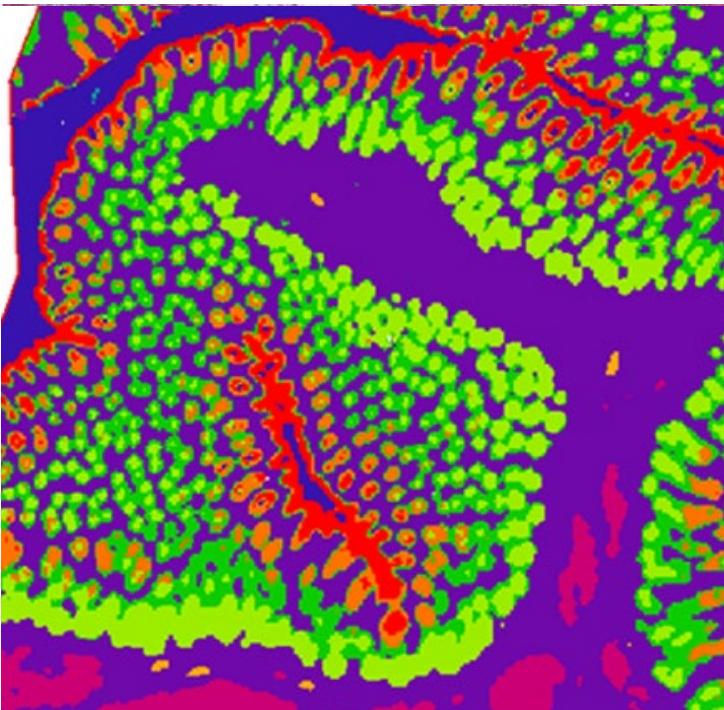
Cells in context behave differently!



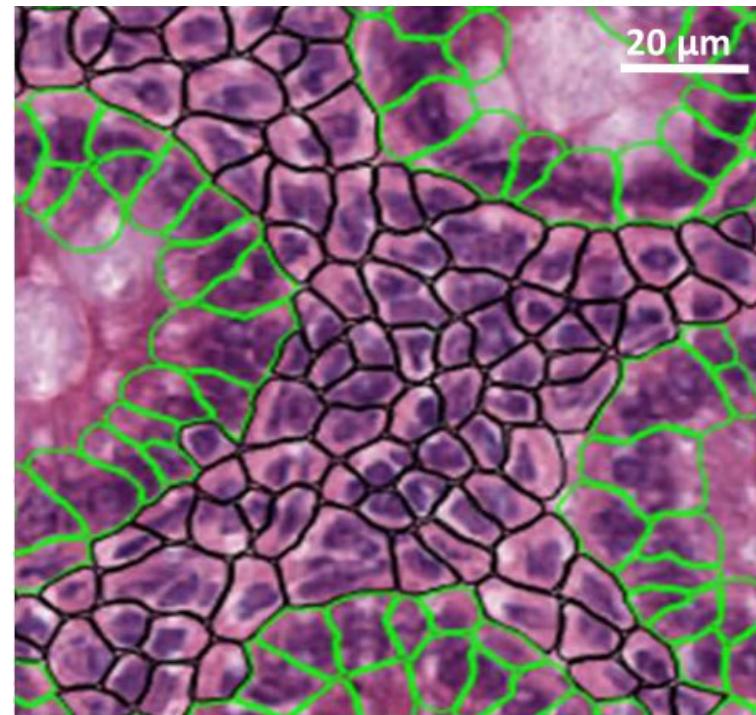


Contextual single cell imaging

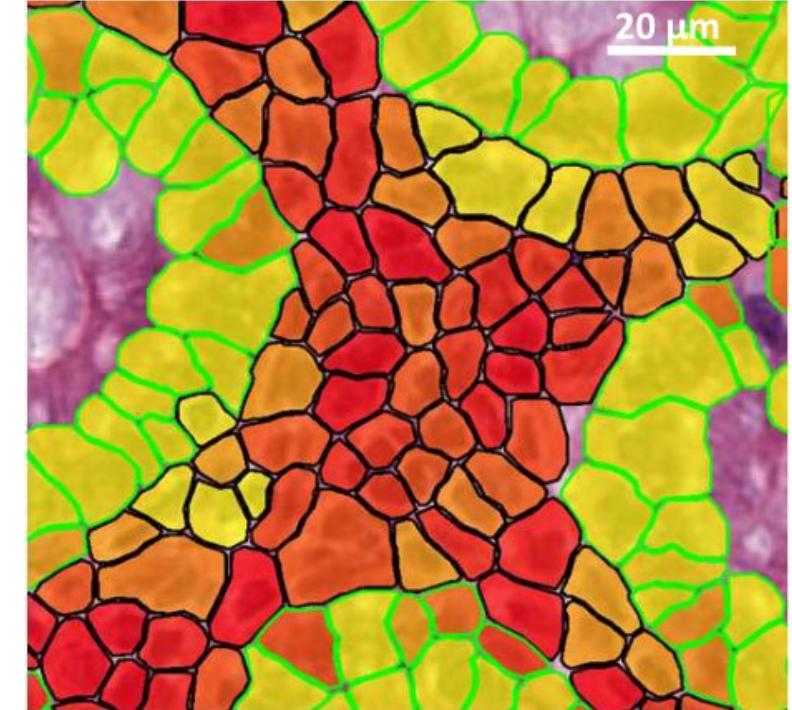
*Optical
Pathology*



*AI
Machine learning*

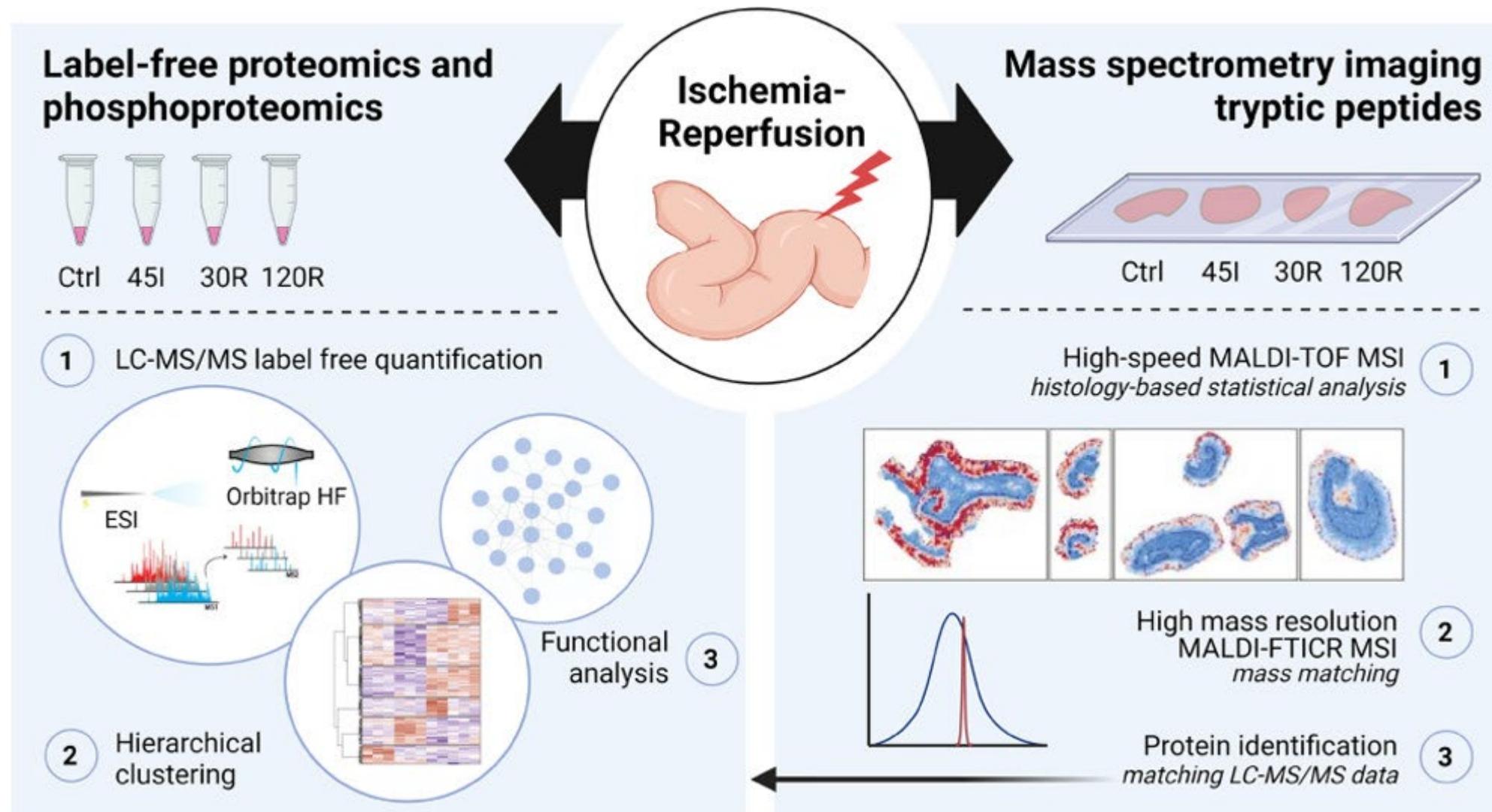


*Molecular
Mass Spectrometry*

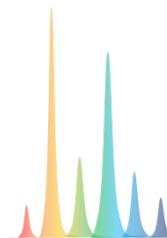


Label-free (!)

