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

@labs_mann

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MAX-PLANCK-GESELLSCHAFT

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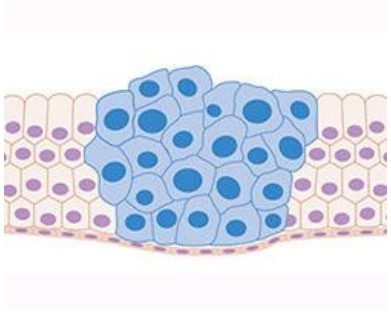
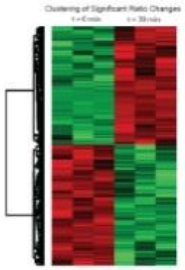
The Novo Nordisk Foundation
Center for Protein Research

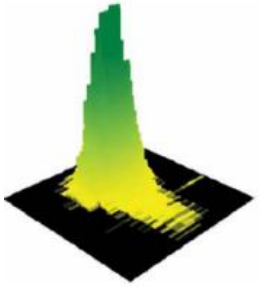
What proteomics can analyze

from Hein … Mann, Handbook of Systems Biology, 2012

Minimalistic proteomics



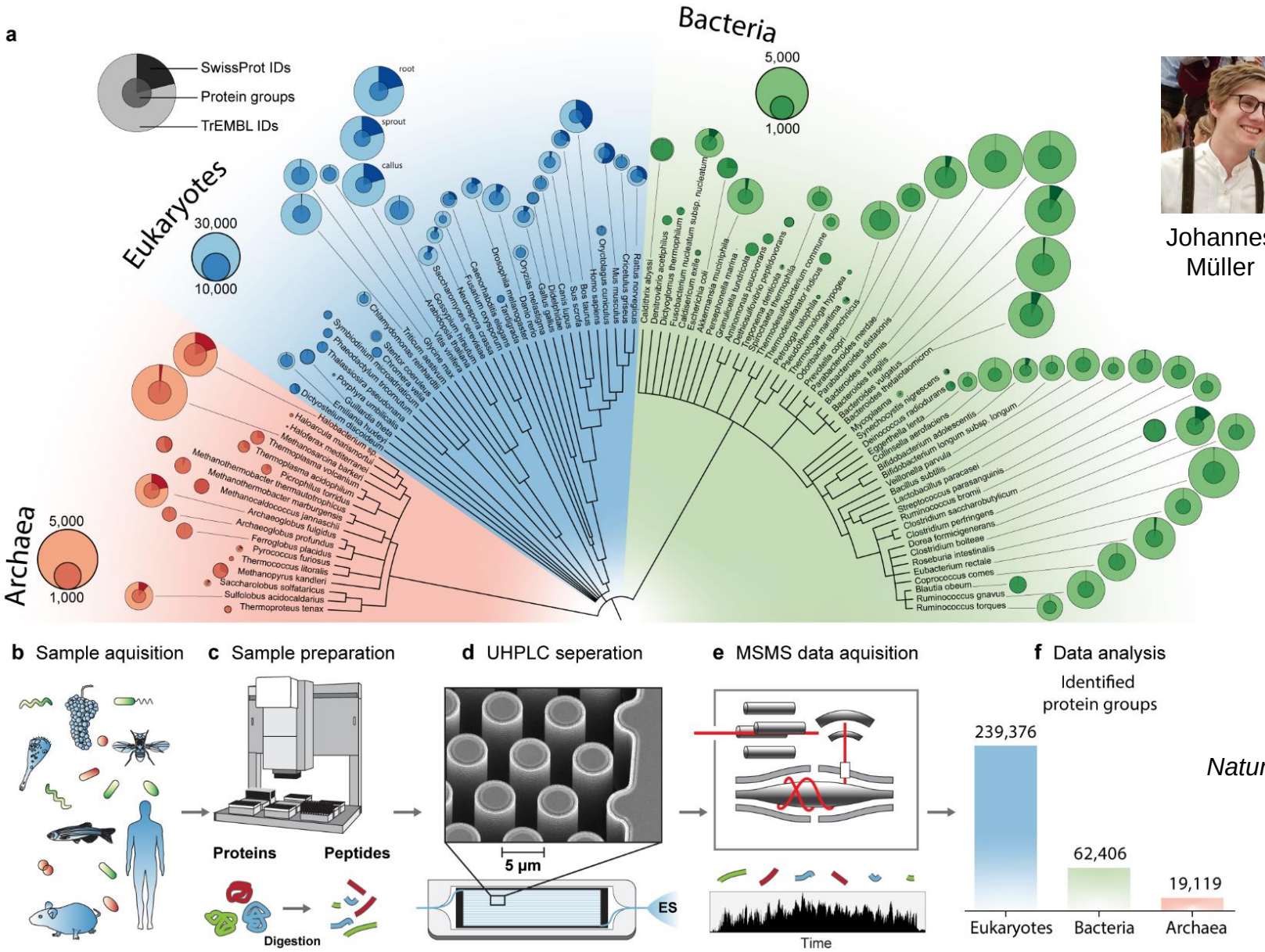




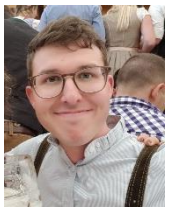
Cellular biology

Clinical applications

The proteome landscape of the kingdoms of life



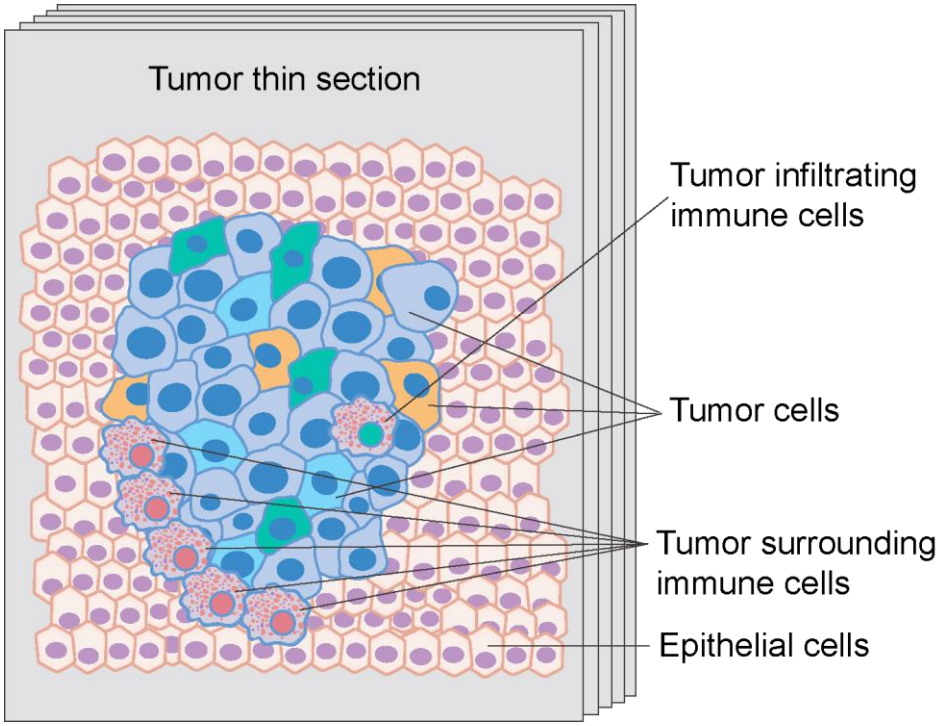
Johannes Müller

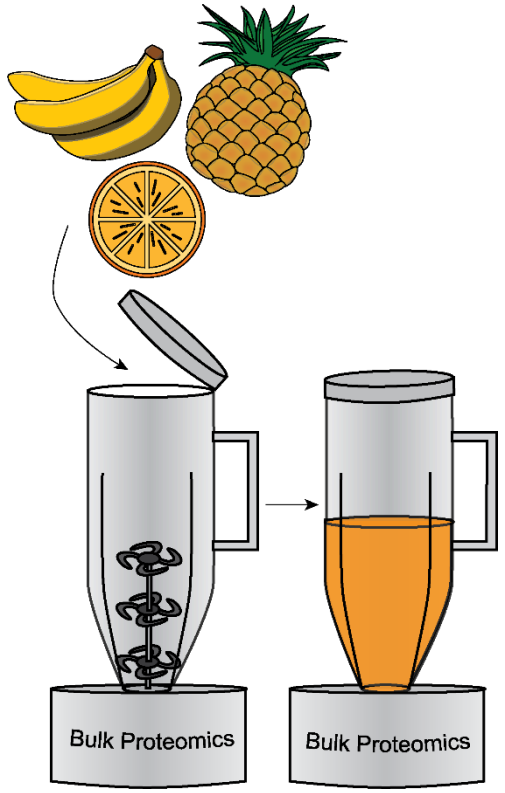


Philipp Geyer

Nature June 2020

Advantages of single cell (type) proteomics





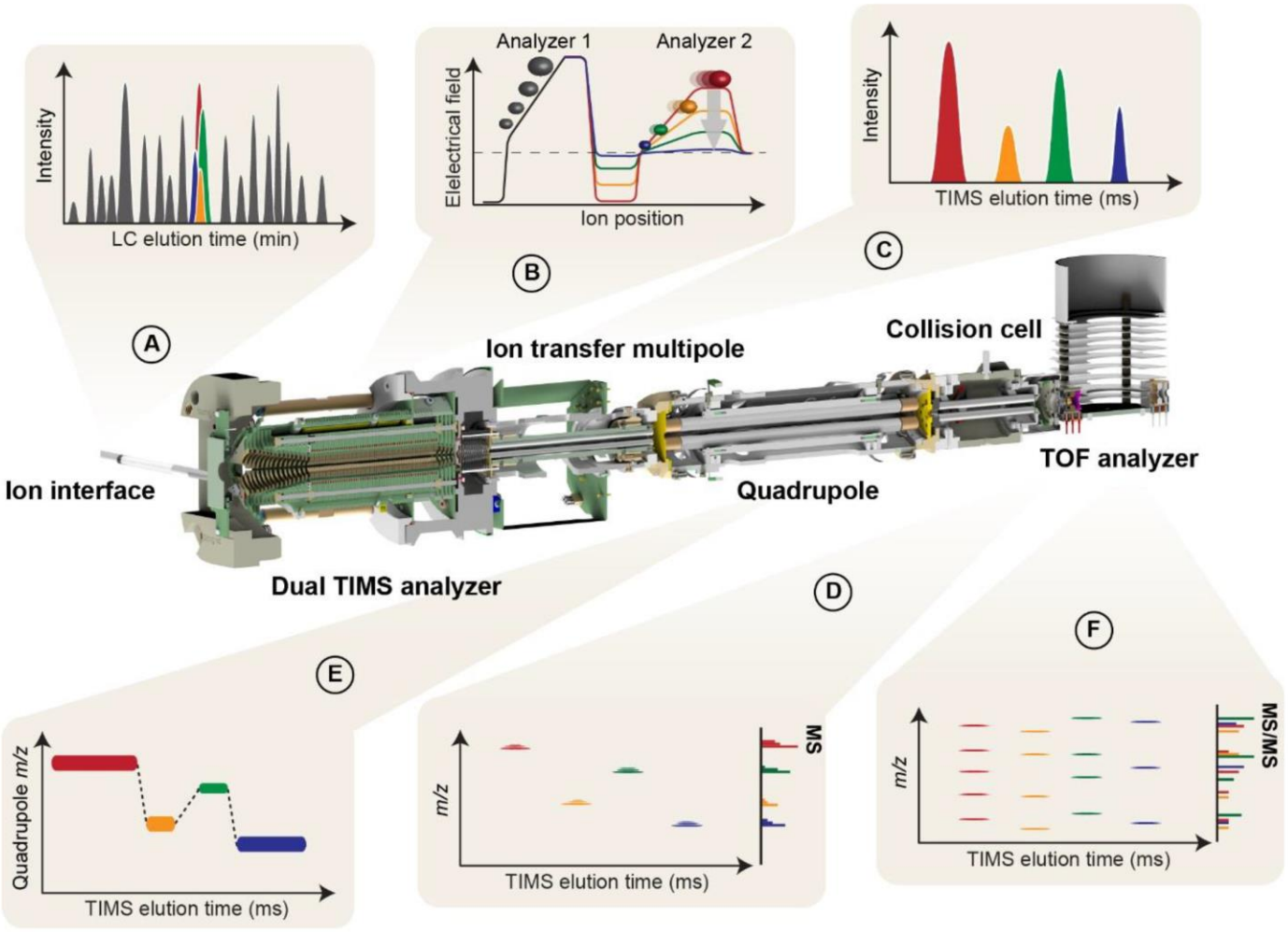
Trapped ion mobility spectrometry (TIMS)

