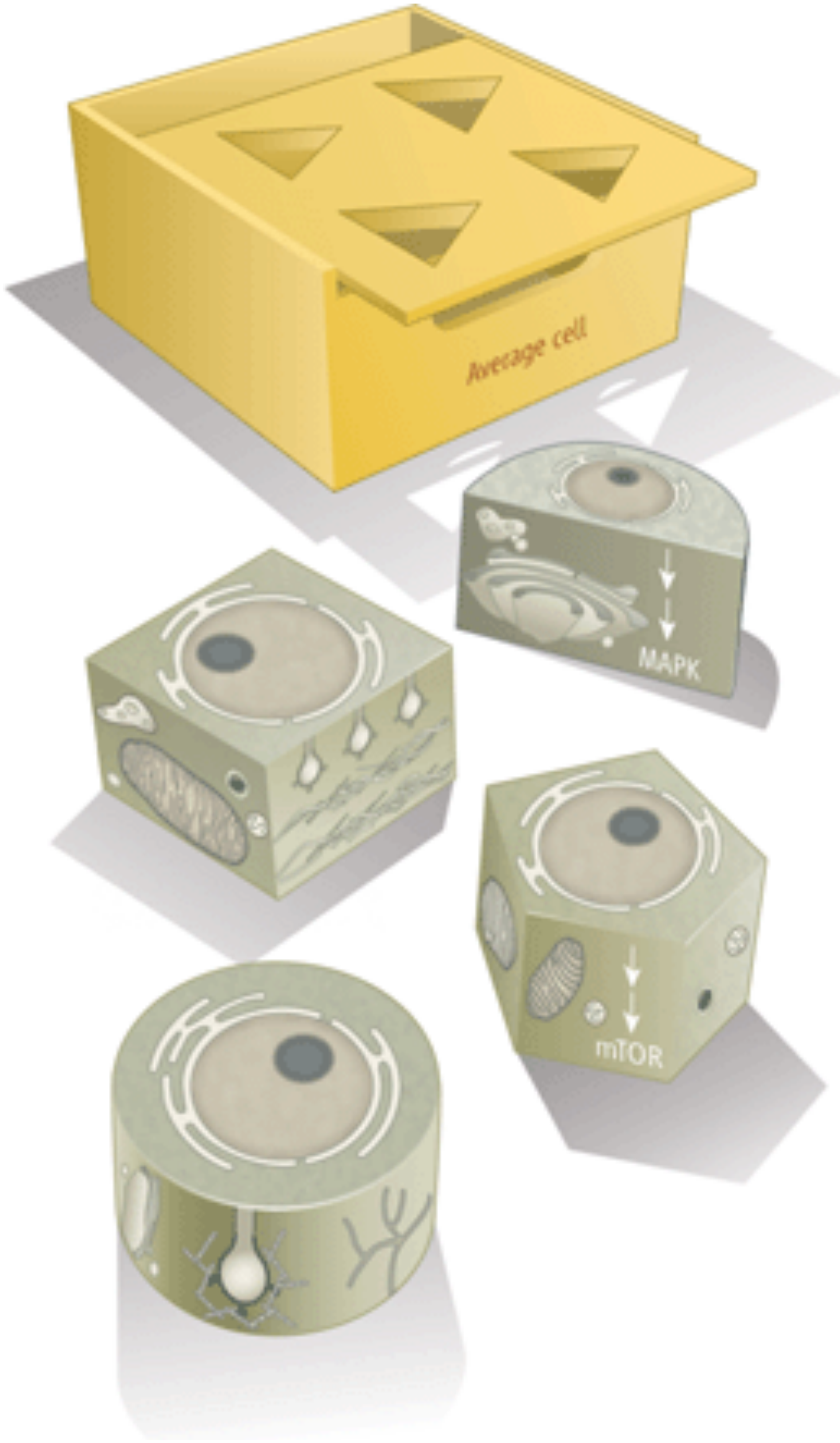
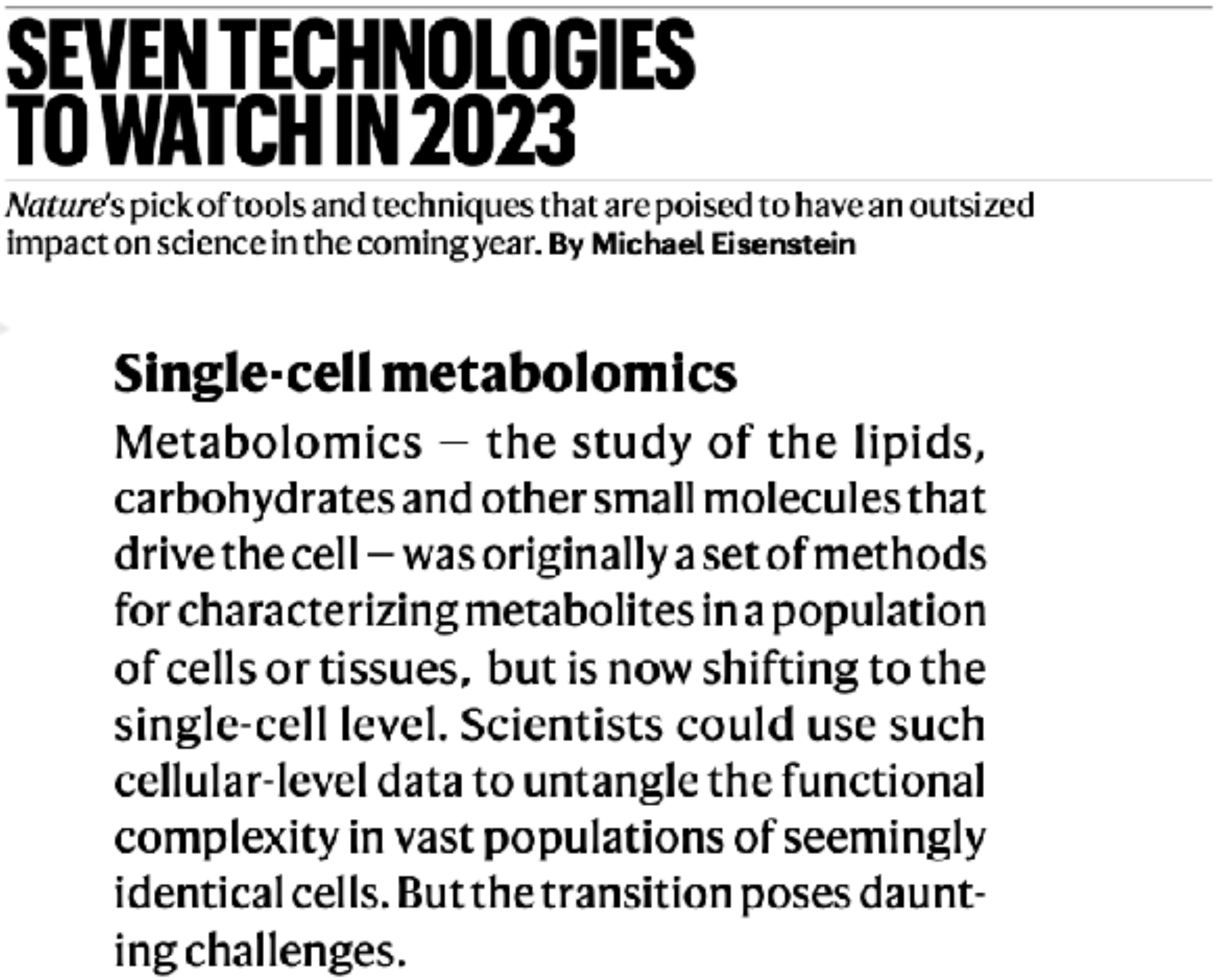
Single-cell biology

Week 7 .

Single cell and Spatial Metabolomics

Single cell Metabolomics





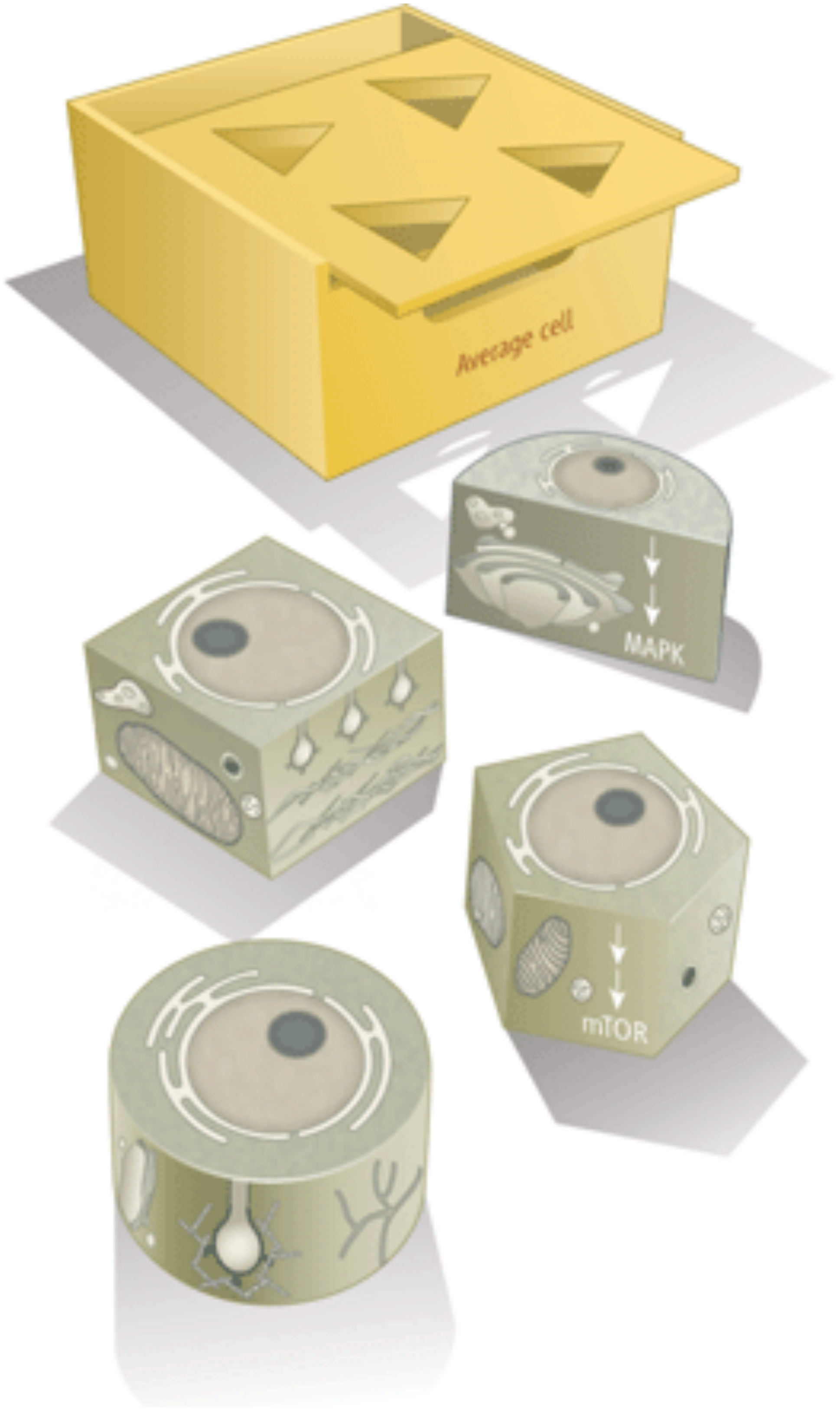
SEVEN TECHNOLOGIES TO WATCH IN 2023

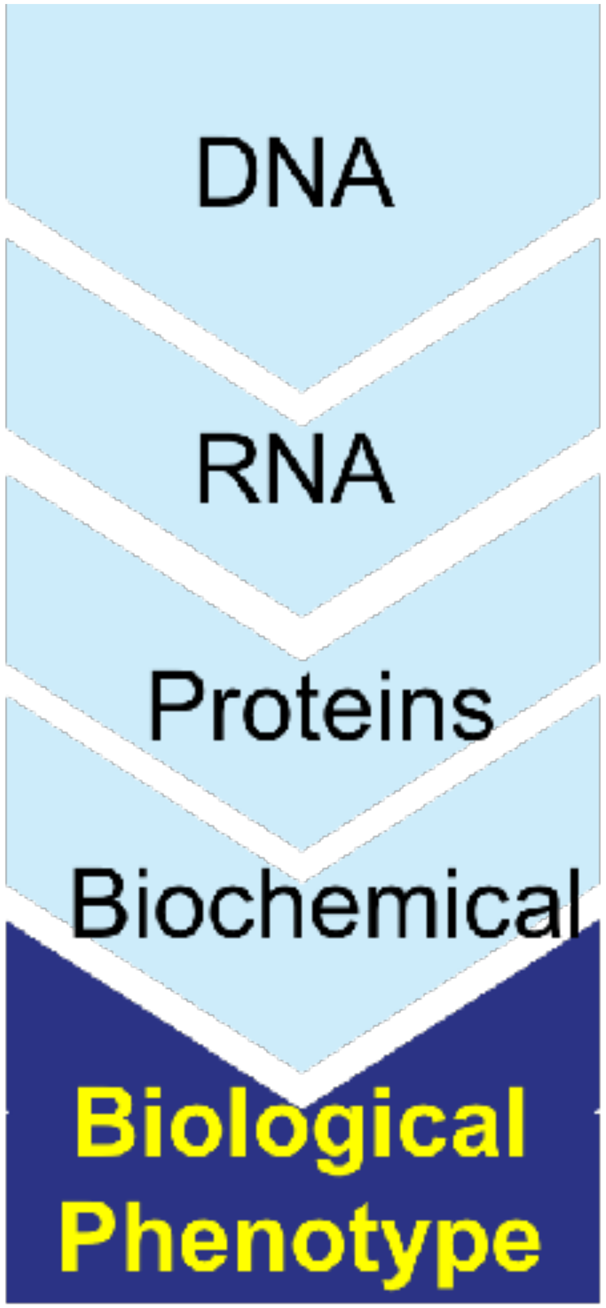
Nature's pick of tools and techniques that are poised to have an outsized impact on science in the coming year. **By Michael Eisenstein**

Single-cell metabolomics

Metabolomics – the study of the lipids, carbohydrates and other small molecules that drive the cell – was originally a set of methods for characterizing metabolites in a population of cells or tissues, but is now shifting to the single-cell level. Scientists could use such cellular-level data to untangle the functional complexity in vast populations of seemingly identical cells. But the transition poses daunting challenges.

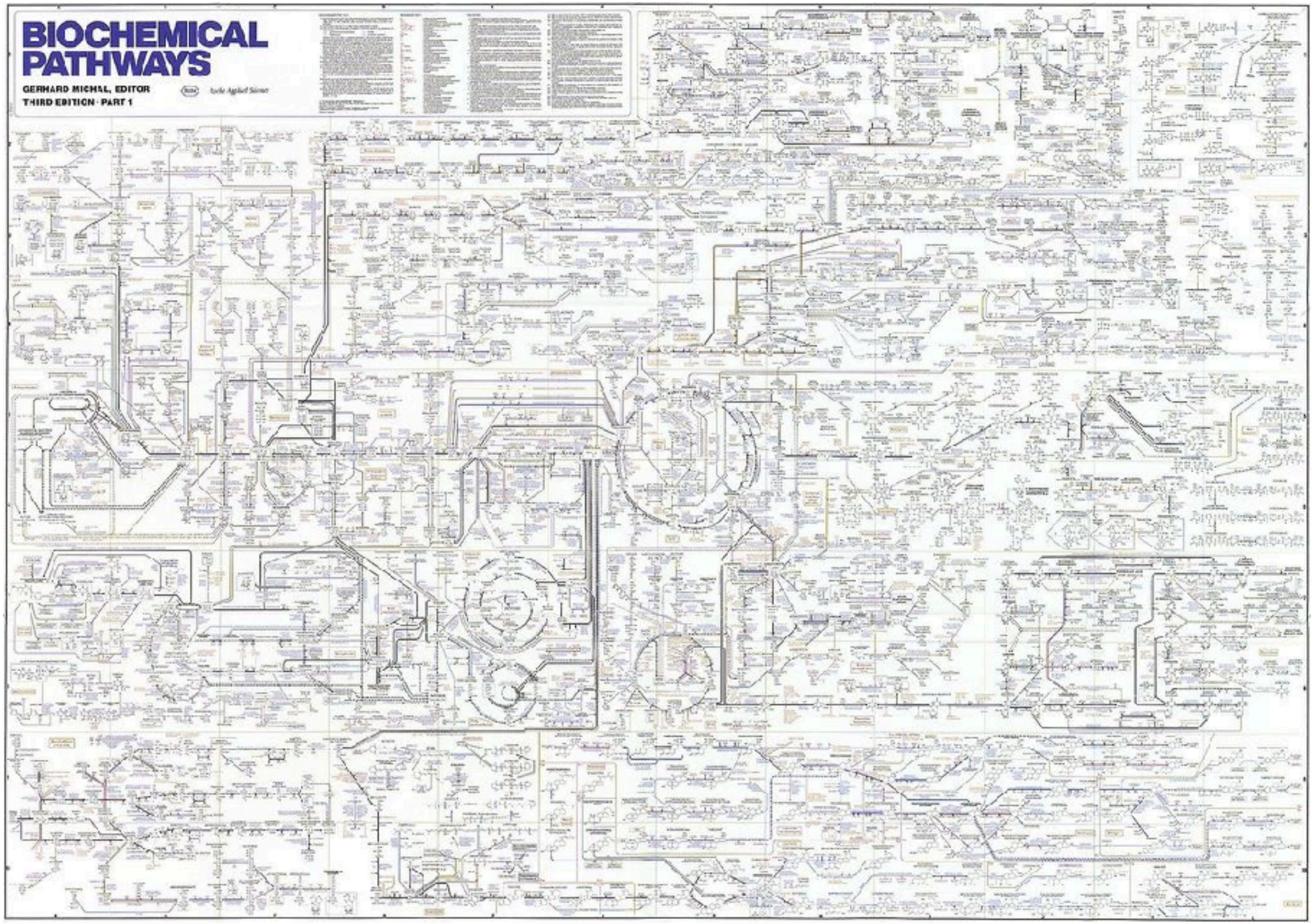
Single cell Metabolomics





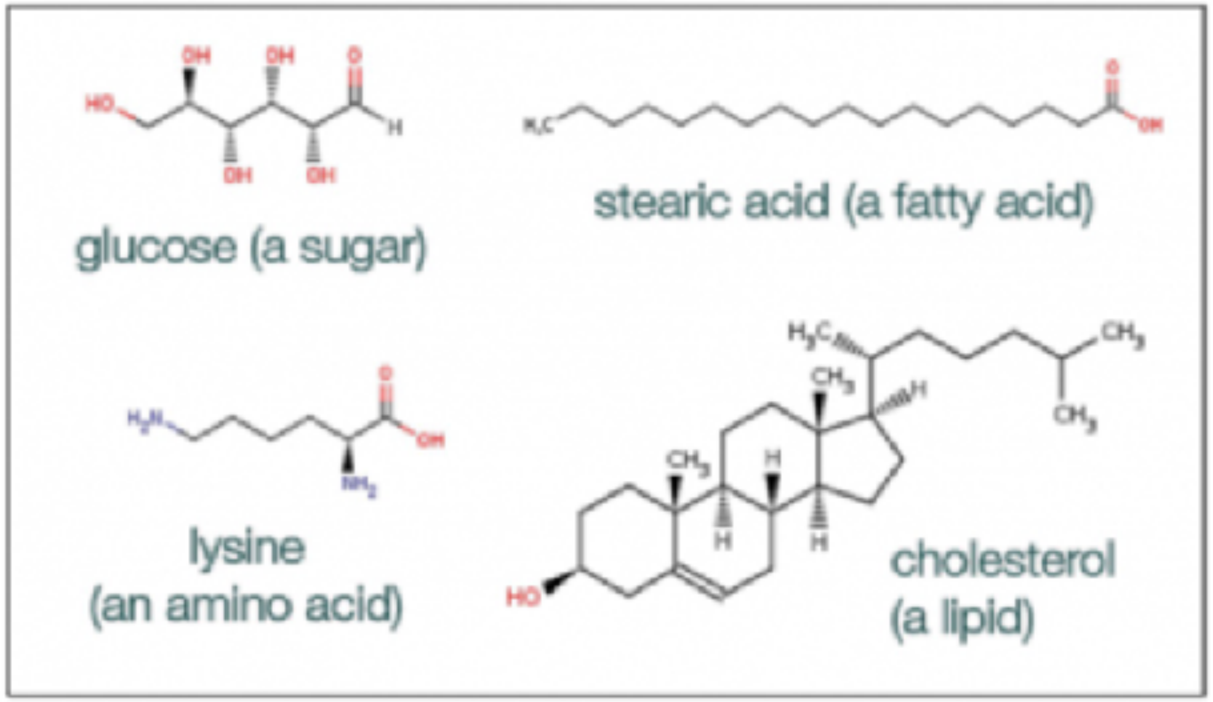
DNA	Genomics
RNA	Transcriptomics
Proteins	Proteomics
Biochemical	**Metabolomics**
Biological Phenotype	

Single cell Metabolomics

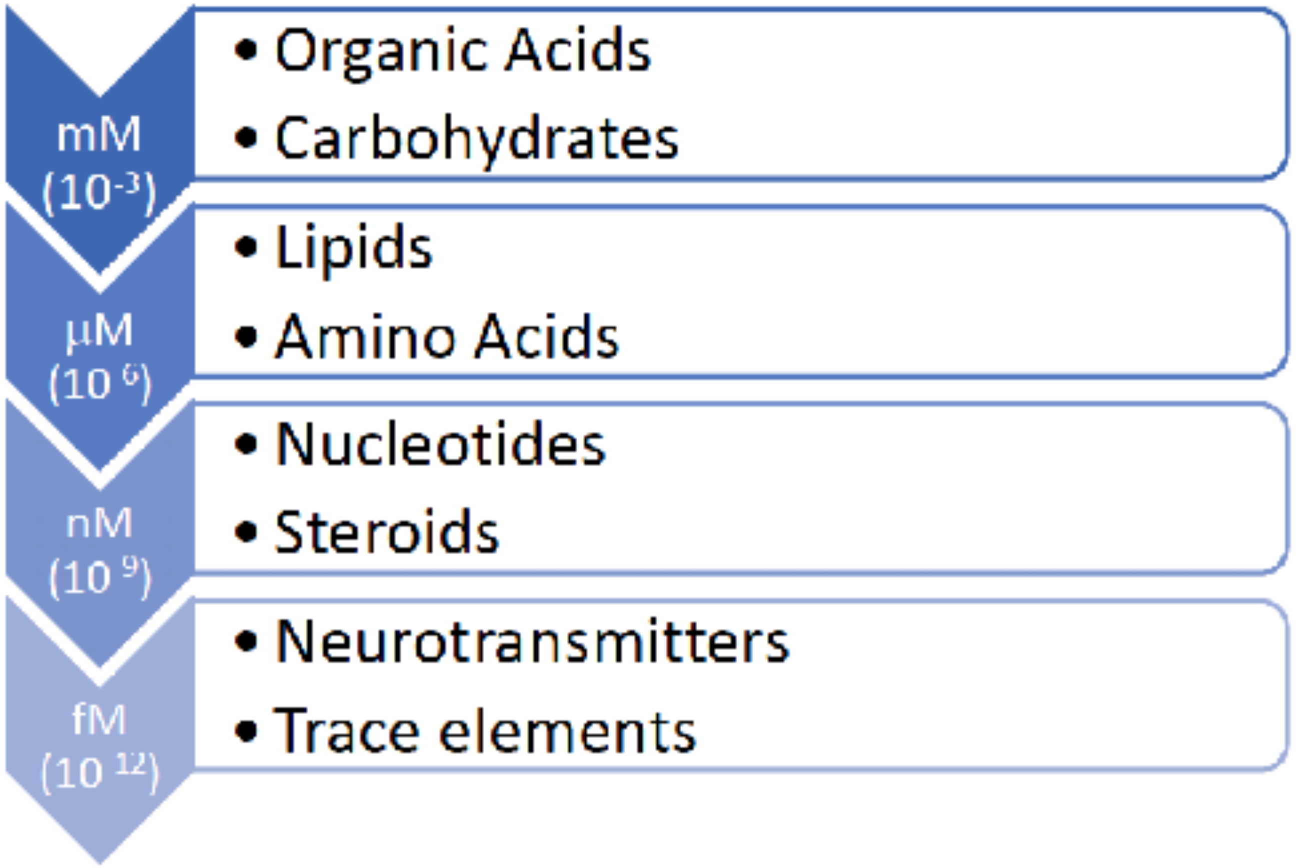


Single cell Metabolomics

Structural diversity



Wide range of concentrations



mM (10^{-3})	Organic Acids Carbohydrates
µM (10^{-6})	Lipids Amino Acids
nM (10^{-9})	Nucleotides Steroids
fM (10^{-12})	Neurotransmitters Trace elements

