

SPATIAL SINGLE-CELL METABOLOMICS

THEODORE ALEXANDROV

UC San Diego

UNIVERSITY OF CALIFORNIA SAN DIEGO
DEPTS. PHARMACOLOGY & BIOENGINEERING

MY CAREER

ST.PETERSBURG



Mathematics, computer science

BREMEN



Mass spectrometry

SAN DIEGO



Metabolomics

HEIDELBERG



Spatial & single-cell metabolomics

COPENHAGEN



Startup on single-cell metabolomics

SAN DIEGO



Single-cell metabolomics, AI/ML, pharmacology

WHY METABOLOMICS?

Metabolites:

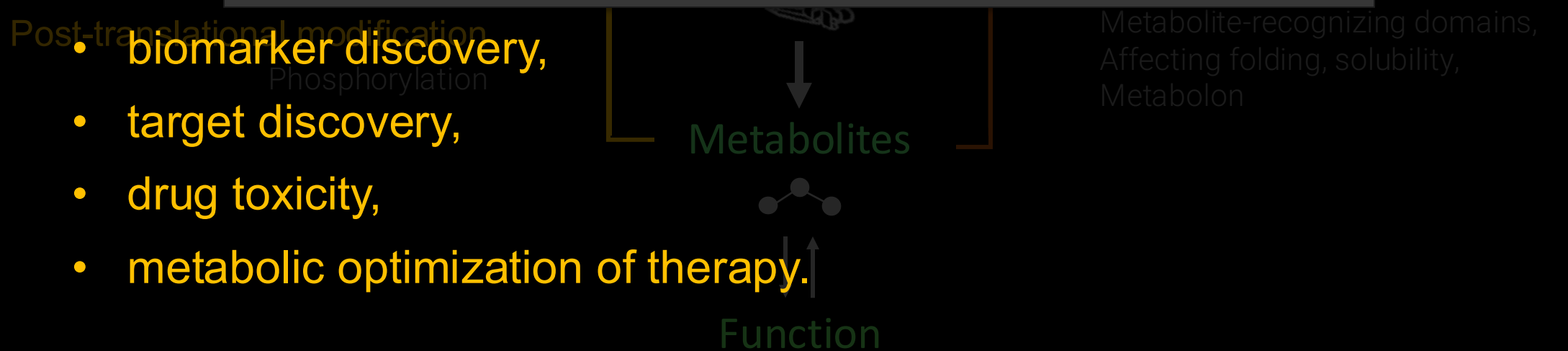
- fill & fuel cells,
- active players of cellular programs,
- biomarkers & influence therapy.



A two-way street: reciprocal regulation of metabolism and signalling

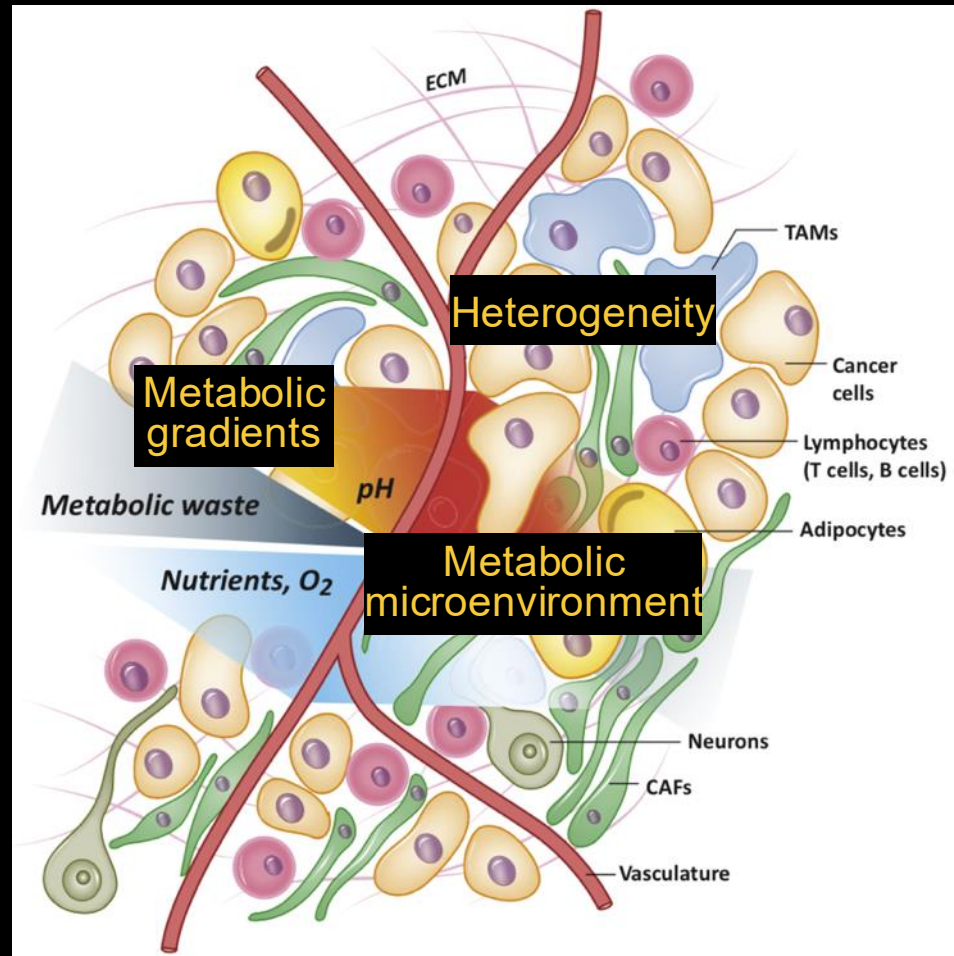
Emerging use of metabolomics in pharmacology:

- metabolic mechanism of action,
- biomarker discovery,
- target discovery,
- drug toxicity,
- metabolic optimization of therapy.



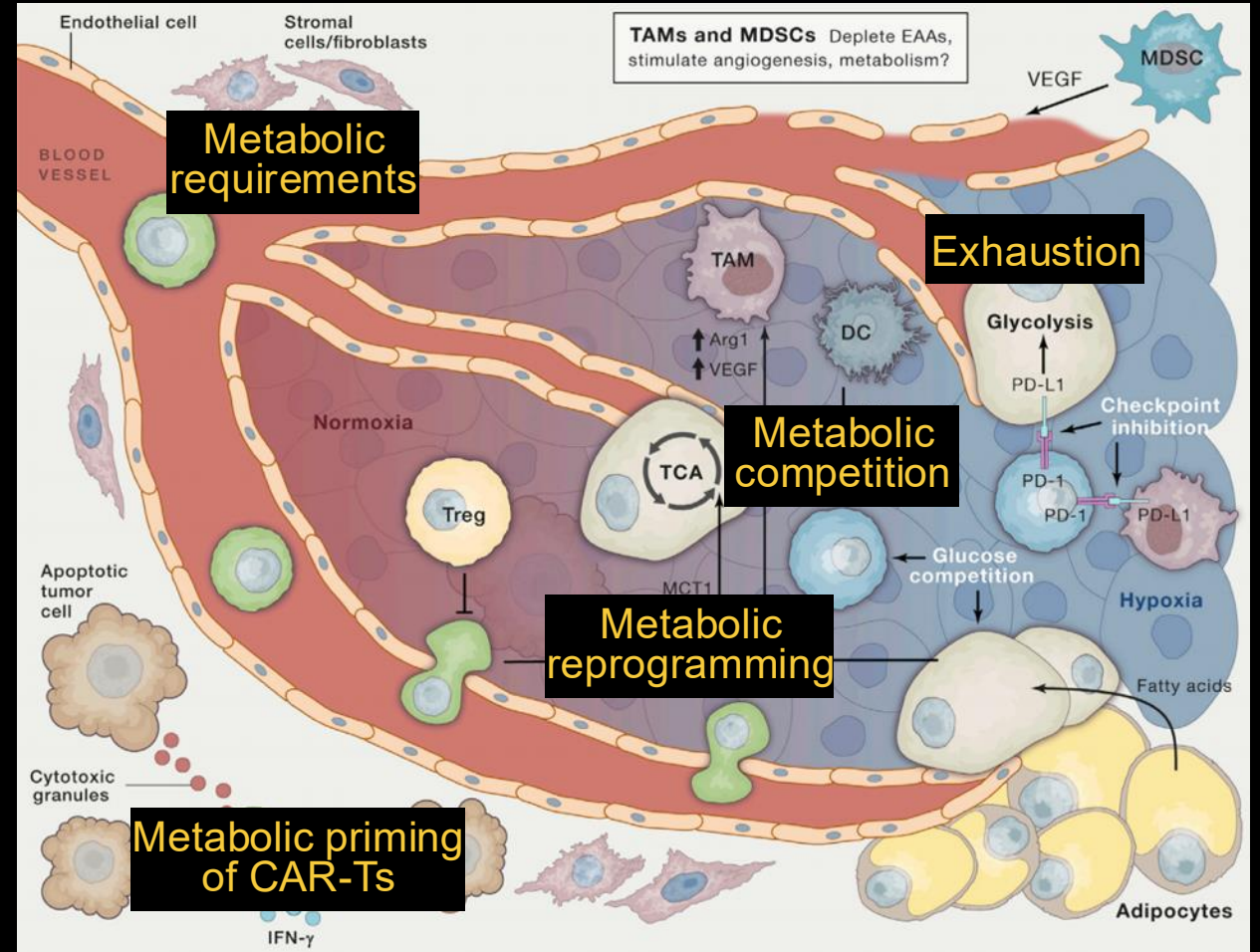
WHY SPATIAL SINGLE-CELL METABOLOMICS?

Tumor microenvironment



Lyssiotis, Kimmelman, *Trends Cell Biology* 2017

Immuno-oncology



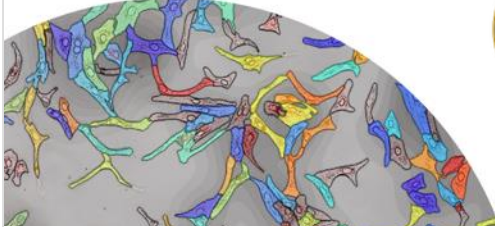
Buck et al, *Cell* 2017

HTTP://ATEAM.UCSD.EDU

**Alexandrov Team**[Home](#)[Research](#)[Team](#)[Projects](#)[Publications](#)[Gallery](#)[Contact Us](#)

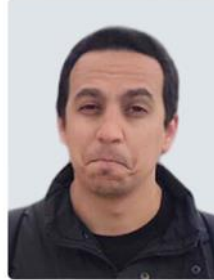
We Develop Technologies for Spatial & Single-Cell Metabolomics

We are scientists & software engineers developing advanced technologies with the mission to address biological, medical & pharmacological challenges



