Single cell-based Analysis of cancer and host proteome interactions by Deep Visual Proteomics

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What proteomics can analyze

from Hein ... Mann, Handbook of Systems Biology, 2012
Minimalistic proteomics

Cellular biology

Clinical applications
The proteome landscape of the kingdoms of life

Nature June 2020
Advantages of single cell (type) proteomics
Trapped ion mobility spectrometry (TIMS)

Florian Meier
Andreas Brunner
Catherine Vasilopoulou
Johannes Müller

Meier, …, Mann, J. Prot. Res. 2015
Meier, …, Mann, Mol. Cell. Prot. 2018
Vasilopoulou, …, Mann, Meier, Nat. Comm. 2020
Parallel Accumulation followed by SErial Fragmentation (PASEF)

PASEF: Multiplying Sequencing Speed and Sensitivity; Meier, ..., Mann; JPR; 2015
Online PASEF with a Novel TIMS; Meier, ..., Mann; MCP; 2018
Parallel Accumulation – Serial Fragmentation (PASEF)
Mass spectrometry-based proteomics to enable single-cell analysis

Fluorescence-activated cell sorting

Laser capture microdissection

Andreas-David Brunner  Marvin Thielert
A modified Trapped Ion Mobility Spectrometer coupled to a Time-of-Flight analyzer
Raw intensity increase
Single cell proteomics on FACS sorted cells

1% PSM and 1% Protein level FDR in MaxQuant
Quantitative reproducibility on protein level

Bulk

8 cells

1 cell

$R = 0.99$

$R = 0.98$

$R = 0.92$
Evosep nanoflow

In EvoTip single cell processing

Advantages
- One-pot reaction
- No transfer step
- Peptides directly immobilized
- Peptide elution in ~20 nl volume
- Peptides pushed by single pump

A novel LC system embeds analytes in pre-formed gradients for rapid, ultra-robust proteomics
Bache, ..., Mann, 2018, MCP

Disclaimer: MM is an indirect investor in Evosep
diaPASEF for increased ion sampling

diaPASEF: Bottom-up proteomics with near optimal ion usage
Florian Meier, Andreas Brunner, ..., Ruedi Abersold, Ben C. Collins, Hannes L. Röst, Matthias Mann, 2019, bioRxiv, accepted in Nature Methods
36 single HeLa cell proteomes
Unsupervised clustering and principal component analysis of 36 single HeLa cell proteomes
Biological process enrichment in the HeLa single cell proteome dataset

KI67 levels across single cells

[Graph showing enrichment factors and KI67 levels across single cells]
Deep Visual Proteomics

- Archived patient tissue samples
- Highly multiplexed microscopy
- Image segmentation using deep learning
- Intelligent image-based single-cell isolation
  - Machine learning algorithms are trained to predict cellular phenotypes
  - Single-cell isolation using laser capture microdissection

Clinical Knowledge Graph

- Ultra-high sensitivity proteomics

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MAX-PLANCK-GESELLSCHAFT
( Archived ) patient tissue samples

**Cancer**
Eckert M, ..., Mann M, Lengyel E 2019, Nature
Coscia F, ..., Mann M, Curtis M 2018, Cell
Doll S, ..., Mann M, 2018, Mol Oncology
Coscia F, ..., Mann M, 2020, J Pathol

**Metabolic diseases**
Niu L, ... Mann M, 2019, Mol Syst Biol.
Niu L, ... Mann M, 2019, in preparation

**Neurodegenerative diseases**
Liu JJ, ... Mann M, 2018, Science
(Archived) patient tissue samples

Cancer
Eckert M, ..., Mann M, Lengyel E 2019, Nature
Coscia F, ..., Mann M, Curtis M 2018, Cell
Doll S, ..., Mann M, 2018, Mol Oncology
Coscia F, ..., Mann M, 2020, J Pathol

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Niu L, ... Mann M, 2019, Mol Syst Biol.