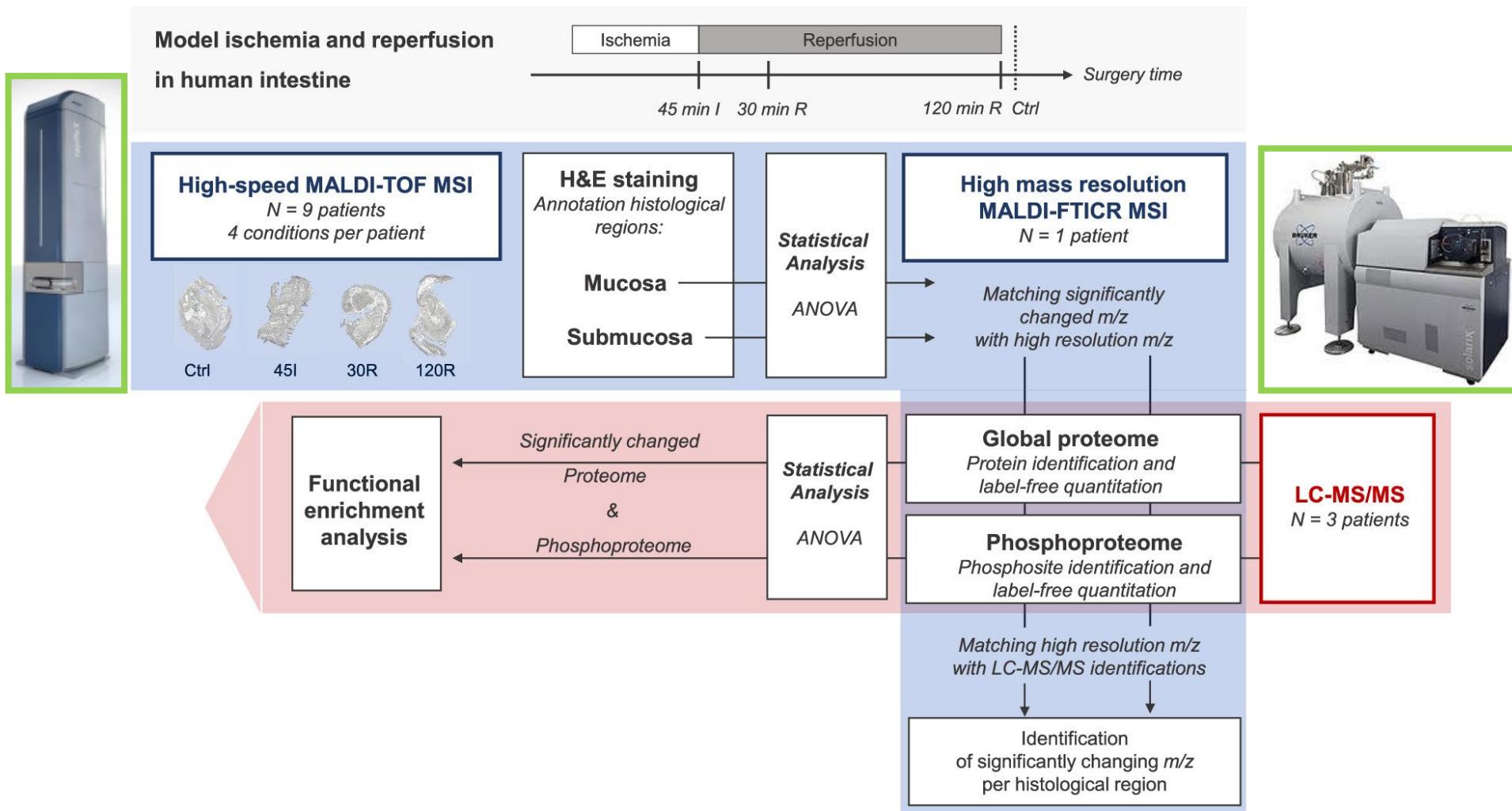
Imaging & Omics in Ischemia-Reperfusion



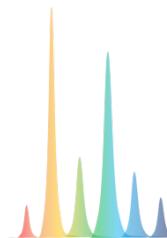
J. Proteome Res. 2022, 21, 1, 49-66



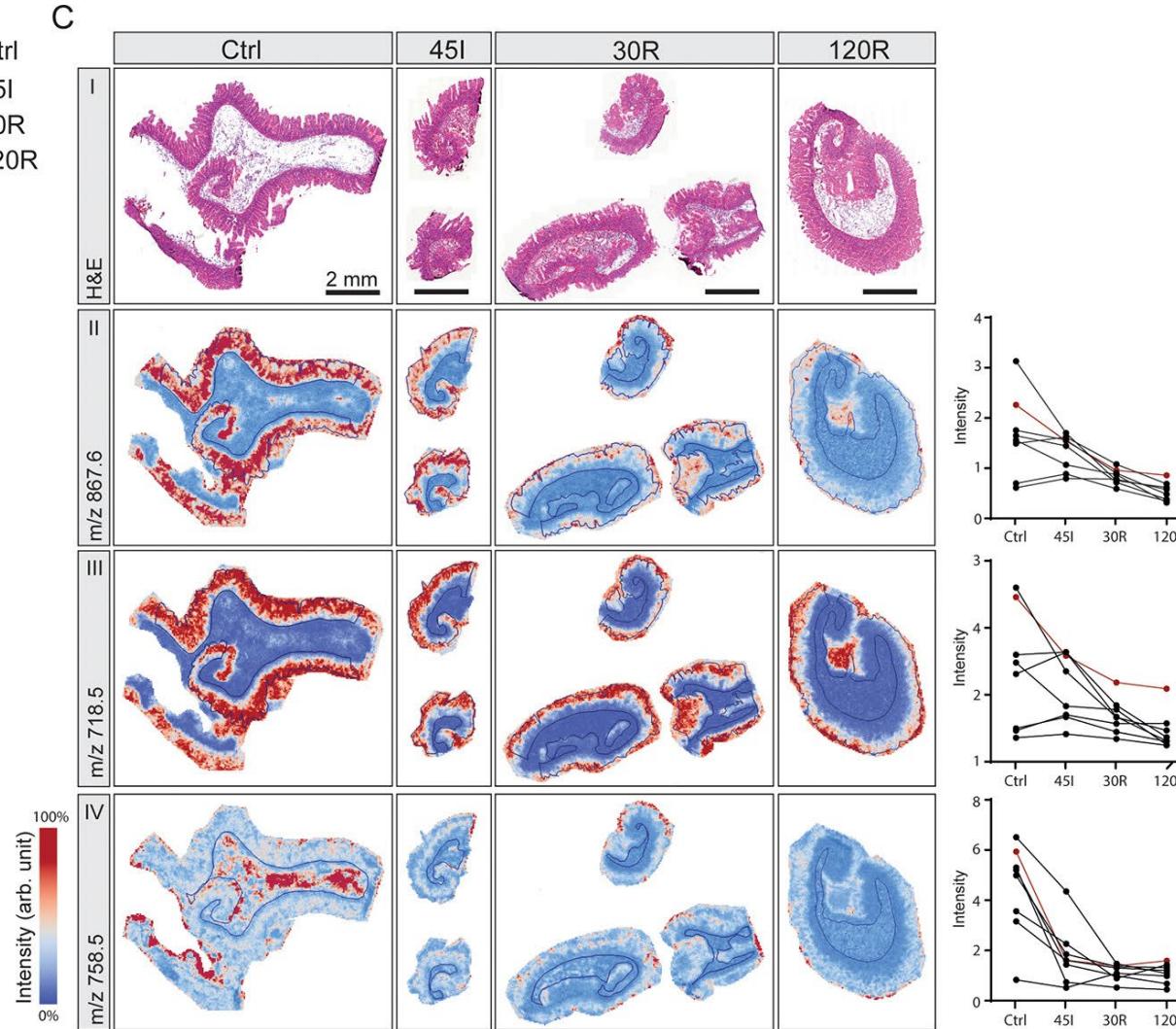
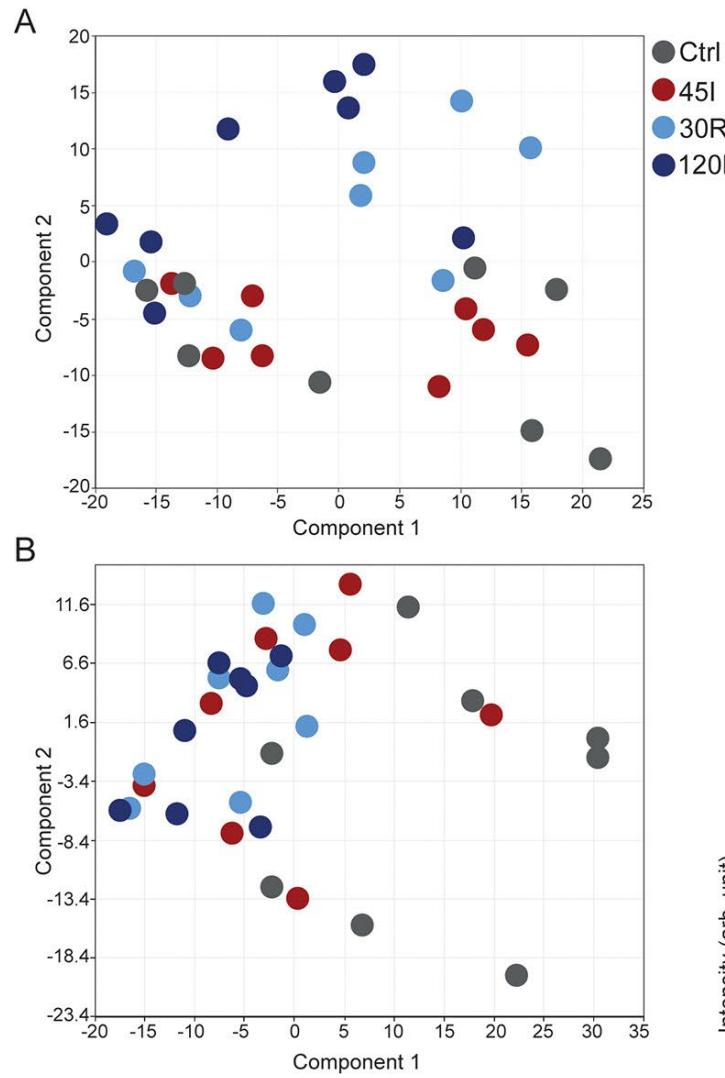
Imaging & Omics in Ischemia-Reperfusion