UC San Diego

SCHOOL OF MEDICINE





Imaging Mass Spectrometry

Foundational technology



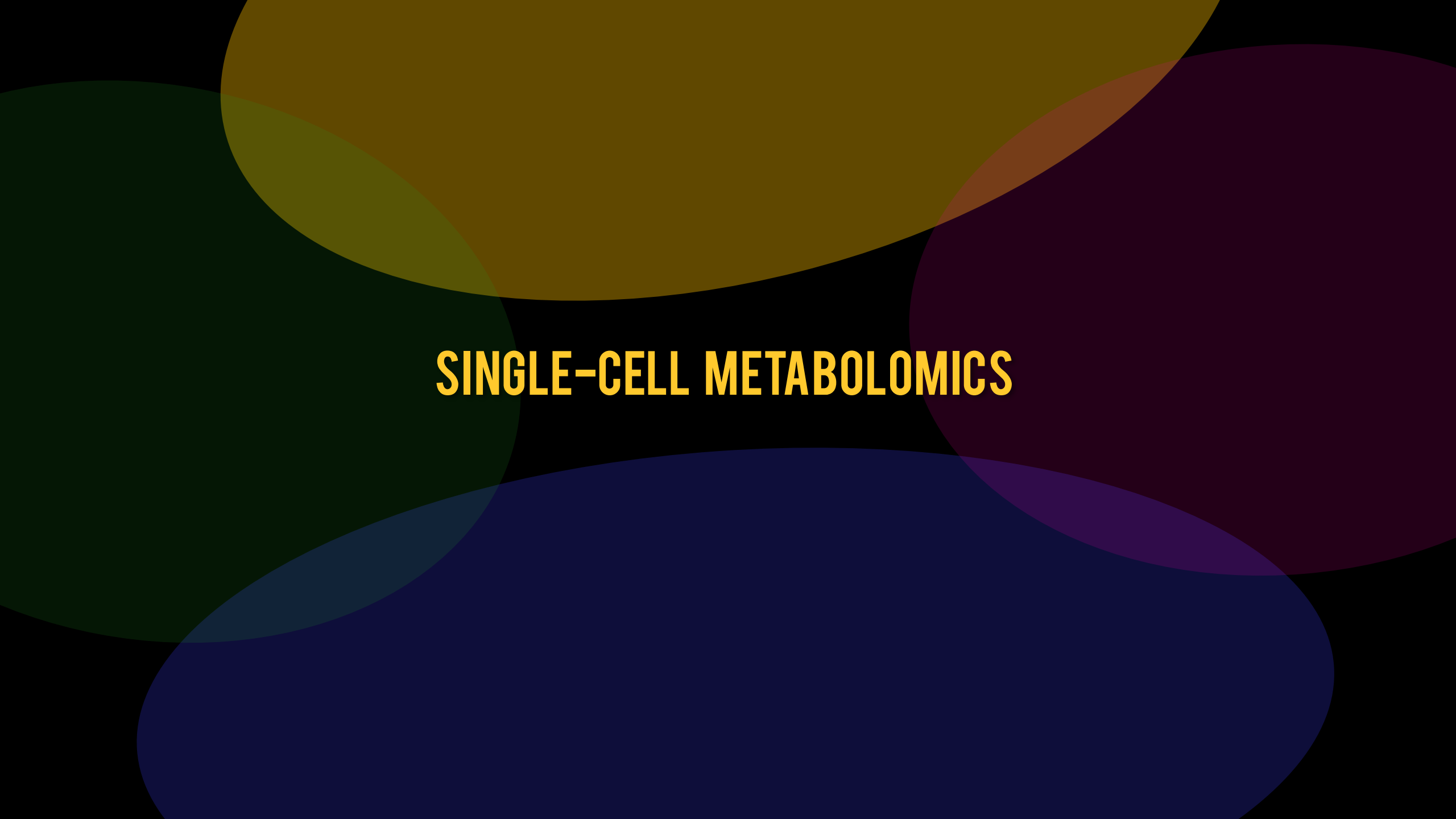
Spatial & Single-Cell Omics

Revealing the heterogeneity of metabolism



Big Data & Software

Mining terabytes of data



SINGLE-CELL METABOLOMICS

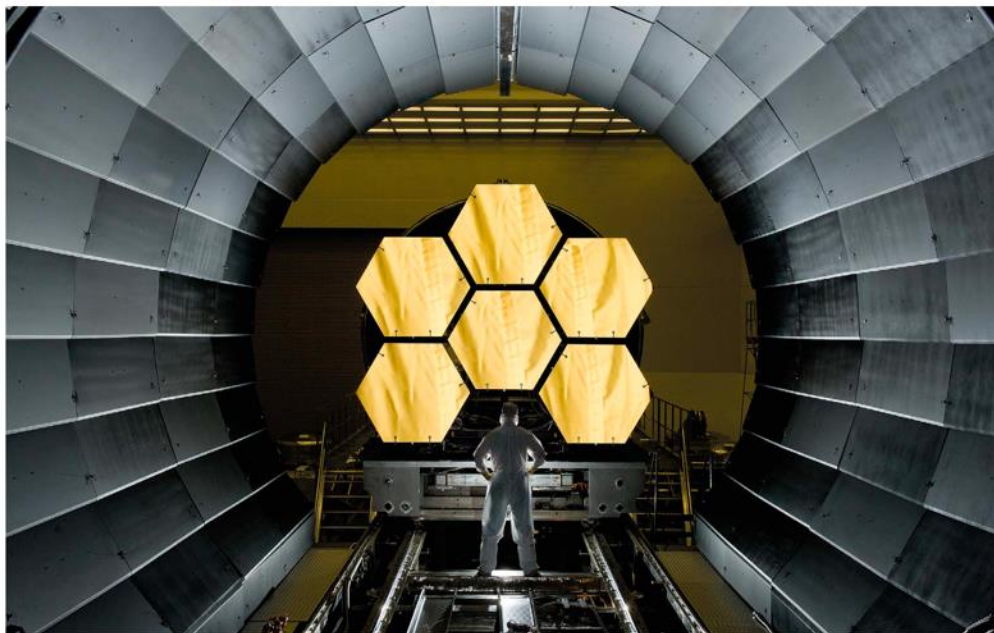
SINGLE



IUPAC Top Ten Emerging Technologies in Chemistry 2021



Work / Technology & tools



The James Webb Space Telescope's 6.5-metre primary mirror (6 of 18 segments shown) can detect objects billions of light years away.

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**



Artificial humus



RNA synthesis



Targeted protein degradation



Single-cell metabolomics

1000

INT. SINGLE-CELL MASS SPEC CONFERENCE

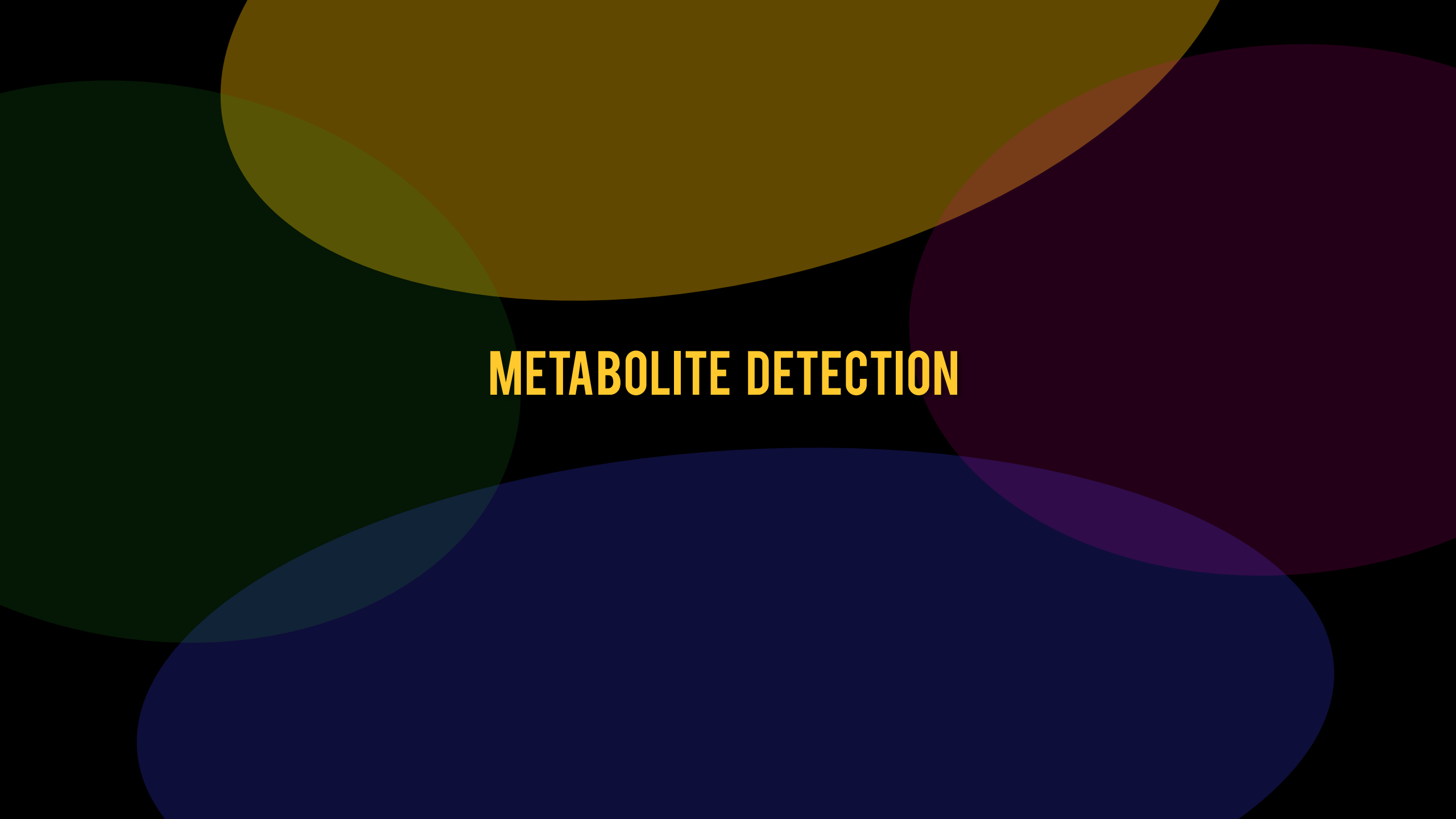
- 2023: Provo, USA
- 2024: Copenhagen, Denmark
- 2025: September-October, to be announced

FIELD,

WE STARTED DOING SINGLE-CELL METABOLOMICS IN 2017

OPEN QUESTIONS BACK THEN

- How to detect metabolites in single cells?
 - Bulk metabolomics requires 1 million cells / sample
- Which metabolites can be detected?
 - Metabolites are structurally diverse & ionize differently
- How to analyze the data?
- Metabolism is so dynamic. Is it even possible to preserve metabolic phenotypes for cells while culturing?



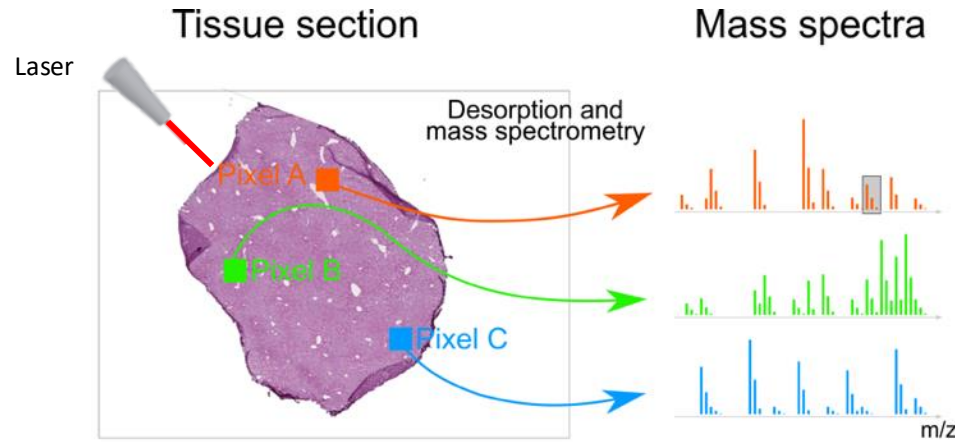
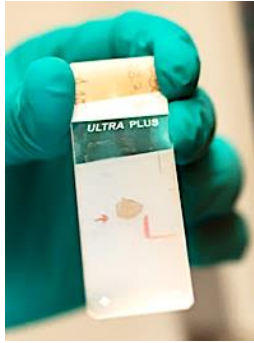
METABOLITE DETECTION

ENABLING TECHNOLOGY: MALDI-IMAGING MASS SPECTROMETRY

Matrix Assisted Laser Desorption Ionization

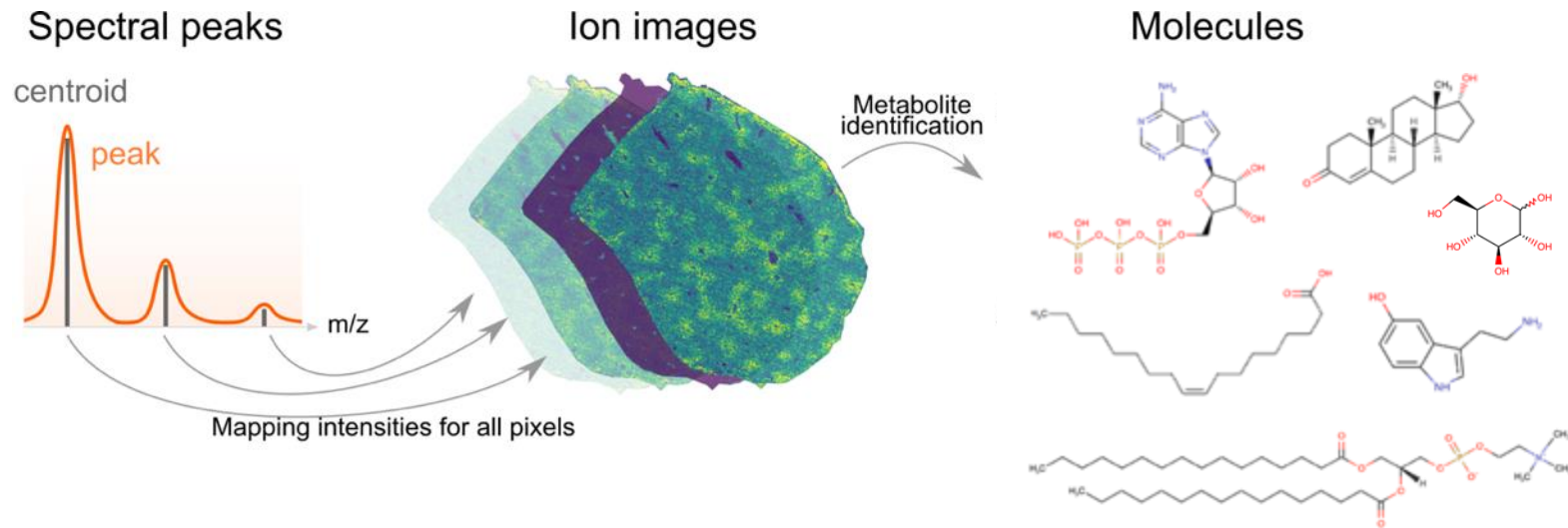


MALDI-IMAGING MASS SPECTROMETRY



Major technology for spatial & single-cell metabolomics

- 5-10 μm pixel size
- 100+ molecules
- Metabolites, lipids, drugs
- 1-100 GB / section



WHICH METABOLITES ARE DETECTABLE BY MALDI-IMAGING?