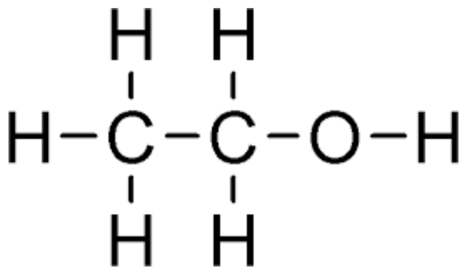
Highly Dynamic

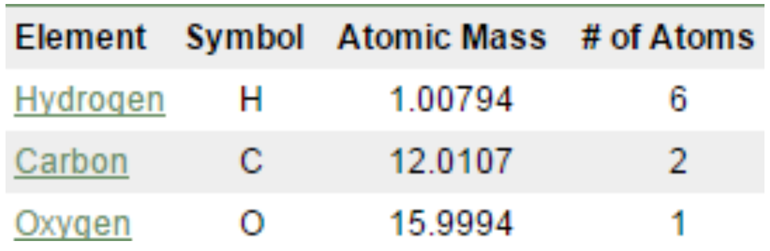
Single cell Metabolomics

Mass Spectrometry

- A compound can be identified by its mass

Example: Ethanol - CH_3CH_2OH, with molecular weight MW = 46.06844 g/mol



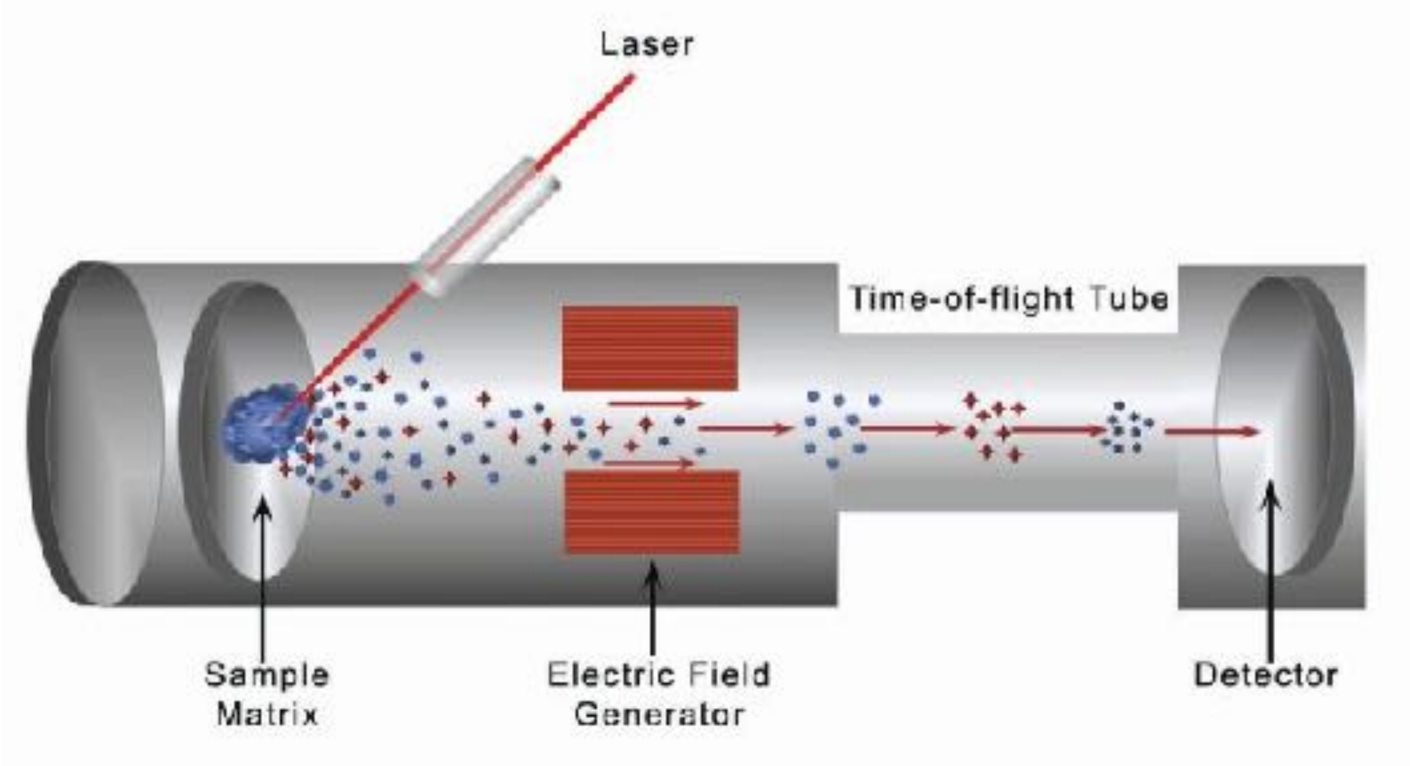


Element	Symbol	Atomic Mass	# of Atoms
Hydrogen	H	1.00794	6
Carbon	C	12.0107	2
Oxygen	O	15.9994	1

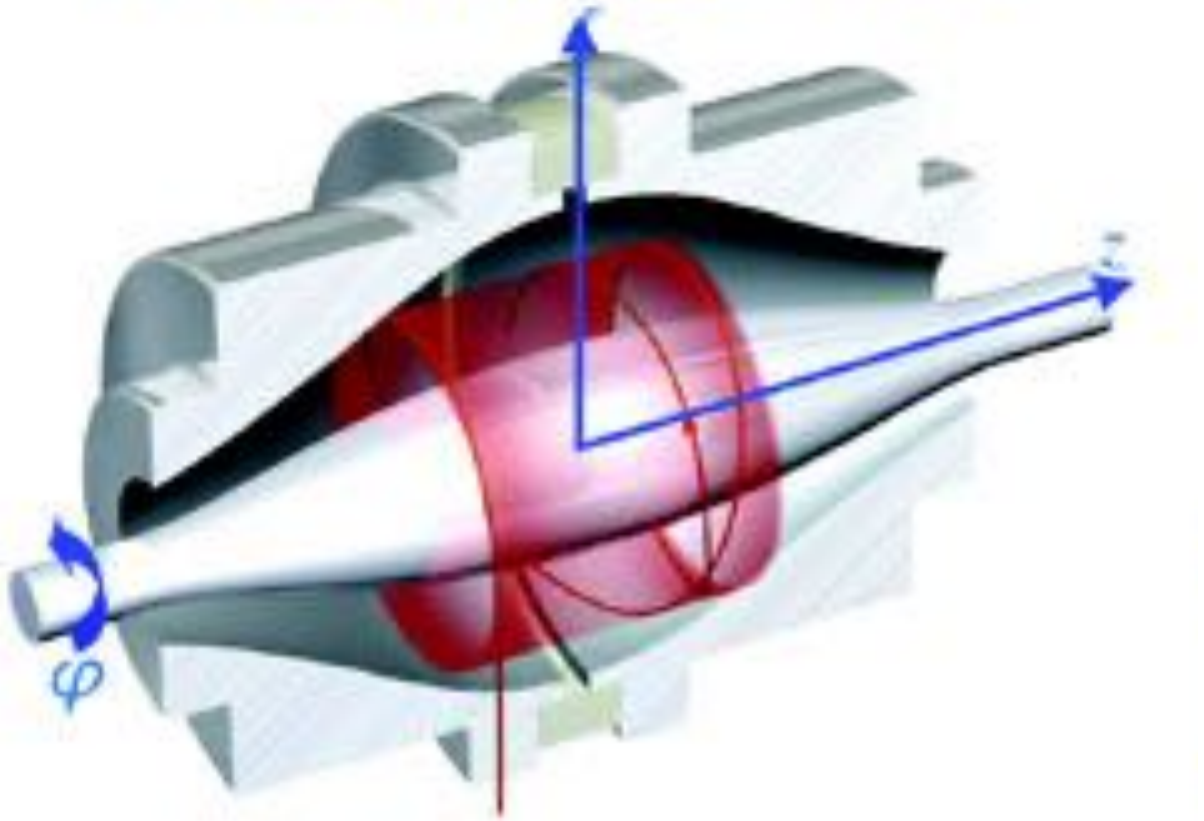
- Mass Spectrometry needs to:
  - Ionize molecules
  - Detect molecules

- MW can be determined with high accuracy
- It can be used for qualitative (untargeted) or quantitative (targeted) analysis

Single cell Metabolomics



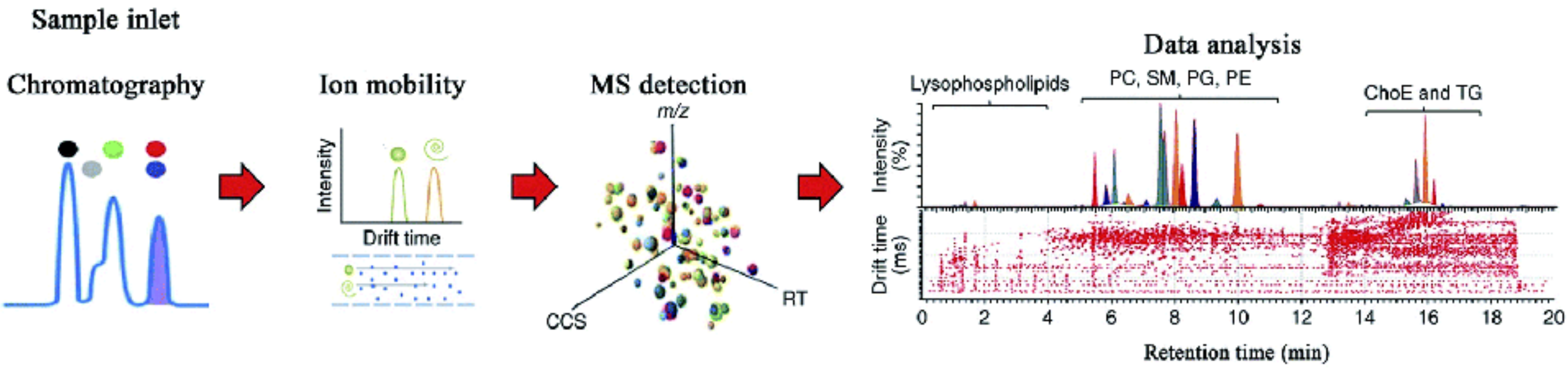
$$\frac{m}{z} = 2e\mathbf{E}s\left(\frac{t}{d}\right)^2$$



$$\omega_z = \sqrt{\frac{k}{m/z}}$$

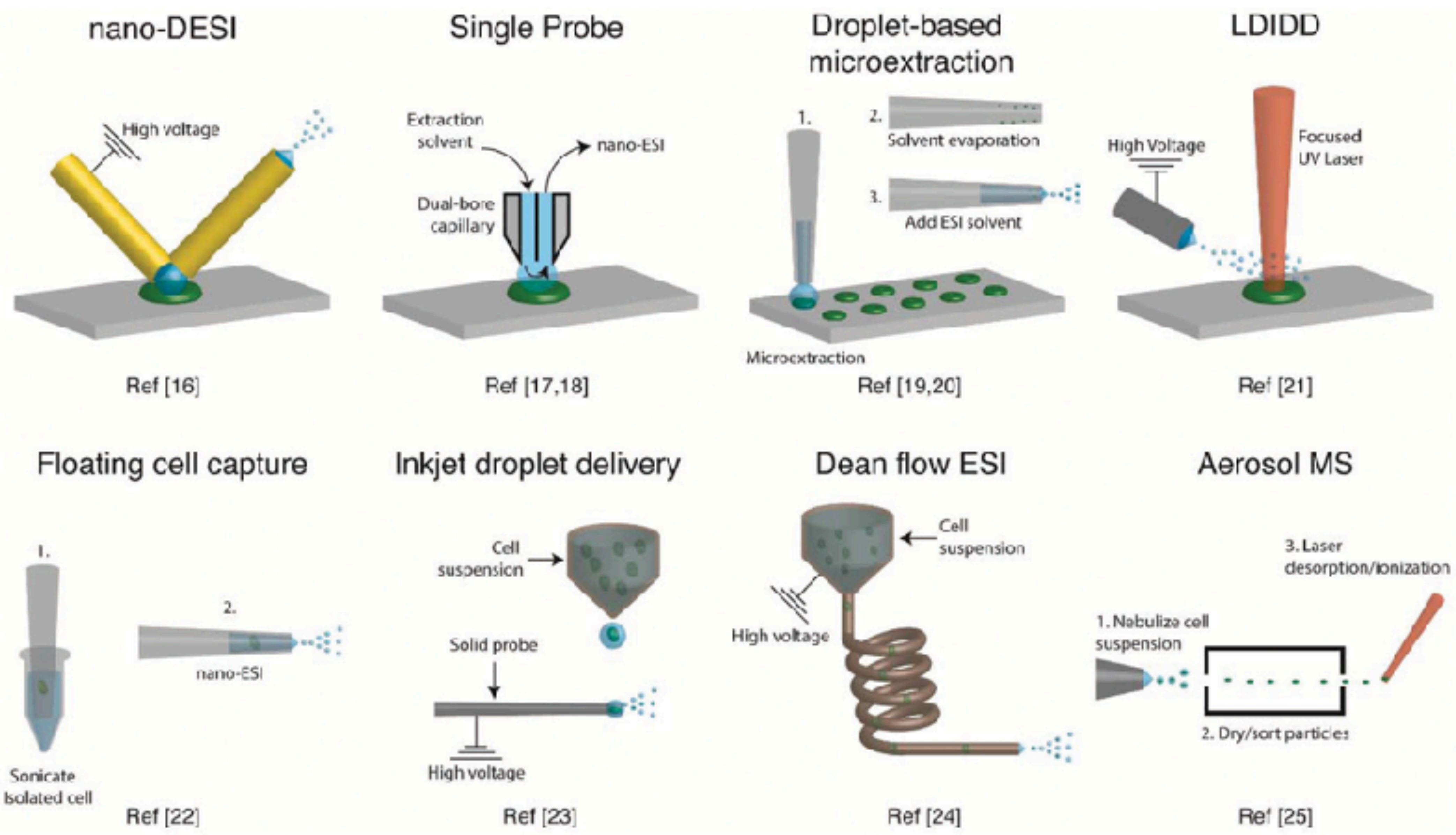
Single cell Metabolomics

Mass spectrometry (**MS**) is an analytical technique that measures the mass-to-charge ratio of ions. The results are typically presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures. MS is used to identify and to quantify metabolites after optional separation. Identification leverages the distinct patterns in which analytes fragment which can be thought of as a mass spectral fingerprint. MS is both sensitive and can be very specific.



With high sensitivity and specificity, wide molecular coverage, relative quantitation, and structural identification capabilities, MS is becoming an important tool for **Single-cell metabolomics.** This, by the way, presents many challenges due to the limited sample volume, low analyte amounts, and rapid turnover rates of the cellular metabolome. Most single cell metabolomics studies are, thus performed using MS in a shotgun-like approach, preferably with high mass resolution.

Single cell Metabolomics



Single cell Metabolomics

Single cell Metabolomics

analytical chemistry

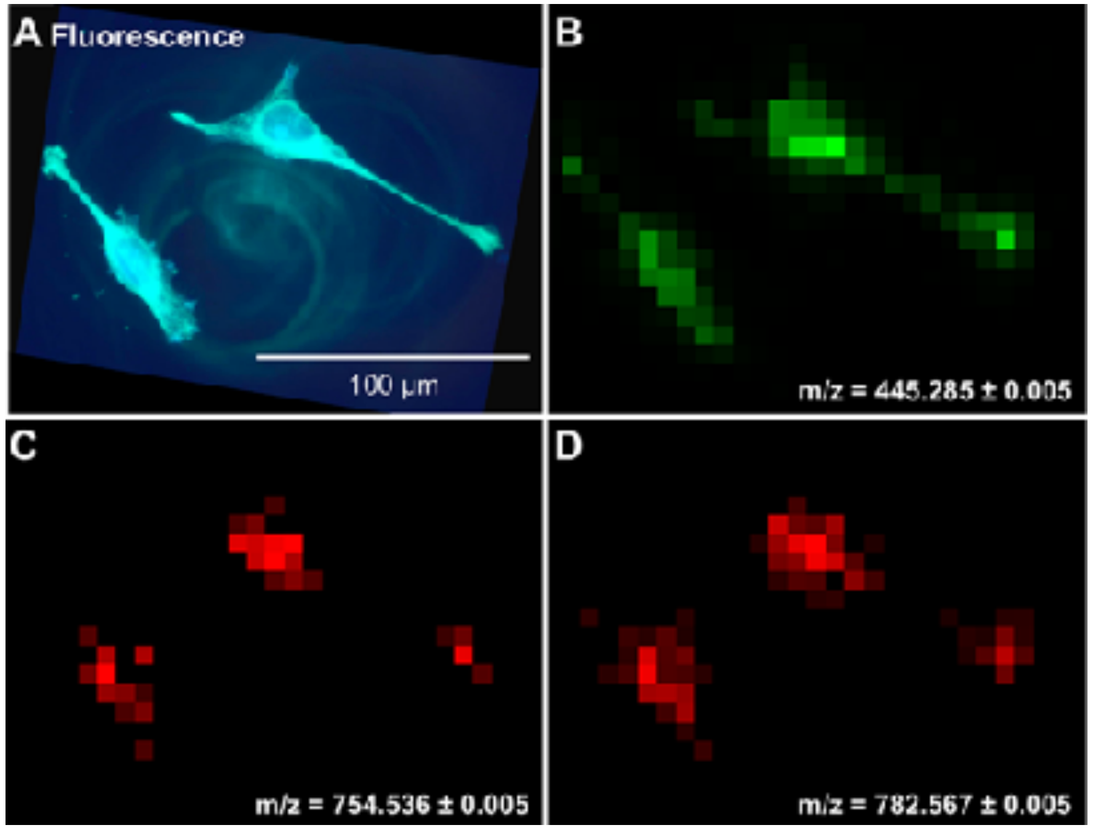
Letter

pubs.acs.org/ac

Single Cell Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging

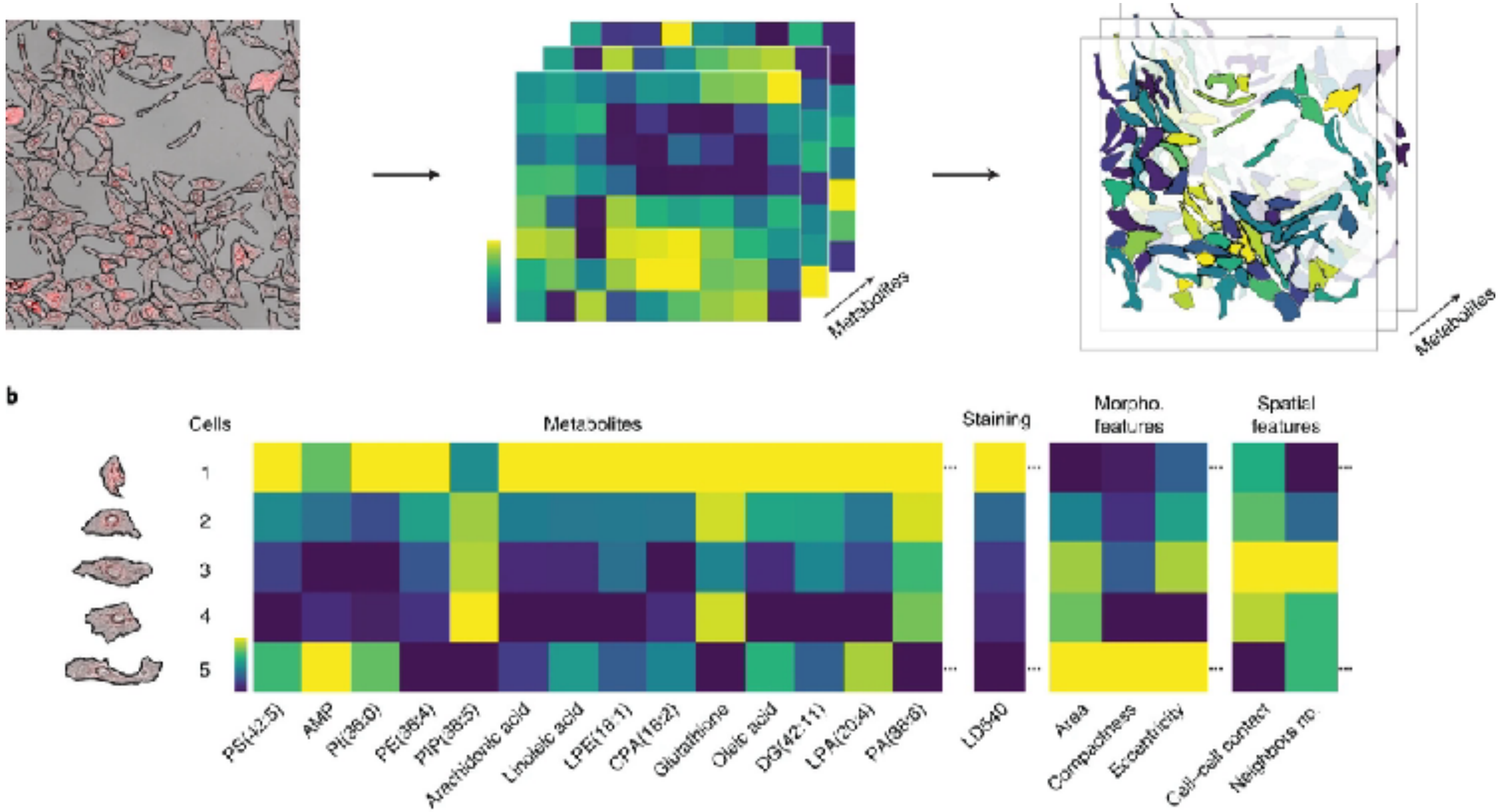
Yvonne Schober, Sabine Guenther, Bernhard Spengler, and Andreas Römpp*

Institute of Inorganic and Analytical Chemistry, Justus Liebig University, Giessen, Germany



Numerous compounds including small metabolites such as adenine, guanine, and cholesterol as well as different lipid classes such as phosphatidylcholine, sphingomyelin, diglycerides, and triglycerides were detected and identified based on a mass spectrum acquired from an individual spot of 7 µm in diameter. These measurements provide molecularly specific images of larger metabolites (phospholipids) in native single cells. The developed method can be used for a wide range of detailed investigations of metabolic changes in single cells.