Florian Meier

Andreas Brunner

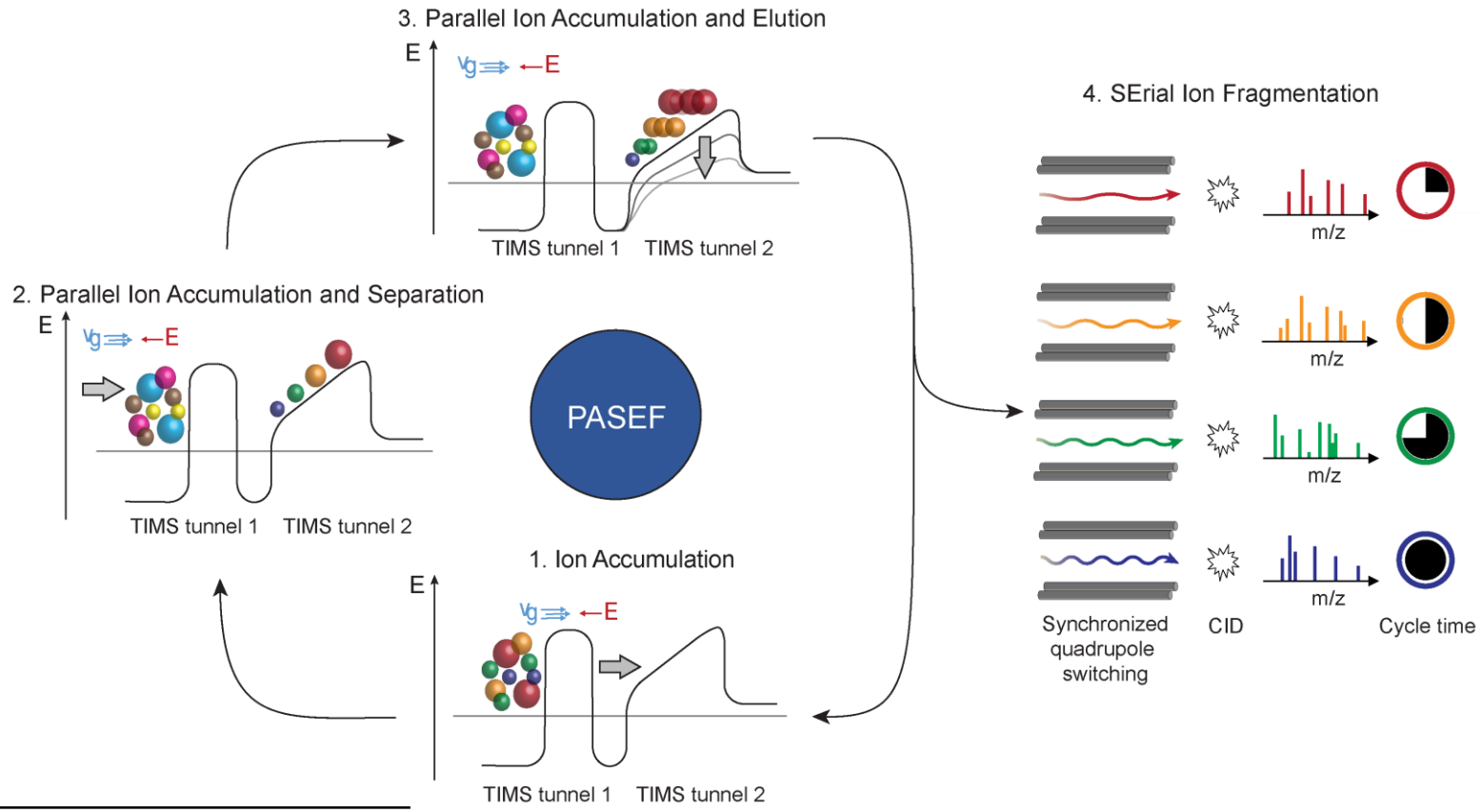
Catherine Vasilopoulou

Johannes Müller



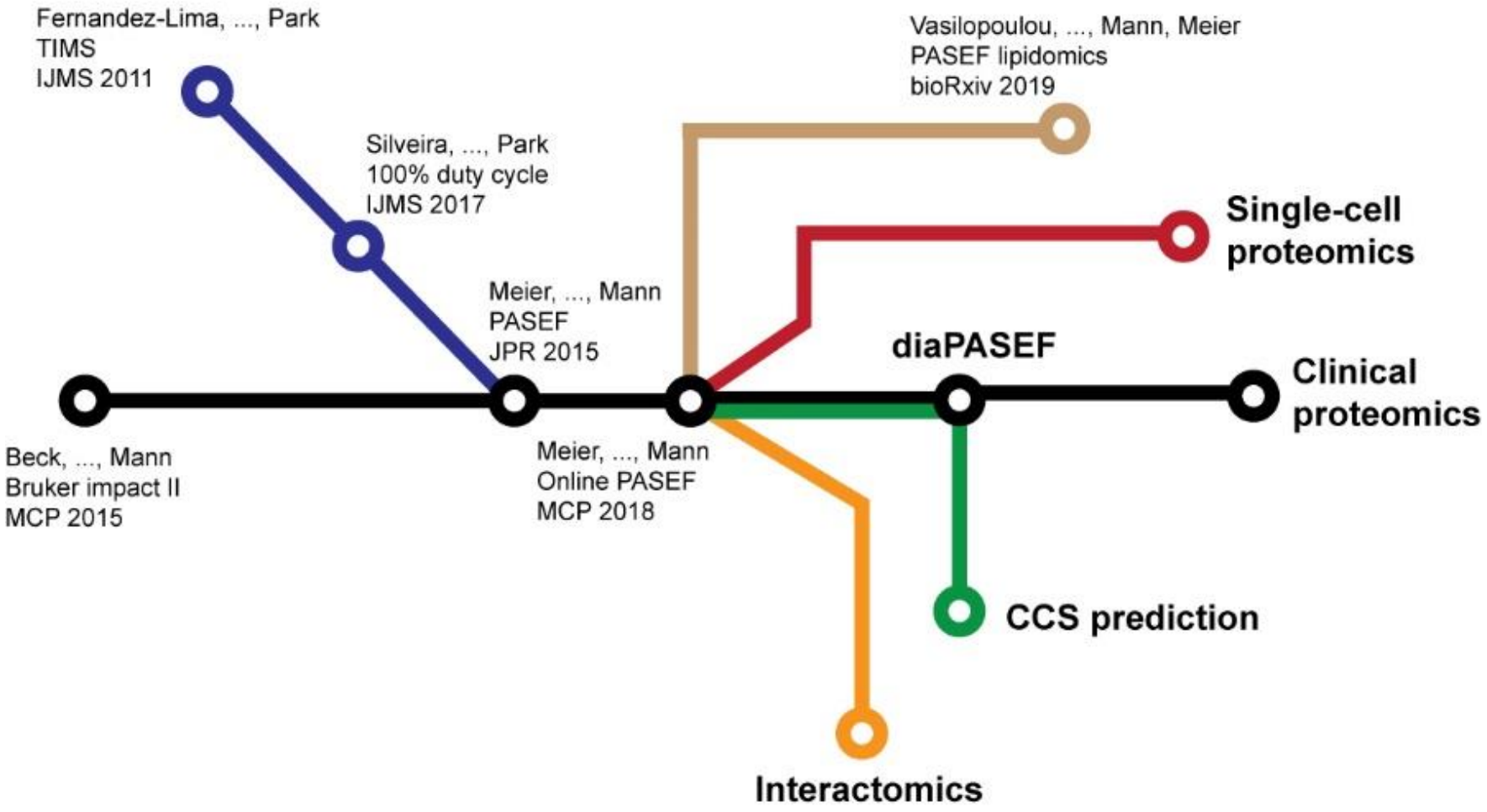
Meier, …, Mann, *J. Prot. Res.* 2015
Meier, …, Mann, *Mol. Cell. Prot.* 2018
Vasilopoulou, …, Mann, Meier, *Nat. Comm.* 2020

Parallel Accumulation followed by SErial Fragmentation (PASEF)

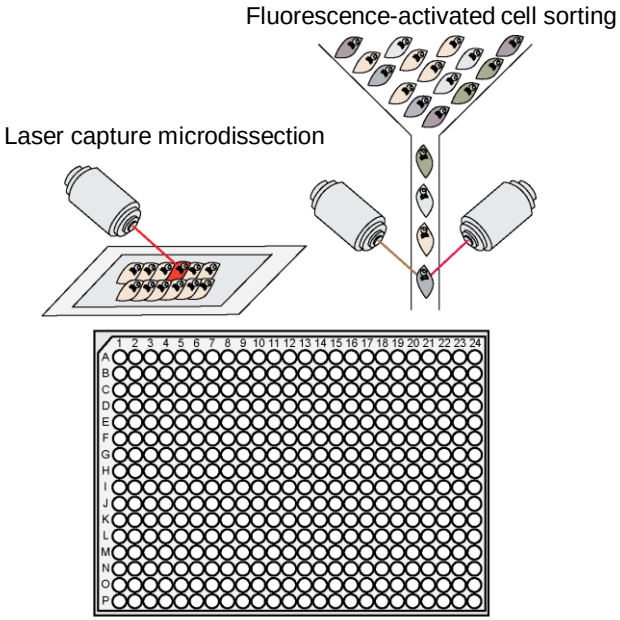


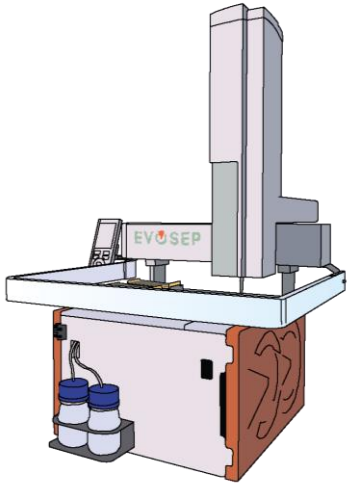
PASEF: Multiplying Sequencing Speed and Sensitivity; Meier, …, Mann; JPR; 2015
Online PASEF with a Novel TIMS; Meier, …, Mann; MCP; 2018

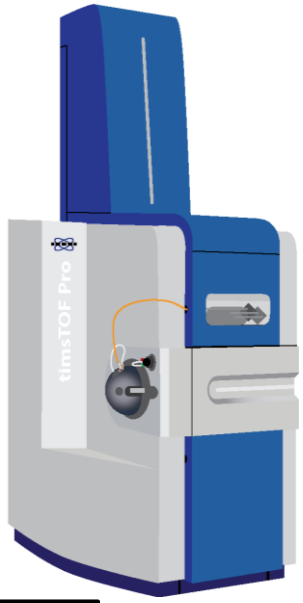
Parallel Accumulation – Serial Fragmentation (PASEF)



Mass spectrometry-based proteomics to enable single-cell analysis







Andreas-David Brunner Marvin Thielert

A modified Trapped Ion Mobility Spectrometer coupled to a Time-of-Flight analyzer

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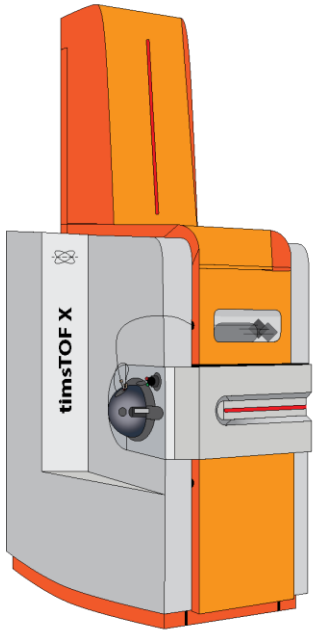
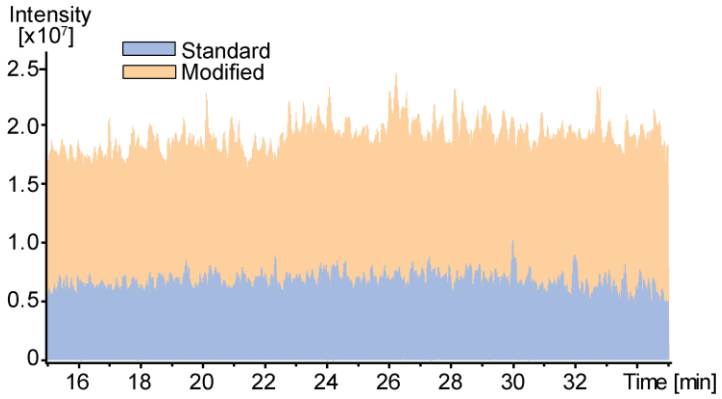
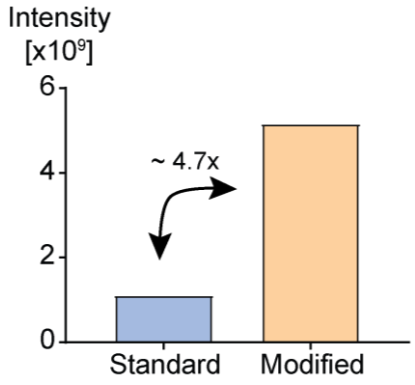
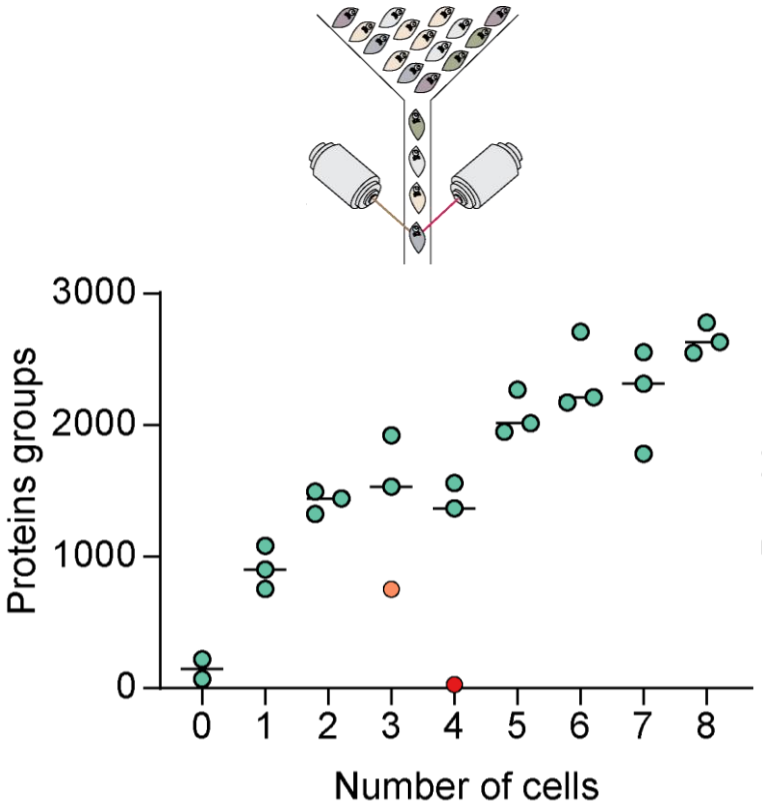


Raw intensity increase



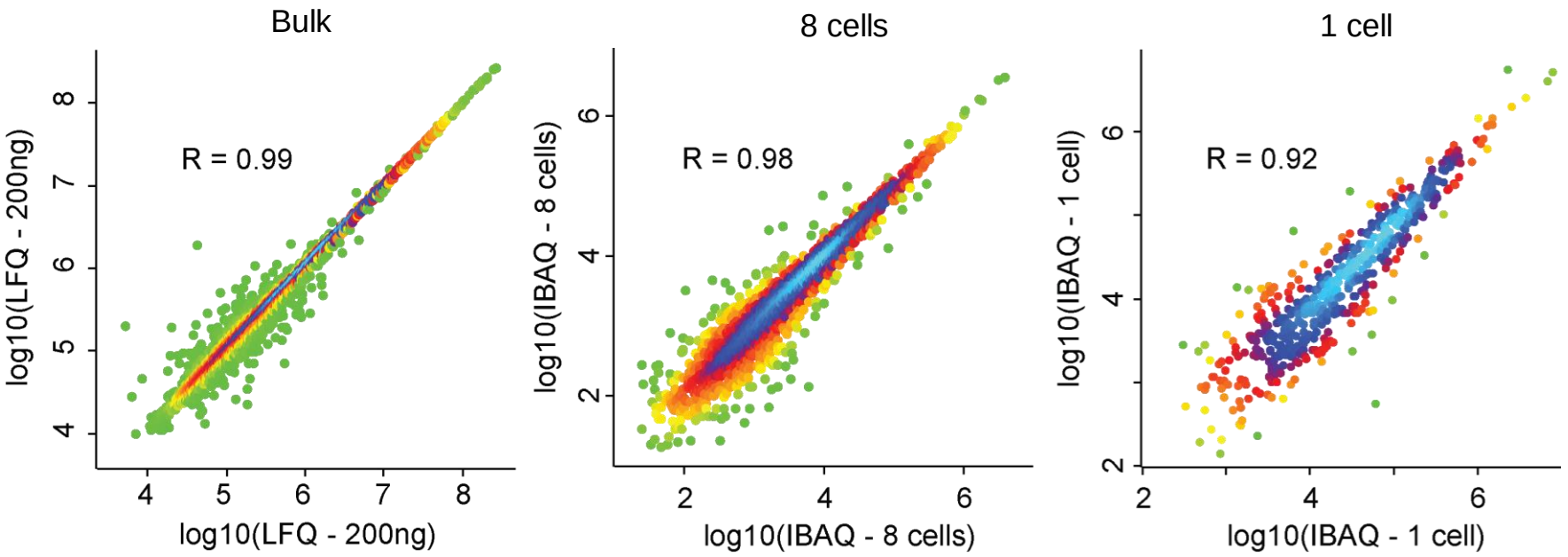


Single cell proteomics on FACS sorted cells

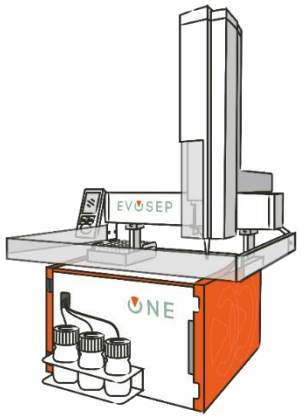


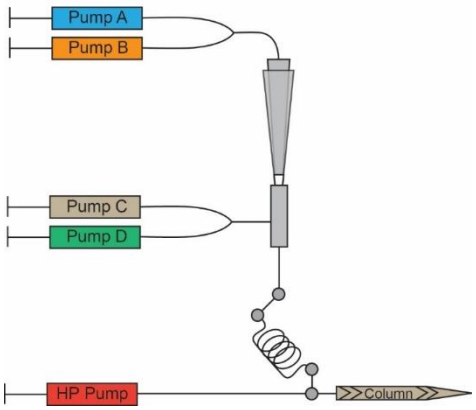
1% PSM and 1% Protein level FDR in MaxQuant