Veronika
Saharuka



Måns
Ekelöf



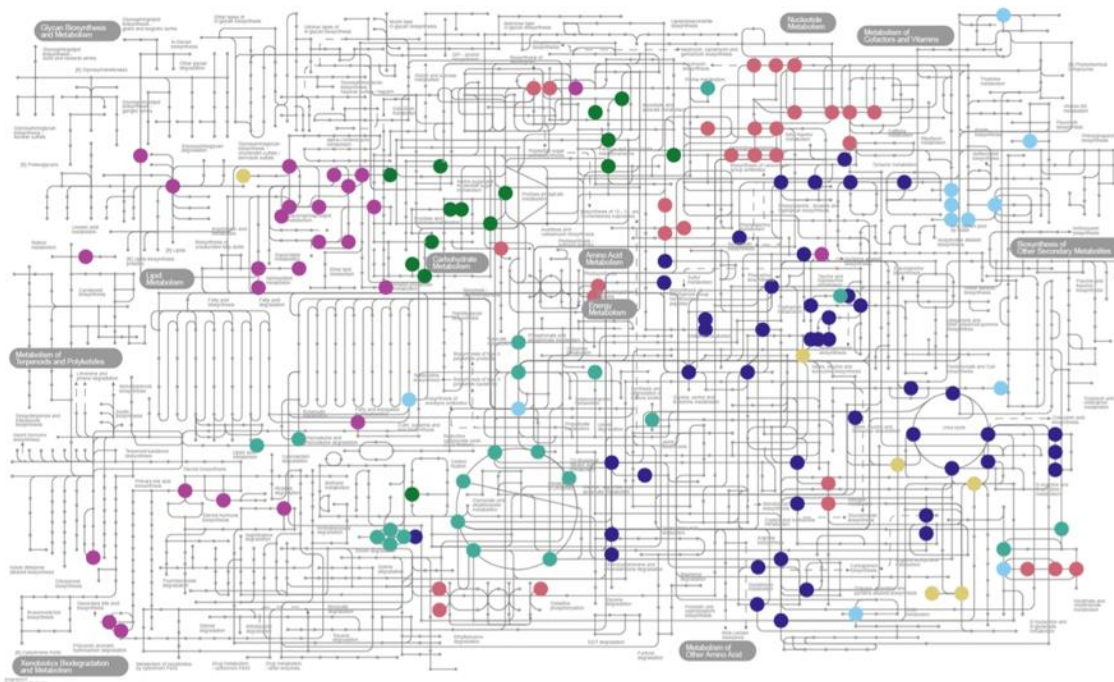
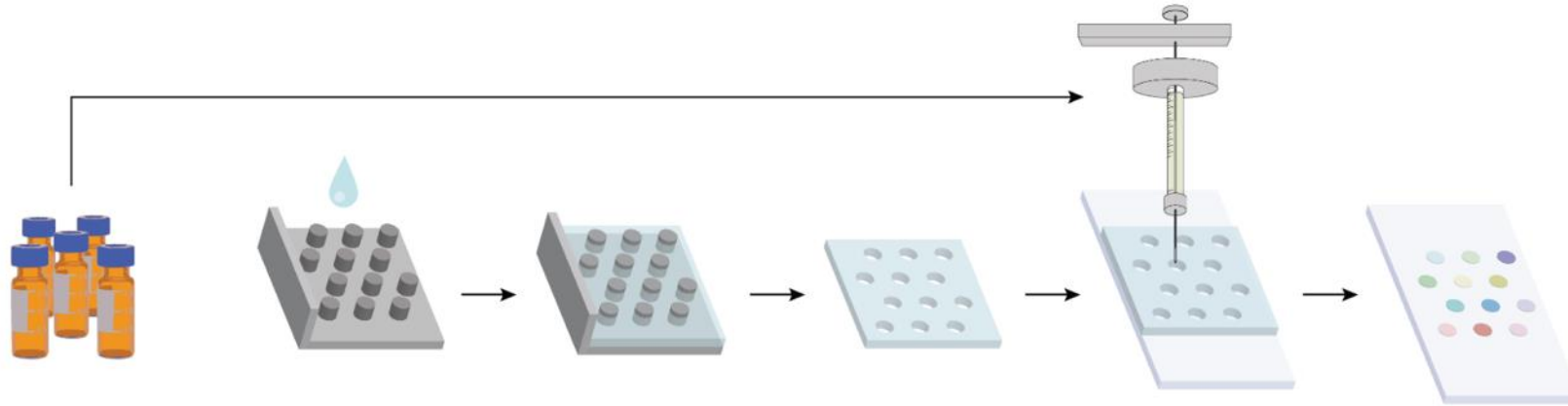
Lucas
Vieira



Lachlan
Stuart

Saharuka et al, *BioRxiv* 2024

STANDARD SAMPLES WITH BIOLOGICALLY-RELEVANT METABOLITES

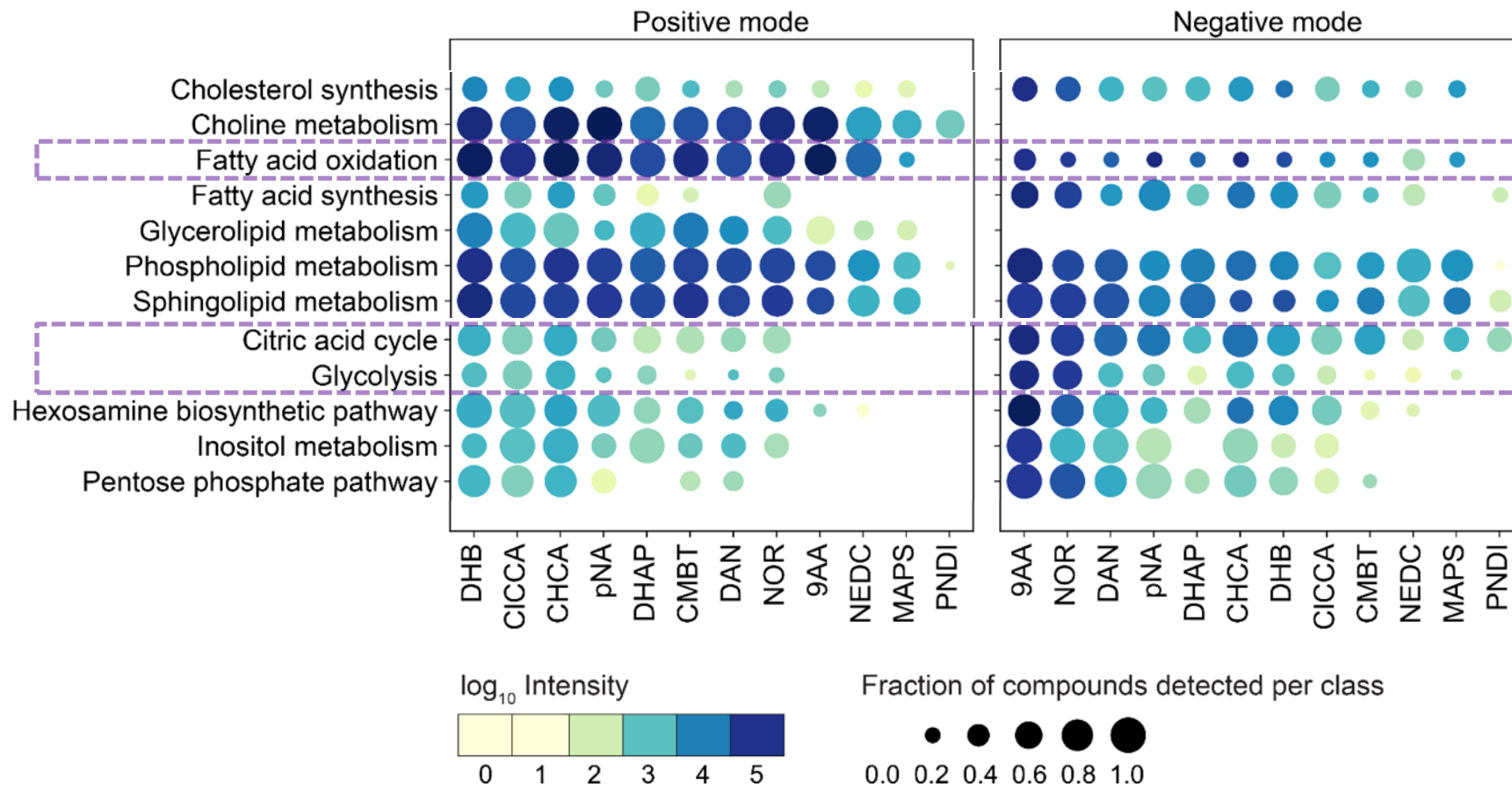


172 metabolites
covering various classes & pathways

- Amines
- Amino acids, peptides, and analogues
- Carbohydrates
- Carboxylic acids
- Lipids and lipid-like molecules
- Nucleosides, nucleotides, and analogues
- Vitamins and cofactors

LARGE-SCALE EVALUATION OF PROTOCOLS & TECHNOLOGIES

24 protocols in our lab



Complementing with
an inter-lab validation
by 10 labs using 12 protocols

USER-FRIENDLY WEBAPP

Showing which metabolites are detectable by which protocols or technologies

The screenshot shows the METASPACE webapp interface. The top navigation bar includes the METASPACE logo and links for Upload, Annotations, Datasets, Projects, Groups, Detectability, Help, Create account, and Sign in. The main interface is divided into several sections:

- Data source:** A dropdown menu with 'EMBL' selected.
- X axis:** A dropdown menu with 'Select axis'.
- Y axis:** A dropdown menu with 'Select axis'.
- Color:** A dropdown menu with 'Select color metric'.
- Sorting:** A dropdown menu with 'Seriate' selected.
- Filters:** Two dropdown menus: 'Polarity' (set to 'positive') and 'Neutral losses' (set to 'None').
- Visualization:** A button labeled 'Visualization' with a grid icon.

Numbered annotations (1-7) are overlaid on the interface to guide the user:

- 1:** Select part of the study (points to the Data source dropdown).
- 2:** Select x-axis for the heatmap (points to the X axis dropdown).
- 3:** Select y-axis (e.g. Class) (points to the Y axis dropdown).
- 4:** Select color (e.g. log10(intensity)) (points to the Color dropdown).
- 5:** How row are sorted (e.g. Seriate) (points to the Sorting dropdown).
- 6:** Modify, add, or remove filters (points to the Filters section).
- 7:** Change between dot- and heat-map (points to the Visualization button).