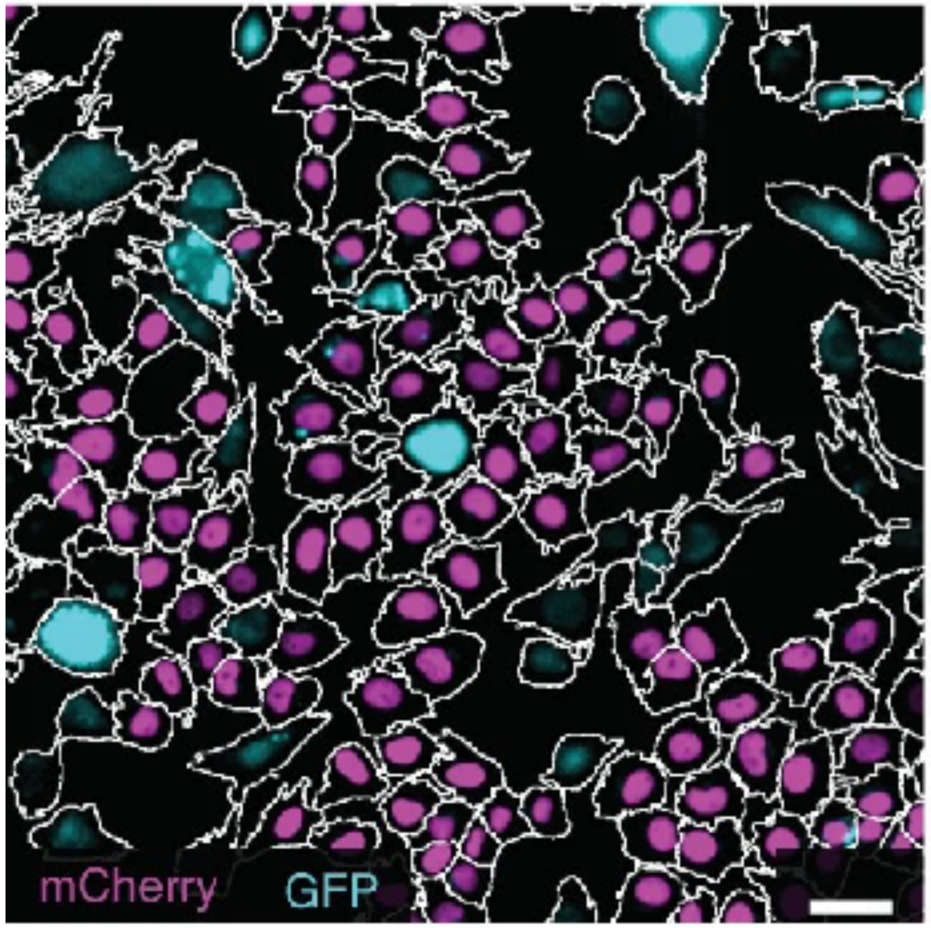
Single cell Metabolomics

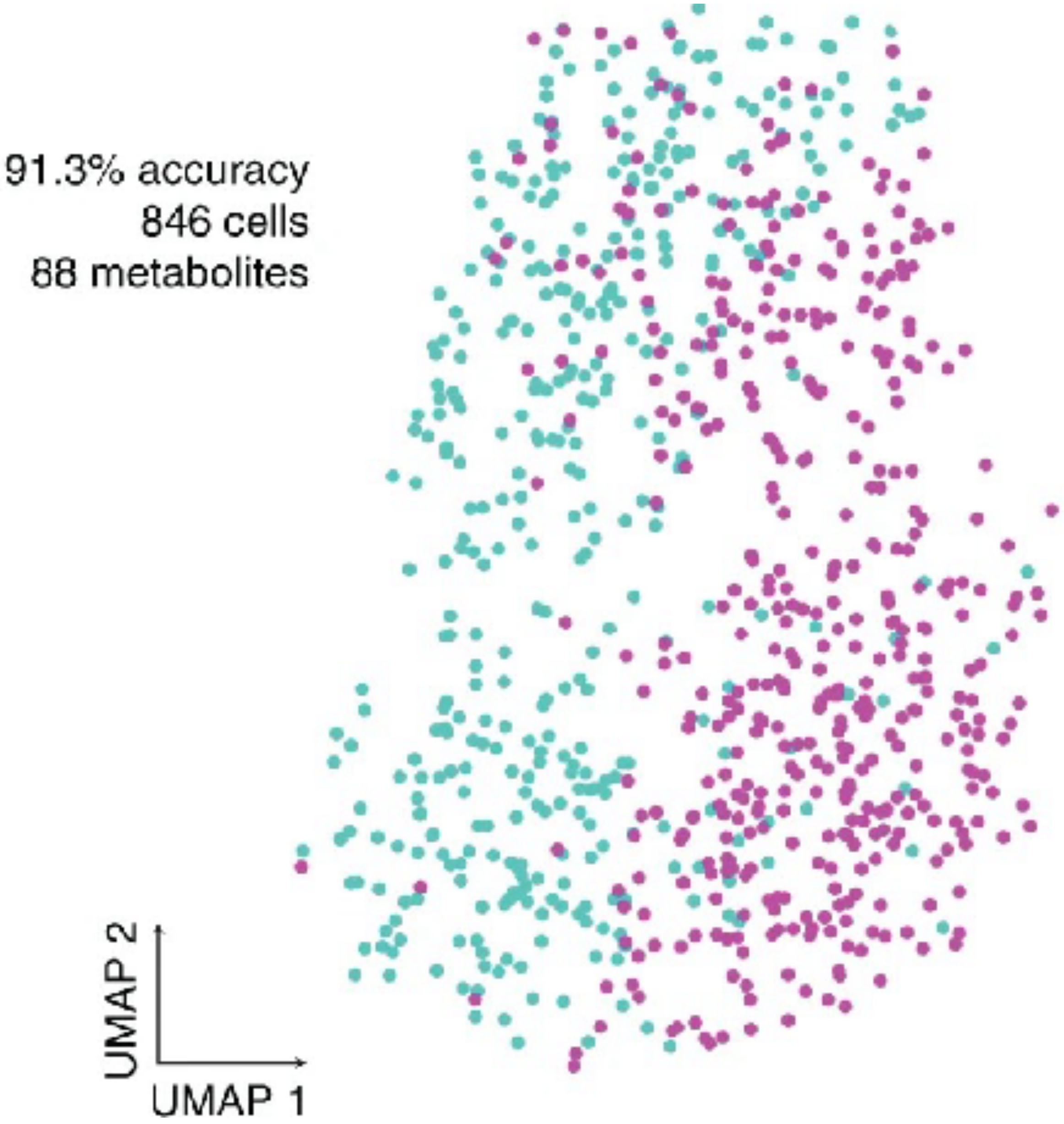


SpaceM reveals metabolic states of single cells

We present SpaceM, an open-source method for in situ single-cell metabolomics that detects >100 metabolites from >1,000 individual cells per hour, together with a fluorescence-based readout and retention of morpho-spatial features. We validated SpaceM by predicting the cell types of cocultured human epithelial cells and mouse fibroblasts.

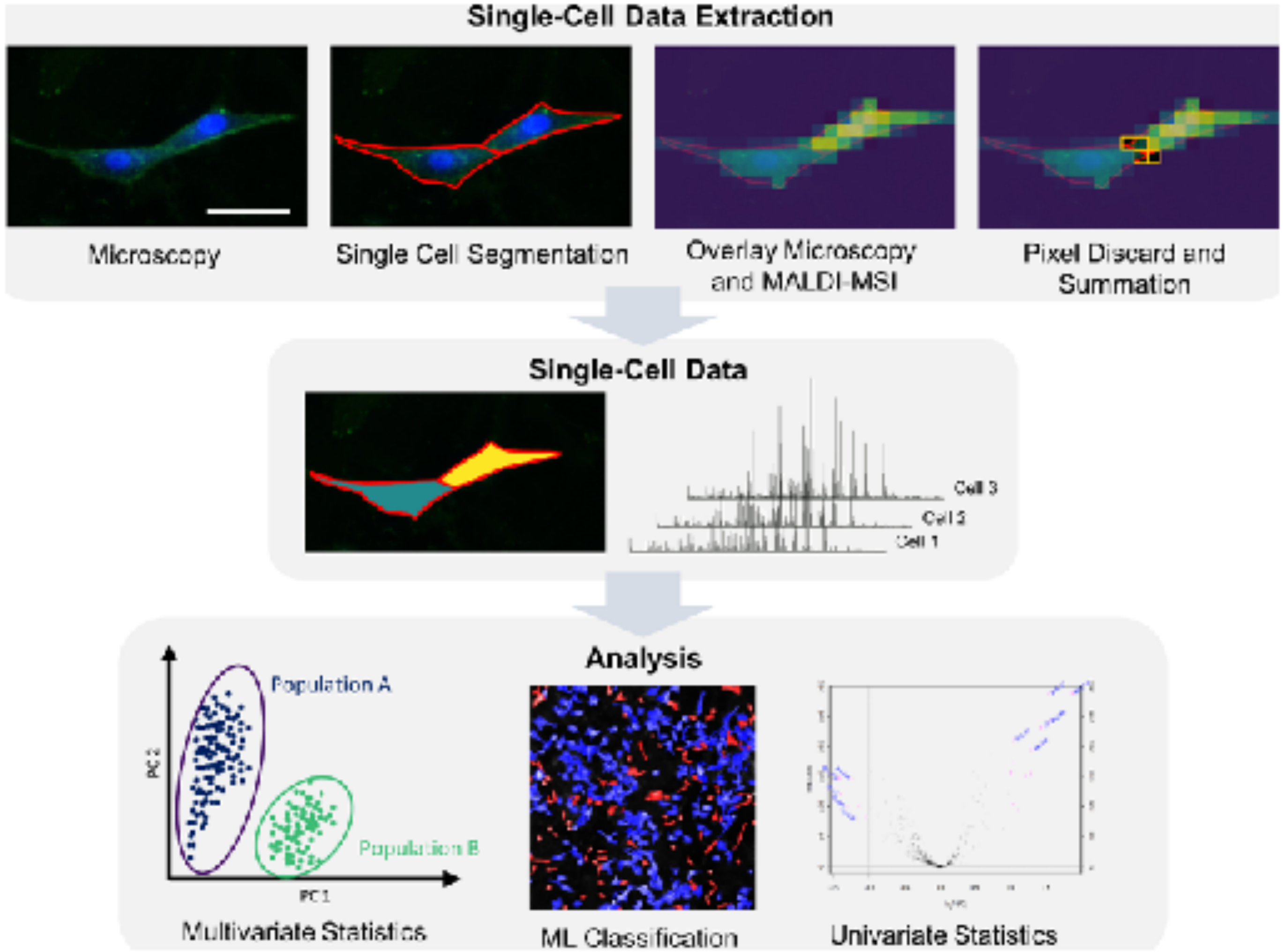
Single cell Metabolomics


HeLa and NIH3T3 coculture


91.3% accuracy
846 cells
88 metabolites

Single cell Metabolomics

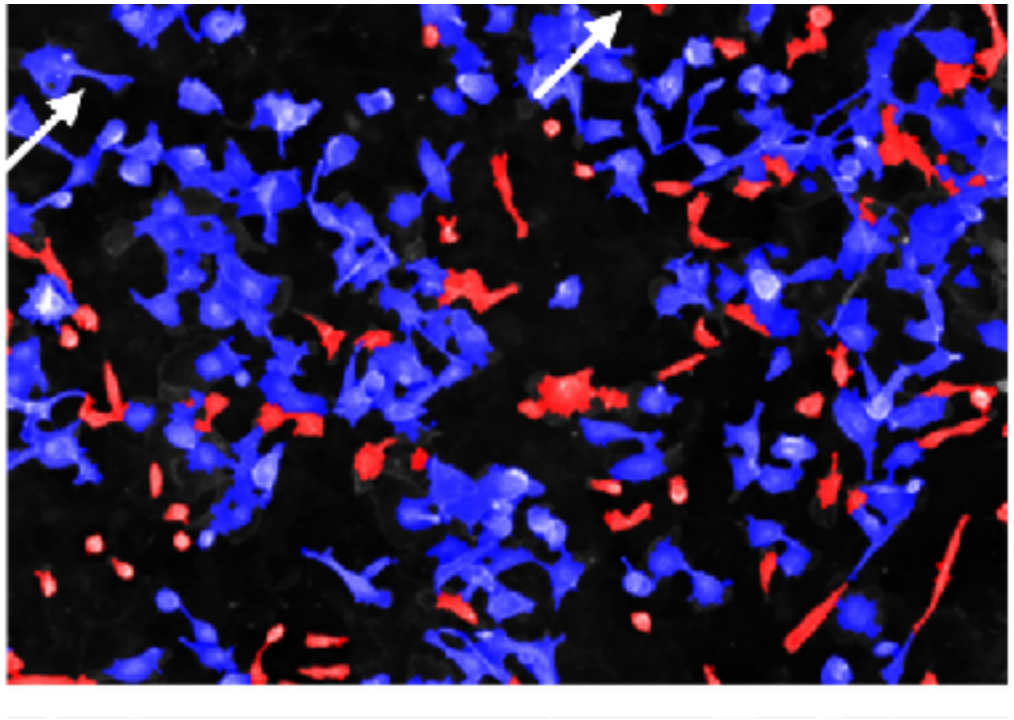
Mass spectrometry imaging to explore molecular heterogeneity in cell culture

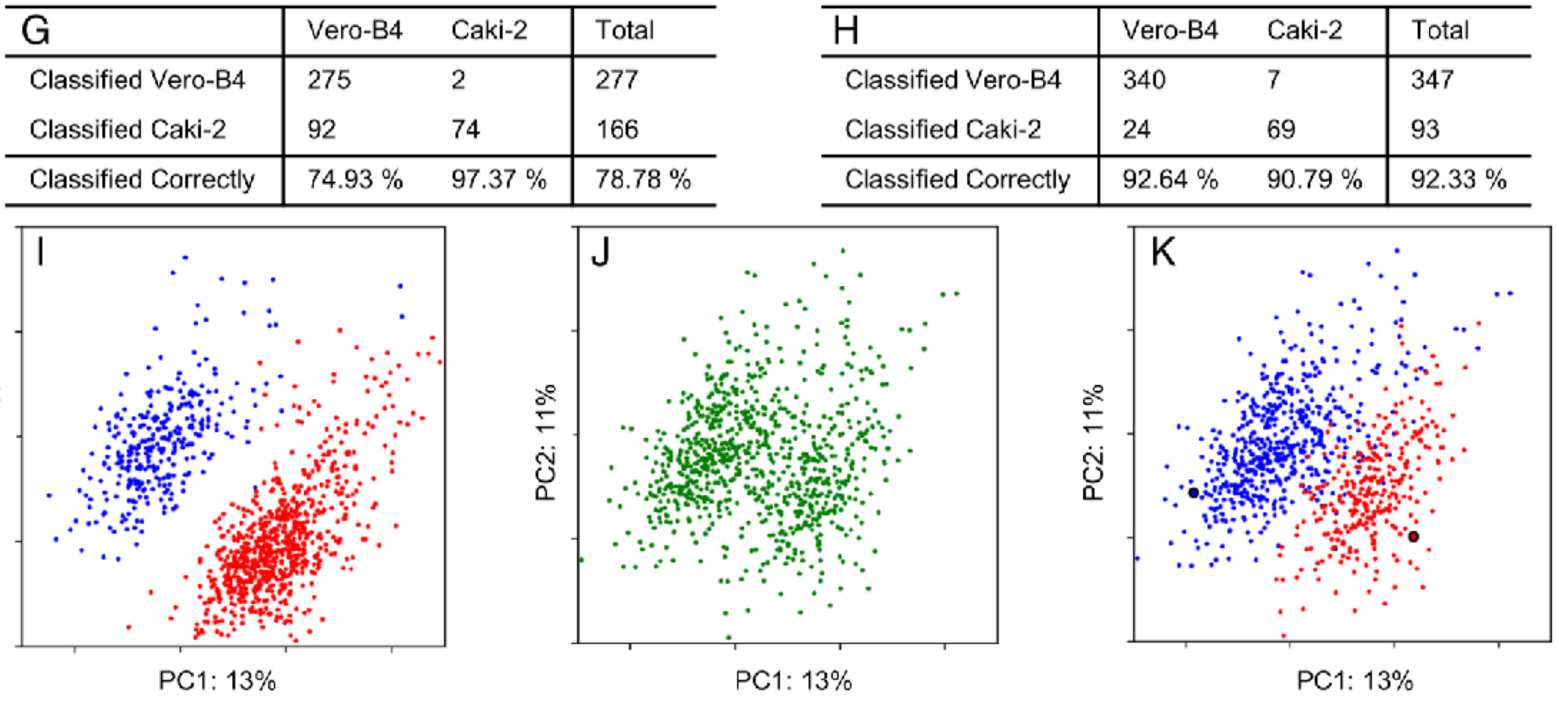


Here, we present a sensitive approach to single-cell MS based on high-resolution MALDI-2-MS imaging in combination with MALDI-compatible staining and use of optical microscopy. Our approach allowed analyzing large amounts of unperturbed cells directly from the growth chamber. Confident coregistration of both modalities enabled a reliable compilation of single-cell mass spectra and a straightforward inclusion of optical as well as mass spectrometric features in the interpretation of data. The resulting multimodal datasets permit the use of various statistical methods like machine learning-driven classification and multivariate analysis based on molecular profile and establish a direct connection of MS data with microscopy information of individual cells.

2022

Single cell Metabolomics

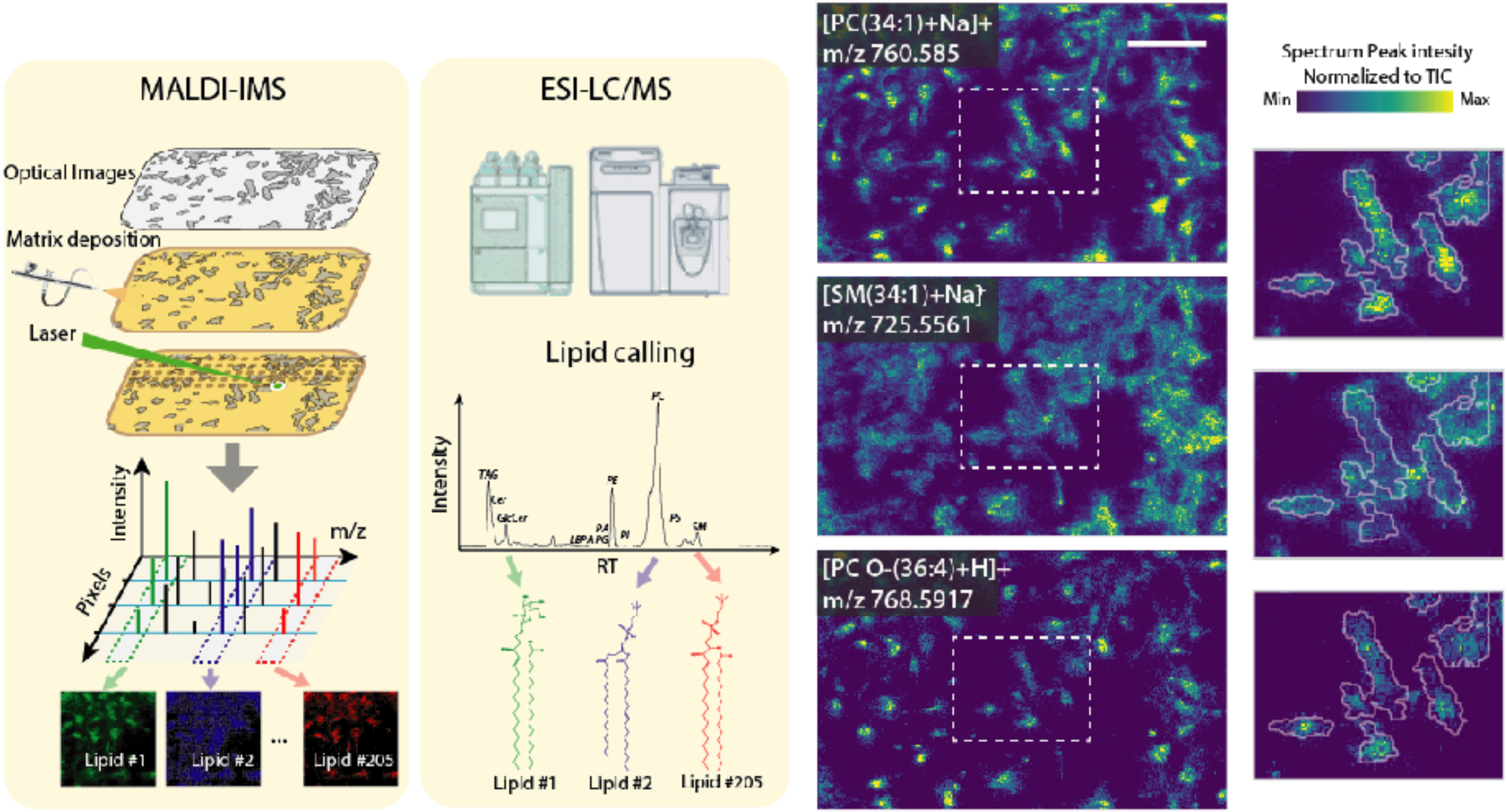




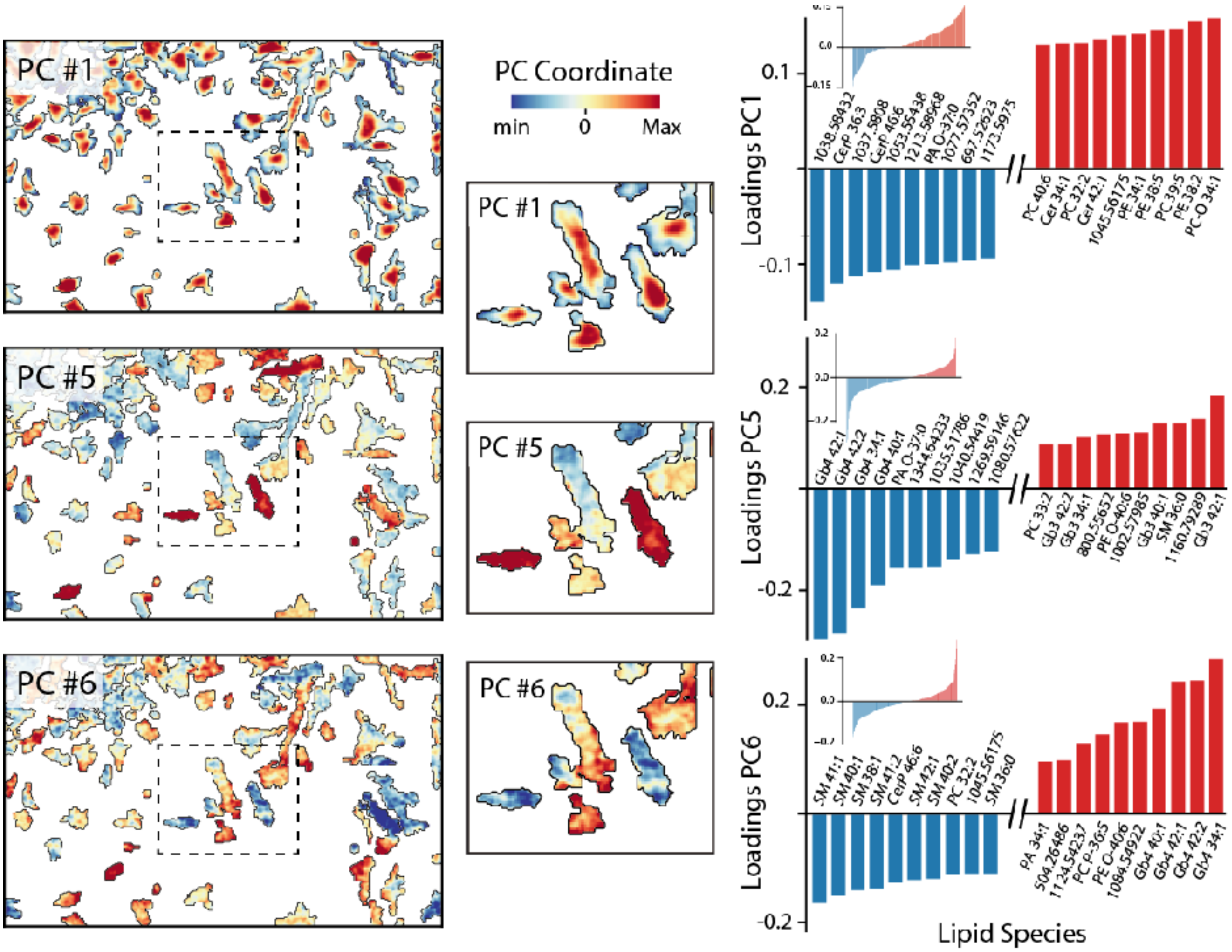
G	Vero-B4	Caki-2	Total
Classified Vero-B4	275	2	277
Classified Caki-2	92	74	166
Classified Correctly	74.93 %	97.37 %	78.78 %

H	Vero-B4	Caki-2	Total
Classified Vero-B4	340	7	347
Classified Caki-2	24	69	93
Classified Correctly	92.64 %	90.79 %	92.33 %