Quantitative reproducibility on protein level

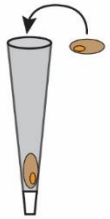


Evosep nanoflow



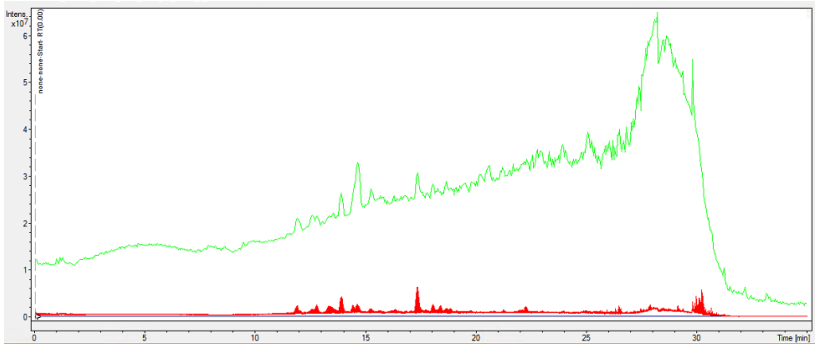


In EvoTip single cell processing



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

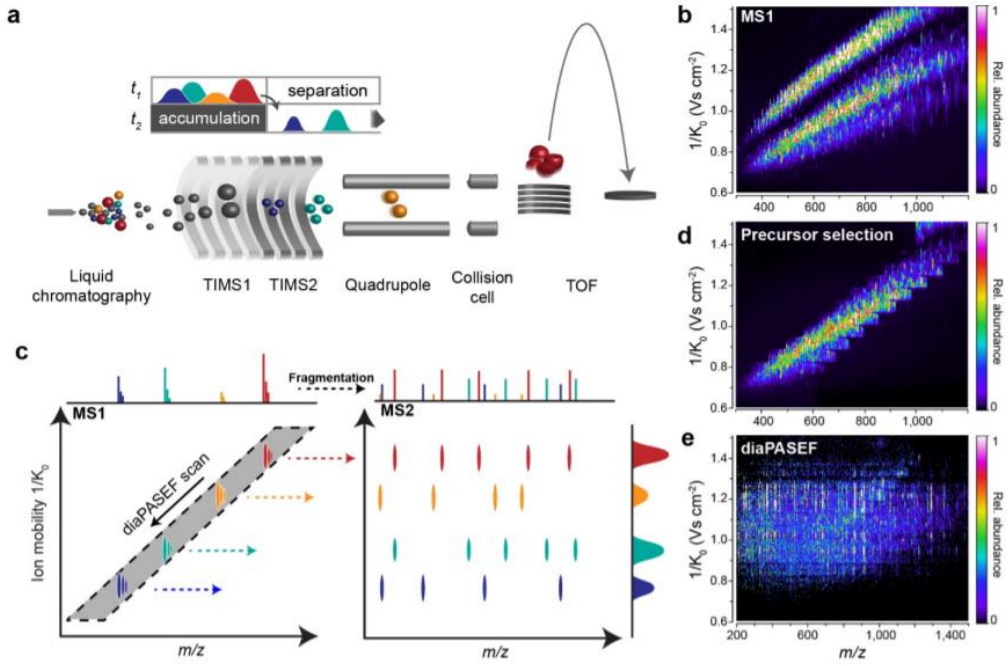
1 cell

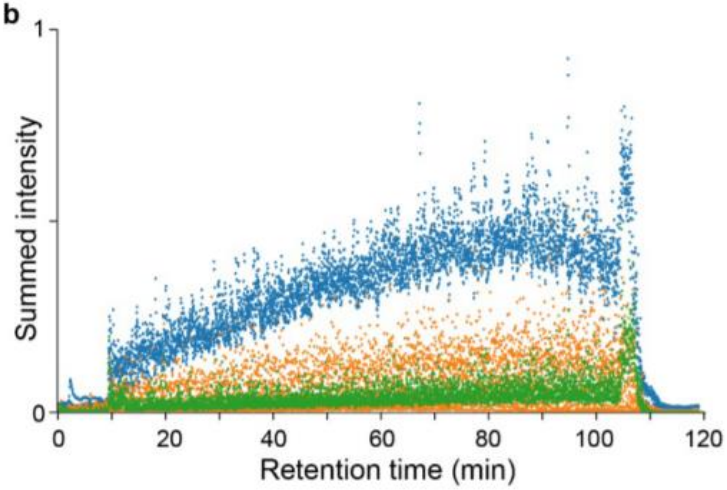


A novel LC system embeds analytes in pre-formed gradients for rapic, ultra-robust proteomics
Bache, ..., Mann, 2018, MCP

Disclaimer: MM is an indirect investor in Evosep

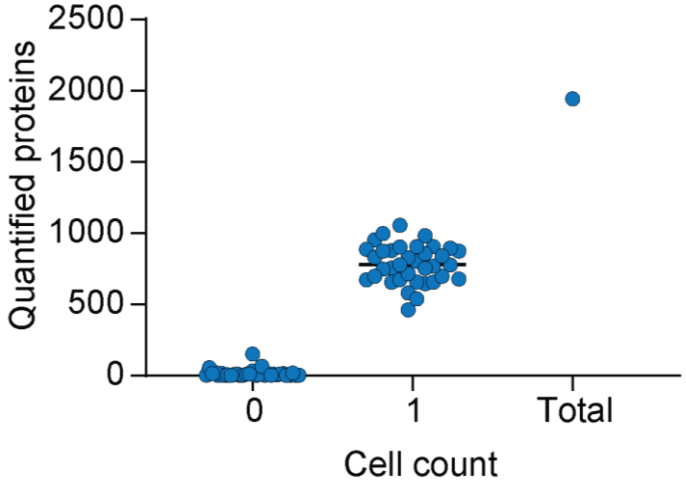
diaPASEF for increased ion sampling

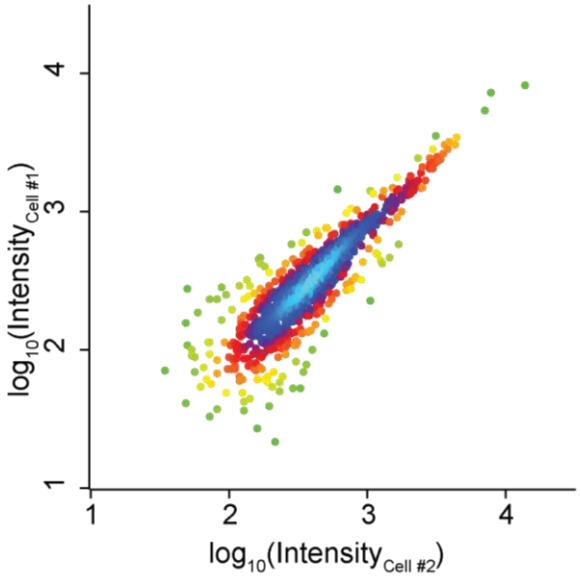




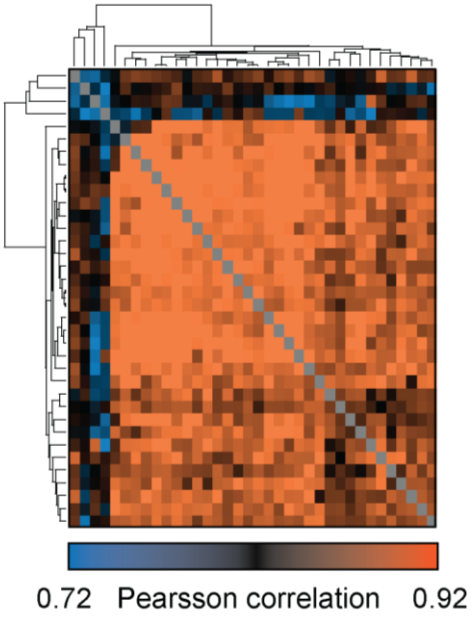
diaPASEF: Bottom-up proteomics with near optimal ion usage
Florian Meier, Andreas Brunner, ..., Ruedi Abersold, Ben C. Collins, Hannes L. Röst, Matthias Mann, 2019, bioRxiv, accepted in Nature Methods

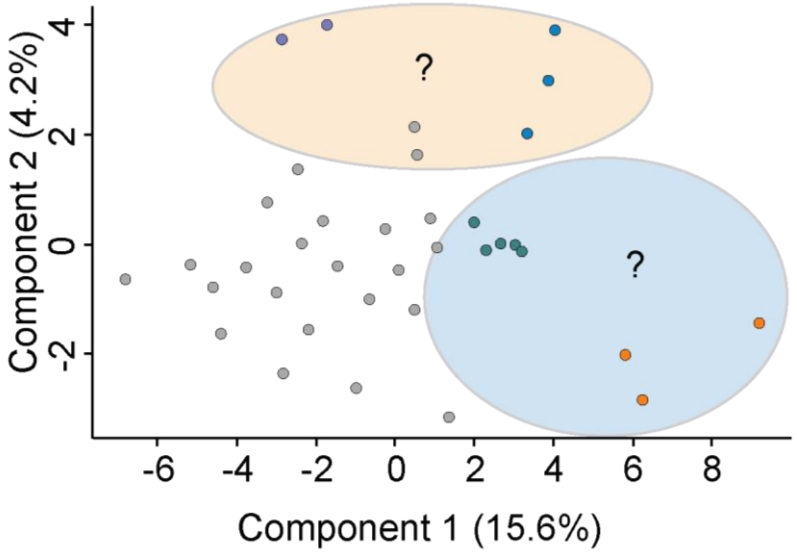
36 single HeLa cell proteomes



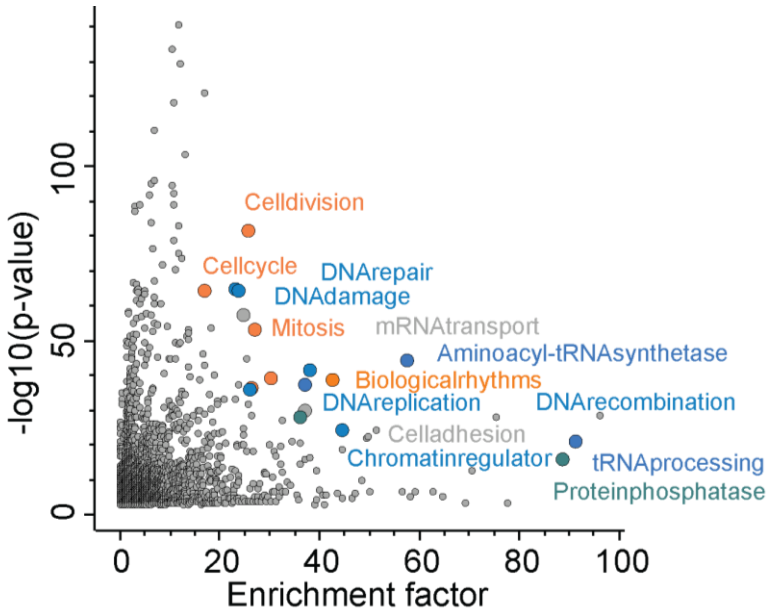


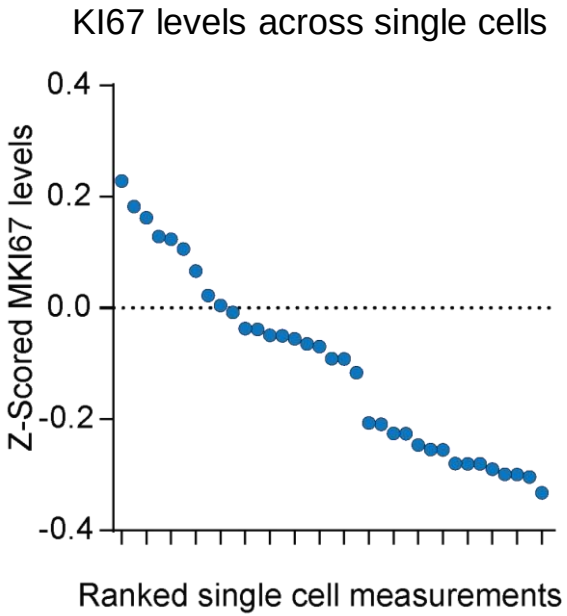
Unsupervised clustering and principal component analyis of 36 single HeLa cell proteomes





Biological process enrichment in the HeLa single cell proteome dataset

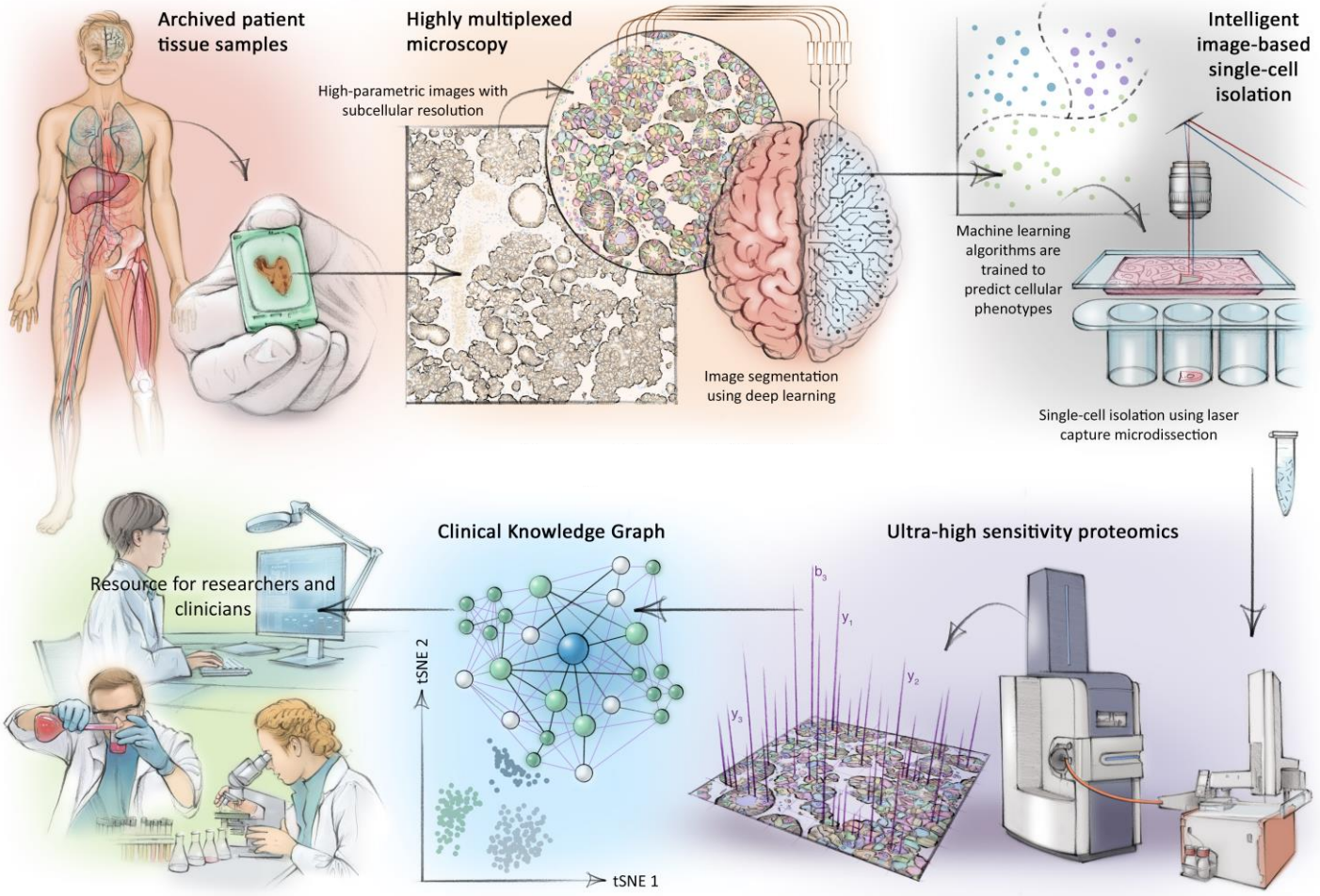




Deep Visual Proteomics

Andreas Mund

Fabian Coscia

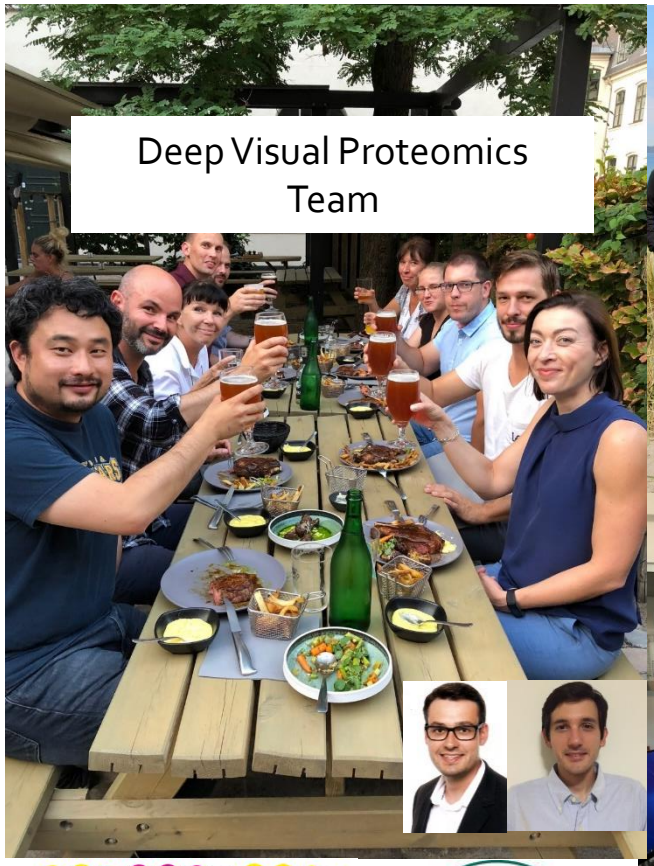


@labs_mann

Mann Lab(s):
Andreas Mund
Fabian Coscia
Andreas-David Brunner
Florian Meier

Biological Research Centre, Szeged
Peter Horvath La
Ferenc Kovacs
Andras Kriston
Réka Hollandi