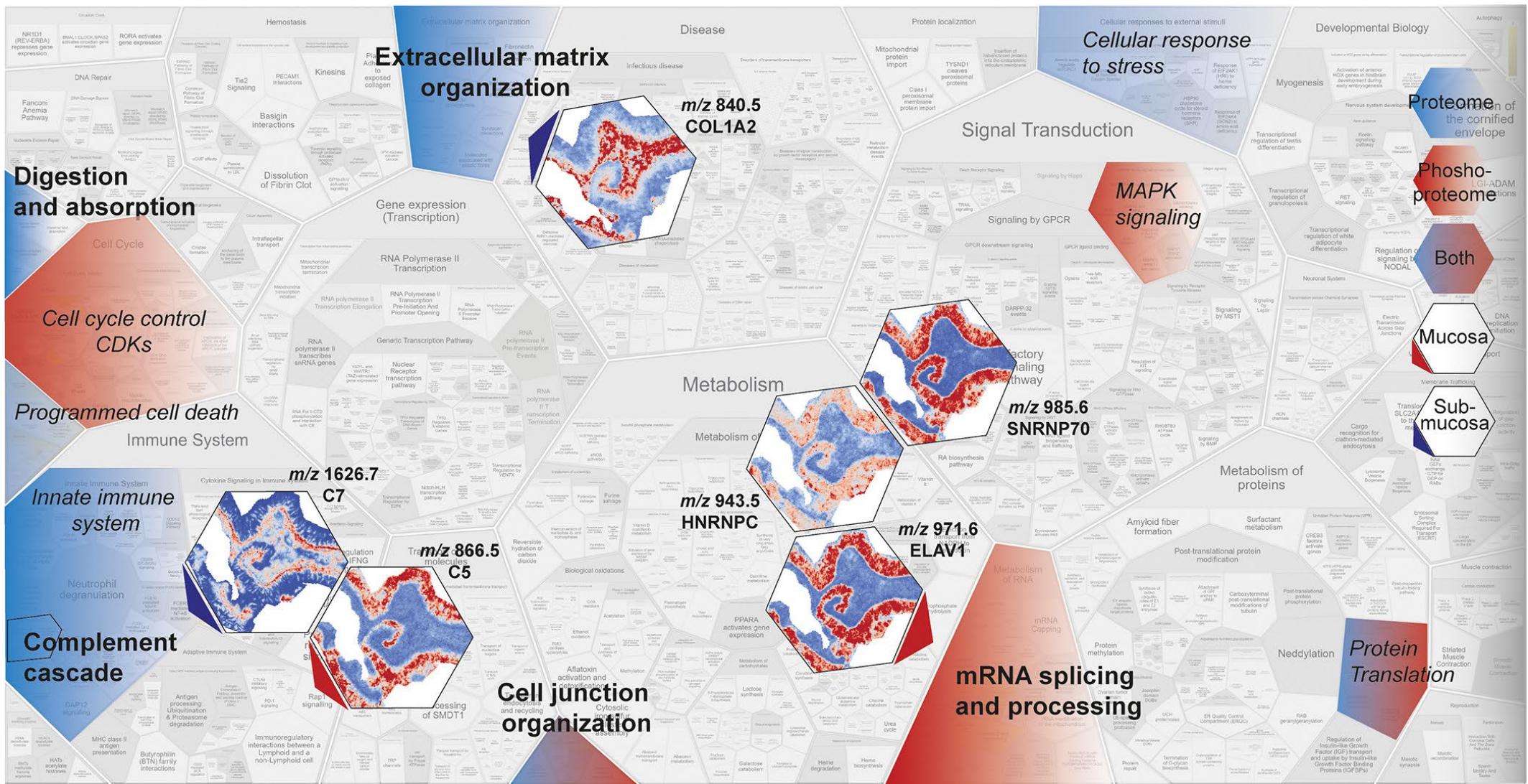
J. Proteome Res. 2022, 21, 1, 49-66



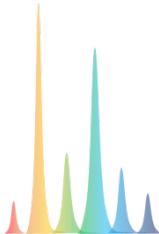
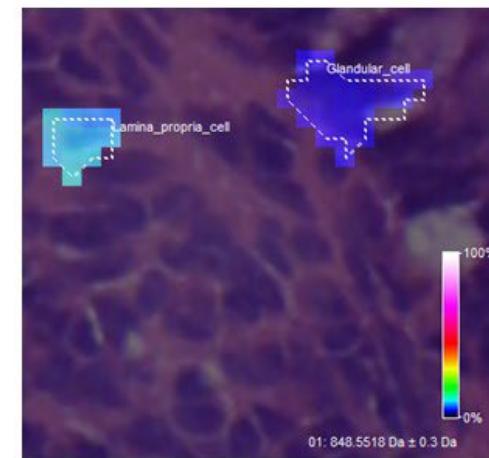
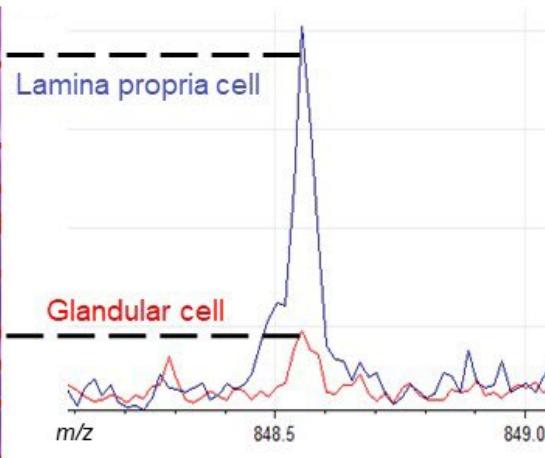
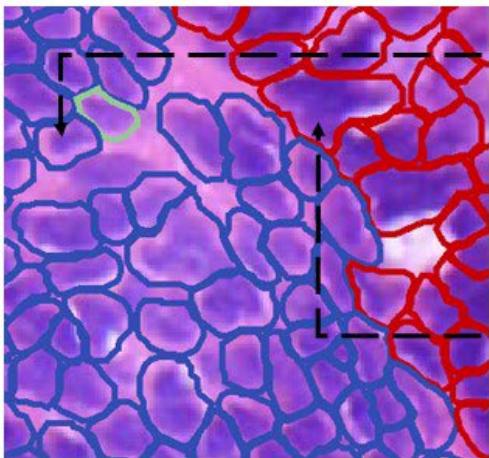
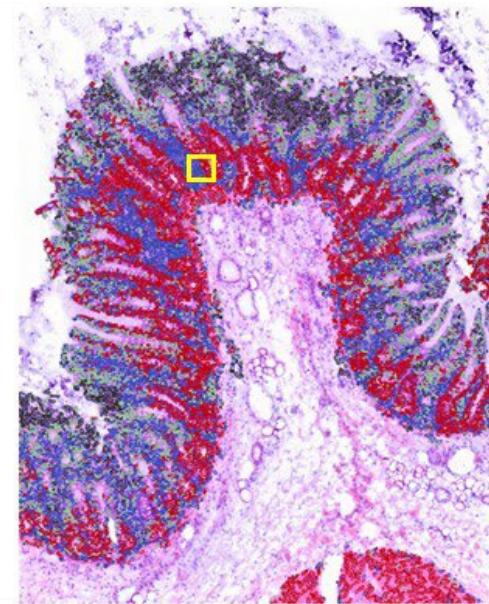
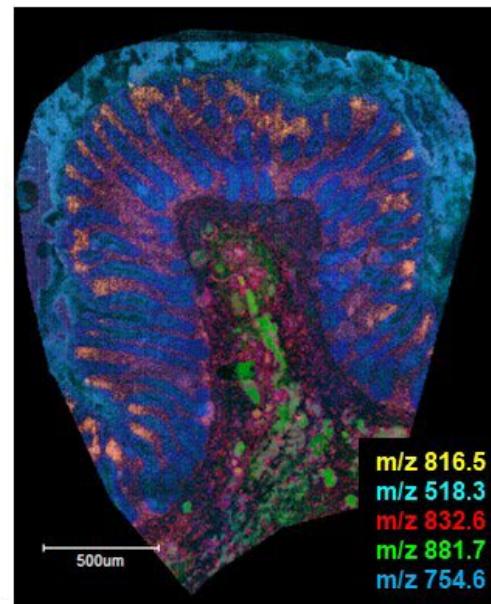
Imaging & Omics in IR



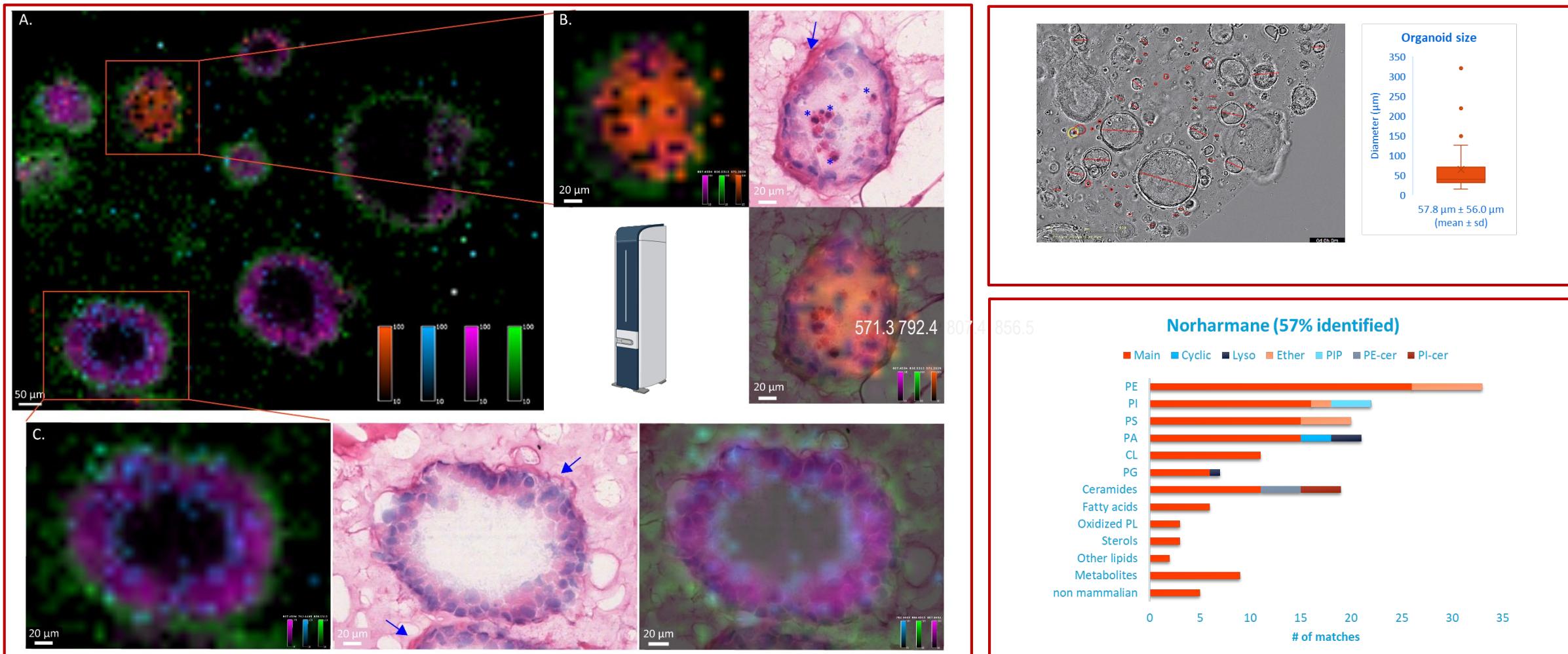
Local Pathways



Single cell MSI annotation



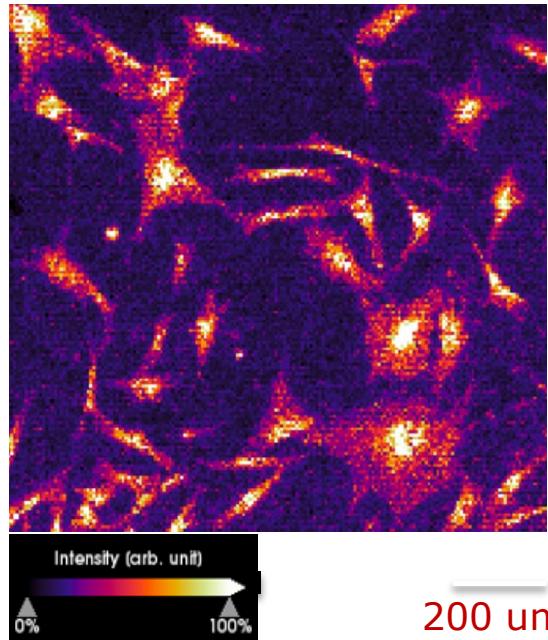
PDAC hollow organoid lipid imaging



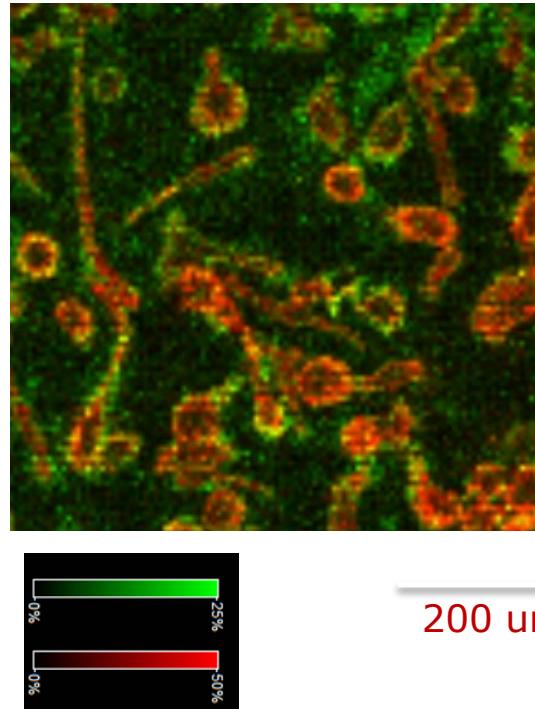
Brenda Bakker et al. (2022) Nat. Prot.
doi.org/10.1038/s41596-021-00661-8

What if we start from cell cultures?