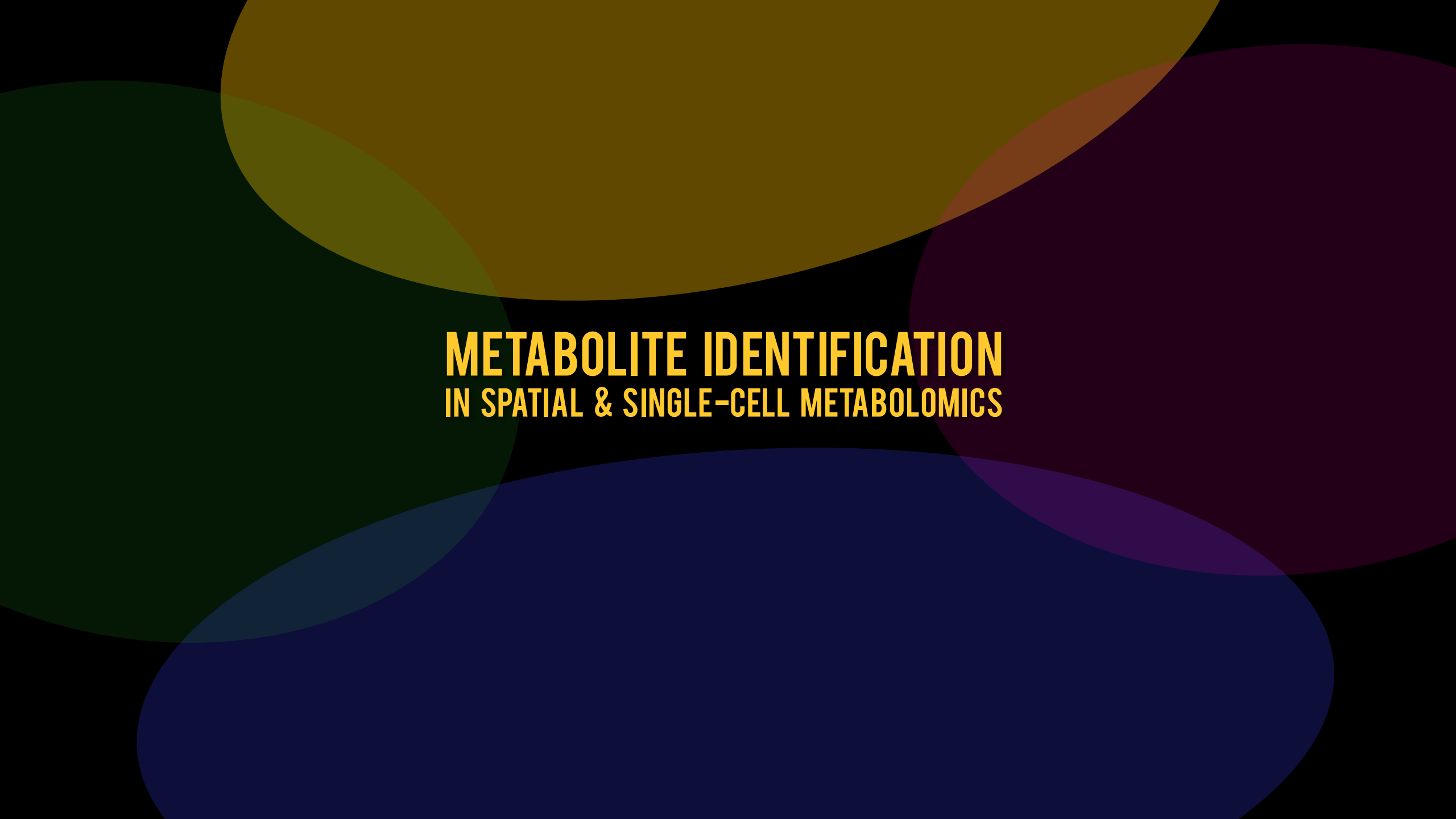
A 'Brief information to get started' section is located at the bottom left, containing a list of steps:

- 1 - Select the x axis metric in the red zone;
- 2 - Select the y axis metric in the green zone;
- 3 - Select the color in the blue zone;
- 4 - Apply the filters you desire.
- 5 - Click on the dots to be redirected to the corresponding annotations in METASPACE.

<http://metaspace2020.eu/detectability>

SUMMARY #1

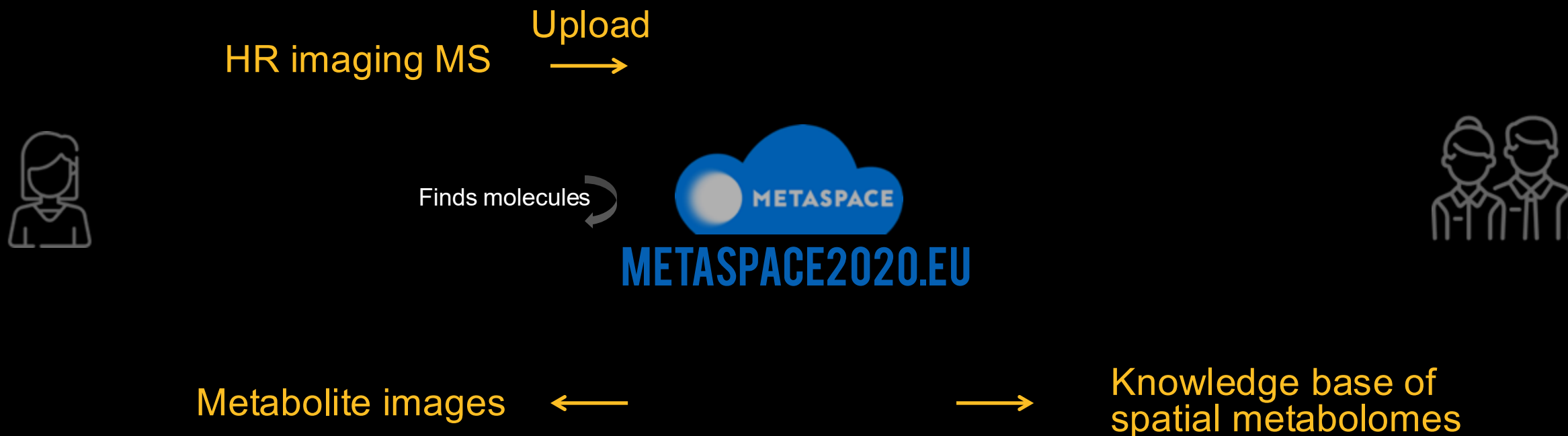
- Addressed a long-standing problem
 - Which metabolites are detectable?
- Most metabolites were detectable
 - Yet, require specific protocols
- Web-app metaspace2020.eu/detectability
 - To find best protocol for metabolites of interest



METABOLITE IDENTIFICATION

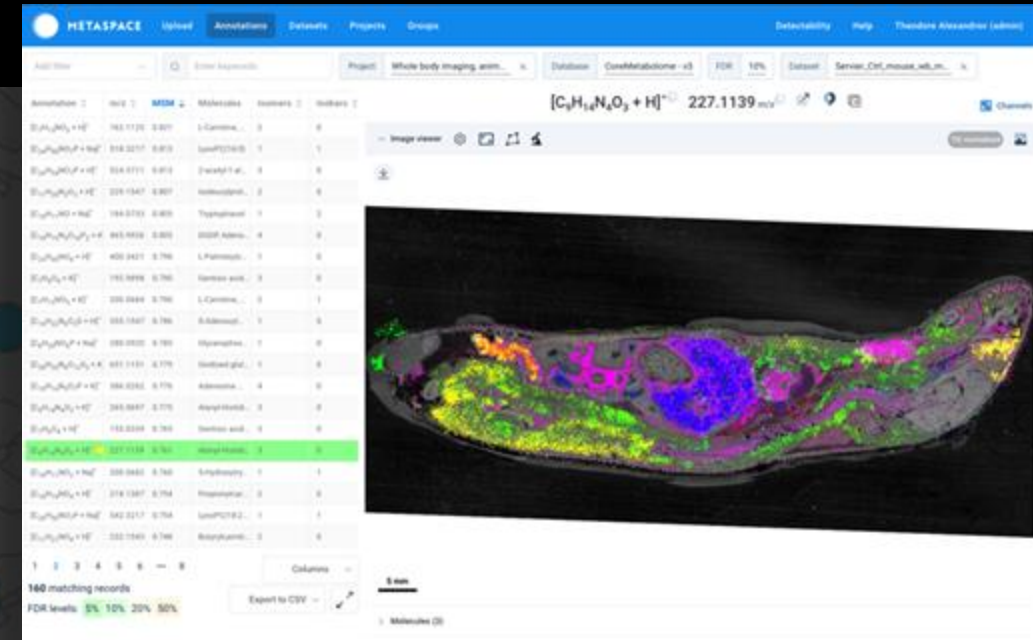
IN SPATIAL & SINGLE-CELL METABOLOMICS

METASPACE: CLOUD SOFTWARE FOR SPATIAL METABOLOMICS



METASPACE

- Metabolite ID
- Data QC, visualization, analysis, sharing
- Knowledge base of spatial metabolomes



Big data

- 50,000+ datasets
- 3,000+ users
- 50+ TB of data

Scientific impact

- 550+ publications

Collaborative

- Free, open source, open data
- NIH atlases (KPMP, LungMAP2)



Lucas Vieira



Bishoy Wadie



Welcome to

METASPACE

Platform for metabolite annotation of imaging mass spectrometry data

Metabolite Annotation

[Submit](#) your high-resolution imaging mass spectrometry data to our high-throughput metabolite annotation engine

Explore the Knowledgebase

[Browse](#) annotations from all datasets using our interactive interface

You can search, filter and compare your annotations alongside those from the whole imaging mass spectrometry community

Get Going Fast

Head to the [upload](#) page to submit a dataset.

We also have interactive [tutorials](#) prepared to help you.

Open Access

All code is open-source, the input format is the [imzML](#) supported by all mass spec major vendors, the metabolite annotations from the community datasets are public and can be browsed or exported.

HOW CAN PUBLIC DATA BE USED?

Submissions to METASPACE → Public data → ?

10,000+ datasets
1,000+ submitters
50+ TB

METASPACE-ML

Problem

- Current method has a number of deficiencies

Solution

- Train an ML-model on public data from METASPACE

Enablers

- Old idea (Silva et al, *Anal Chem* 2018)
- Big, representative, heterogeneous public data



Bishoy Wadie



Lachlan Stuart

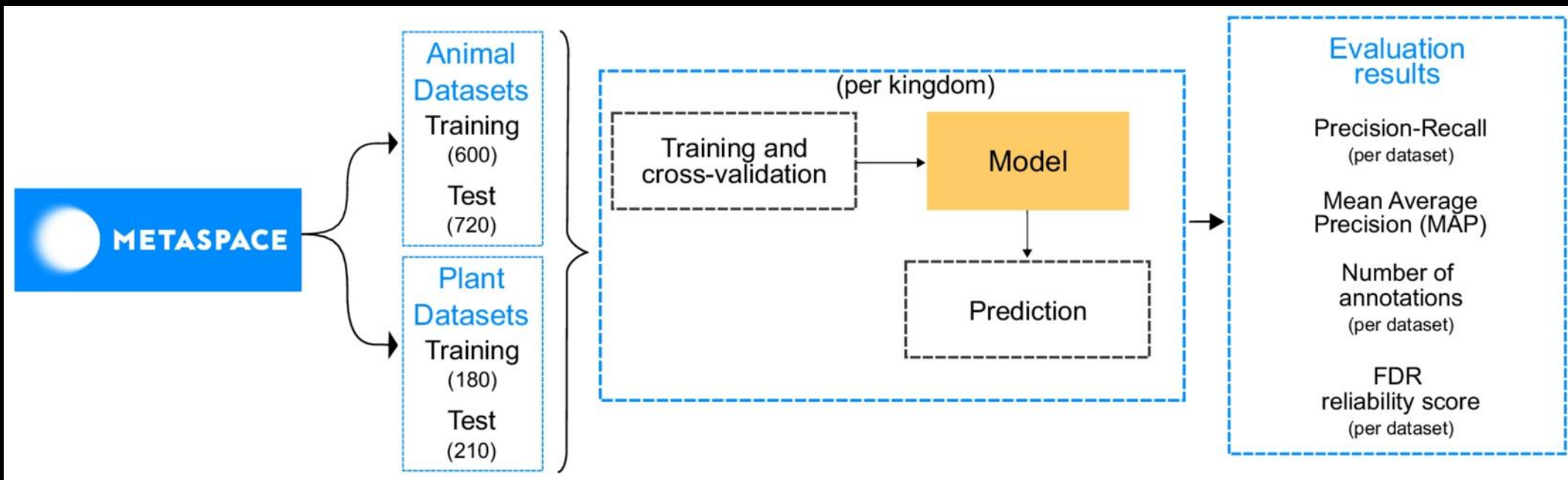
nature communications

METASPACE-ML: Context-specific metabolite annotation for imaging mass spectrometry using machine learning

