Spatial Biology for Immunologists

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Sanofi US R&D

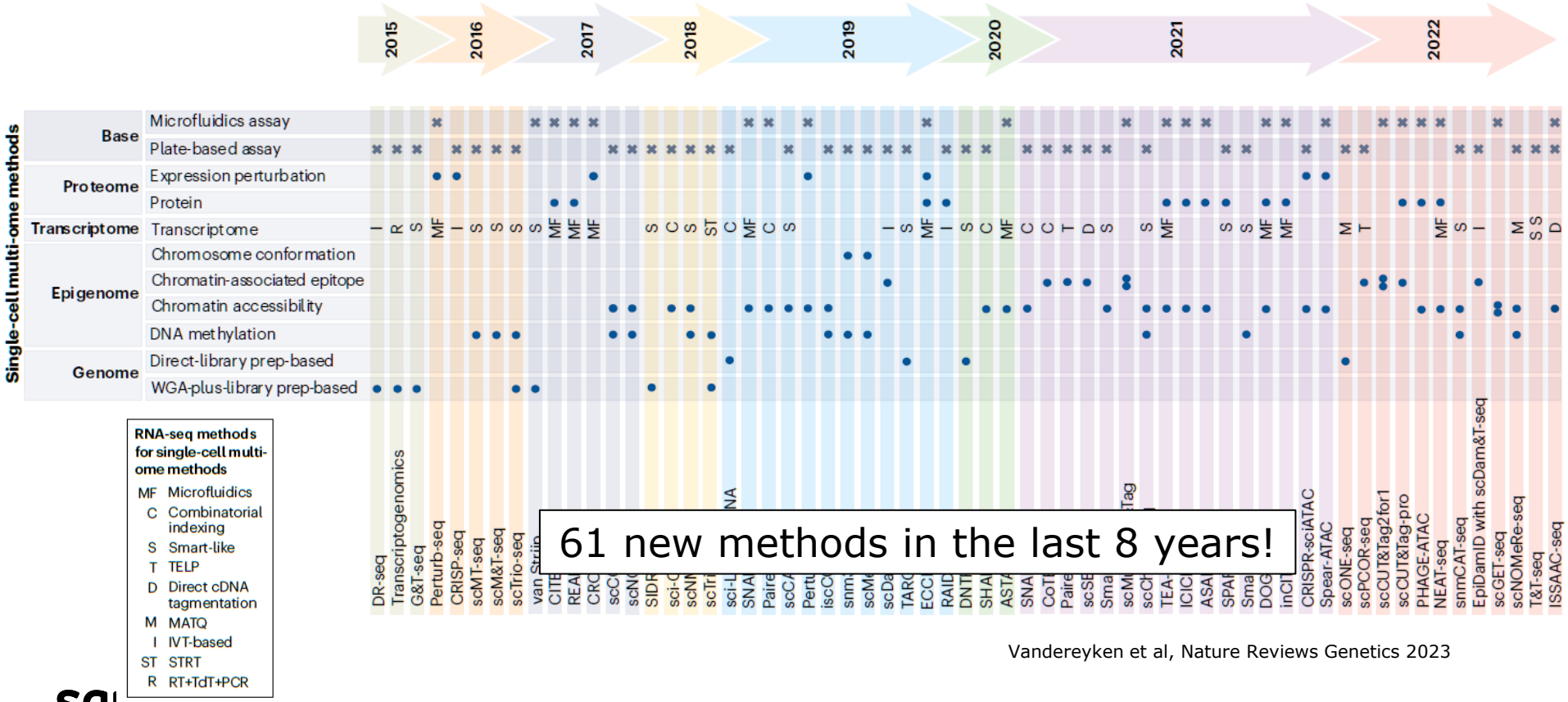
Outline for today

- Introduction to spatial biology technologies
- Overview of spatial biology data analysis
- Deep dive into spatial transcriptomics
  → Paper #1 – Meylan et al, Immunity 2022
- Deep dive into multiplexed spatial proteomics
  → Paper #2 – Gaglia, Burger et al, Cancer Cell 2023

Learning objectives:

- How to choose the correct spatial technology
- What insight can we get and how
- What are the current limitations & future developments of the field

The evolution of **single cell** vs spatial transcriptomics



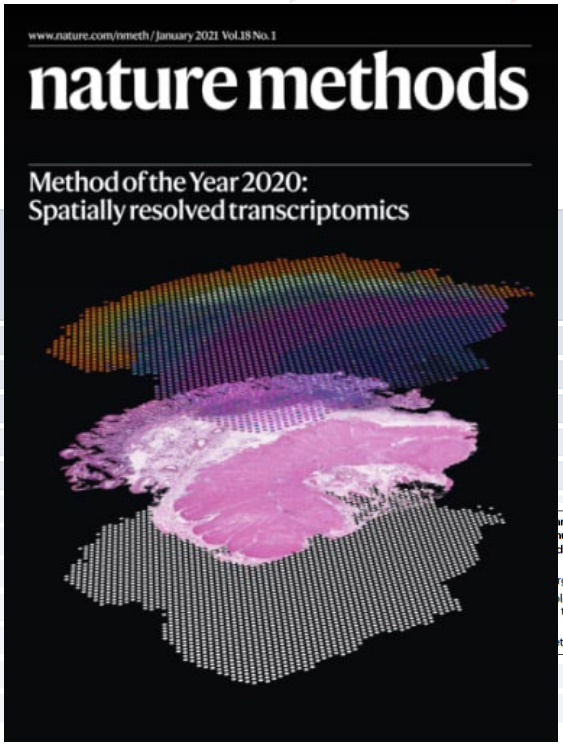
61 new methods in the last 8 years!

Vandereyken et al, Nature Reviews Genetics 2023

The evolution of single cell vs **spatial transcriptomics**

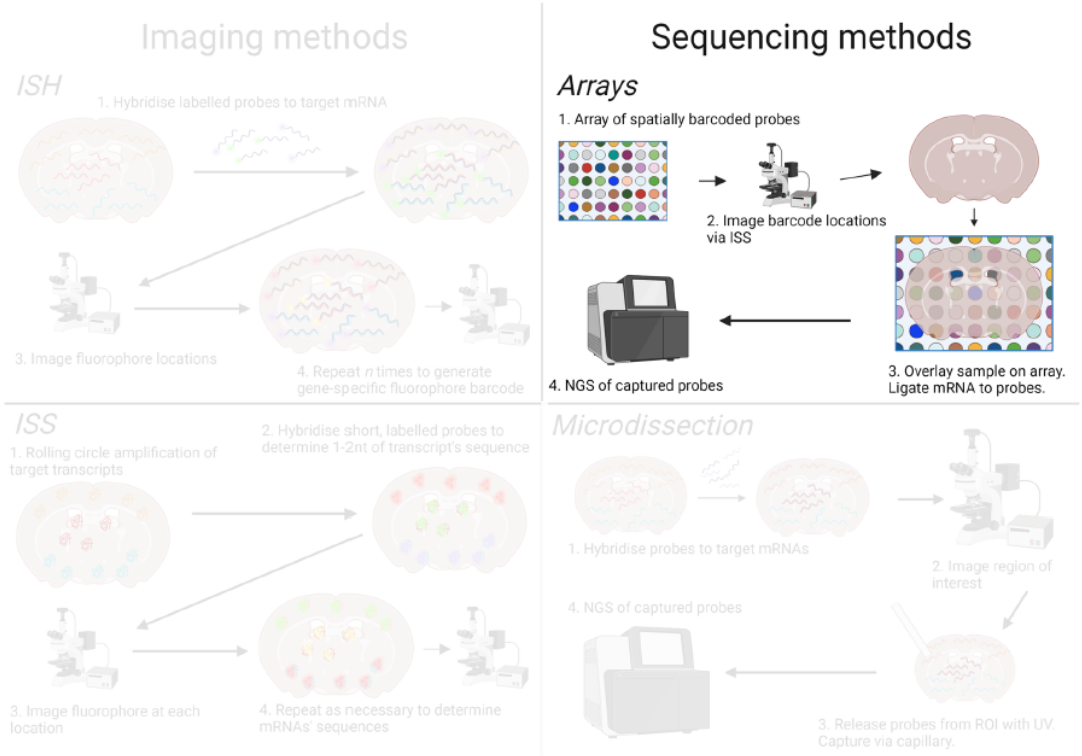
- Technologies are in flux
- Spatial biology is 5-10 years behind single cell
- Analytical challenges are emerging

Spatial multi-ome methods		secFISH	MERFISH	DNA-MERFISH	OligoFISSEQ	DBiT-seq	Visium	GeoMx DSP
Resolution		Subcellular	Subcellular	Subcellular	Single-cell	10–25 µm	55 µm	Single-cell
Sample	FFPE tissue section						✖	✖
	Fresh-frozen tissue section					✖	✖	✖
	Cultured cells	✖	✖	✖	✖			
Order	In parallel		✖			✖		
	Sequential	✖		✖	✖		✖	✖
Proteome	DNA-conjugated antibody NGS readout					●		●
	DNA-conjugated antibody fluorescent readout		●	●				
	Fluorescent antibody readout	●			●		●	
Transcriptome	Probe hybridization-based	●	●	●				●
	Poly(A) capture-based					●	●	
Epigenome/ genome	Probe hybridization-based			●	●			
	(Open) chromatin							



What can we measure?

- Genes

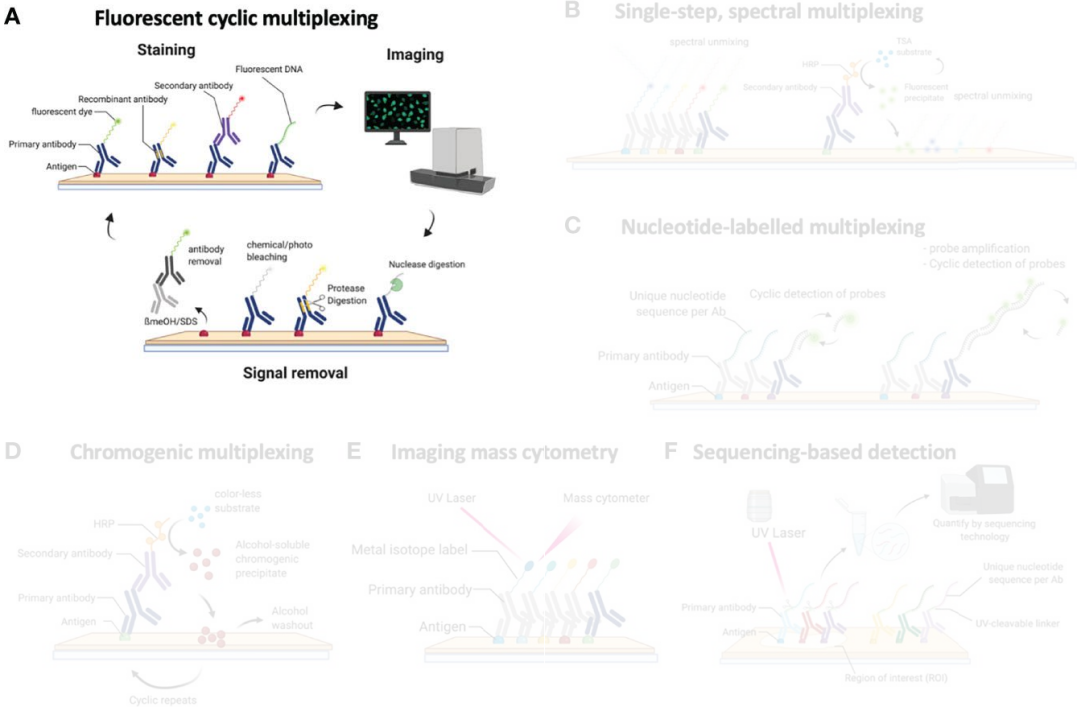


Williams et al,
Genome Medicine 2022

What can we measure?

- Genes
- Proteins

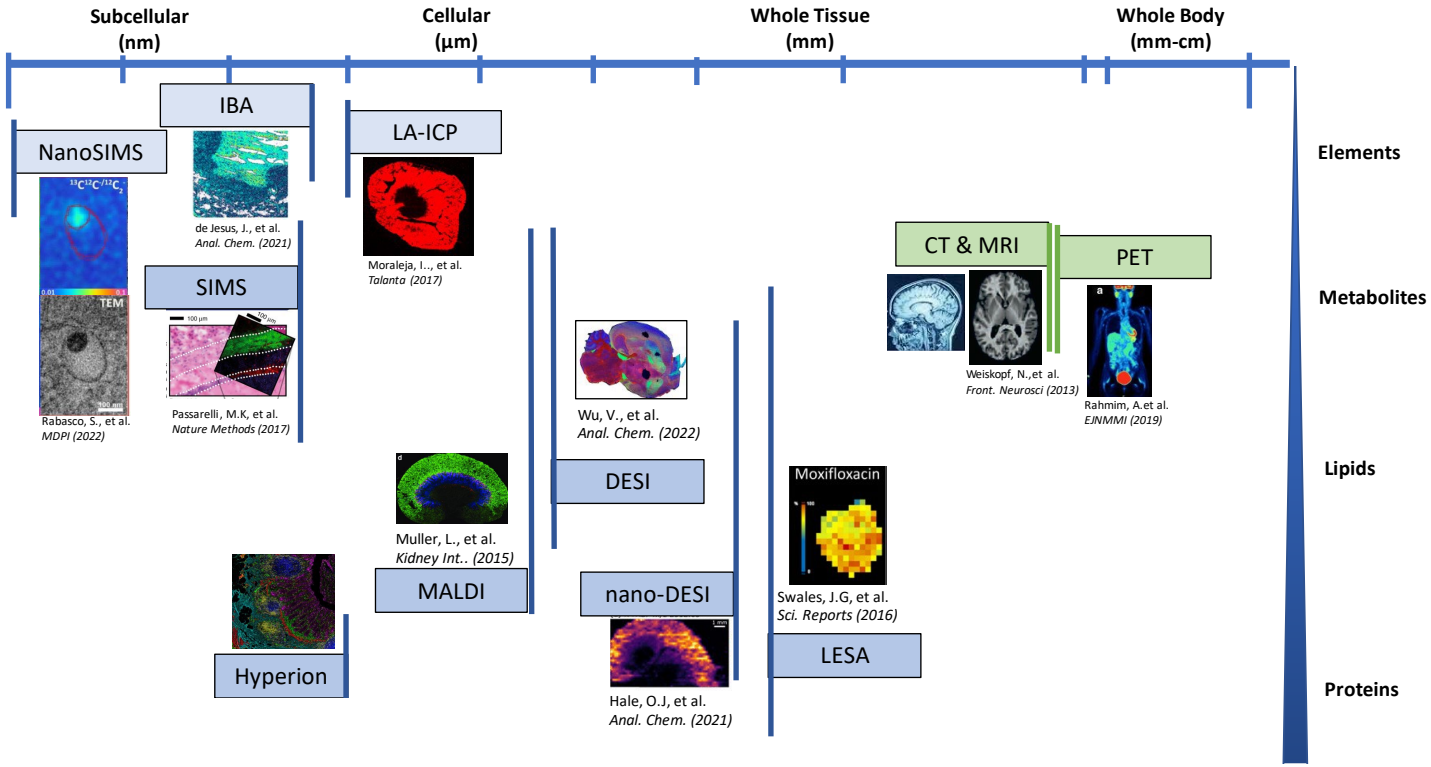
Biased - Antibody-based detection



Moffitt et al, Nat Rev Gen 2022

What can we measure?