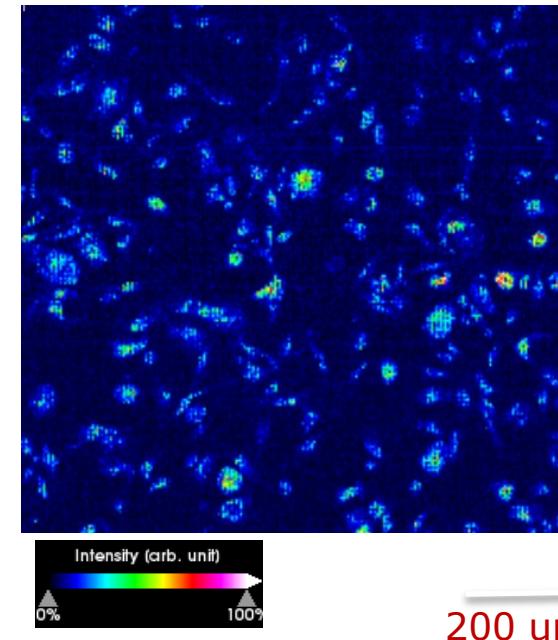
fibroblasts



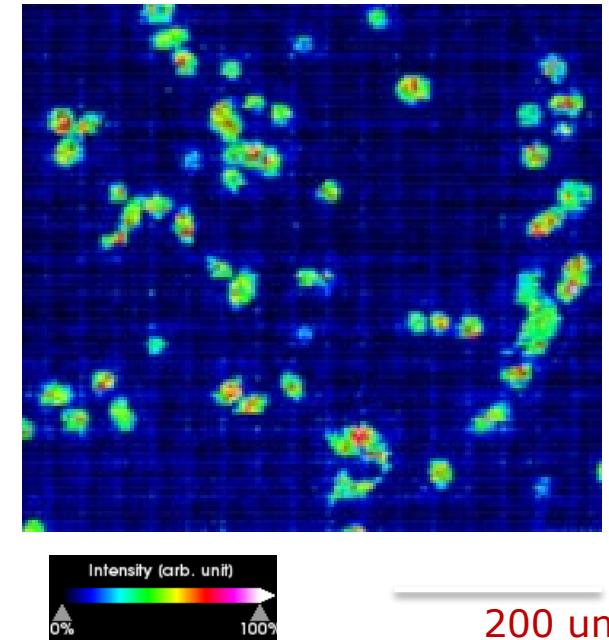
macrophages



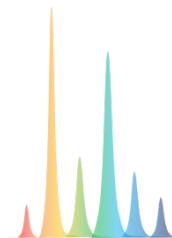
cardiomyocytes



breast cancer

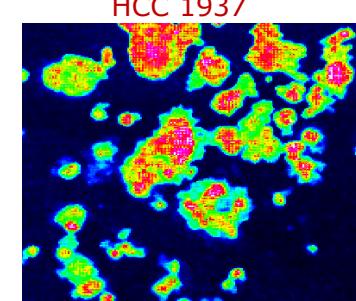
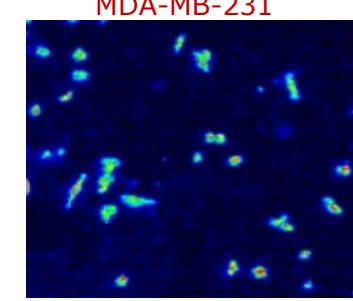
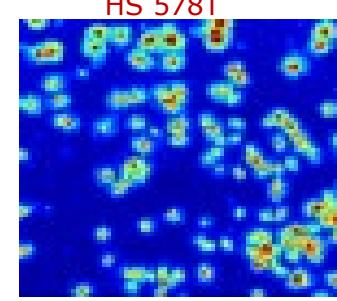
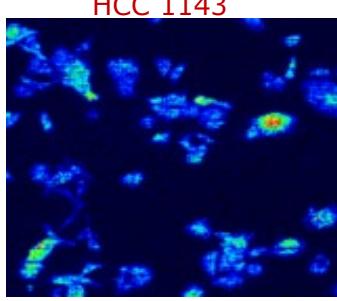
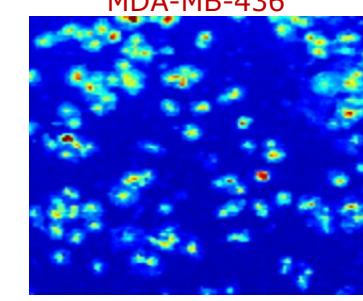
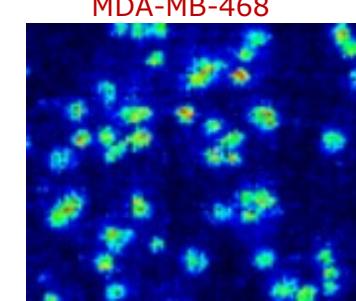
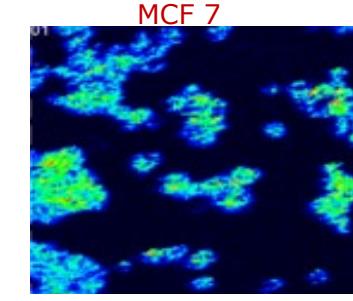
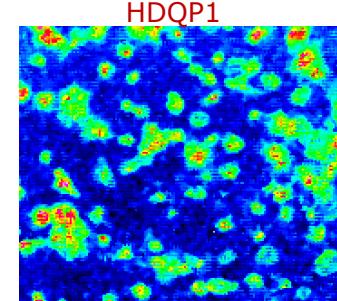
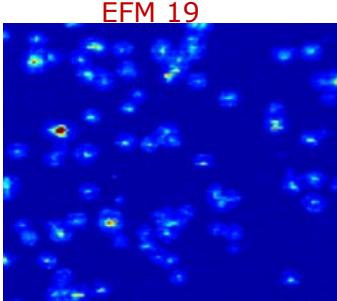
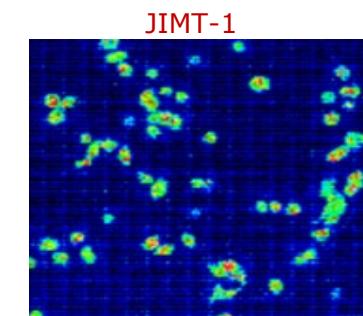
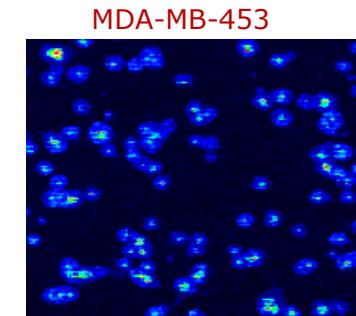
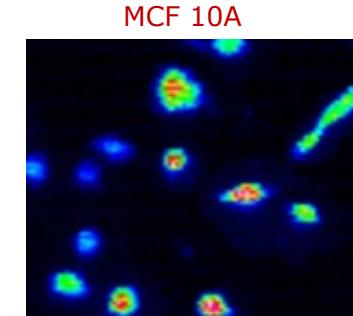
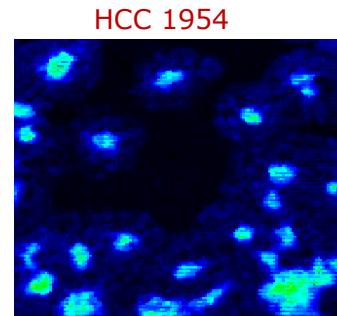
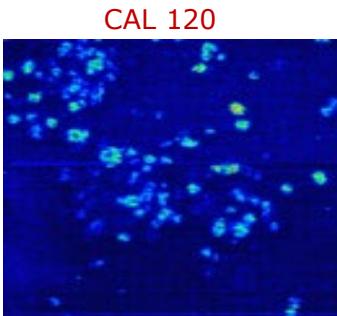


Eva Cuypers, Anal. Chem. (2022) Accepted

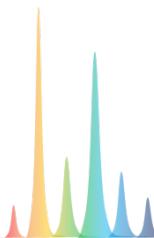


Single cell Imaging Mass Spectrometry

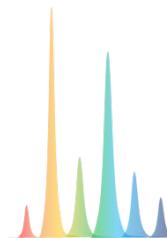
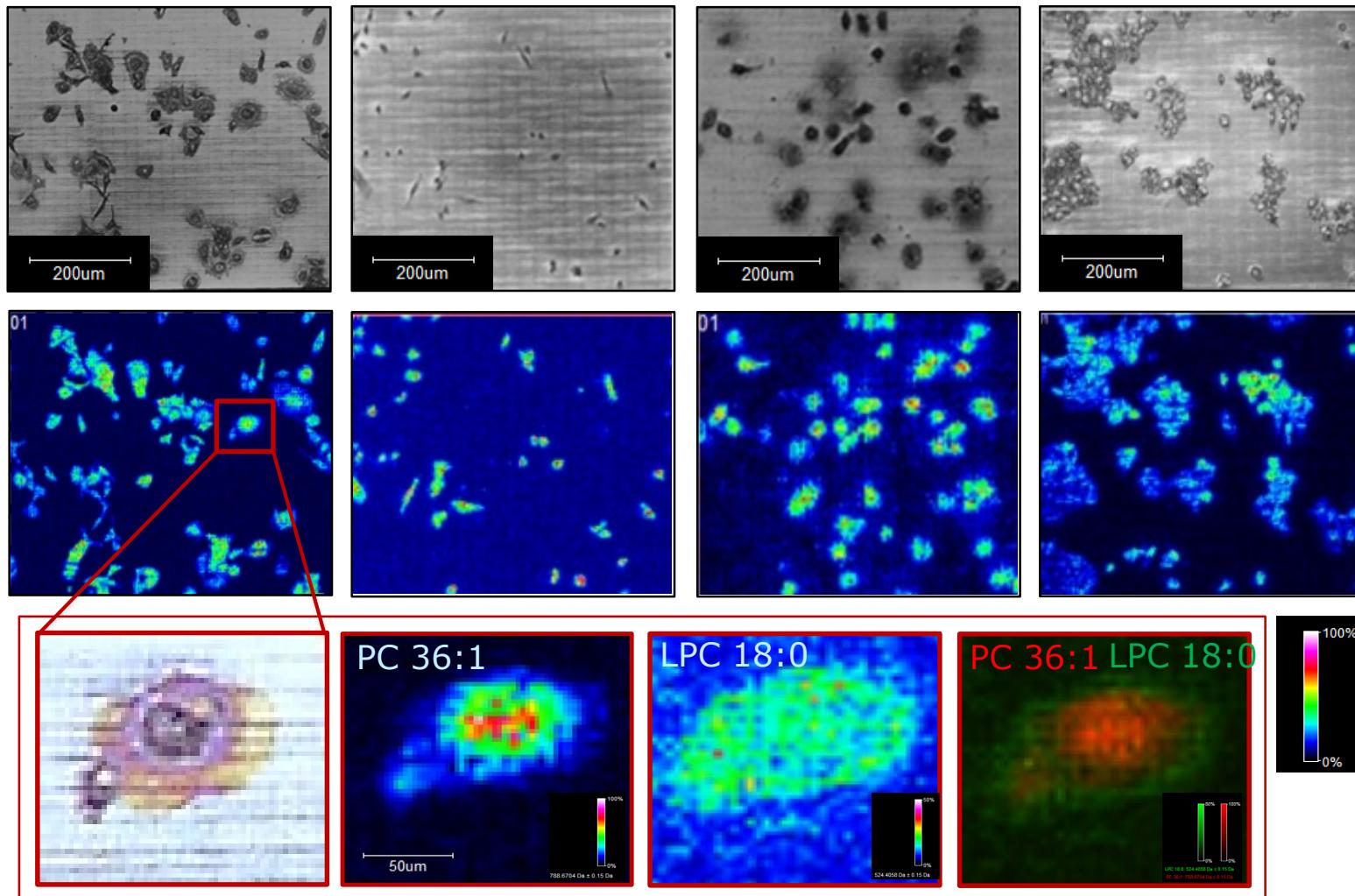
breast cancer



