Spatially guided omics analyses of the tumour microenvironment

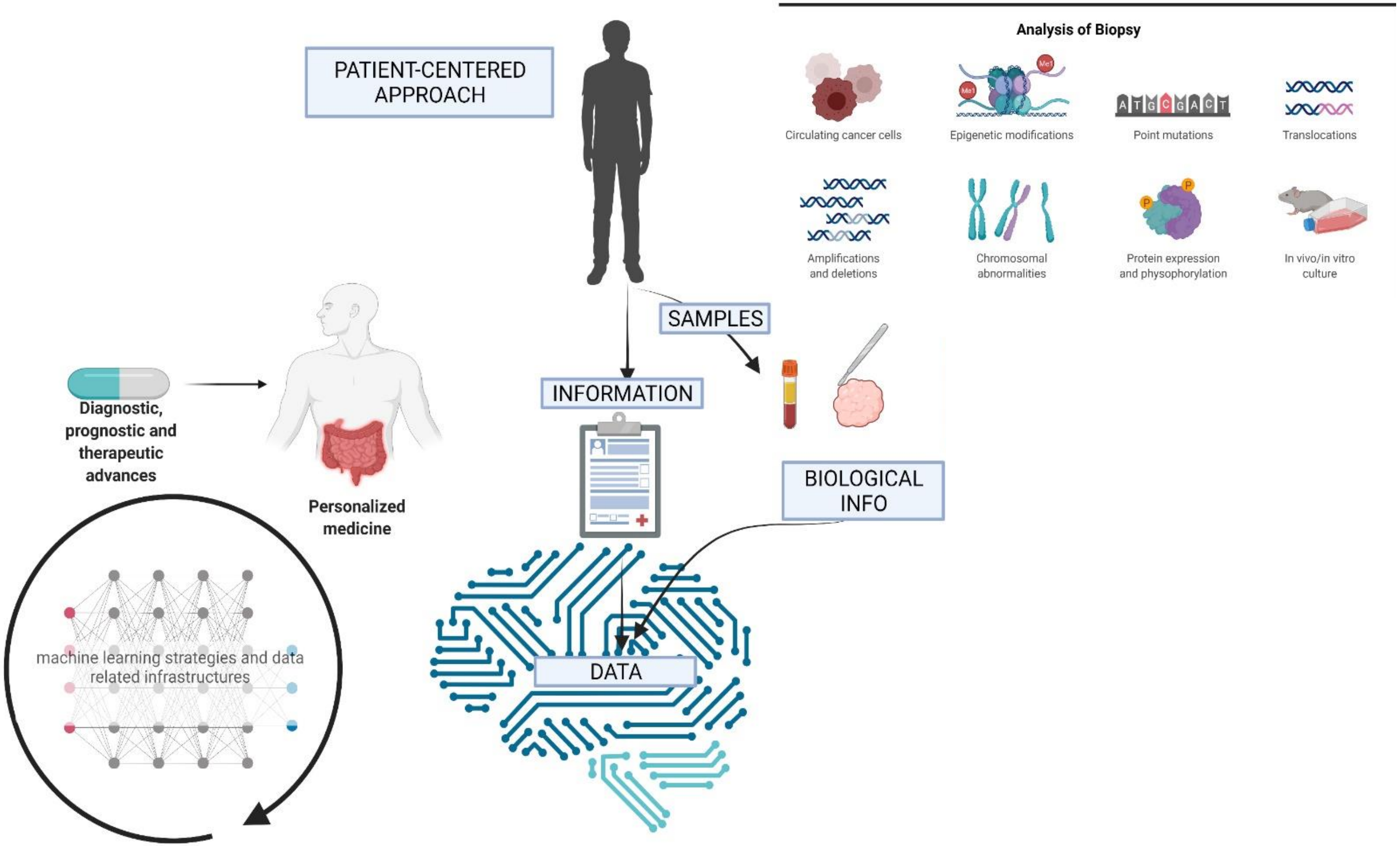
- Background
- Spatial omics
- Challenges

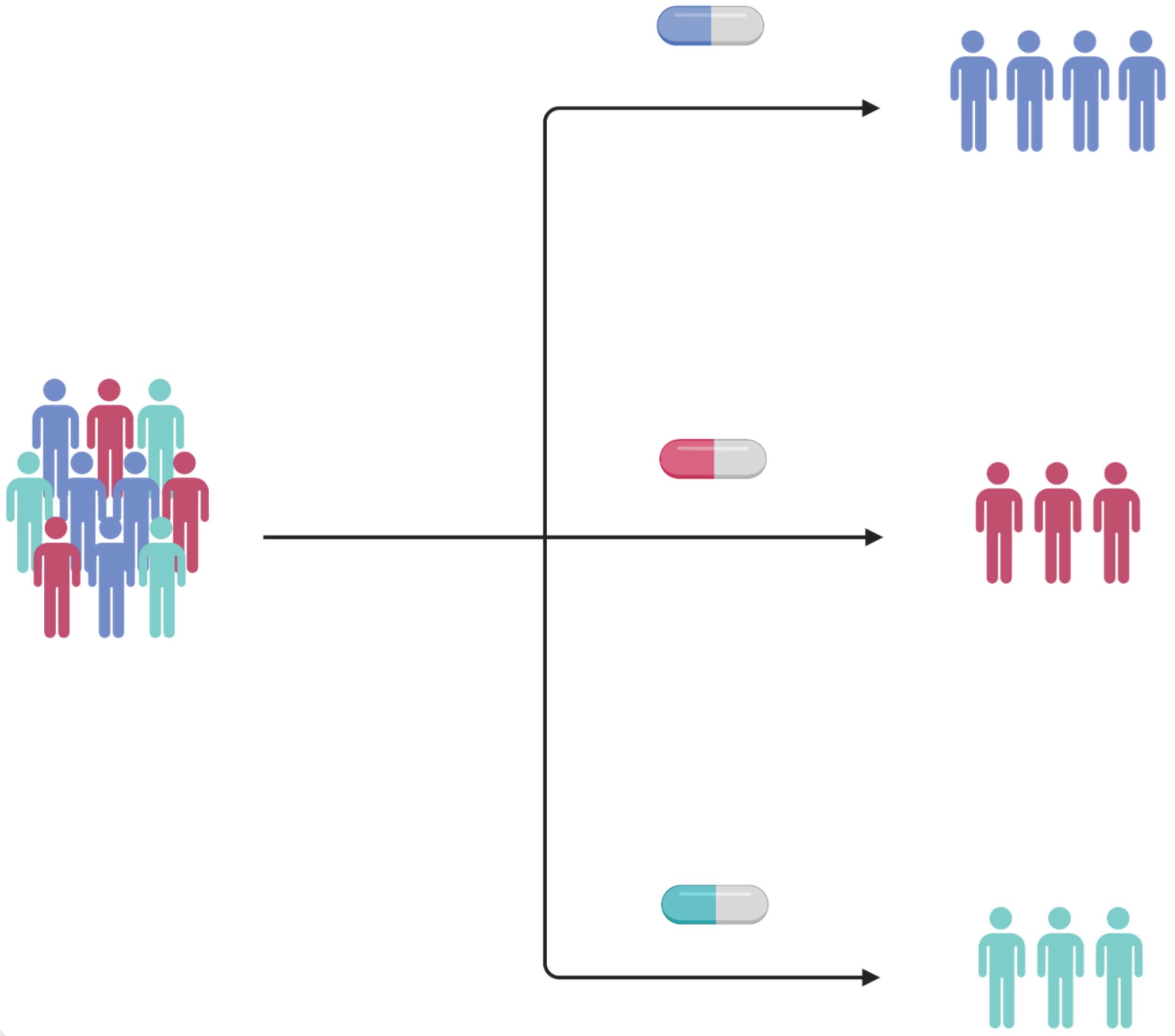
Sara Ek, professor Department of Immunotechnology

LUND UNIVERSITY

LTH

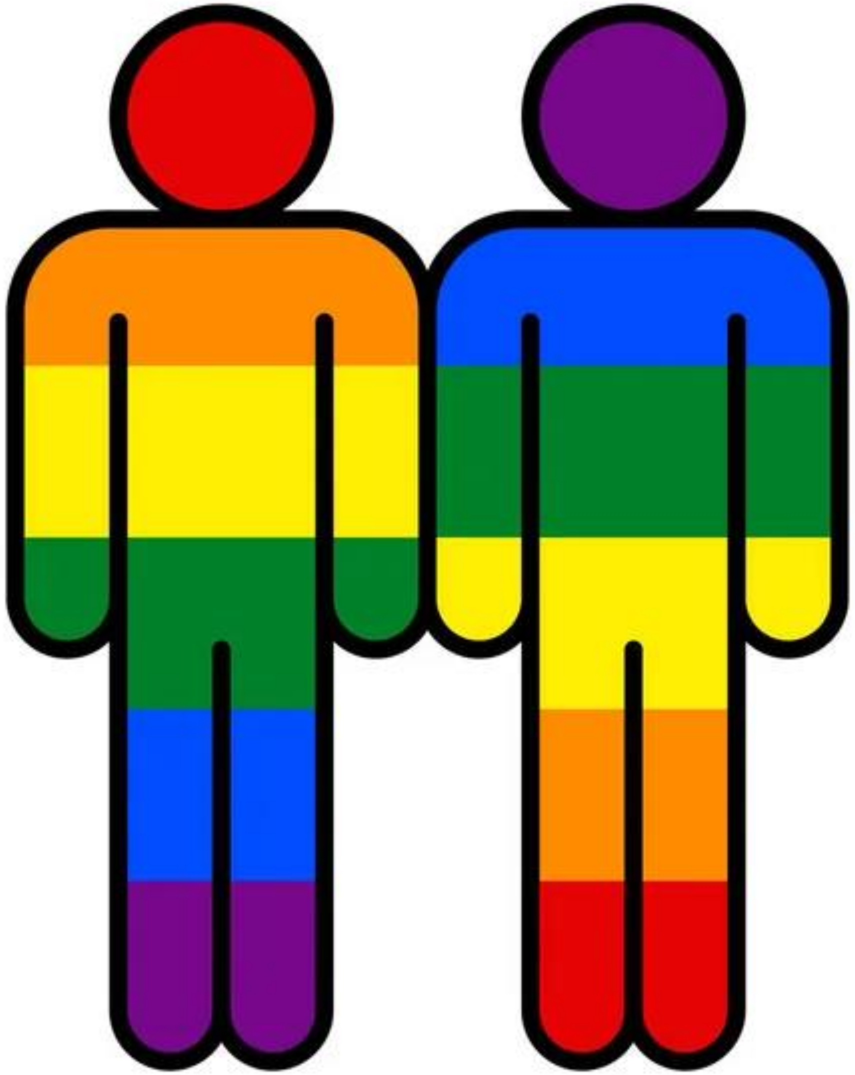
FACULTY OF ENGINEERING


PATIENT-CENTERED APPROACH
Analysis of Biopsy
Circulating cancer cells
Epigenetic modifications
Point mutations
ATGCGACT
Translocations
Amplifications and deletions
Chromosomal abnormalities
Protein expression and physophorylation
In vivo/in vitro culture
SAMPLES
INFORMATION
Diagnostic, prognostic and therapeutic advances
Personalized medicine
BIOLOGICAL INFO
machine learning strategies and data related infrastructures
DATA