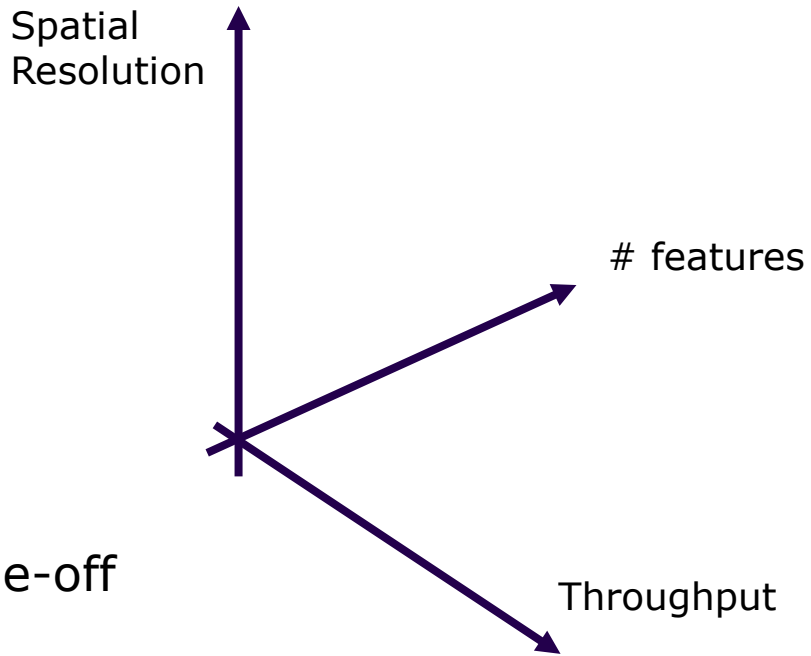
- Genes
- Proteins
- Metabolites
- Lipids



Slide courtesy of Chelsea Nikula, Sanofi US R&D

What variables are important in spatial biology

- Spatial resolution
- Number of features
- Throughput (area x time)



Each technology has its trade-off

Which technology should I use and when?

Discussion points:

1. **What's the question – exploratory vs confirmatory**

➡ How "well know"?
Are antibodies available?
Is it multicellular, cellular or intra-cellular?

2. **What is the variability of the phenotype?**

Toolbox

- **Spatial Transcriptomics with Visium 10X**
  - Experimental method
  - Cell type imputation
  - Clonotype imputation

- **Single Cell Spatial Data Analysis**
  - Mathematical representation
  - Spatial metrics
  - Triangulation & networks

- **Mapping phenotypic space to tissue space**

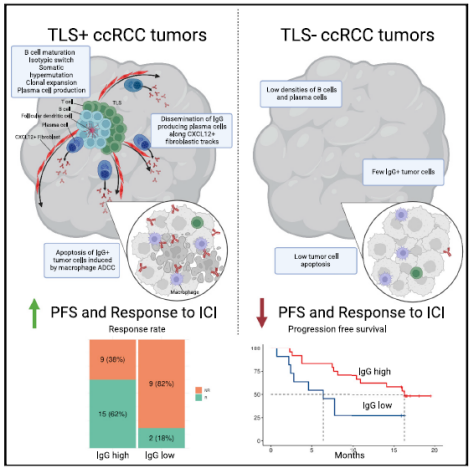
Let's get to science!

Investigating immune structures in cancer with spatially resolved methods

Immunity

Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer

Graphical abstract



Authors

Maxime Meylan, Florent Petitprez, Etienne Becht, ..., Aurélien de Reyniès, Catherine Sautès-Fridman, Wolf Herman Fridman

Correspondence

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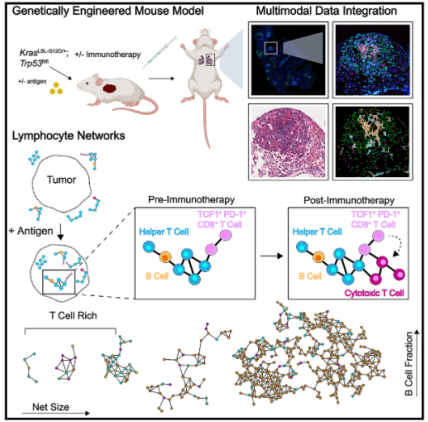
In brief

Meylan et al. show that tertiary lymphoid structures found in tumors are sites of generation of fully mature B cell immunity. Plasma cells disseminate into tumor beds, producing antibodies that bind to tumor cells and initiate their apoptosis, providing a mechanism to support cancer immunotherapies that modulate the tumor microenvironment.

Cancer Cell

Lymphocyte networks are dynamic cellular communities in the immunoregulatory landscape of lung adenocarcinoma

Graphical abstract



Authors

Giorgio Gaglia, Megan L. Burger, Cecily C. Ritch, ..., Peter K. Sorger, Tyler Jacks, Sandro Santagata

Correspondence

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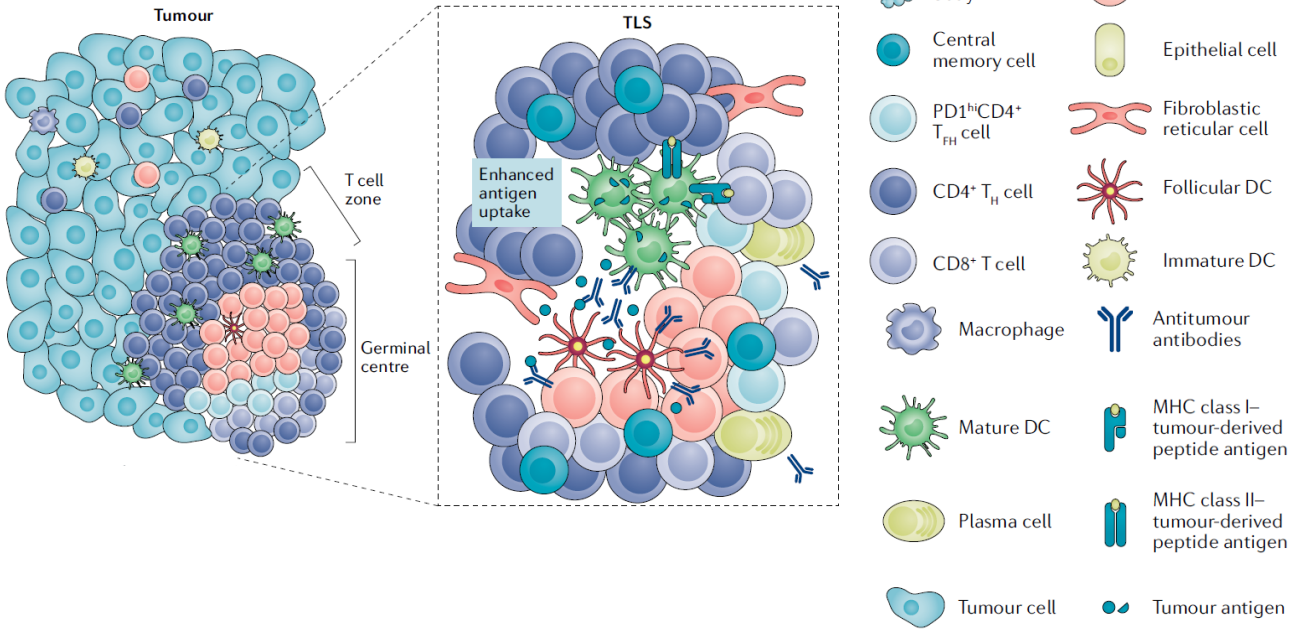
In brief

Gaglia et al. find striking changes in the spatial arrangement of immune cells in response to tumor antigens. T and B cells are recruited in lymphocyte networks ("lymphonets"), which contain progenitor T cells. After immunotherapy, lymphonets gain cytotoxic T cells, likely due to progenitor cell differentiation and activation in this distinct immune environment.

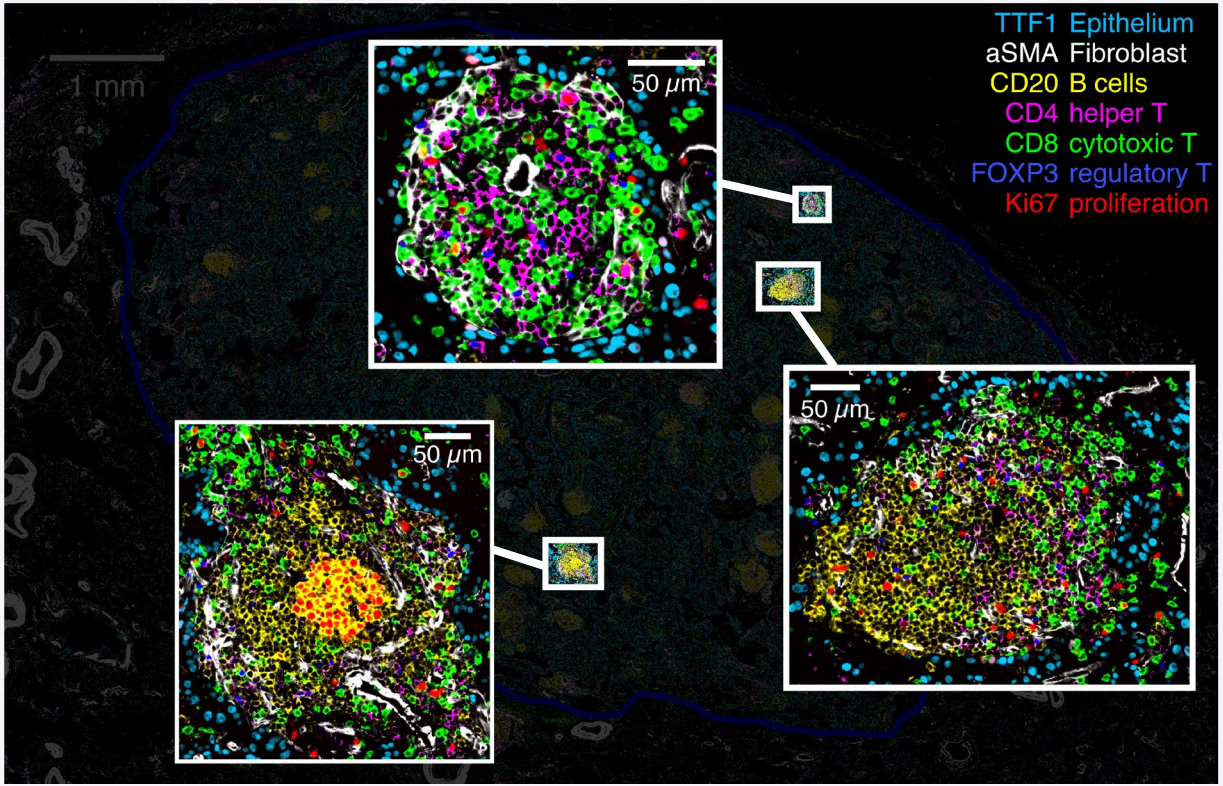
Introduction to Tertiary Lymphoid Structures (TLS)

- Immune structures composed of multiple cell types
- Potential sites for local and antigen presentation and clonal selection
- Form in chronic inflammation and cancer
- Similarities and differences from primary secondary lymphoid organs



Sautès- Fridman et al,
Nat Rev Cancer 2019

Tertiary Lymphoid Structures in Lung Tumors



Human lung adenocarcinoma in multiplexed IF
Gaglia et al Cancer Cell 2023

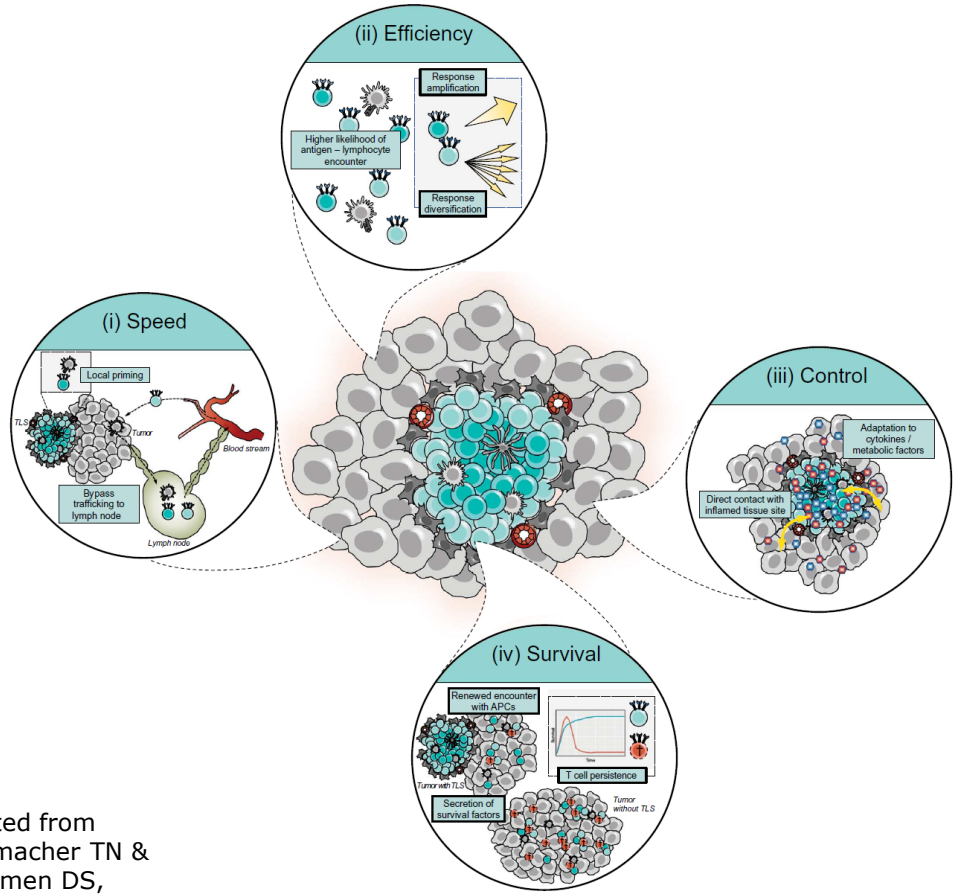
Fundamental questions

1) How is the spatial patterning of the immune cells in tumors established?

