INTEGRATIVE TOOLS FOR SPATIAL AND MULTIOMICS DATA

eSSENCE project 2023-2024

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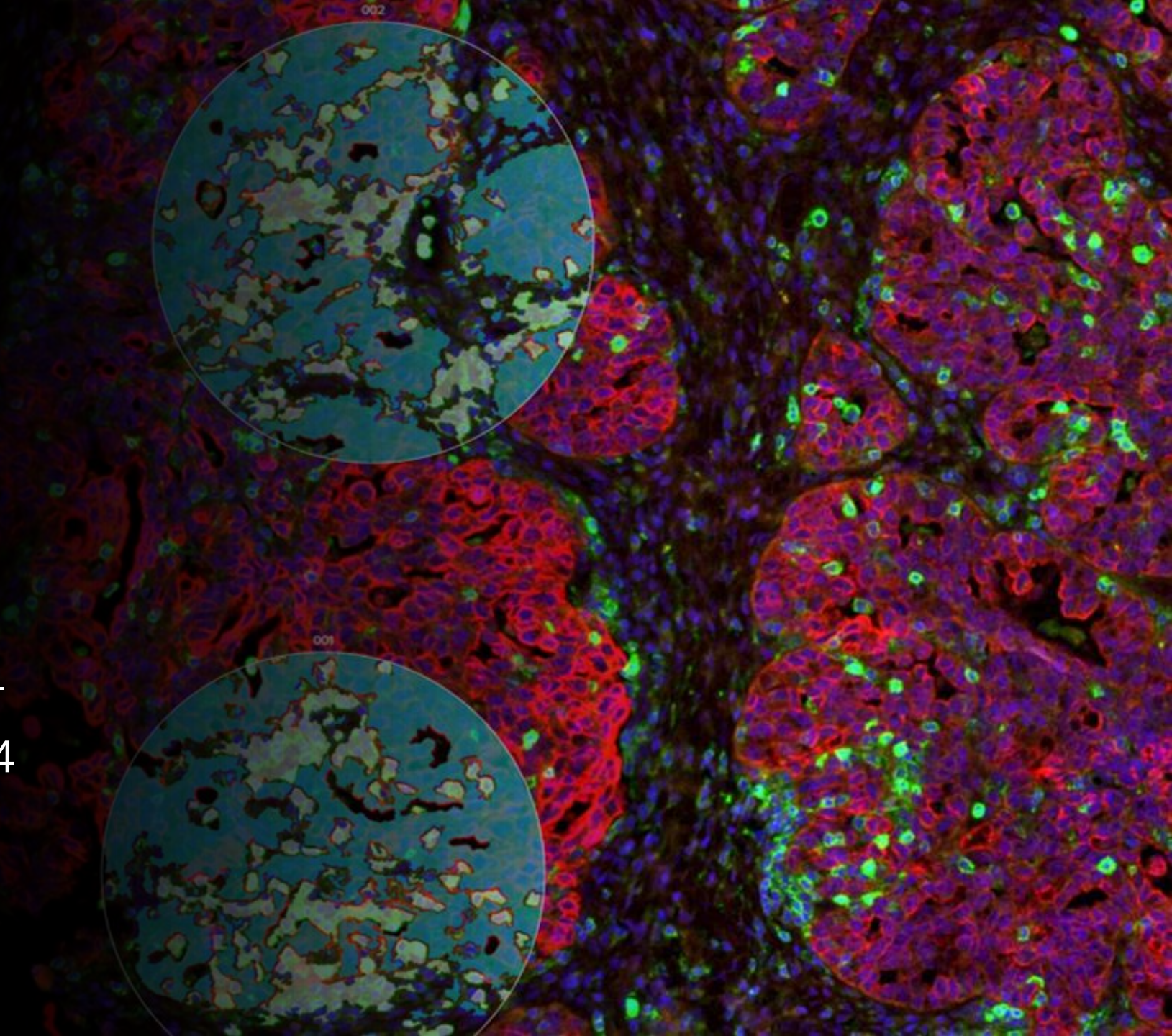
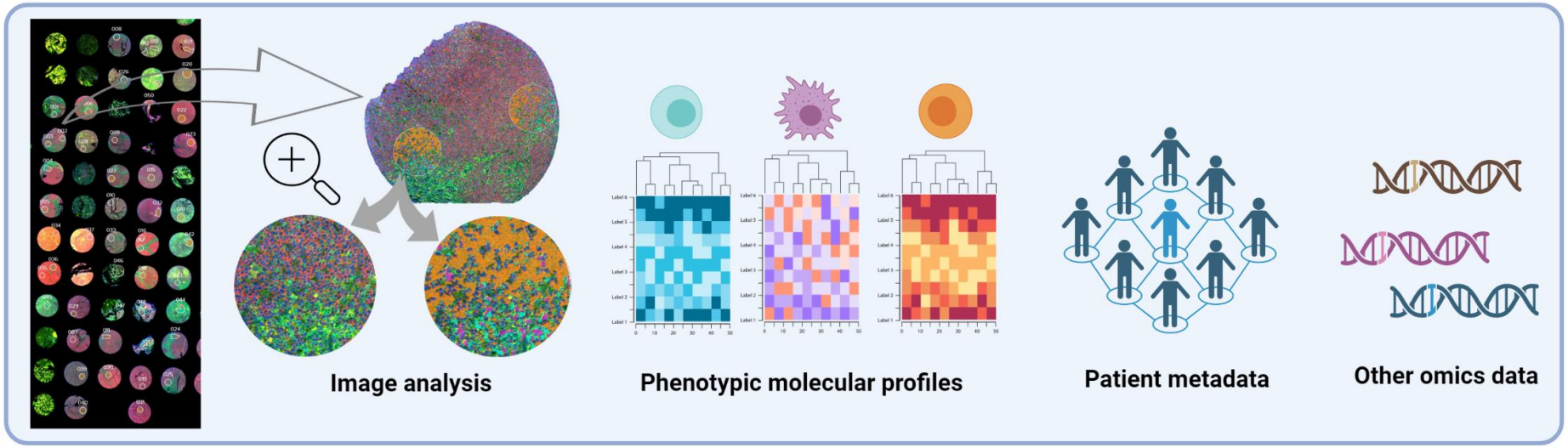
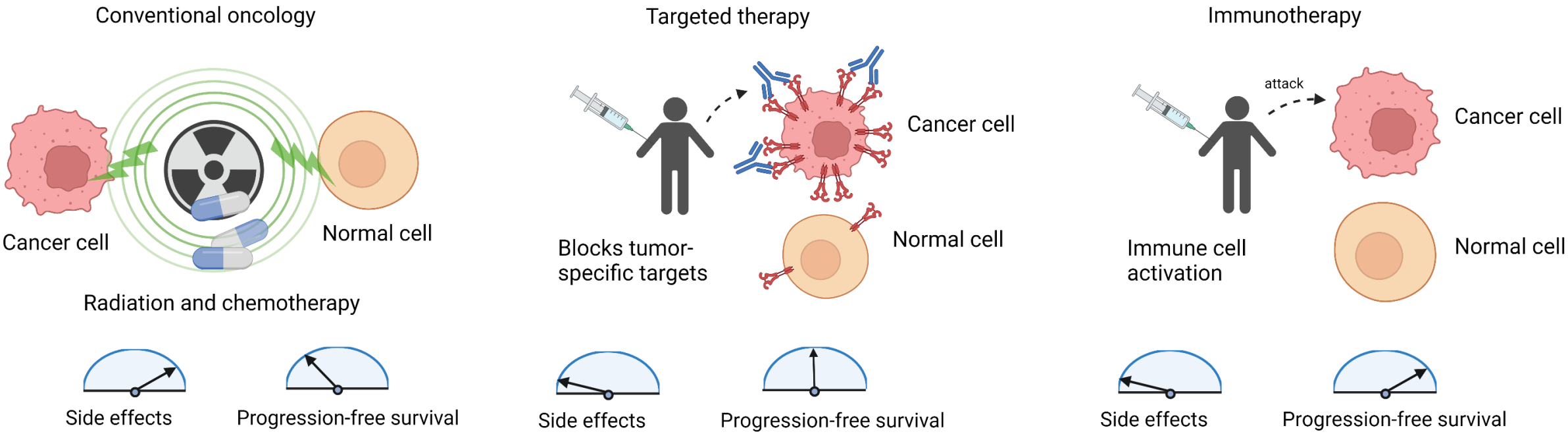


Integration of spatial and multiomics data to provide models for tumor stratification