Leica
Florian Hoffmann
Christoph Greb
Falk Schlaudraff



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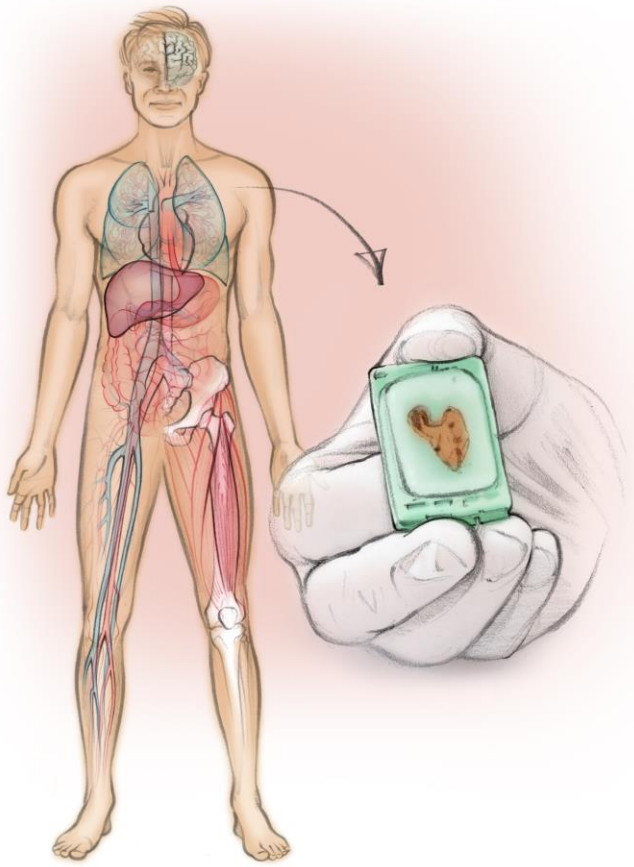
(Archived) patient tissue samples



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

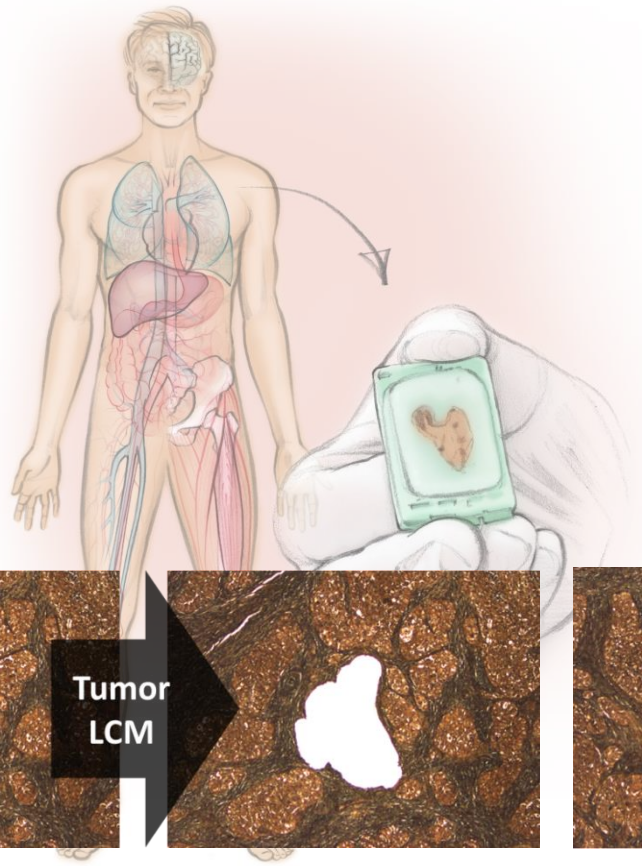
Niu L, … Mann M, 2019, Mol Syst Biol.

Niu L, … Mann M, 2019, in preparation

Neurodegenerative diseases

Liu JJ, … Mann M, 2018, Science

(Archived) patient tissue samples



Cancer

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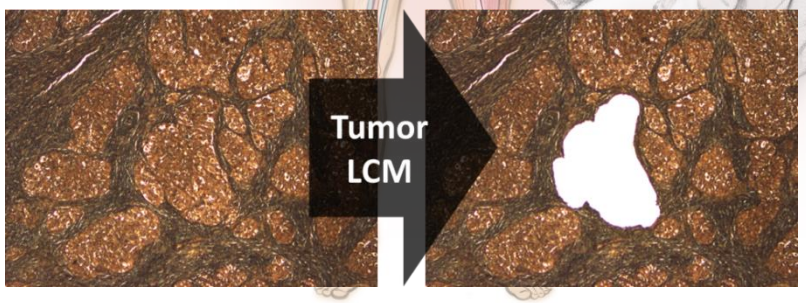
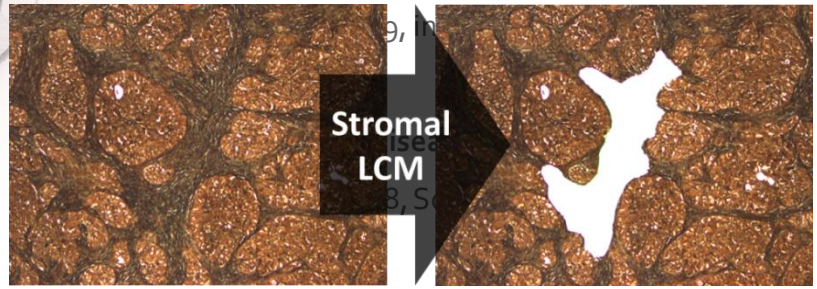




Eckert MA, Coscia F … Mann M, Lengyel E, 2019, Nature

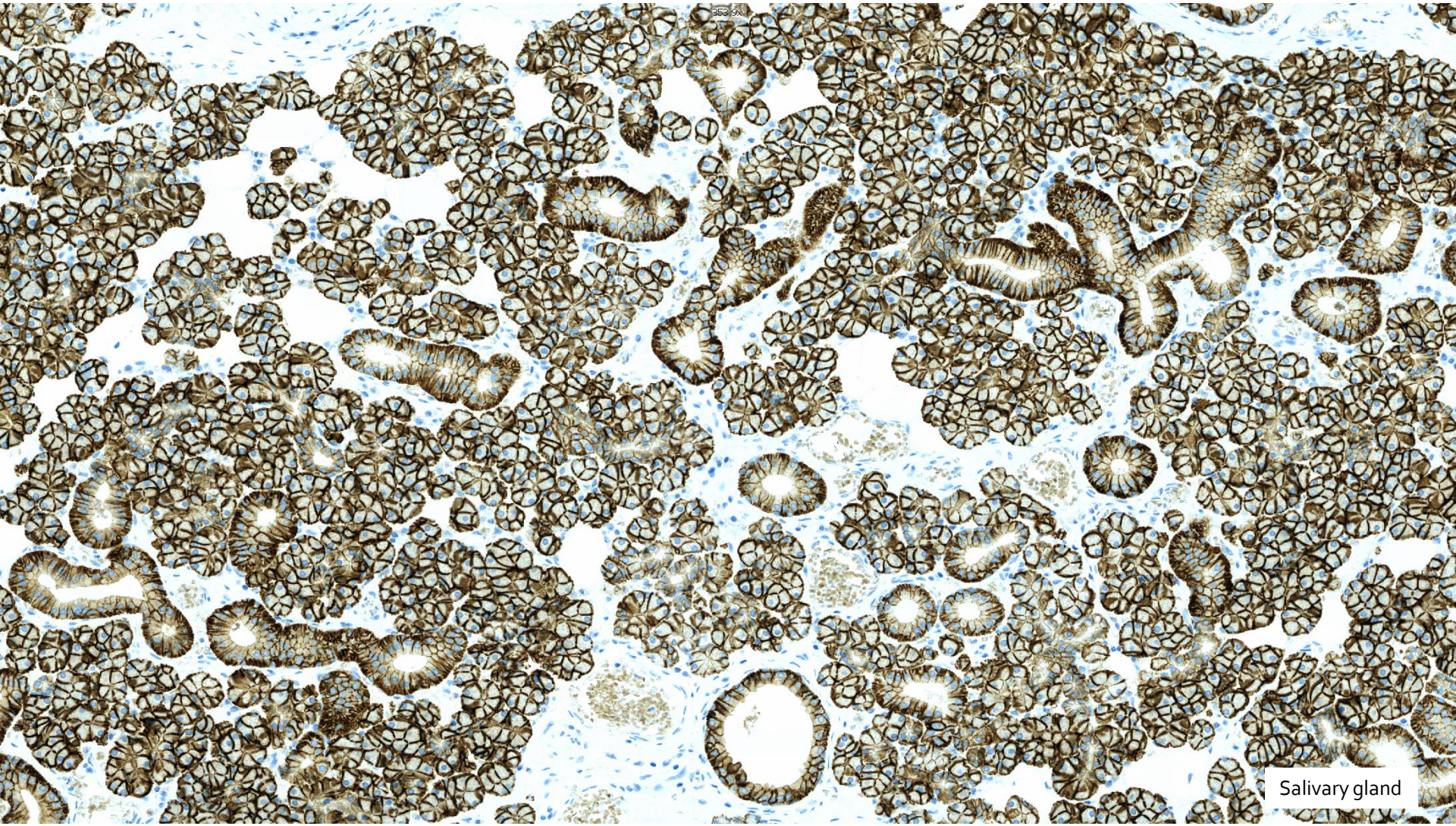
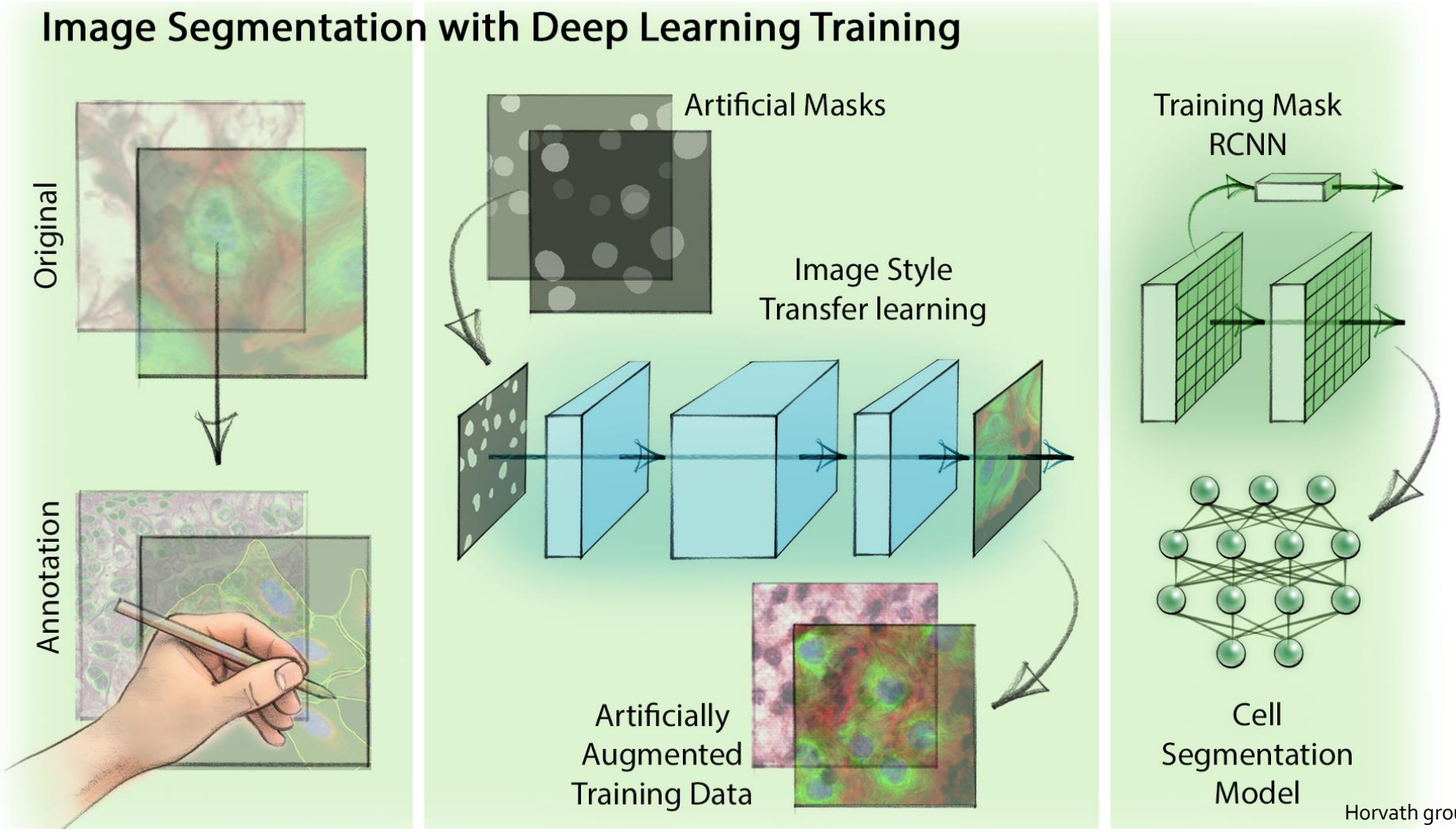