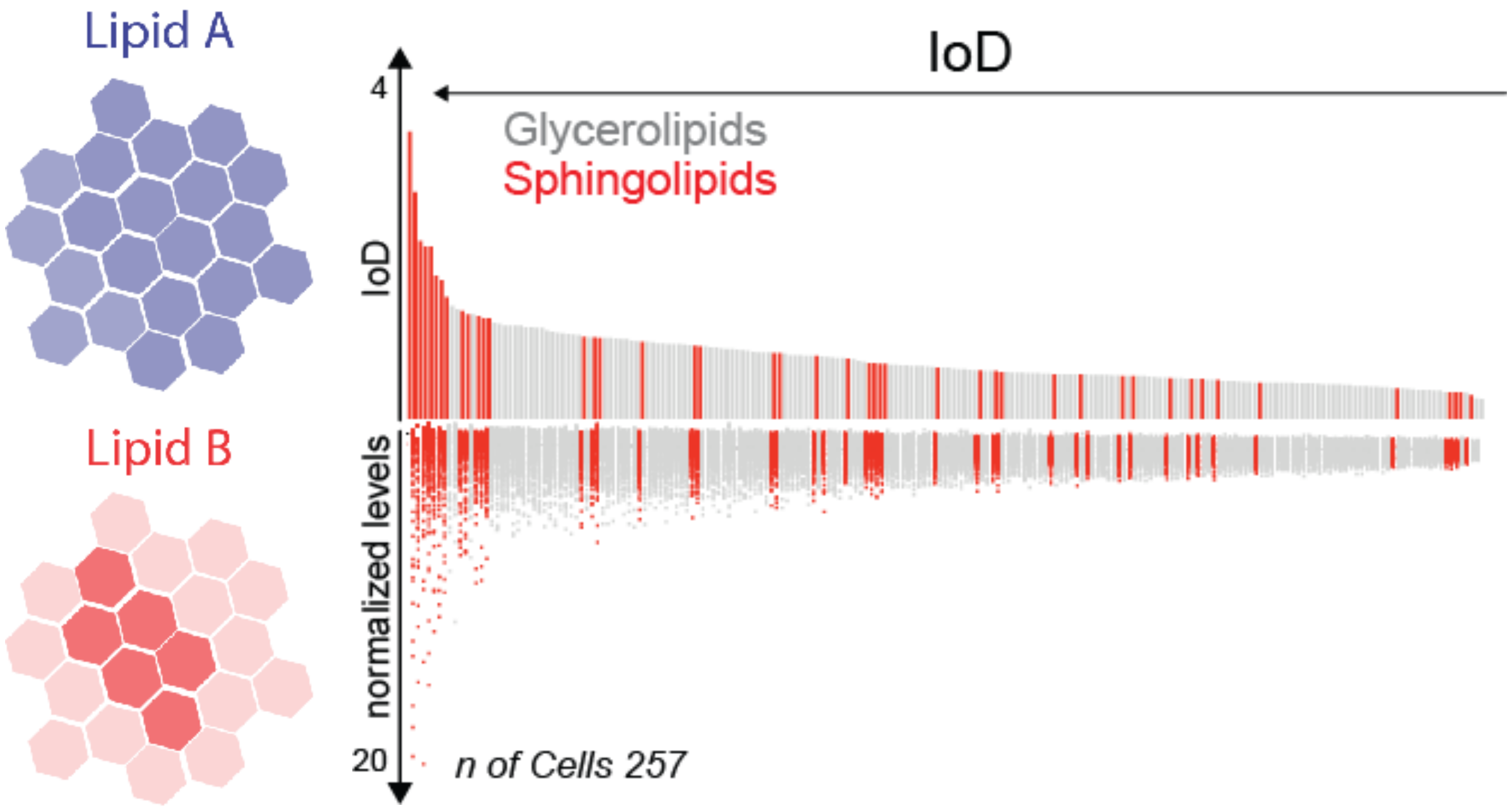
Single cell Metabolomics



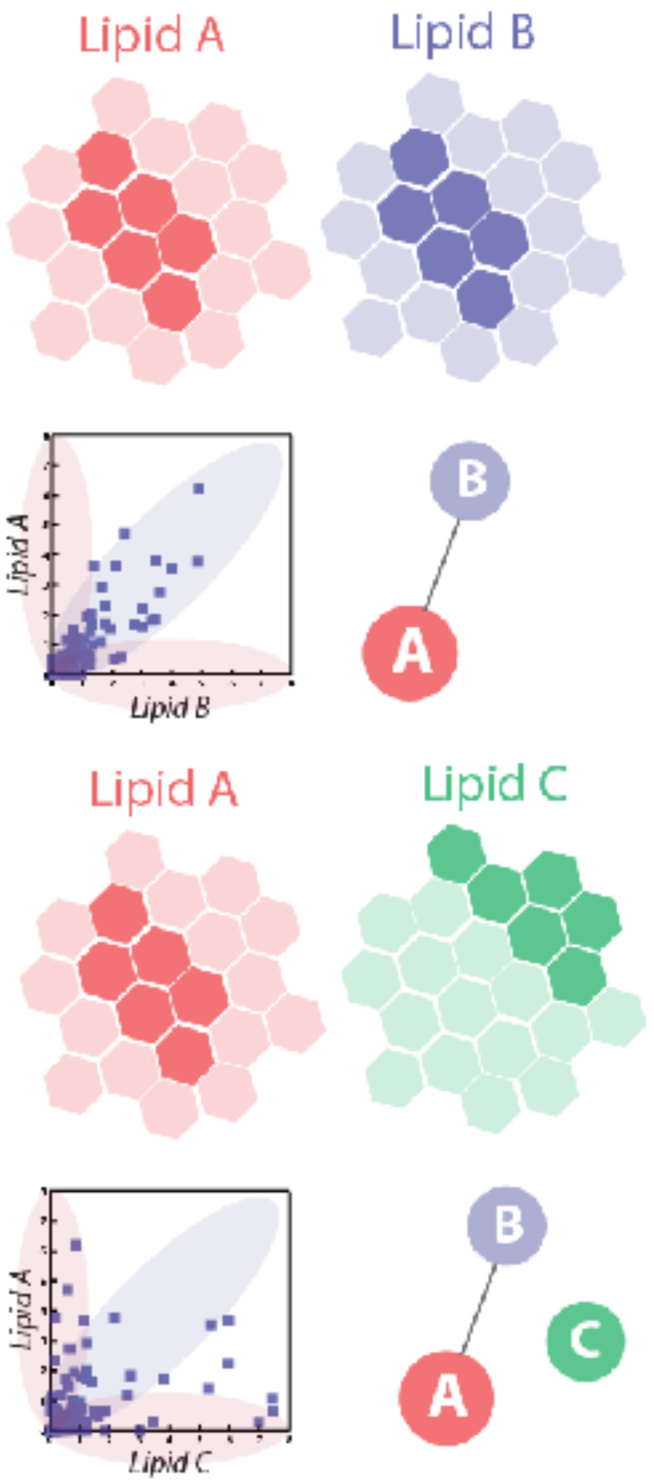
Single cell Metabolomics

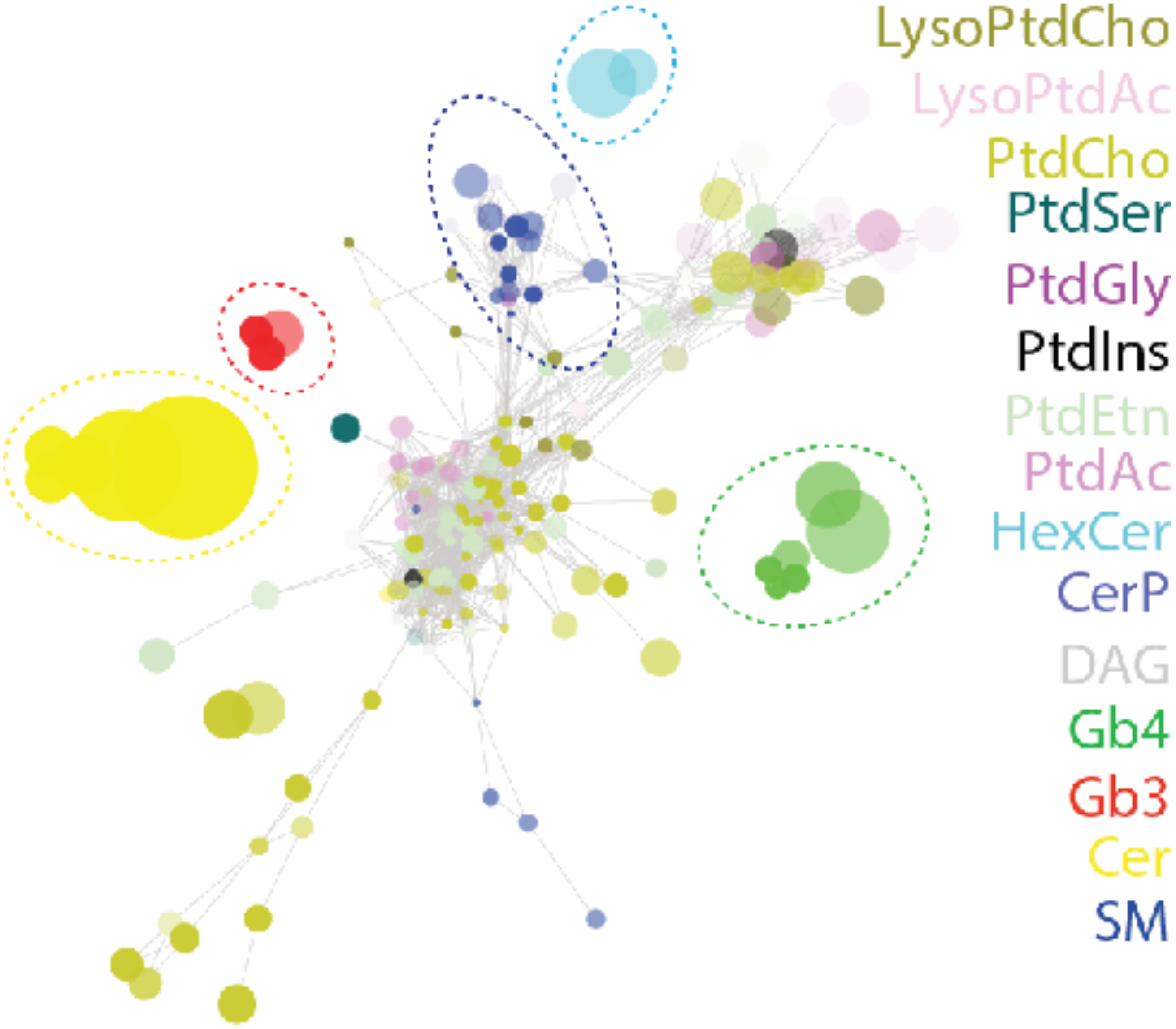


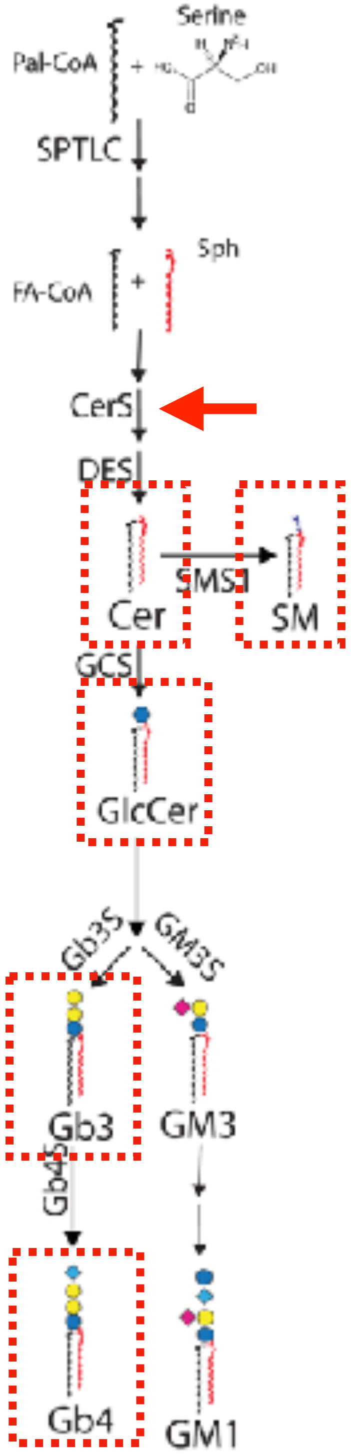
Single cell Metabolomics



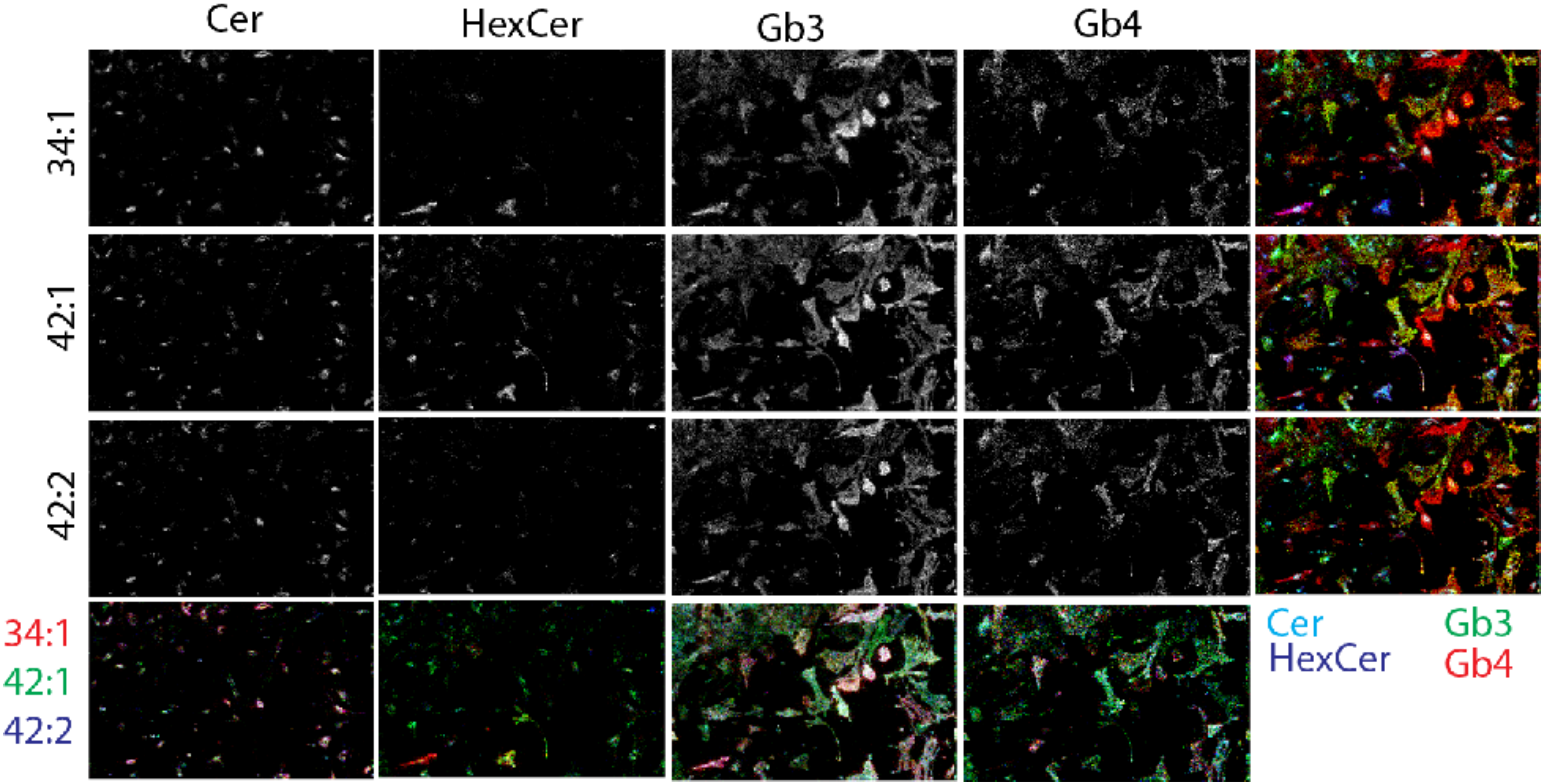
Single cell Metabolomics



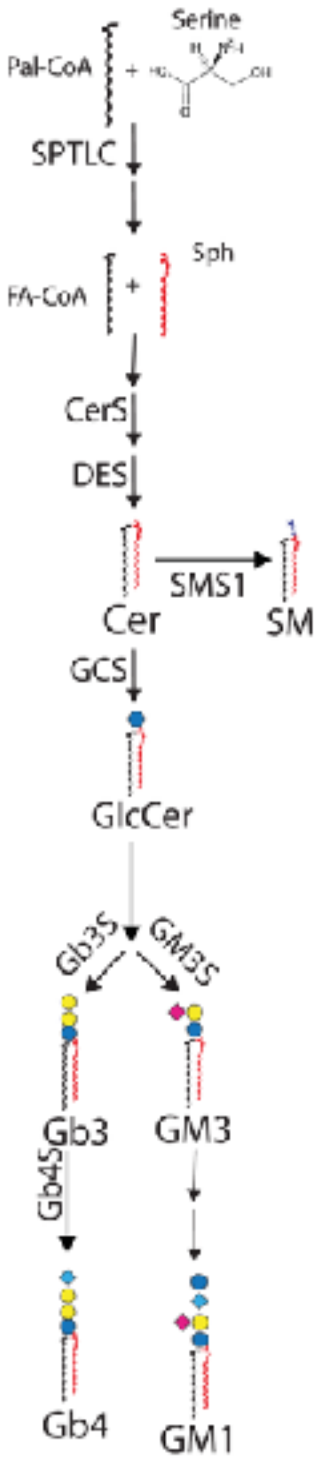


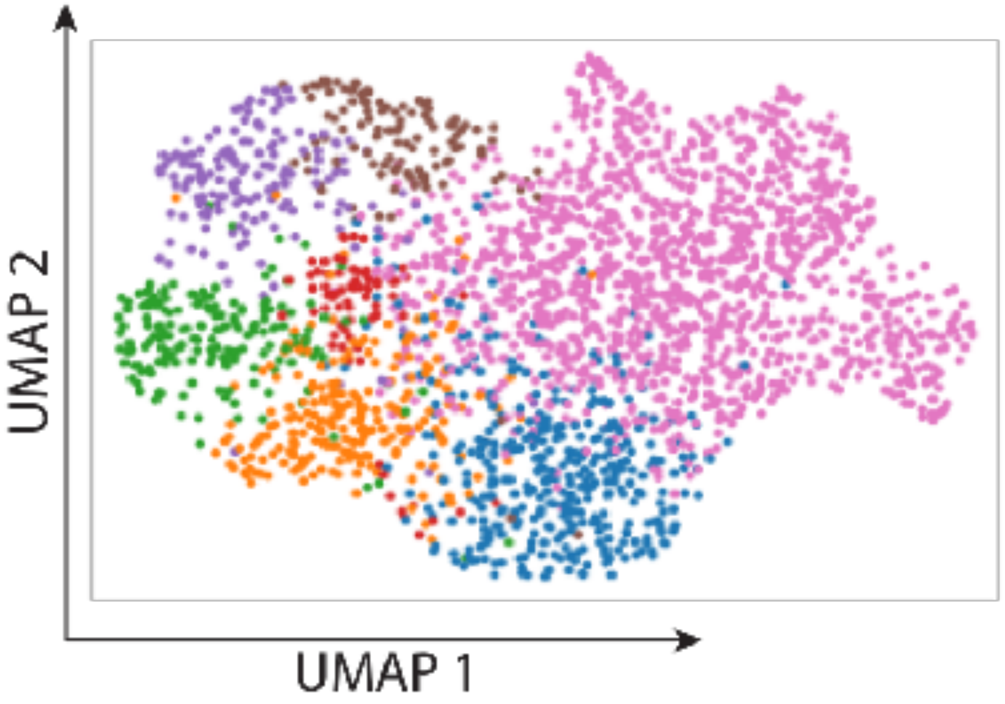


Single-cell Lipidomics



Single cell Metabolomics





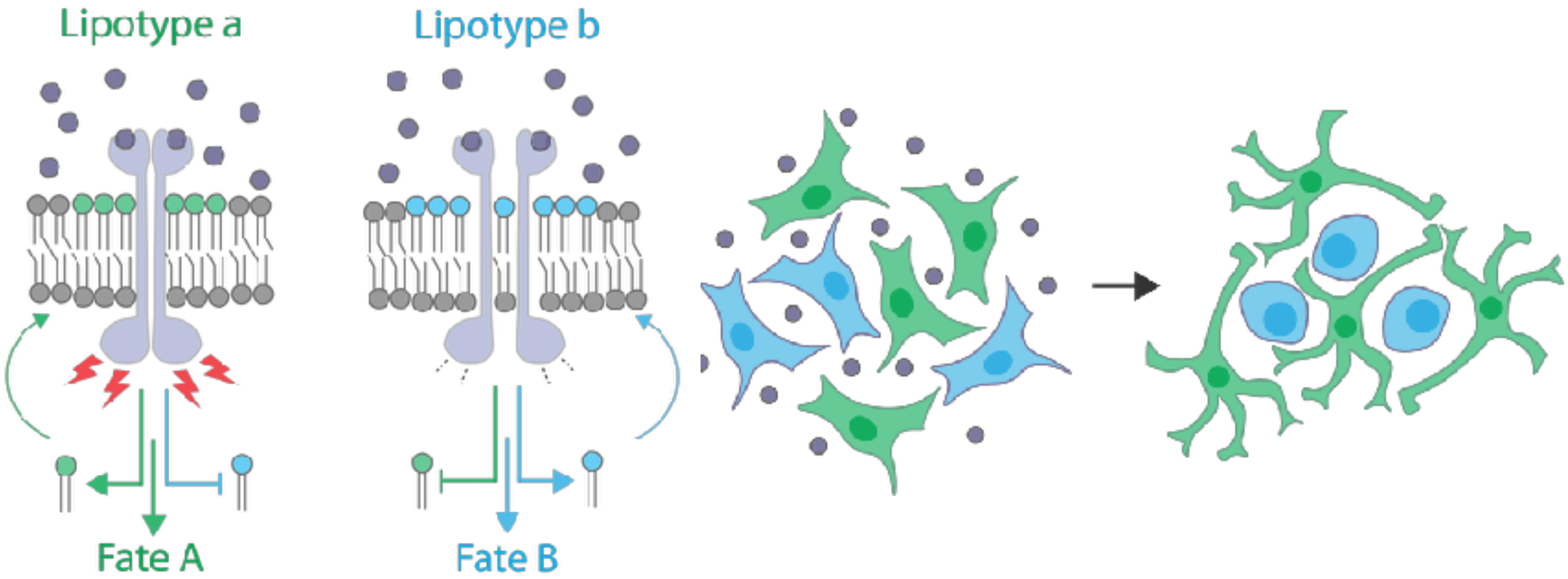
Single cell Metabolomics

Lipotypes represent local energetic minima of the lipid compositional landscape.

Lipotypes are both a consequence and a component of differentiation programs that lead to cell state emergence.

Cells populating different regions in tissues and organs are likely to belong to different *lipotypes* and *lipotypes* to mark different anatomical structures

Lipid composition is a major driver for the establishment of cell identity



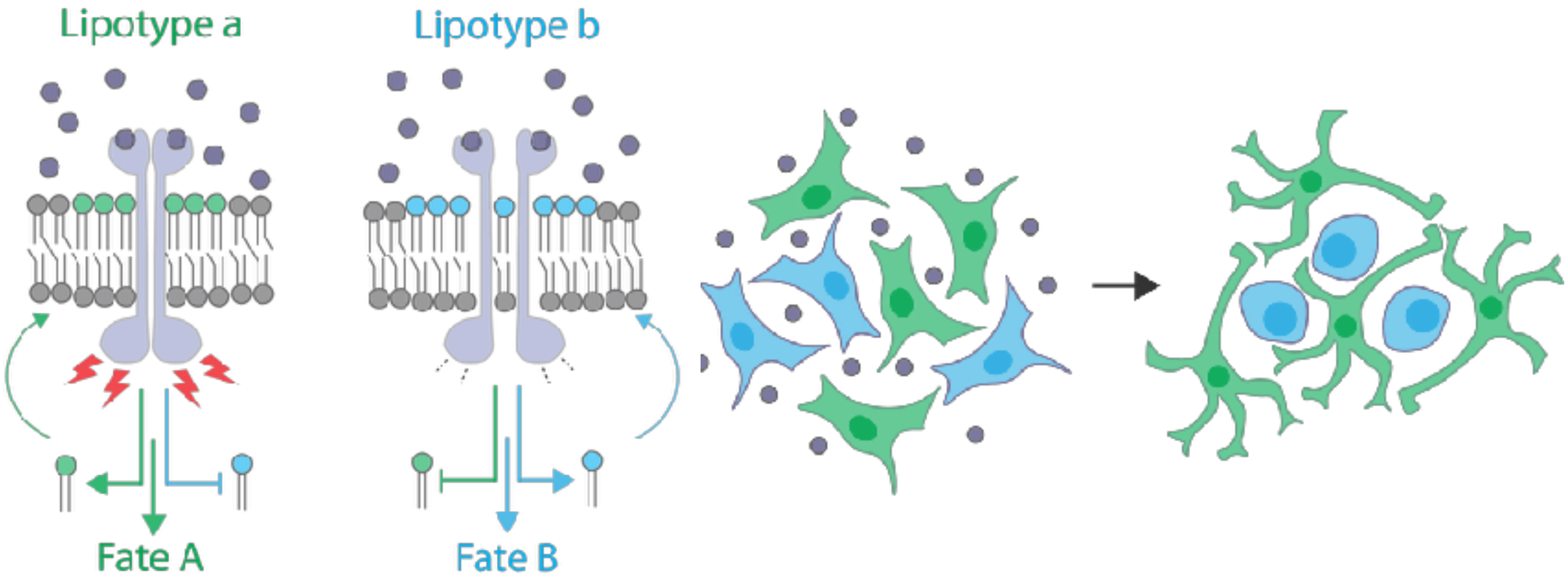
Single cell Metabolomics

Lipotypes represent local energetic minima of the lipid compositional landscape.