2) How are the phenotypes of immune cells impacted by the spatial organization?

3) What information about tumor growth and response to therapy can we glean from spatial analysis?

Potential contributions of TLS to antitumor immunity & treatment



Tumor with no TLS

Vax or ICB

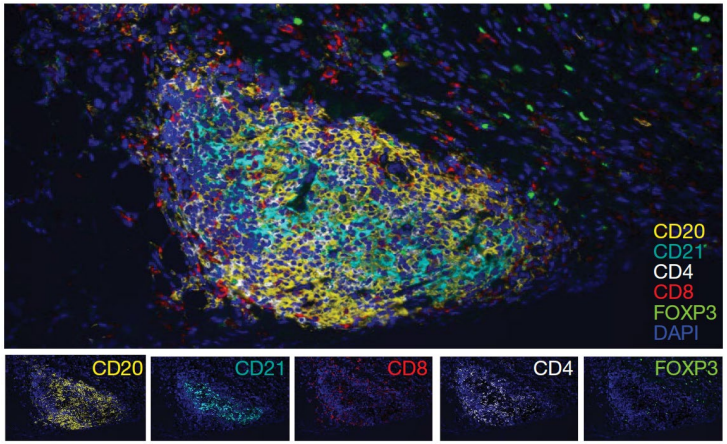
TLS induction

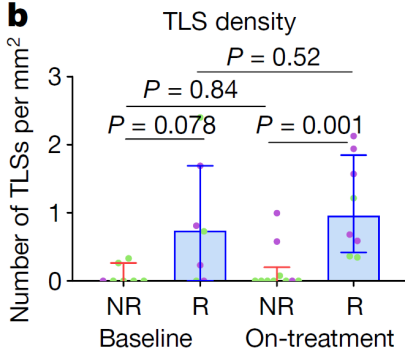
ICB

TLS maturation, increase or enhancement

Adapted from Schumacher TN & Thommen DS, Science 2022

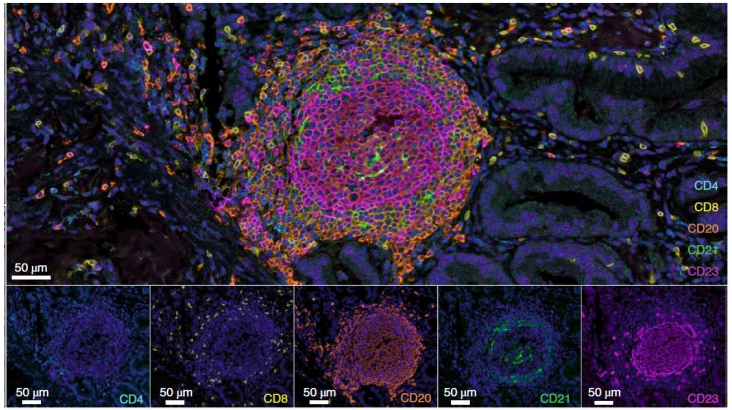
TLS promote of immunotherapy response

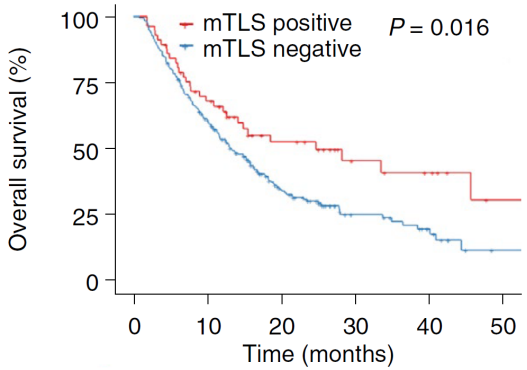




Melanoma treated with treated with neo-adjuvant Ipilimumab and Nivolumab NCT02437279

Helmink et al. Nature 2020

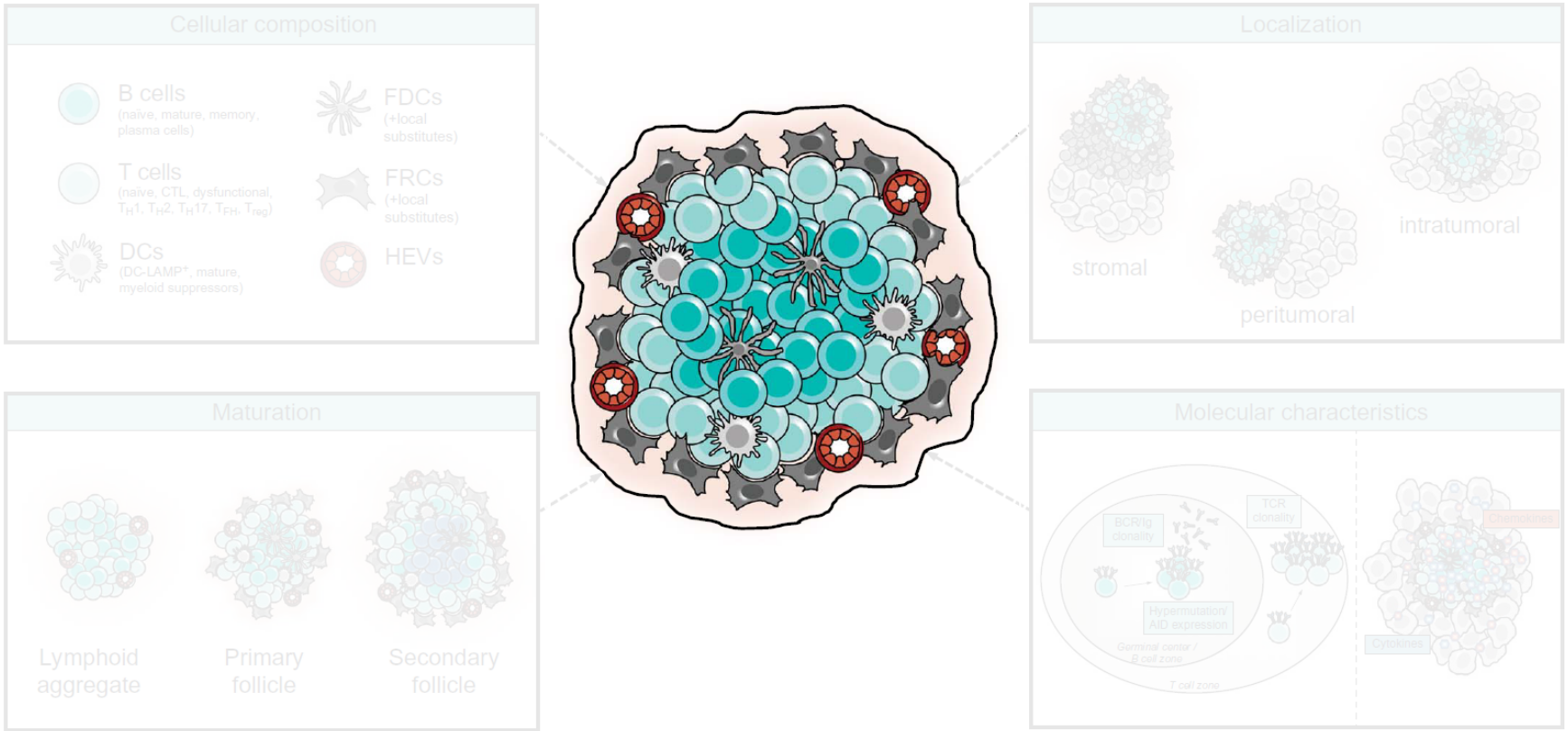




Multi-tumor analysis

Vanhersecke et al. Nature 2020

What are the open questions?



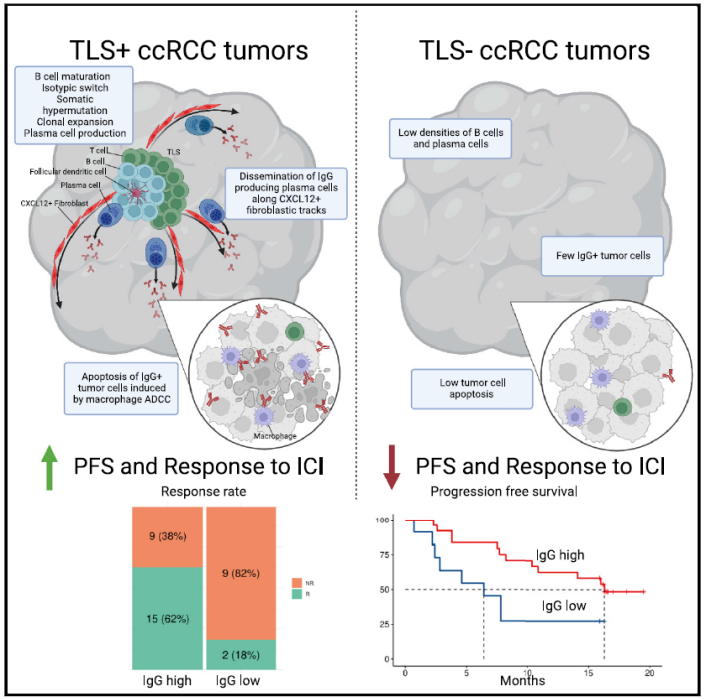
Schumacher TN and Thommen DS, Science 2022

Immunity

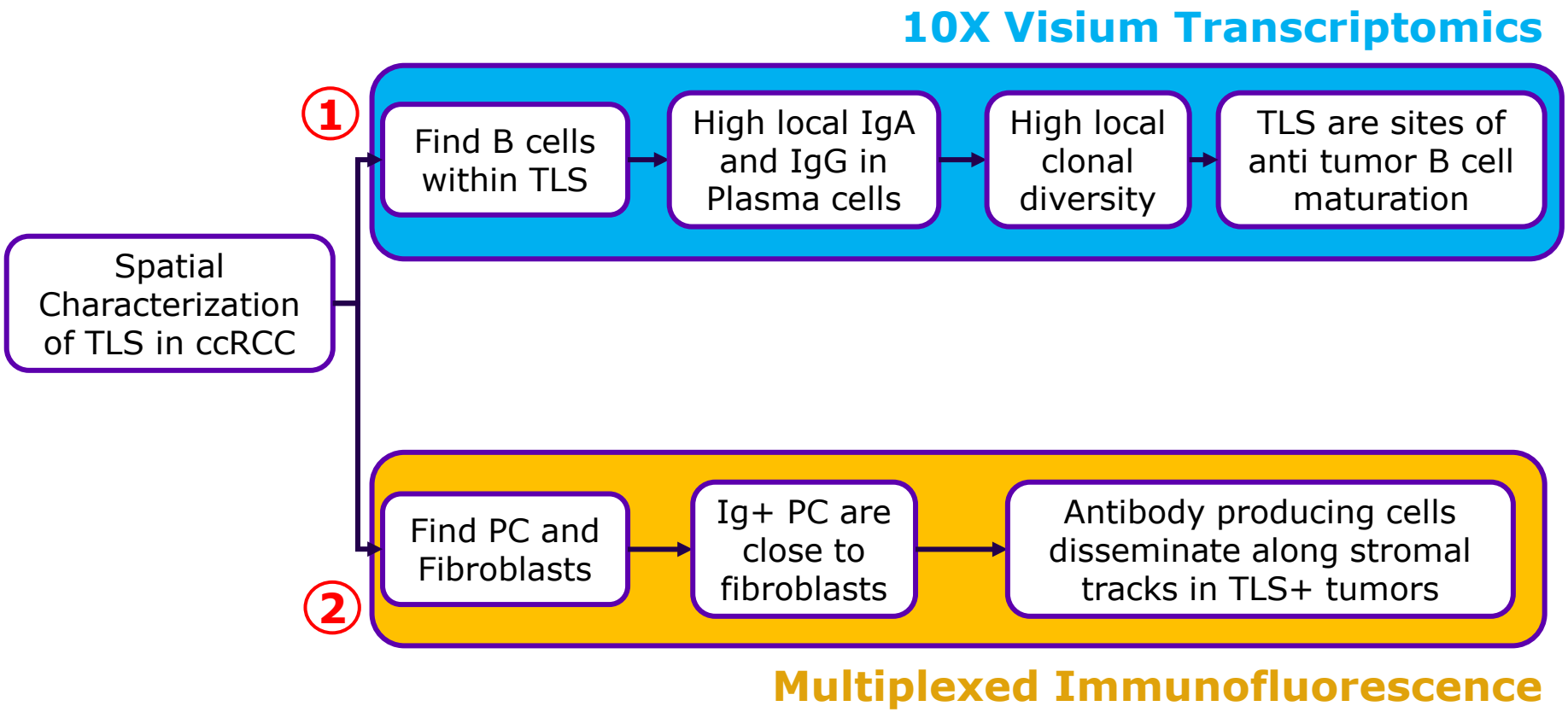
Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer

Highlights

- Tertiary lymphoid structures are sites of *in situ* B cell maturation toward plasma cells
- IgG+ and IgA+ plasma cells disseminate into the tumor tissue along fibroblastic tracks
- Tumor cells are labeled by locally produced IgG
- Patients with IgG-labeled tumor cells have high response rate to ICI and prolonged PFS



Paper flow


10X Visium Transcriptomics
1
Find B cells within TLS
High local IgA and IgG in Plasma cells
High local clonal diversity
TLS are sites of anti tumor B cell maturation
Spatial Characterization of TLS in ccRCC
2
Find PC and Fibroblasts
Ig+ PC are close to fibroblasts
Antibody producing cells disseminate along stromal tracks in TLS+ tumors
Multiplexed Immunofluorescence