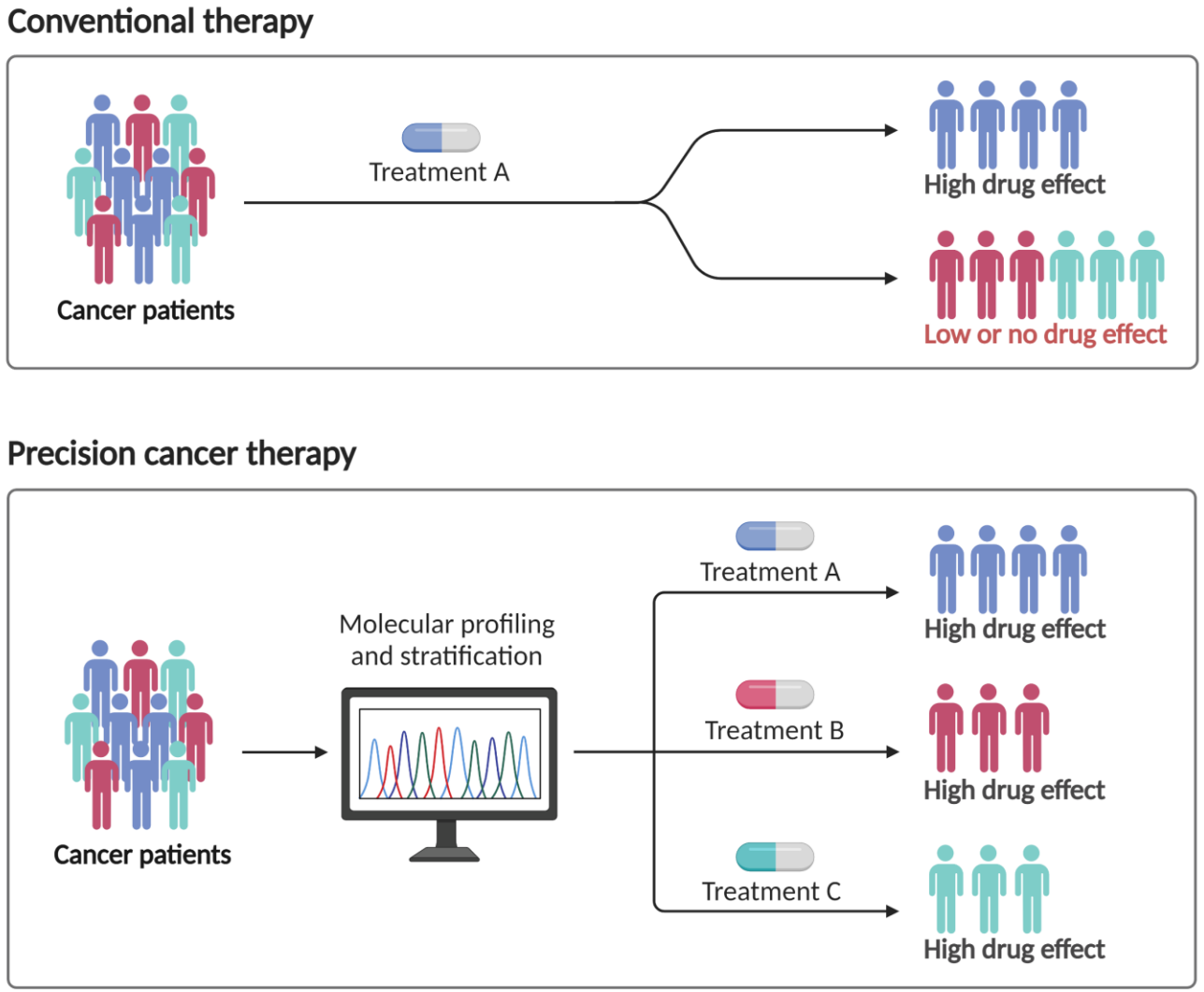


Targeted therapies, including immunotherapy, has led to increased survival rate for cancer patients

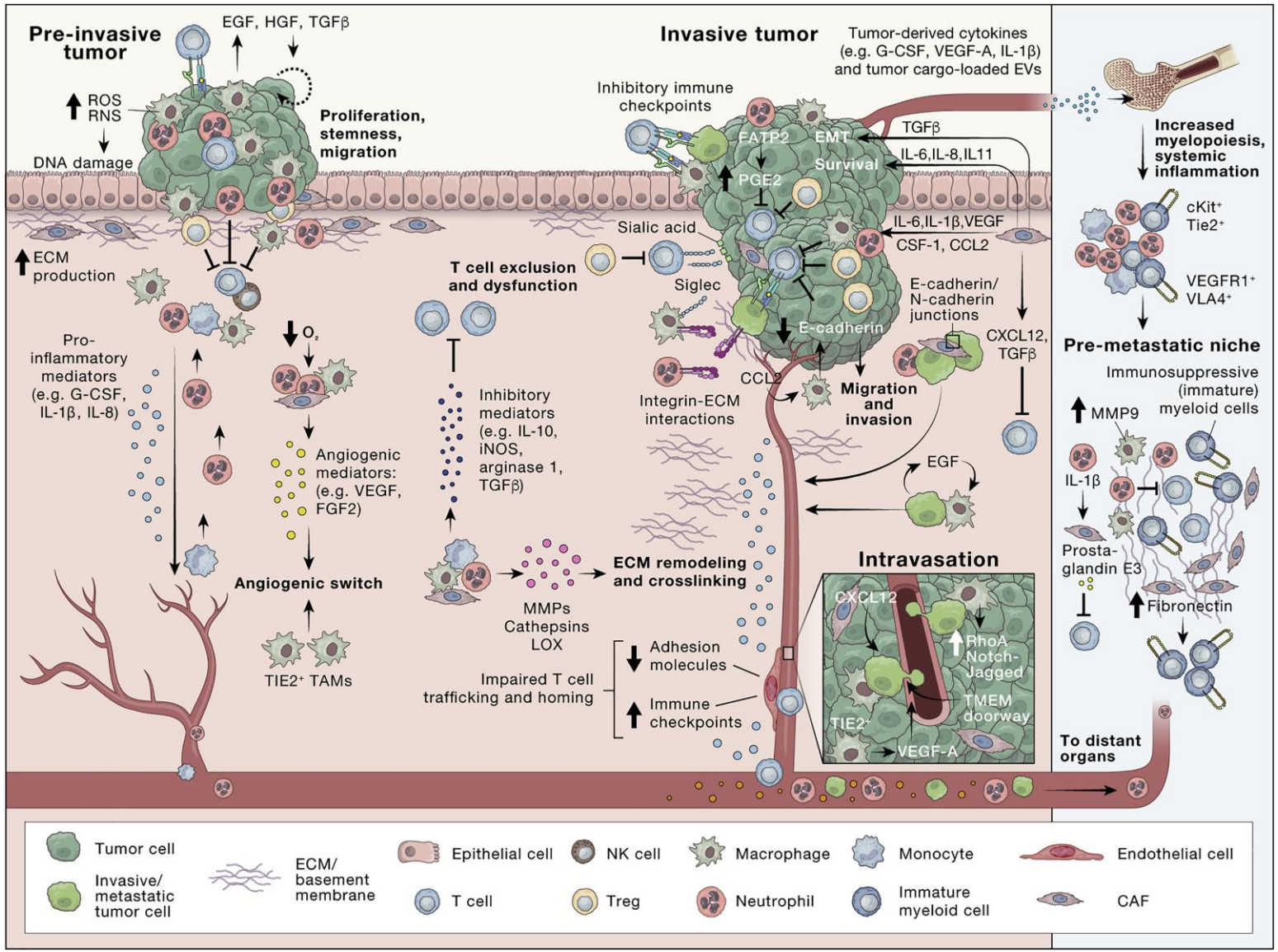


- Immunotherapy provides longer PFS (and even cure!) in responders
- For most cancers, only 15-20% of patients respond to available check point blockers

Precision medicine by molecular profiling of tumors



The complex tumor microenvironment



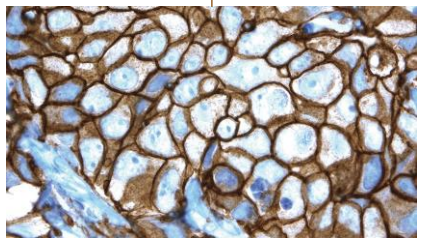
The complex composition of tumor microenvironments needs to be understood in order to make optimal treatment selection for individual patients

Karin E. de Visser, Johanna A. Joyce,
The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth,
Cancer Cell, Volume 41, Issue 3, 2023