[Bishoy Wadie](#), [Lachlan Stuart](#), [Christopher M. Rath](#), [Bernhard Drotleff](#), [Sergii Mamedov](#) & [Theodore Alexandrov](#) 

[Nature Communications](#) **15**, Article number: 9110 (2024) | [Cite this article](#)

METASPACE-ML



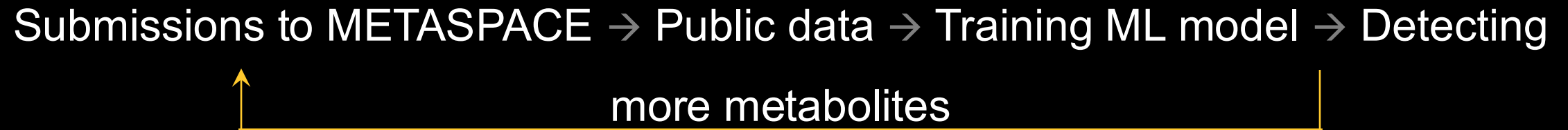
EVALUATION

On 920 public METASPACE
datasets:

- Annotating +100 metabolites
- Annotating metabolites of low intensity

THE POWER OF PUBLIC DATA

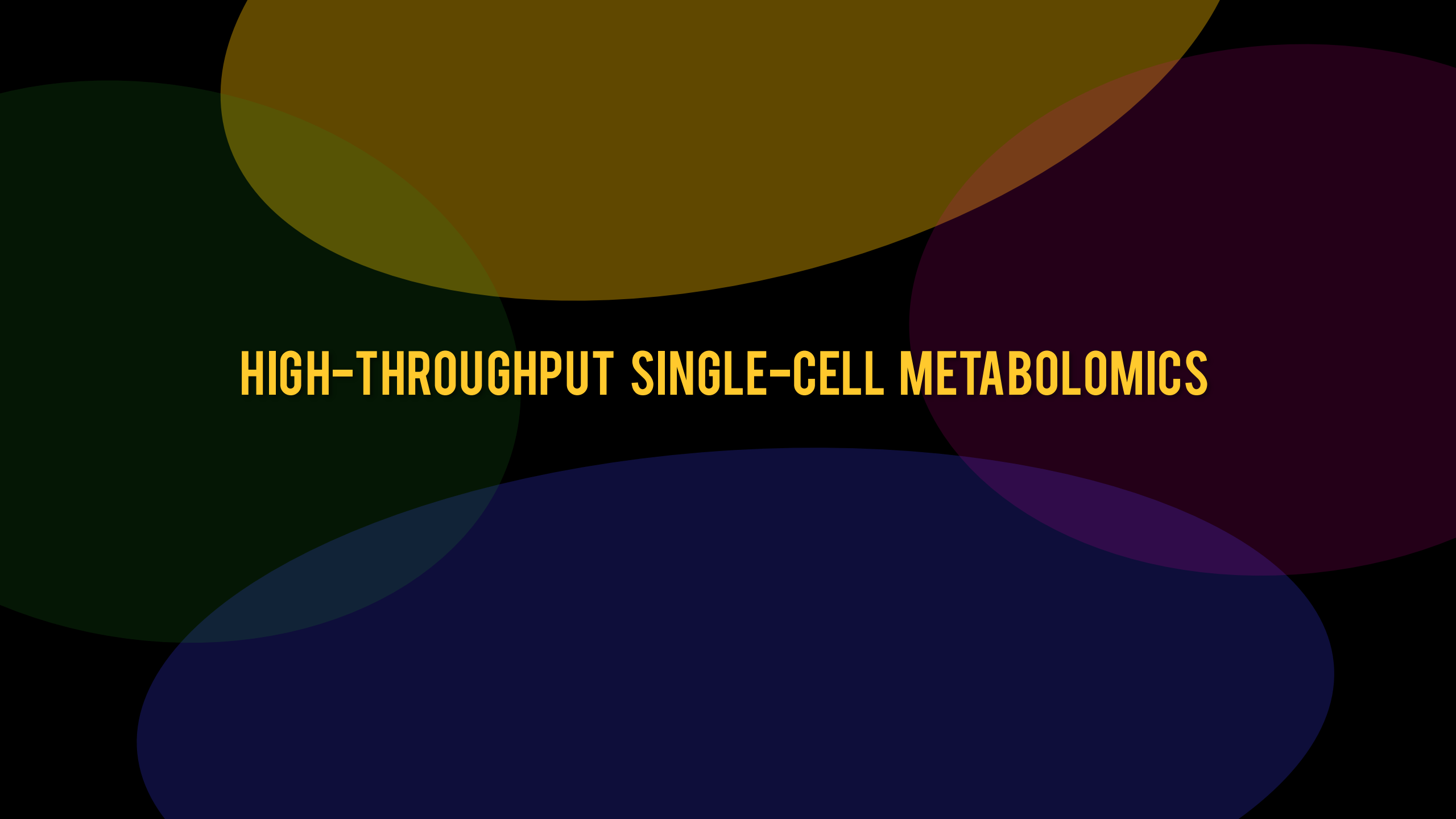
Submissions to METASPACE → Public data → Training ML model → Detecting
↑
more metabolites



Cycle of improvement for METASPACE & the field

SUMMARY #2

- **MALDI imaging mass spectrometry**
 - Foundational tech for spatial (and now single-cell) metabolomics
- **METASPACE: a cloud platform for spatial metabolomics**
 - For metabolite ID, and beyond
- **METASPACE-ML: ML-based metabolite identification**
 - Trained on big public data
 - Finds +100 metabolites, low-intensity

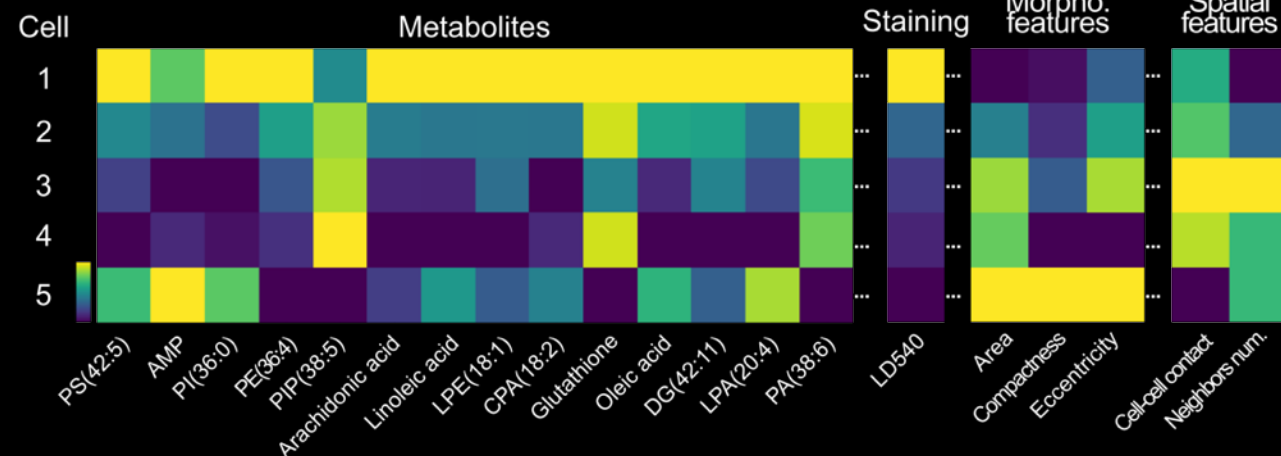
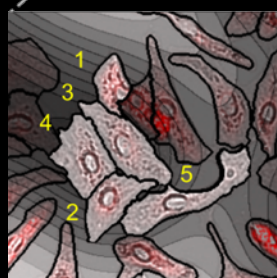
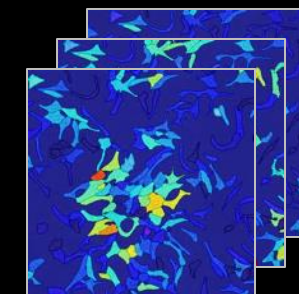
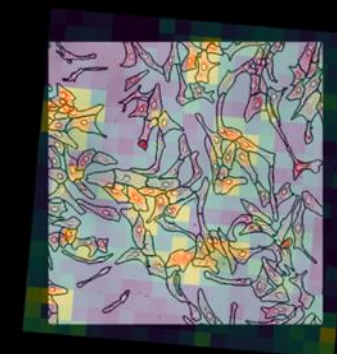
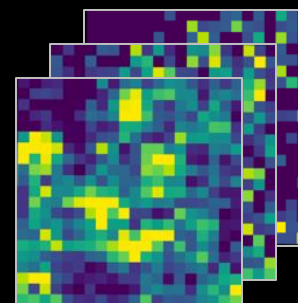
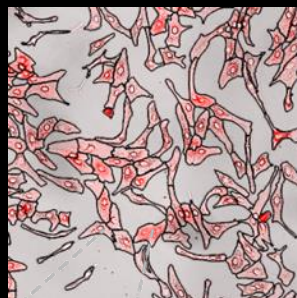
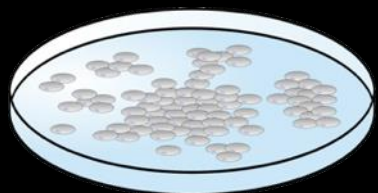
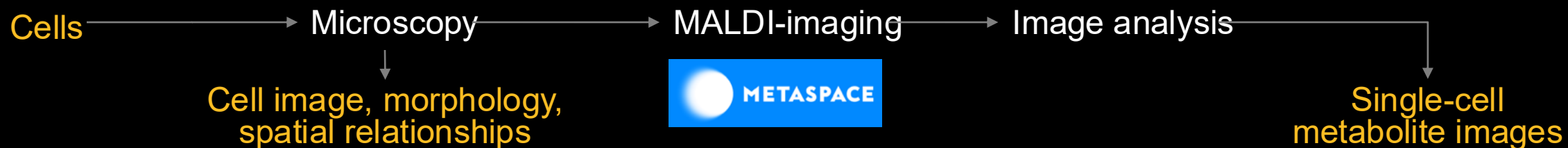