Lipotypes are both a consequence and a component of differentiation programs that lead to cell state emergence.

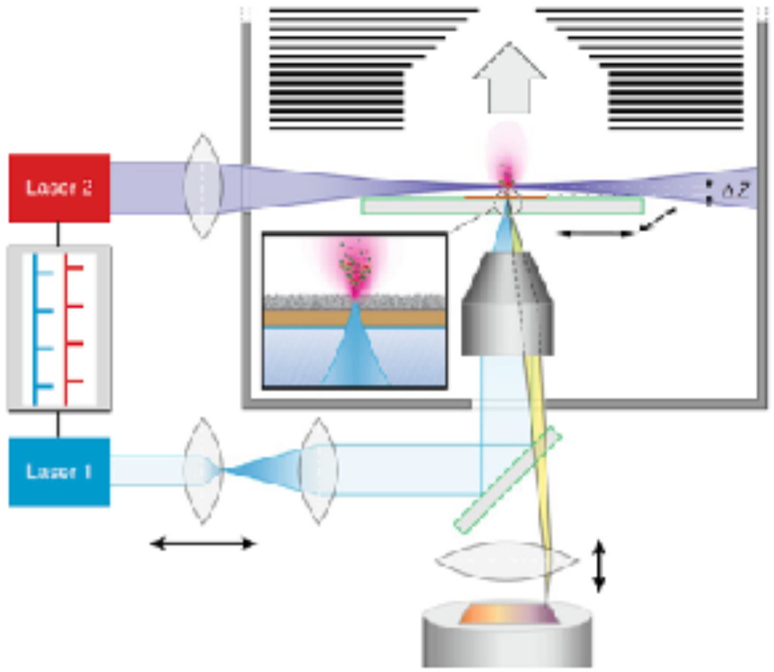
Cells populating different regions in tissues and organs are likely to belong to different *lipotypes* and *lipotypes* to mark different anatomical structures

Lipid composition is a major driver for the establishment of cell identity

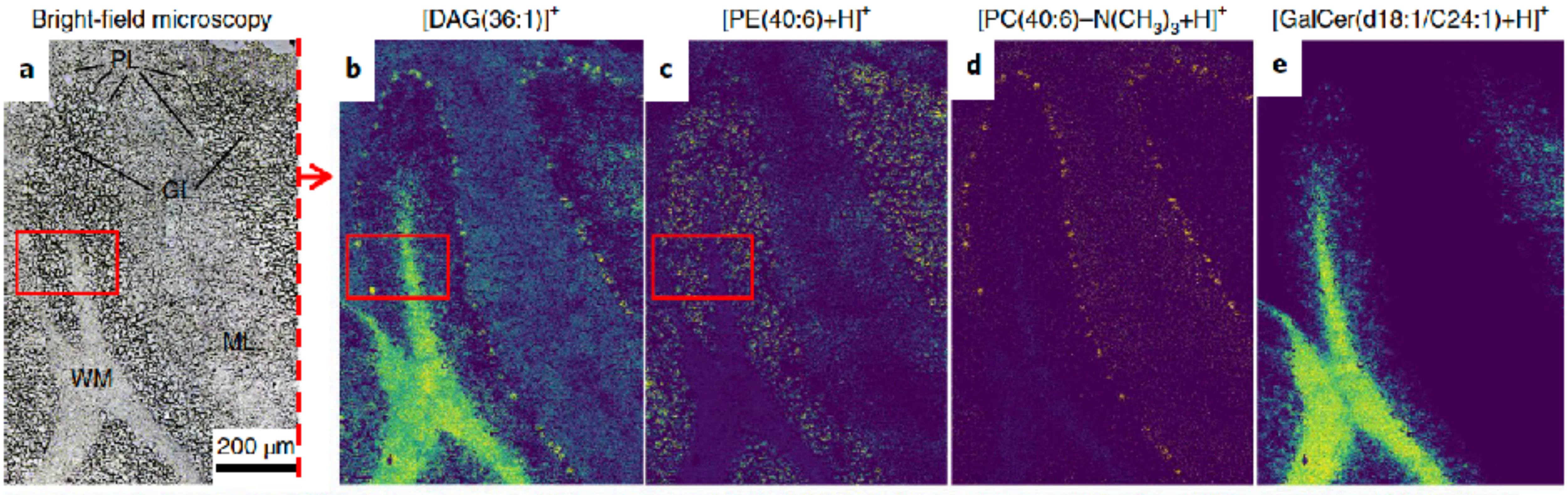


Spatial metabolomics

MALDI-2-IMS

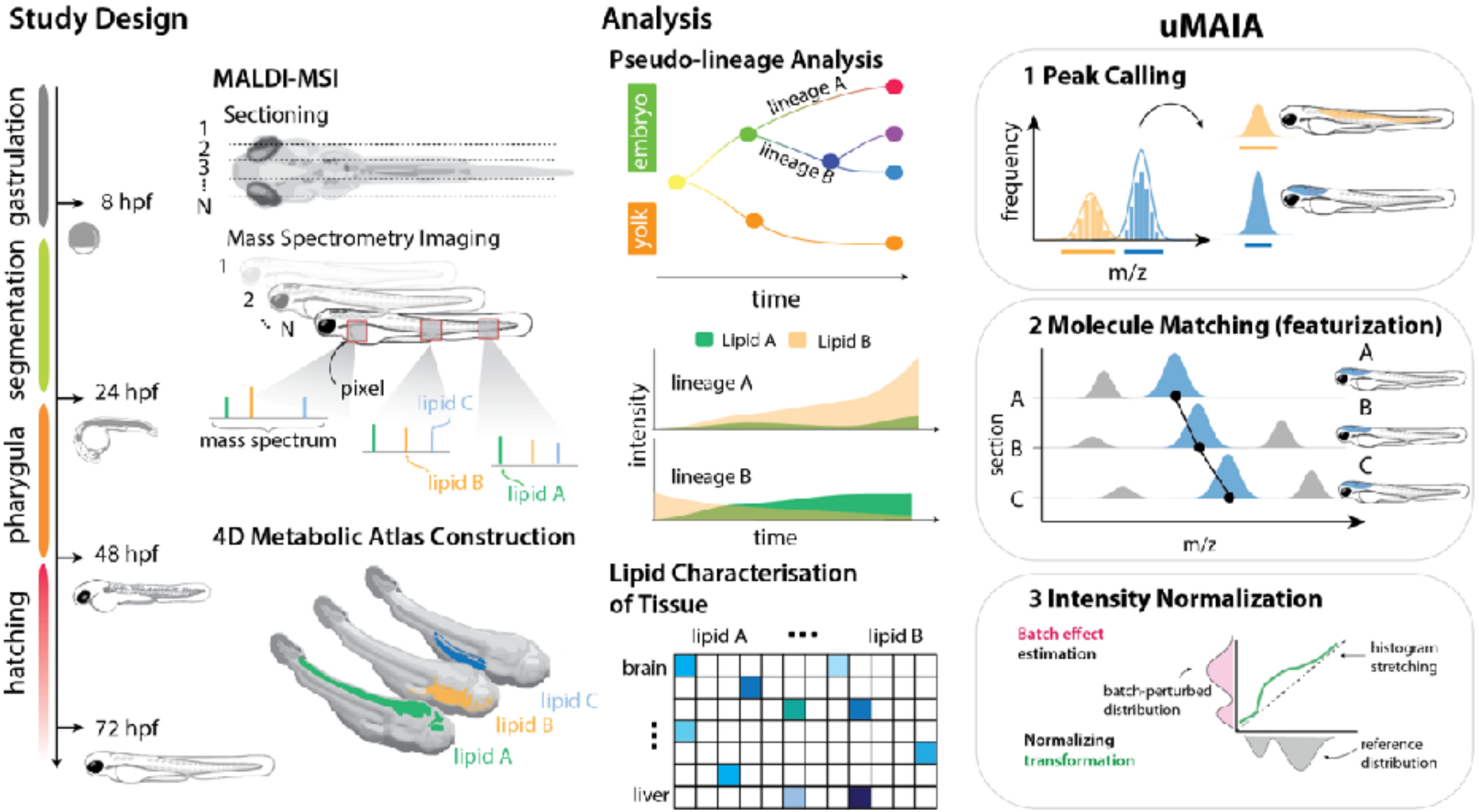


Matrix-assisted laser desorption–ionization mass spectrometry imaging in transmission-mode geometry (t-MALDI–MSI) can provide molecular information with a pixel size of 1 µm and smaller, which makes this label-free method highly interesting for characterizing the chemical composition of tissues and cells on a (sub)cellular level. laser-induced postionization (MALDI-2) increase the sensitivity of this technique to allow in situ single cell metabolomics



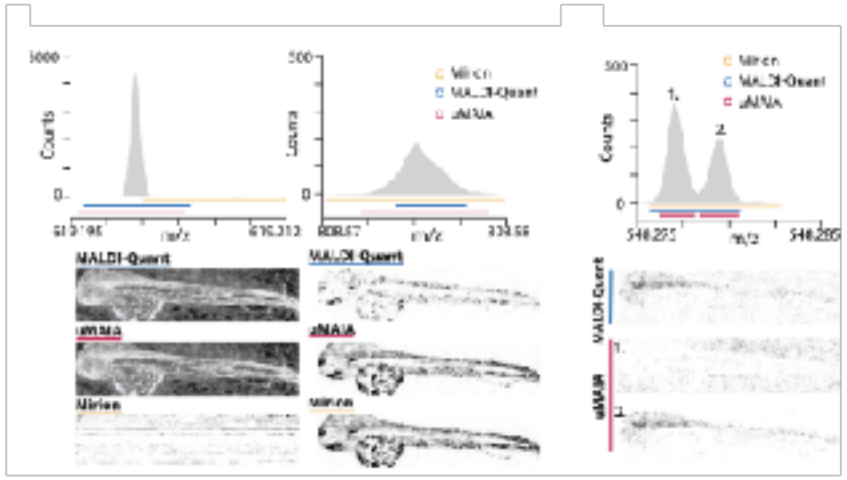
Niehaus et al Nat Met 2019

Spatial metabolomics

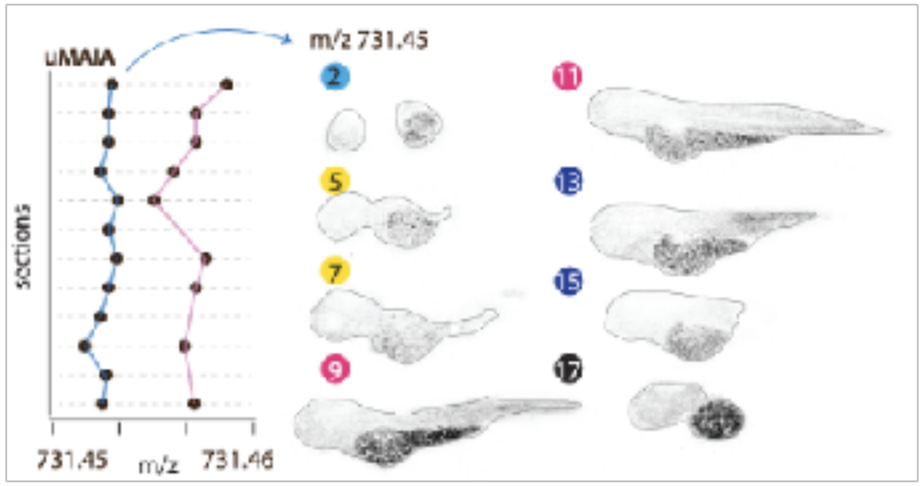


Schede, Alieh et al. bioRXiv, 2024

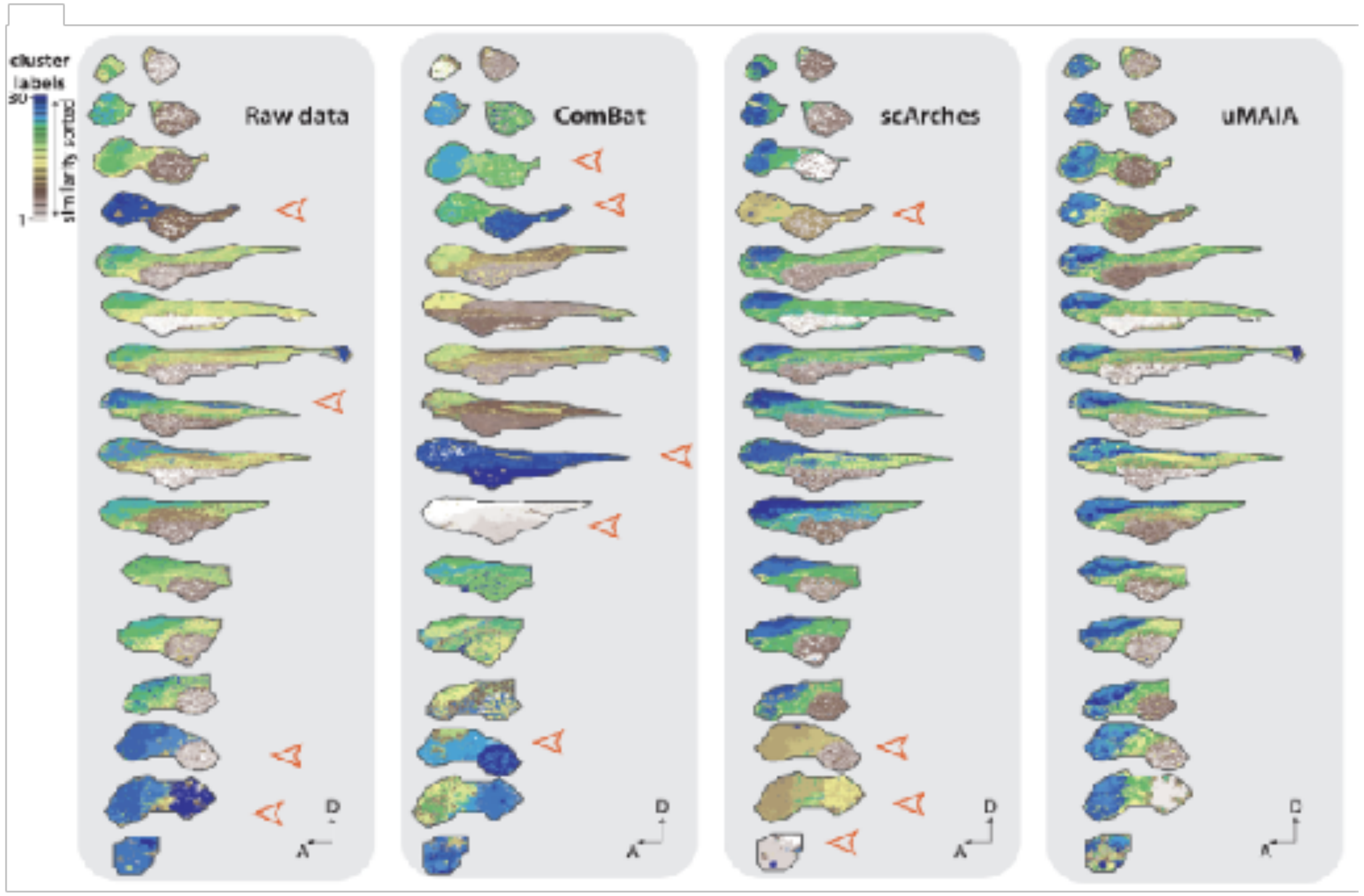
Spatial metabolomics



Bring in the same feature space

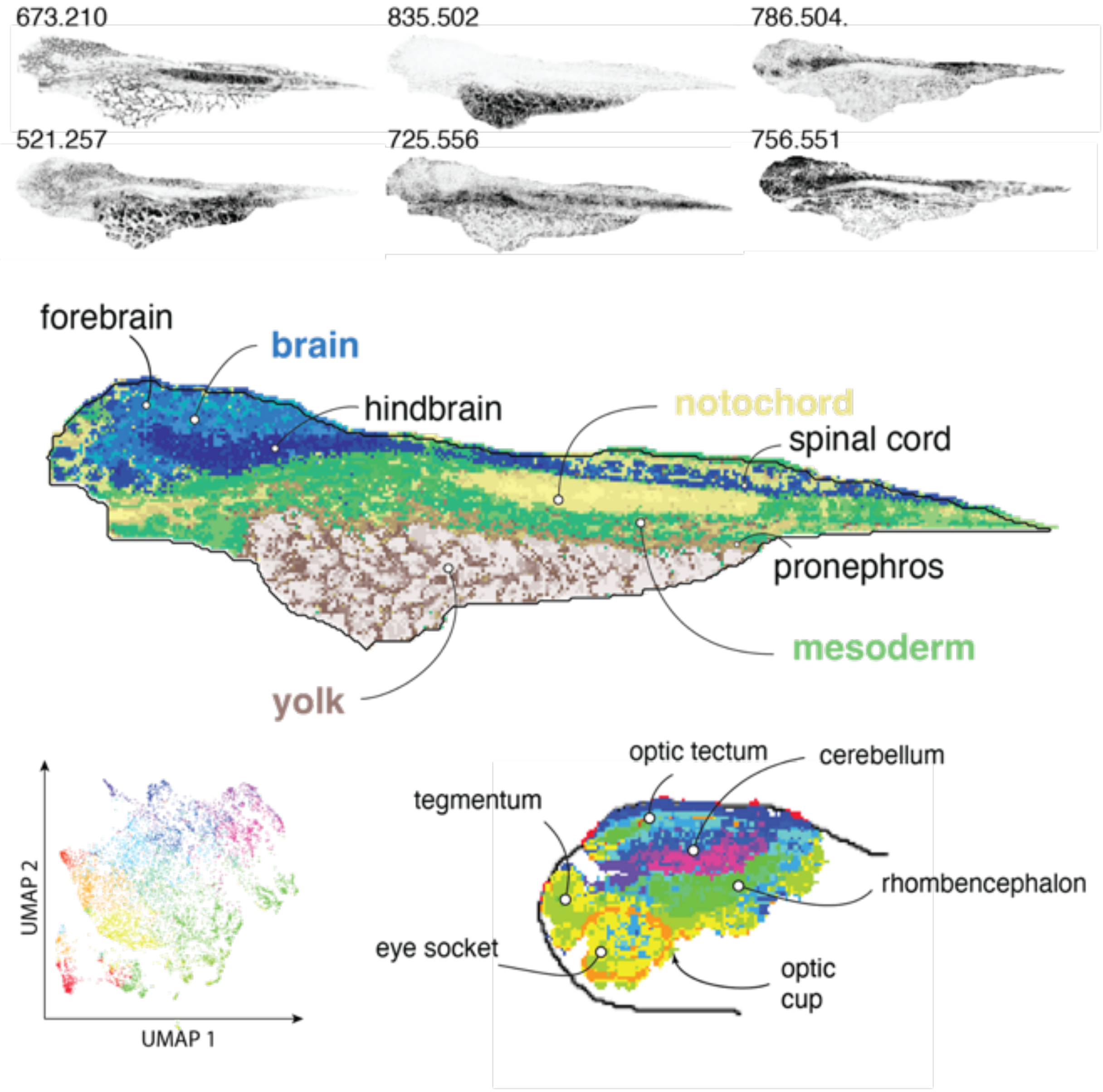


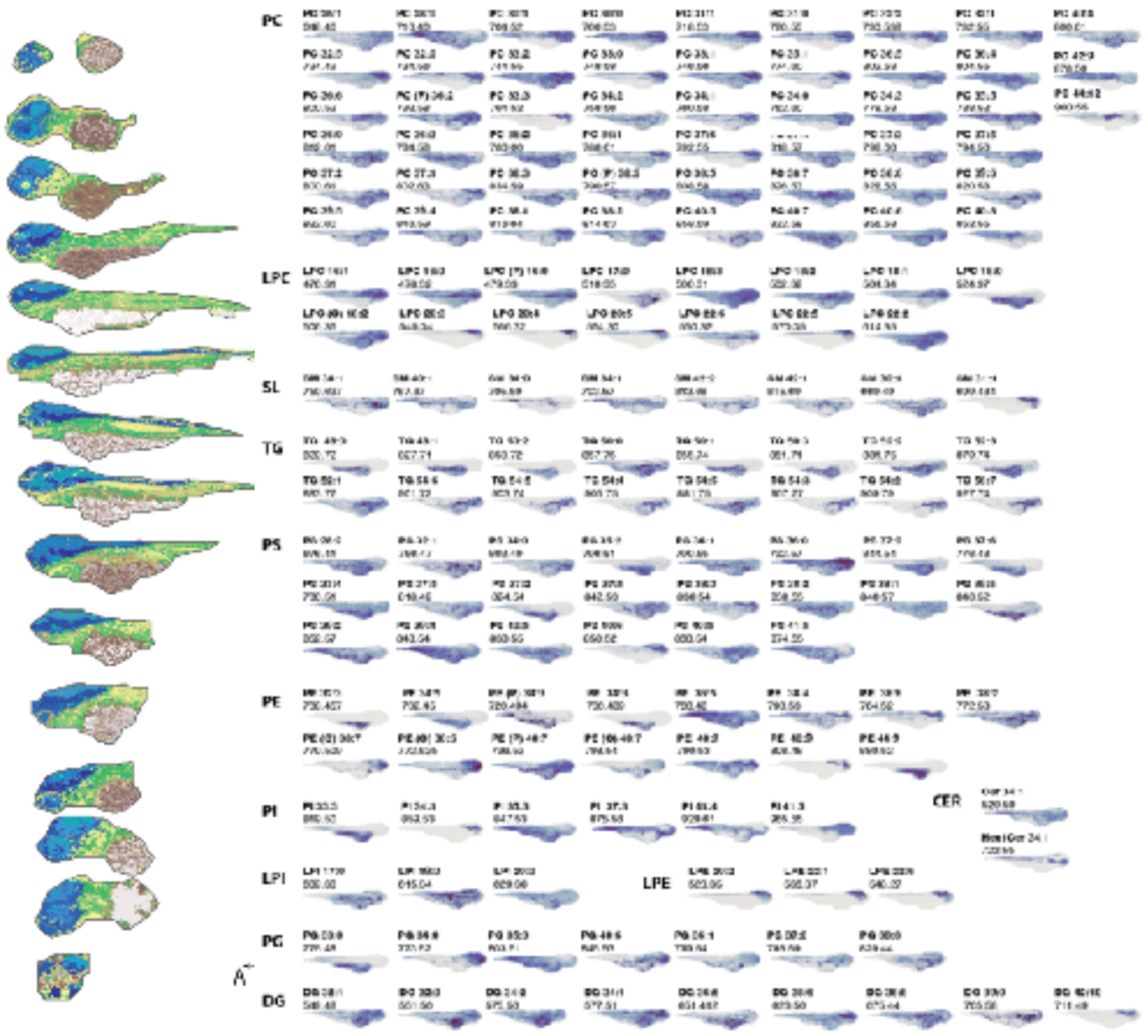
Normalize
(Regularized histogram matching)