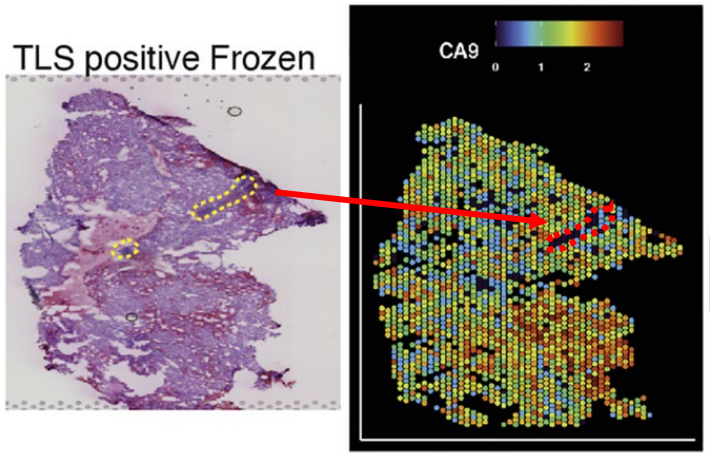
Main workflow combines H&E annotation by pathologist and spatial transcriptomics

H&E Reviewed by Pathologist
→ Manual annotation of TLS

<u>All spatial analysis and insights are based on this annotation</u>

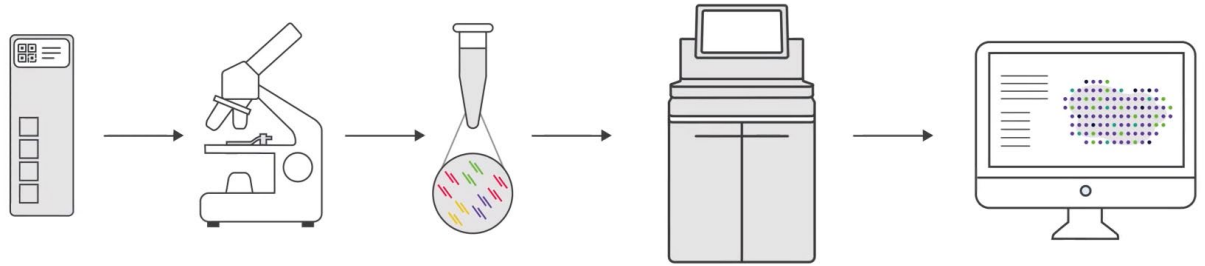
Generalization of labels by ML/AI (next talk!)

Figure 1A-B

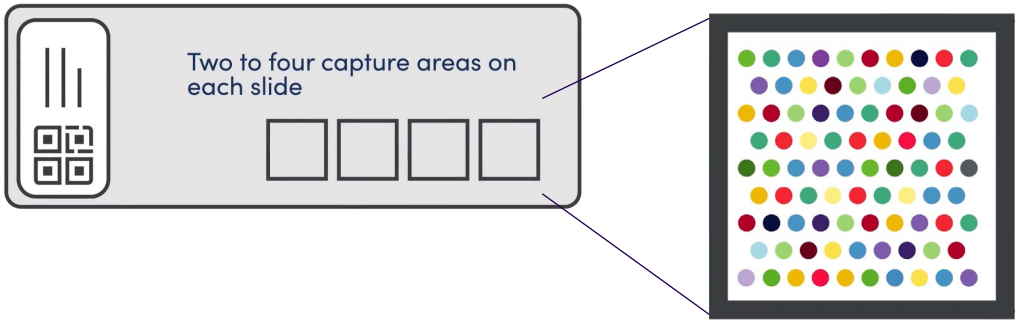


Spatial Transcriptomics by 10X Visium
→ Quantify gene expression
→ Impute cell types by signatures (MCP-Counter)
→ Impute B cell clonality (MiXCR)

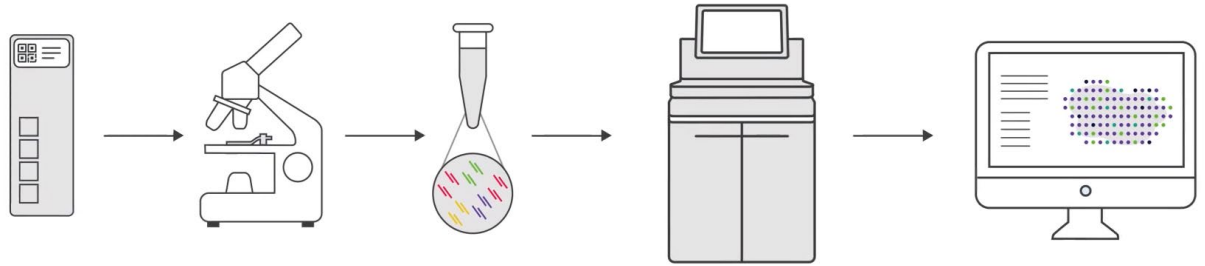
Deep dive into 10X Visium Technology

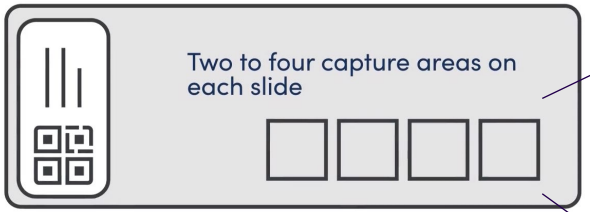


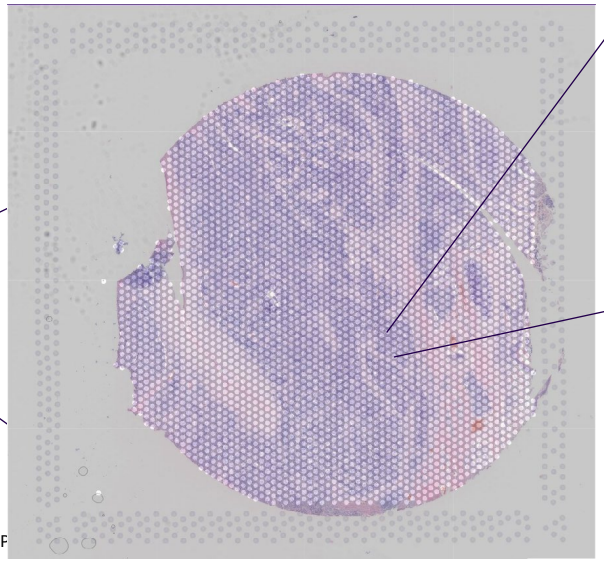
5000 spots
10^6 probes/spot
55uM diameter

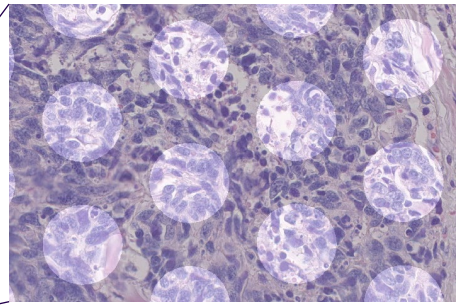


Deep dive into 10X Visium Technology



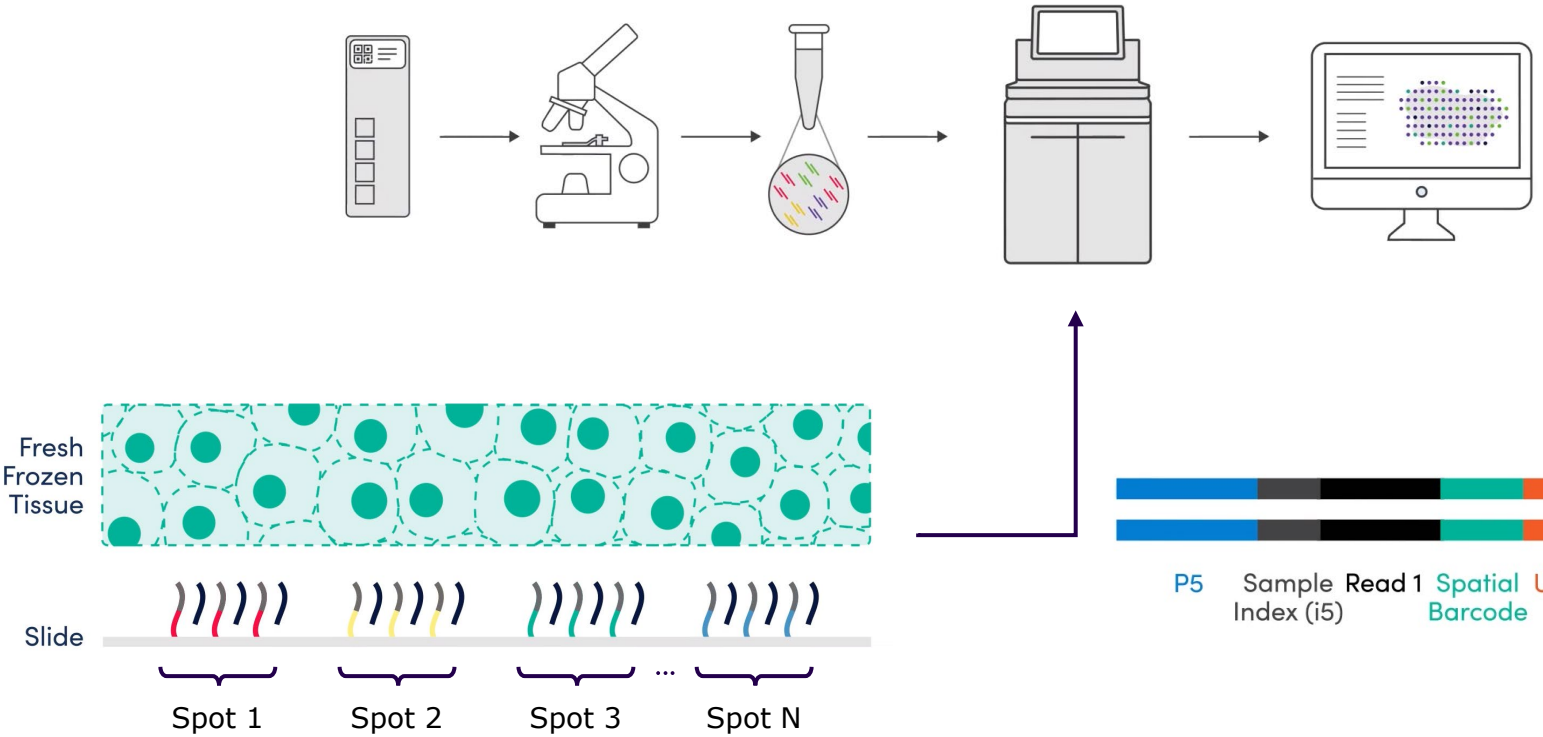






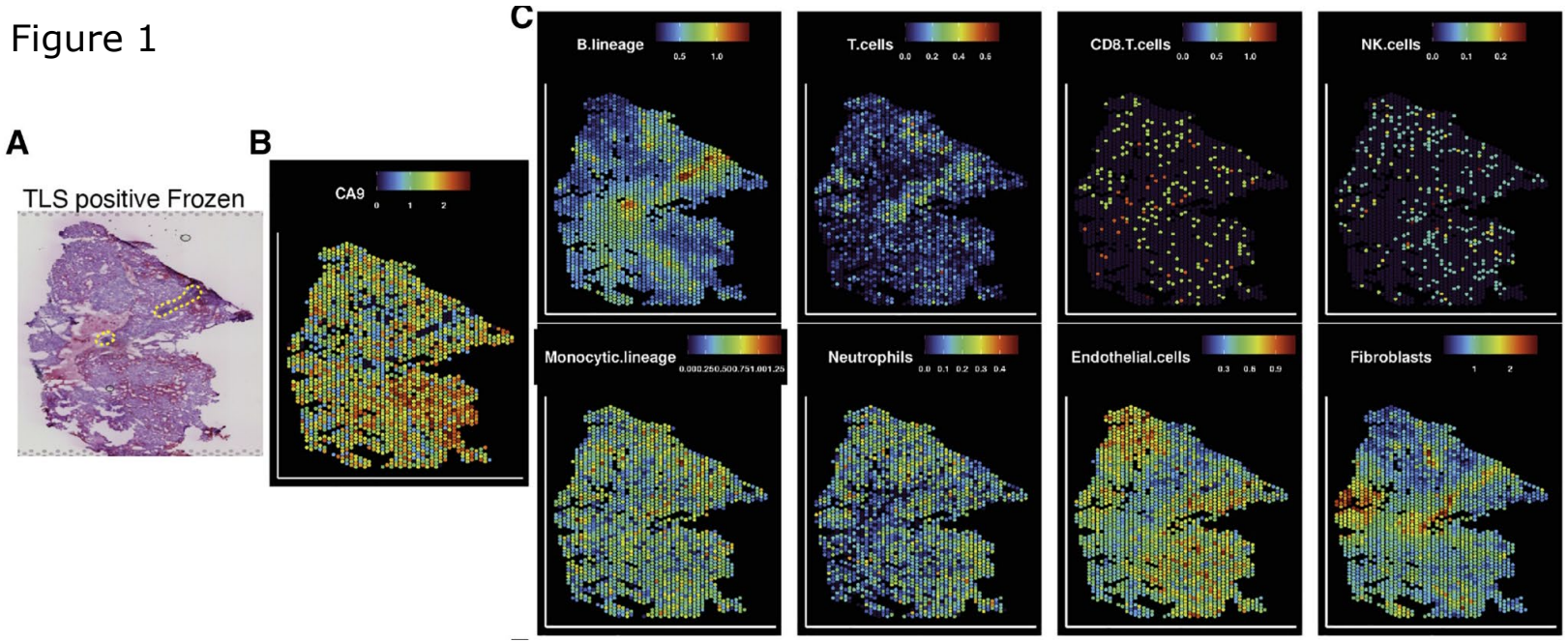
One sequencing reaction per spot
→ Multicell average
→ Substantial gaps

Deep dive into 10X Visium Technology



Spatial transcriptomics can detect cell types (?)