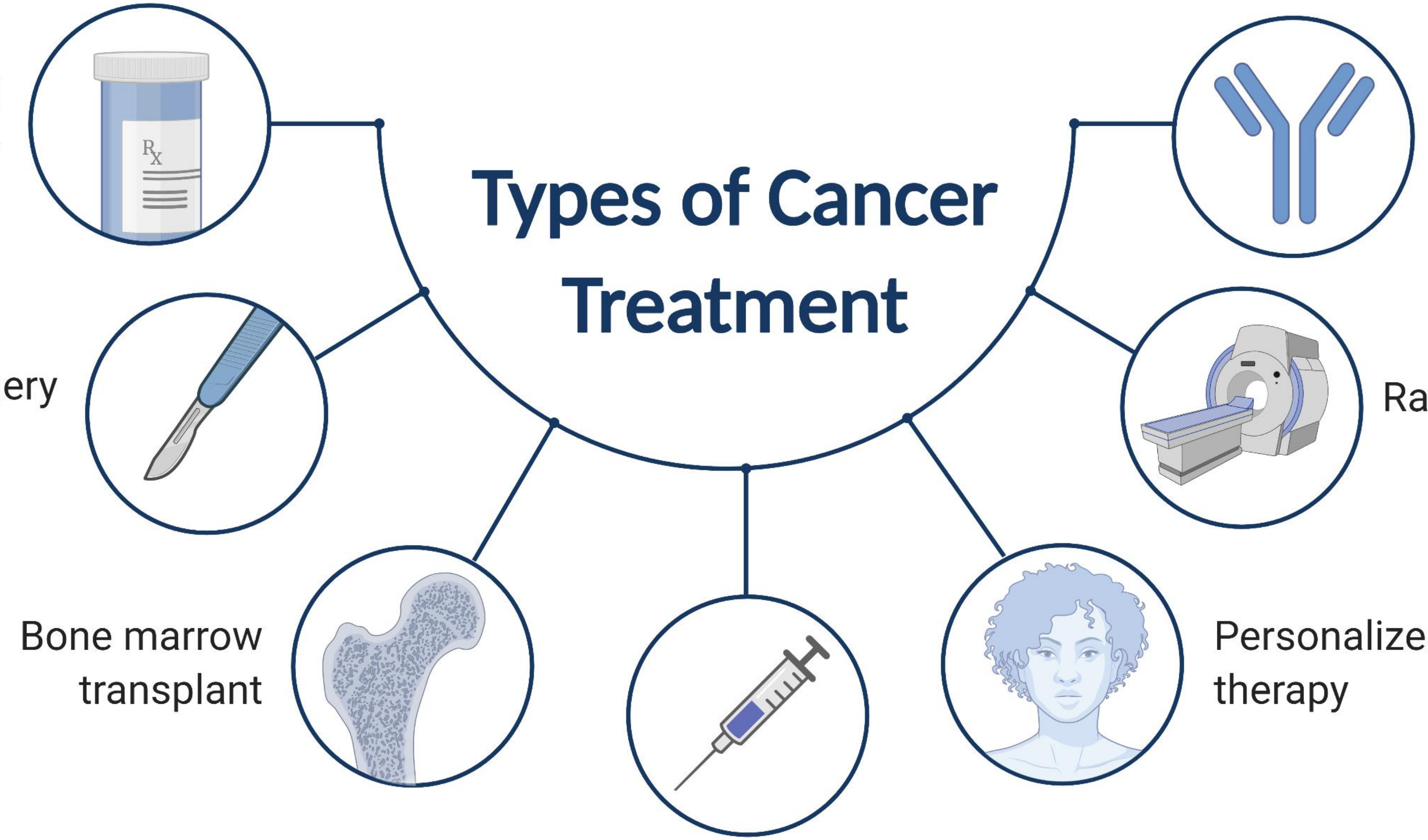
**PREDICTION/
STRATIFICATION**

Diversity



Dynamics

Types of Cancer Treatment



Hormonal therapy

Surgery

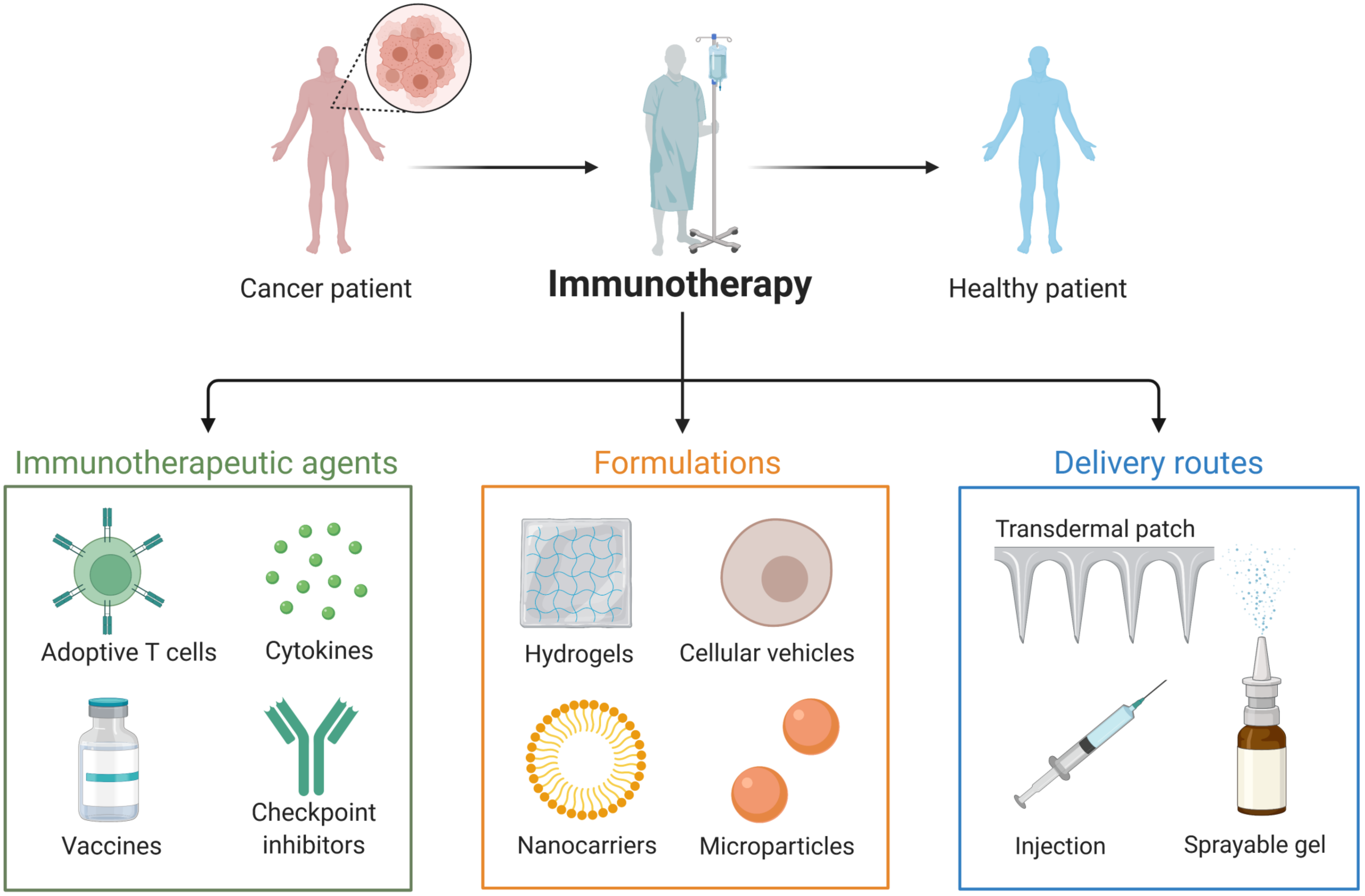
Bone marrow transplant

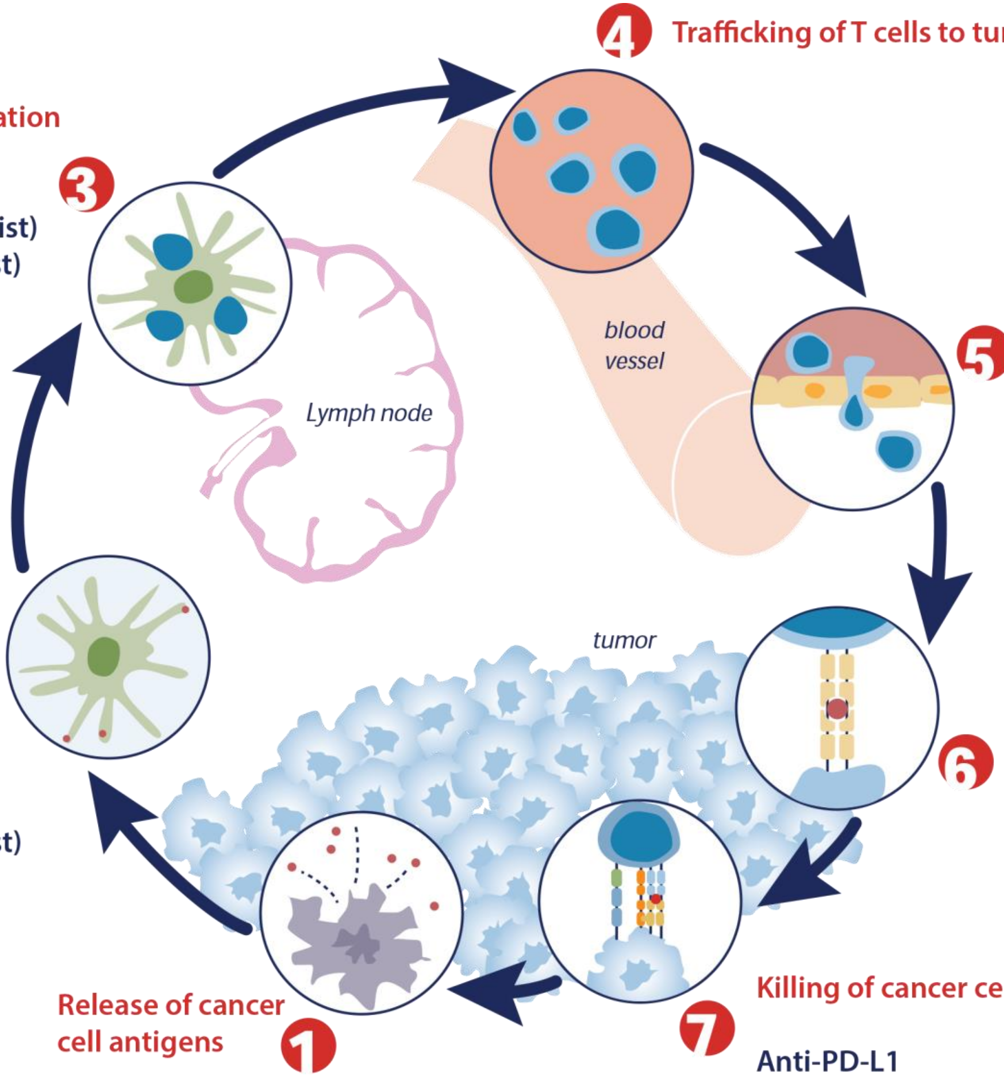
Chemotherapy

Personalized therapy

Radiotherapy

Immunotherapy


Cancer patient
Immunotherapy
Healthy patient
Immunotherapeutic agents
Adoptive T cells
Cytokines
Vaccines
Checkpoint inhibitors
Formulations
Hydrogels
Cellular vehicles
Nanocarriers
Microparticles
Delivery routes
Transdermal patch
Injection
Sprayable gel



① Release of cancer cell antigens

- Chemotherapy
- Radiation Therapy
- Targeted Therapy

② Cancer antigen presentation

- Vaccines
- IFN-α
- GM-CSF
- Anti-CD40 (agonist)
- TLR agonists

③ Priming and activation

- Anti-CTLA4
- Anti-CD137 (agonist)
- Anti-OX40 (agonist)
- Anti-CD27
- IL-2
- IL-12

④ Trafficking of T cells to tumors

⑤ Infiltration of T cells into tumors

- Anti-VEGF

⑥ Recognition of cancer cells by T cells

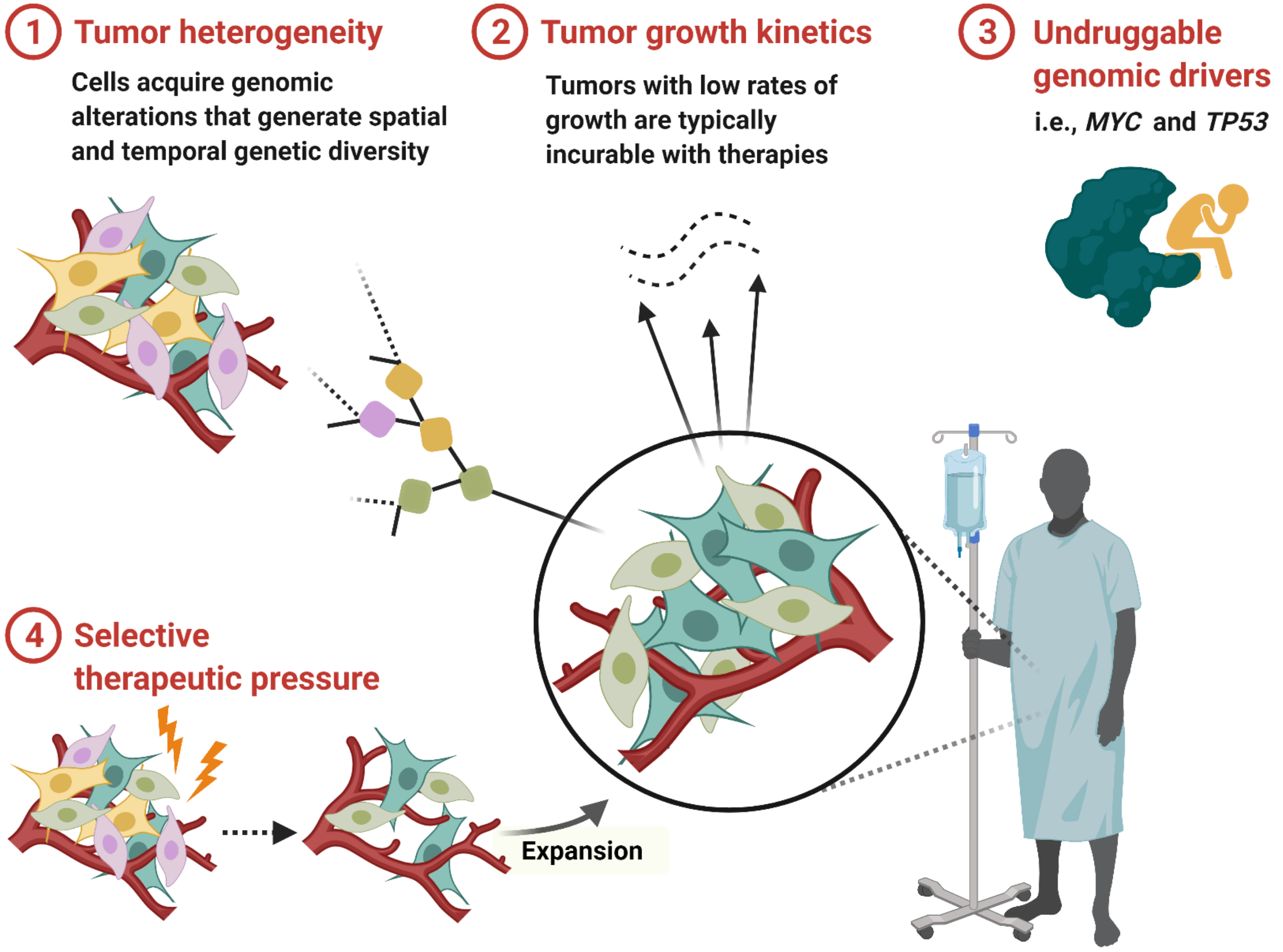
- CARs

⑦ Killing of cancer cells

- Anti-PD-L1
- Anti-PD-1
- IDO inhibitors

Abbviescience.com

Tumour heterogeneity - a challenge also in the immuno-oncology era



① **Tumor heterogeneity**

Cells acquire genomic alterations that generate spatial and temporal genetic diversity

② **Tumor growth kinetics**

Tumors with low rates of growth are typically incurable with therapies

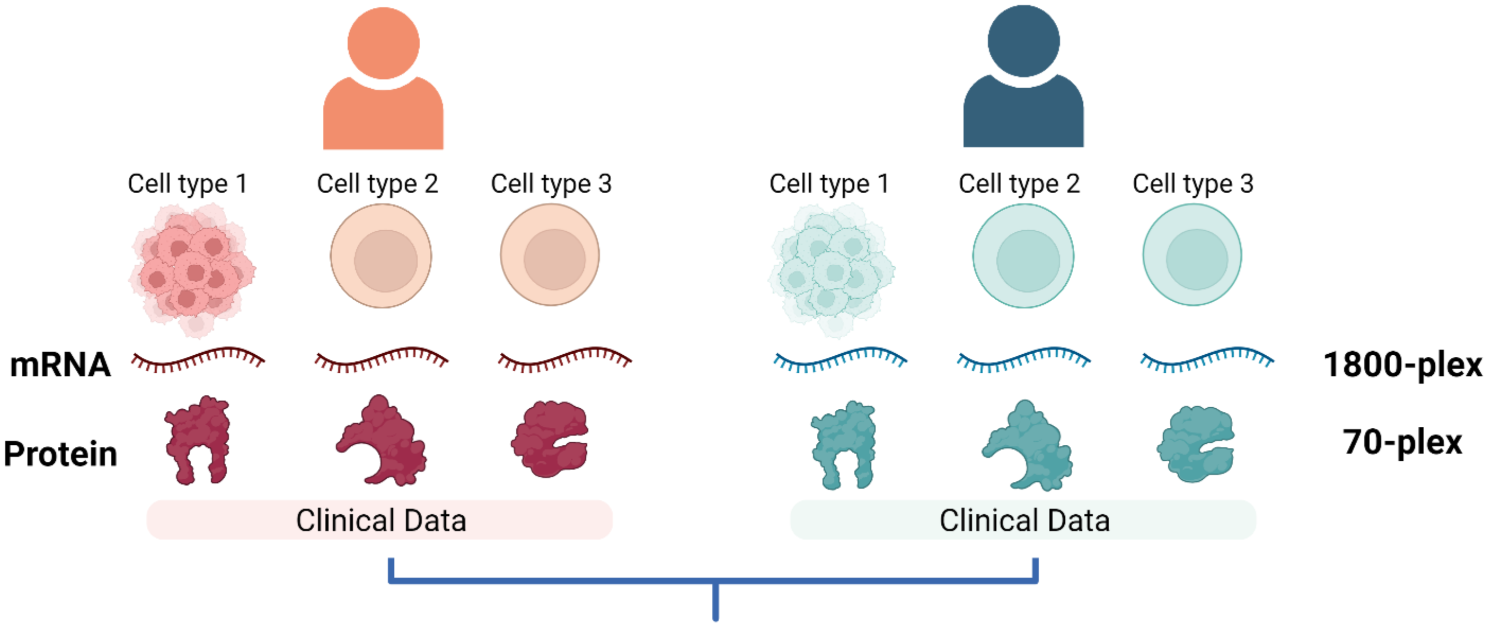
③ **Undruggable genomic drivers**

i.e., *MYC* and *TP53*

④ **Selective therapeutic pressure**

Expansion

Improved companion diagnostics – when more complex molecular traits are measured



Cell type 1 Cell type 2 Cell type 3

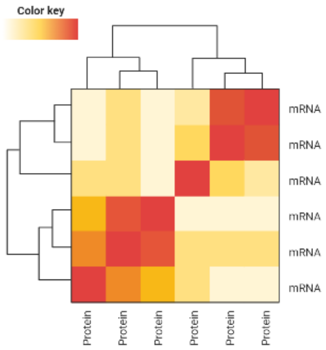
Cell type 1 Cell type 2 Cell type 3

mRNA **1800-plex**

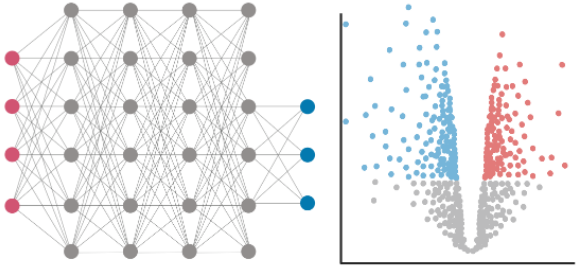
Protein **70-plex**

Clinical Data

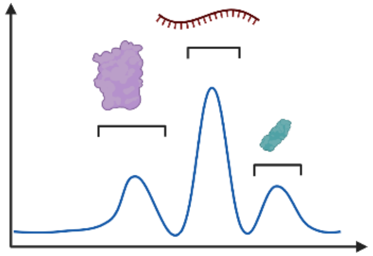
Clinical Data



Integrated Analysis



Prediction and classification models

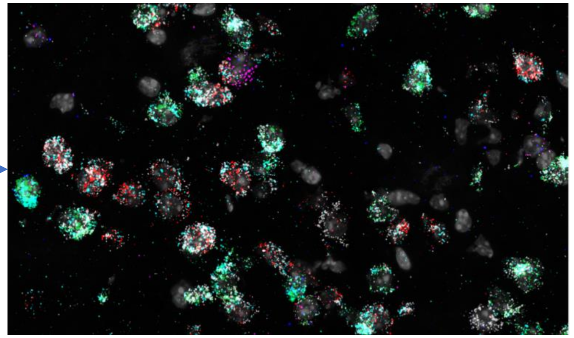


Biomarker discovery

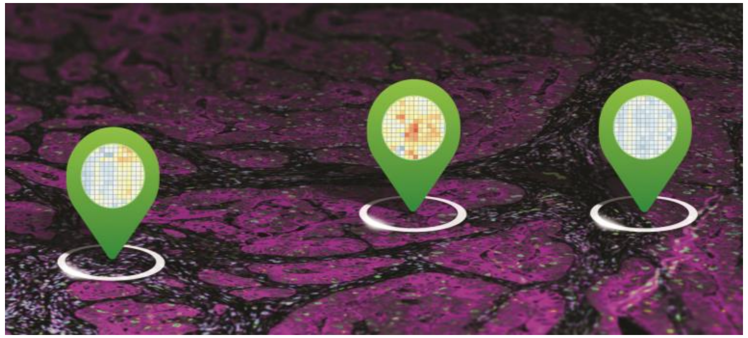
Why use spatially-guided omics analyses?

FRESH FROZEN TISSUE

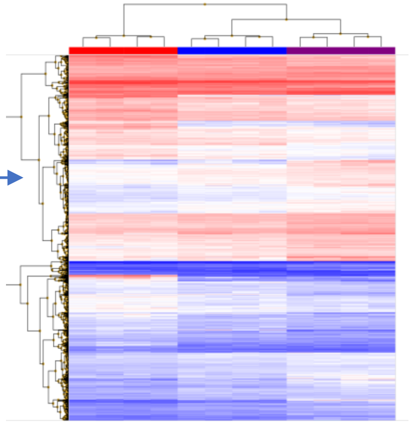
FFPE TISSUE BLOCK



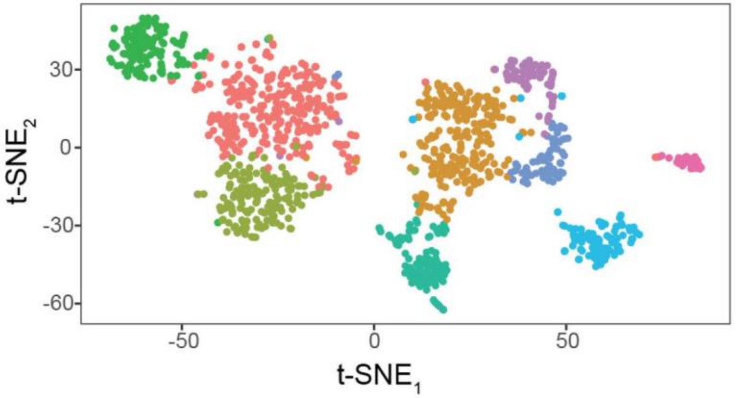
Low plex: IHC, (F)ISH



High plex analyses and spatially-resolved individual cell types



High plex but no cell type specific info: bulk RNA-seq



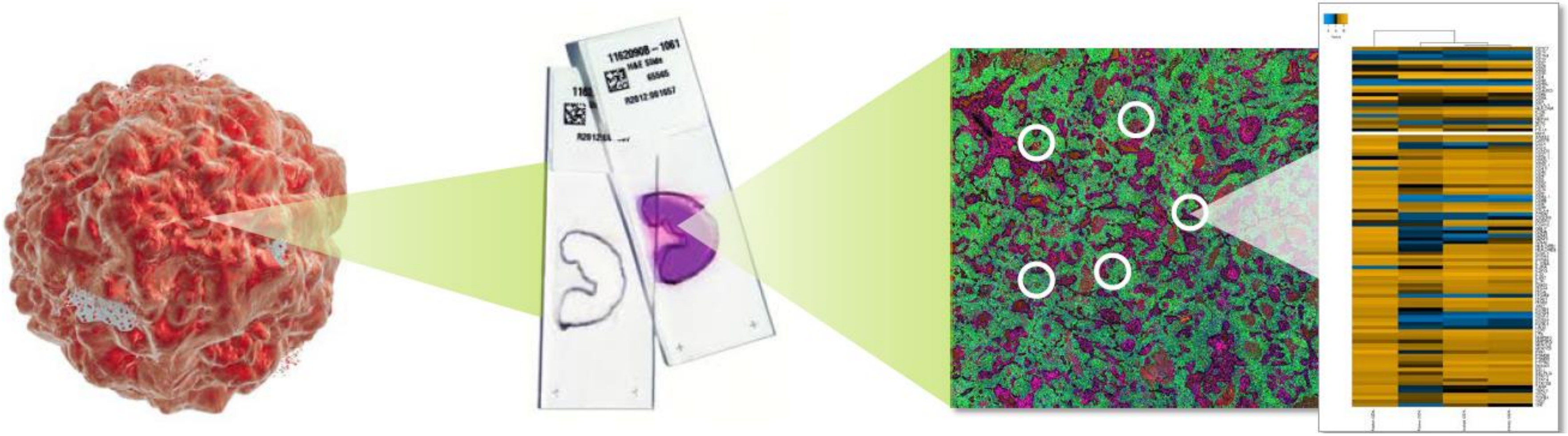
High plex and individual cell types resolved but no info on spatial localization: scRNA-seq