The emerge of Spatial omics

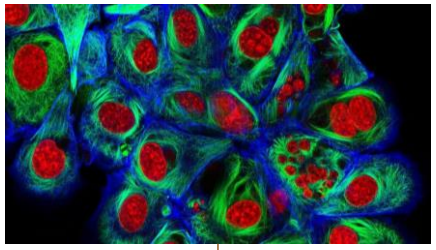
1940

Immunohistochemistry



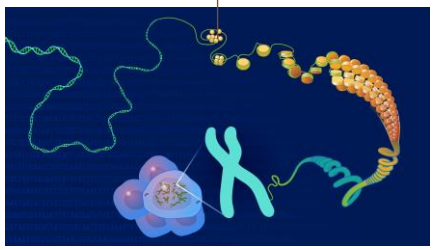
1990

Immunofluorescence



2000

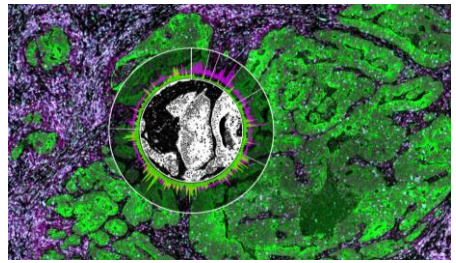
Bulk omics



2010

Single-cell omics

Spatial omics



Combines high-resolution imaging with high-plex molecular profiling

Digital Spatial Profiling – protein or RNA signatures of spatially defined regions

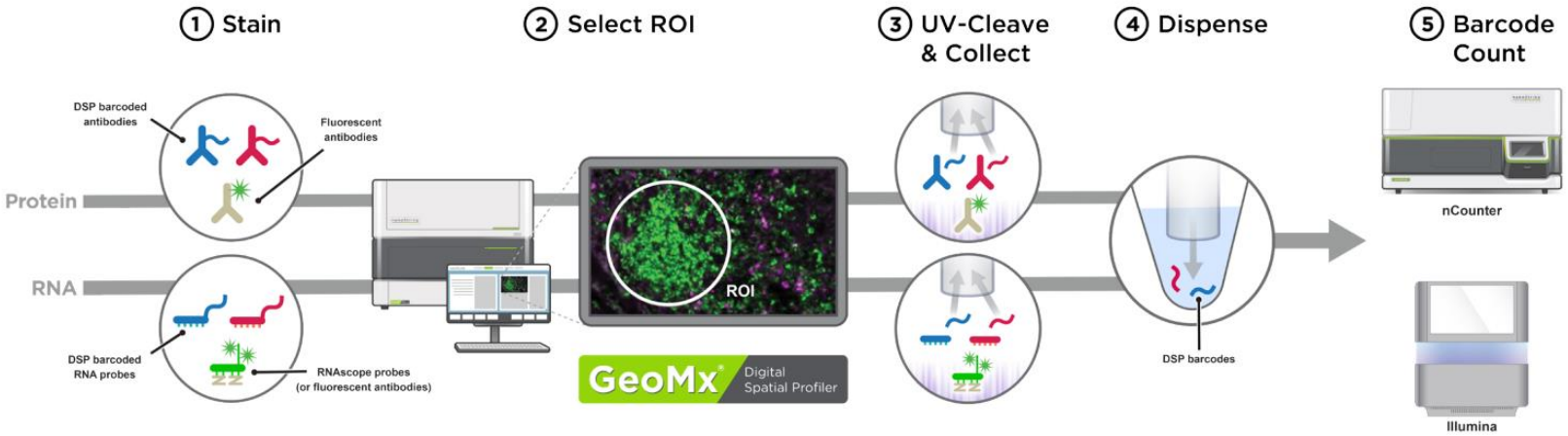
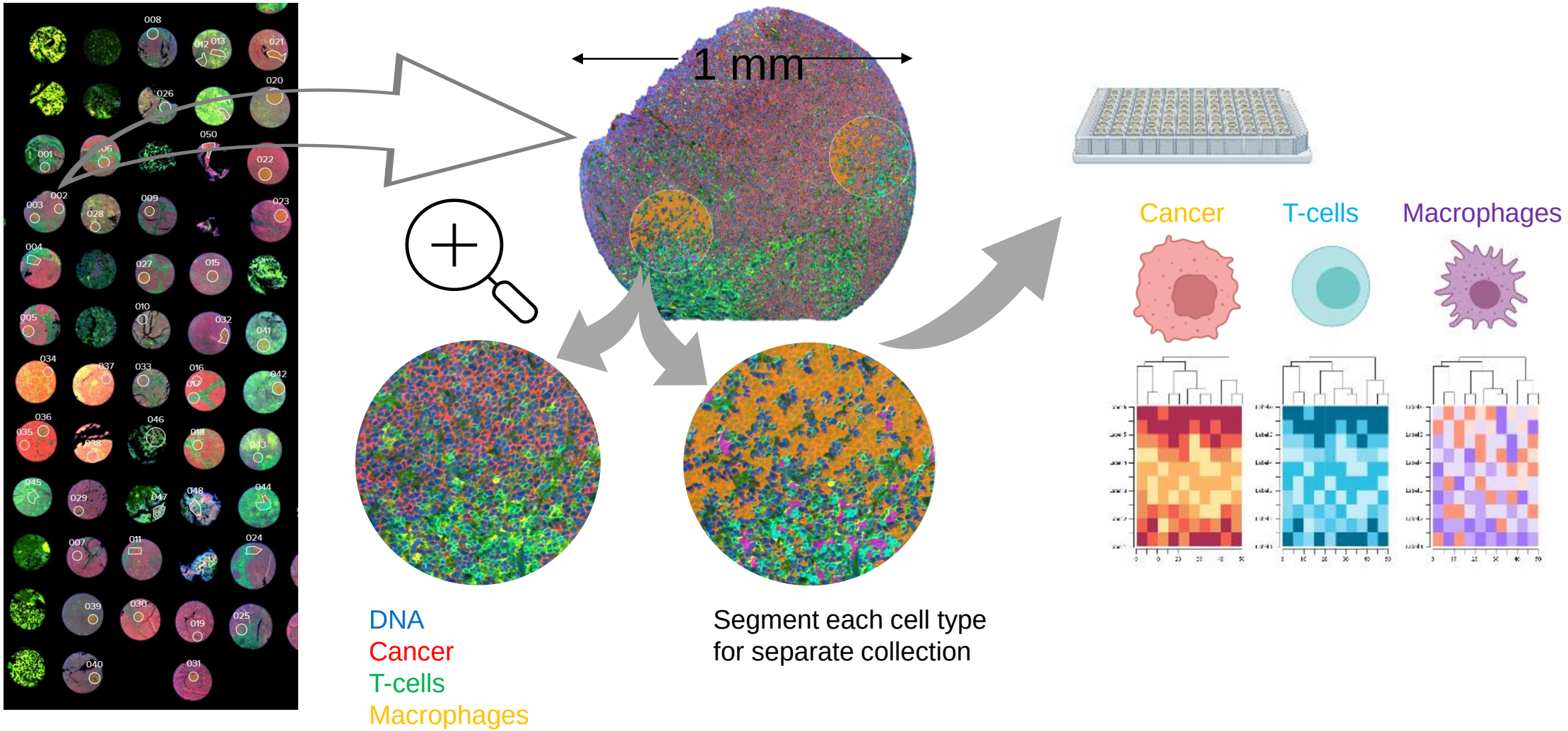


Figure from Nanostring

- The first Spatial Omics technology allowing for both protein and RNA profiling of FFPE tissues
- FFPE is the standard format for tissue diagnostics and archiving
  - Opportunities to explore biobanks for high-resolution characterization of cells and interactions in tissues
  - Facilitates direct transfer of predictive image- and biomarker-based models to the clinical settings
- DSP links molecular data to regions of interest, but does not (inherently) provide image-based metrics such as cell-to-cell distances or densities
- Frameworks for integrative analysis merging DSP data and data from other modalities have yet to be developed