Figure 1



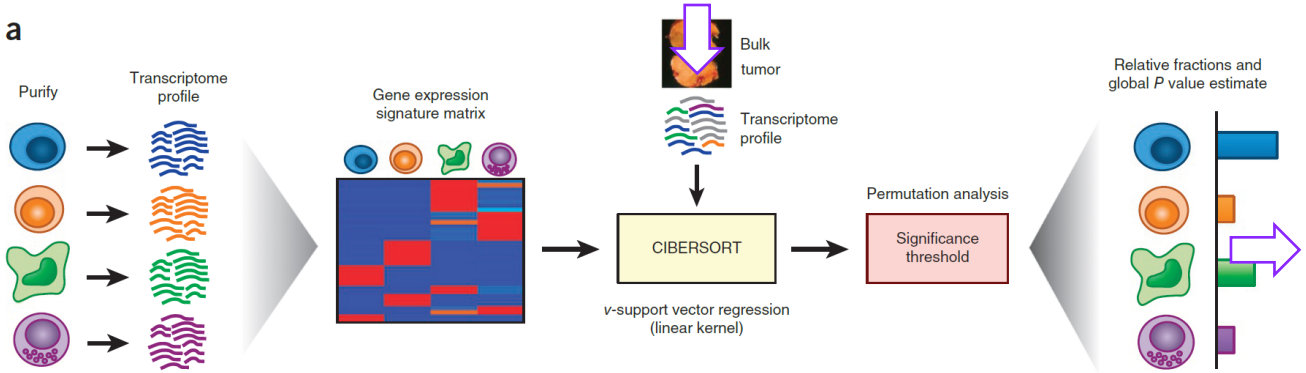
Cell types are **imputed** at the **spot level** by averaging set of genes (**signature**)

Cell type imputation strategy – estimate fractions

Estimate fraction of cells in bulk RNA-seq

INPUT:
Sequences from each spot of 10X Visium

CIBERSORT
Newman AN et al.
Nat Methods, 2015

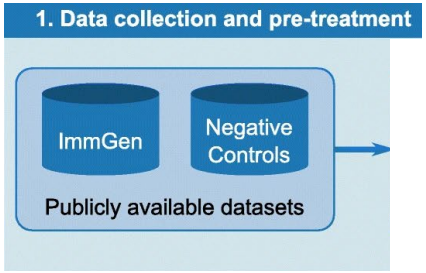


OUTPUT:
Proportion of cells in spot

- Agility
  → user can input list of genes for each cell types of interest, change it and re-run

- Proportion have pros and cons
  → useful if cell counts are available
  → risky if there are unknown cell types

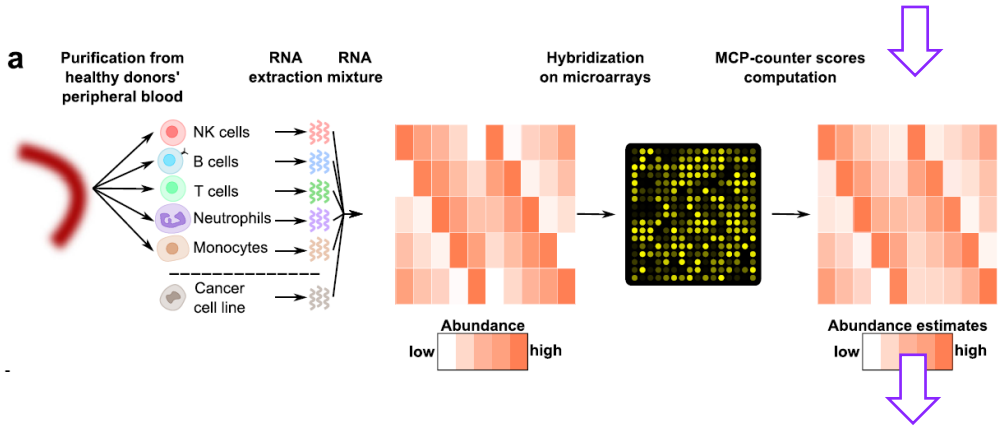
Cell type imputation strategy – signature detection

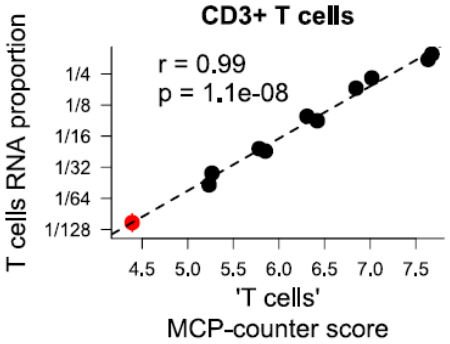


MCP-counter – human, Becht et al. Genome Biology, 2016
mMCP-counter – mouse, Petitprez et al. Genome Medicine, 2020

Cell type imputation strategy – signature detection

INPUT:
Sequences from each spot of 10X Visium

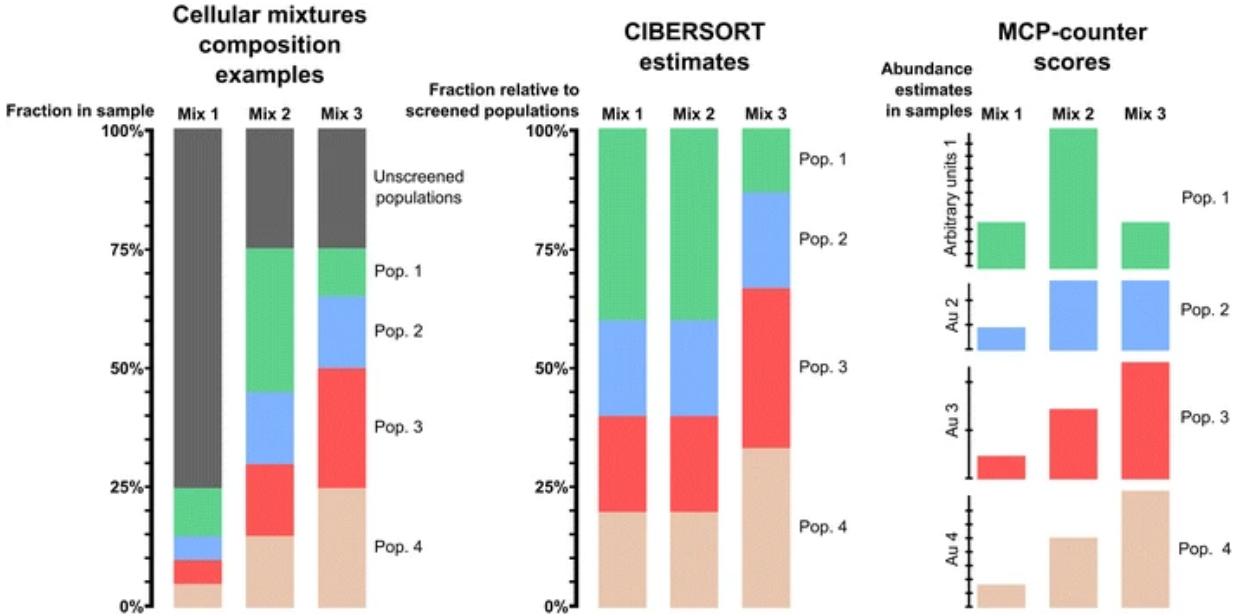




Becht et al. Genome Biology, 2016

OUTPUT:
Cell type signature score
→ proportional to cell type abundance

Cell type imputation strategy – comparison



Petitprez F et al. Cancer Immunol Immunother 2018
https://doi.org/10.1007/s00262-017-2058-z

Spatial characterization of B cell phenotypes

- Detection of subtypes of B cells by signature
- Spatial analysis limited to H&E annotation of TLS

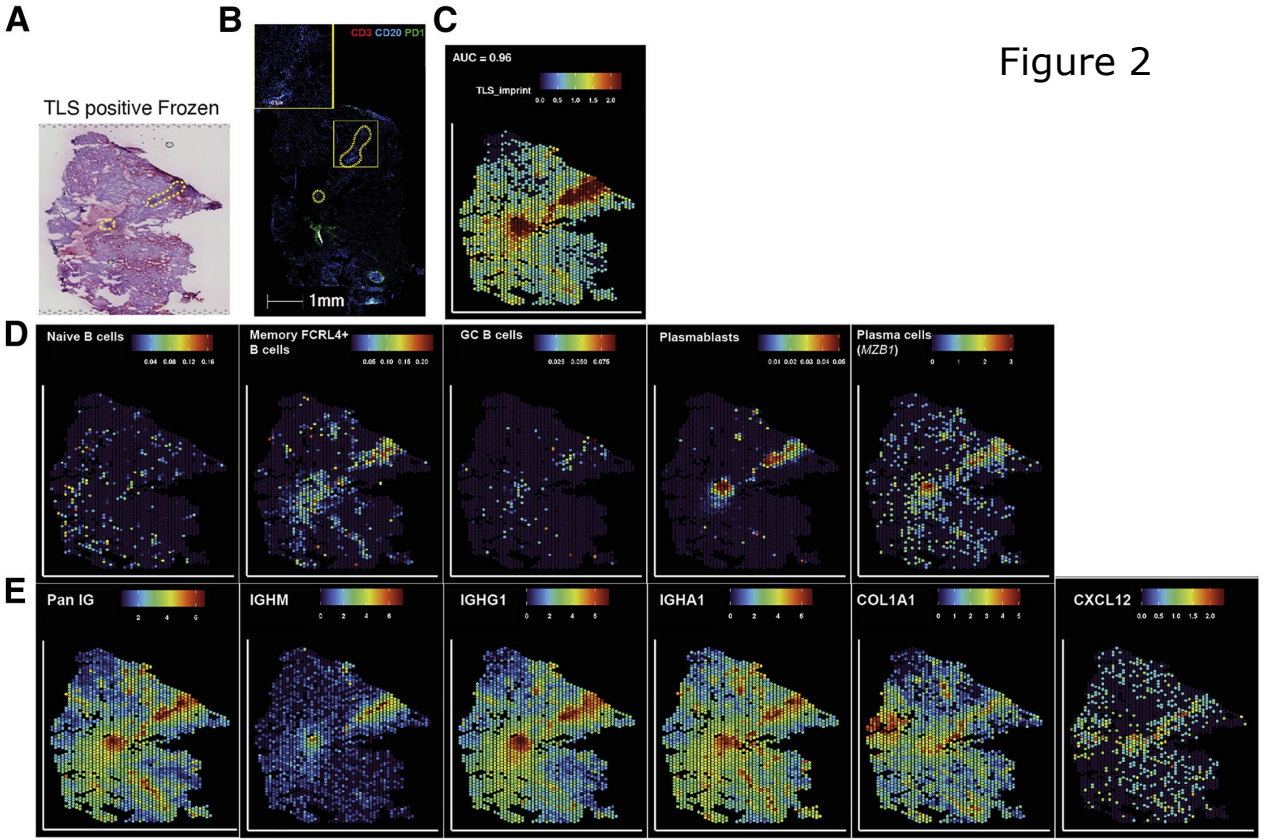


Figure 2

Paper flow

10X Visium Transcriptomics

1

Spatial Characterization of TLS in ccRCC

Find B cells within TLS

High local IgA and IgH in Plasma cells

High local clonal diversity

TLS are sites of anti tumor B cell maturation

2

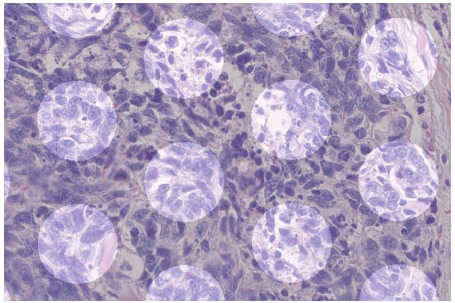
Find PC and Fibroblasts

Ig+ PC are close to fibroblasts

Antibody producing cells disseminate along stromal tracks in TLS+ tumors

Multiplexed Immunofluorescence

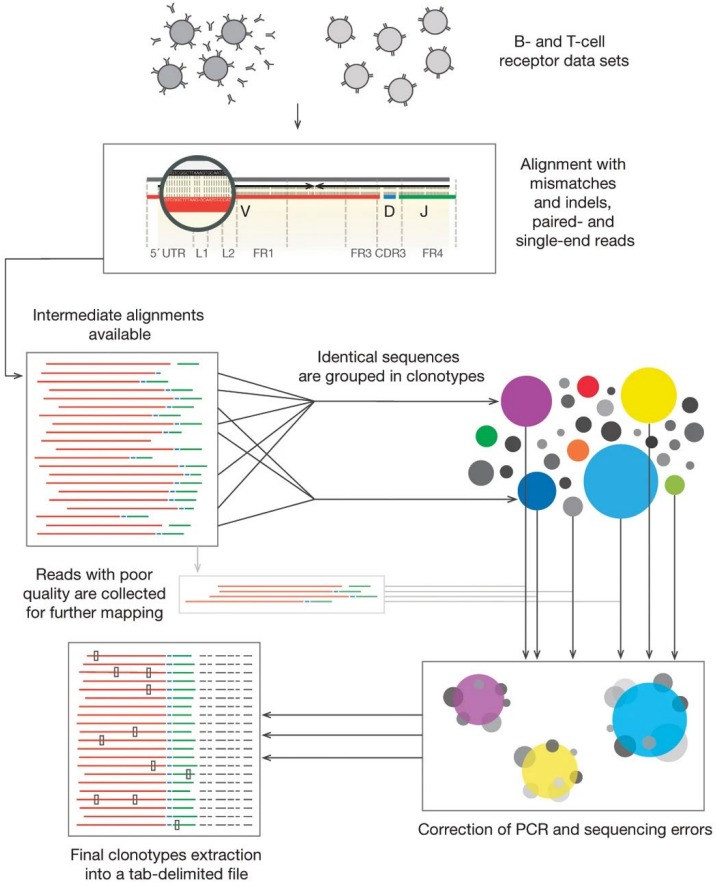
Cell averaging allows for application bulk RNA-seq algorithms



MIXCR – Bolotin et al, Nat Meth 2015

software for fast and accurate analysis of raw T- or B- cell receptor repertoire sequencing data

(requires 3X deeper sequencing)



Spatial characterization of B cell clonotypes

- Highest BCR clonotype diversity is observed in TLS → number of different clones