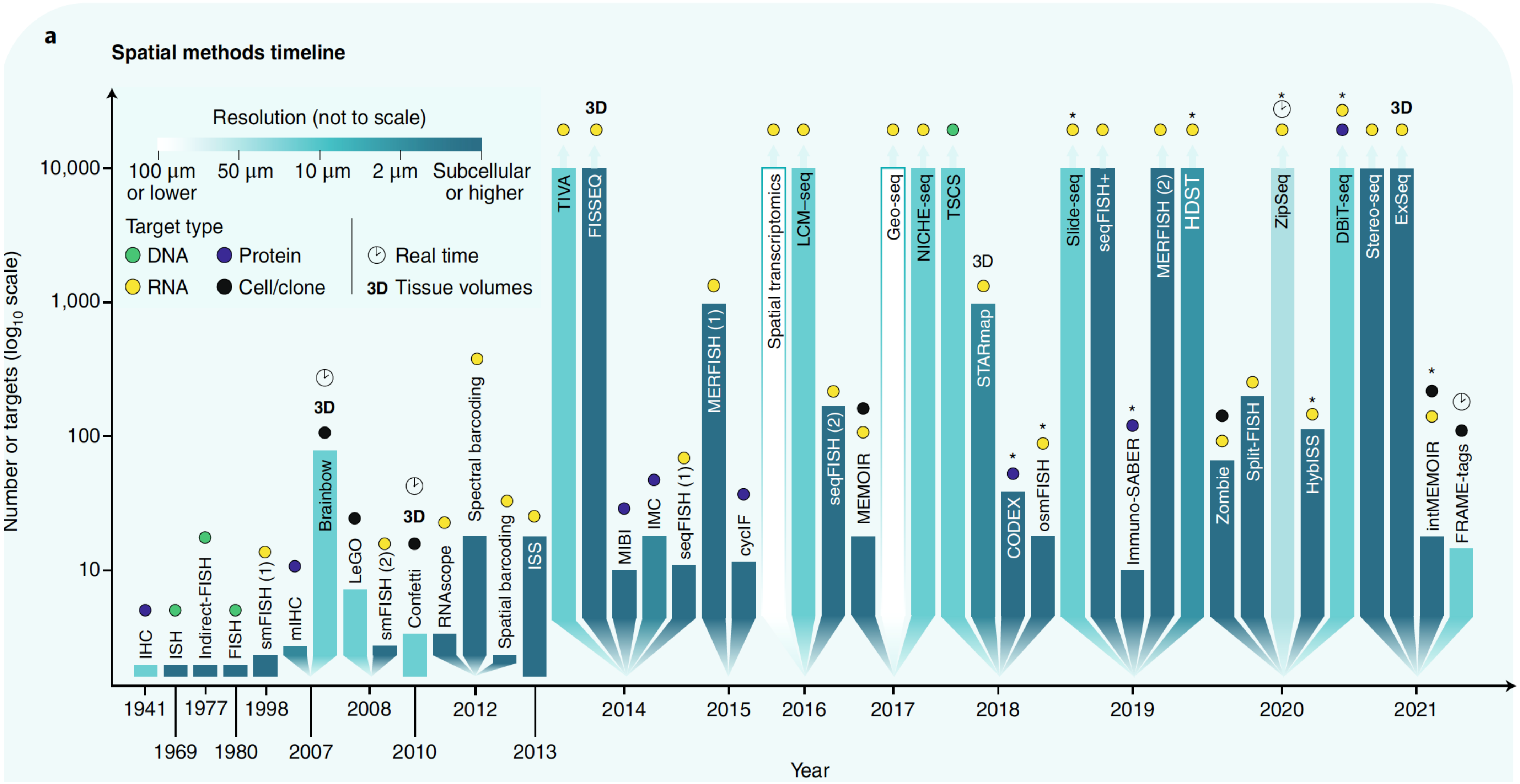
Method of the Year: spatially resolved transcriptomics

Nature Methods has crowned spatially resolved transcriptomics Method of the Year 2020.

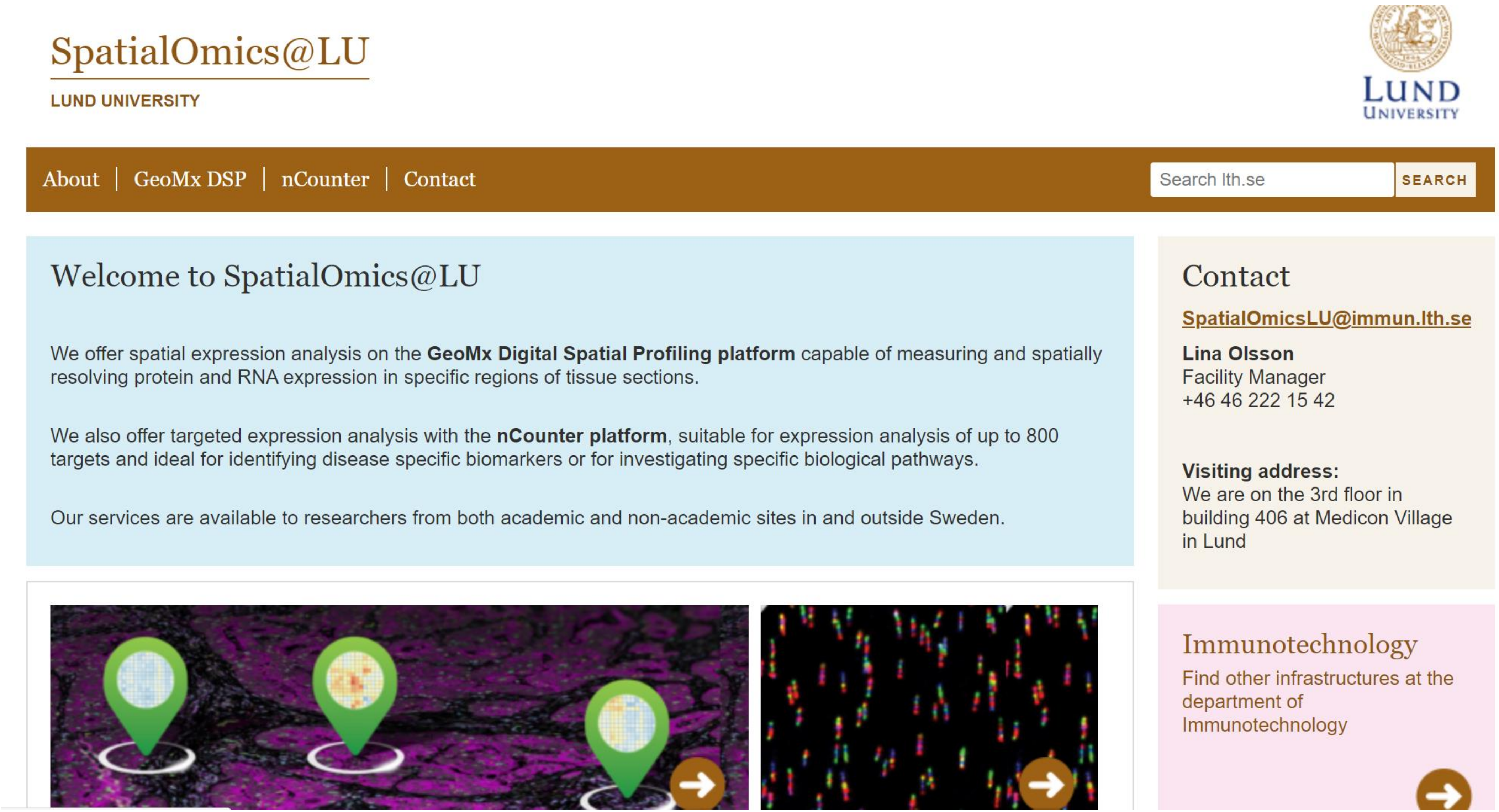
Vivien Marx

NATURE METHODS | VOL 18 | JANUARY 2021 | 9–14 | www.nature.com/naturemethods




a Spatial methods timeline

Lewis et al, Spatial omics and multiplexed imaging to explore cancer biology. Nature Methods 2021



SpatialOmics@LU

LUND UNIVERSITY

Welcome to SpatialOmics@LU

We offer spatial expression analysis on the **GeoMx Digital Spatial Profiling platform** capable of measuring and spatially resolving protein and RNA expression in specific regions of tissue sections.

We also offer targeted expression analysis with the **nCounter platform**, suitable for expression analysis of up to 800 targets and ideal for identifying disease specific biomarkers or for investigating specific biological pathways.

Our services are available to researchers from both academic and non-academic sites in and outside Sweden.

Contact

SpatialOmicsLU@immun.lth.se

Lina Olsson
Facility Manager
+46 46 222 15 42

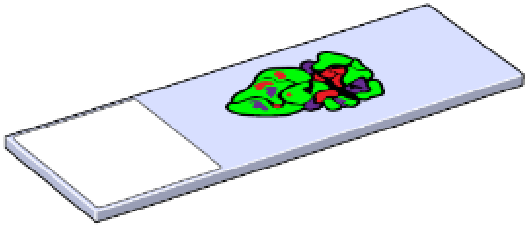
Visiting address:
We are on the 3rd floor in building 406 at Medicon Village in Lund

Immunotechnology

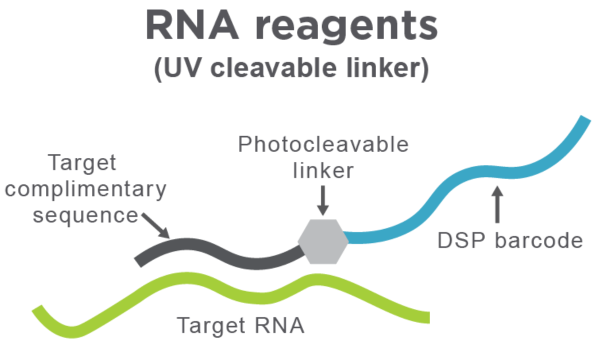
Find other infrastructures at the department of Immunotechnology

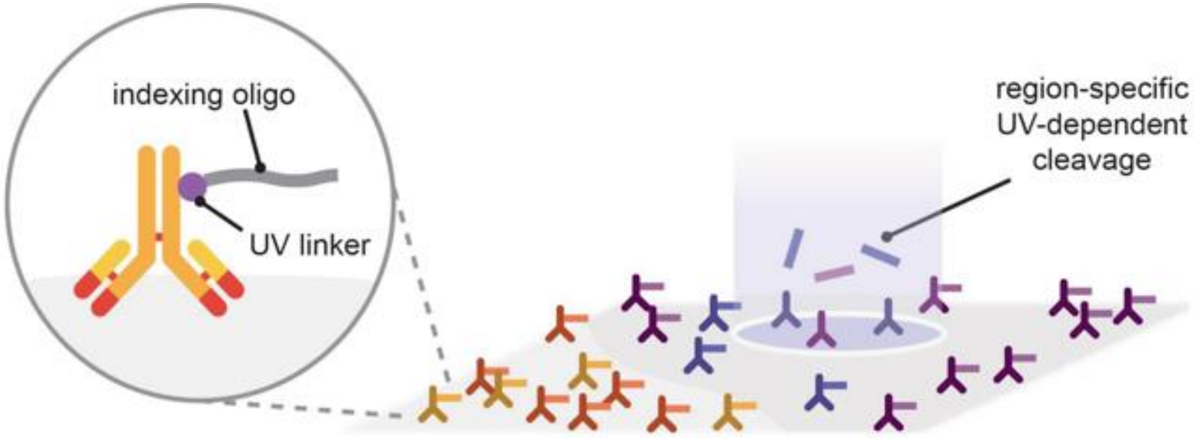
Principles of the GeoMx DSP technology

Tissue section is stained with up to three antibodies to visualize morphology



Protein or RNA detection antibodies/probes are also added and bind to the tissue

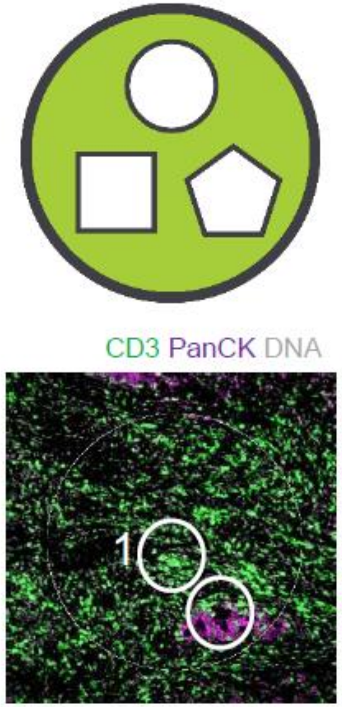




Selected tissue regions are exposed to UV light –> indexing oligos/barcodes are released, collected and quantitated

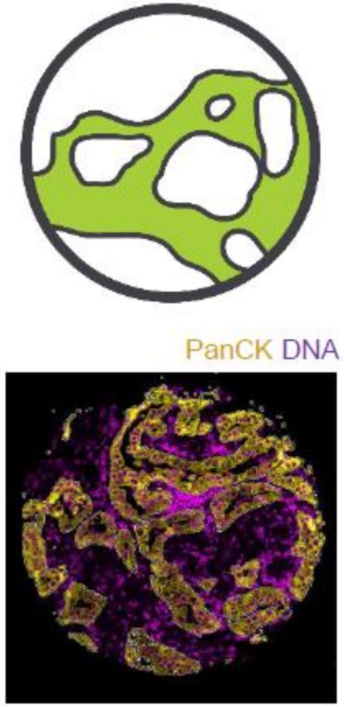
Selection of regions of interest (ROI)

Geometric



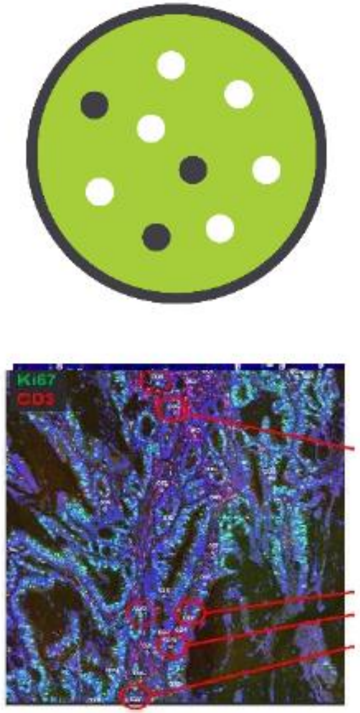
What is the heterogeneity of expression in different regions of my tissue?

Segmentation



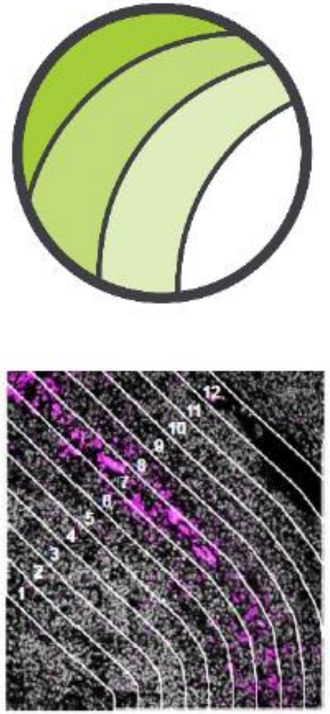
What is the expression profile of distinct biological compartments (e.g., Tumor-TME)?

Cell Type Specific



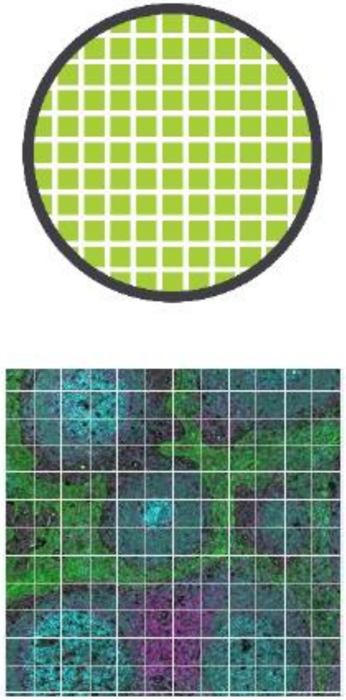
What is the expression profile of a specific cell population in my tissue?

Contour

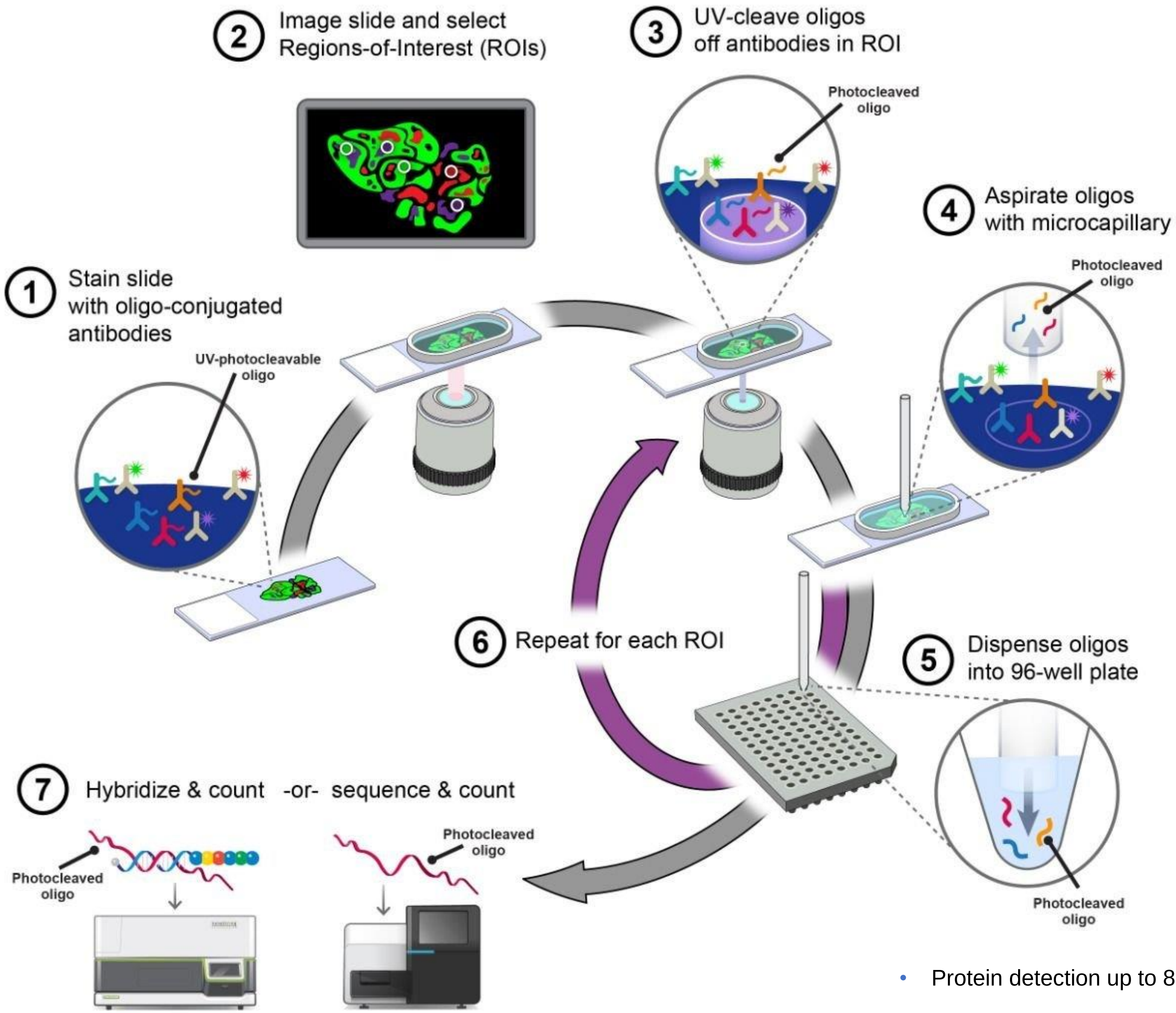


How does the immune environment change on either side of an infiltrate boundary?