Salivary gland

Image Segmentation with Deep Learning Training



Horvath group

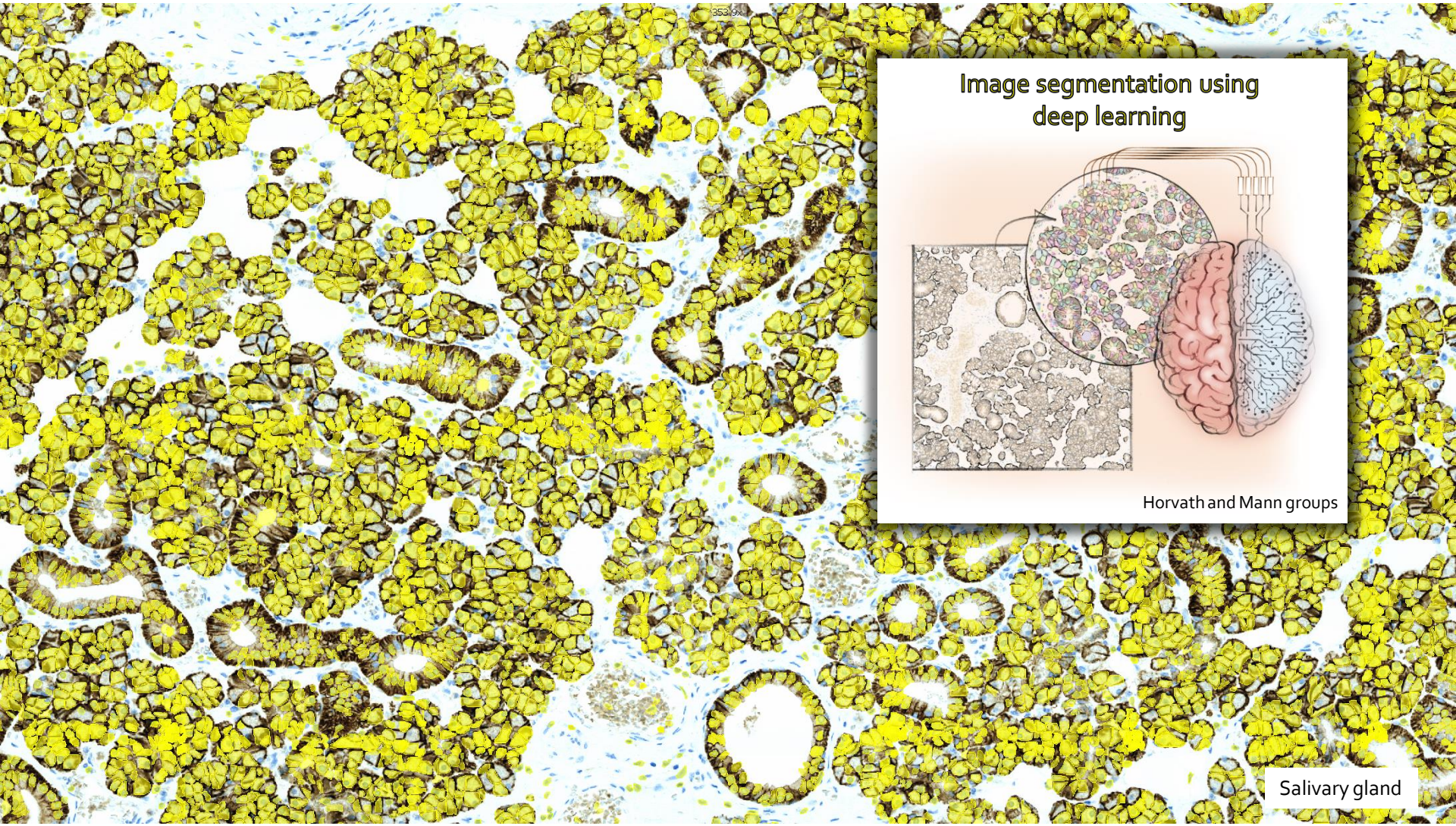
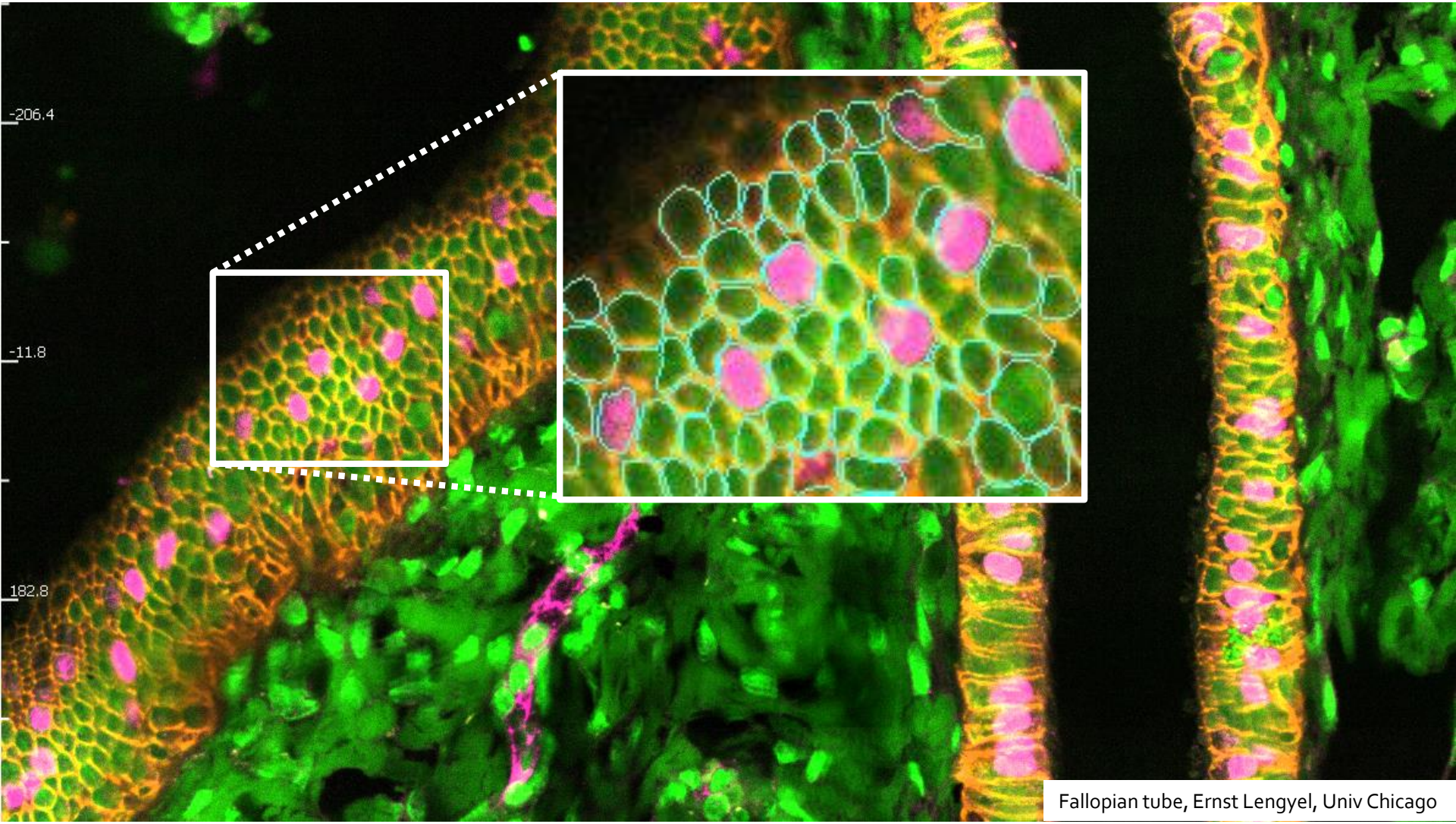
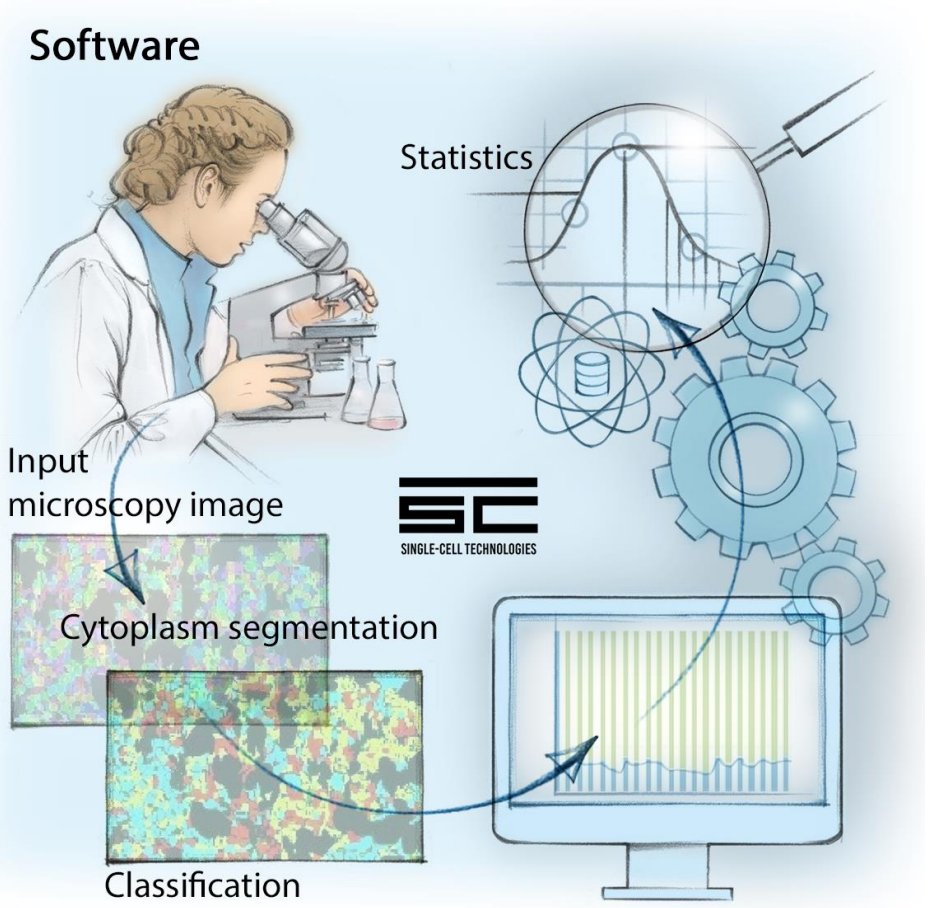


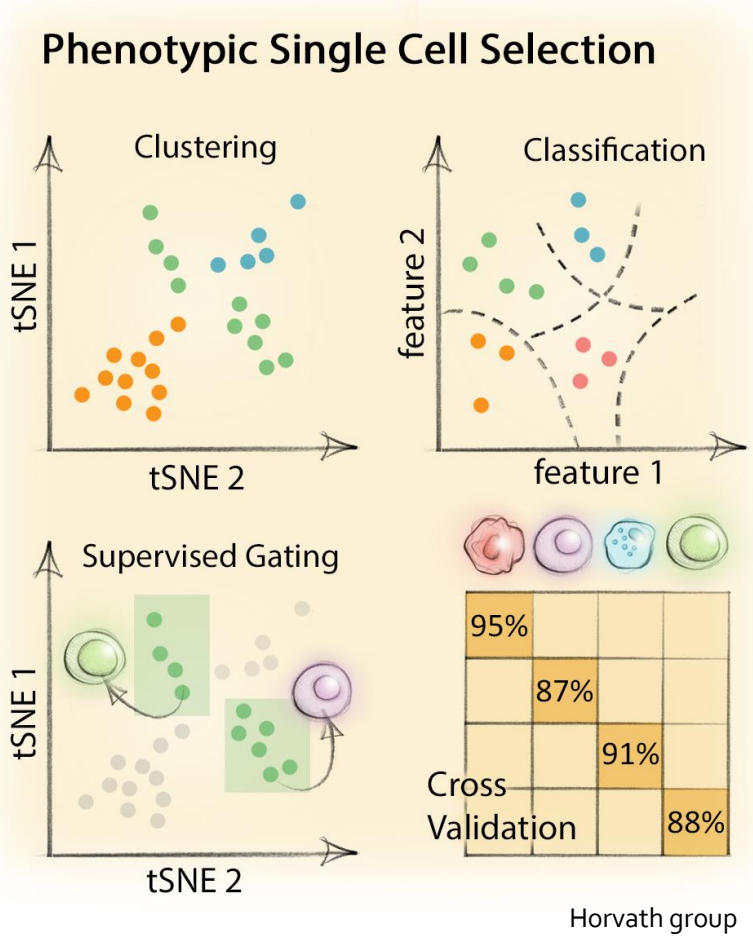
Image segmentation using
deep learning
Horvath and Mann groups
Salivary gland



Fallopian tube, Ernst Lengyel, Univ Chicago

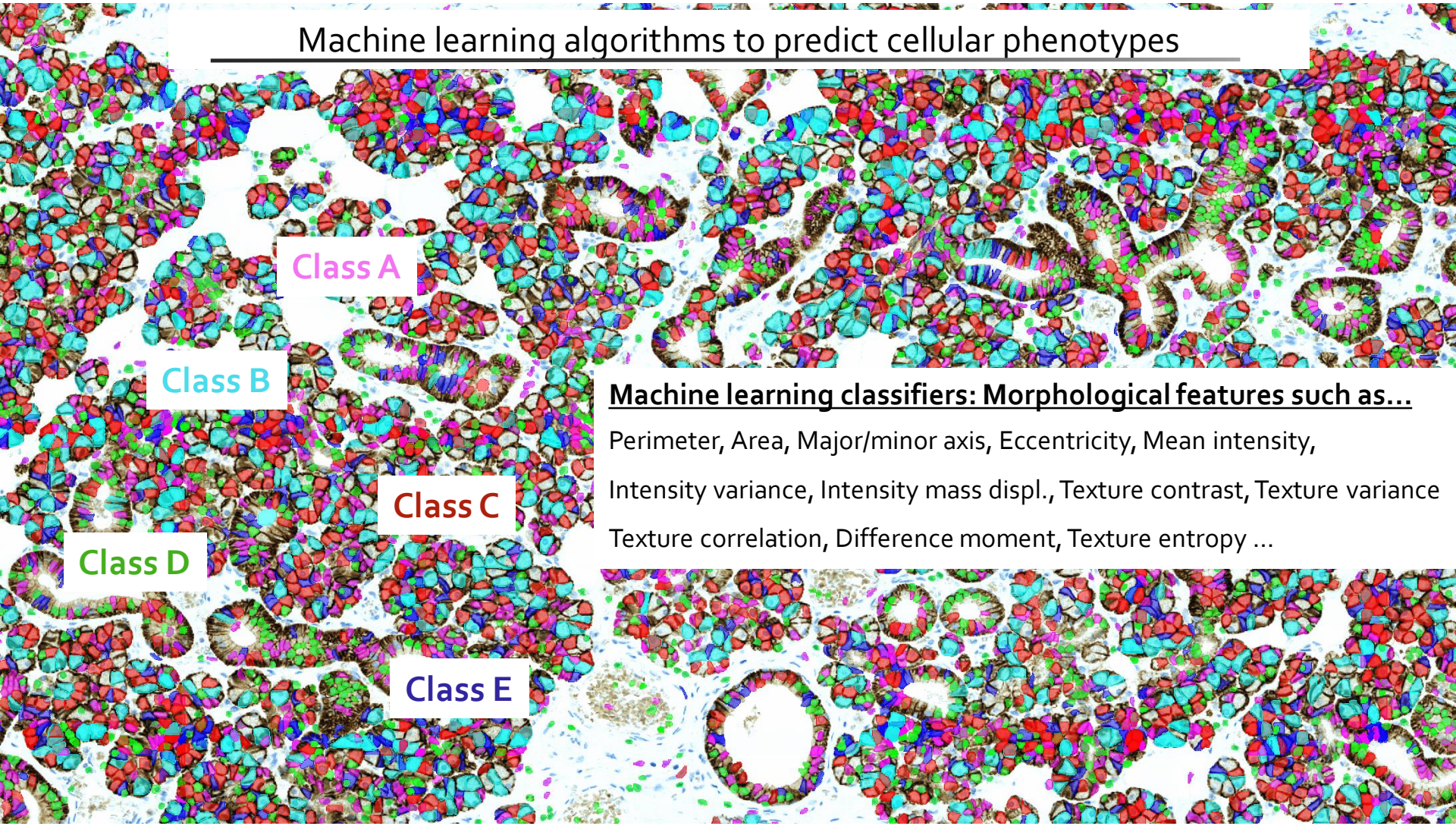
Machine learning algorithms to predict cellular phenotypes





Horvath group

Machine learning algorithms to predict cellular phenotypes



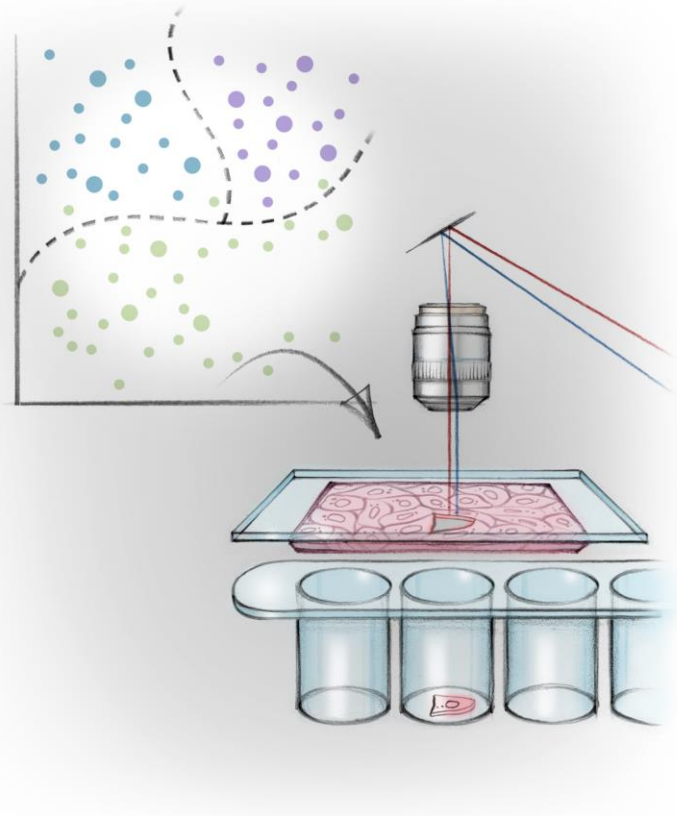
Machine learning classifiers: Morphological features such as…