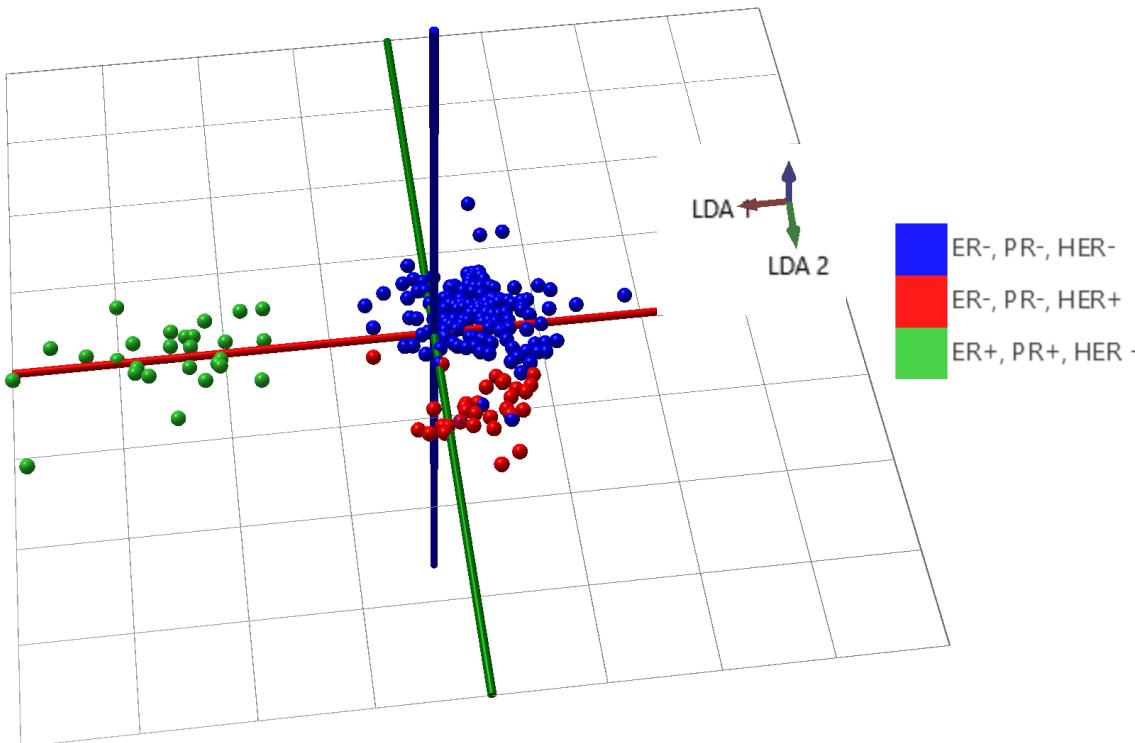
200 µm



Subcellular spatial distributions



Single cell receptor expression model



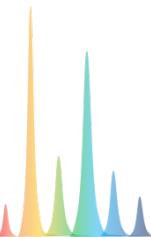
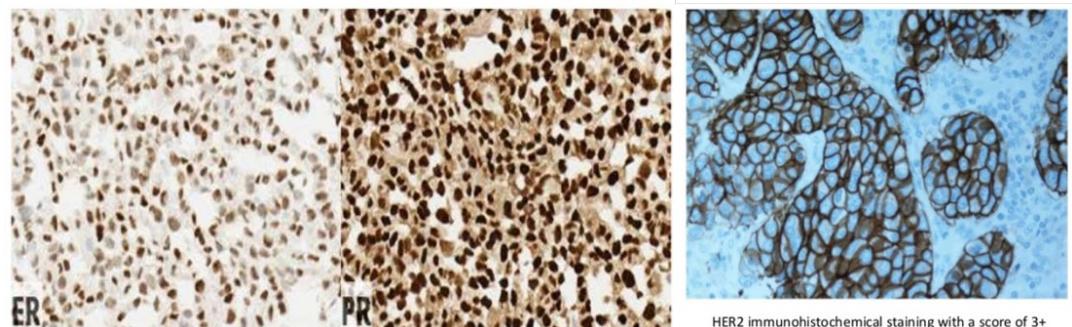
Validation Type	20% out
Model type	Lda
Number of Pca dimensions	25
Number of Lda dimensions	2
Outlier Type	Based on standard deviation
Parameter	3

Group result:

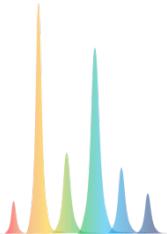
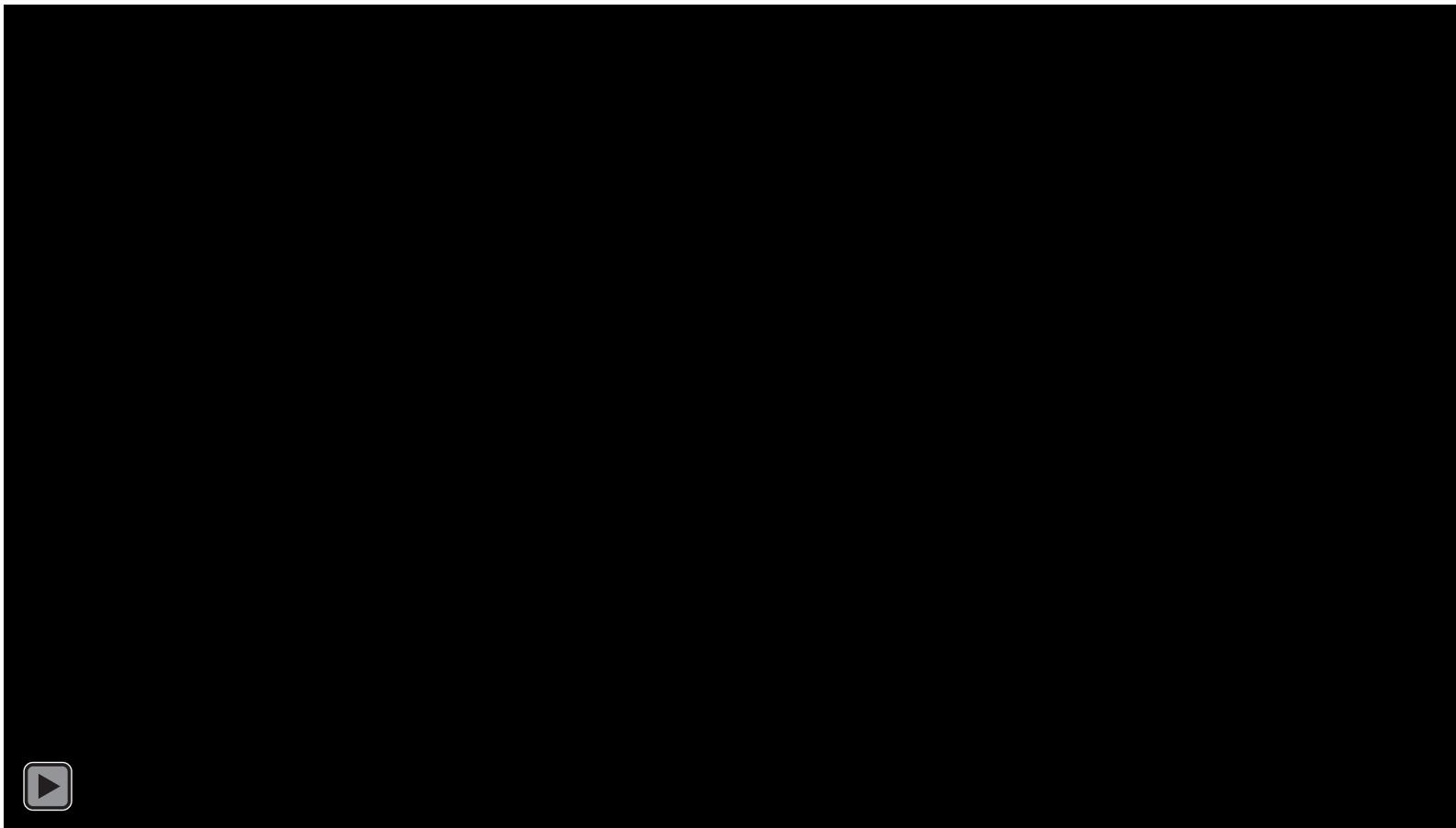
Group	Number of spectra	Number of passes	Number of failures	Number of outliers	Correct Classification Rate	
					Excluding outliers	Including outliers
Total	229	203	13	13	93.98%	88.65%

Confusion matrix:

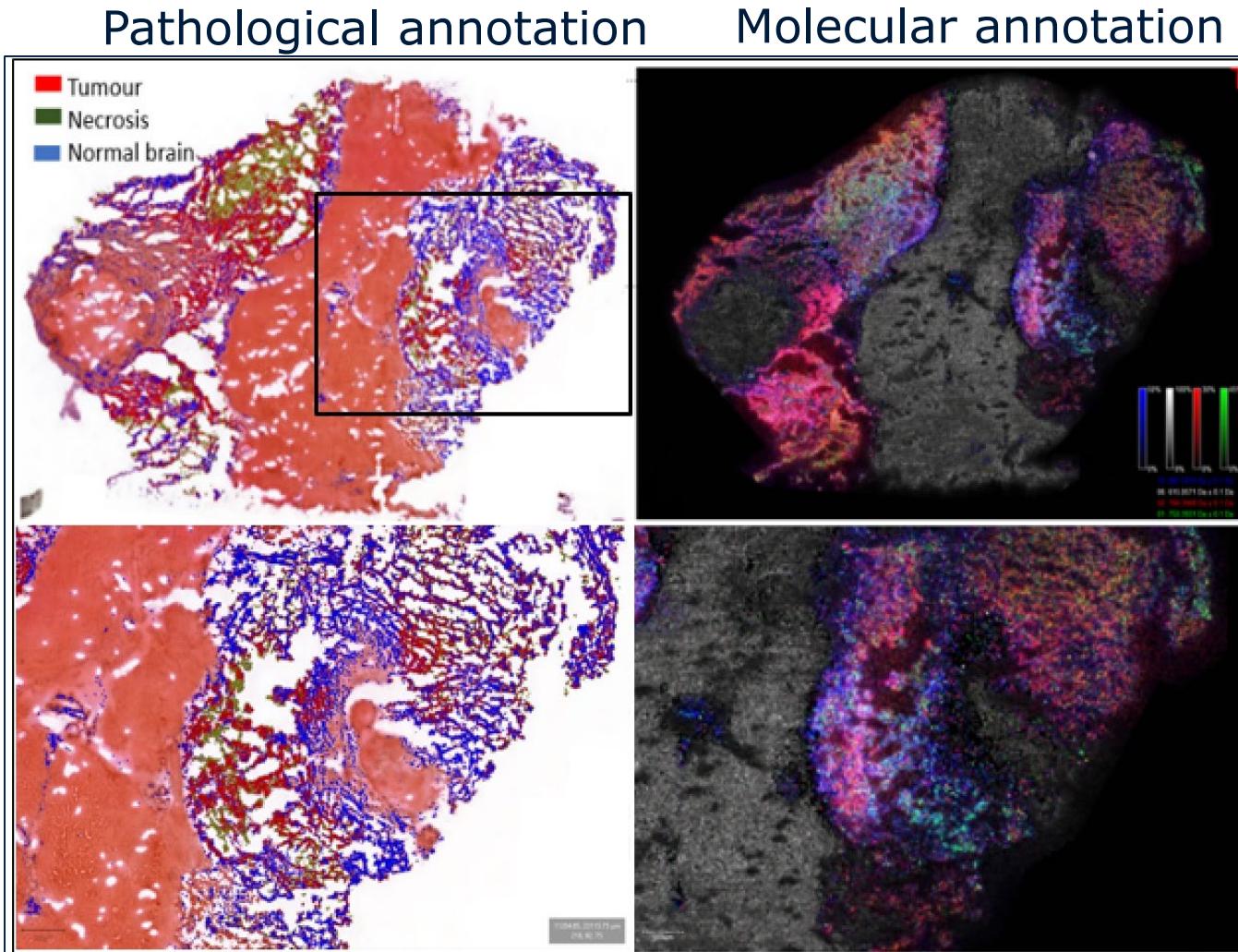
	er-, pr-, her-	er-, pr-, her+	er+, pr+, her -	Outlier	Total
er-, pr-, her-	143	12	0	9	164
er-, pr-, her+	1	31	0	1	33
er+, pr+, her -	0	0	29	3	32
Total	144	43	29	13	229



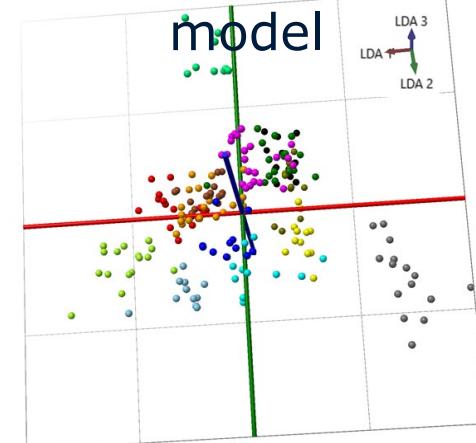
On-the-fly cell recognition



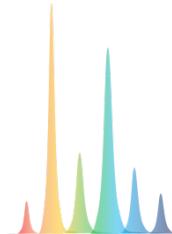
Digital cell biology & pathology platform