Gridded

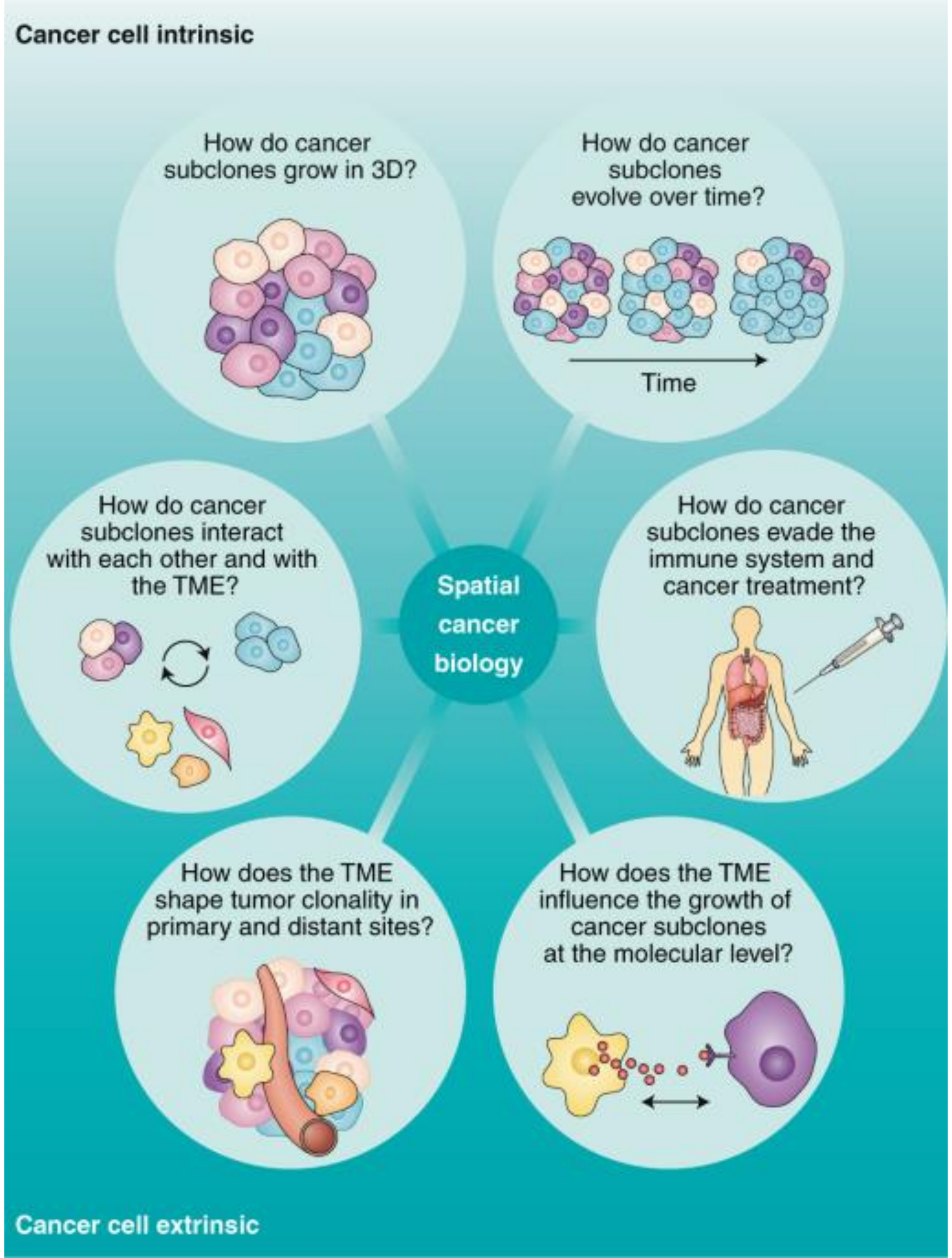


What novel targets are uncovered with deep mapping of a specific tissue region?

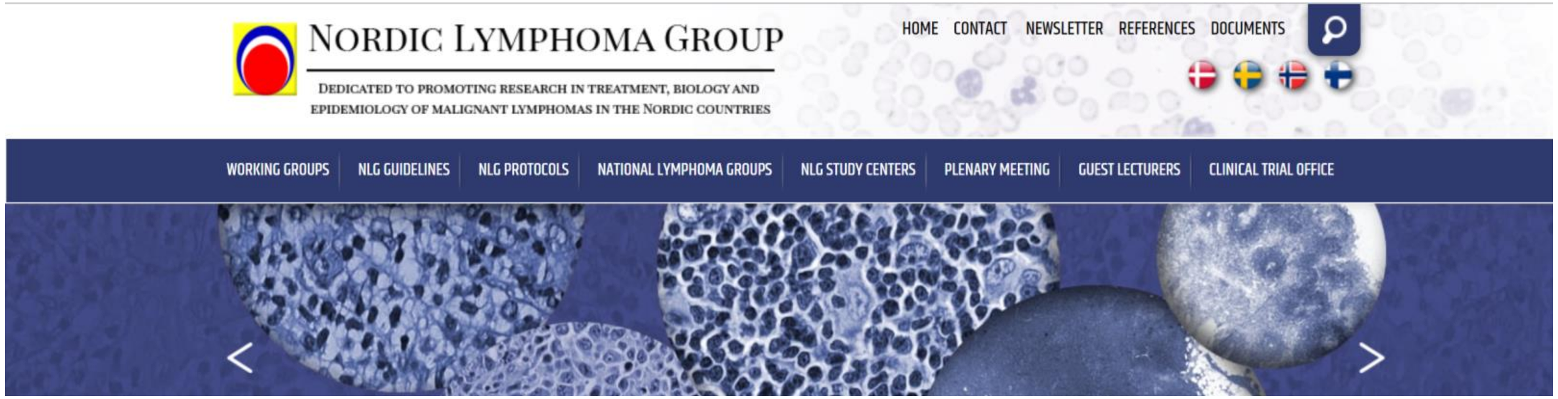


- Protein detection up to 84 plex
- RNA detection ~1800 plex (CTA) or 20 000 plex (whole transcriptome)

Why use spatially-guided omics analyses?



Lewis et al, Spatial omics and multiplexed imaging to explore cancer biology. Nature Methods 2021



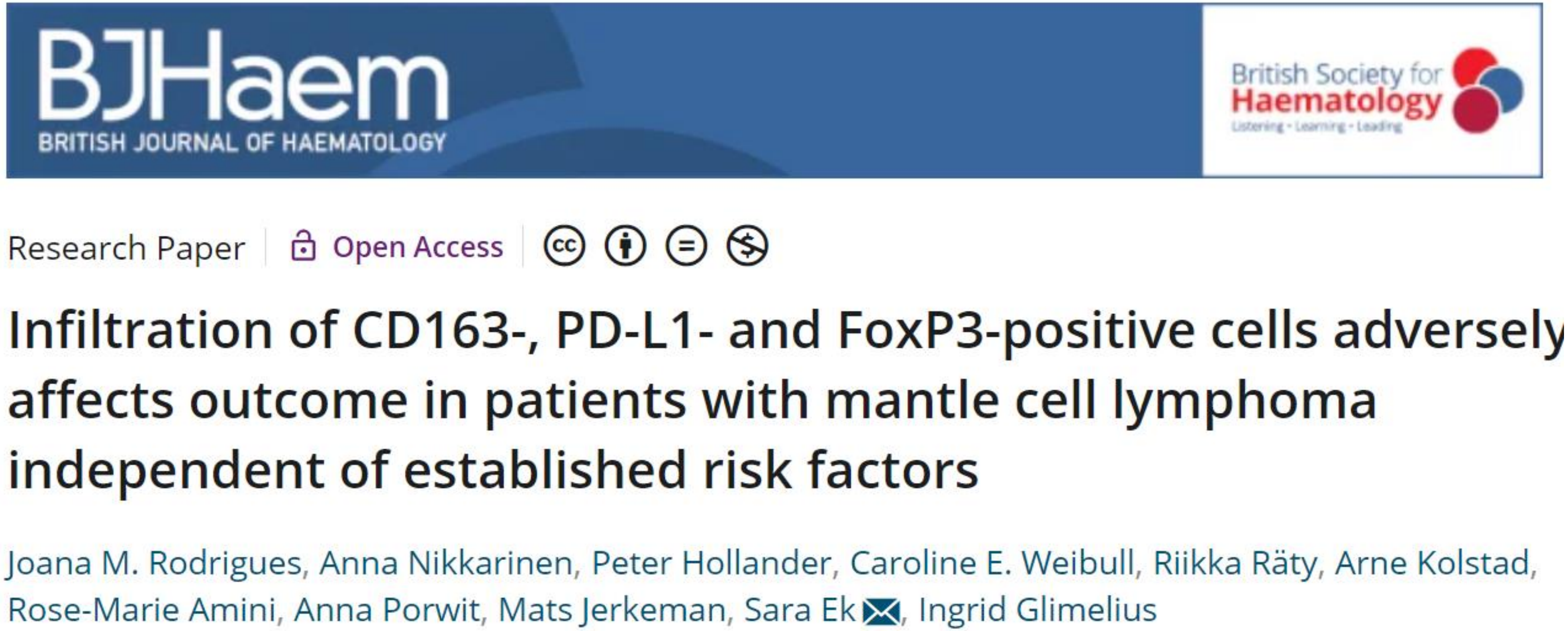
NORDIC LYMPHOMA GROUP

DEDICATED TO PROMOTING RESEARCH IN TREATMENT, BIOLOGY AND EPIDEMIOLOGY OF MALIGNANT LYMPHOMAS IN THE NORDIC COUNTRIES

BIO-MUSE

- **Predictive and prognostic <u>BIO</u>markers in patients with**
- **<u>M</u>ycosis F<u>U</u>ngoides and <u>S</u>ézary syndrom<u>E</u>**

Individual collaborations on solid cancer:
Lung cancer – Patrick Micke, UU
Ovarian cancer – Karin Sundfeldt, GU



BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

British Society for Haematology
Listening • Learning • Leading

Research Paper | Open Access

Infiltration of CD163-, PD-L1- and FoxP3-positive cells adversely affects outcome in patients with mantle cell lymphoma independent of established risk factors

Joana M. Rodrigues, Anna Nikkarinen, Peter Hollander, Caroline E. Weibull, Riikka Räty, Arne Kolstad, Rose-Marie Amini, Anna Porwit, Mats Jerkeman, Sara Ek, Ingrid Glimelius

Digital spatial profiling of mantle cell lymphoma

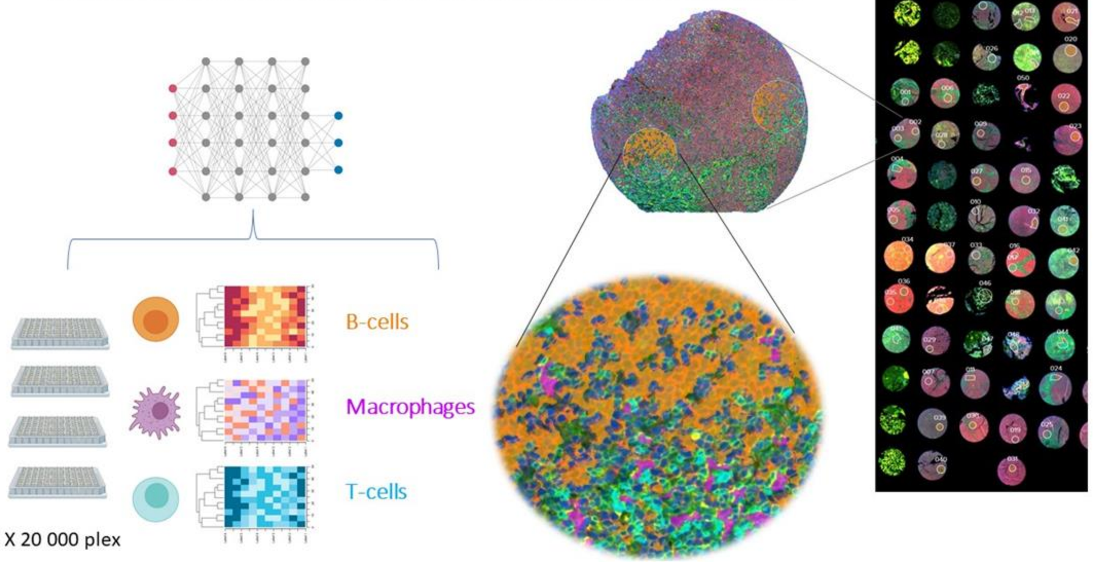

Lavanya Lokhande


Joana de Matos Rodrigues

In collaboration with Mats Jerkeman and Anna Porwit

Background

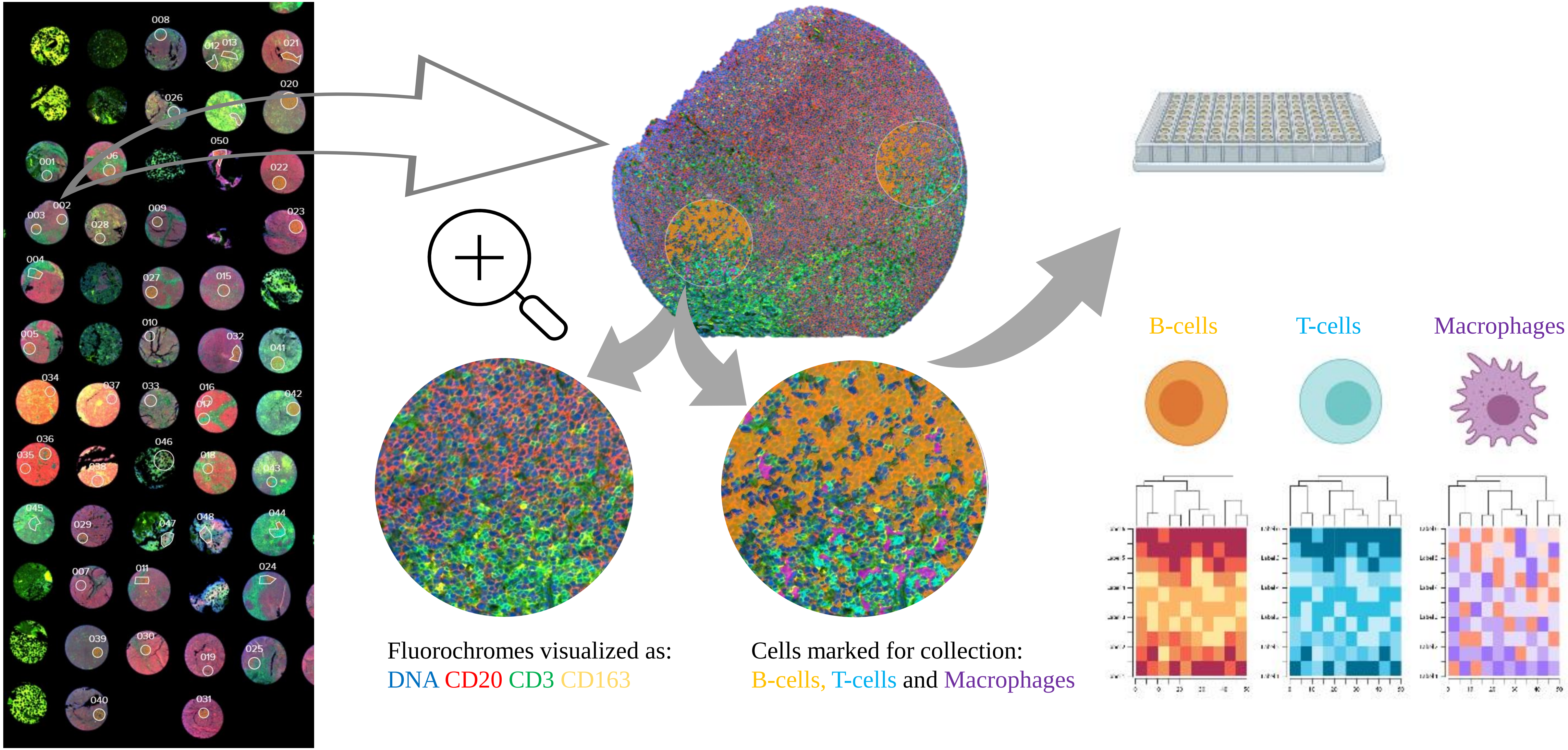
- Increased interest for immune-modulatory treatment in MCL – companion diagnostics are lacking.
- We recently showed that M2 macrophages have a poor prognostic impact- but the function in MCL is not described.