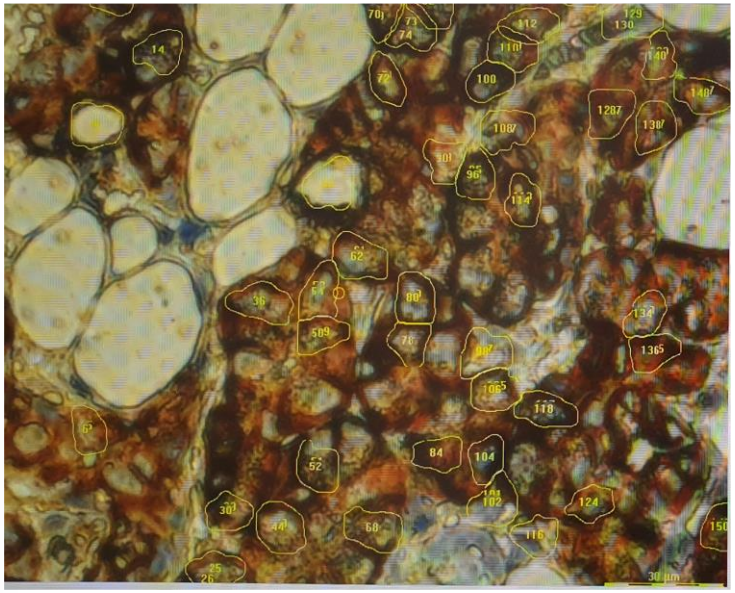
Perimeter, Area, Major/minor axis, Eccentricity, Mean intensity,

Intensity variance, Intensity mass displ., Texture contrast, Texture variance

Texture correlation, Difference moment, Texture entropy …

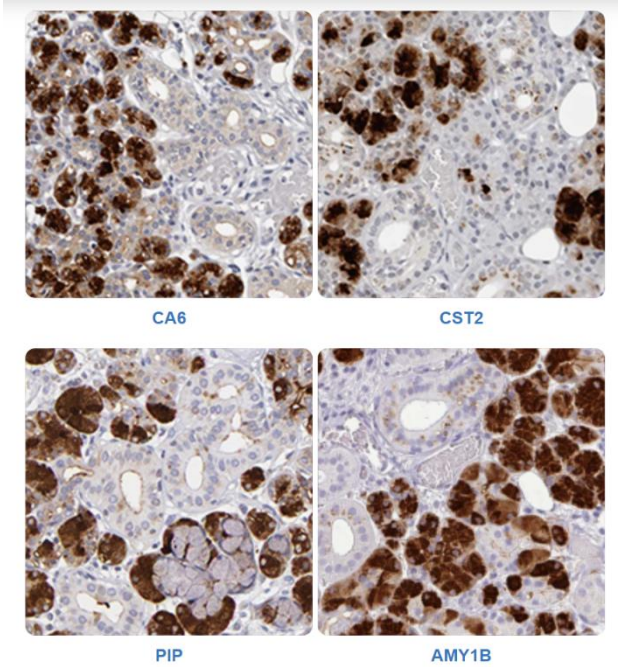
Laser Microdissection | Precise single cell isolation



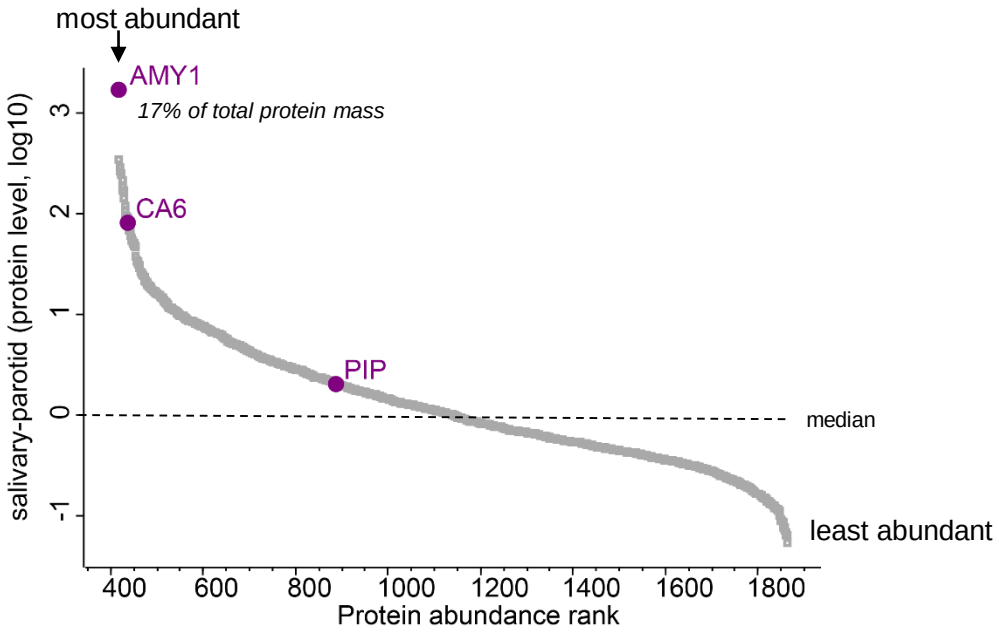


approx.30.000 cells/day

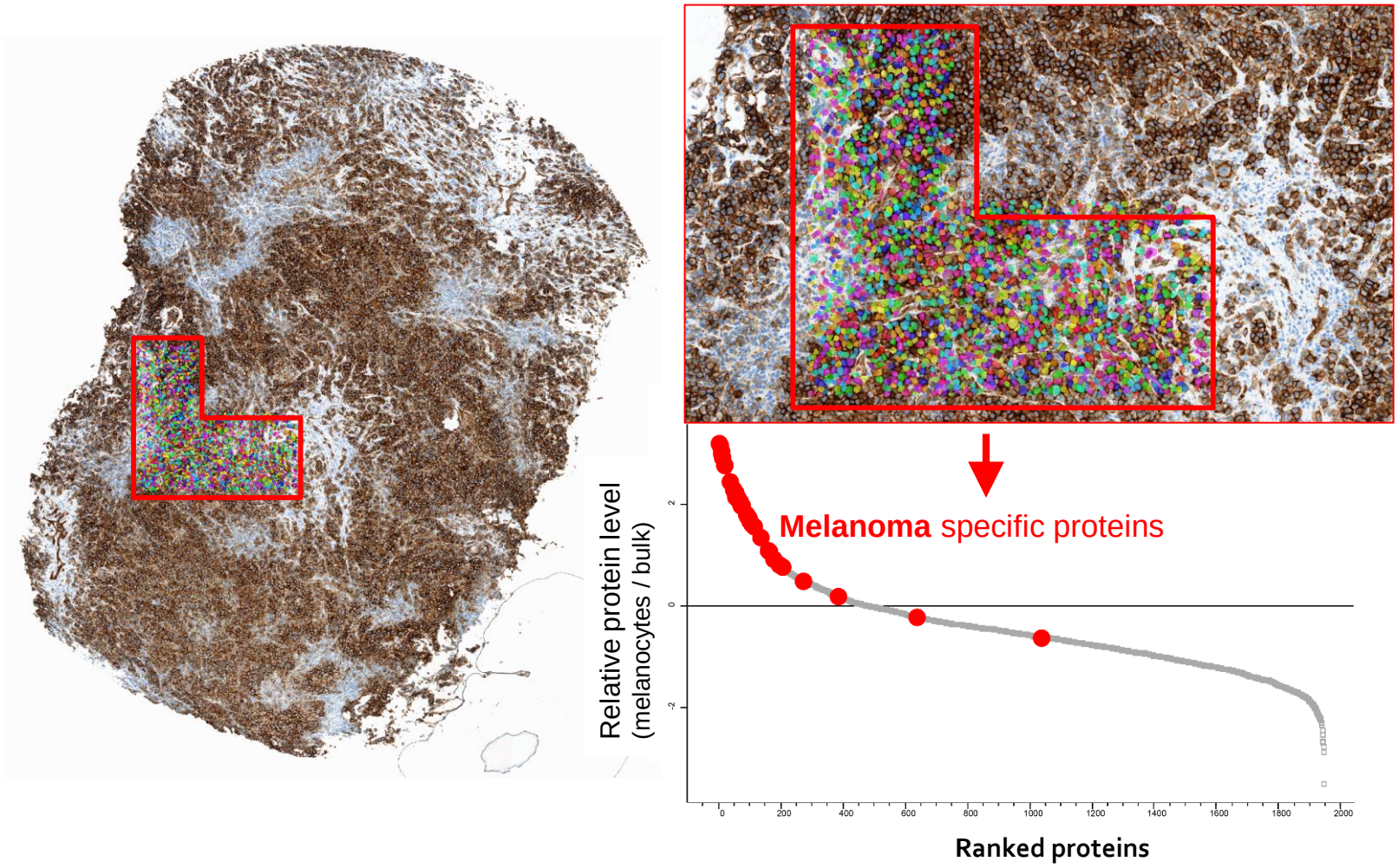
Proteins specifically expressed in serous salivary glands



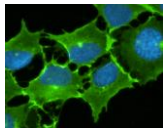
https://www.proteinatlas.org/humanproteome/tissue/salivary+gland



Cell type specific proteomes



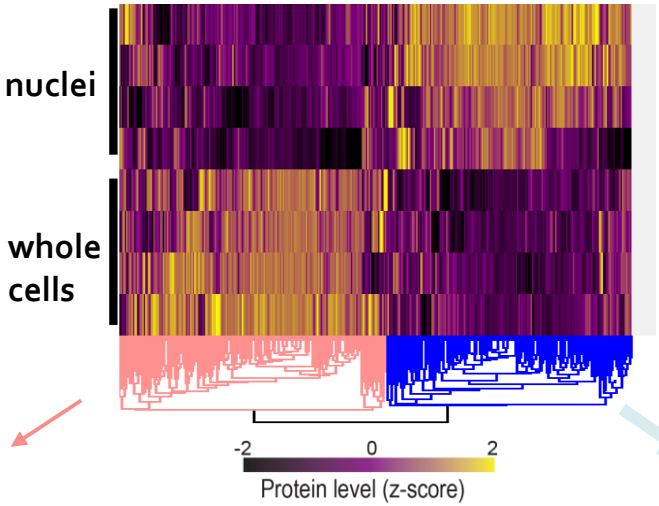
Subcellular proteomics of individual cells

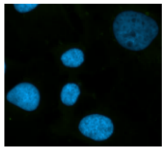


Top enriched in whole cells

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

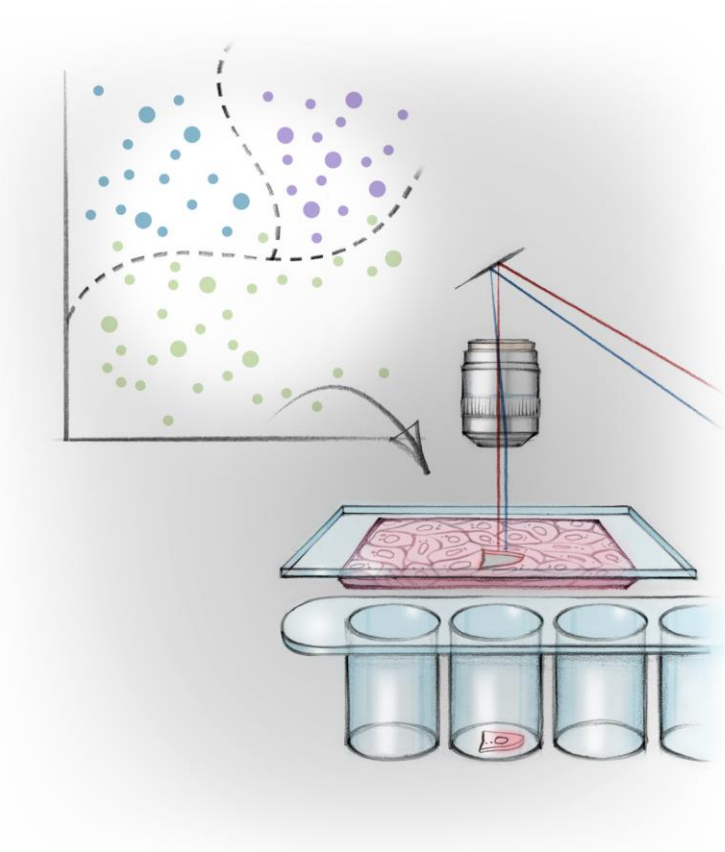


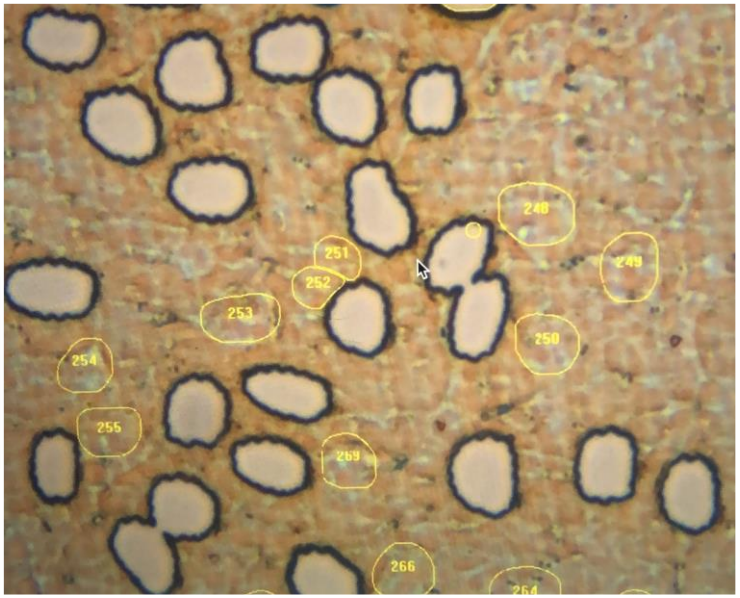


Top enriched in nuclei

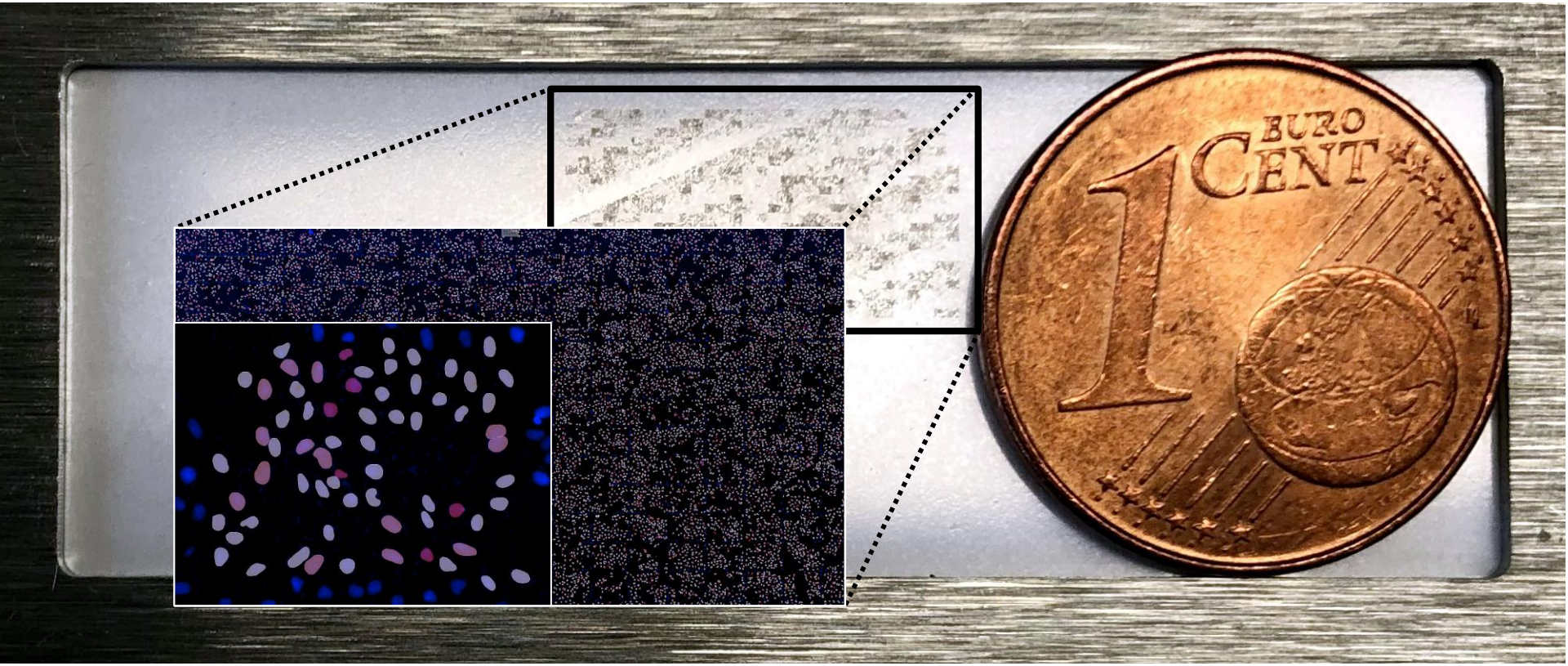
nucleobase-containing compound metabolic process
RNA metabolic process
nucleoplasm
cellular nitrogen compound metabolic process
nitrogen compound metabolic process
RNA processing
macromolecule metabolic process
RNA splicing
mRNA processing
chromosome organization
spliceosomal complex
nucleolus
DNA metabolic 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