Molecular profiling of distinct cellular phenotypes

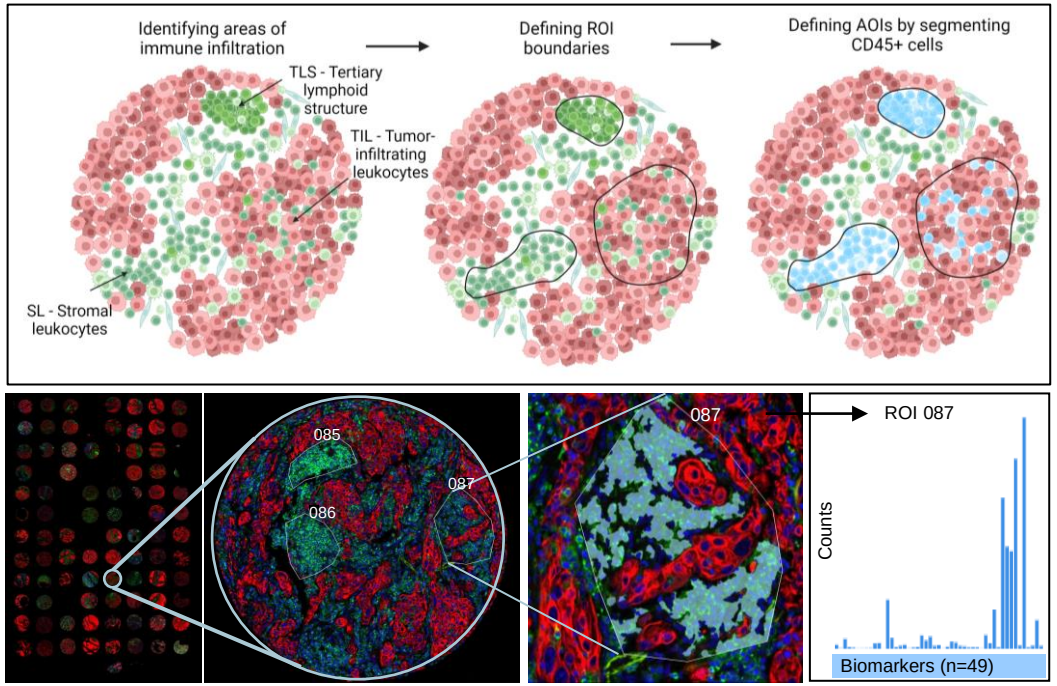


Examples of studies

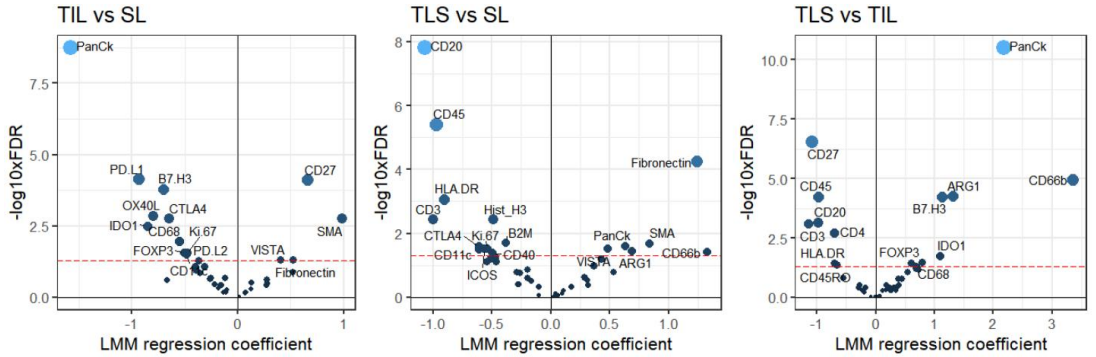
1. Protein profiles of immune regions in lung cancer
2. Protein profiles of immune and tumor regions in ovarian cancer
3. Gene profiles of macrophages, neutrophils and cancer cells in pancreatic cancer

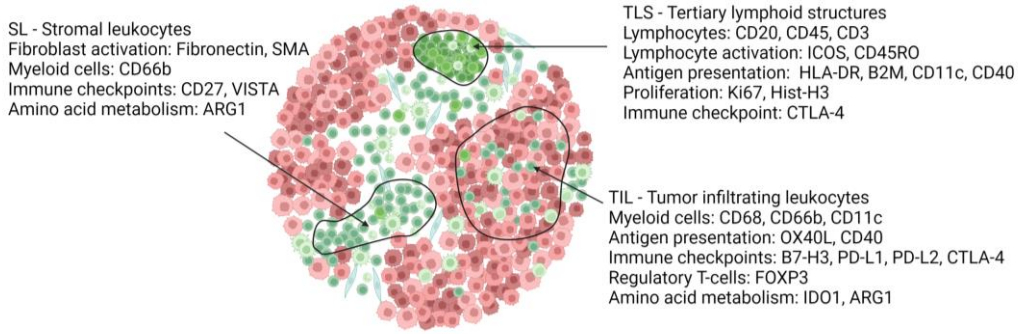
1. Phenotypic characterization of spatial immune infiltration niches in non-small cell lung cancer

Workflow



Linear mixed model analysis



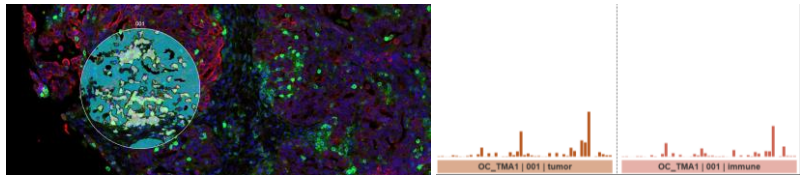


- Functional profiles of distinct immune niches, independent of the overall level of immune infiltration
- Potential immuno-oncology targets

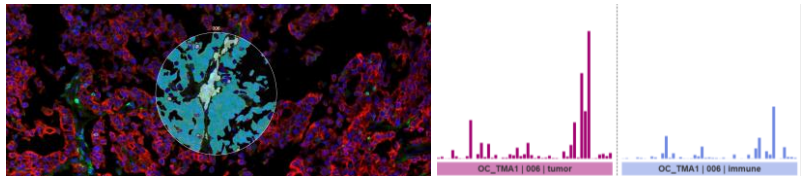
2. Spatial tumor immune phenotypes in ovarian cancer

1. Protein profiling of tumor and immune compartments in each region of interest

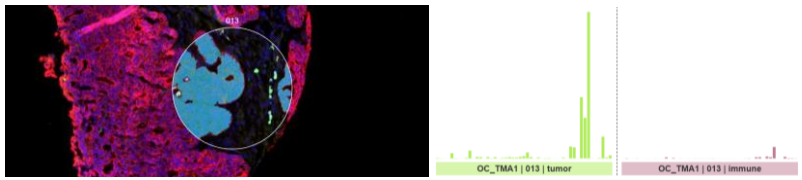
Diffuse immune infiltration



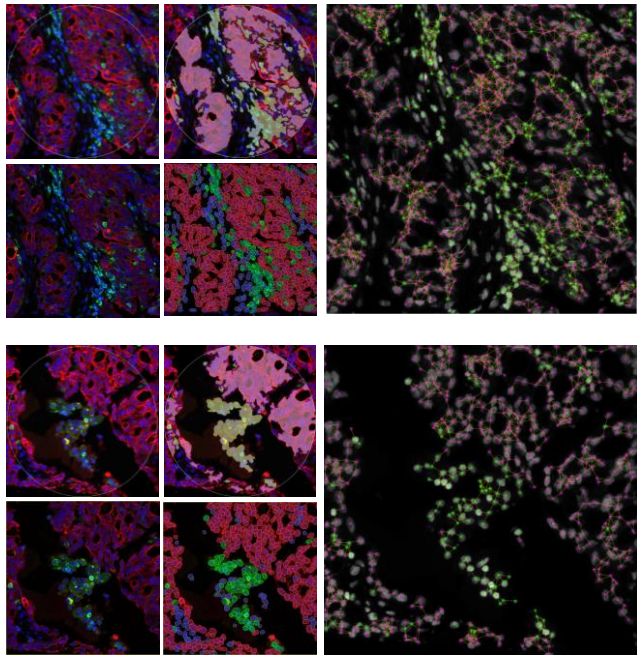
Focal immune infiltration



Immune-ignored

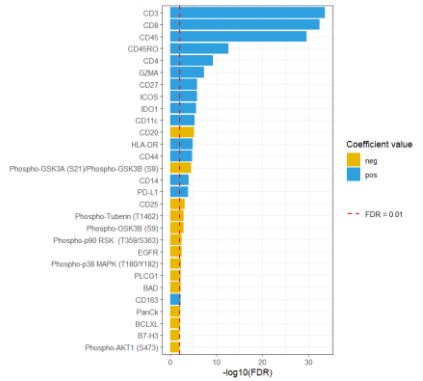


2. Image processing and spatial metrics analysis



3. Combined spatial metrics and protein profiling analysis

High vs low GDC (fraction of tumor cells connected to at least one immune cell within a 12 µm distance) compared using linear mixed models