Spatially-resolved omics analyses combined with machine learning strategies identifies disease-associated targets and improve clinical decision making

Main objectives

1. T-cell and tumor cell adaptation in the presence of macrophages. *Guide therapeutic strategies*
2. Detailed investigations of T-cell subtype functionality (mRNA profiling of four subsets) in relation to genetic and clinicopathological parameters. *Companion diagnostic insight*
3. Inter and intra patient tumor hetrogeneity (mRNA profiling for target discovery)

In depth analysis of the microenvironment

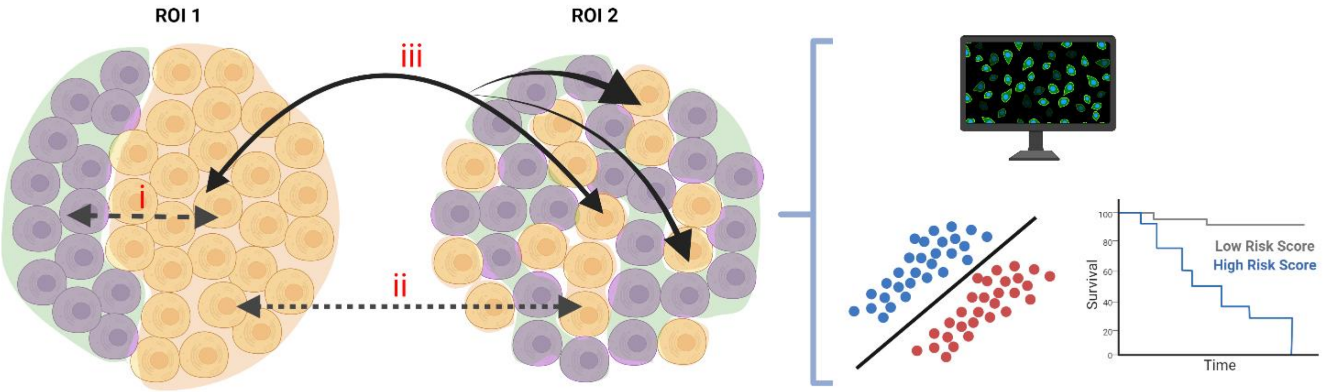


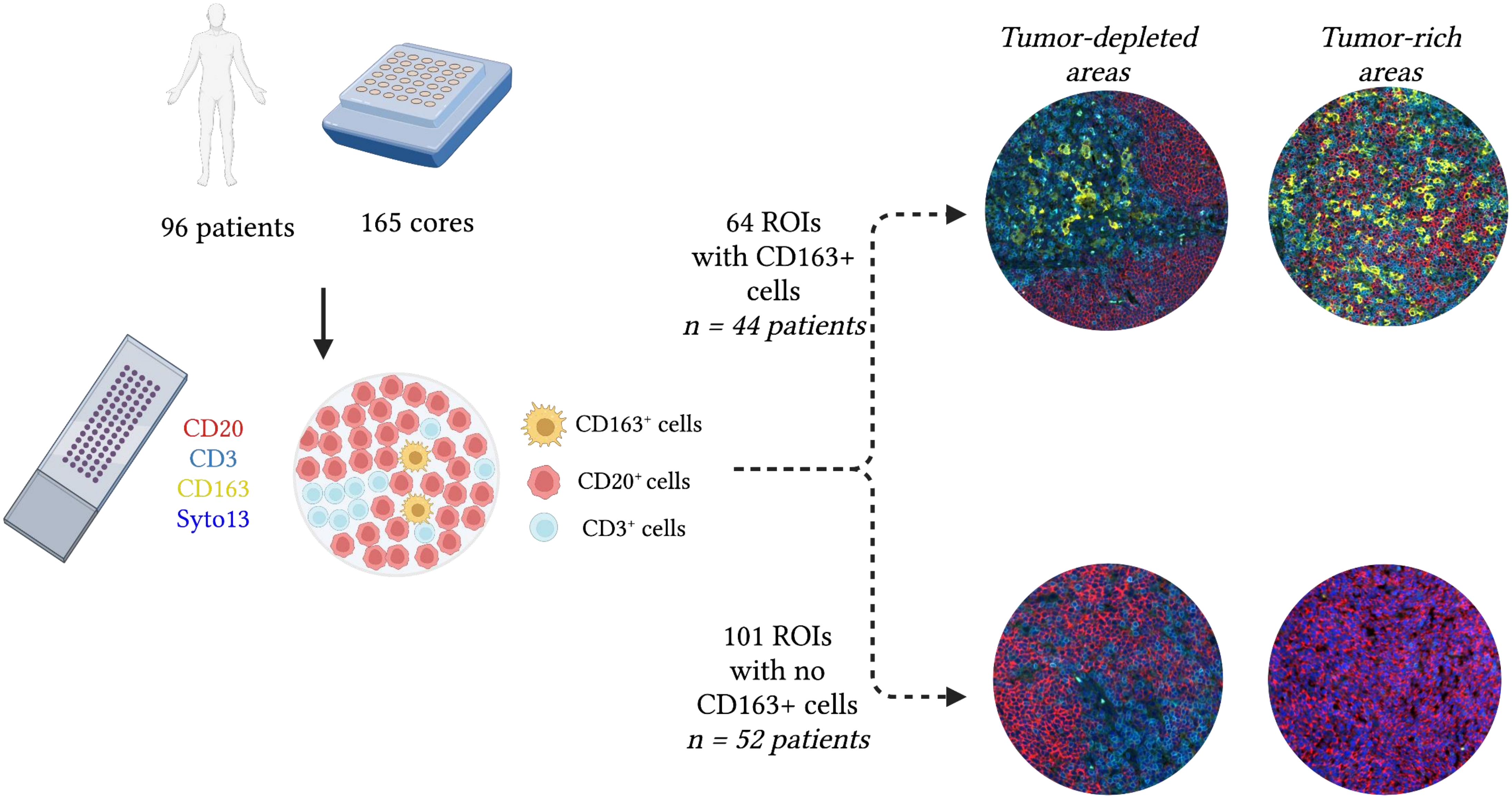
Fluorochromes visualized as:
DNA CD20 CD3 CD163

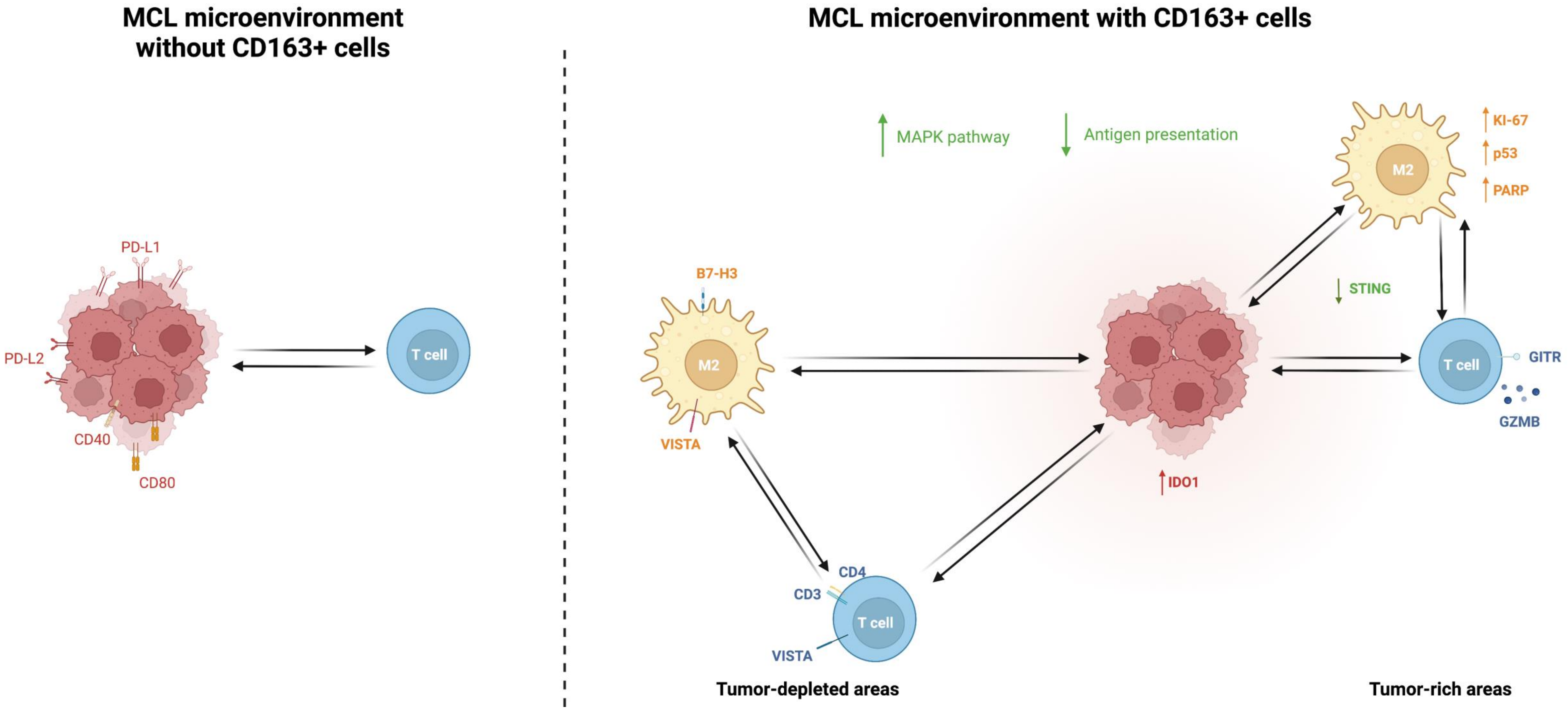
Cells marked for collection:
B-cells, T-cells and Macrophages

How does the distance between macrophages and tumor cells affect the molecular profile of the cells?

Have the immune-composition/proximity between tumor and immune cells an impact on outcome?




96 patients
165 cores
CD20
CD3
CD163
Syto13
CD163+ cells
CD20+ cells
CD3+ cells
64 ROIs with CD163+ cells
n = 44 patients
101 ROIs with no CD163+ cells
n = 52 patients
Tumor-depleted areas
Tumor-rich areas


MCL microenvironment without CD163+ cells
PD-L1
PD-L2
CD40
CD80
T cell
MCL microenvironment with CD163+ cells
MAPK pathway
Antigen presentation
M2
KI-67
p53
PARP
B7-H3
M2
VISTA
STING
T cell
GITR
GZMB
IDO1
CD4
CD3
T cell
VISTA
Tumor-depleted areas
Tumor-rich areas

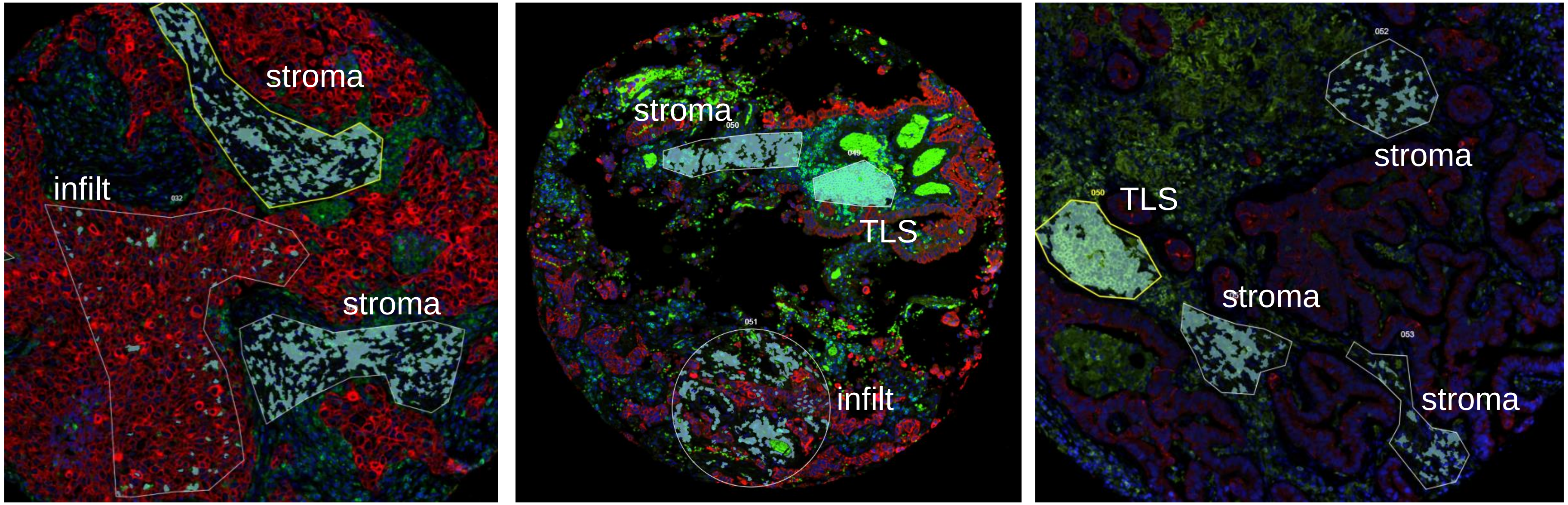
Digital spatial profiling of NSCLC

In collaboration with Professor Patrick Micke, Uppsala University

Ass. Professor Anna Gerdtsson

Background

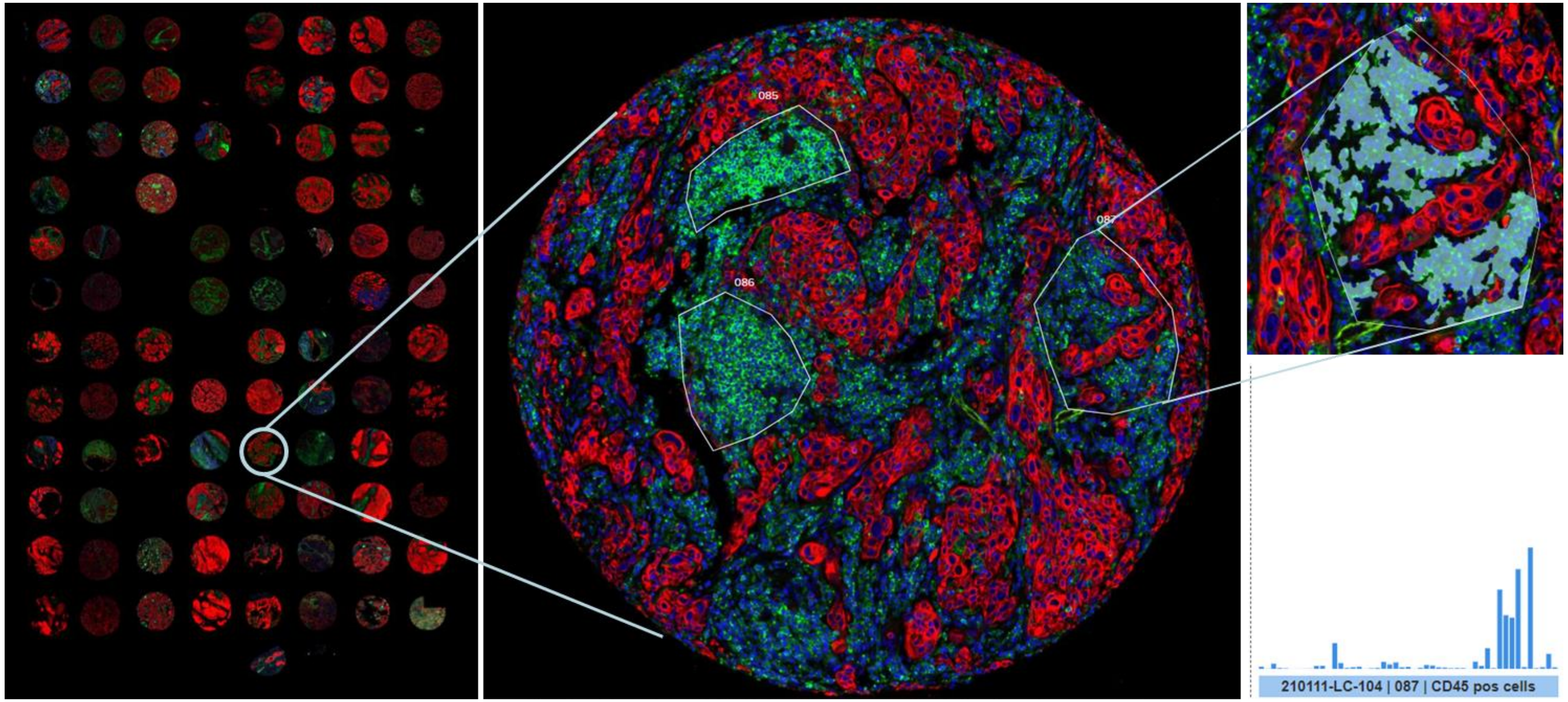
- Checkpoint inhibition approved for first- and second line treatment of advanced stage NSCLC
- Limited performance of PD-L1 as predictive biomarker



Objectives

1. Deconvolute composition of spatial CD45 niches (infiltrating, stroma, TLS)
2. Assess spatial phenotypes in relation to PD-L1 status and survival
3. Characterize spatial heterogeneity of immune infiltration within and across tumors

Selection and segmentation of CD45 regions

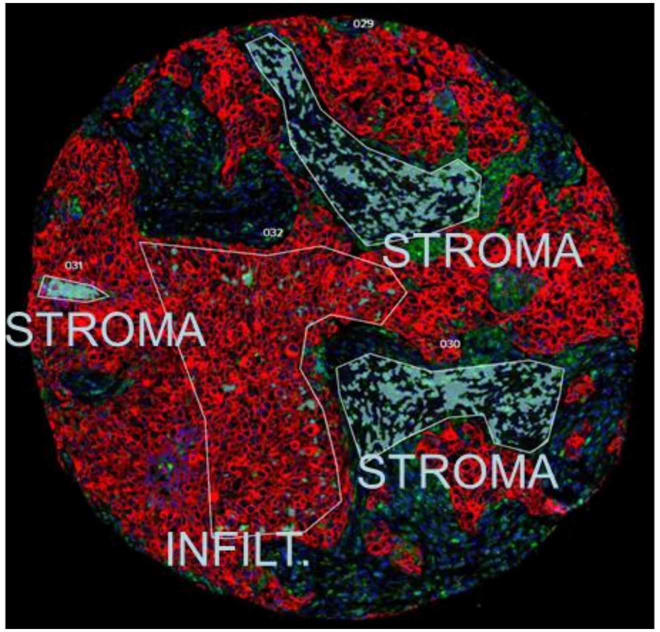


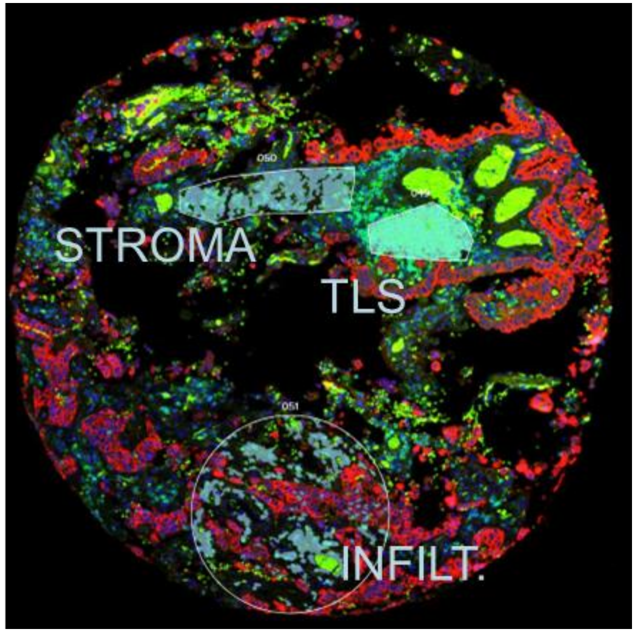
- TMA with duplicate 1mm cores
- Stained with Syto13, Pan-CK, CD45

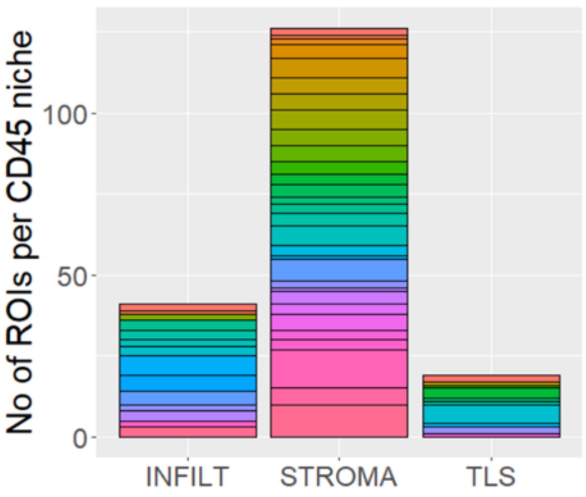
- AOIs defined by segmentation of CD45+, Syto13+ cells.
- Antibody-coupled oligo identifiers released from each AOI by UV illumination
- Collected in separate plate wells, and quantified after hybridization to color-coded barcodes.