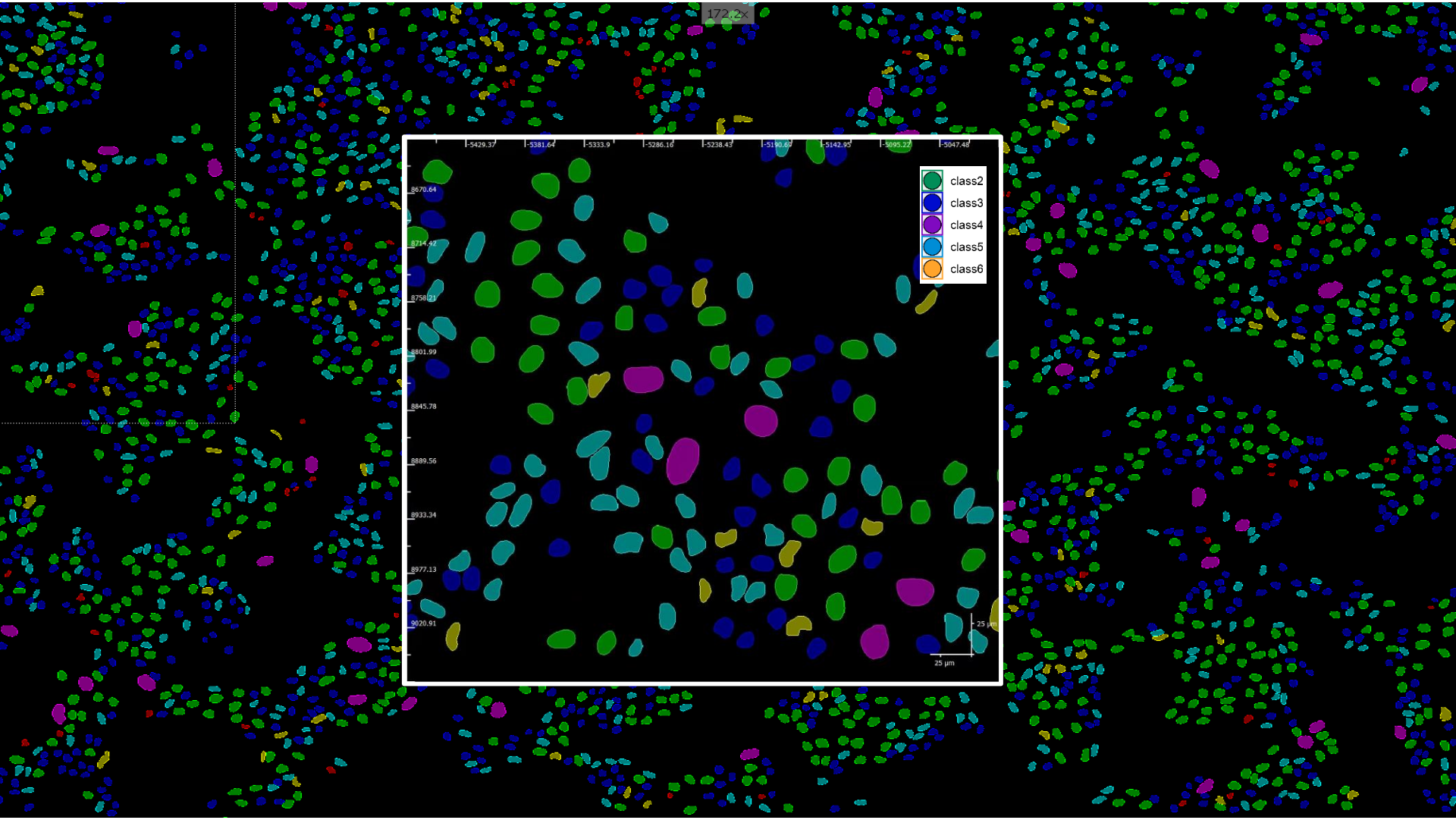
Fully automated single-nuclei isolation



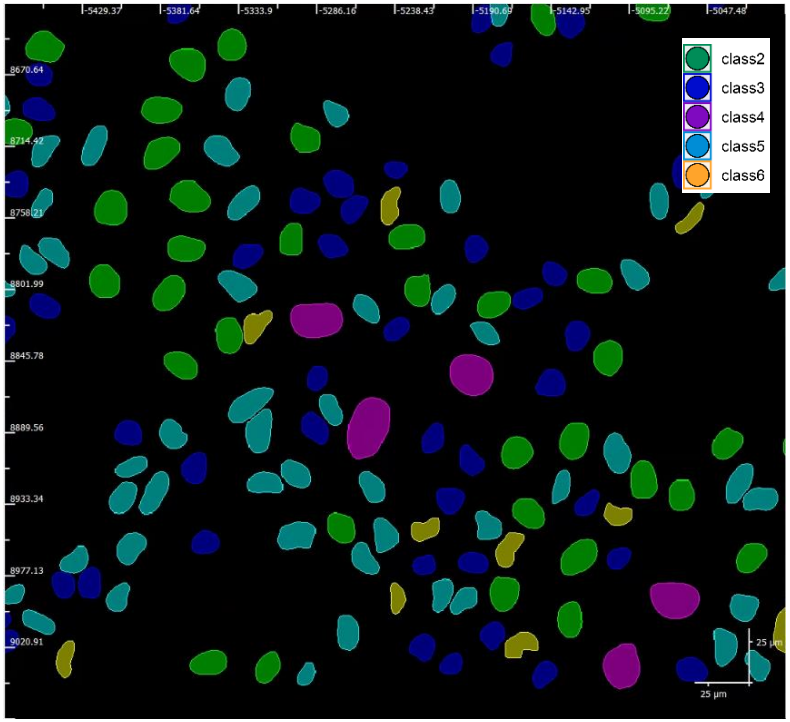


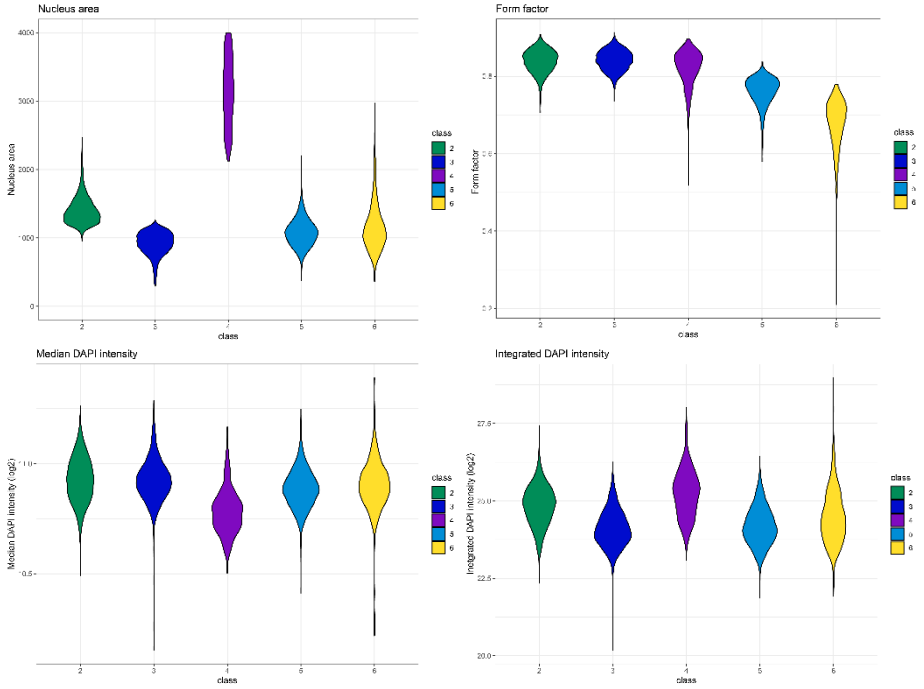
Single cell isolation with subcellular spatial resolution



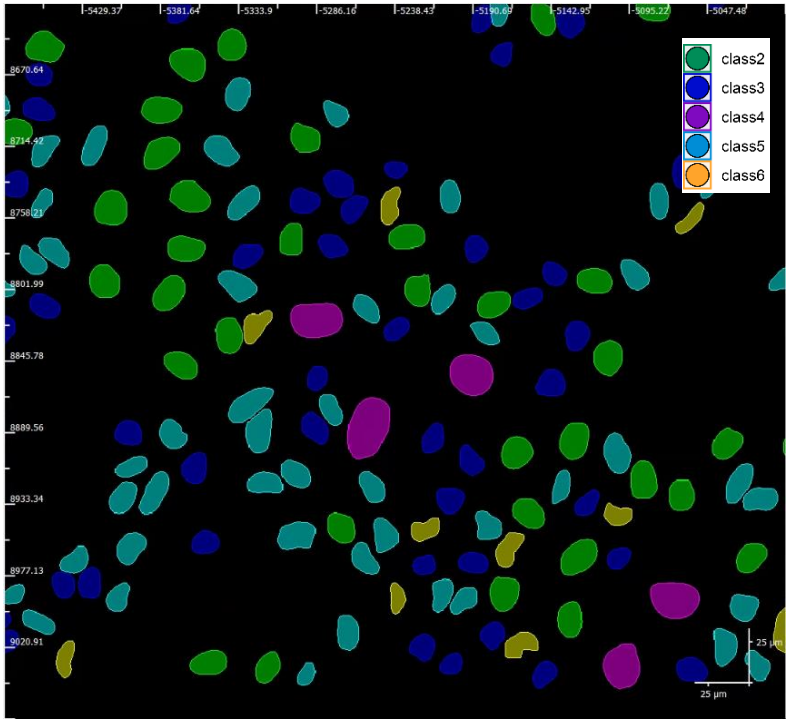

172.2x
-5429.37
-5381.64
-5333.9
-5286.16
-5238.43
-5190.69
-5142.95
-5095.22
-5047.48
8670.64
8714.42
8758.21
8801.99
8845.78
8889.56
8933.34
8977.13
9020.91
class2
class3
class4
class5
class6
25 µm
25 µm

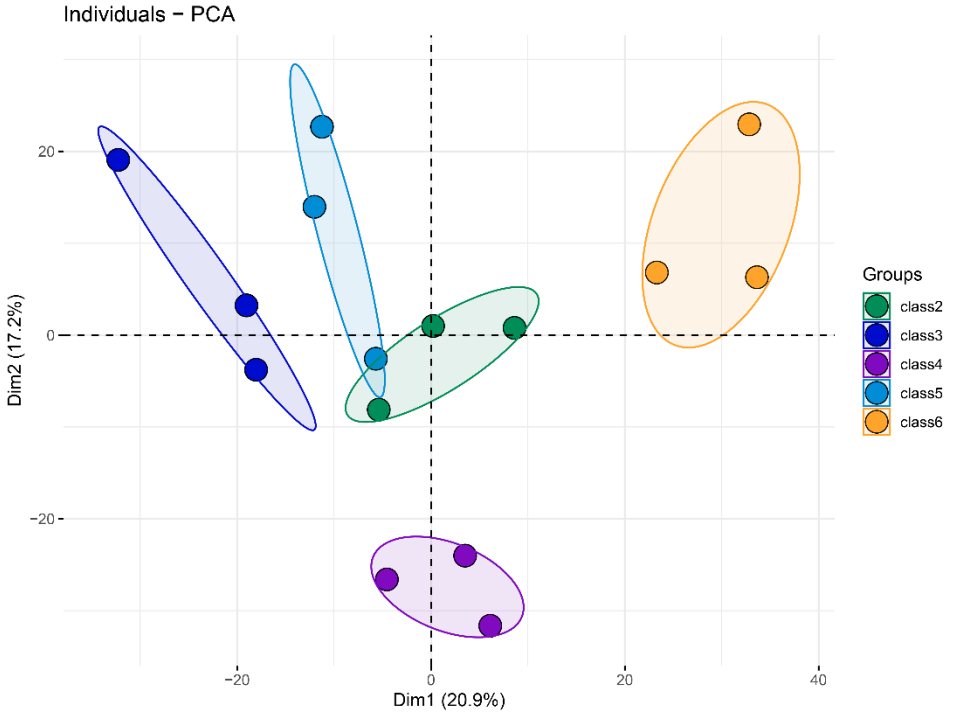
Phenotypic differences of 5 nuclei classes (DAPI dense regions) used for unsupervised clustering



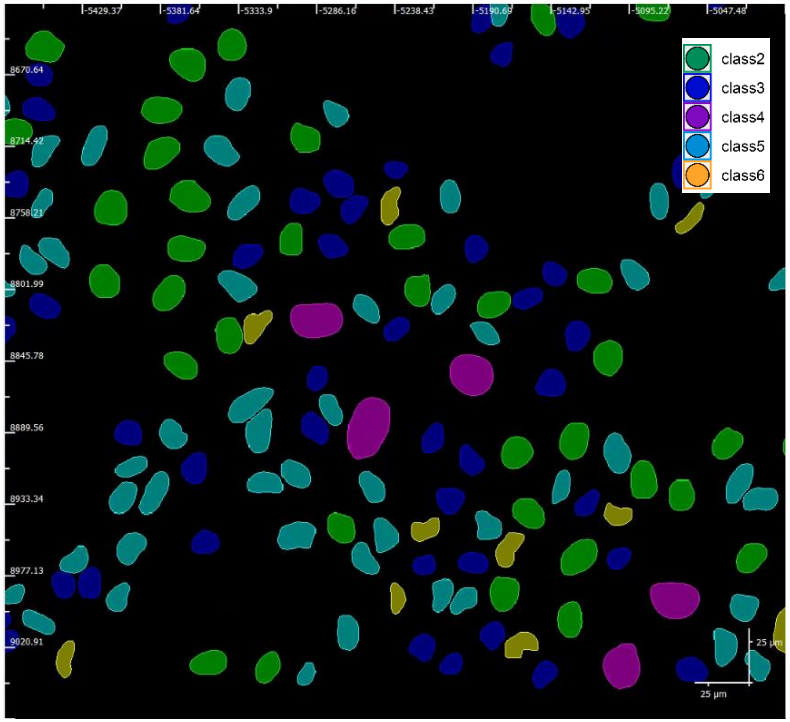


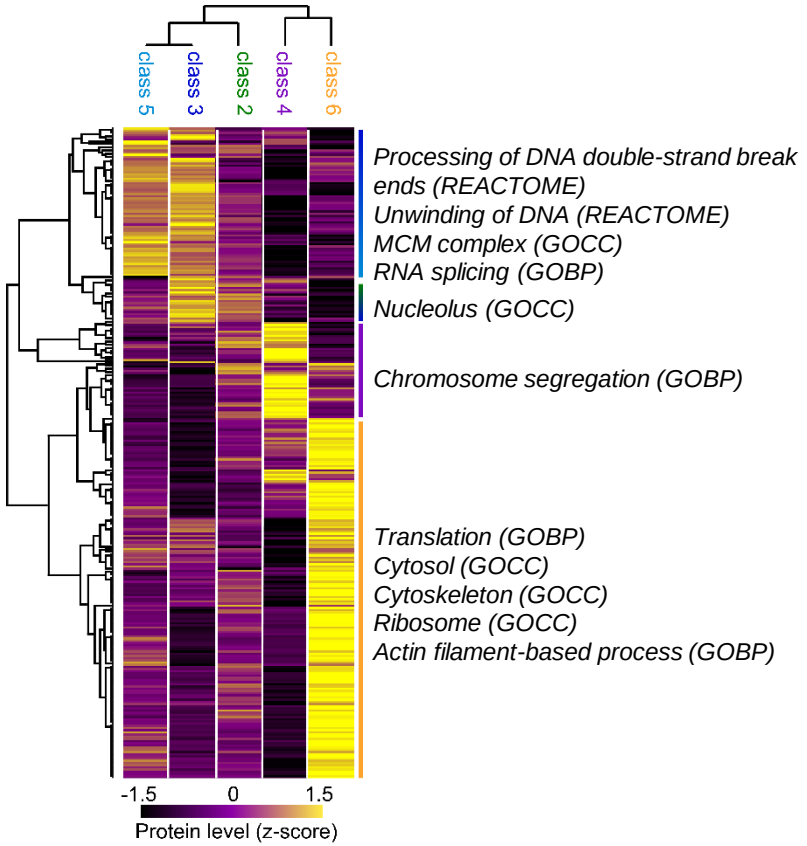
5 nuclei classes (DAPI dense regions) show distinct proteomics profiles