Schede, Alieh et al. bioRXiv, 2024

Spatial metabolomics

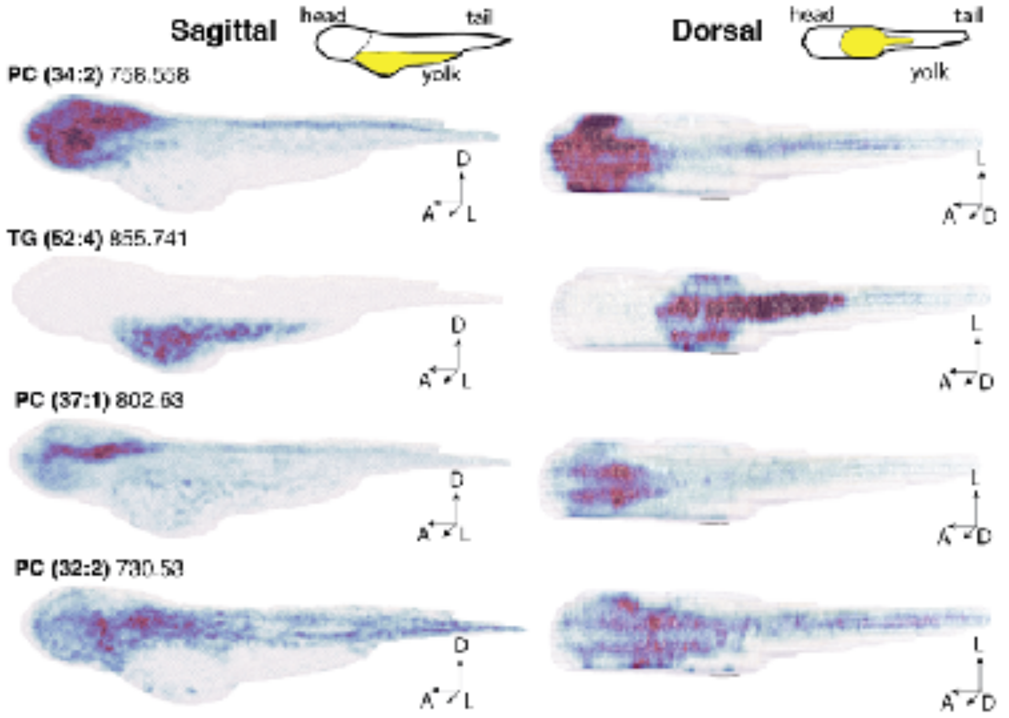


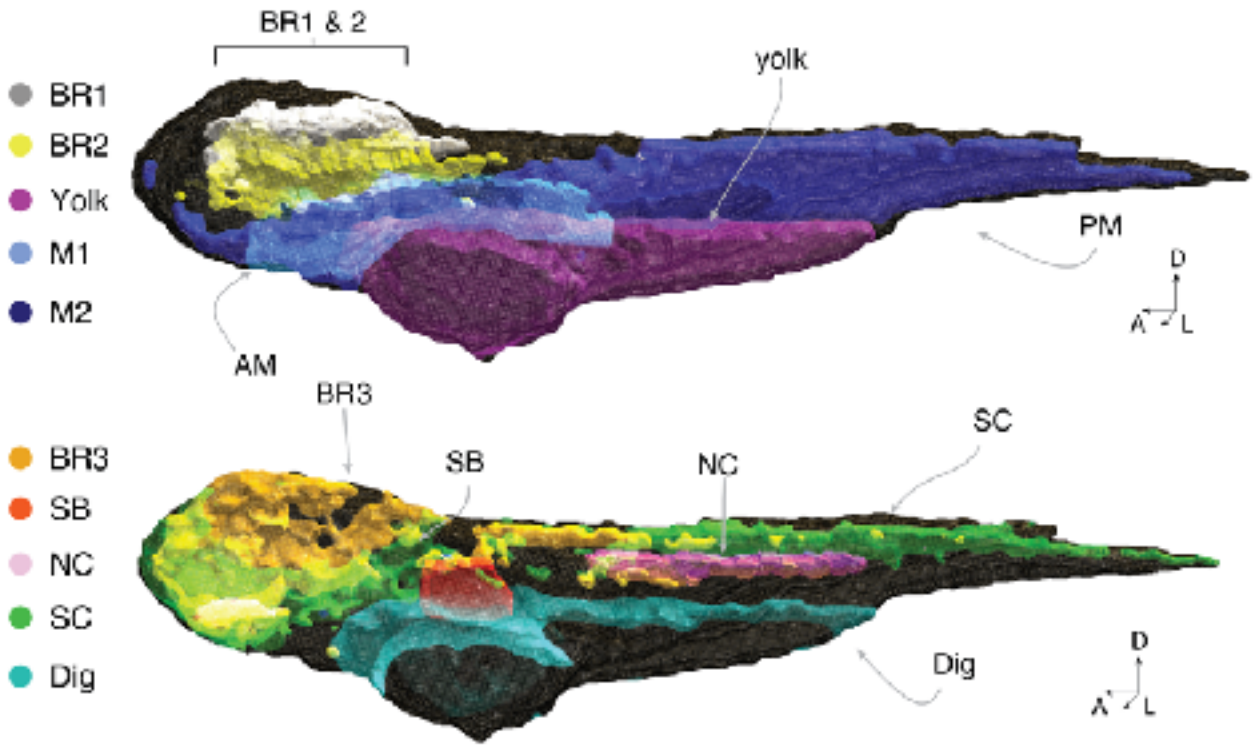


Schede, Alieh et al. bioRXiv, 2024

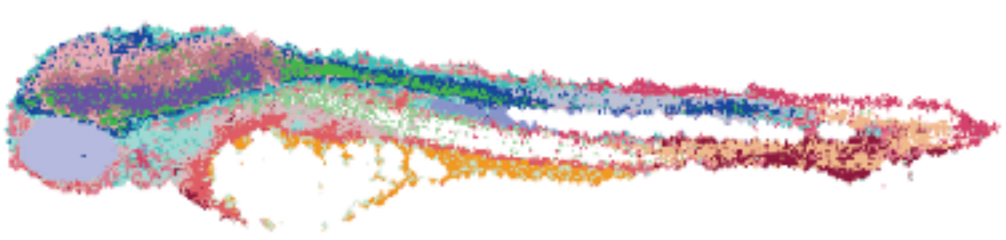
Spatial metabolomics

3D reconstruction of Lipid Distributions

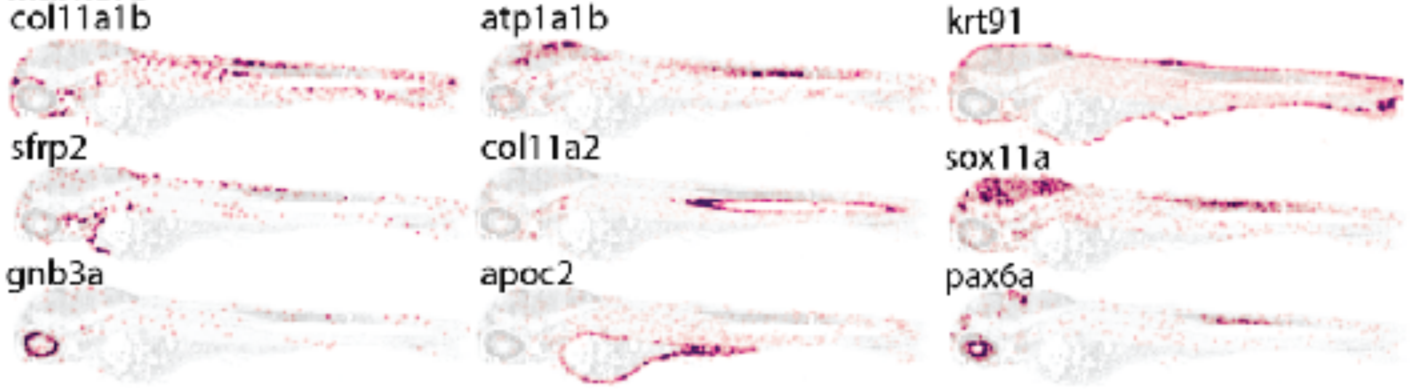




Lipid Clustering

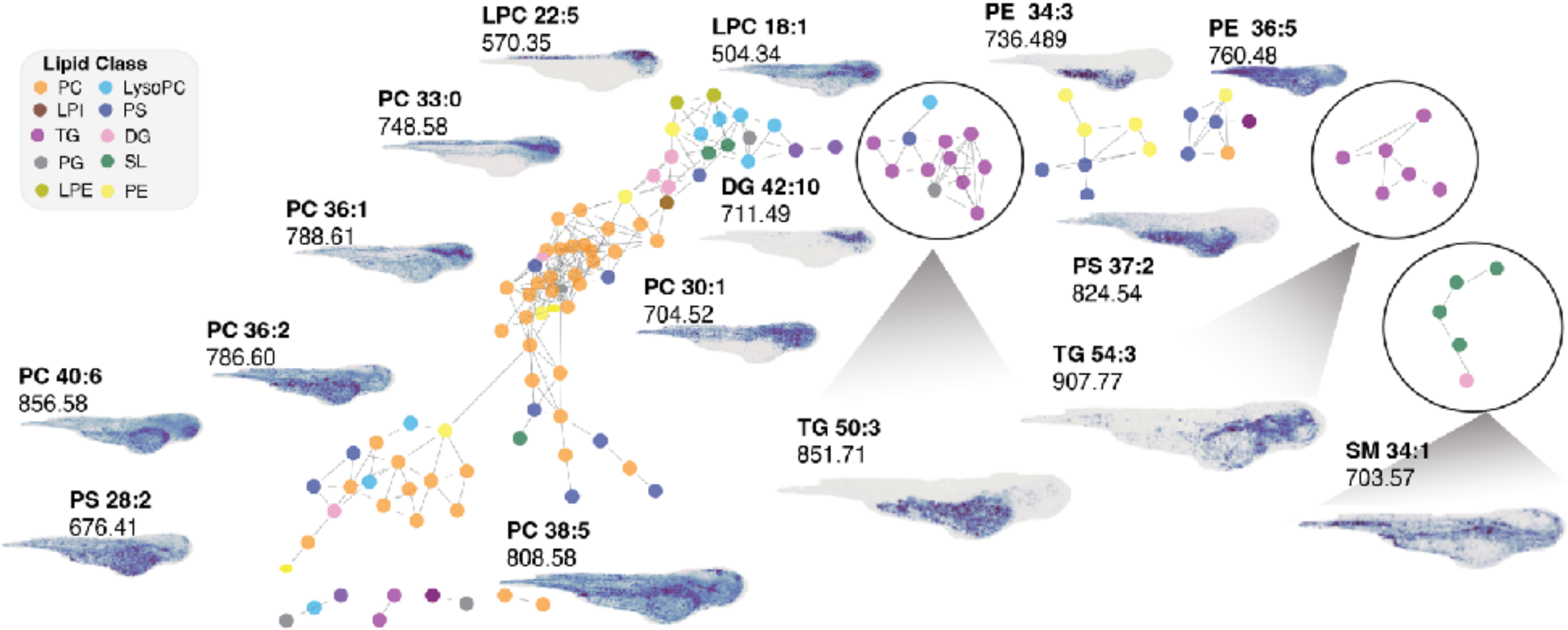


Markers



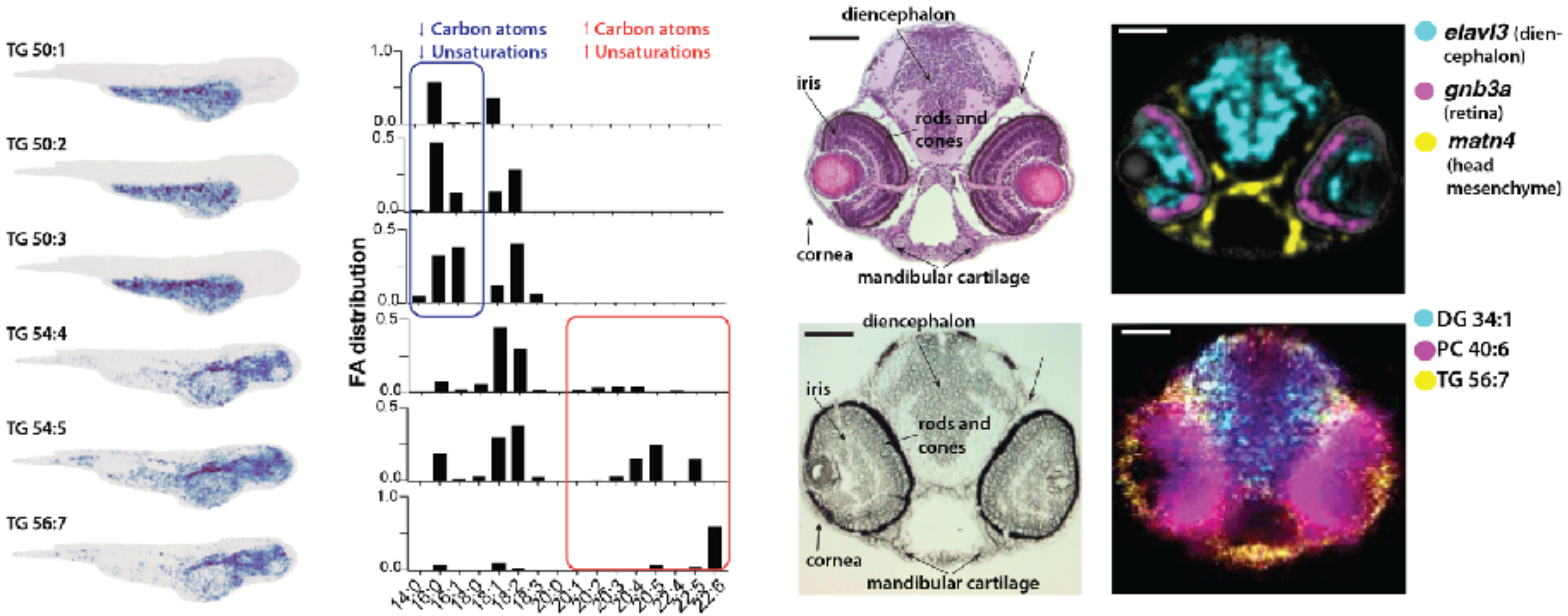
Schede, Alieh et al. bioRXiv, 2024

Spatial metabolomics



Schede, Alieh et al. bioRXiv, 2024

Spatial metabolomics



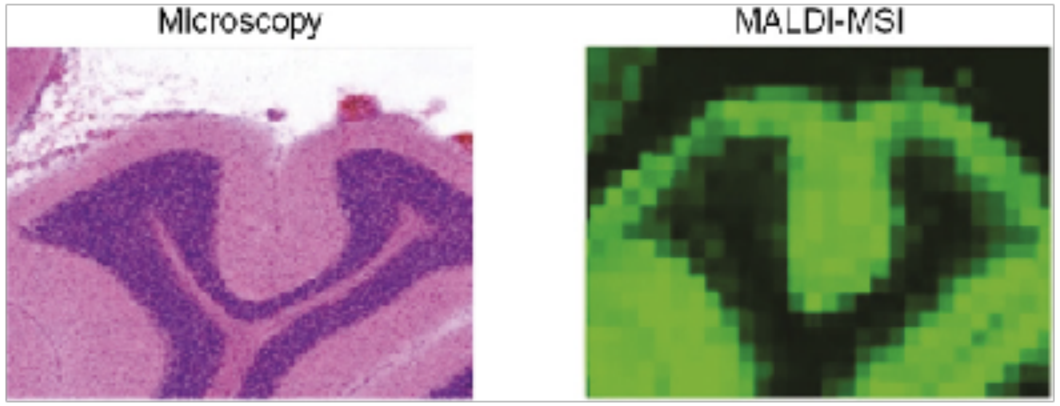
Schede, Alieh et al. bioRXiv, 2024

Spatial metabolomics

Overcoming the resolutions limits of MSI instruments

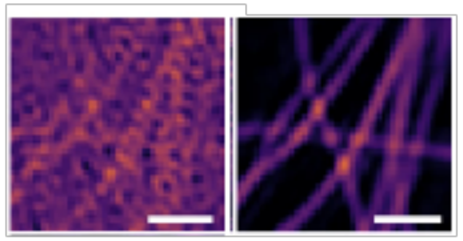
Resolution limits:

- Laser focus: ~4 µm
- Minimum step: ~5 µm
- Matrix crystal size: ~1-2 µm

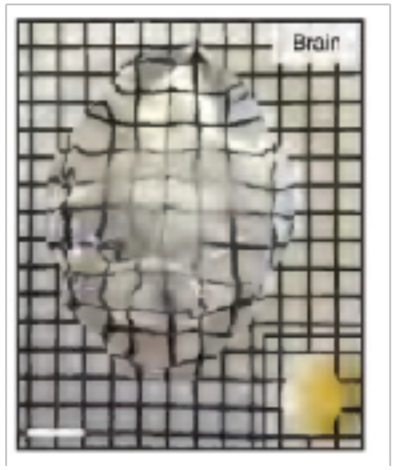


Borrowing ideas from super-resolution Microscopy:

- Computational methods:
    - Deep learning



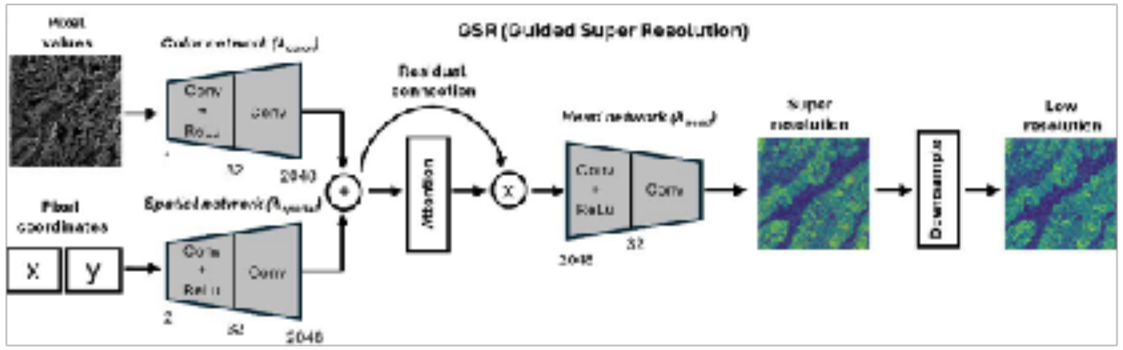
- Tissue-prep methods
    - Tissue Expansion

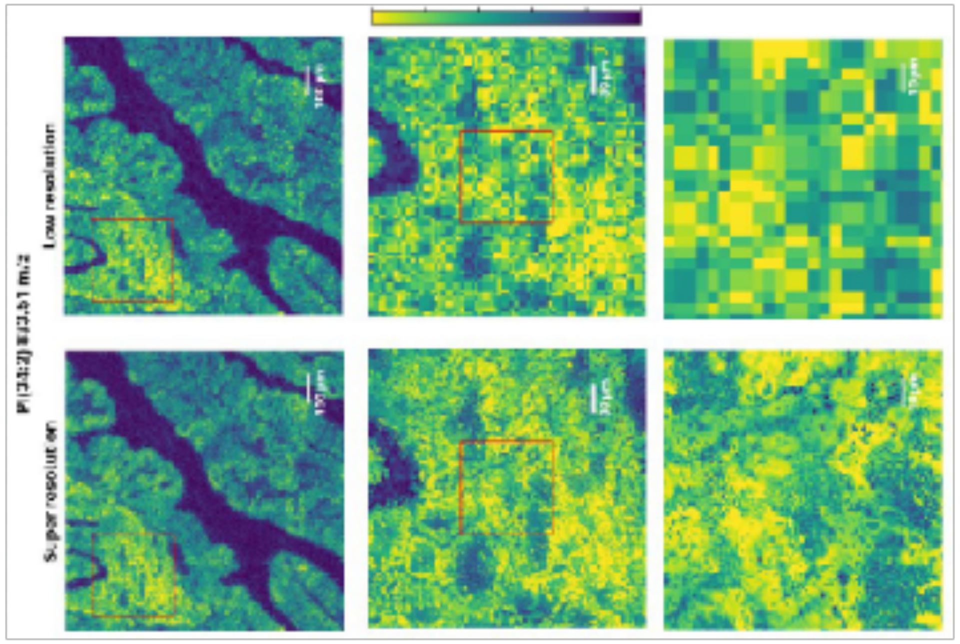


Spatial metabolomics

Computational methods based on deep inference procedures

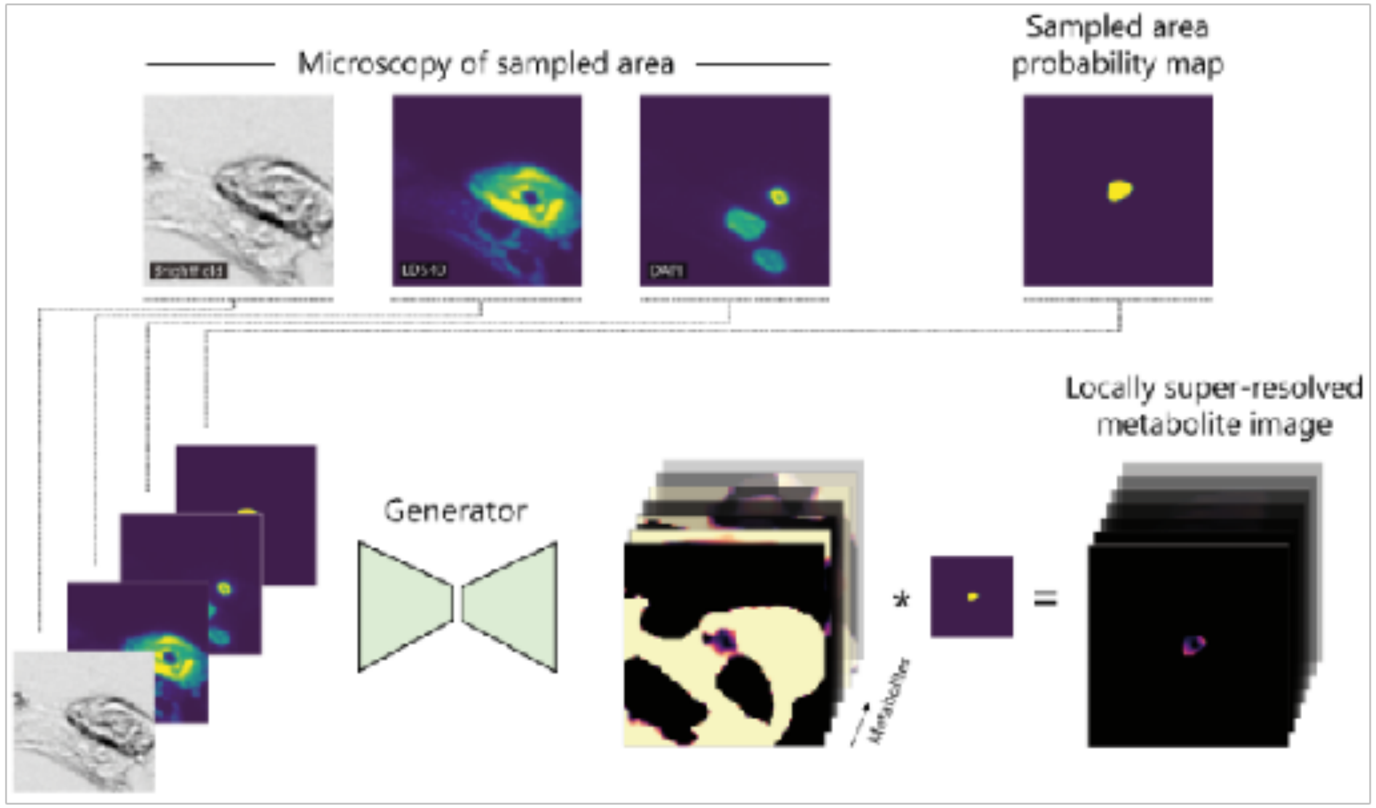
Guided super resolution





Super resolved single-cell spatial… Oztruk et al. 2024

Weakly supervised propagation “MetaLens”