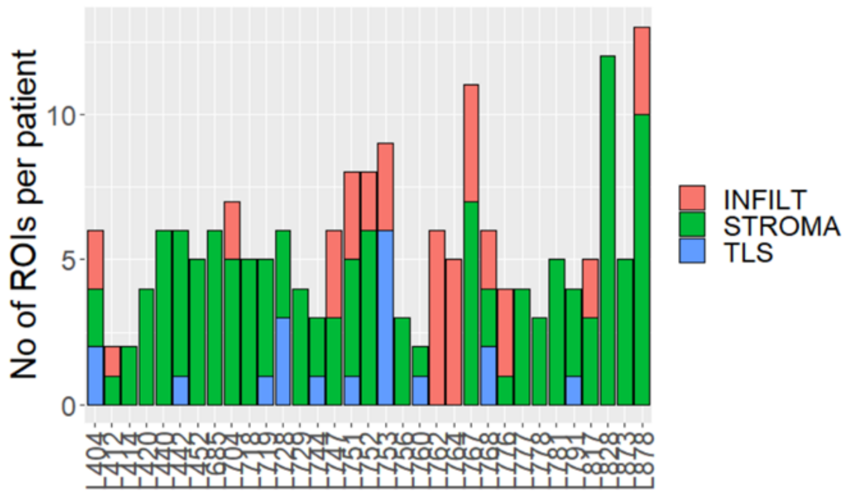
Annotation of distinct spatial immune ROIs

- ROIs classified by spatial distribution:
  - stromal CD45
  - infiltrated CD45 (dispersed among tumor cells)
  - TLS (dense stromal CD45 compartments)
- Multiple ROIs, frequently of different spatial types, were selected per patient.
- ROIs also classified by distance to tumour region







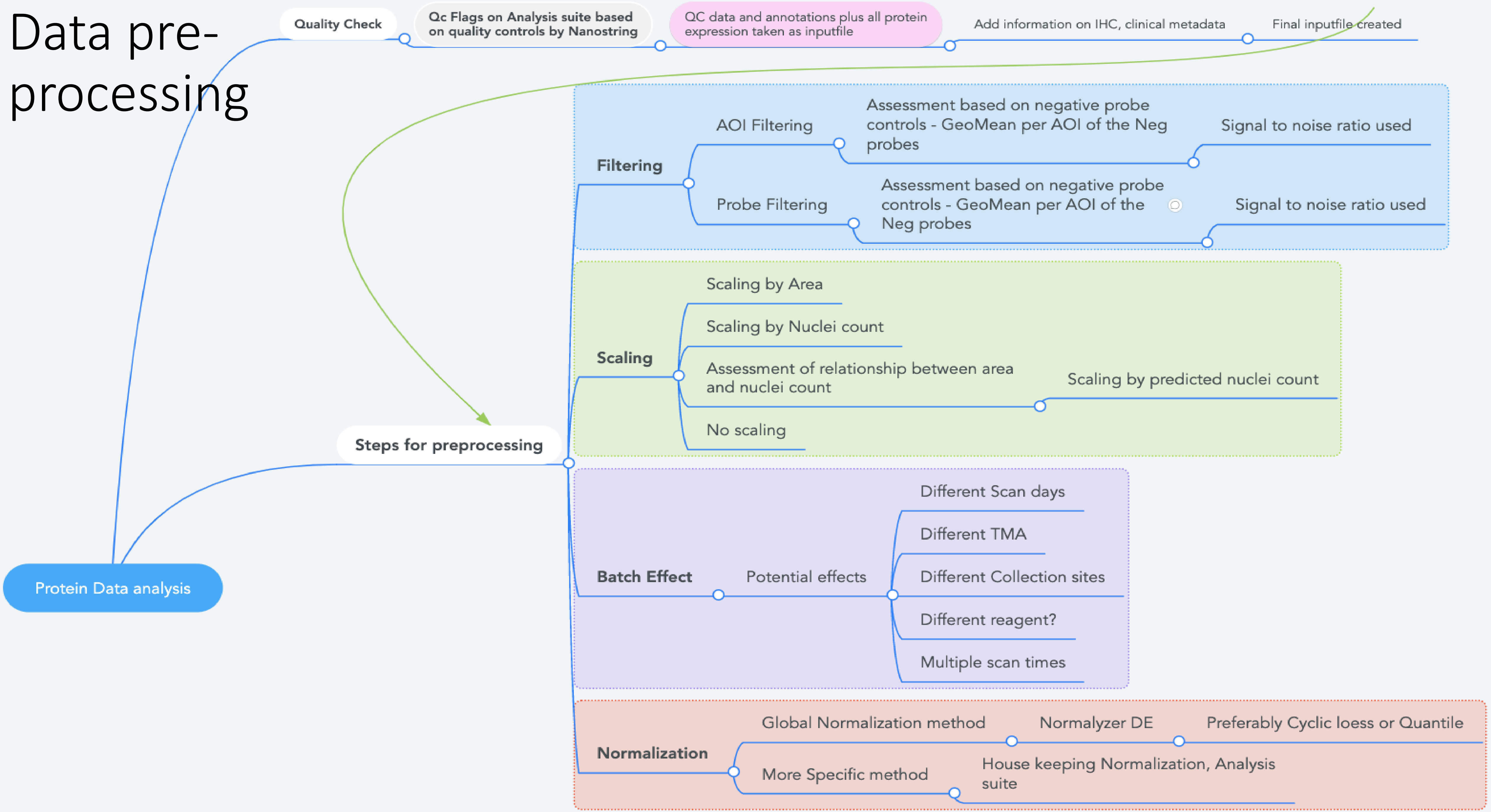


General conclusions so far

- Spatial proteomics can reveal functional differences in immune cells based on their location and proximity to other cells

- Spatial proteomics reveal tumor heterogeneity related to immune infiltration of specific cells

Data pre-processing



Protein Data analysis

Quality Check

Qc Flags on Analysis suite based on quality controls by Nanostring

QC data and annotations plus all protein expression taken as inputfile

Add information on IHC, clinical metadata

Final inputfile created

Steps for preprocessing

Filtering

- AOI Filtering
  - Assessment based on negative probe controls - GeoMean per AOI of the Neg probes
    - Signal to noise ratio used
- Probe Filtering
  - Assessment based on negative probe controls - GeoMean per AOI of the Neg probes
    - Signal to noise ratio used

Scaling

- Scaling by Area
- Scaling by Nuclei count
- Assessment of relationship between area and nuclei count
  - Scaling by predicted nuclei count
- No scaling

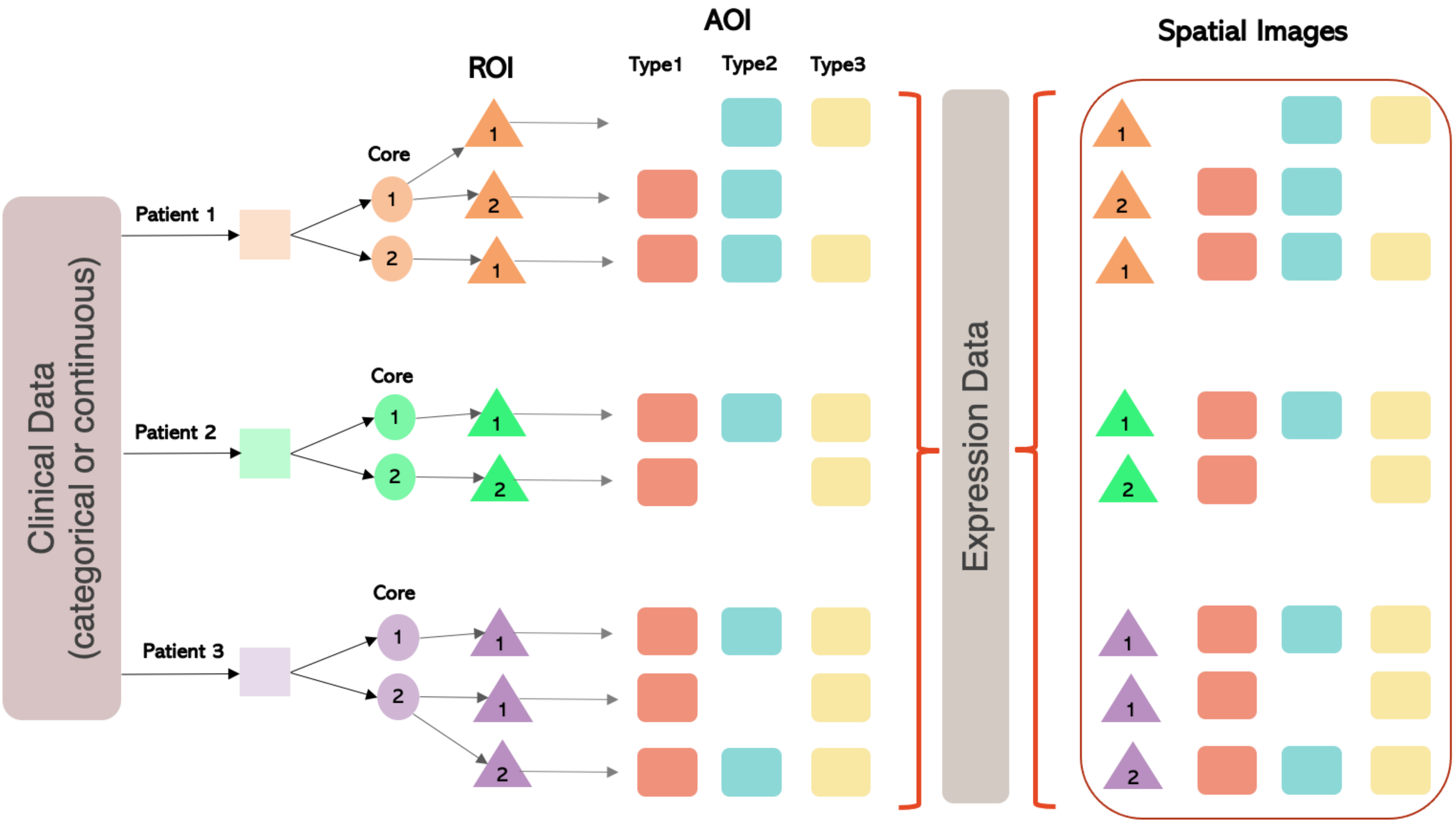
Batch Effect

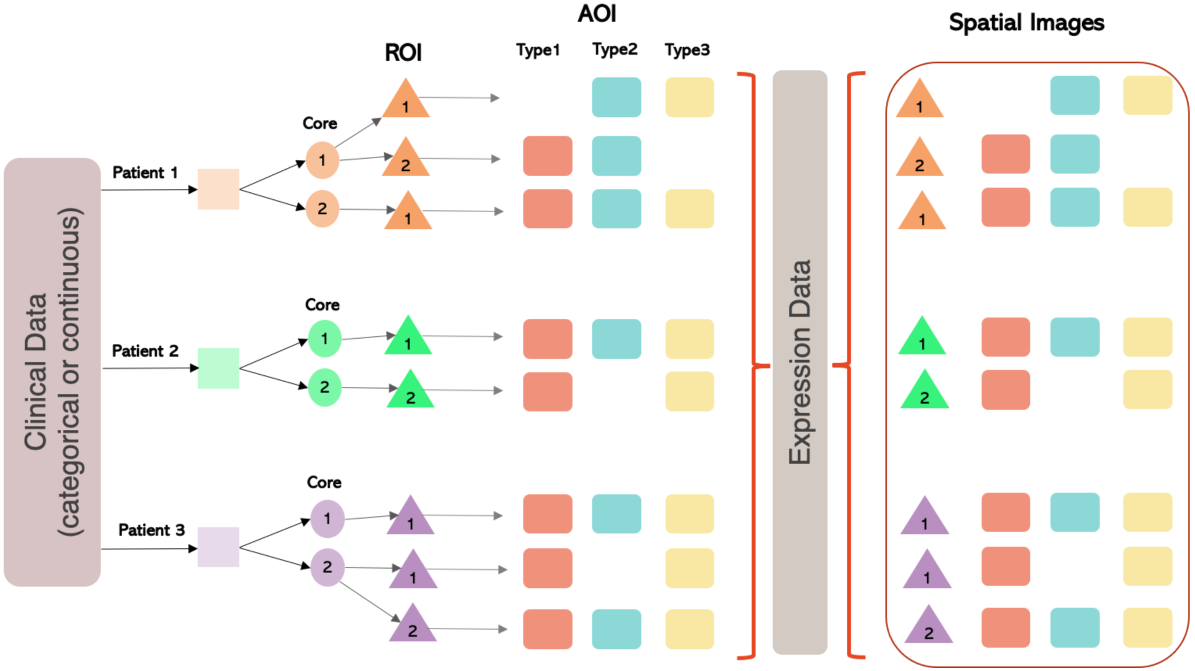
- Potential effects
  - Different Scan days
  - Different TMA
  - Different Collection sites
  - Different reagent?
  - Multiple scan times

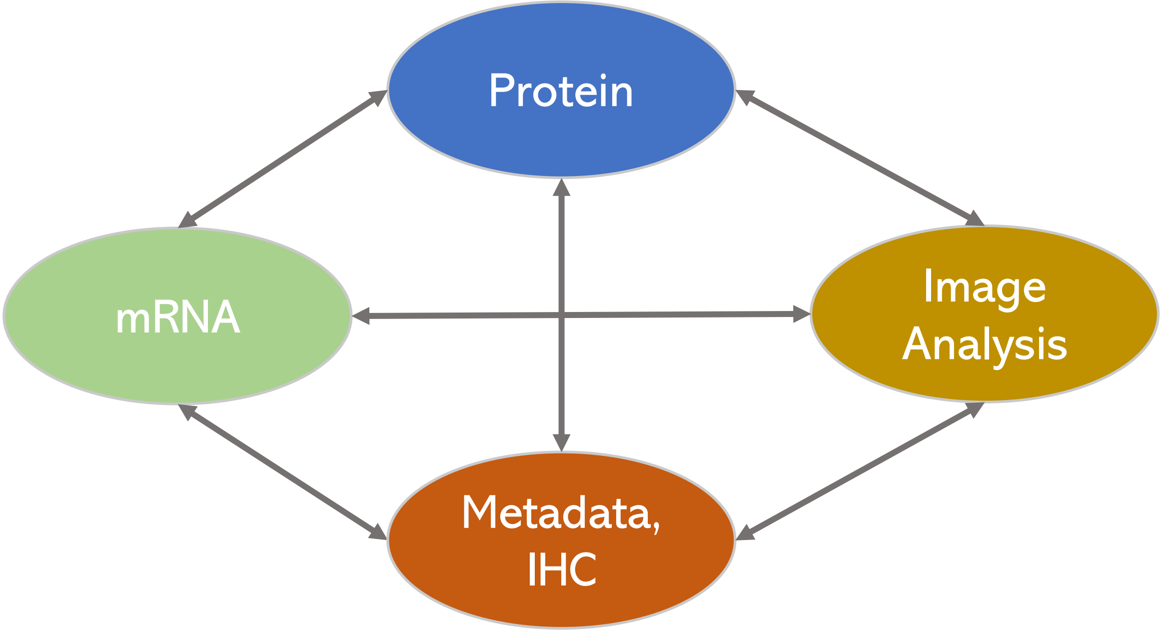
Normalization

- Global Normalization method
  - Normalyzer DE
    - Preferably Cyclic loess or Quantile
- More Specific method
  - House keeping Normalization, Analysis suite

