SPATIAL SINGLE-CELL METABOLOMICS

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SPATIAL METABOLOMICS

**Molecular cartography**
Protsyuk et al, *Nature Protocols* 2018

**Metabolite ID**
Alexandrov et al, *BioRxiv* 2019

**Spatial single-cell metabolomics**
Rappez et al, *BioRxiv* 2019

**Tools:**
- Mass spectrometry
- Computational biology
- AI
Tumor microenvironment

Lyssiotis, Kimmelman, *Trends Cell Biology* 2017

Immuono-metabolism in time and space

Buck et al, *Cell* 2017

**WHY SPATIAL METABOLOMICS?**
**Why Spatial Metabolomics?**

Bulk metabolomics

- TUMOR
- FATTY LIVER
- CONTROL

**Turnover time**
Arrivault et al 2009

- NADP
- ADP
- DHAP
- 3PGA
- RUBP
- F6P
- UDPG

**Cellular pool half-life**
Shamir et al Cell 2016

- Metabolite 1 min
- mRNA 10 h
- Protein 1 day
Molecular cartography
Protsyuk et al, Nature Protocols 2018

Metabolite ID
Palmer et al, Nature Methods 2017
Alexandrov et al, BioRxiv 2019

Spatial single-cell metabolomics
Rappez et al, BioRxiv 2019
MALDI-IMAGING MASS SPECTROMETRY

5 μm pixel size (2 μm in 2020)
100+ molecules
1-100 GB / sample

2 labs in Lille: Uni, ImaBiotech
EXAMPLE: BRAIN TISSUE

Mouse brain WT MALDI-imaging
25 μm pixel size
Adenine
N-Acetylaspartylglutamic acid
LysoPE(18:1)
Citrate*

Shahraz Mohammed
Detectable

Amino acids, fatty acids, lipids

- Adenine
- N-Acetylaspartylglutamate
- LysoPE(18:1)

Glutaminolysis

- Glutamine
- Glutamate
- Aspartate

Glycolysis/TCA

- Hexose
- Hexose-phosphate
- Citrate*

Amino acids
- Citrate
- Malate
- Oxaloacetate
- Fumarate
- α-KG
- Carnitine
- Spermine
- Spermidine
- Creatine
- GSH, GSSG
- Bile acids
- Fatty acids
- PE, PC, PS, PI
- MG, DG, TG
- Cholesterol, CE

Fatty acids

Glycolysis/TCA

- Hexose
- Hexose-phosphate
- Citrate*

Glycolysis/TCA

- Hexose

Metabolites: Adenine, N-Acetylaspartylglutamate, LysoPE(18:1), Glutamine, Glutamate, Aspartate, Citrate, Hexose, Hexose-phosphate, Citrate*.
TECHNOLOGICAL PROGRESS OF IMAGING MASS SPEC

Spatial resolution
2-10 μm

Number of molecules
~100 @ FDR 10%

Throughput
1-50 pixels/sec

Sensitivity
μmol–fmol

Quantitation
90% agreement with HPLC

Data analysis
1–100 GB

KEY CHALLENGE: METABOLITE IDENTIFICATION
METASPACE: METABOLITE ID FOR IMAGING MS

Palmer et al, Nat Methods 2017
Alexandrov et al, BioRxiv 2019

HR imaging MS
Upload
Finds molecules
METASPACE
METASPACE2020.EU
Metabolite images
Metabolite imaging knowledge base

EU
NIH
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.
METASPACE: ENGINE + GLOBAL KNOWLEDGE BASE

- 5000+ submissions
- 80% of them public
- 100+ labs
- 200+ users
- 30+ publications in '18-'19

Alexandrov et al, BioRxiv 2019
PART 1: SUMMARY

• Metabolomics demands to be done in situ

• Imaging mass spectrometry is perfect for spatial metabolomics

• METASPACE
  – provides metabolite ID for imaging mass spectrometry
  – is a community knowledge base of spatial metabolomics
**FRONTIER: SINGLE CELLS**

- **Organism**
  - 1 m
  - Molecular cartography
    - Protsyuk et al, *Nature Protocols* 2018

- **Tissues**
  - 1 cm
  - Metabolite ID

- **Single Cells**
  - 1 μm
  - Spatial single-cell metabolomics
**SPACEM: SPATIAL SINGLE-CELL METABOLOMICS**

Adherent cells ➔ Microscopy ➔ MALDI-imaging ➔ Image analysis ➔ Single-cell metabolite images

- Cell image, morphology, spatial relationships
- Adherent cells ➔ Microscopy ➔ Image analysis
- Metabolite images
- Cell image, morphology, spatial relationships
- Cell image, morphology, spatial relationships


Patent application
VALIDATION

HeLa
NIH3T3 murine fibroblasts
METABOLOME IS PREDICTIVE OF CELL TYPE

92% accuracy of cell type prediction

Metabolic intermixing upon co-culture

Most intermixing among neighbors of different type
NON-ALCOHOLIC STEATOHEPATITIS
MOST COMMON FACTOR FOR LIVER CANCER

Anstee, ..., Heikenwalder, Nat Rev Gastroenterol Hepatol, 2019
SINGLE-CELL ANALYSIS OF NASH IN VITRO

Human hepatocytes dHepaRG + oleic acid + palmitic acid + TNF

With Heikenwaelder lab, DKFZ
Wolf et al, Cancer Cell, 2014
**STEATOTIC CELLS ACCUMULATE LONG LIPIDS**

Human hepatocytes dHepaRG + oleic acid + palmitic acid + TNFα

Fenland and NSHD studies
Sanders et al, *Genome Biol*, 2018

Lipid droplet

Triglyceride TG(50:1)
TG(16:0_16:0_18:1)

Palmitic acid

Oleic acid
METABOLIC STATES OF INDIVIDUAL HEPATOCYTES

Lipid accumulation

NASH metabolic state

NASH cell subpopulations

CTRL

+FA

+IL17A

+TPCA1

CTRL

+FA

+IL17A

+TPCA1
PART 2: SUMMARY

• Single-cell revolution is happening

• SpaceM is a method for spatial single-cell metabolomics

• SpaceM
  – discriminate cell types
  – characterizes spatial effects of neighboring cells
  – helps discover metabolic states, subpopulations
  – link phenotype-metabolome

searching for collaborators for single-cell metabolomics of macrophages, eg. BMDMs
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Veronika Saharuka, Lachlan Stuart
Sergio Triana, Vitaly Kovalev
Mohammed Shahraz, Mans Ekelof, Andreas Eisenbarth, Luisa Abreu
Prasad Phapale, Rose Gathungu
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