HIGH-THROUGHPUT SINGLE-CELL METABOLOMICS

SPACeM: SPATIAL SINGLE-CELL METABOLOMICS



Rappez et al, *Nat Methods* 2021
Patented

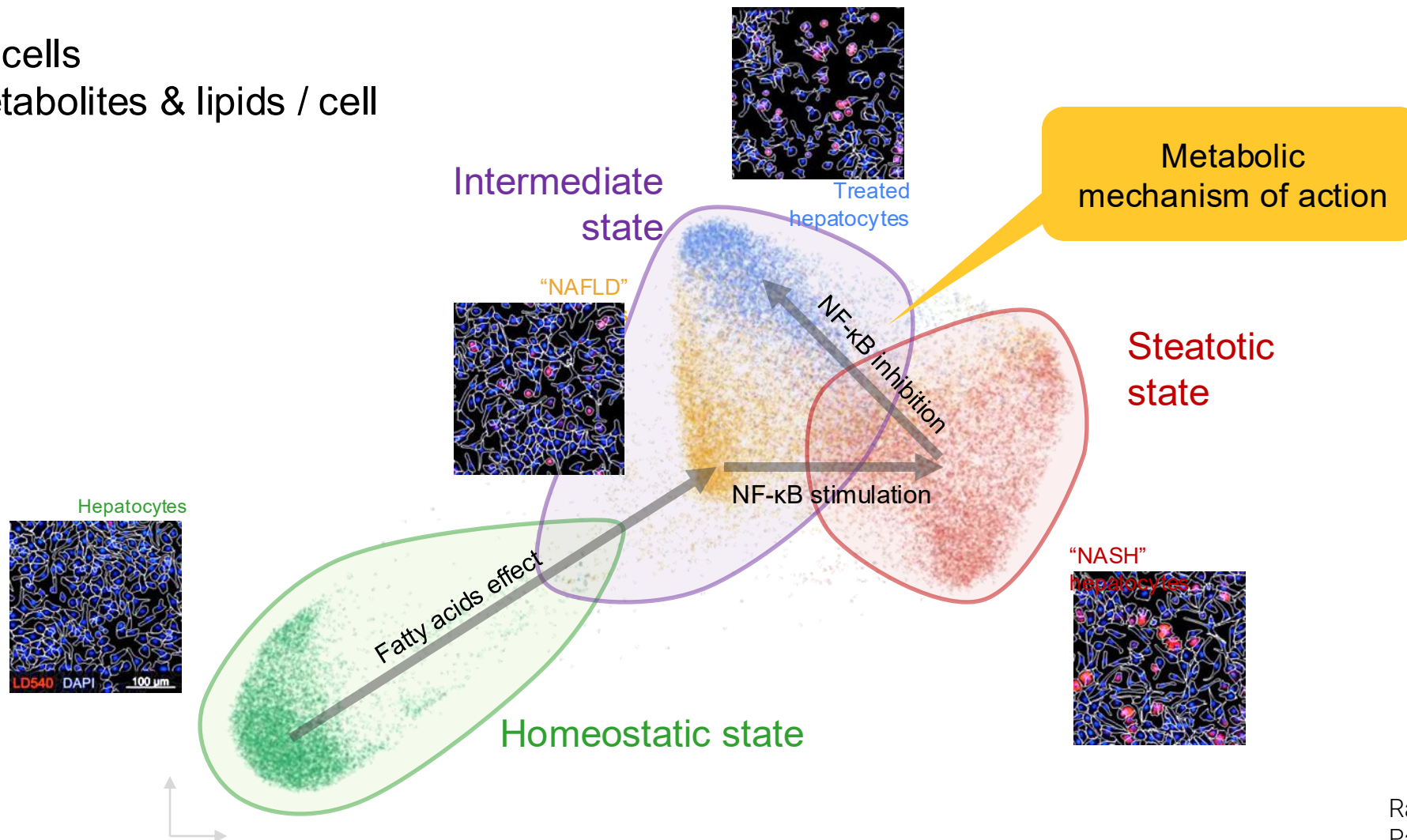


100+ metabolites
500+ lipids

SINGLE-CELL METABOLOMICS OF NAFLD/NASH

27.000+ cells

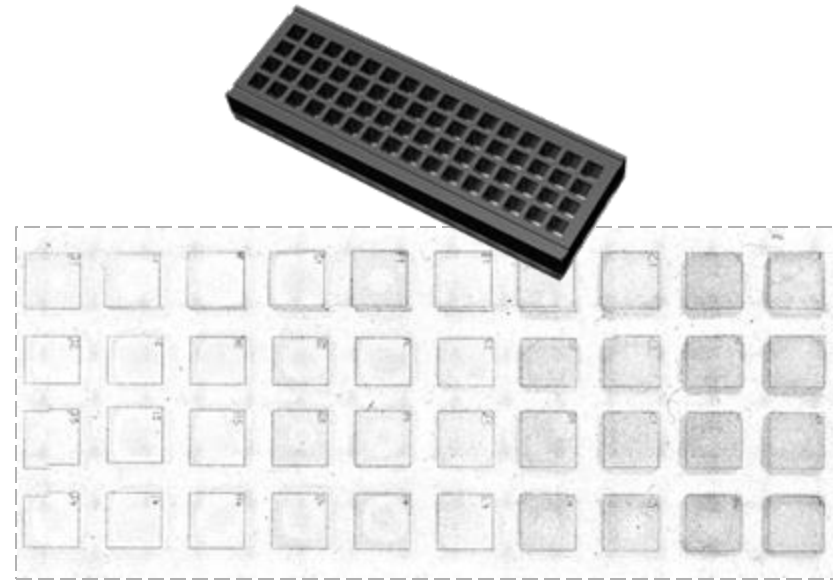
700+ metabolites & lipids / cell



HIGH-THROUGHPUT SPACEM

High-throughput SpaceM

- 40+ samples on a slide
- Protocol for small-molecule metabolites
- Custom glass slides
- Batch processing software



Jeany Delafiori



Shahrar
Mohammad

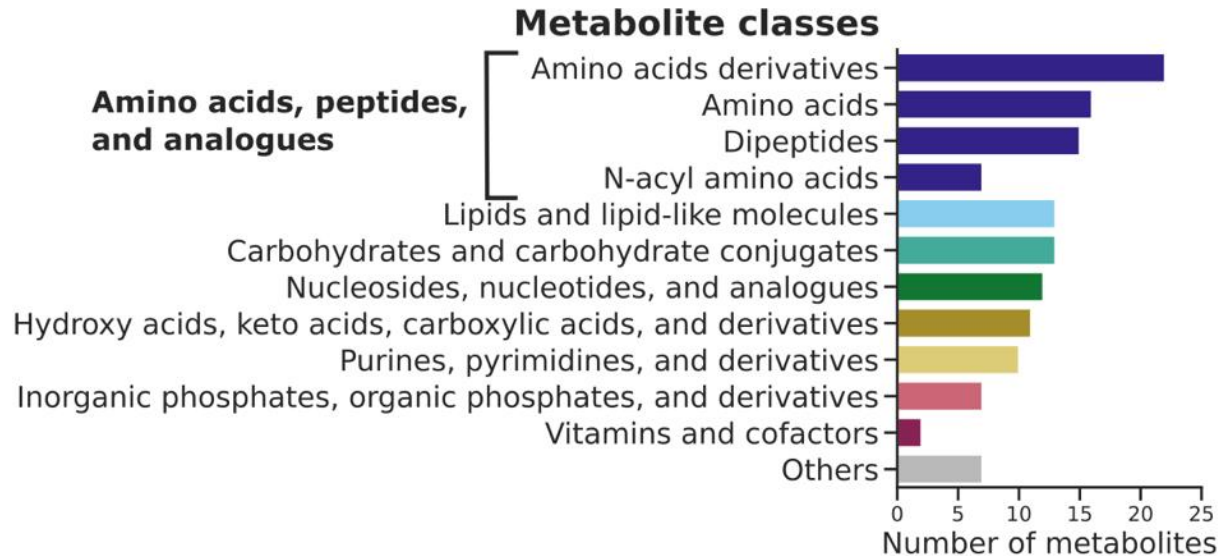
BioRxiv 2024

METABOLITE COVERAGE

Cells

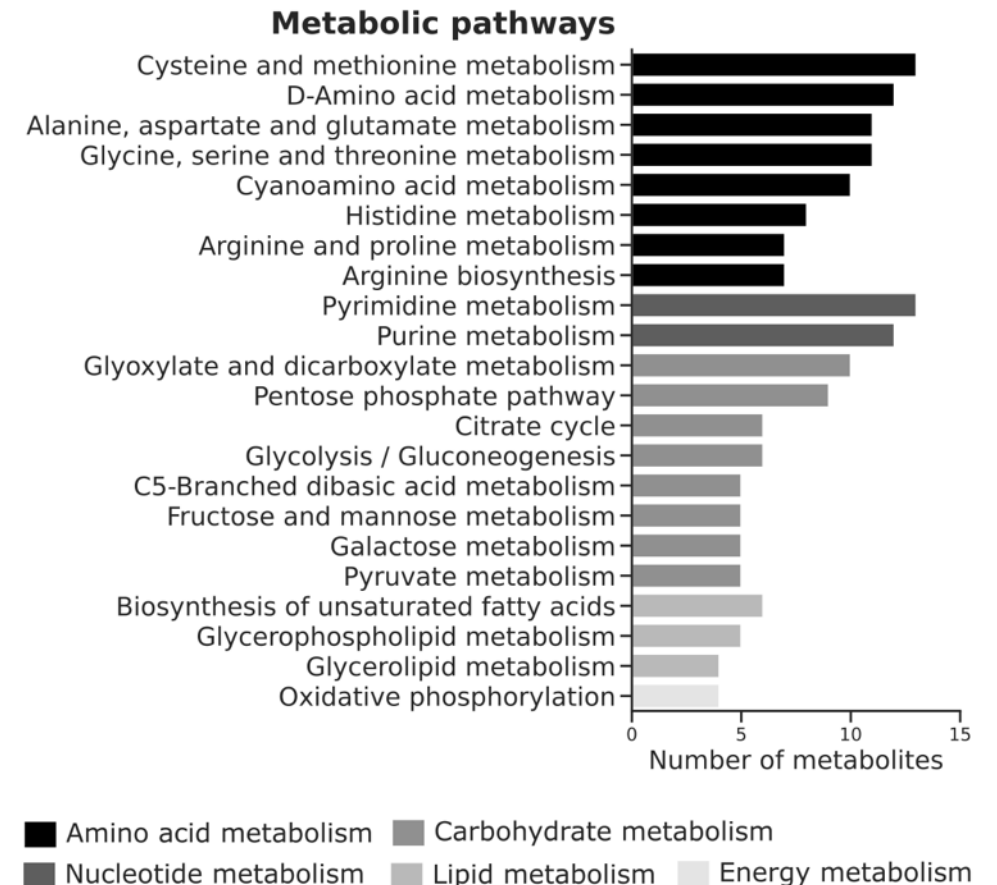
- HeLa, NIH3T3
- 3 slide-replicates, 72 well-replicates

135 metabolite ions



Biologically relevant

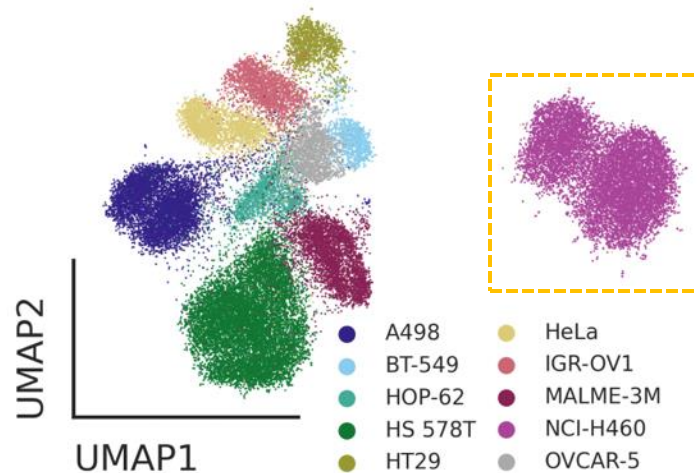
- 56% metabolites differ between HeLa vs NIH3T3



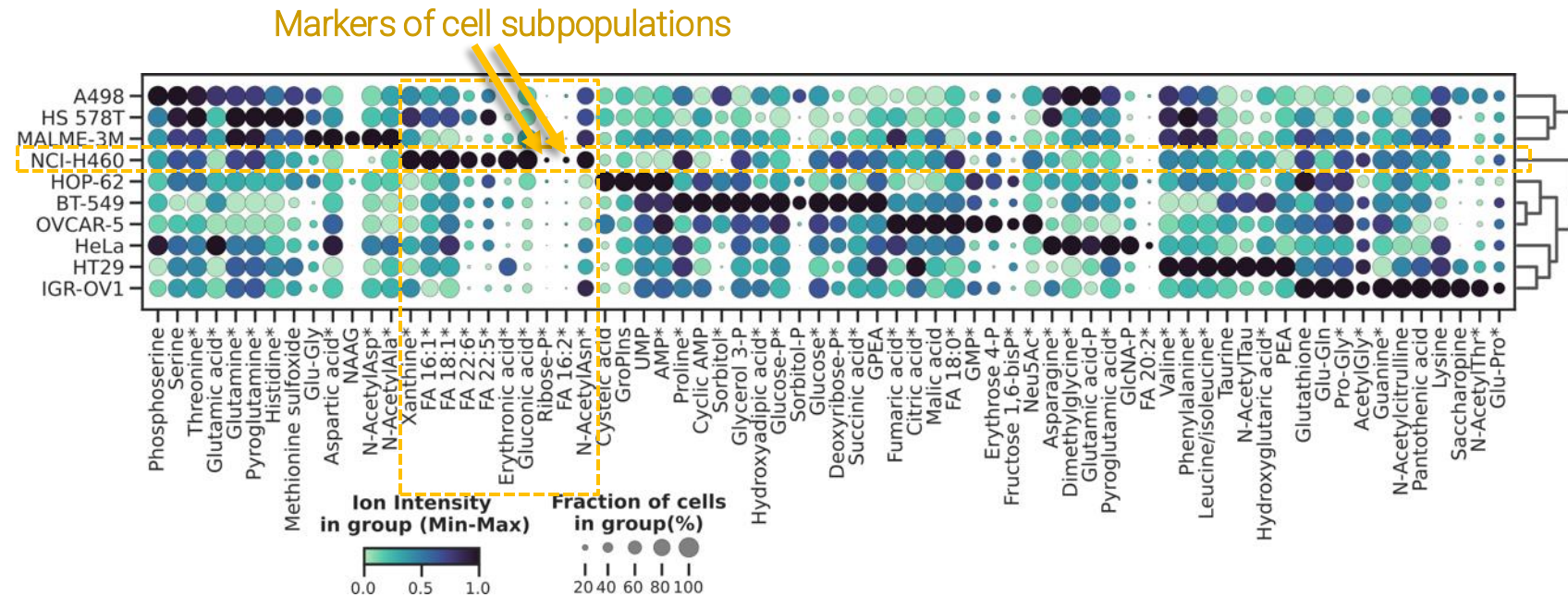
CASE STUDY: 9 CANCER LINES FROM NCI-60 + HELA