Eckert MA, Coscia F ... Mann M, Lengyel E, 2019, Nature
Salivary gland
Image Segmentation with Deep Learning Training

- Original Image
- Annotation
- Artificial Masks
- Image Style Transfer Learning
- Artificially Augmented Training Data
- Training Mask RCNN
- Cell Segmentation Model

Horvath group
Image segmentation using deep learning

Horvath and Mann groups

Salivary gland
Machine learning algorithms to predict cellular phenotypes

Software

Input microscopy image -> Cytoplasm segmentation -> Classification

Statistics

Phenotypic Single Cell Selection

Clustering

Classification

Supervised Gating

Cross-Validation

95%

87%

91%

88%
Machine learning algorithms to predict cellular phenotypes

Machine learning classifiers: Morphological features such as...
Perimeter, Area, Major/minor axis, Eccentricity, Mean intensity,
Intensity variance, Intensity mass dispt., Texture contrast, Texture variance
Texture correlation, Difference moment, Texture entropy...
Laser Microdissection | Precise single cell isolation

approx. 30,000 cells/day
Proteins specifically expressed in serous salivary glands

https://www.proteinatlas.org/humanproteome/tissue/salivary-gland

Most abundant protein: AMY1 (17% of total protein mass)

CA6

CST2

PIP

AMY1B

Least abundant protein: PIP
Cell type specific proteomes

Ranked proteins

Relative protein level (melanocytes / bulk)

Melanoma specific proteins

Ranked proteins
Subcellular proteomics of individual cells

**Top enriched in whole cells**
- membrane
- transport
- establishment of localization
- translational initiation
- translational elongation
- protein transport
- vesicle-mediated transport
- cellular ketone metabolic process
- translation
- organic acid metabolic process
- protein targeting
- small molecule metabolic process
- endoplasmic reticulum
- generation of precursor metabolites and energy
- vesicle
- cellular component disassembly
- heterocycle metabolic process
- plasma membrane
- nucleotide metabolic process
- ribosome

**Unsupervised proteome clustering**

**Top enriched in nuclei**
- nucleobase-containing compound metabolic process
- RNA metabolic process
- nucleoplasm
- cellular nitrogen compound metabolic process
- nitrogen compound metabolic process
- RNA processing
- macromolecule metabolic process
- RNA splicing
- mRNA processing
- chromosome organization
- spliceosomal complex
- nucleolus
- DNA metabolic process
- primary metabolic process
- chromatin organization
- response to DNA damage stimulus
- DNA repair
- chromatin modification
- metabolic process
- cellular metabolic process
- mRNA metabolic process
- nucleus
- chromatin remodeling complex
Fully automated single-nuclei isolation
Single cell isolation with subcellular spatial resolution
Phenotypic differences of 5 nuclei classes (DAPI dense regions) used for unsupervised clustering
5 nuclei classes (DAPI dense regions) show distinct proteomics profiles
5 nuclei classes (DAPI dense regions) show distinct proteomics profiles

- Processing of DNA double-strand break ends (REACTOME)
- Unwinding of DNA (REACTOME)
- MCM complex (GOCC)
- RNA splicing (GOBP)
- Nucleolus (GOCC)
- Chromosome segregation (GOBP)
- Translation (GOBP)
- Cytosol (GOCC)
- Cytoskeleton (GOCC)
- Ribosome (GOCC)
- Actin filament-based process (GOBP)
Integrating image data with protein abundance for (sub)cellular phenotyping

Protein atlas

[Image of Protein atlas with KIF23]

Deep visual proteomics

[Image of Deep visual proteomics with KIF23]

https://www.proteinatlas.org/

Systems biology at the imaging and at the proteomic levels

Protein atlas

KIF23

https://www.proteinatlas.org/

Deep visual proteomics

Protein or pathway of choice

MS signal

low

high
Next directions...
It is going to be a lot of fun...
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Scarlet Koch
Heiner Koch
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Ole Bjeld Horning
Peter Sondergaard
Dorte Bekker-Jensen