Single cell
Molecular recognition
model



Data Eva Cuypers on Human GBM



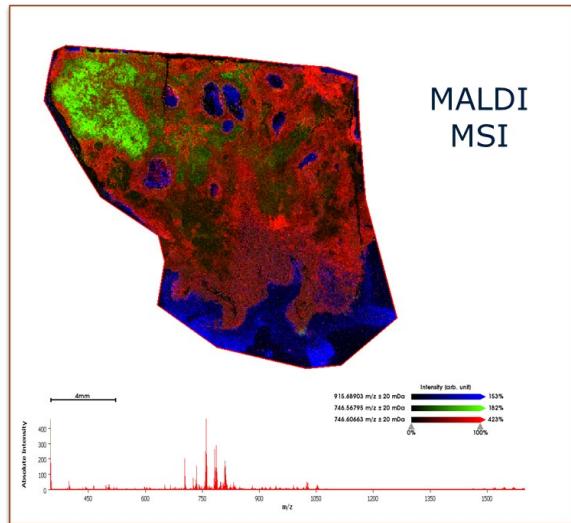
Breast cancer SpatialOMX & pathways



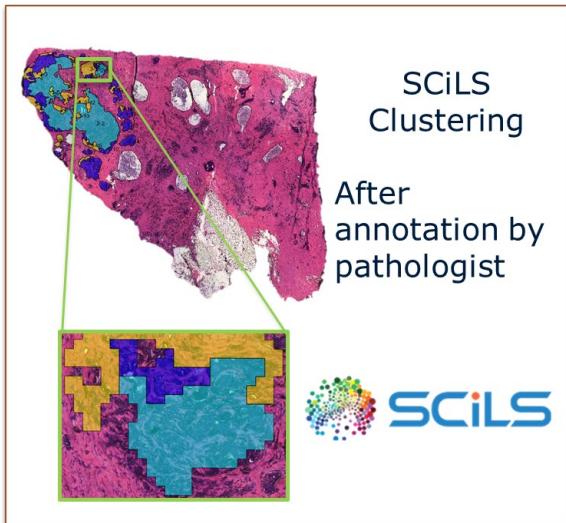
SpatialOMX on the same instrument: timsTOF flex



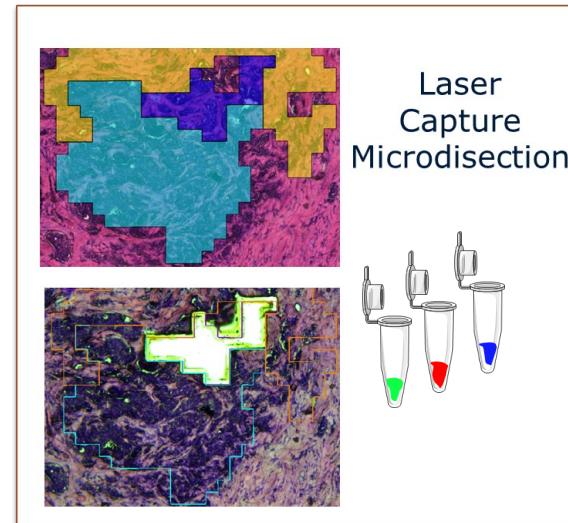
Step 1



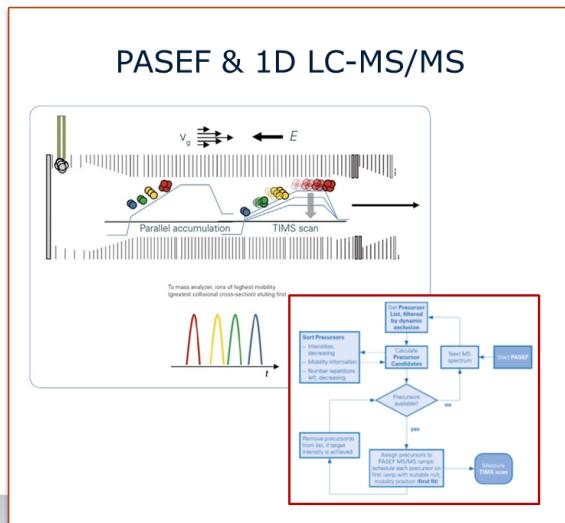
Step 2



Step 3

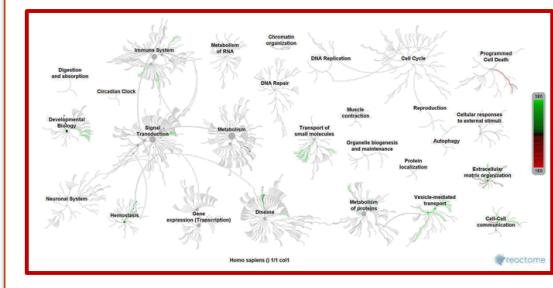


Step 4

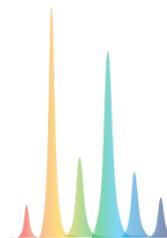


Step 5

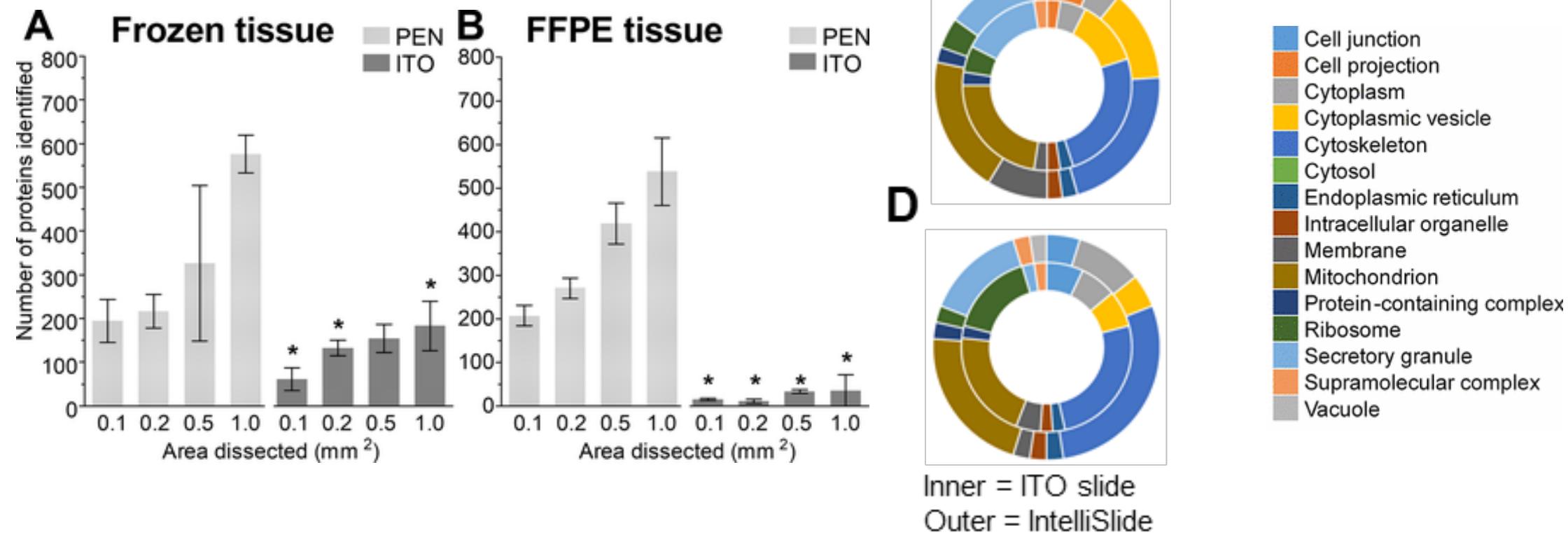
MASCOT 2.6 Protein ID's
followed by
Pathway analysis



Dewez et al.
Proteomics (2020) 201900369



Protein identification and cellular components



Stephanie Mezger et al. Anal. Chem. 2021, 93, 4, 2527–2533

The search results (the numbers game)

Breast tumor samples Mascot 2.6 database search results

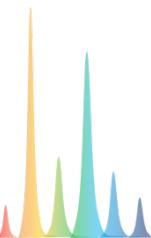
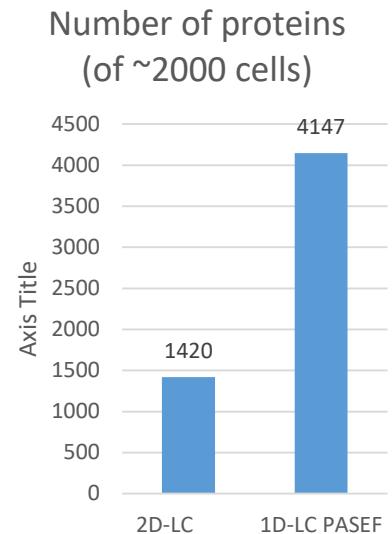
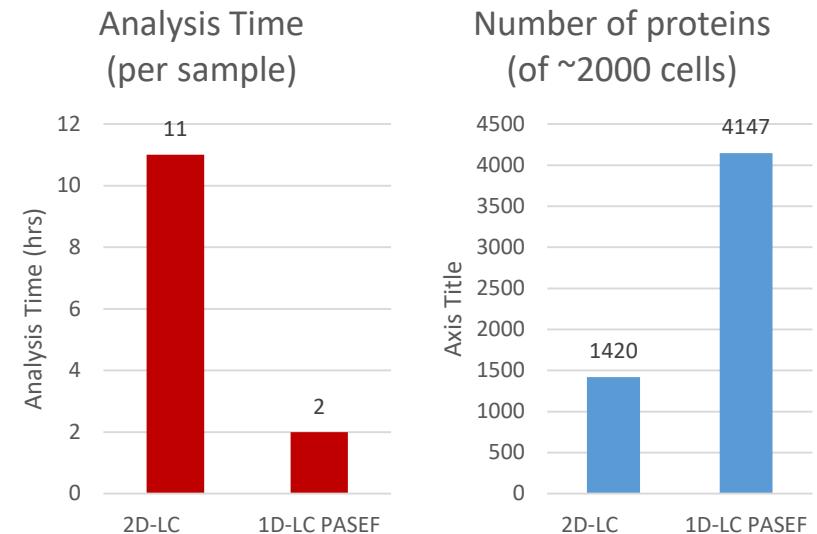
Result table:

Sample ID	PSM	No Compounds	% spectra matching	Proteins	Protein Families	Sequences
111 2000 Cells_Slot1-5_1_28_5.3.0.mgf	54538	254320	21.4	4147	3814	29011
211 2000 Cells_Slot1-6_1_30_5.3.0.mgf	12478	193357	6.5	1431	1316	6773
311 2000 Cells_Slot1-7_1_32_5.3.0.mgf	15905	240261	6.6	1566	1408	8539
20000 Cells in 20ul Slot1-8_1_34_5.3.0.mgf	63550	203275	31.3	4467	4079	34998