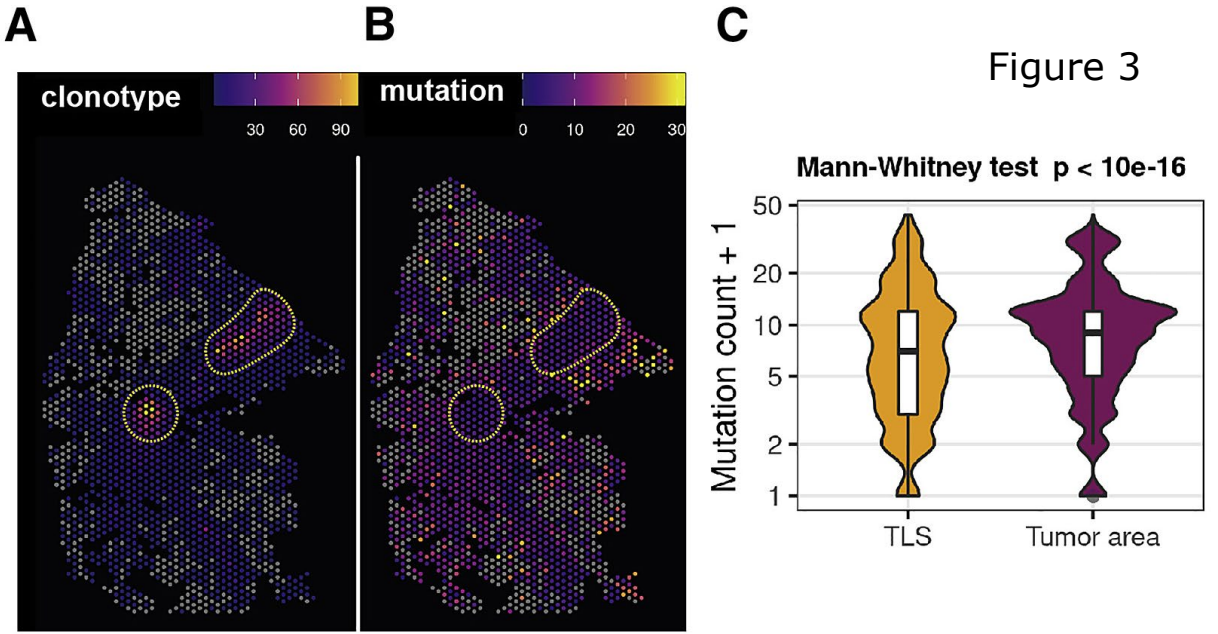
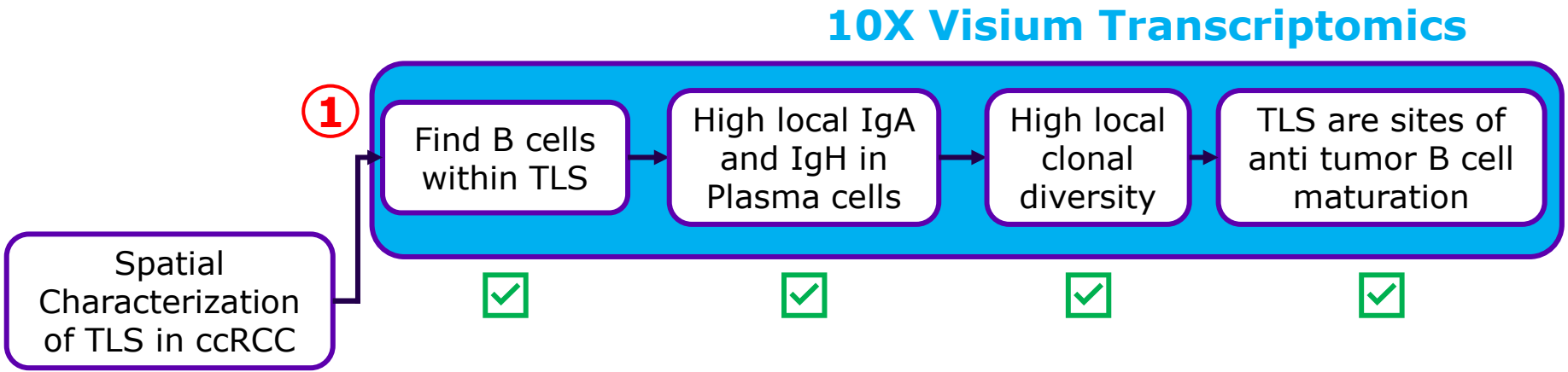


Figure 3

Paper #1: Summary and conclusions



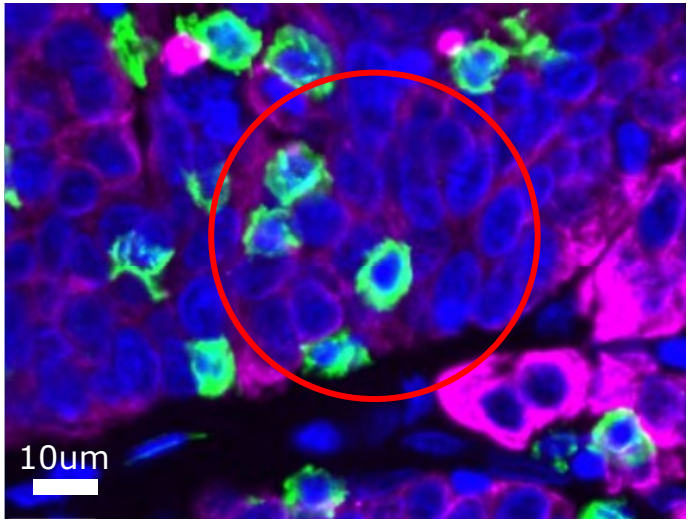
Overall conclusions:

- Sequencing-based spatial transcriptomics enables huge depth of analysis
- The actual spatial analysis is limited to definition of broad region of interest (such as TLS)
- Technical aspects are the bottleneck
    1. Lack of single cell resolution and averaging across cell types
    2. Substantial gaps in the tissue sampling

Detour: Comparison between transcriptomics and proteomics

- Spatial resolution

Colonrectal Carcinoma
Keratin = Tumor Cell
CD8 = T cell

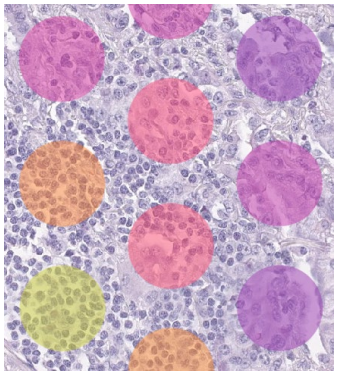


Example of Visium 10X Spot >20 cells

What variables are important in spatial biology

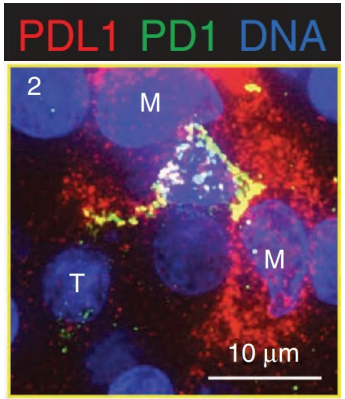
- Spatial resolution Ref: Cell size (diameter) range = 5um – 20um

Multicellular



RNA Sequencing

Subcellular



Multiplexed Immunofluorescence

Nirmal AJ et al. Cancer Discov, 2022

What variables are important in spatial biology

- Spatial resolution
- Number of features Ref: 20K genes, 20-100K proteins

Unbaised	**Baised panel**
~2-4K genes (average)	40-60 antibodies/proteins
RNA Sequencing	Multiplexed Immunofluorescence

What variables are important in spatial biology

- Spatial resolution
- Number of features
- Throughput (area x time)

Ref: single operator on one machine

Large ROI

A: ~1cm^2
T: 4 days

~4 samples

RNA Sequencing

Large ROI

A: ~10cm^2
T: 10 days

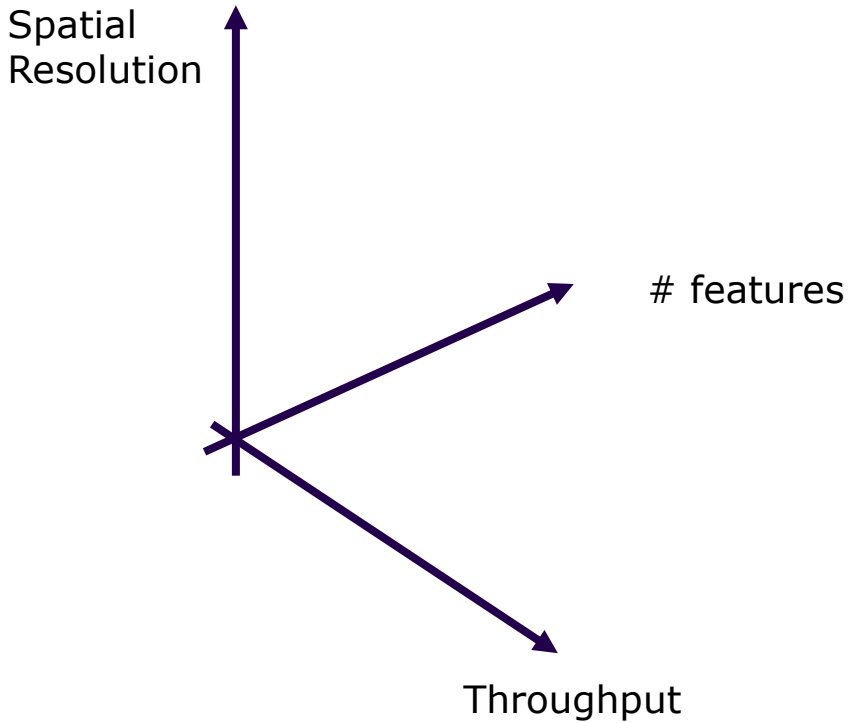
~20 samples

Multiplexed
Immunofluorescence

Discussion points – how to study spatial biology of TLS?

Variables
- Spatial resolution
- Number of features
- Throughput (area x time)

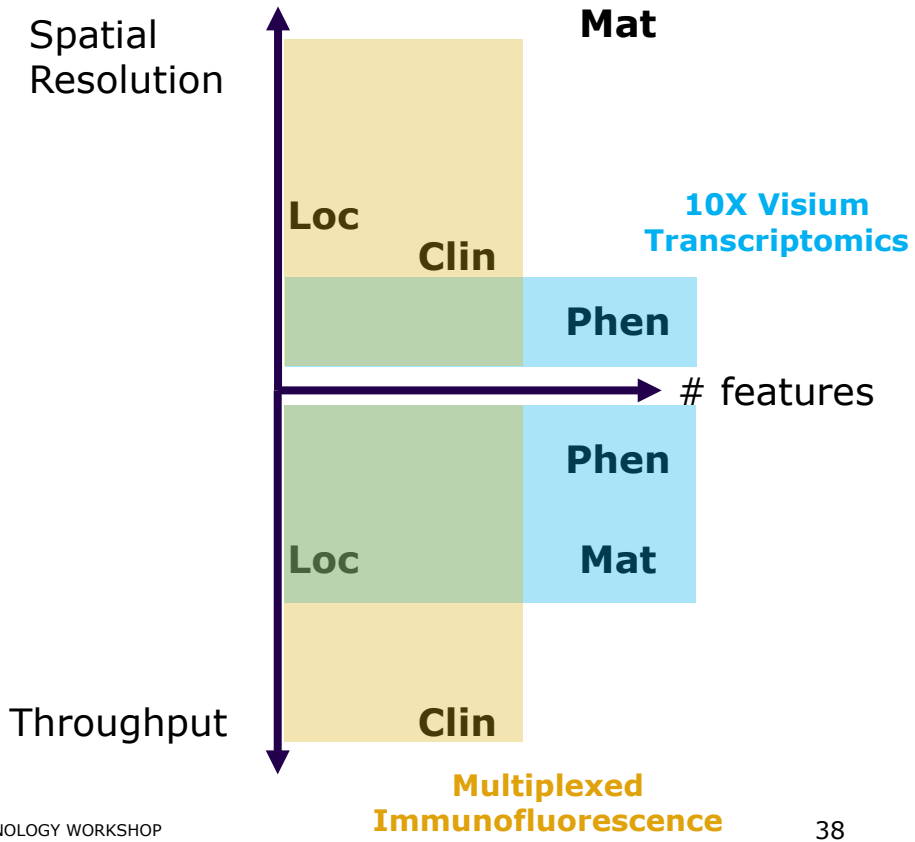
Questions:



Discussion points – how to study spatial biology of TLS?