Data Processing requires development of novel bioinformatic workflow







Lavanya Lokhande

In house development, with support from collaborators

NATIONAL BIOINFORMATICS INFRASTRUCTURE SWEDEN

Louella Vasquez and Paul Theodor Pyl

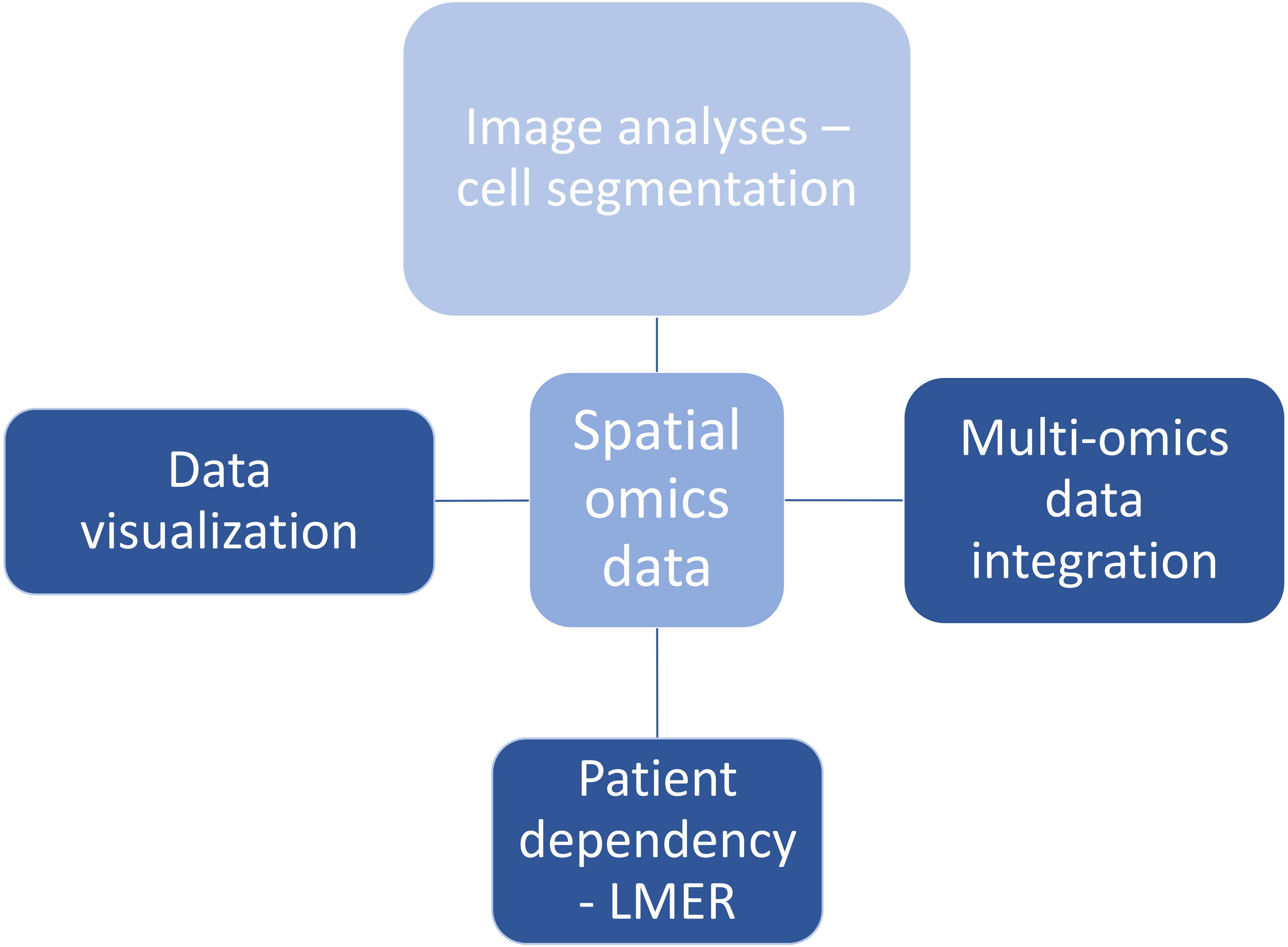


Image analyses – cell segmentation

Data visualization

Spatial omics data

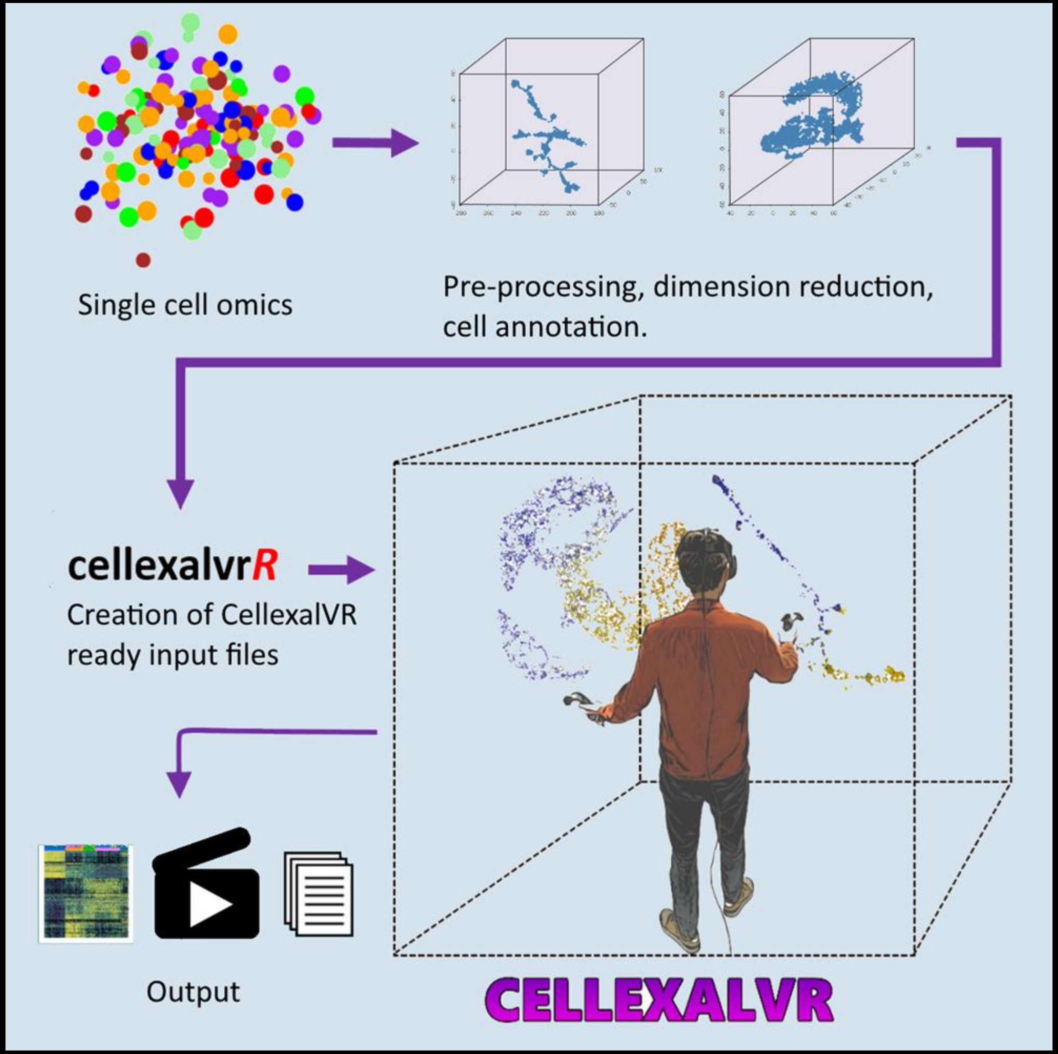
Multi-omics data integration

Patient dependency - LMER

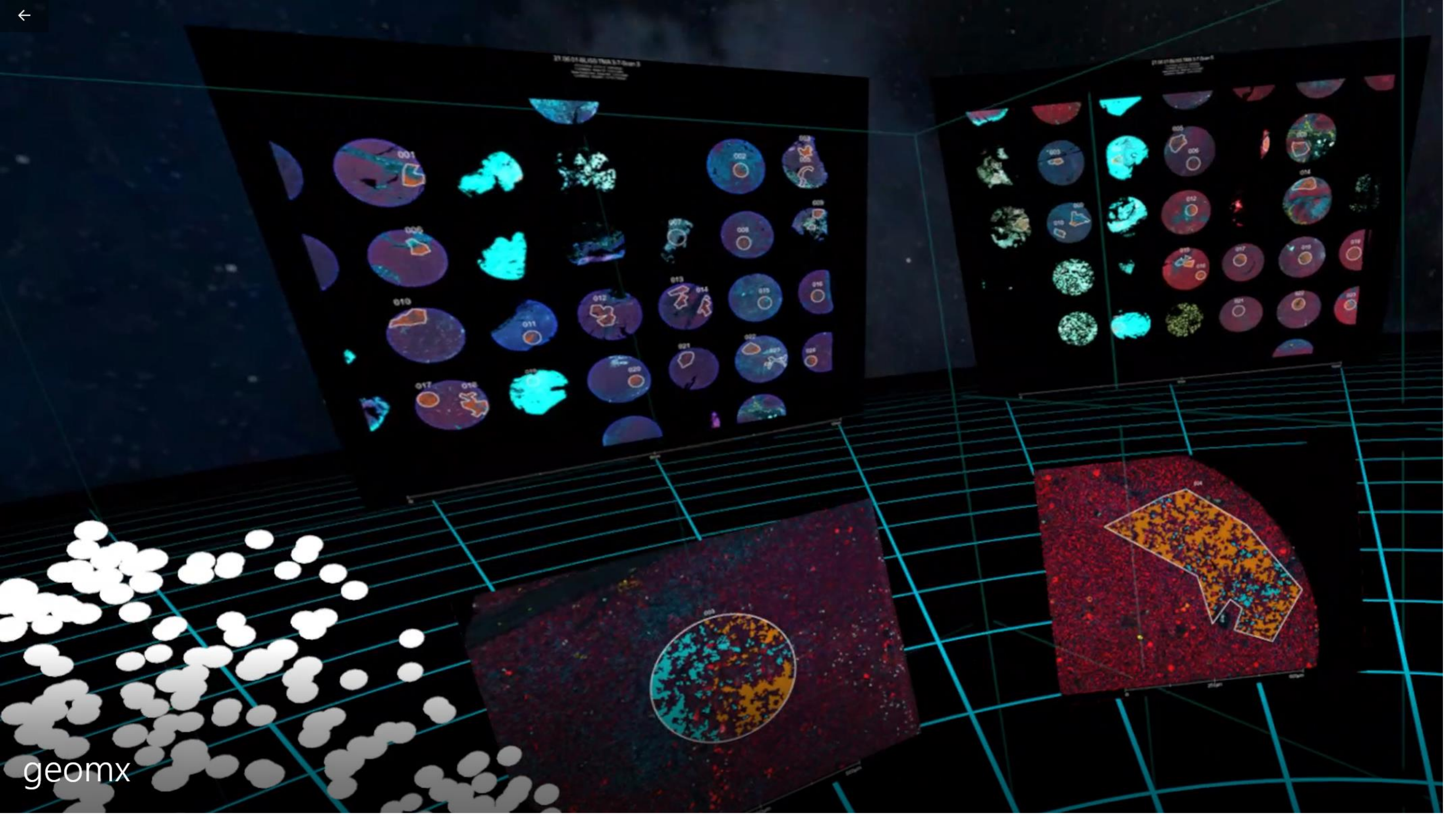
CellexalVR: A virtual reality platform to visualize and analyze single-cell omics data

Oscar Legetth, Johan Rodhe, Stefan Lang, Parashar Dhapola, Mattias Wallergård, Shamit Soneji

iScience
Volume 24 Issue 11 (November 2021)
DOI: 10.1016/j.isci.2021.103251



iScience 2021 24DOI: (10.1016/j.isci.2021.103251)