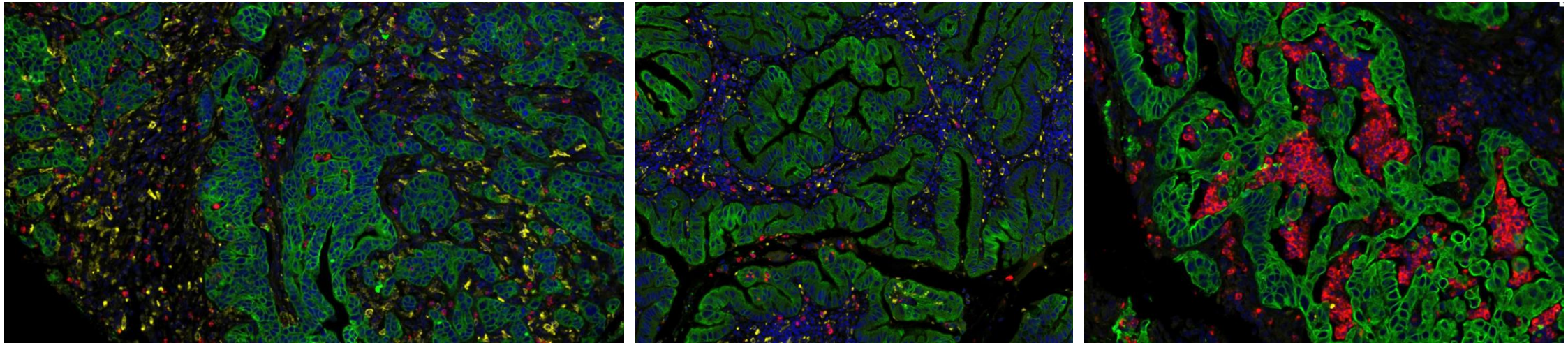
High GDC/diffuse infiltration: Activated cytotoxic and helper T-cells, immune suppression markers, antigen presentation

Low GDC/focal infiltration: B-cells, M2 macrophages, Tregs, PI3K/AKT signaling

- Breaking down heterogeneity – stratification by tumor immune phenotypes
- Immuno-oncology target activity in OC histology subtypes

3. Effect of macrophage and neutrophil infiltration in pancreatic tumors

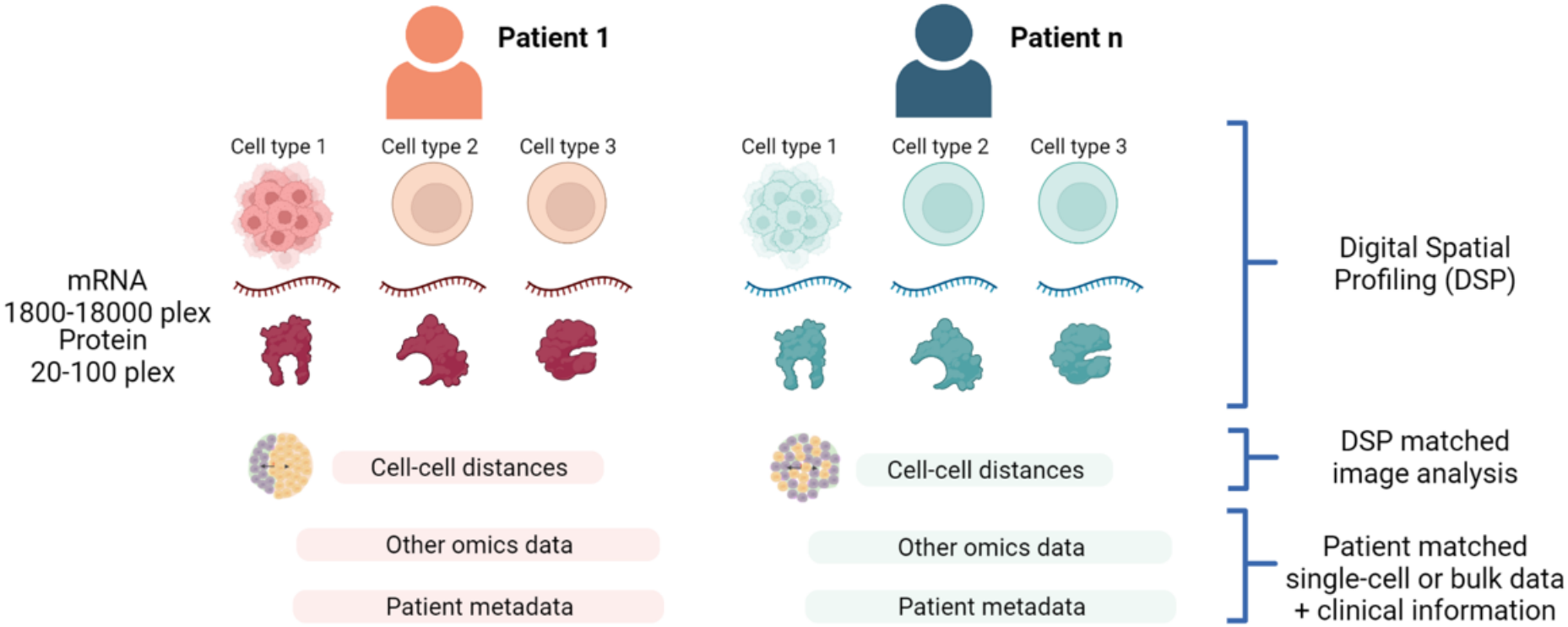
1. Image analysis – spatial distribution of TAMs, TANs and cancer cells in relation to cancer stage, subtype and outcome
2. Gene profiling (18 000-plex) of TAMs, TANs and tumor cells to identify gene programs associated with immune suppression



Cancer cells, M2 macrophages, Neutrophils

Jakob Blom

Multiple layers of data per patient



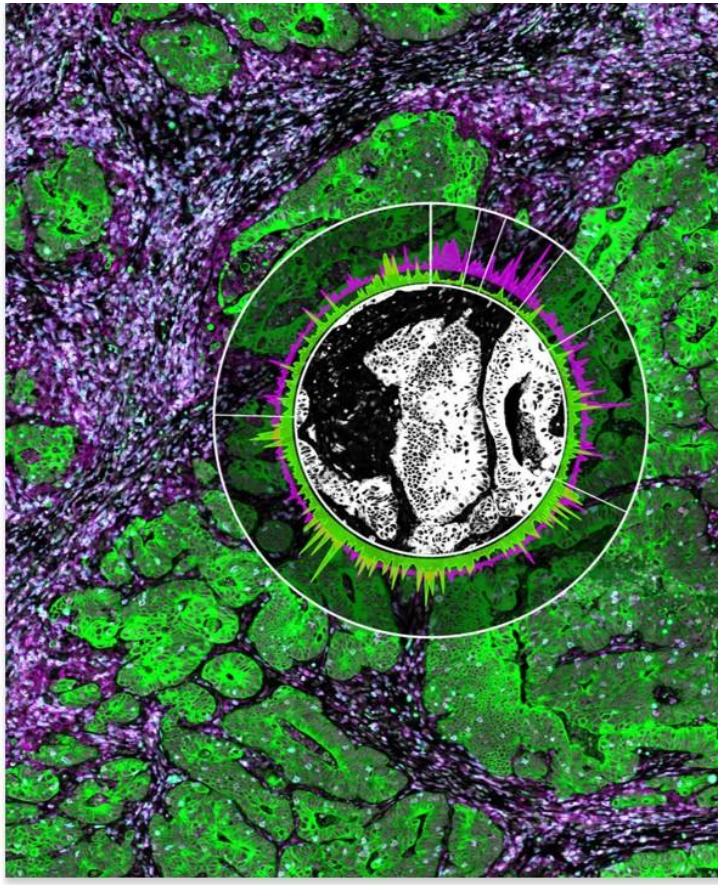
Scope of project

Spatial Omics provides high-resolution depictions of tumors, but the vast increase in measurements does not automatically translate into more clinically actionable information

We will generate workflows for extracting and integrating image-derived spatial metrics with spatial omics, genomic data and clinical metadata, to facilitate the development of integrated prediction models to guide choice of therapy.