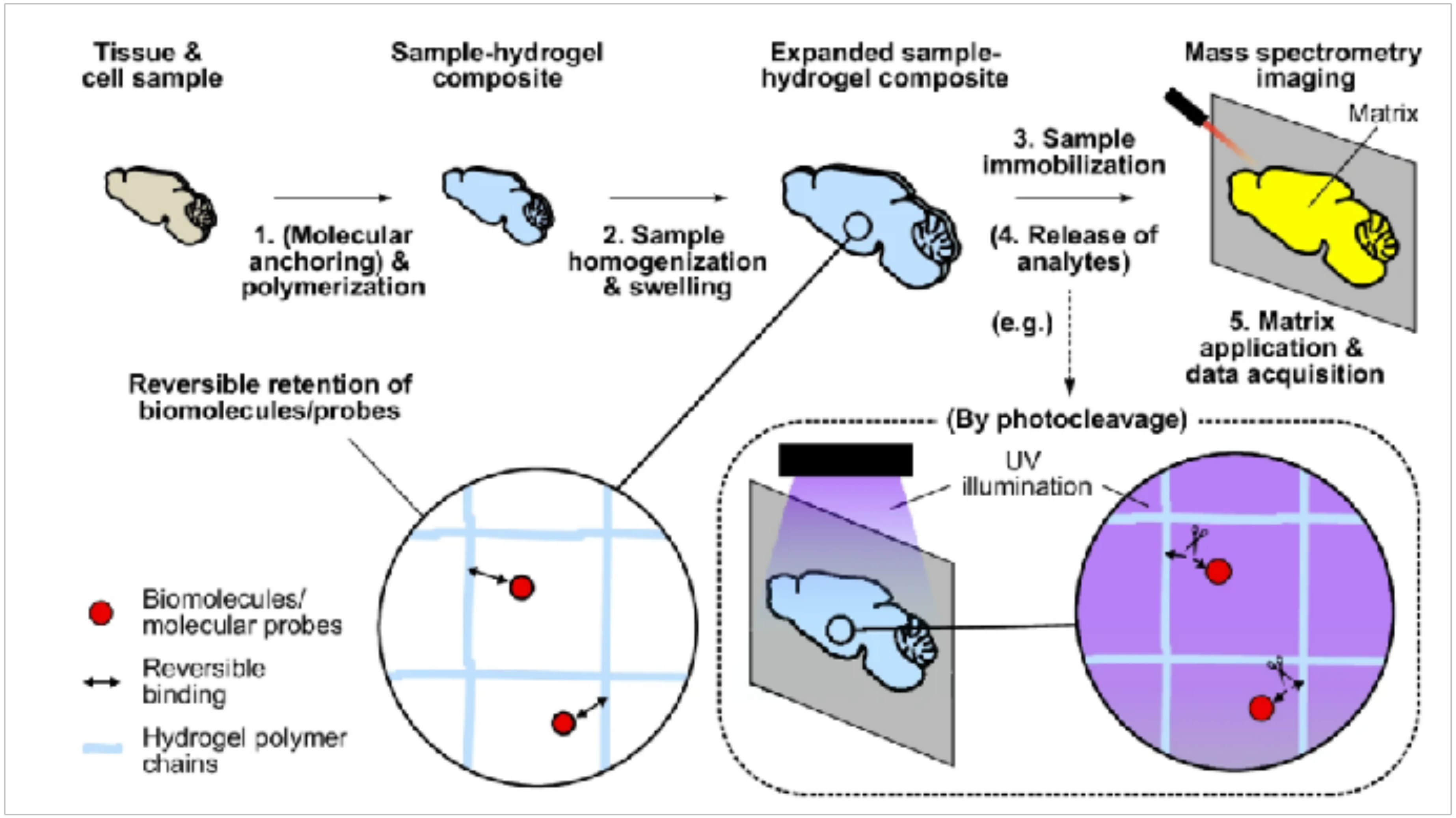


Inferring super-resolved spatial… Rappez et al. 2024

Spatial metabolomics

Gel-assisted expansion for super-resolved MALDI-MSI

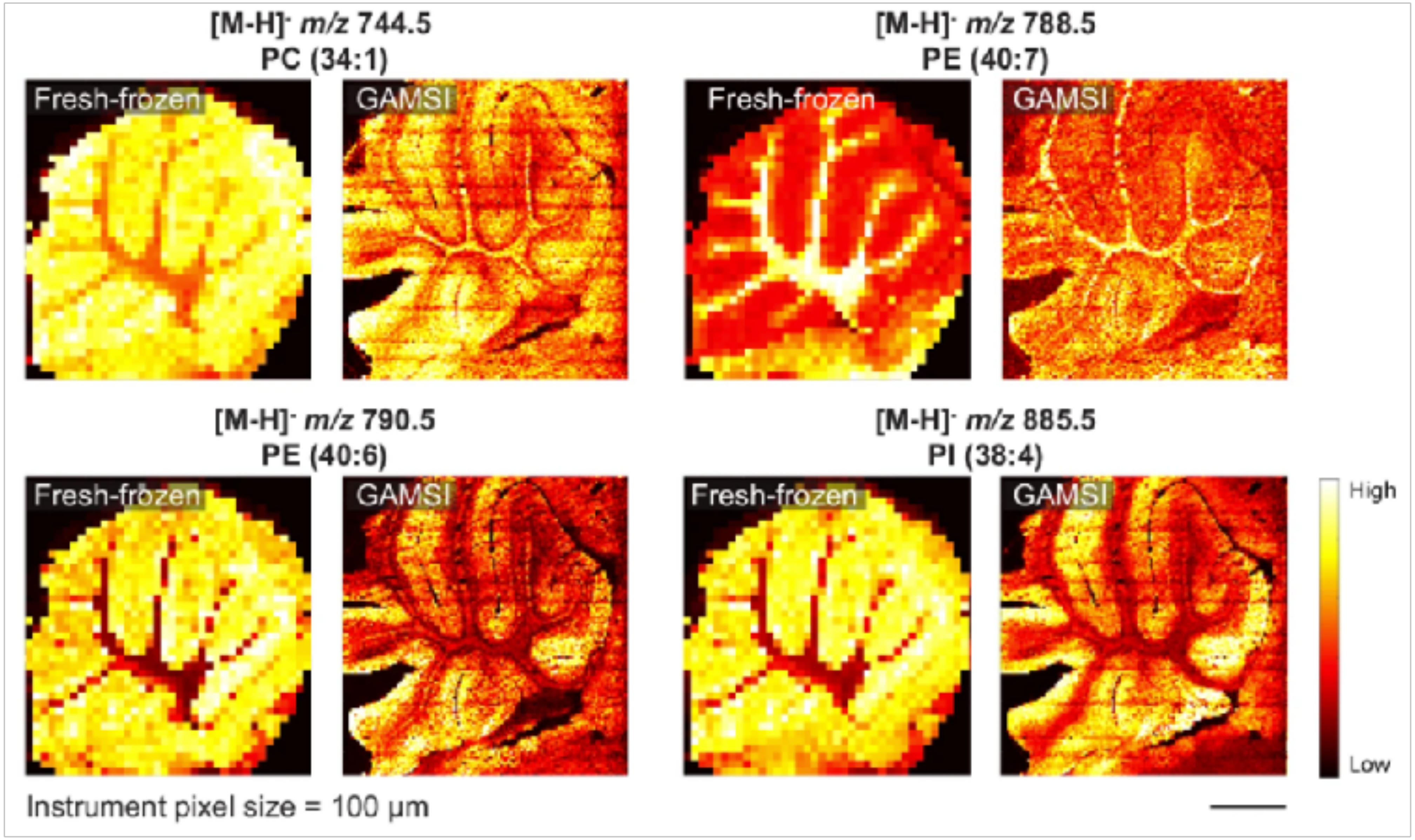
Expanding the sample to increase resolution



Gel-assisted mass spectrometry… Chan et al. 2024

Spatial metabolomics

Results of GEMSI with 100 um pixel size

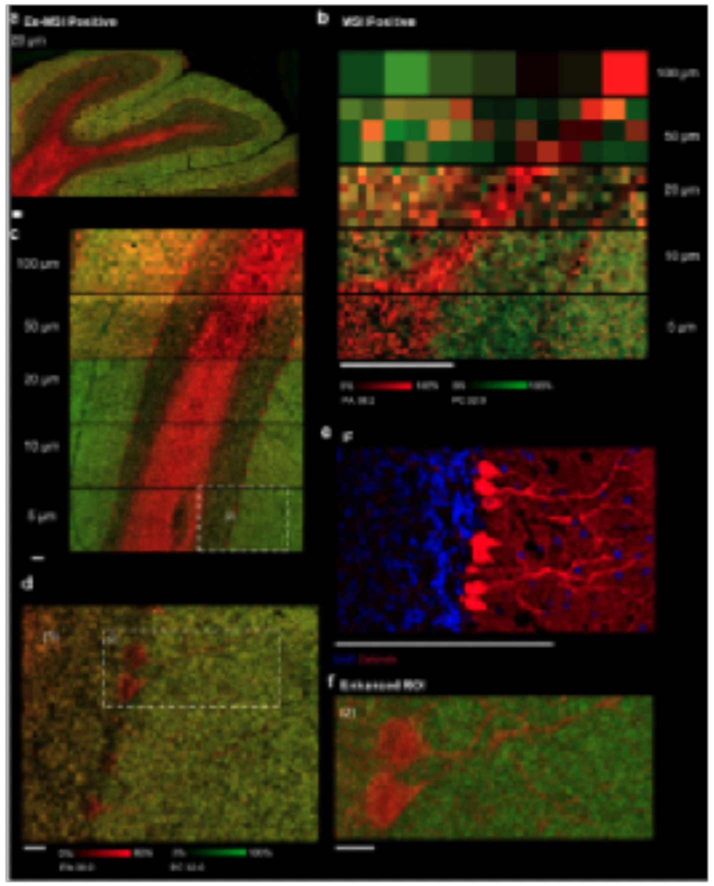


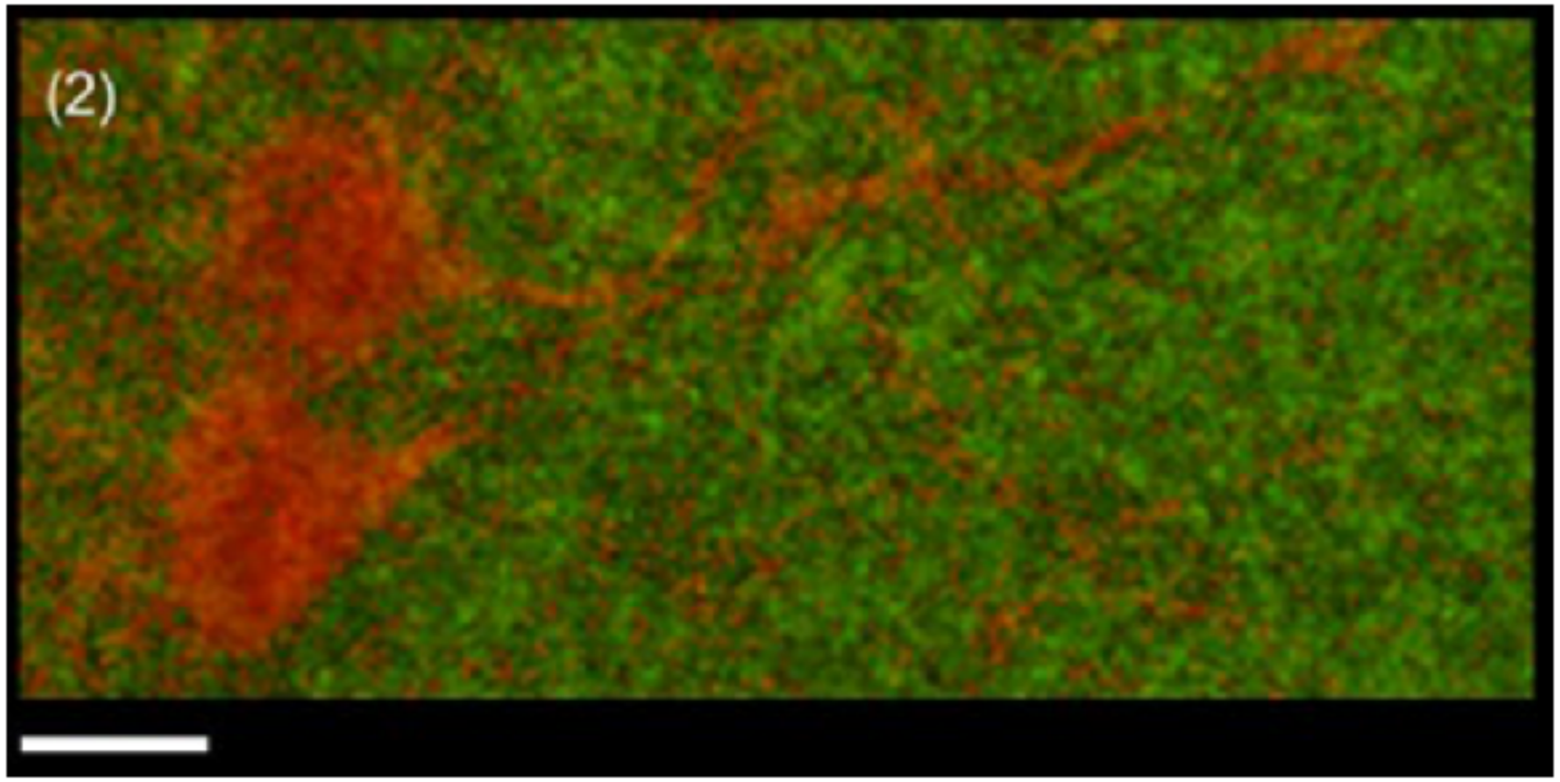
Gel-assisted mass spectrometry… Chan et al. 2024

Spatial metabolomics

Observing single-cells and subcellular structures in tissue

Mass Image of Purkinje cells in the cerebellum and of their neurites



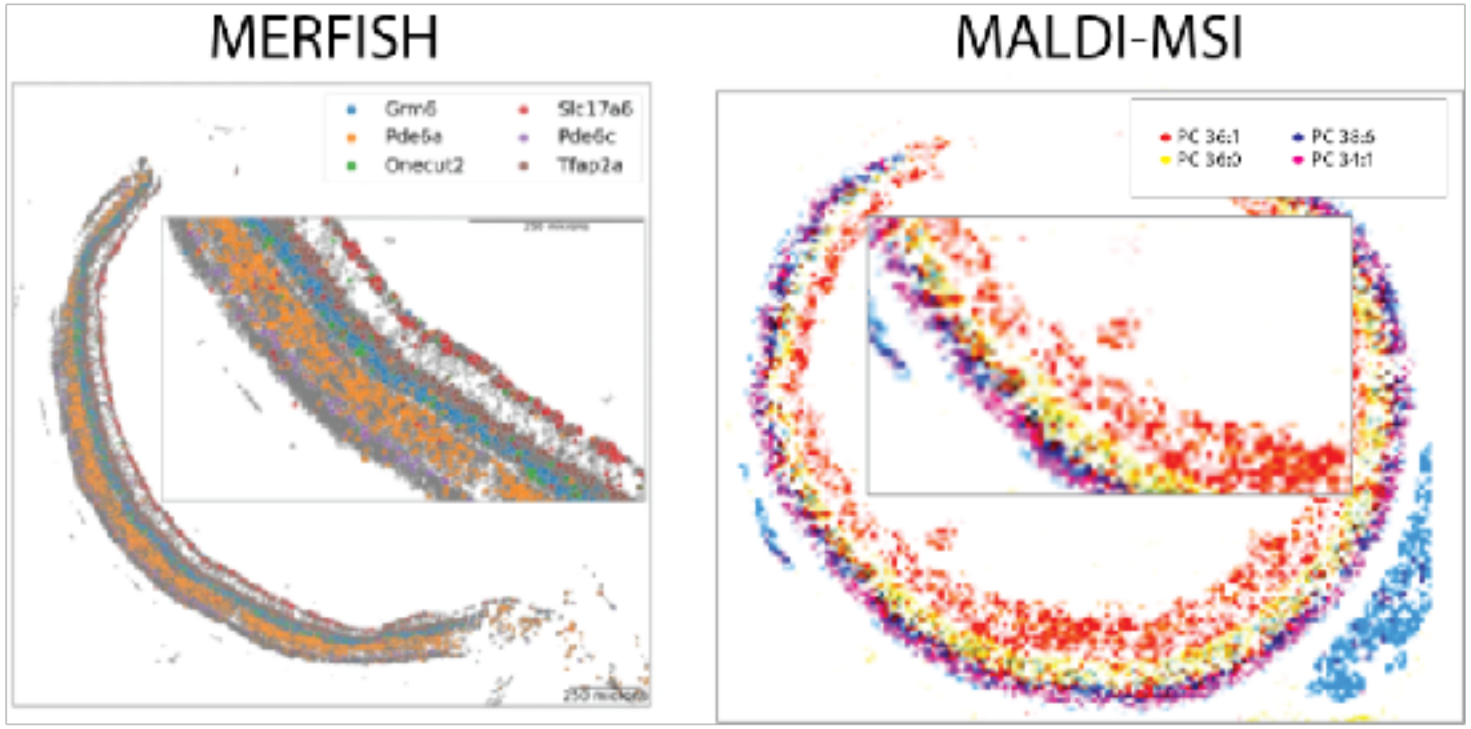


Ten-Fold Expansion MALDI Mass… Xie et al. 2024

Spatial metabolomics

What's about context, type and function? Other modalities!

Mouse Retina



Choi et al. 2023

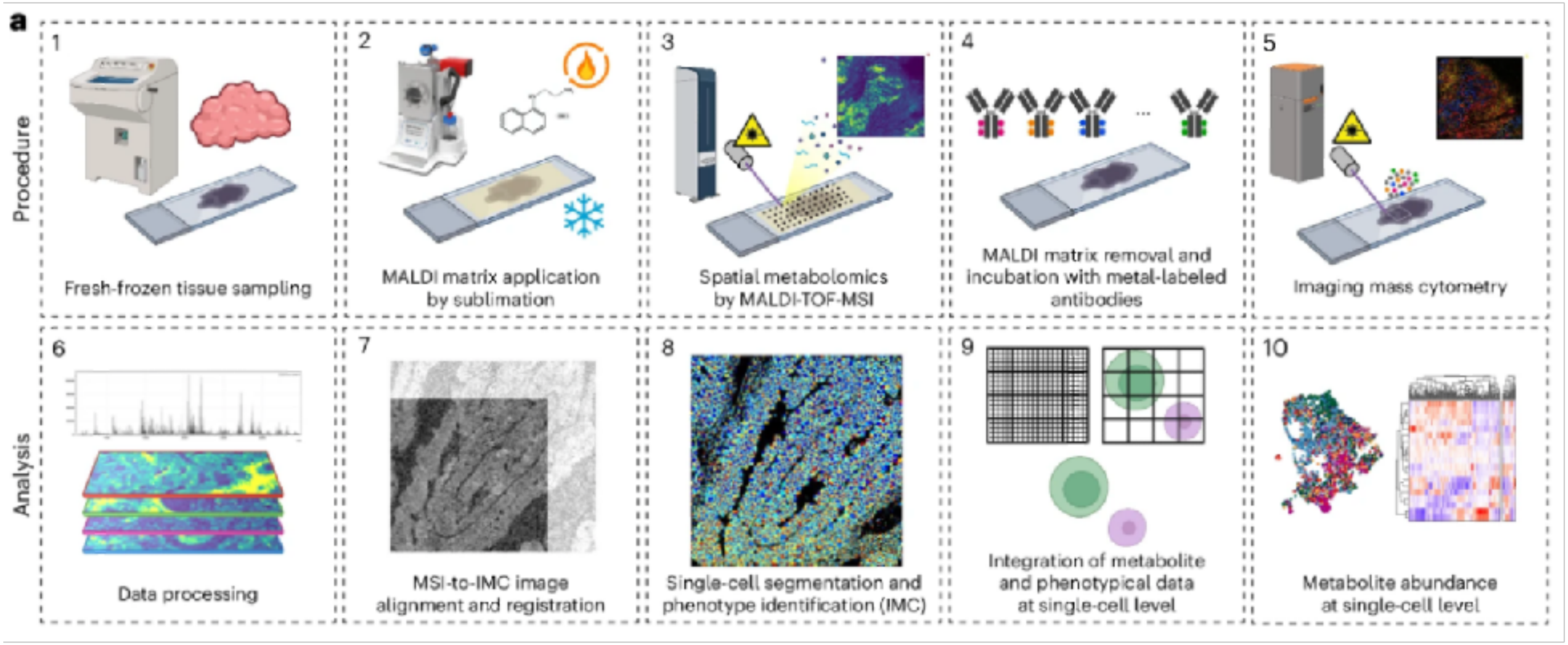
Our Data

Even in the most simple cases It can be difficult to tell what cells we are imaging by MSI.

Concatenating different modalities brings data stratification and correlation and cross-explanatory power.

Spatial metabolomics

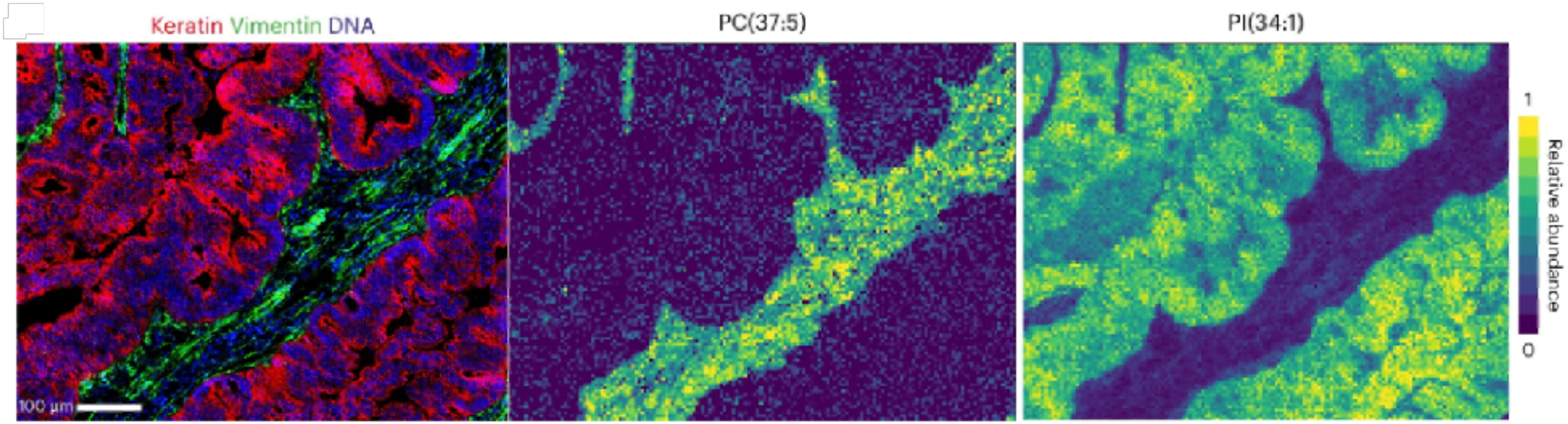
Coupling Spatial Proteomics and Metabolomics (MALDI)



Integration of Mass Cytometry… Nunes et al. 2024

Spatial metabolomics

Coupling Spatial Proteomics and Metabolomics (MALDI)

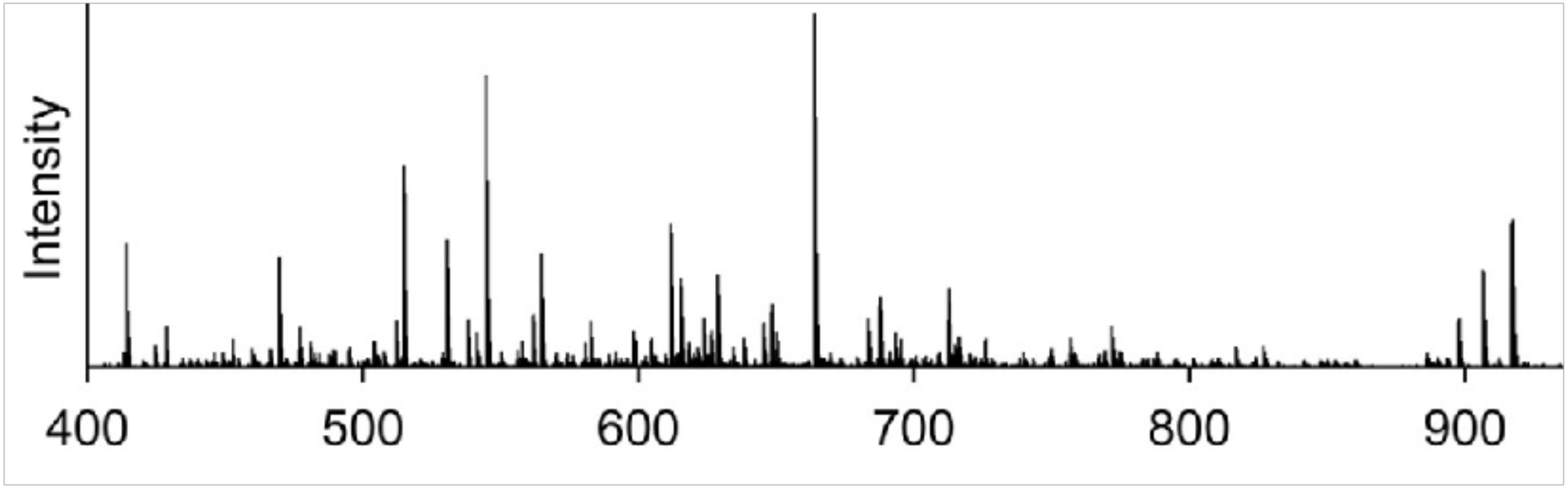


Integration of Mass Cytometry… Nunes et al. 2024

Spatial metabolomics

How to molecularly interpret MSI spectra?