42K cells
202 metabolite ions
39-147 ions/cell

Unsupervised characterization

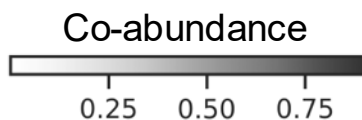
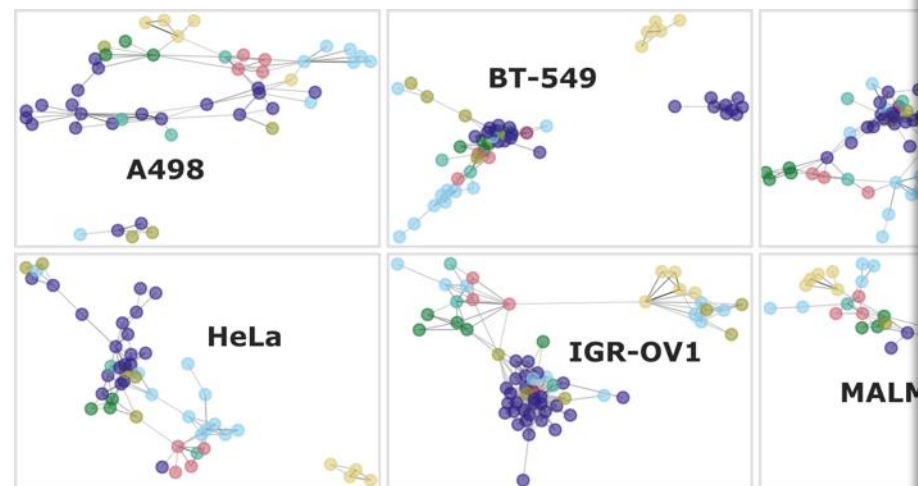


Cell-type specific markers



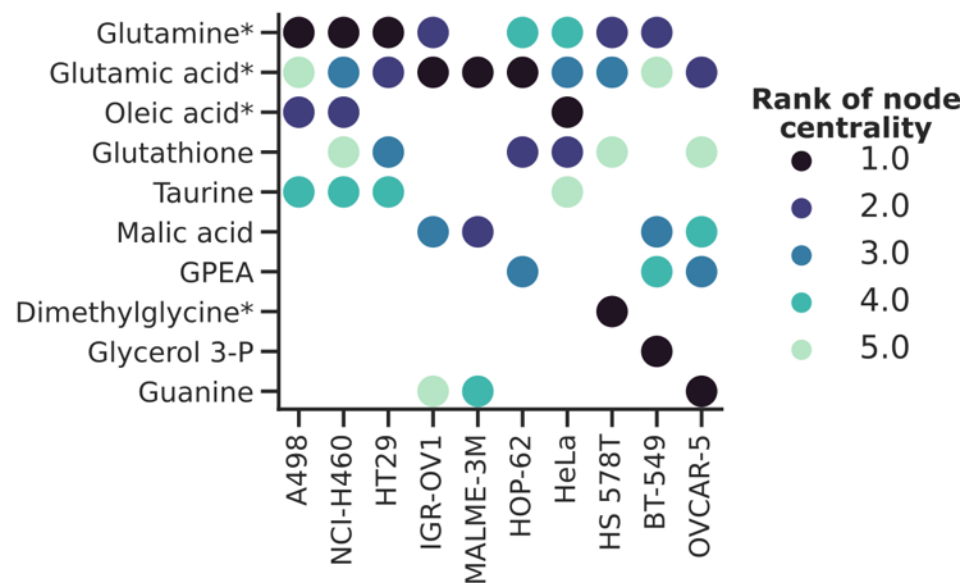
METABOLITE CO-REGULATION: CELL-TYPE SPECIFIC

Single-cell co-abundance metabolite networks



- Amino acids, peptides, and analogues
- Lipids and lipid-like molecules
- Inorganic phosphates, organic phosphates, and derivatives
- Hydroxy acids, keto acids, carboxylic acid, and derivatives
- Carbohydrates and carbohydrate conjugates
- Nucleosides, nucleotides, and analogues
- Vitamins and cofactors
- Purines, pyrimidines, and derivatives

Metabolic hubs



Can be used for target discovery

SUMMARY #3

- **HT SpaceM: high-throughput single-cell metabolomics**
 - High-throughput, reproducible, of high-coverage
 - Fast (1 cell/sec), affordable (x10-x100 cheaper cf. scRNAseq)
- **Reveals**
 - Markers of cell types, subpopulations
 - Cell-type-specific metabolite regulation – can be used for target discovery

SINGLE-CELL LIPIDOMICS FOR PDAC



University Hospital Essen



Jeany Delafiori



Shahrar
Mohammad



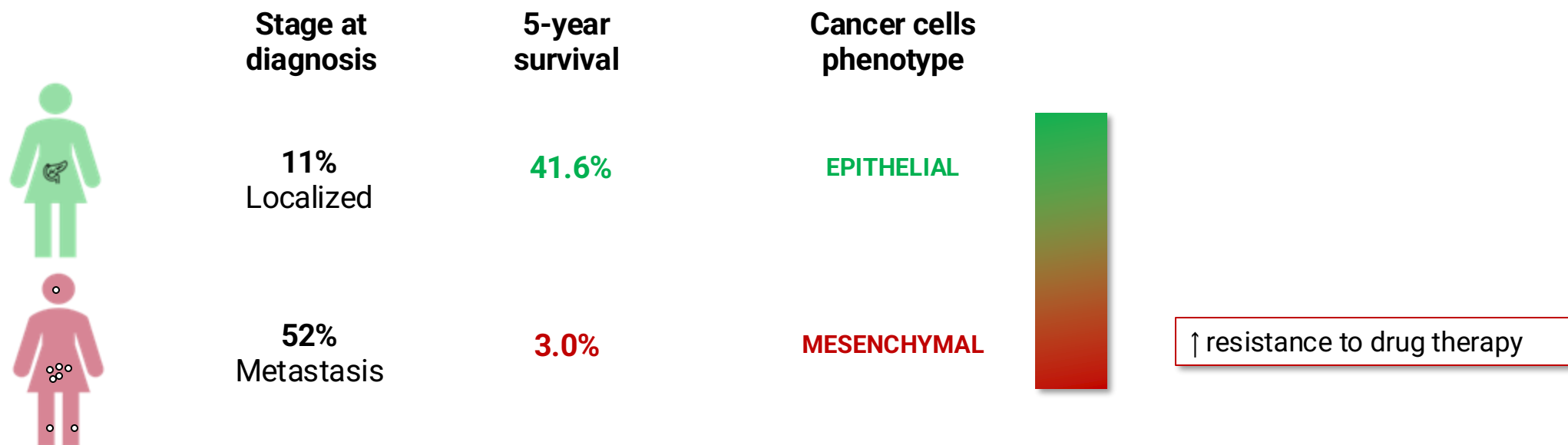
Marija
Trajkovic-Arsic



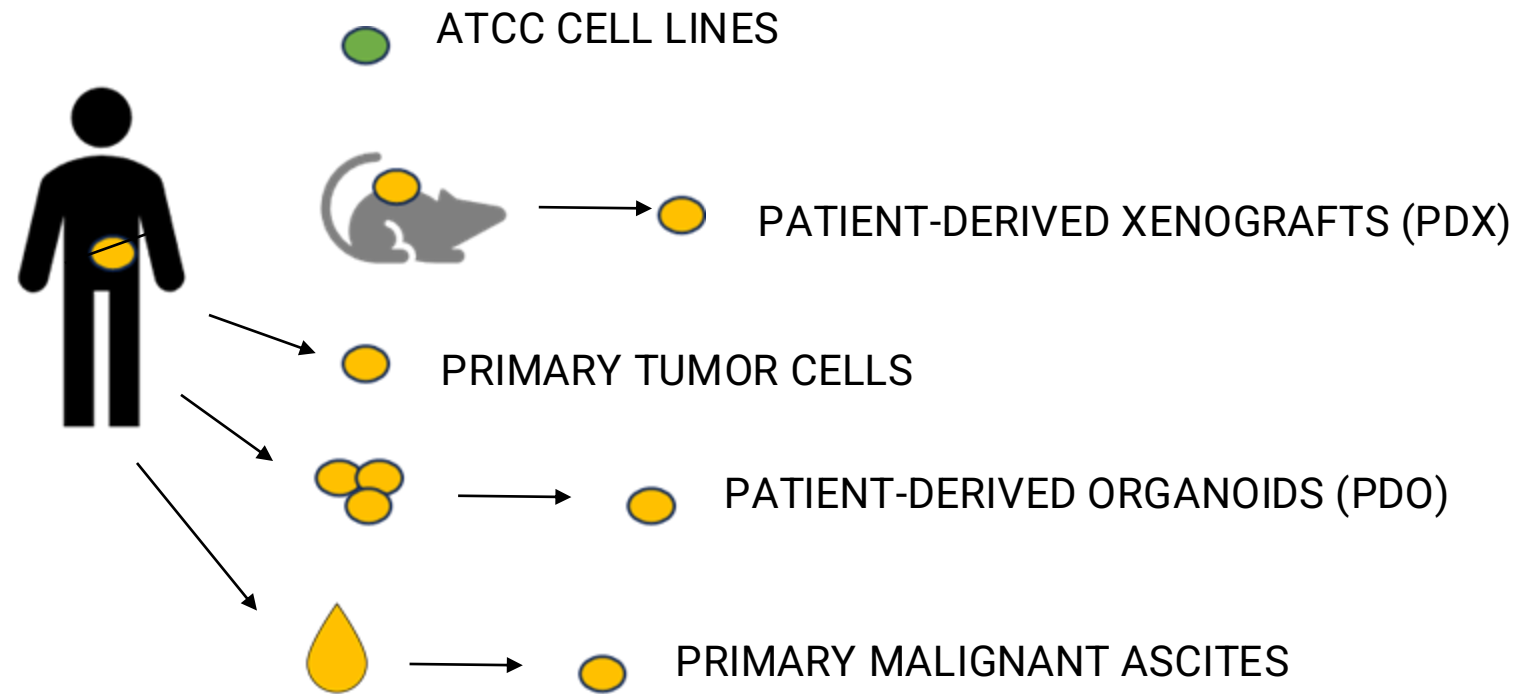
Jens
Siveke

PANCREATIC DUCTAL ADENOCARCINOMA (PDAC)