Work packages:

1. Spatial metrics
2. Multi-omics data integration
3. Prediction models
4. User application

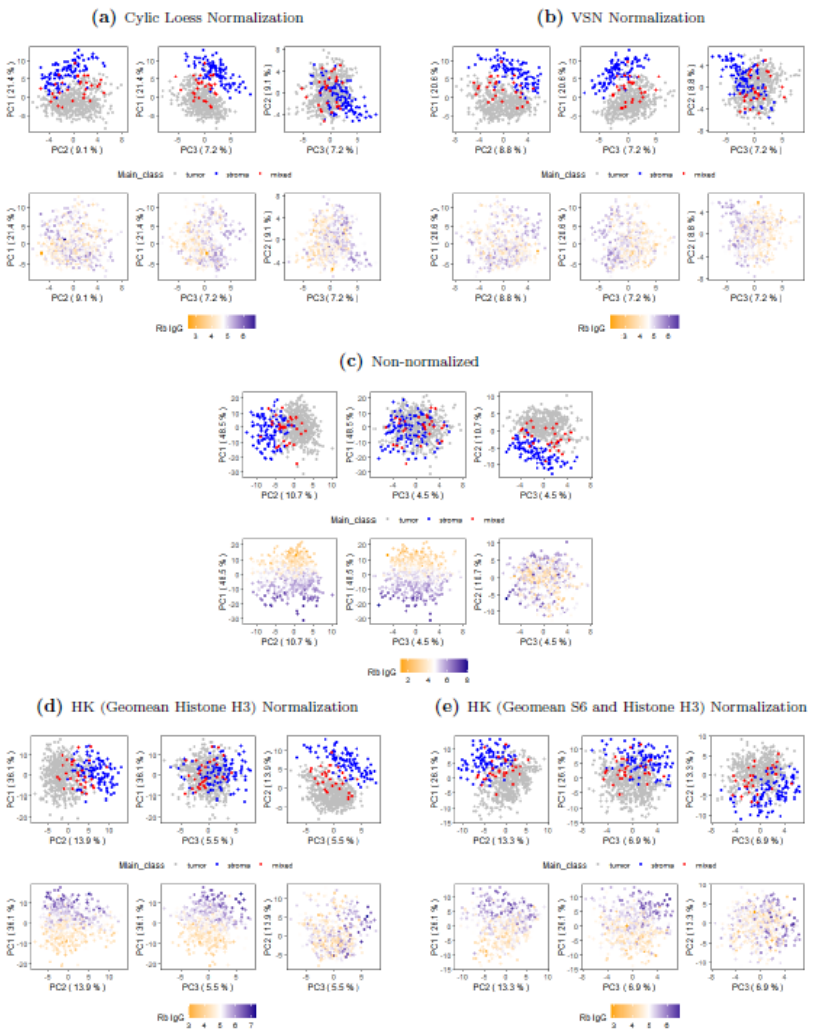


Preliminary results

Elias Carlsson, Mattis Knulst

Established workflows for

1. preprocessing of spatial omics data – QC and evaluation of normalization approaches
2. biomarker signature identification through mixed model regression with tumor/patient as random effect, for binary, ordinal and continuous response variables
3. image processing and graph analytics - extraction of spatial metrics for two classes
   - ratio, density, group degree centrality, assortativity index (relative connection to same class vs different class), cluster co-occurrence (relative connection between nodes vs random within and between classes)

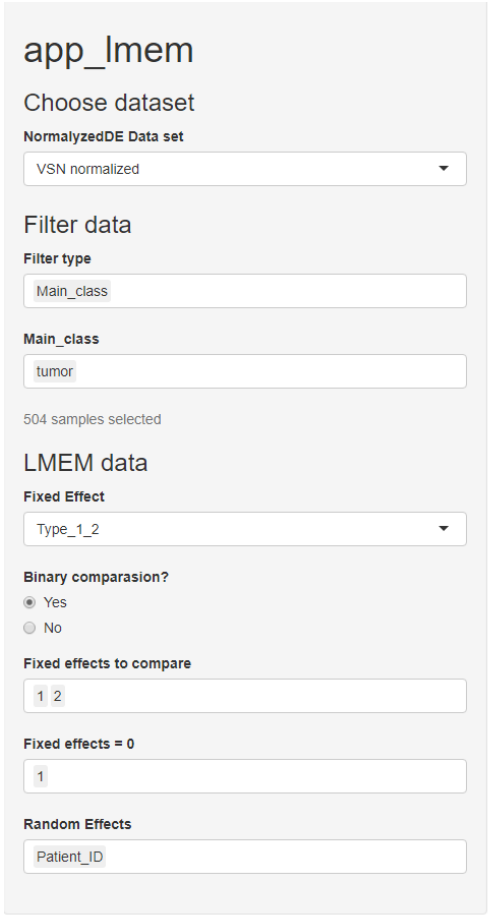


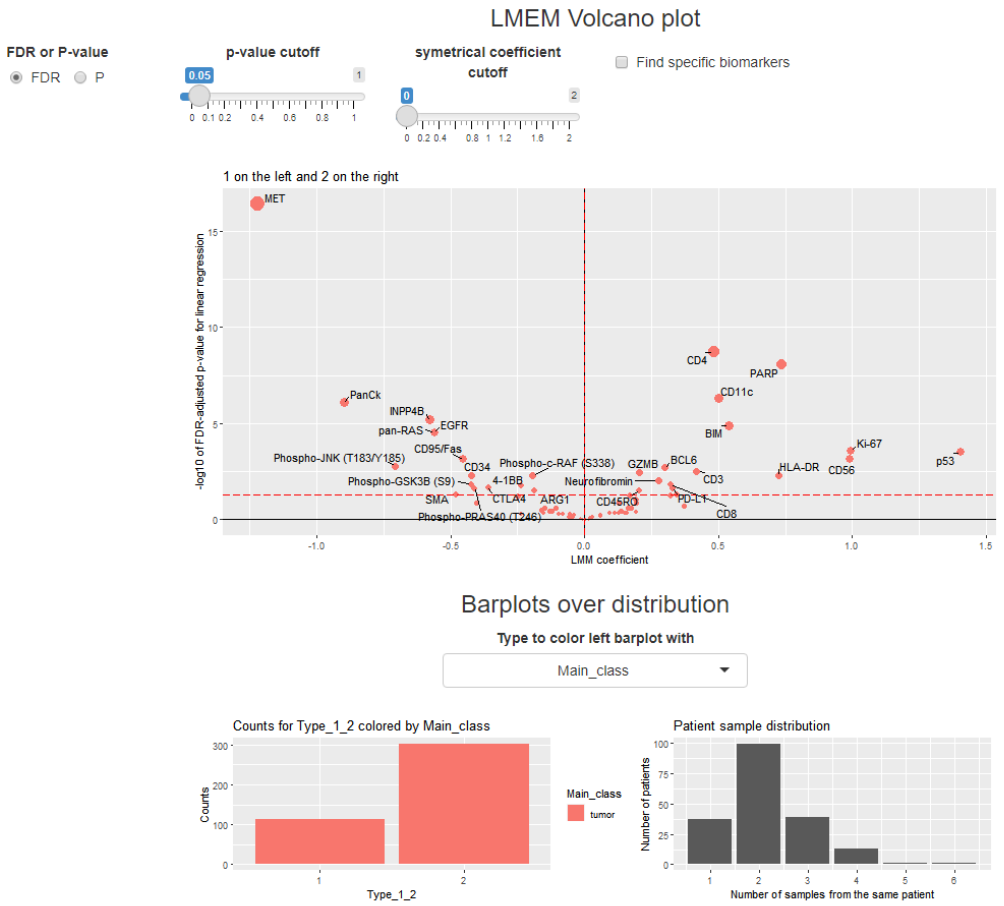
Evaluation of normalization methods. Top three plots are colored by ROI-type (tumor in grey, stroma in blue and mixed in red). Bottom three plots are colored by negative control (Rb IgG) intensity.