Spatial and single-cell metabolomics data are often challenging to annotate

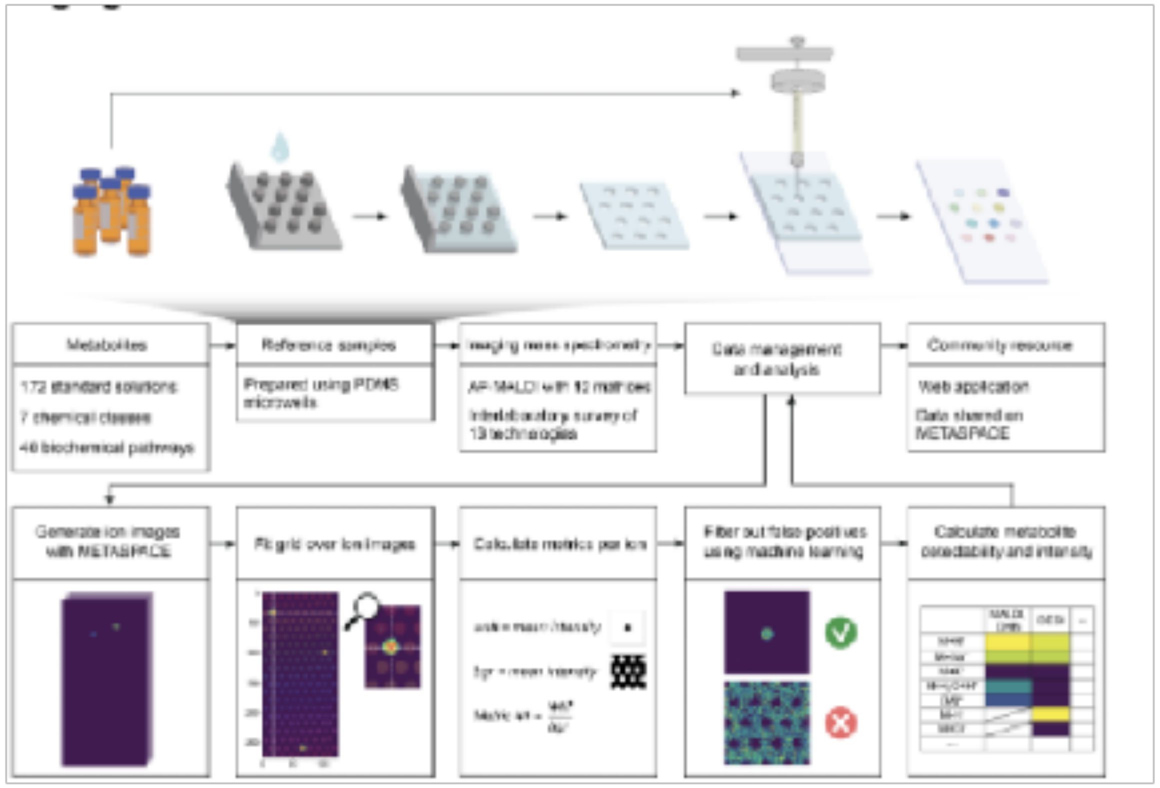


Increase the dimensions for systematic molecular identification

Spatial metabolomics

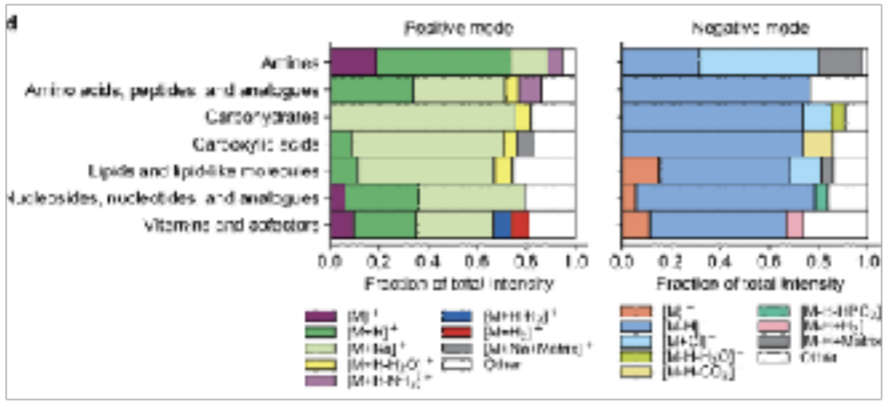
Foundational benchmarking: 24 untargeted MSI protocols

Experimental and computational workflow to measure detectability



Coverage

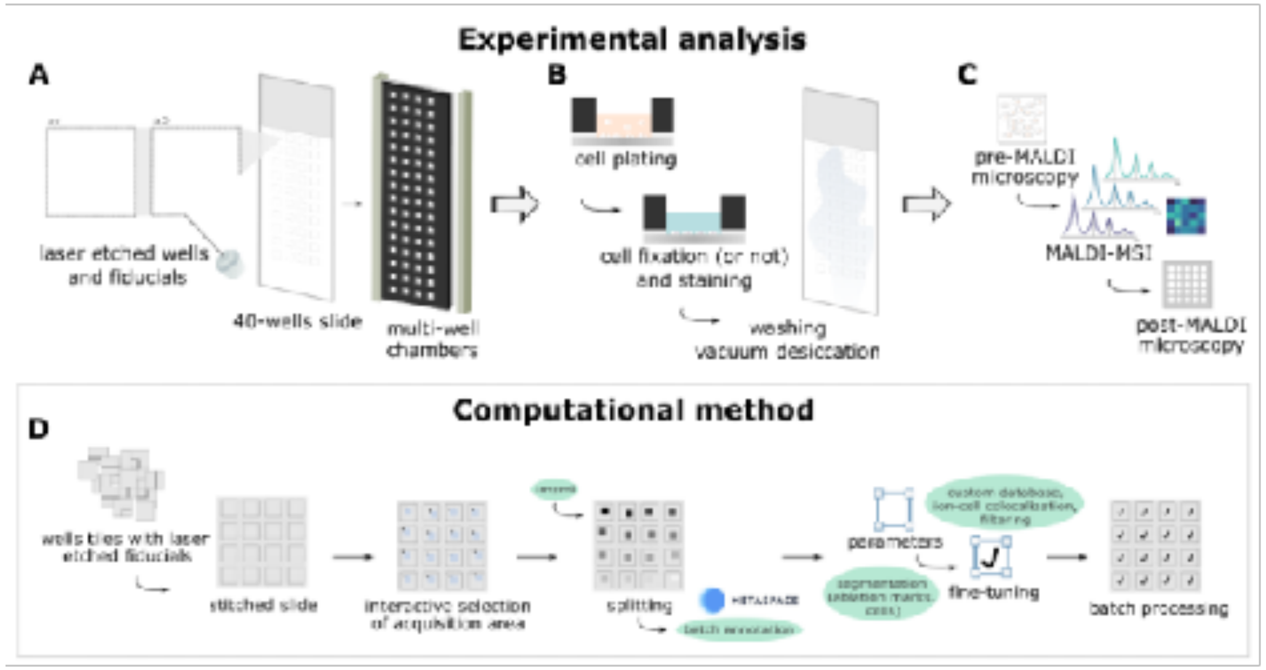
Interlaboratory survey

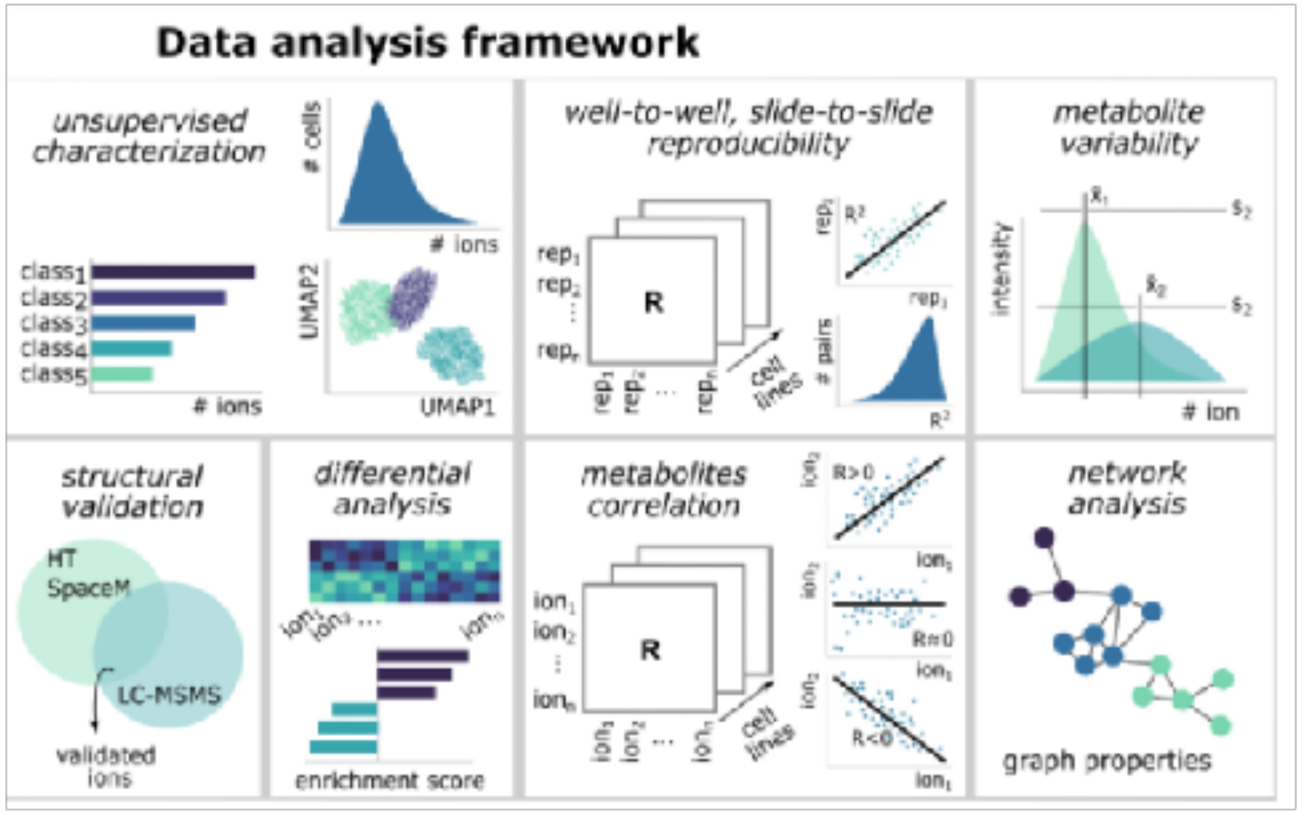


Large-Scale Evaluation of Spatial… Saharuka et al. 2024

Spatial metabolomics

Workflow small molecules - single cells - large datasets

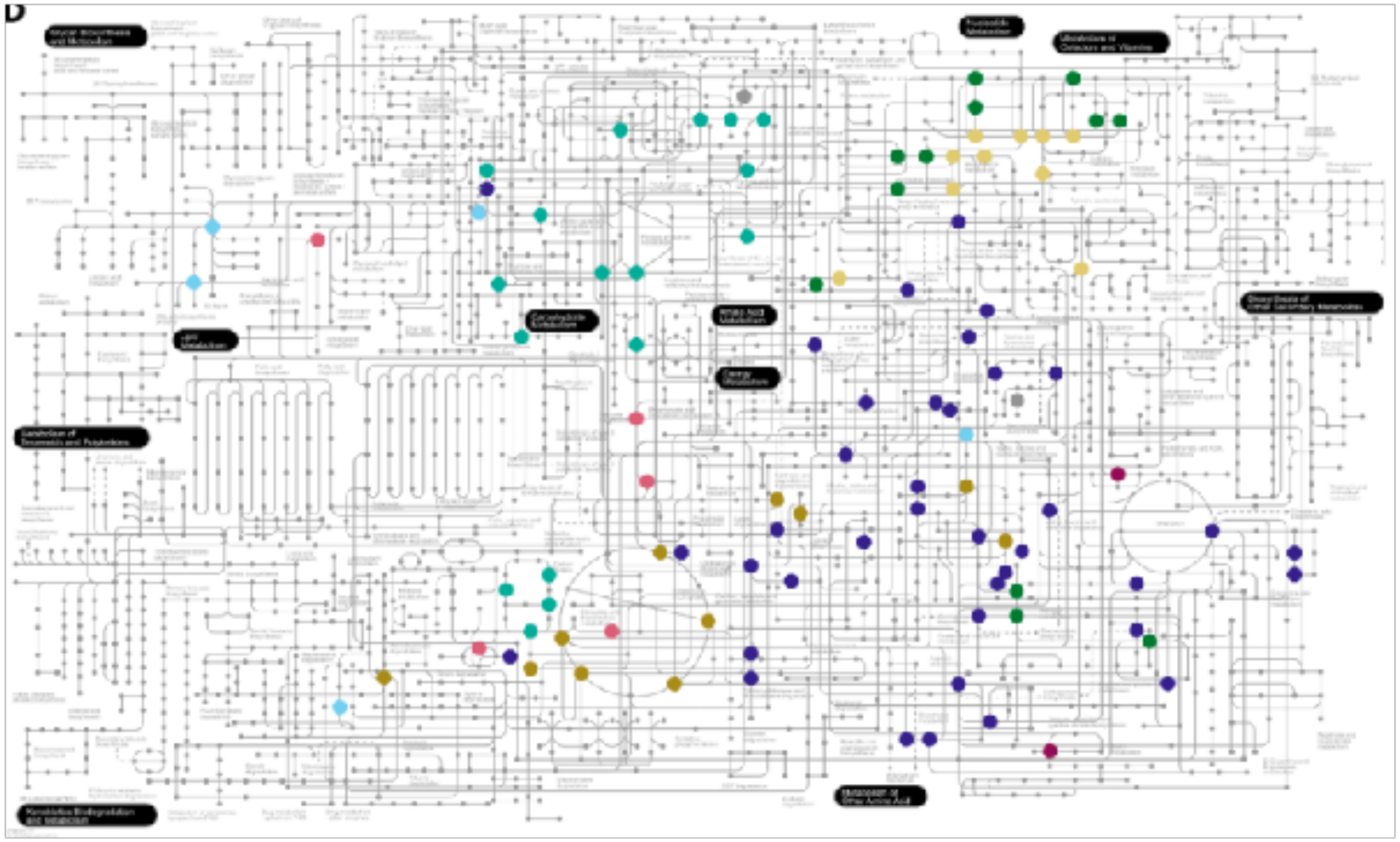


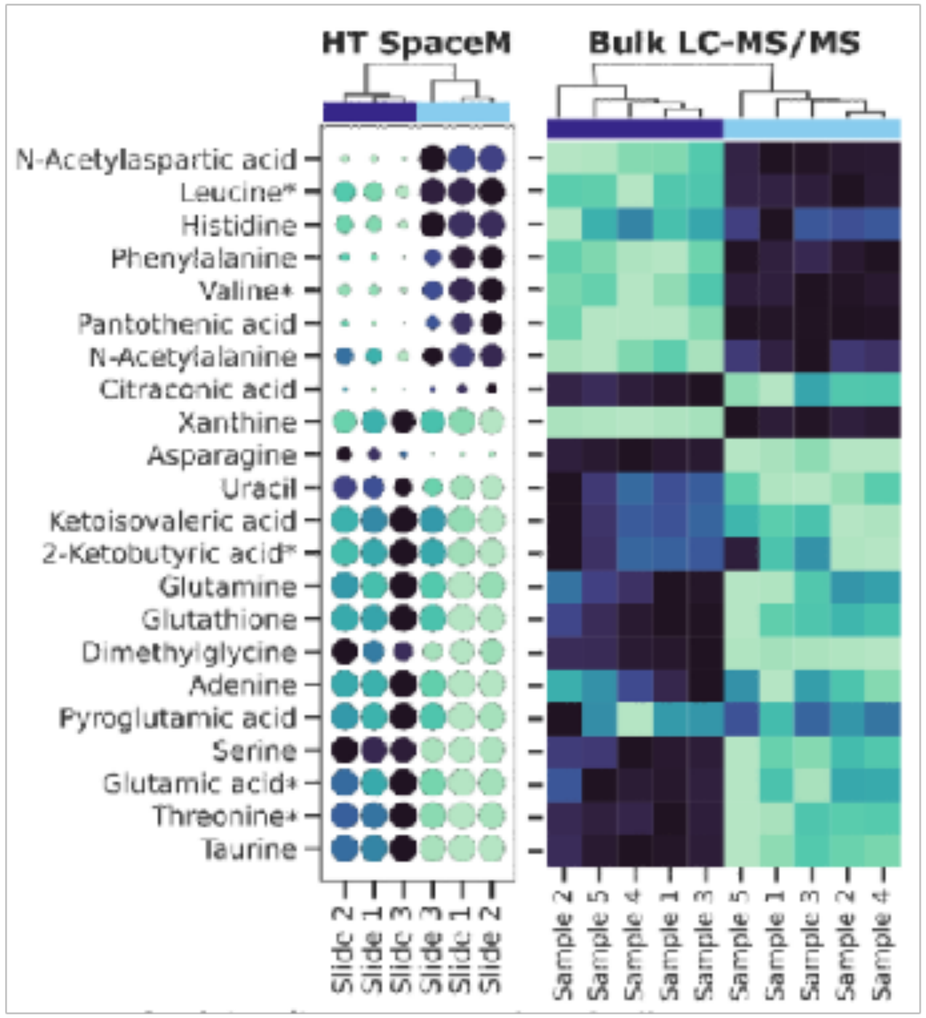


HT SpaceM: A High-Throughpt and… Delafiori et al. 2024

Spatial metabolomics

Towards a more complete metabolic network coverage



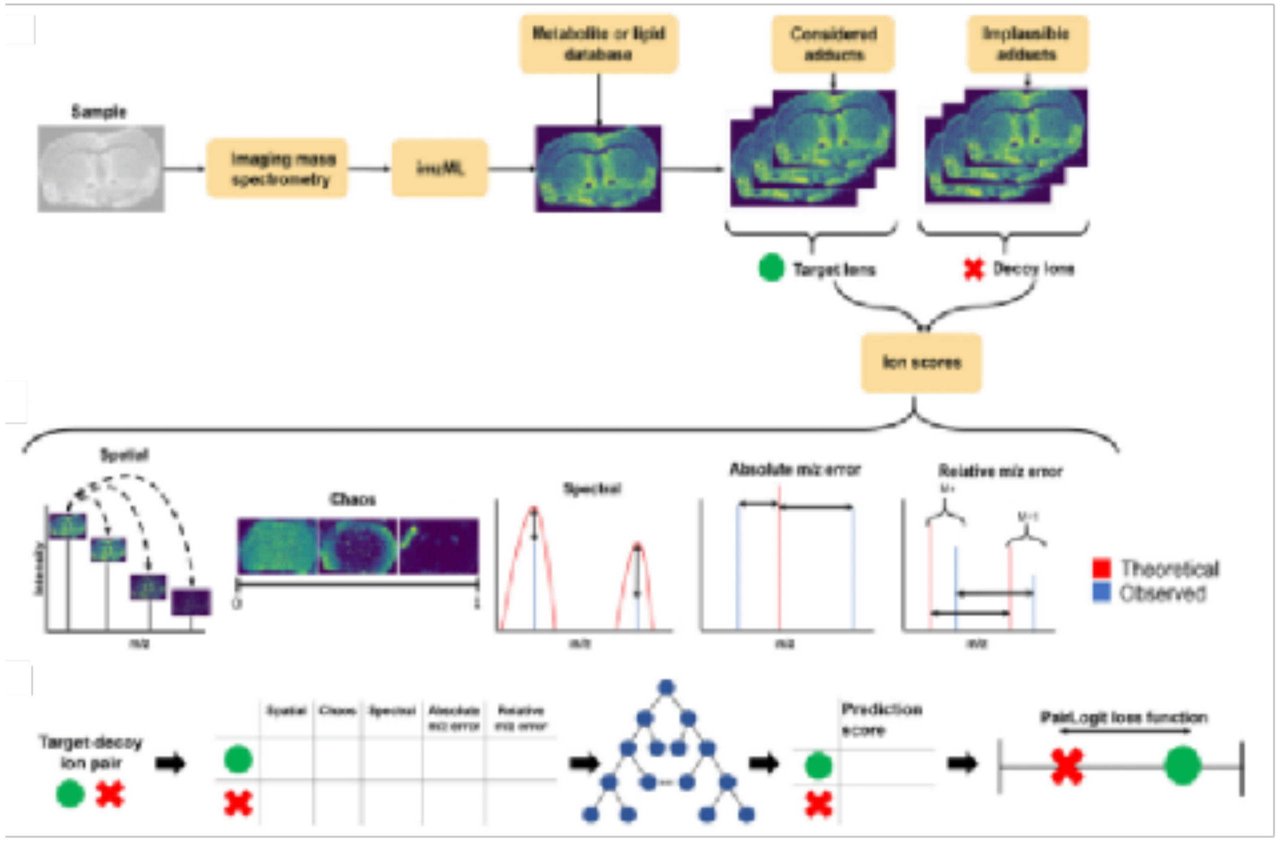


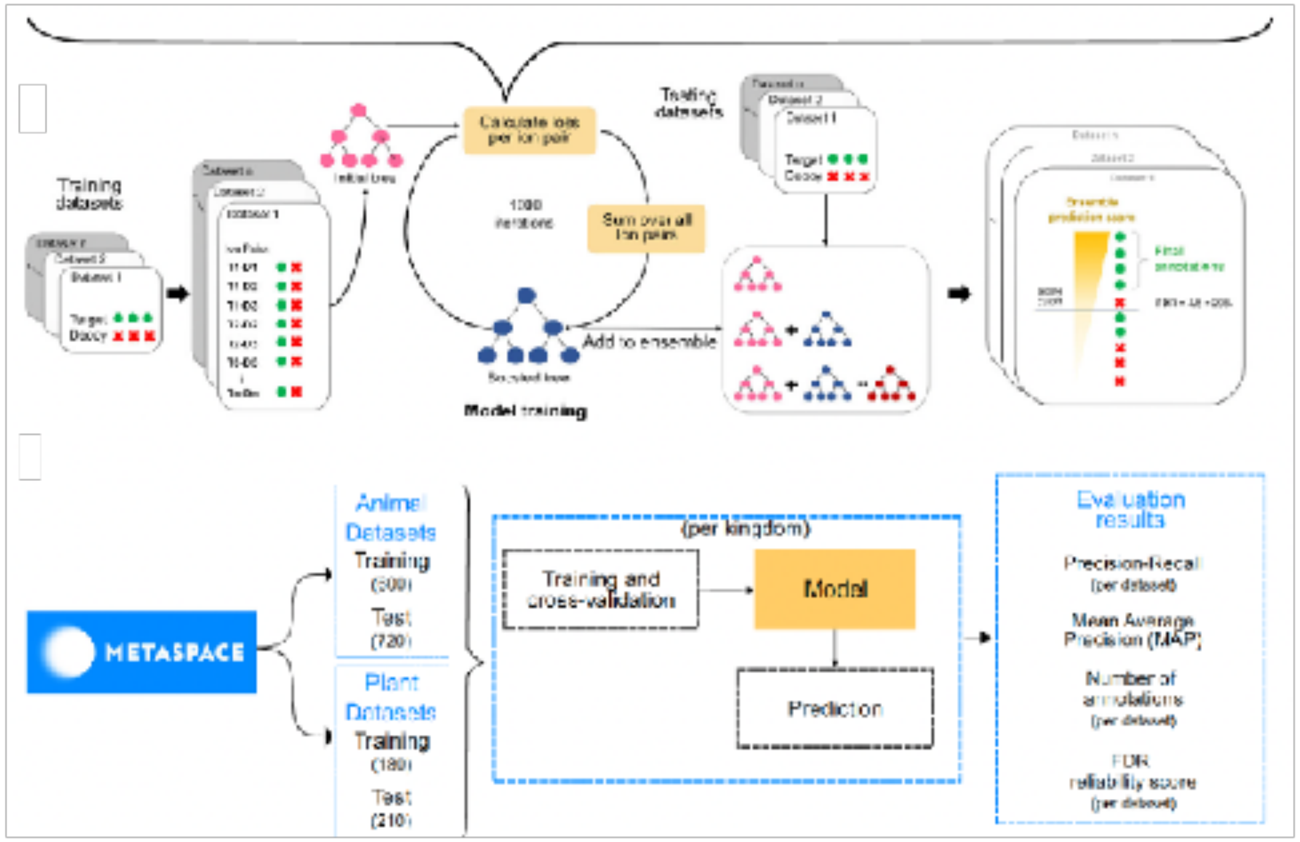
HT SpaceM: A High-Throughpt and… Delafiori et al. 2024

Spatial metabolomics

Computational methods based metabolite annotation

Important idea: Systematically prioritise plausible annotations





METASPACE-ML: Context Specific … Wadie et al. 2024

Spatial metabolomics