Variables
- Spatial resolution
- Number of features
- Throughput (area x time)

Questions:

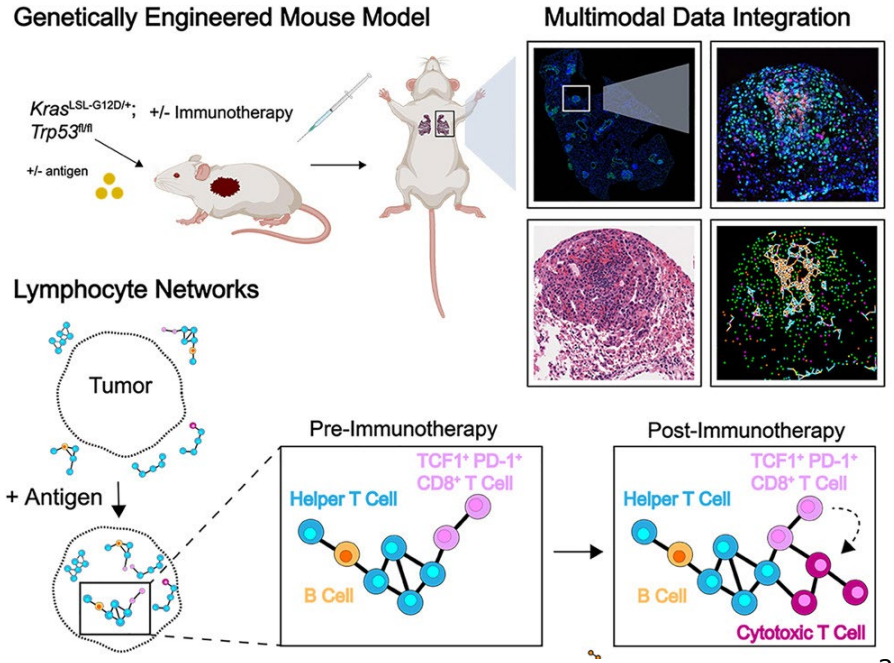


Cancer Cell

Lymphocyte networks are dynamic cellular communities in the immunoregulatory landscape of lung adenocarcinoma

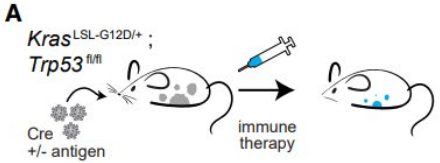
Highlights

- Interacting networks of lymphocytes (lymphonets) from in the KP GEMM of lung cancer
- Small lymphonets have mostly T cells, and B cell fraction rises as networks enlarge
- A key feature of lymphonets is that they contain TCF1^{+}PD-1^{+}CD8^{+} T cell progenitors
- Lymphonets gain cytotoxic CD8^{+} T cells after immunotherapy



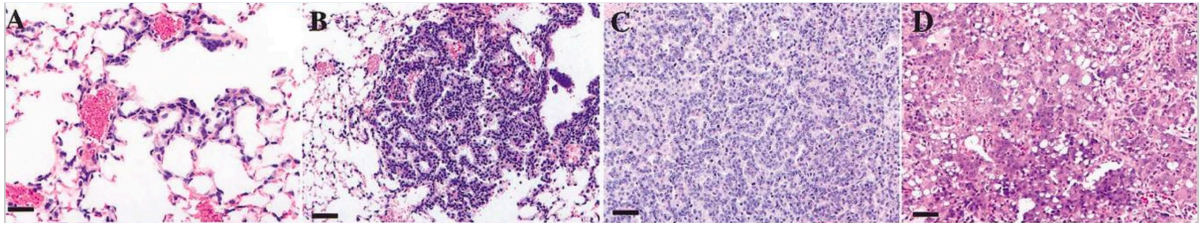
Paper #2: Key aspects

1. Multimodal data integration
   - Pathology
   - Proteomics
   - RNA

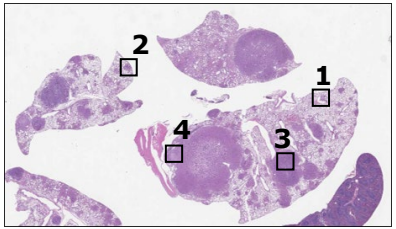


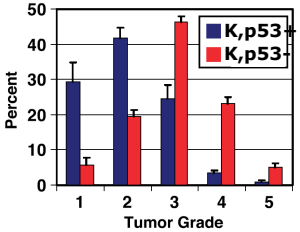
GEMMs are a tractable of model of spatial heterogeneity

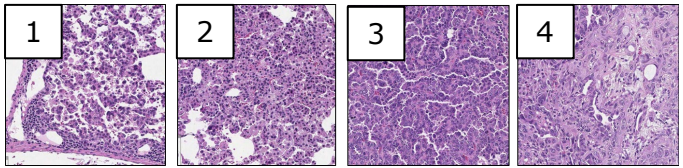
"KP" lung cancer model
K-ras$^{LSL-G12D/+}$ p53$^{fl/fl}$

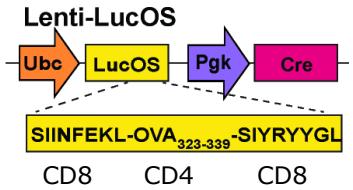


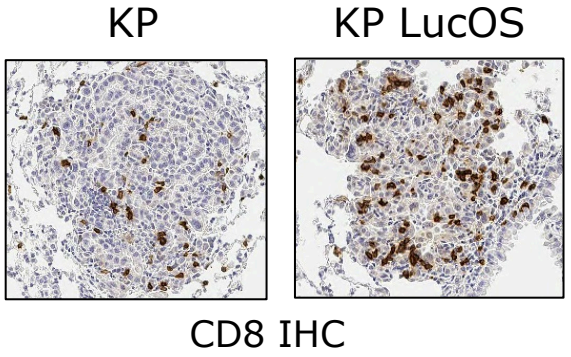
Intra-mouse tumor heterogeneity







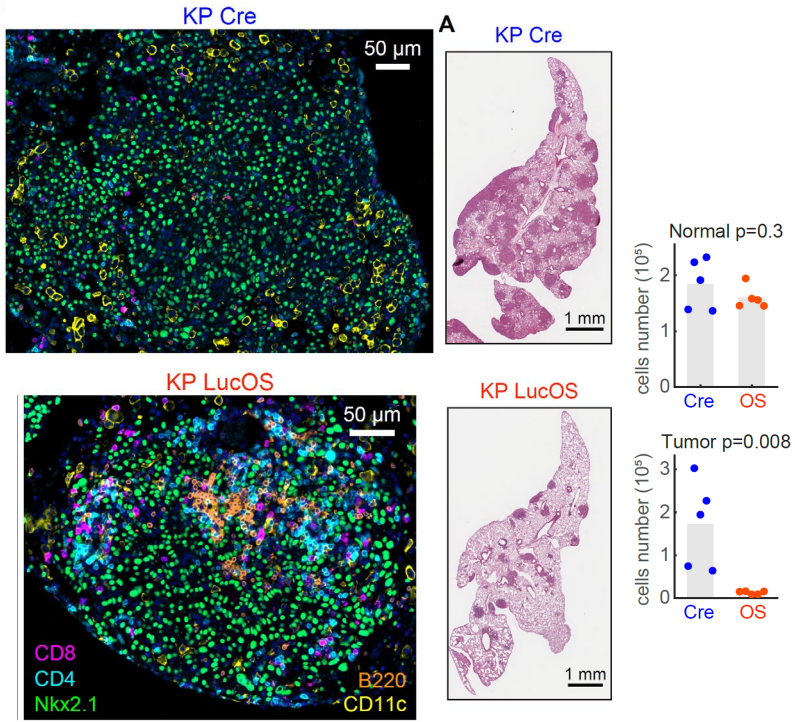


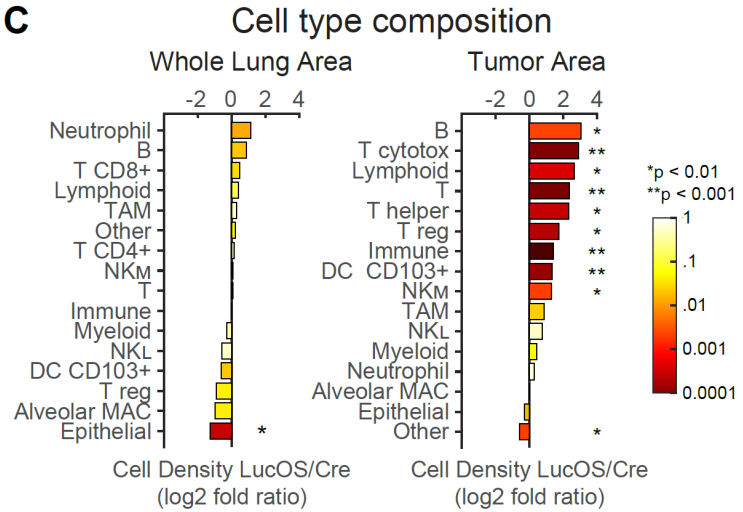


Jackson et al. Cancer Res 2005
DuPage et al. Nat Protocols, 2009
DuPage et al. Nature 2012

Antigen expression *locally* reshapes the lung immune landscape

KP = Kras G12D/p53-/-, LucOS = CD8 T neo-antigen

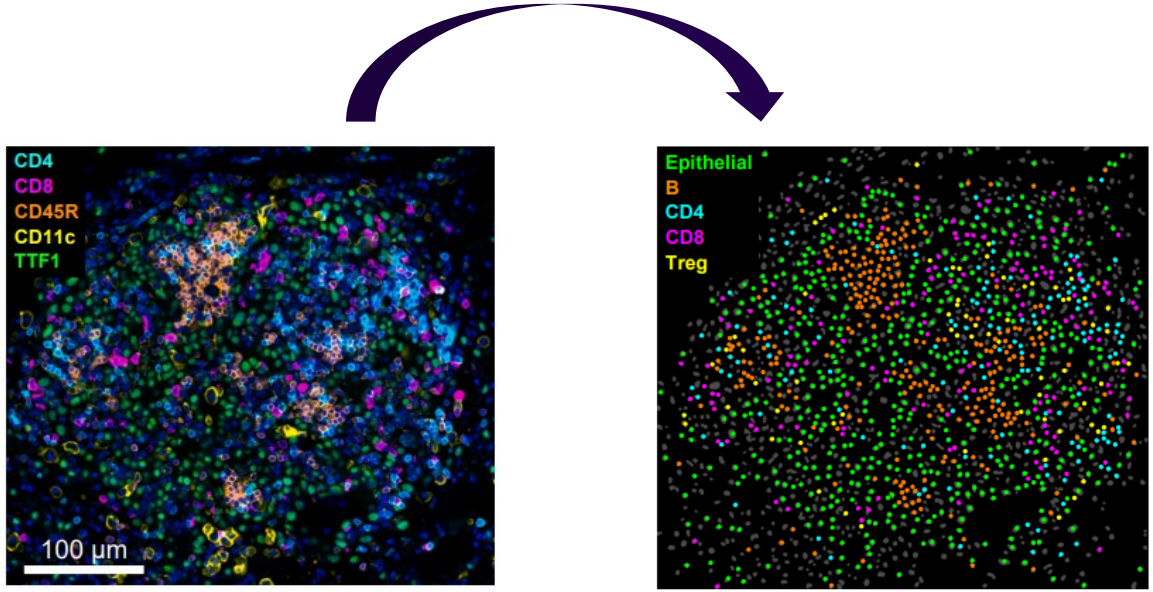




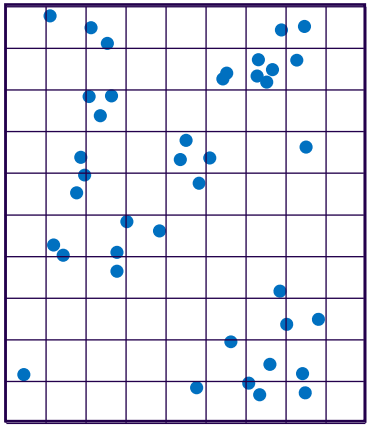
Neo-antigen presentation does not alter <u>whole lung</u> immune landscape

→ **Spatial resolution needed!**

Basic transformation from cells to.. points with labels

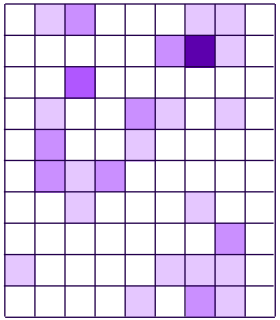


Mathematical representation



- Agent = tree, cell, mRNA

Gridded process

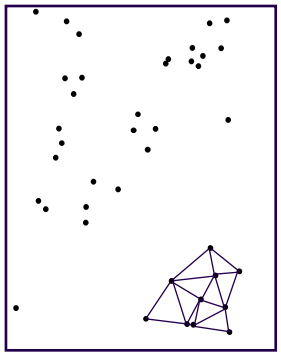


Small & Dense
"Count" Matrix

```
0 1 2 0 0 0 1 1 0
0 0 0 0 0 2 4 0
0 0 3 0 0 0 0 0 0
0 1 0 0 2 1 0 1 0
0 2 0 0 1 0 0 0 0
0 2 1 2 0 0 0 0 0
0 0 1 0 0 0 1 0 0
0 0 0 0 0 0 0 2 0
1 0 0 0 0 1 1 1 0
0 0 0 0 1 0 2 1 0
```

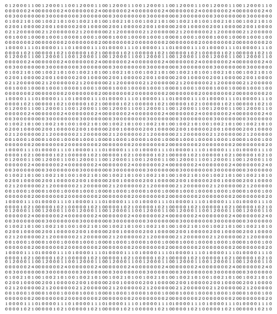
Each entry of the matrix is one square

Point process



Network?

Large, Binary &
Sparse Matrix



Each entry of the matrix is one cell → the x,y are the cell location

First-order global metric: aggregation indices

Ripley's K index

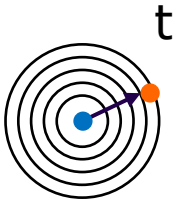
I = 1
If condition
is true

d = distance

Radius of
search

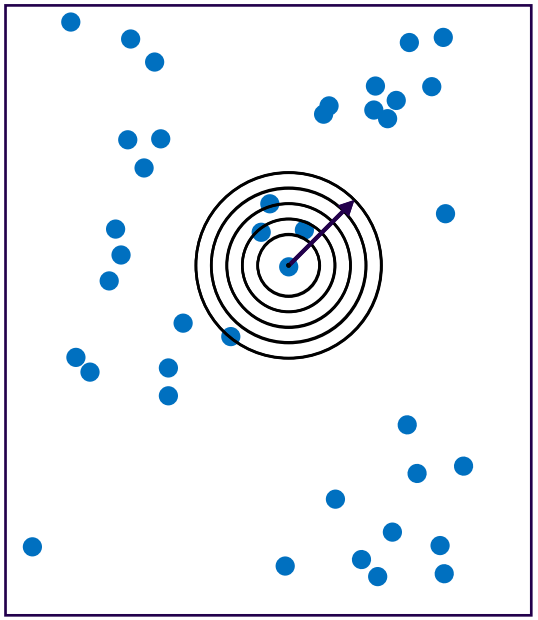
$$\widehat{K}(t) = \lambda^{-1} \sum_{i \neq j} \frac{I(d_{ij} < t)}{n}$$

t



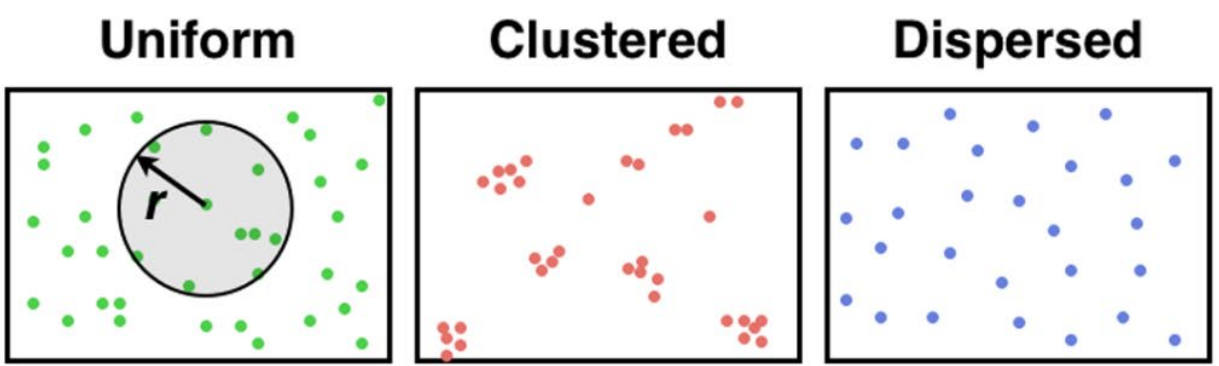
Density & number of cells
→ Averaging factors