Note:
between each sample one "blank" in injection was done, results not shown

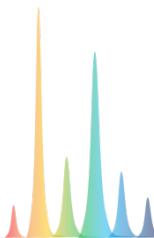
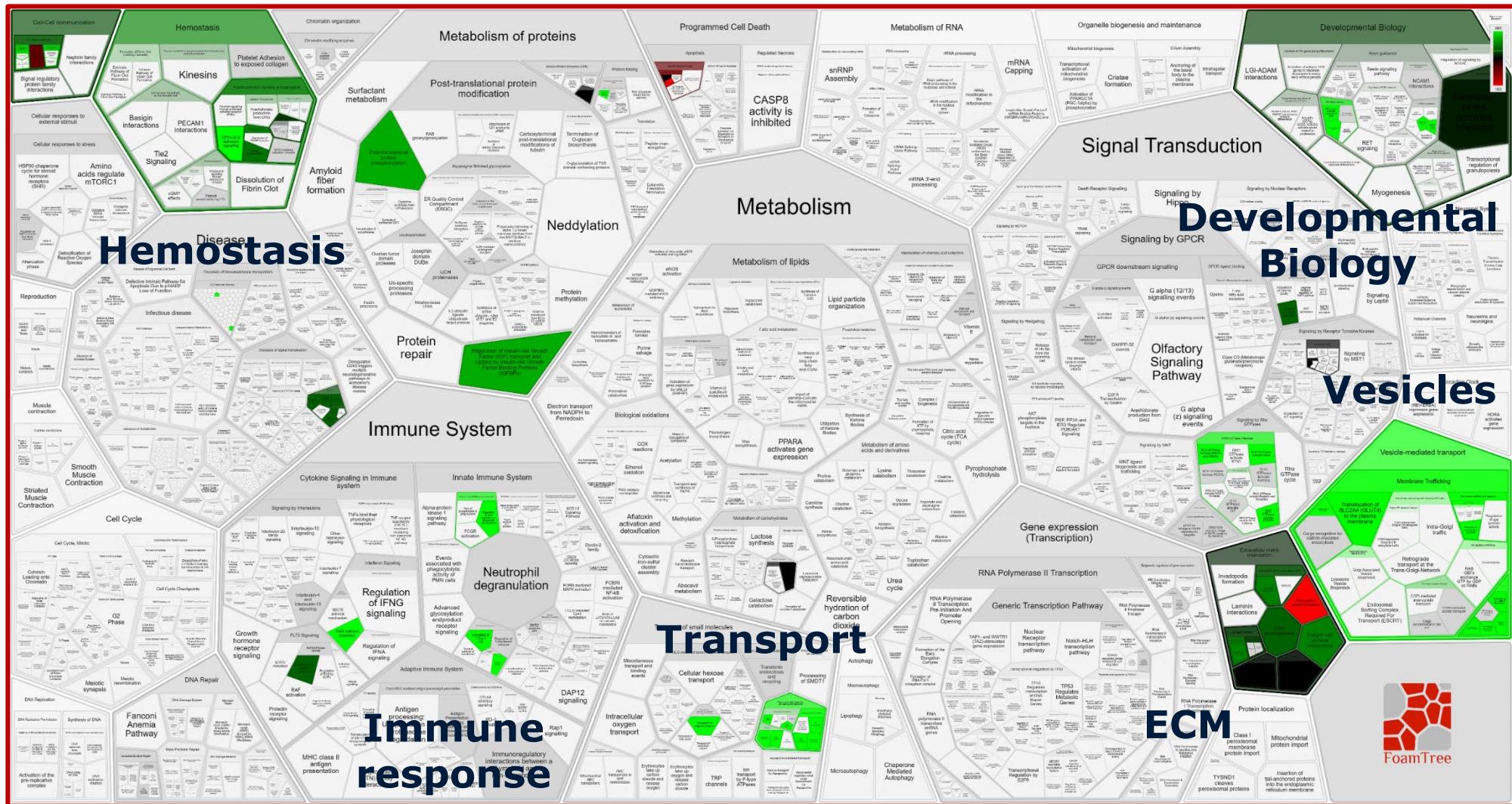
Mascot DB Search
Method:
• Database: SwissProt HumanIsoforms

• Taxonomy: all entries
• Enzyme: Trypsin, Allow up to 1 missed cleavage
• Fixed Modifications: Carbamidomethyl (C)
• Variable Modifications: Acetyl (N-term), Oxidation (M), Deamidation (NQ)
• Peptide mass tolerance: 15 ppm
• Fragment mass tolerance: 0.05 Da
• Peptide Decoy (Mascot), adjust FDR to 1%, use Percolator

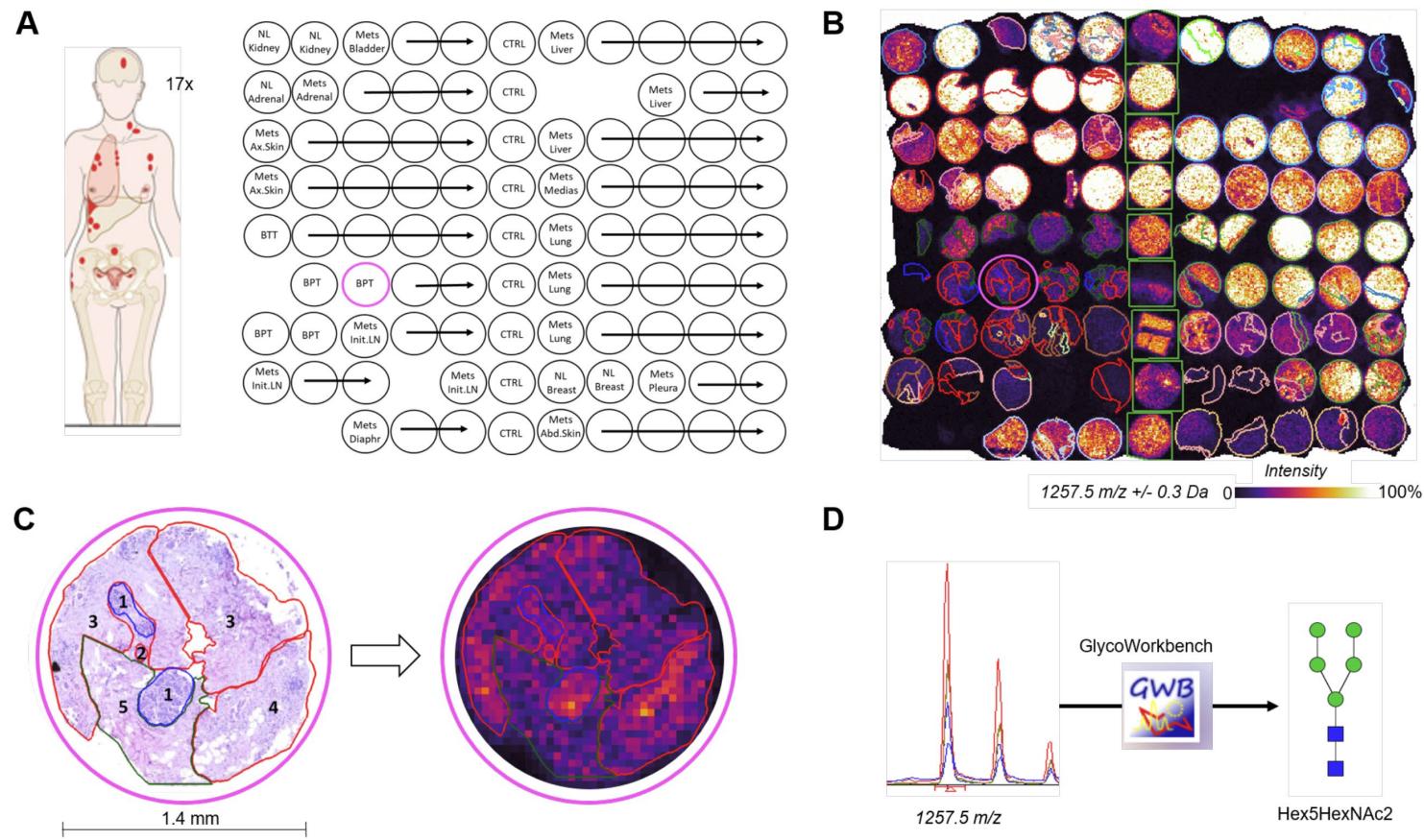


Cell-Cell Communication

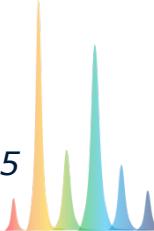
Part of the proteome (~4000 protein ID's)



TMA's from warm autopsy program at JHU: *Tissue specific N-glycan patterns*



- Explore the molecular profile between the primary BC and the metastatic sites.
- Investigate the inter- and intra- patient heterogeneity.

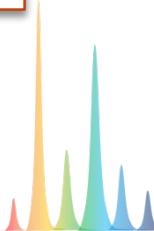
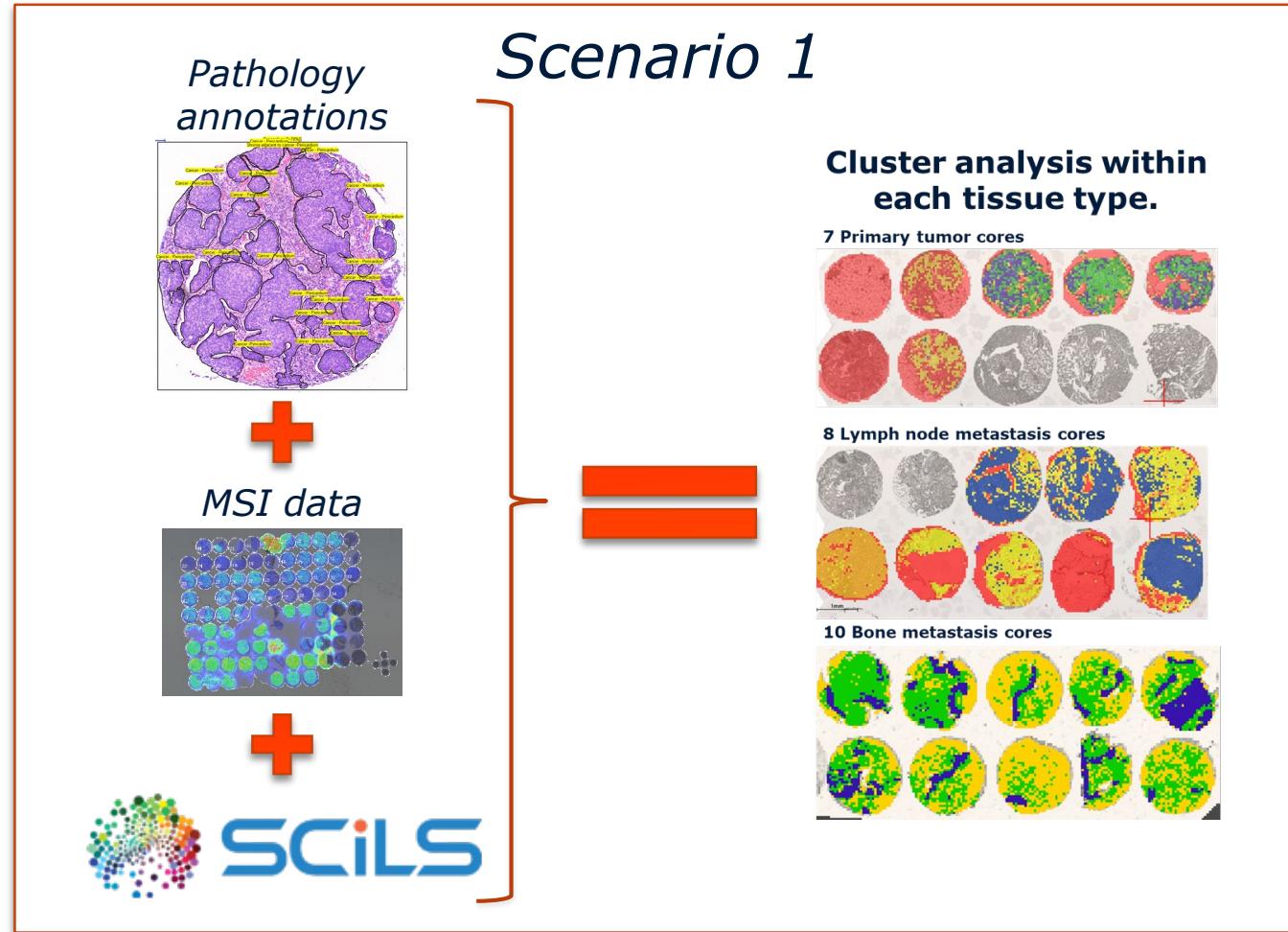