geomx

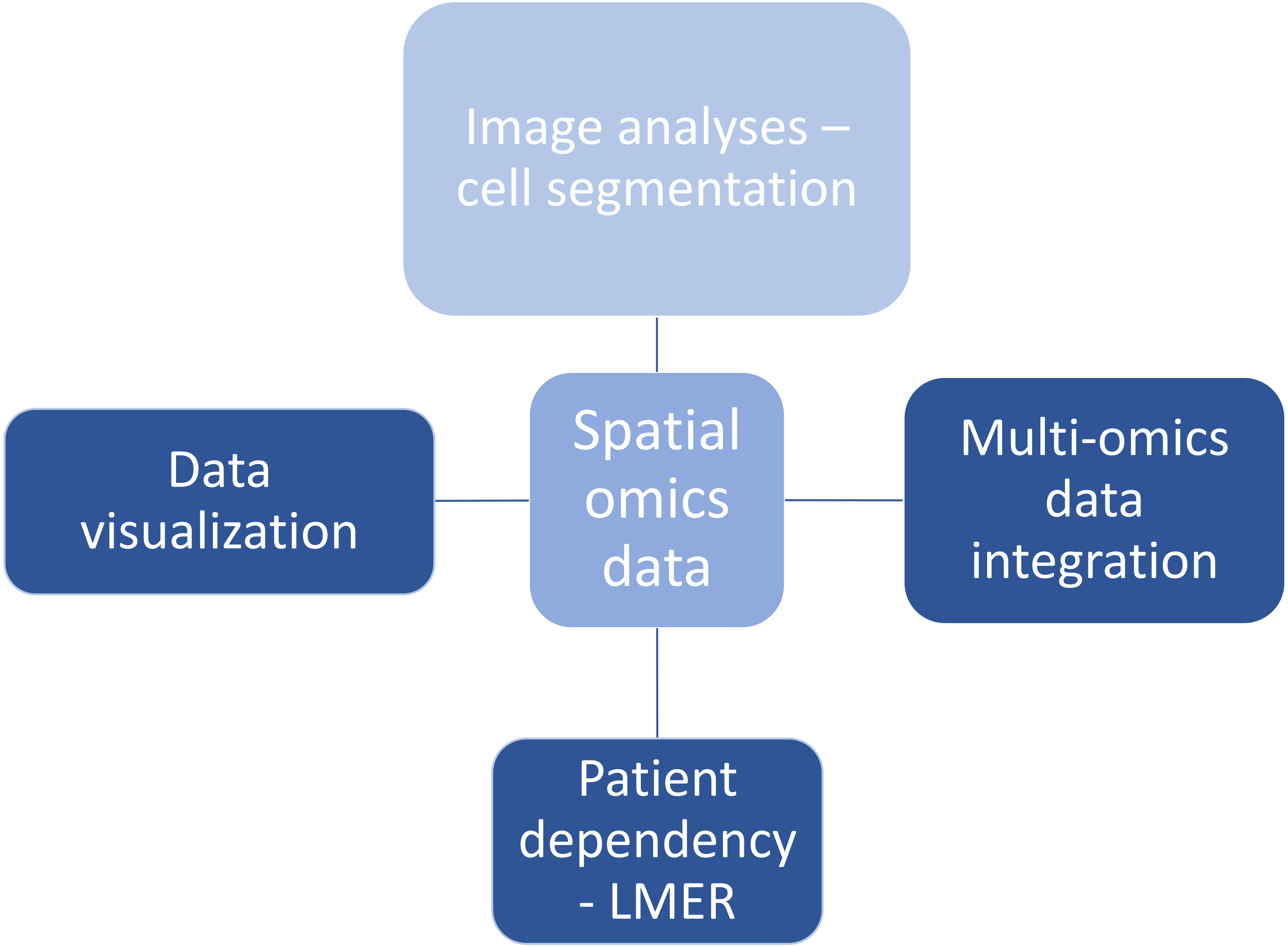


Image analyses – cell segmentation

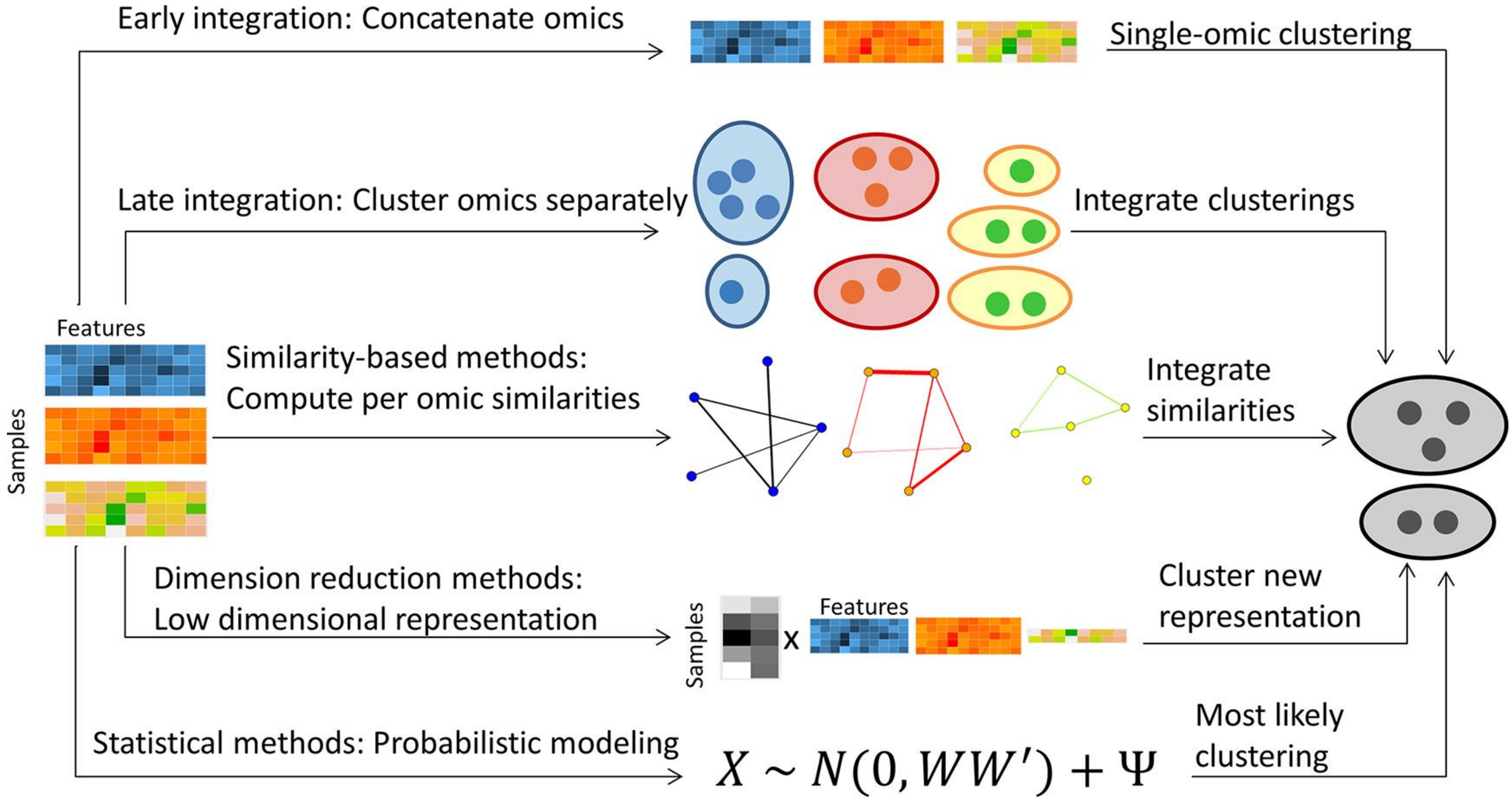
Data visualization

Spatial omics data

Multi-omics data integration

Patient dependency - LMER

Data integration



Early integration: Concatenate omics

Single-omic clustering

Late integration: Cluster omics separately

Integrate clusterings

Similarity-based methods:
Compute per omic similarities

Integrate
similarities

Dimension reduction methods:
Low dimensional representation

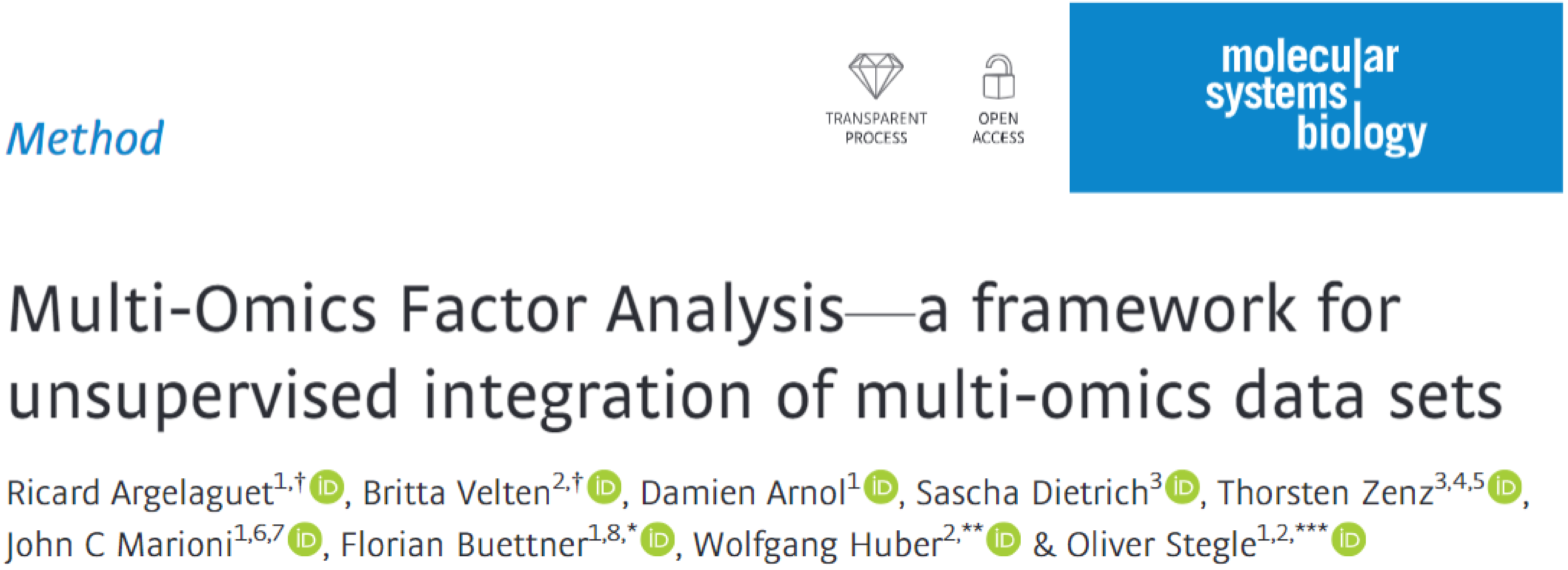
Cluster new
representation

Statistical methods: Probabilistic modeling

$X \sim N(0, WW') + \Psi$

Most likely
clustering

Rappoport et al, 2018



Method

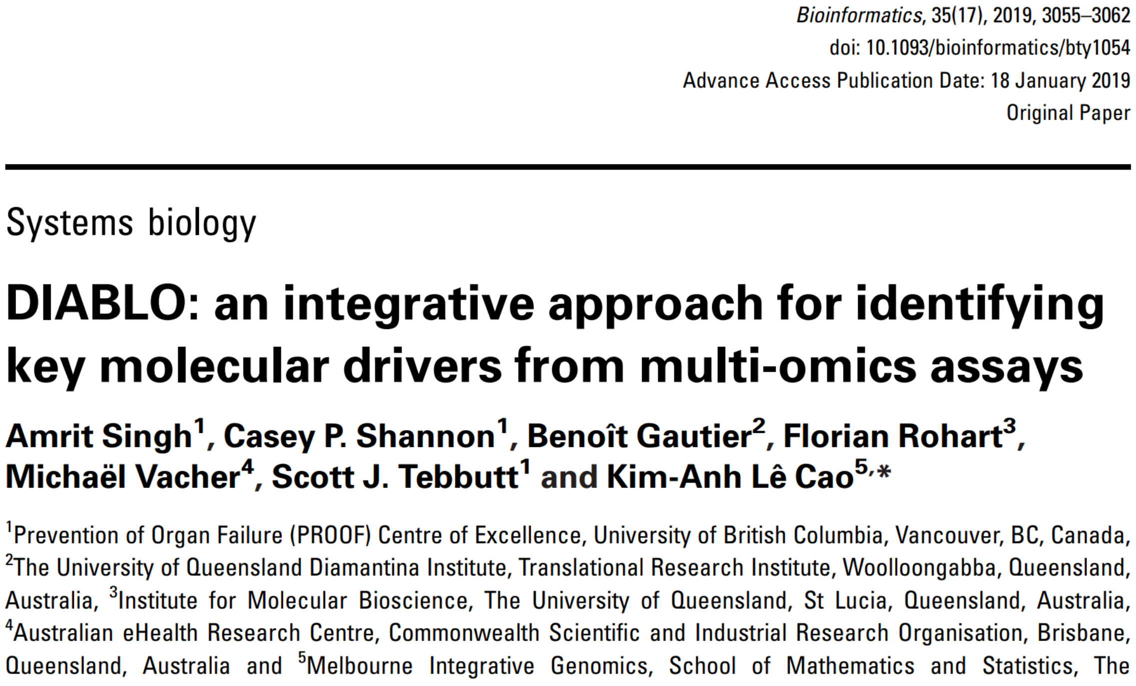
TRANSPARENT PROCESS

OPEN ACCESS

molecular systems biology

Multi-Omics Factor Analysis—a framework for unsupervised integration of multi-omics data sets

Ricard Argelaguet[1,†], Britta Velten[2,†], Damien Arnol[1], Sascha Dietrich[3], Thorsten Zenz[3,4,5], John C Marioni[1,6,7], Florian Buettner[1,8,*], Wolfgang Huber[2,**] & Oliver Stegle[1,2,***]



Bioinformatics, 35(17), 2019, 3055–3062
doi: 10.1093/bioinformatics/bty1054
Advance Access Publication Date: 18 January 2019
Original Paper

Systems biology

DIABLO: an integrative approach for identifying key molecular drivers from multi-omics assays

Amrit Singh[1], Casey P. Shannon[1], Benoît Gautier[2], Florian Rohart[3], Michaël Vacher[4], Scott J. Tebbutt[1] and Kim-Anh Lê Cao[5,*]

[1]Prevention of Organ Failure (PROOF) Centre of Excellence, University of British Columbia, Vancouver, BC, Canada, [2]The University of Queensland Diamantina Institute, Translational Research Institute, Woolloongabba, Queensland, Australia, [3]Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland, Australia, [4]Australian eHealth Research Centre, Commonwealth Scientific and Industrial Research Organisation, Brisbane, Queensland, Australia and [5]Melbourne Integrative Genomics, School of Mathematics and Statistics, The

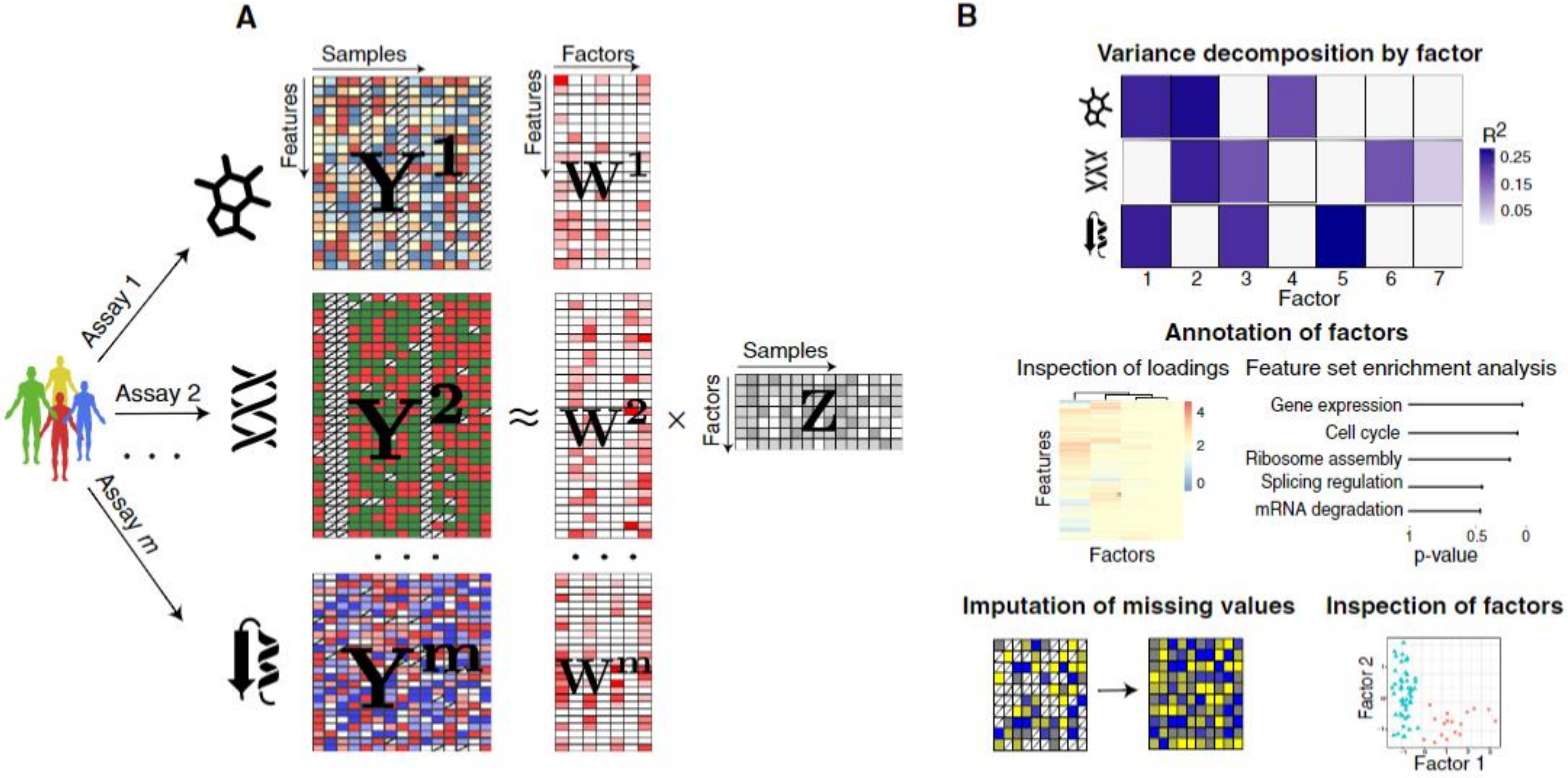


Figure 1. Multi-Omics Factor Analysis: model overview and downstream analyses.

A Model overview: MOFA takes M data matrices as input ($\mathbf{Y}^1, \ldots, \mathbf{Y}^M$), one or more from each data modality, with co-occurrent samples but features that are not necessarily related and that can differ in numbers. MOFA decomposes these matrices into a matrix of factors (**Z**) for each sample and *M* weight matrices, one for each data modality ($\mathbf{W}^1, \ldots, \mathbf{W}^M$). White cells in the weight matrices correspond to zeros, i.e. inactive features, whereas the cross symbol in the data matrices denotes missing values.

B The fitted MOFA model can be queried for different downstream analyses, including (i) variance decomposition, assessing the proportion of variance explained by each factor in each data modality, (ii) semi-automated factor annotation based on the inspection of loadings and gene set enrichment analysis, (iii) visualization of the samples in the factor space and (iv) imputation of missing values, including missing assays.

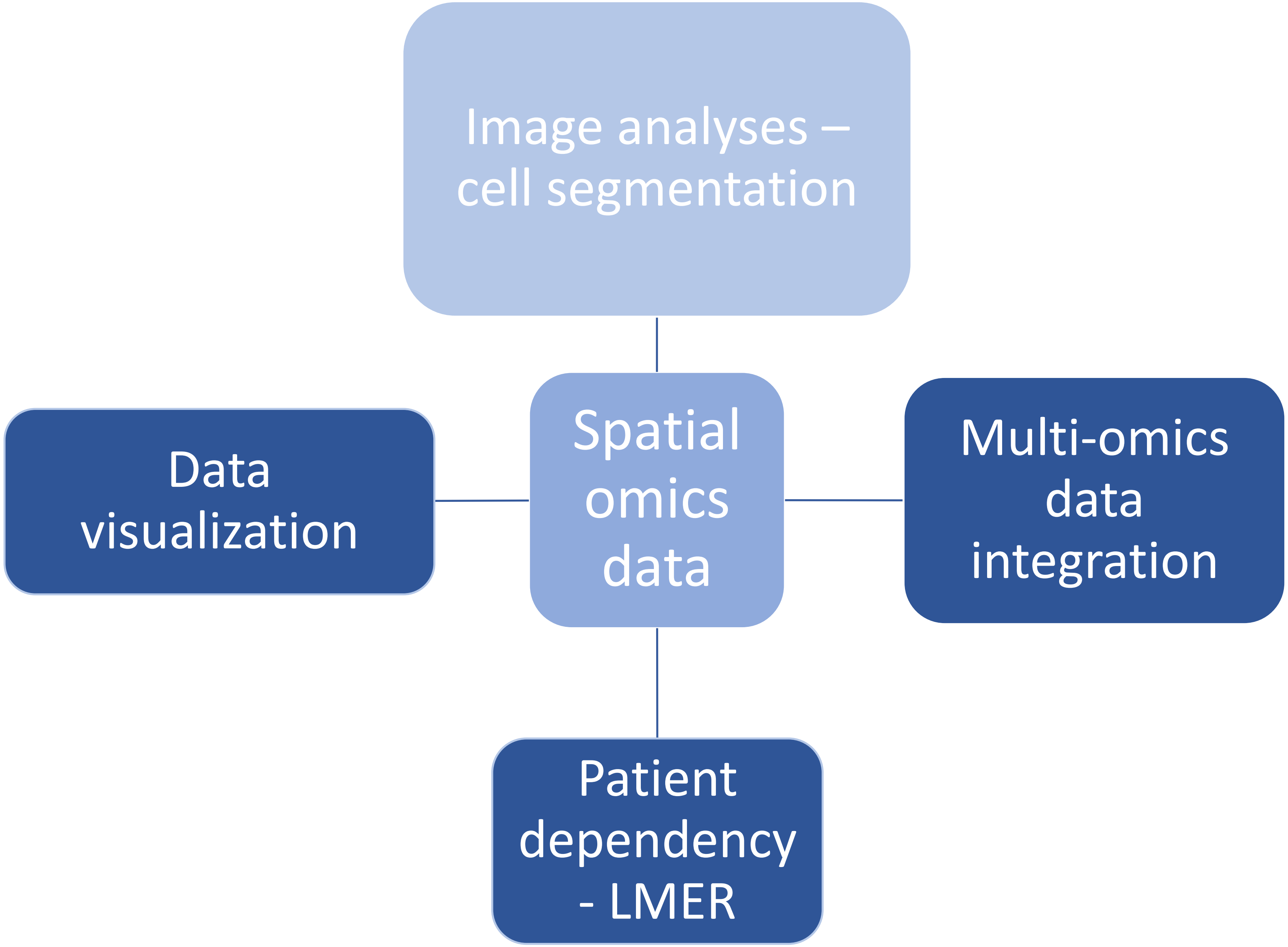


Image analyses – cell segmentation

Data visualization

Spatial omics data

Multi-omics data integration

Patient dependency - LMER

Acknowledgement

Clinical collaborators in B-cell lymphoma projects
Mats Jerkeman, Department of Oncology, Lund
Anna Porwit, Department of Pathology, Lund
Ingrid Glimelius, Department of Oncology, Uppsala
Anna Nikkarinan, Department of Oncology, Uppsala
Nordic MCL groups in Norway, Finland and Denmark

Clinical collaborators in Lung Cancer project
Patrick Micke, Department of Pathology, Uppsala University

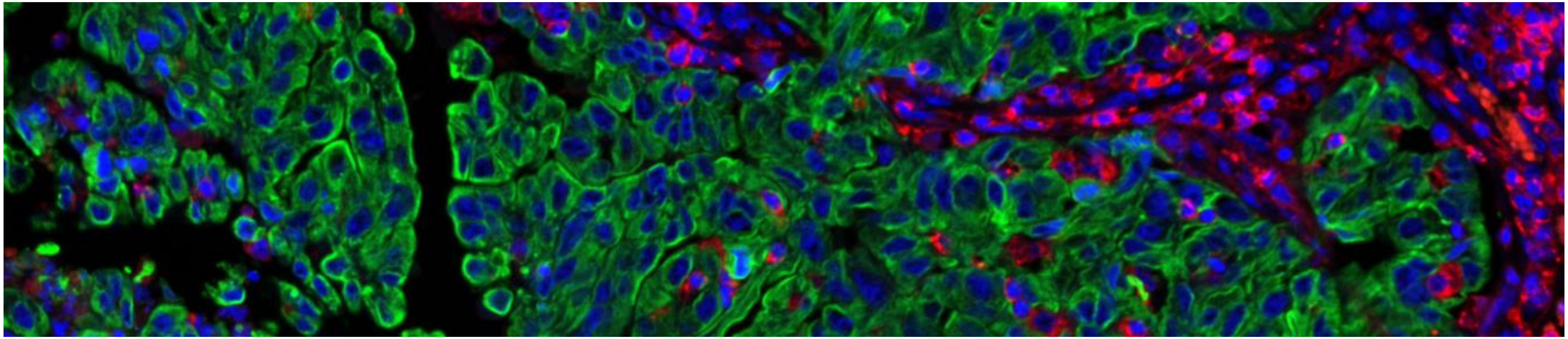
Mathematical Statistics
Andreas Jakobsson

Clinical translational genomics - LUND

SpatialOmics@LU facility
Lina Olsson
May Hassan

NBIS long term support
Paul Theodor Pyl
Louella Vasquez

The CancerTarget group researchers and students
Anna Gerdtsson, Lavanya Lokhande, Joana Rodrigues, Eirini Kalliara, Angelica Johansson, Jana Hladílková and Mattis Knulst
Affiliated guest researchers/students: Eirinaios Gkika, Christine Coffey, Daniel Nilsson, Aura Zelco, Xinning Luan, Elias Carlsson, Huaqiang Ouyang



LUND UNIVERSITY
LTH
FACULTY OF ENGINEERING

LUND UNIVERSITY
LUCC
CANCER CENTRE

Stefan Paulssons Cancerfond

Mats Paulssons stiftelse
för forskning, innovation och samhällsbyggande

CANCERFONDEN

Fru Berta Kamprads stiftelse
för utforskning och bekämpning av cancersjukdomar

Stiftelsen Cancera

Co-Funded by the European Union
This project has received co-funding from the European Union's Horizon 2020 Framework Programme for Research and Innovation under grant agreement 754299

Co-Funded by the European Union
This project has received co-funding from the European Union's Horizon 2020 Framework Programme for Research and Innovation under grant agreement 847583

NBIS
NATIONAL BIOINFORMATICS INFRASTRUCTURE SWEDEN

L M K
STIFTELSEN