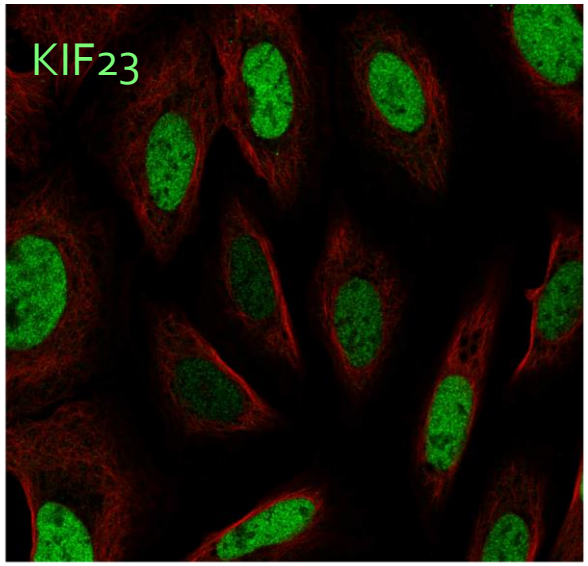
5 nuclei classes (DAPI dense regions) show distinct proteomics profiles





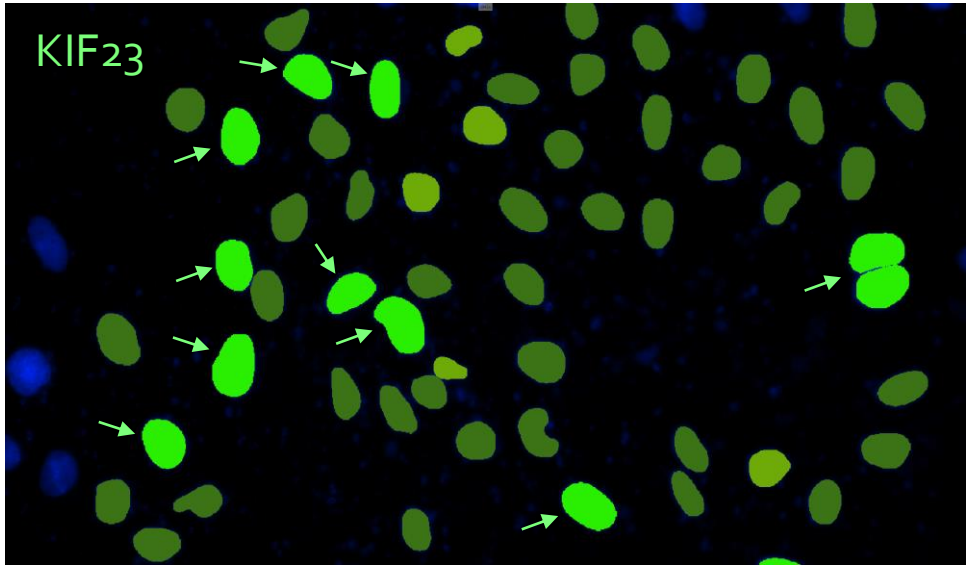
Integrating image data with protein abundance for (sub)cellular phenotyping

Protein atlas



https://www.proteinatlas.org/

Deep visual proteomics



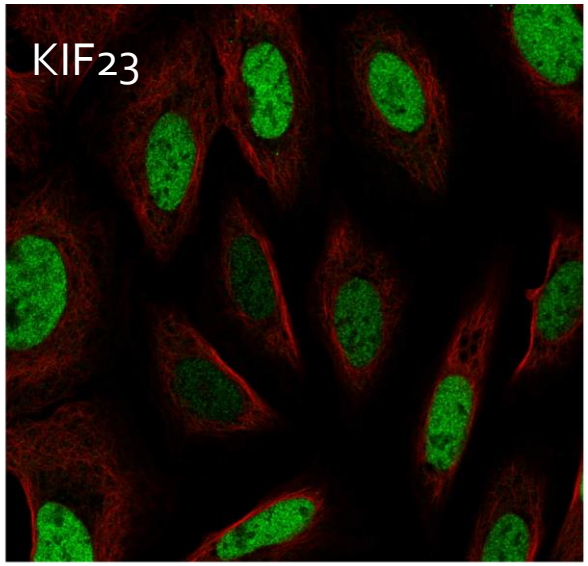
MS signal

low high

Thul PJ…Uhlén M, Lundberg E., 2017. **A subcellular map of the human proteome.** *Science.*

Systems biology at the imaging and at the proteomic levels

Protein atlas



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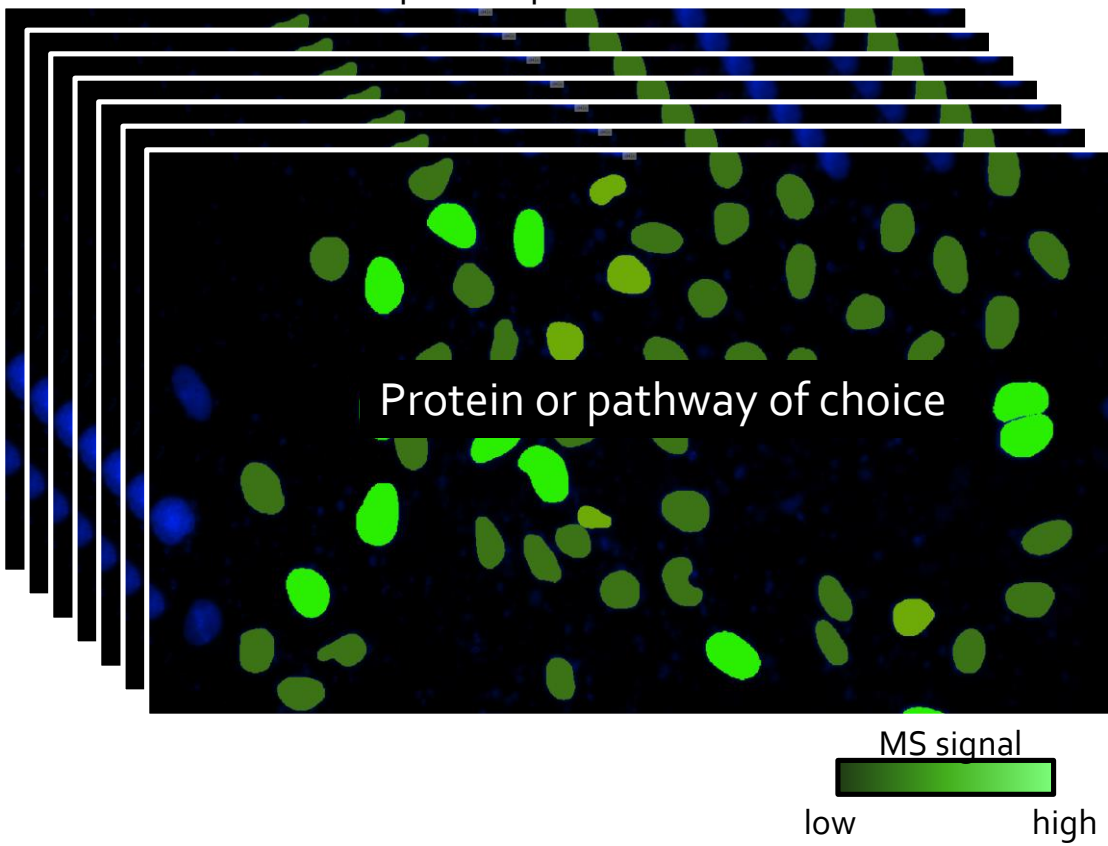
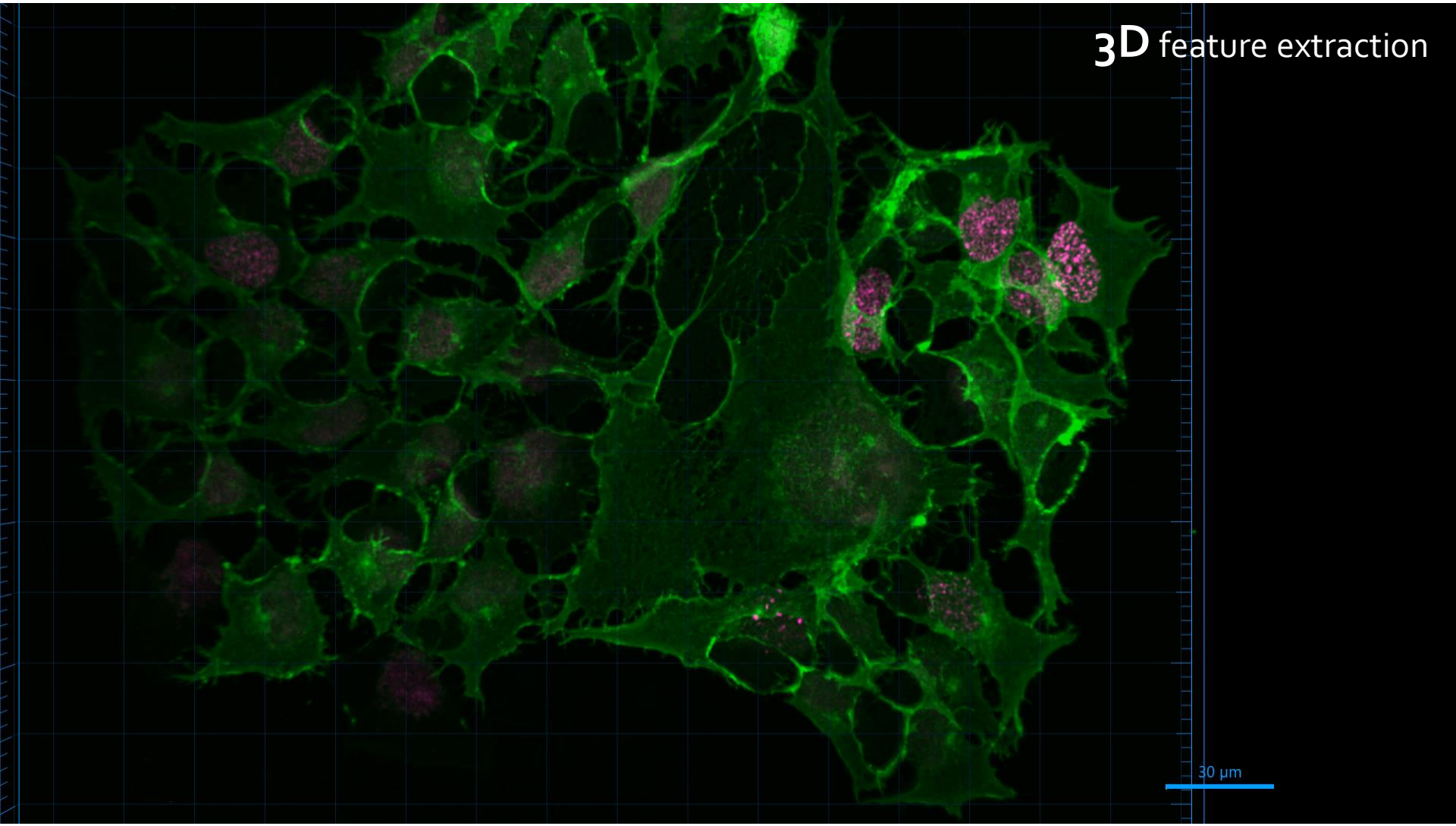
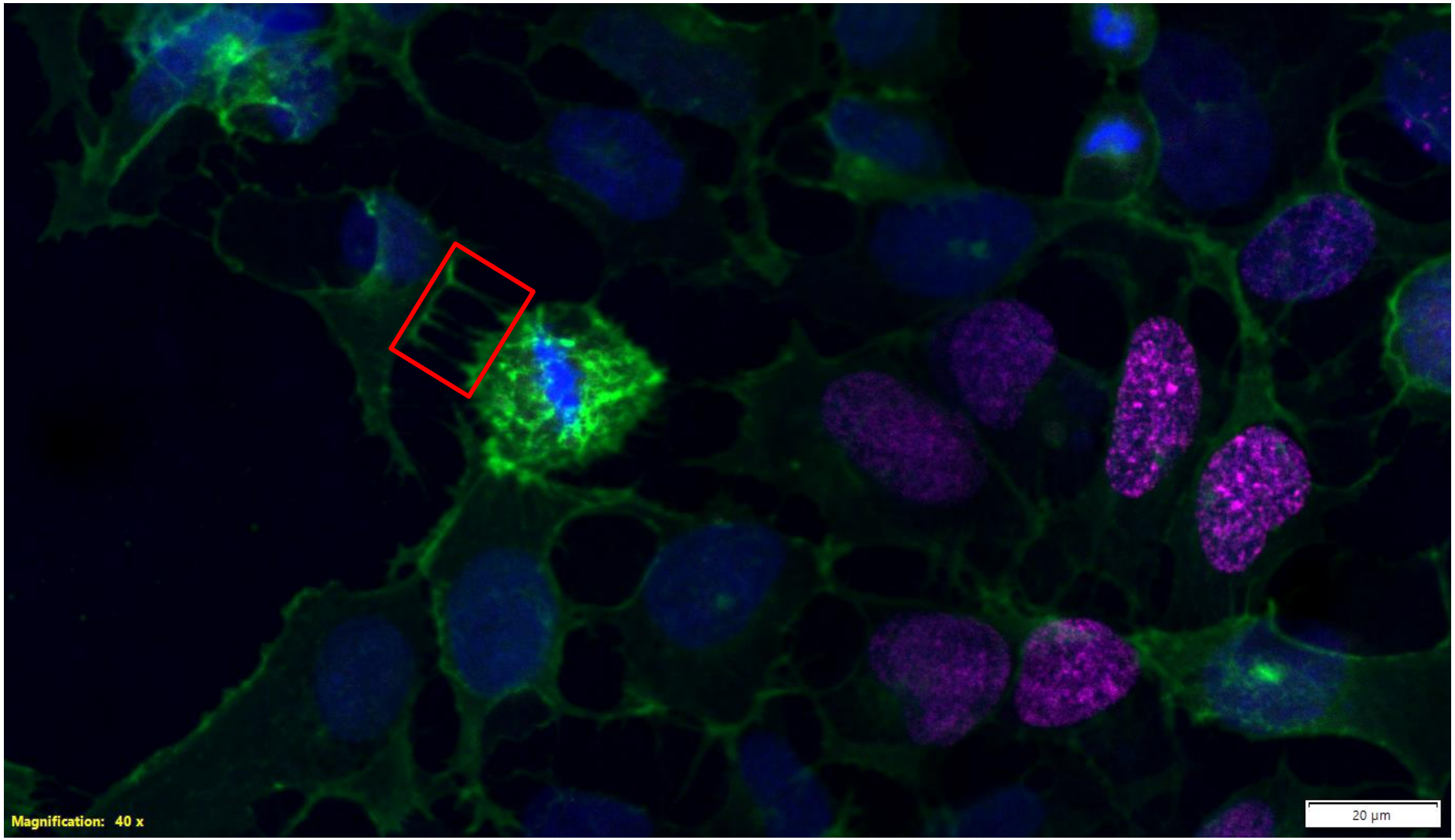
Deep visual proteomics



Next directions...



It is going to be a lot of fun…



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