Interactive applications to facilitate DSP analysis

Elias Carlsson

1. Normalization
2. Linear mixed models
3. Survival analysis (CoxME and KM)
4. Random forest models



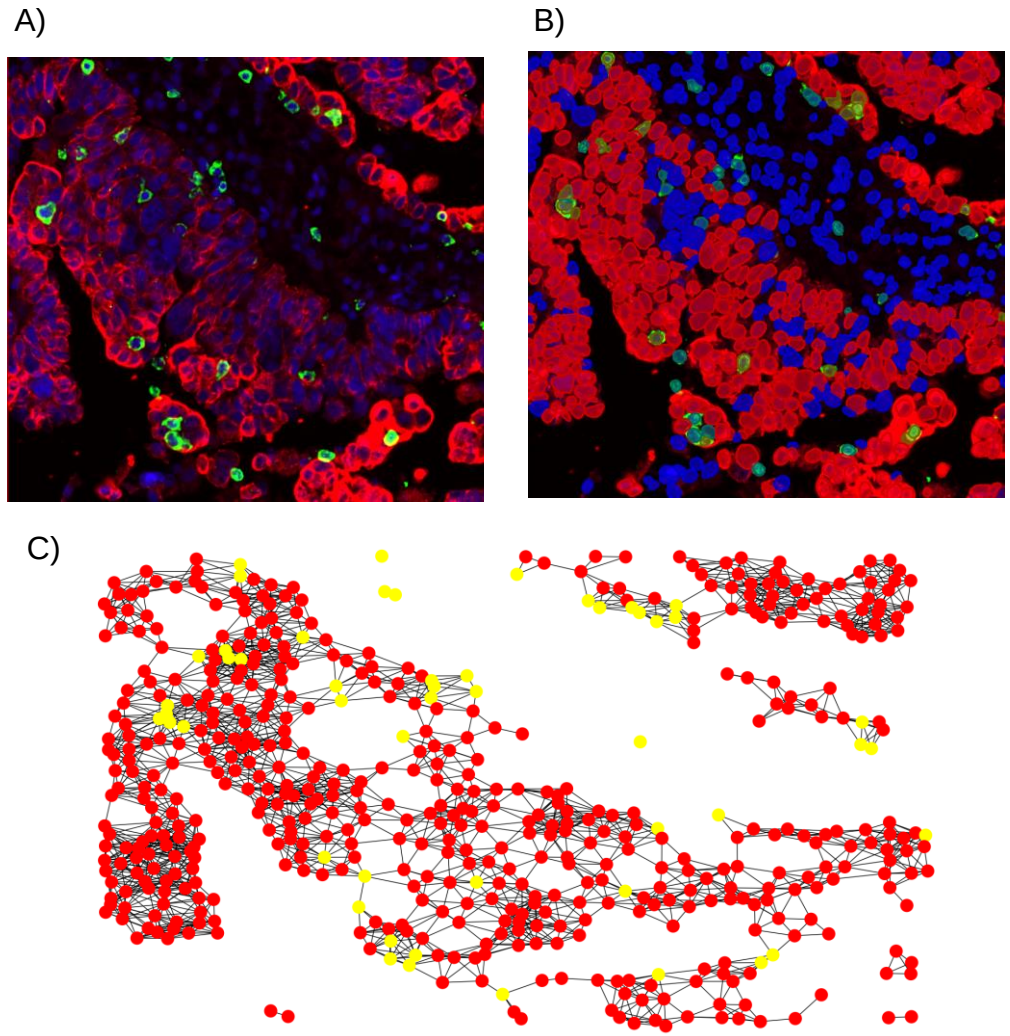


1. Spatial metrics

- Single or cyclic 4-plex immunofluorescence

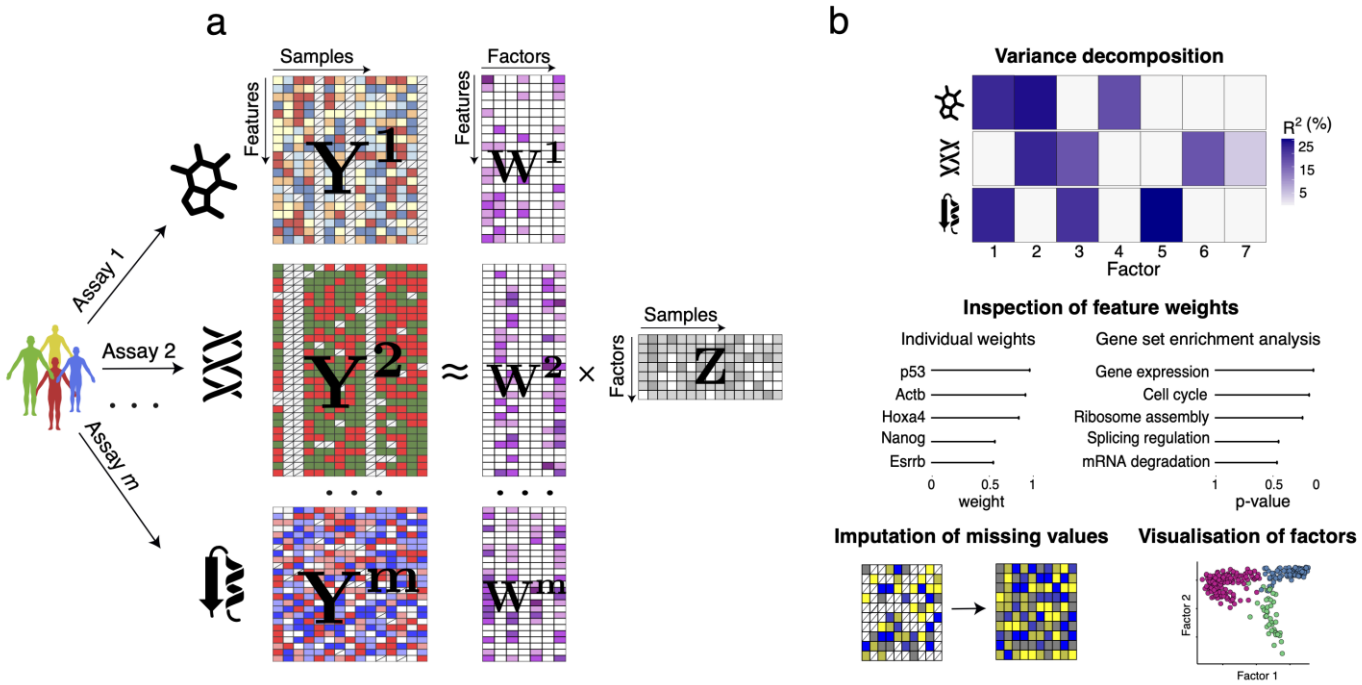
Generate workflows for

- Image processing (background subtraction, stitching, …)
- DL-based segmentation and cell classification
- Neighborhood analysis and graph analytics to describe tissue structures and spatial relation between cell types
- Spatial metrics: gdc, aac, ccr, entropy indices



2. Multi-omics data integration

- Currently working on merging protein and RNA data generated from sequential tissue sections using DSP
- Yet to define methods for integrating spatial omics data with image metrics and other omics datasets available for the same samples
- To be evaluated: matrix factorization, Bayesian consensus clustering, dimensionality reduction

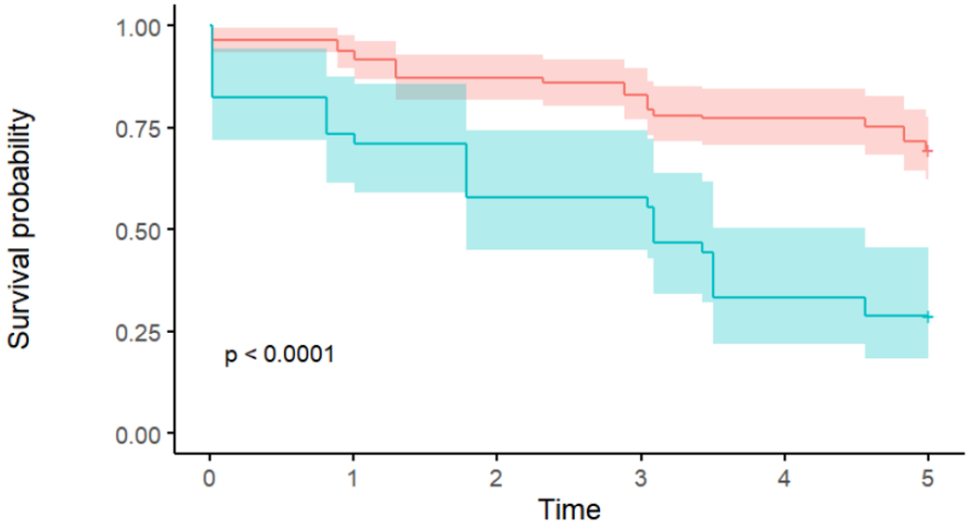


Multi-Omics Factor Analysis (MOFA): unsupervised discovery of the principal axes of biological and technical variation when multiple omics assays are applied to the same samples.