10th most common cancer among men and women



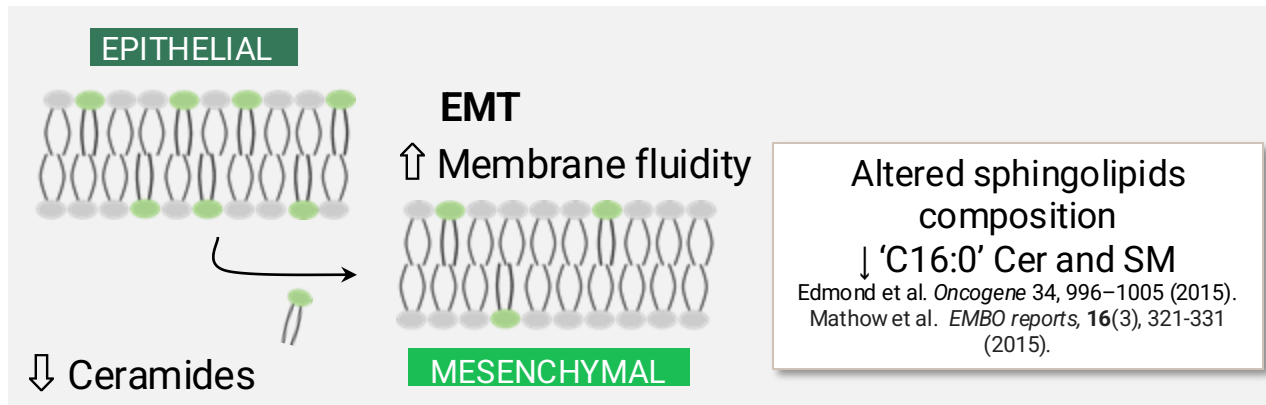
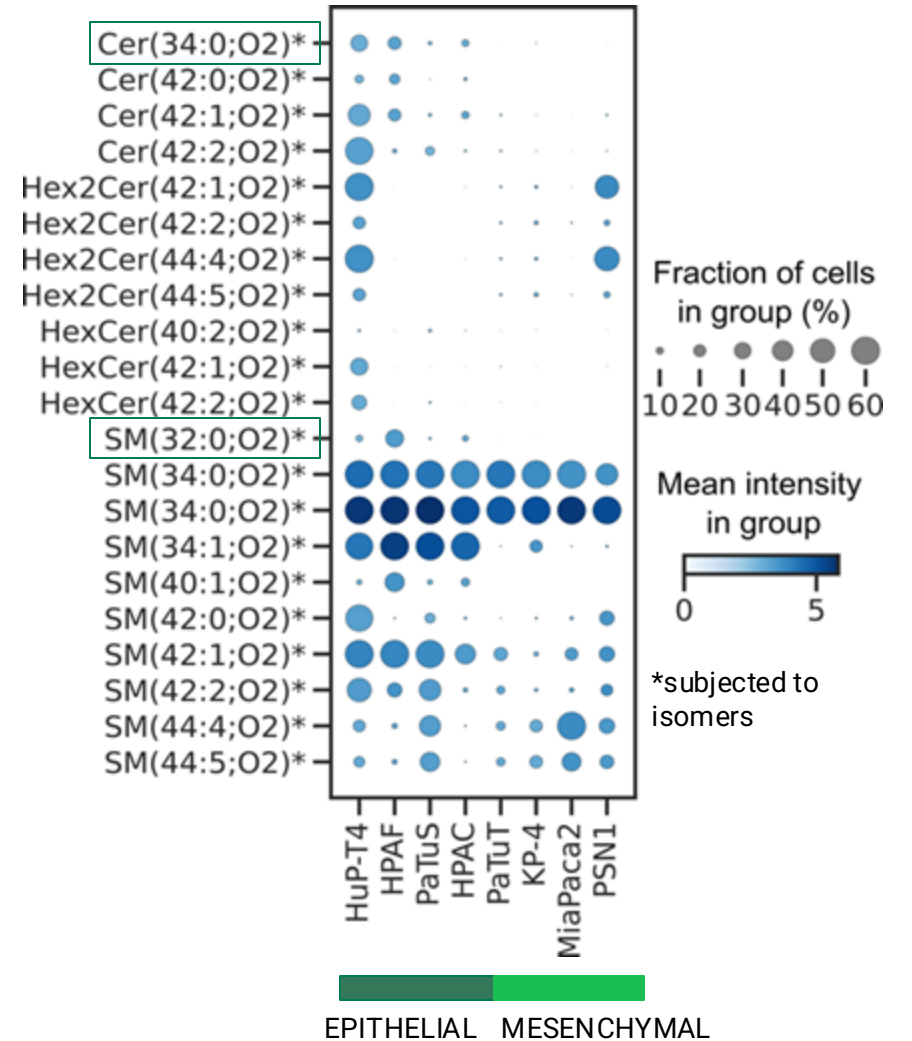
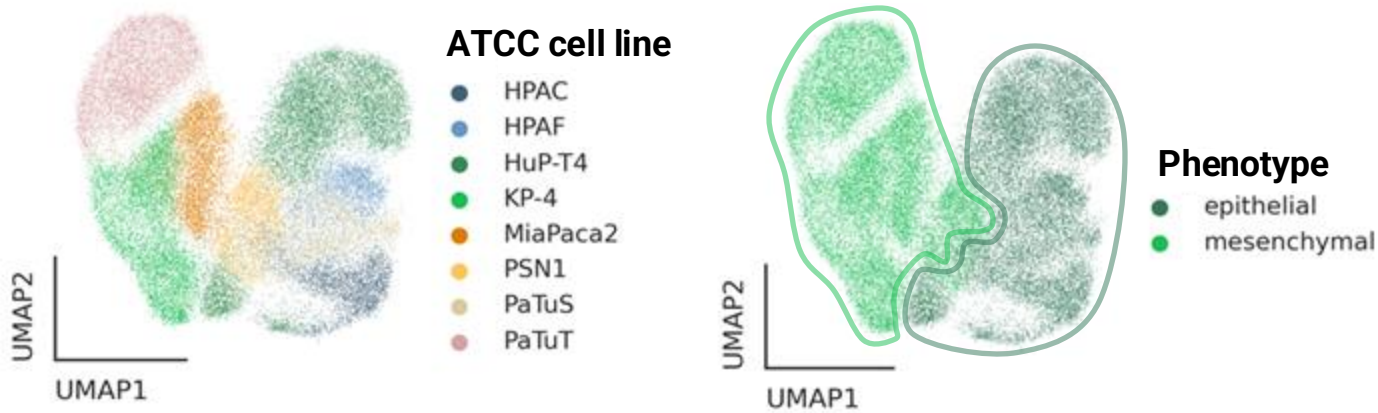
***EX VIVO* MODELS**



EPITHELIAL VS MESENCHYMAL ATCC CELL LINES

ALTERATIONS IN SPHINGOLIPIDS METABOLISM

Single-cell lipidomics analysis



CONSIDERING ALL CELL LINES & *EX VIVO* MODELS TOGETHER

8 ATCC cell lines

10 patient-derived samples

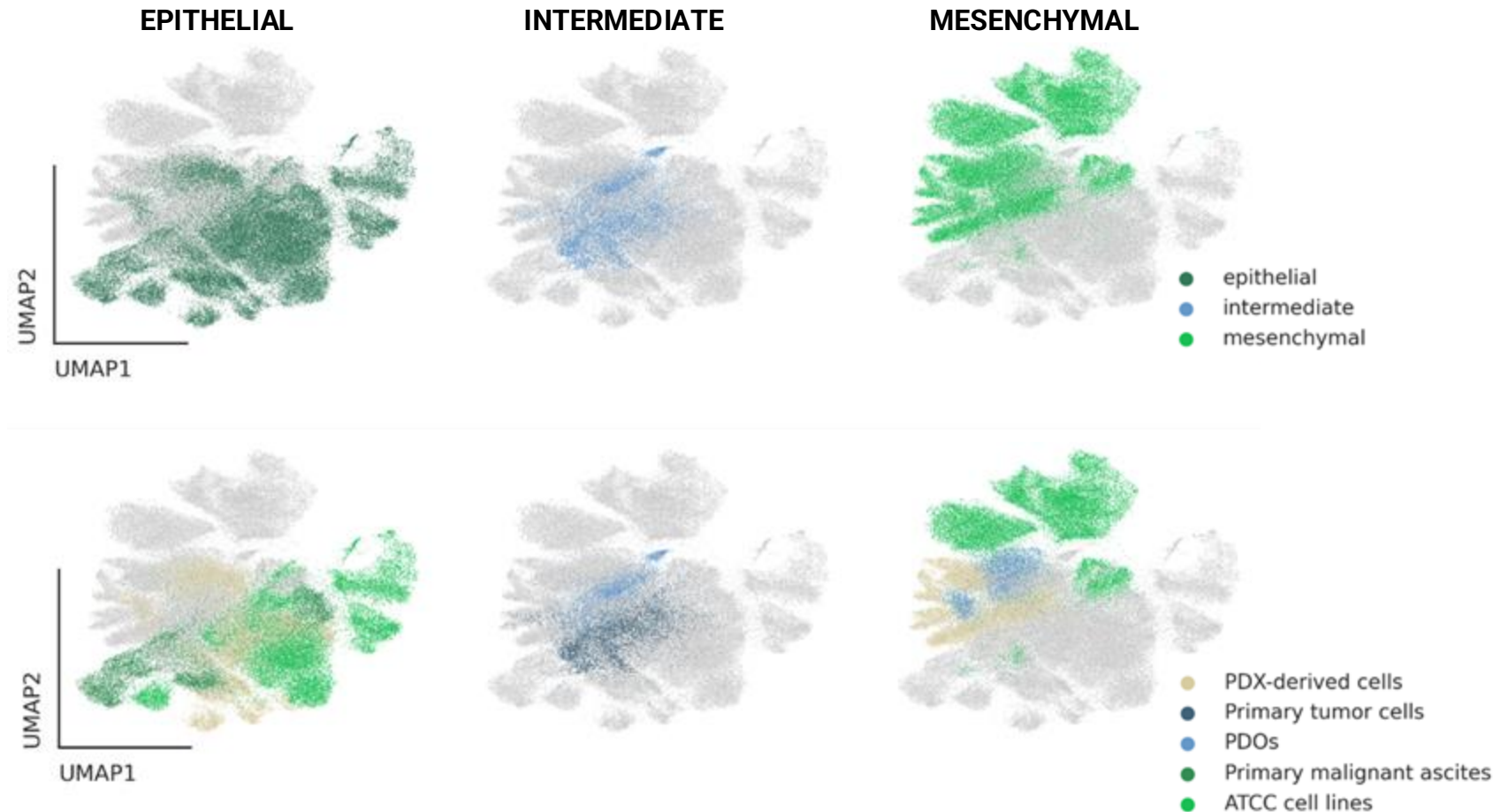
- 5 PDXs
- 2 PDOs
- 1 primary tumor
- 2 ascites samples

Phenotypes

- Epithelial
- Mesenchymal (aggressive)
- Intermediate

146K cells

1400 lipid ions



Metabolic memory of epithelial vs mesenchymal cell phenotypes
preserved through the process of *ex vivo* culturing

SUMMARY #4

- Single-cell lipidomics discerns epithelial vs mesenchymal phenotypes
 - Markers are in line with literature & lipid functions
- Metabolic patterns are consistent for all considered *ex vivo* models
 - Justifies using *ex vivo* models for metabolomics-based phenotyping

SUMMARY: ANSWERED QUESTIONS

- How to detect metabolites in single cells?
 - Using MALDI-imaging mass spectrometry, we developed the SpaceM method
- Which metabolites can be detected?
 - Most of metabolites, yet requires knowledge of protocols & software
- How to analyze the data?
 - METASPACE is a cloud software developed by our team
- Do *ex vivo* models preserve metabolic phenotypes?
 - Yes, at least in some cases (epithelial vs mesenchymal)

FUTURE

TECHNOLOGY

- Adding detection of proteins & genes
- Single-cell atlases of metabolic states & drug toxicity profiles
- AI/ML for data interpretation

APPLICATION AREAS

- Drug toxicity
- Cell therapies
- Target discovery
- Therapy response & monitoring
- Patient diagnosis, prognosis, phenotyp

COMMERCIALIZATION

- Deep-tech platform for drug & therapy development



→ UC San Diego

<http://ateam.ucsd.edu>

**University Hospital
Essen**

Marija Trajkovic-Arsic
Jens Siveke

Shahraz Mohammed
Lucas Vieira
Jeany Delafiori
Luisa Abreu
Bishoy Wadie
Sharath Menon
Amandine Prats

Selected alumni

Veronika Saharuka
Tim Rose
Alyona Buglakova
Mans Ekelof
Lachlan Stuart
Alex Mattausch
Andreas Eisenbarth
Sergii Mamedov
Sergio Triana
Luca Rappez
Vitaly Kovalev
Andrew Palmer



Open Targets



Chica and Heinz
Schaller Foundation