Tissue classification

Spatial annotation scenarios

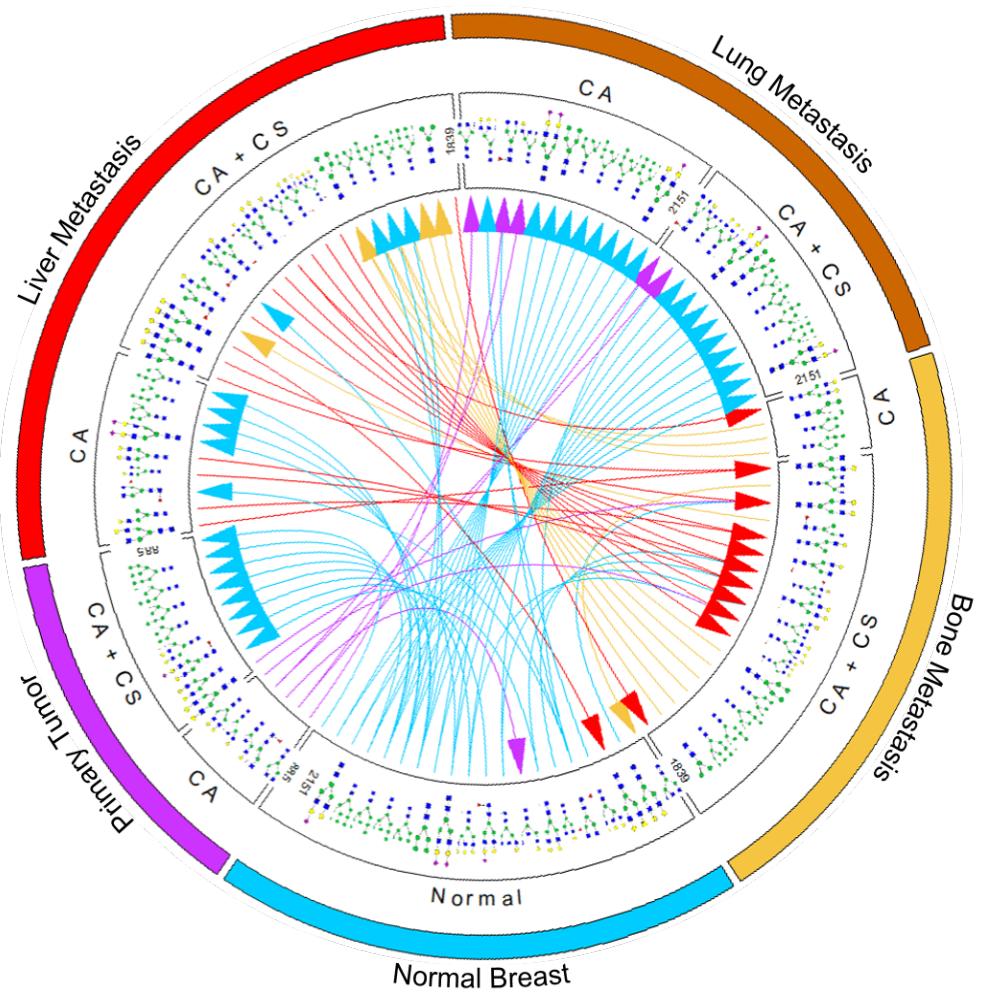
1. Pathological annotation available for tissue classification (You get your spatial annotations)

2. No available annotations
 - A. You need to do it yourself using spectral correlation
 - B. You need to do it yourself using morphological correlation

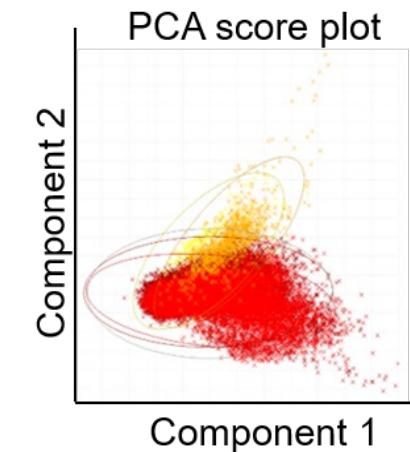


Glycan connections

A

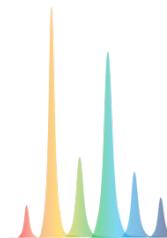
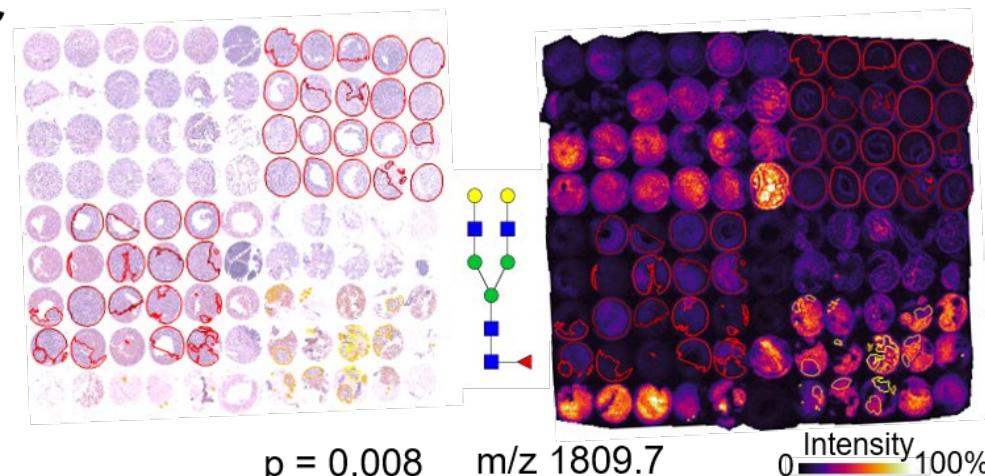


B

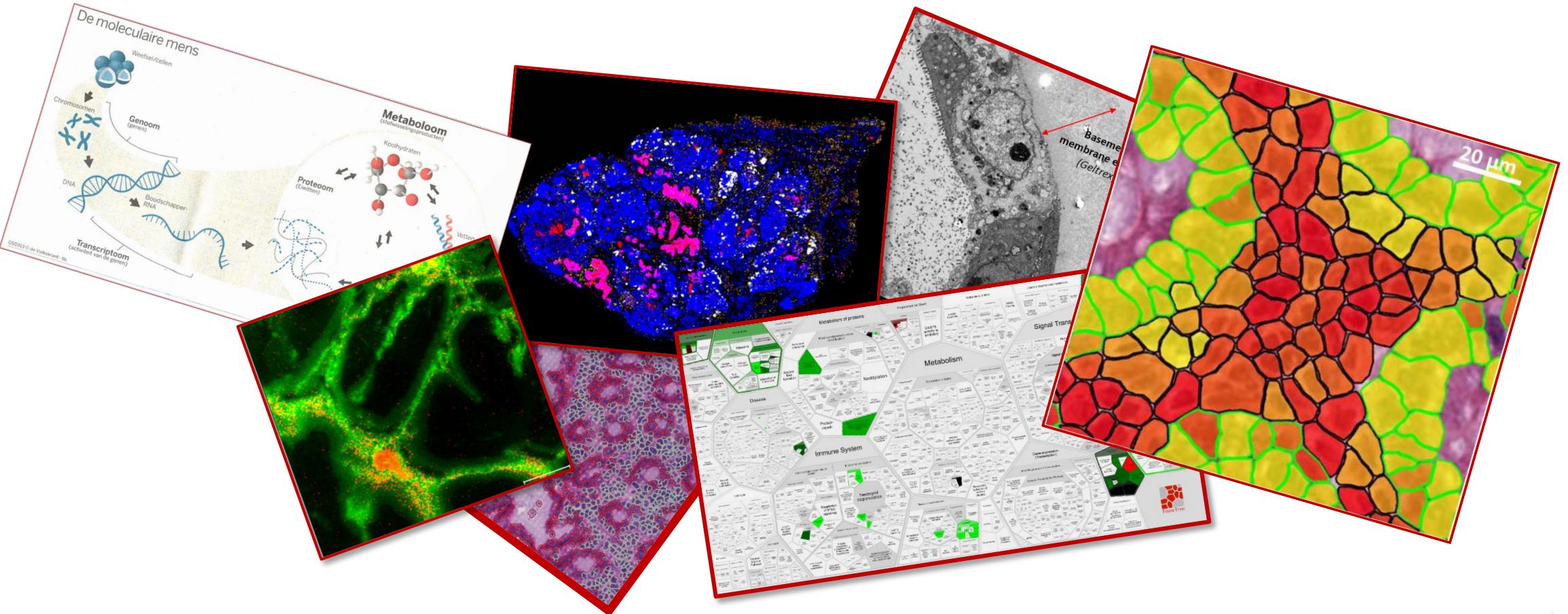


- Liver – Cancer and Cancer + CS
- Bone – Cancer and Cancer + CS

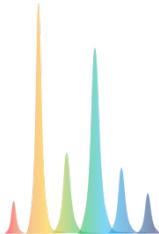
C



MultiModal Cellular Molecular Analysis



Imaging \longleftrightarrow Omics \longleftrightarrow Pathways \longleftrightarrow Context



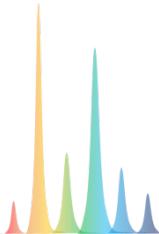
Summary

- Contextual imaging essential
- Innovations in single cell molecular imaging
- Technology drives sciences
- Translation digital pathology
- MultiModal profiles are the future



An interdisciplinary, multimodal, single cell specific “omics” based contextual analysis brings personalized diagnostics within reach

Omics without boundaries !!



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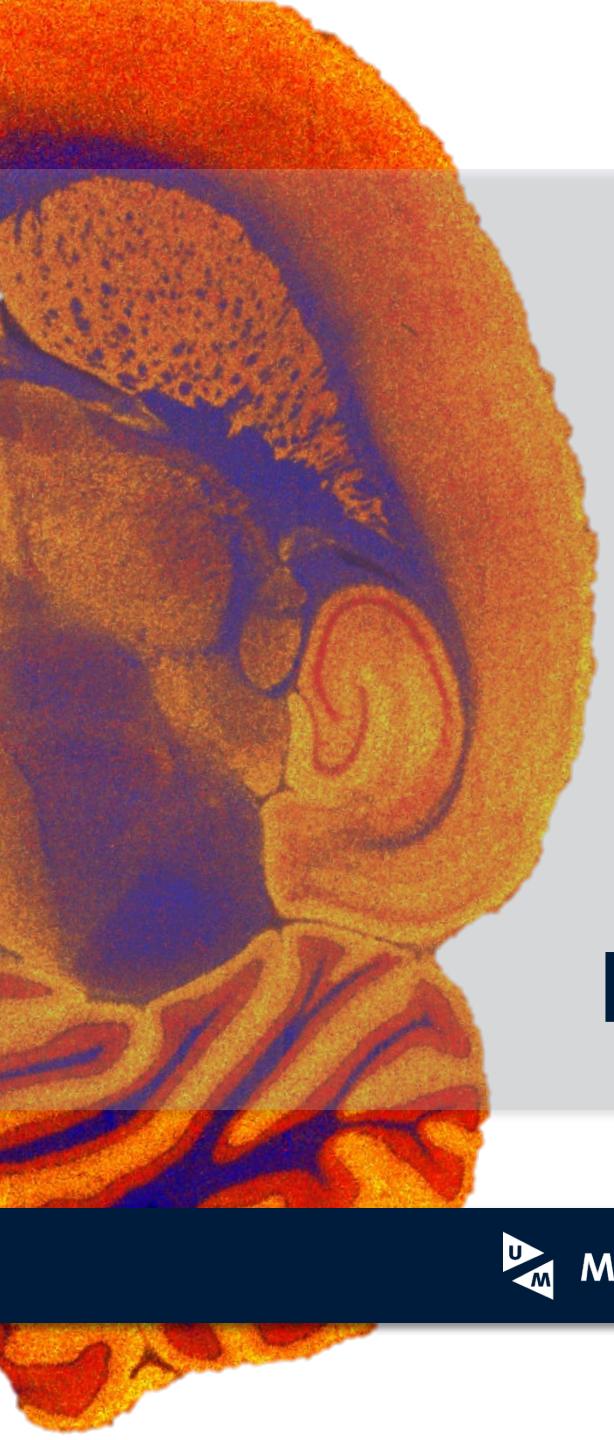
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