Ricard Argelaguet, et al. Multi-Omics Factor Analysis—a framework for unsupervised integration of multi-omics data sets, Mol Syst Biol. (2018) 14: e8124

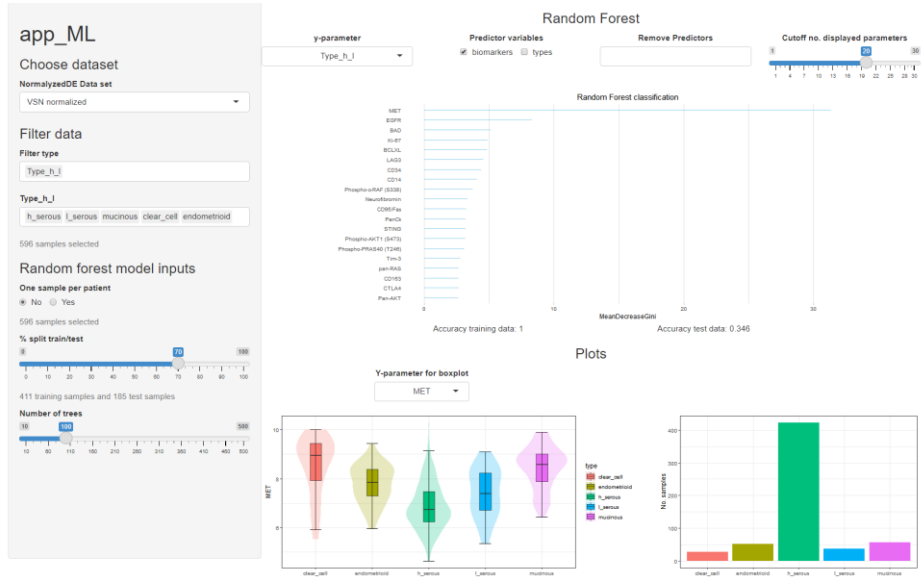
3. Prediction models

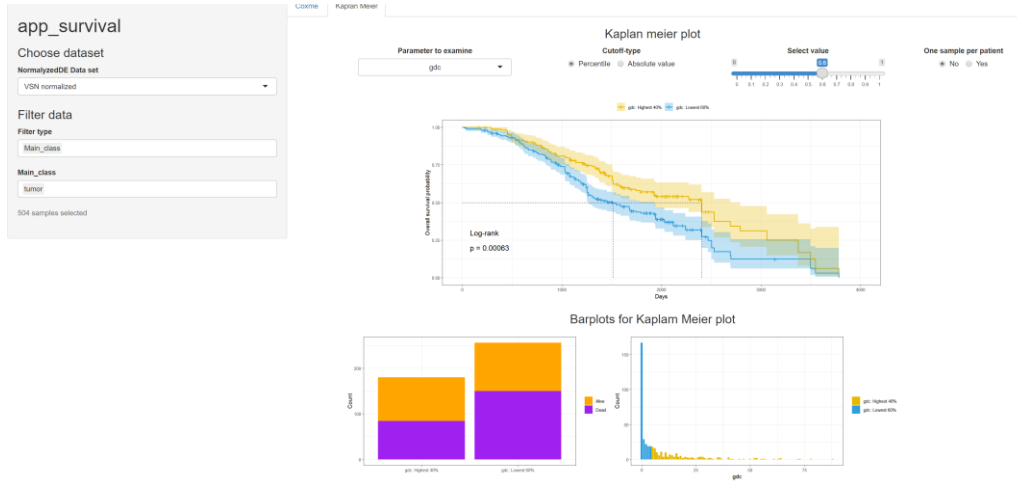
- Developing tools for building prediction models based on integrated spatial and multi-omics data
- Categorical (e.g. treatment outcome) or continuous (e.g. time of progression-free survival) response variables
- Supervised ML models in conjunction with feature selection
- Testing by cross-validation or independent data
- The goal is to define biomarker signatures to stratify patients by e.g. survival or response to therapy
- Maximal condensation of features to facilitate clinical implementation



4. Interactive user application

- To expedite utility for non-bioinformatician users
- Interactive visualization based on dimensionality reduction / clustering to depict spatial heterogeneity, cellular communities and interactions
- Tools to assess feature selection and performance of prediction models
- TBD – embedding DSP data processing (normalization), image analysis, integration between data points and link to original images





Data and applications

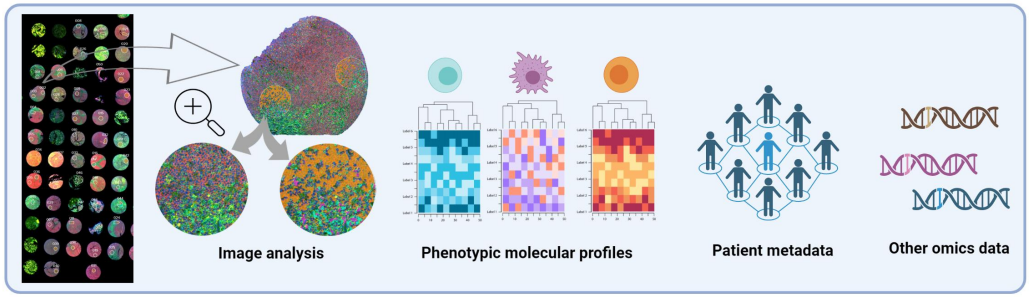
Datasets available for the project

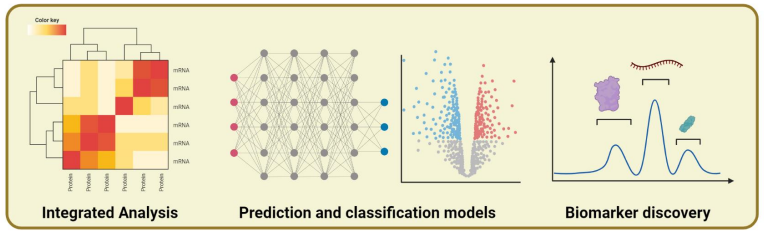
Dataset	Type, plexity	No of patients	No of AOIs	Type of AOIs	Scope	Other data available	Clinical collaborator	PI
Ovarian cancer 1	Protein 49-plex	52	144	Immune and tumor	Composition of spatial immune niches	Clinical metadata	Karin Sundfeldt, Gothenburg University	ASG
Ovarian cancer 2	Protein 78-plex	259	654	Immune and tumor	Immune infiltration predictive of tumor subtype and survival	Clinical metadata, plasma affinity proteomics, Plasma mass-spec proteomics	Karin Sundfeldt, Gothenburg University	ASG
Lung cancer	Protein 49-plex	33	183	Immune	Characterizing spatial immune niches and heterogeneity	Clinical metadata, RNAseq, IHC scores, multiplex immunofluorescence	Patrick Micke, Uppsala University	SE
Mantle cell lymphoma 1	Protein 69-plex	131	606	Tumor, macrophages, T-cells	Tumor, macrophage and T-cell interaction	Clinical metadata, mutational status, IHC scores, bulk transcriptomics	Mats Jerkeman, Lund University	SE
Mantle cell lymphoma 2	Protein 69-plex	104	602	4 different T-cell types	Profiling T-cell subsets with spatial resolution	Clinical metadata, mutational status, IHC scores, bulk transcriptomics	Mats Jerkeman, Lund University	SE
Mantle cell lymphoma 3	RNA 1811-plex	104	600	Tumor, 2 different T-cell types	Lymphocyte tumor interactions with spatial resolution	Clinical metadata, mutational status, IHC scores, bulk transcriptomics	Mats Jerkeman, Lund University	SE
Pancreatic cancer	RNA 1811-plex	TBD	TBD	Tumor, macrophages, neutrophils	Spatial interplay of tumor and myeloid cells	Clinical metadata, multiplex immunofluorescence	Karin Jirström, Lund University	ASG

- Recently submitted work on protein profiling of spatial immune niches in NSCLC
- New workflows may be applied on a larger cohort of 712 NSCLC patients
  - Additional data: Genome-wide sequencing and copy number analysis, or RNA sequencing (400 patients)
- Tumor immune microenvironment interactions related to treatment outcome, and proposal of new therapeutic strategies
- Tentatively – immuno-oncology clinical trial-TIME signatures related to immunotherapy response

Summary

- Immunotherapy has the opportunity to improve survival for cancer patients
- Tumor immune microenvironments are complex and heterogeneous – need for precision medicine tools
- Spatial Omics provide unprecedented resolution of molecular profiling of tumors but frameworks to integrate omics data with image-derived metrics and data from other modalities are lacking





- Benefit for patients and oncologists – models for tumor stratification in relation to targeted cancer therapies
- Benefit for the growing Spatial Omics research community - metrics for characterizing tumor immune microenvironment structures, integrative tools for processing, visualizing and interpreting spatial and multimodal data, insight into optimal design of Spatial Omics

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