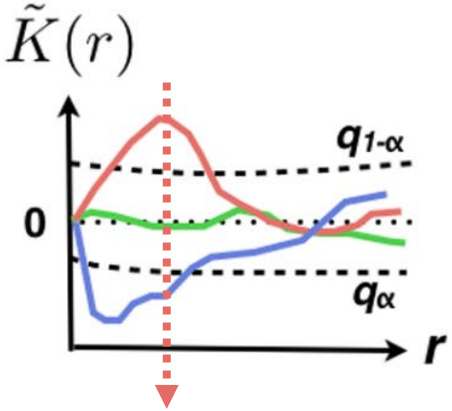
Single value for the whole field
→ Aggregation index



First-order global metric: aggregation indices

Ripley's K index





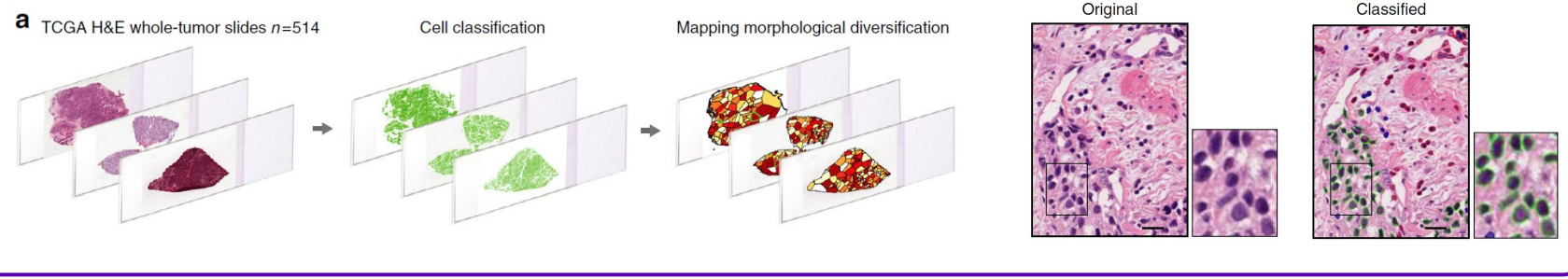
"Characteristic size" of aggregation

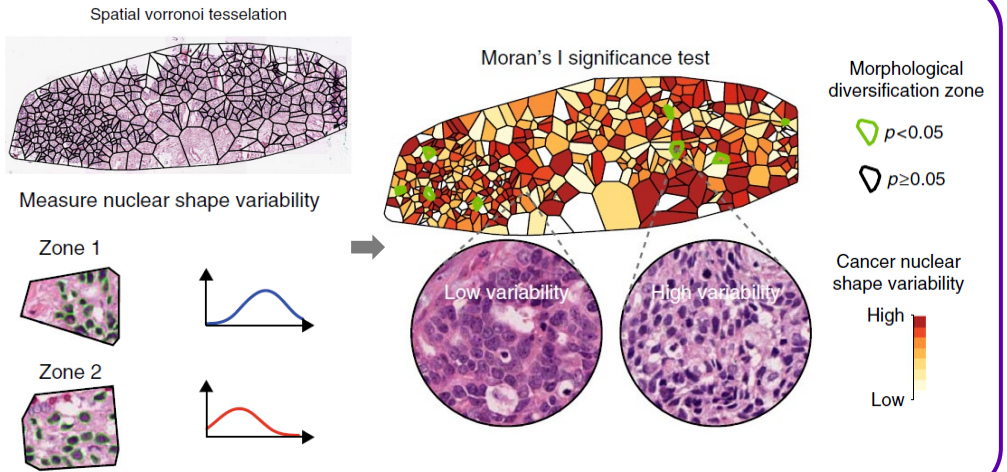
Other common aggregation indices:

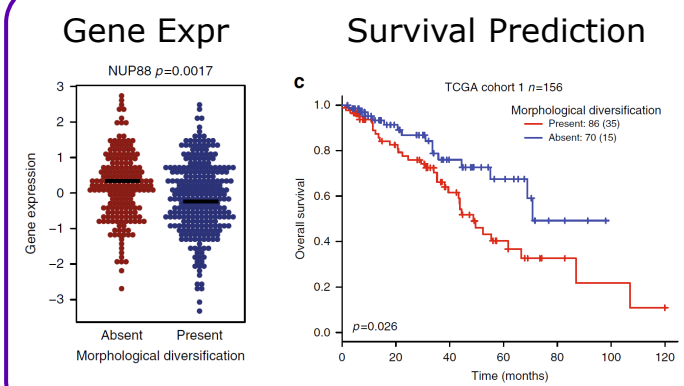
- Moran's I
- Geary's C
- Pair Correlation

Lagache T et al, PLoS One 2013

Application of Moran's I to nuclear shape in cancer







Heindl A et al, Nat Comm 2018

Historical detour

Other major academic scientific fields have investigated spatial organization:

- Statistical Physics
- Astronomy

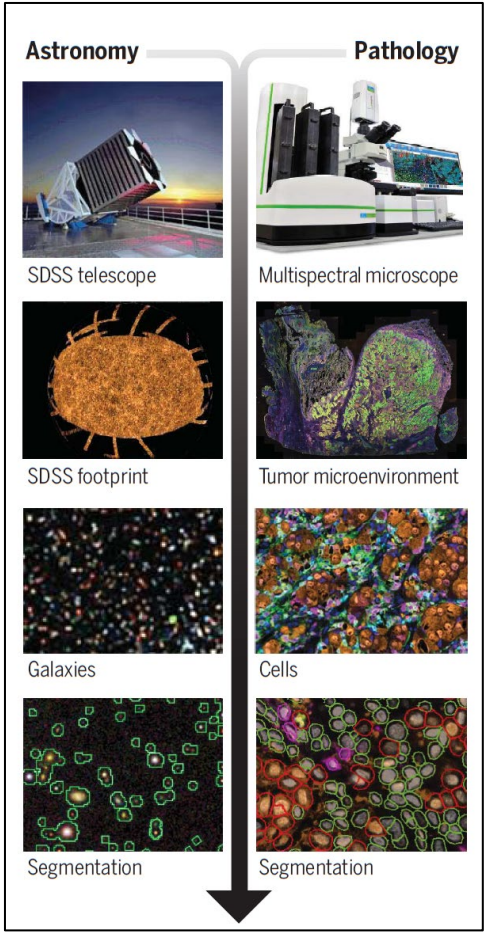
AstroPath - Berry et al, Science 2021

RESEARCH ARTICLE SUMMARY

CANCER

Analysis of multispectral imaging with the AstroPath platform informs efficacy of PD-1 blockade

Fig 1 **Strong parallels between multispectral analyses in astronomy and emerging multiplexing platforms for pathology.** The next generation of



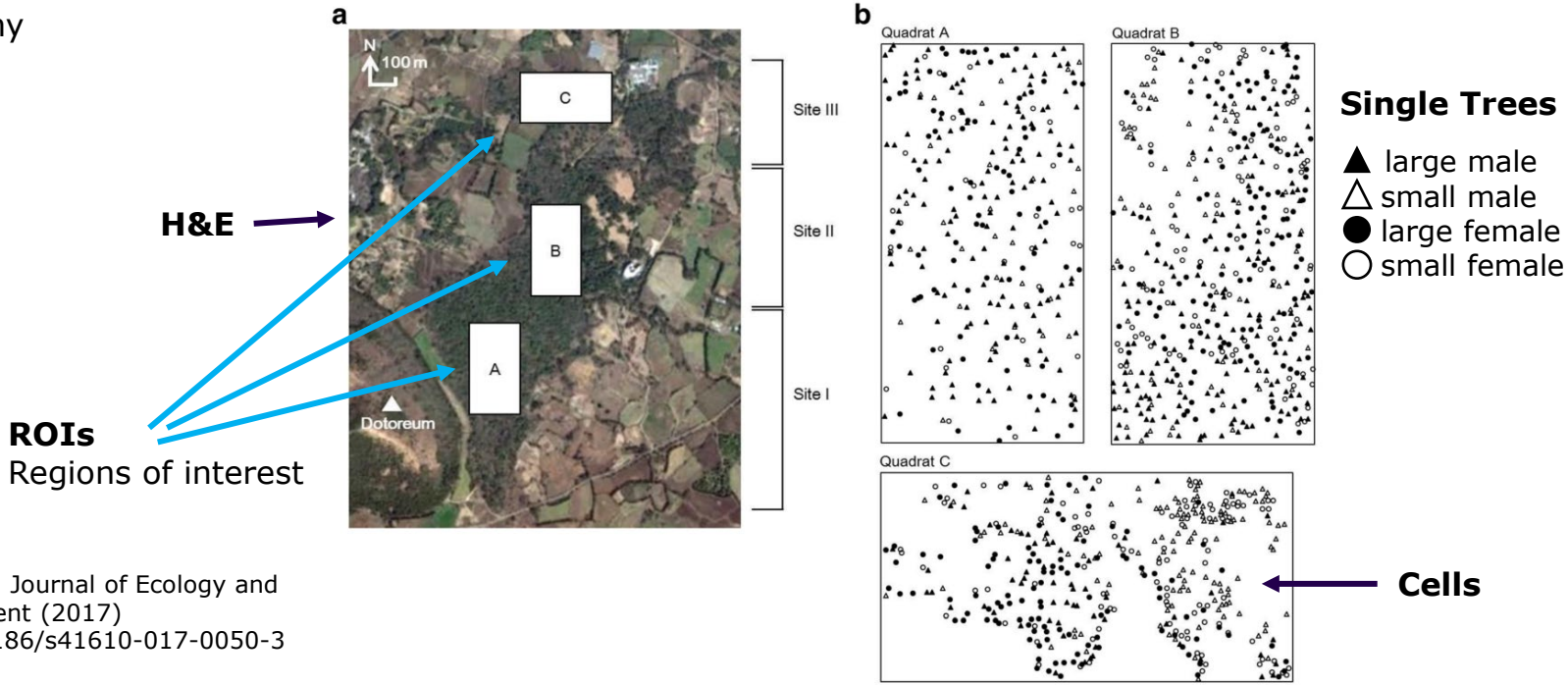
Inspiring analogy.. but the stellar and tissue context have extremely **different density and heterogeneity**

Historical detour

Other major academic scientific fields have investigated spatial organization:

- Statistical Physics
- Astronomy
- **Ecology**

Tree dynamics distribution



Shin et al. Journal of Ecology and Environment (2017)
DOI 10.1186/s41610-017-0050-3

Fig. 3 a Location of the three quadrats (160 × 300 m) in Torreya Forest. **b** Distribution of T. nucifera trees according to sex and size groups in each quadrat: small males (DBH < 50 cm, △), large males (DBH ≥ 50 cm, ▲), small females (DBH < 50 cm, ○), and large females (DBH ≥ 50 cm, ●)

Historical detour

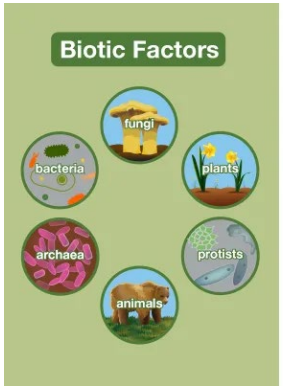
Other major academic scientific fields have investigated spatial organization:

- Astronomy
- Statistical Physics
- Ecology

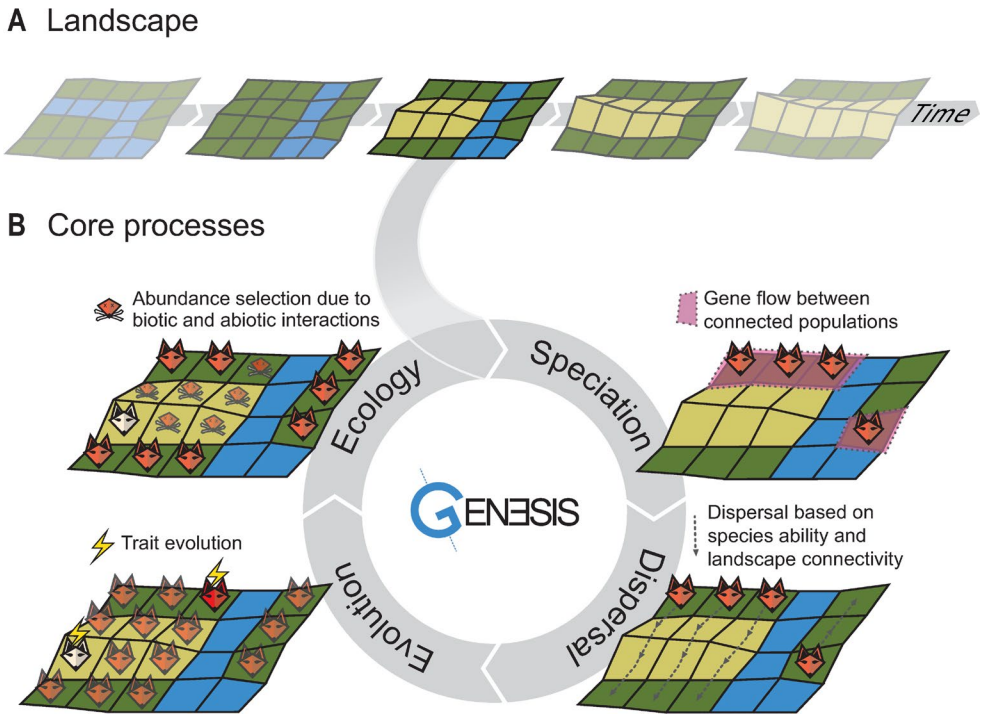
Key Similarities between Ecology and Immunology :

(1) Temporal and spatial problems & observations
(2) Length scale of complexity
(3) Overlay of environment and "agent" interactions

Abiotic vs Biotic contribution parallels tissue architecture

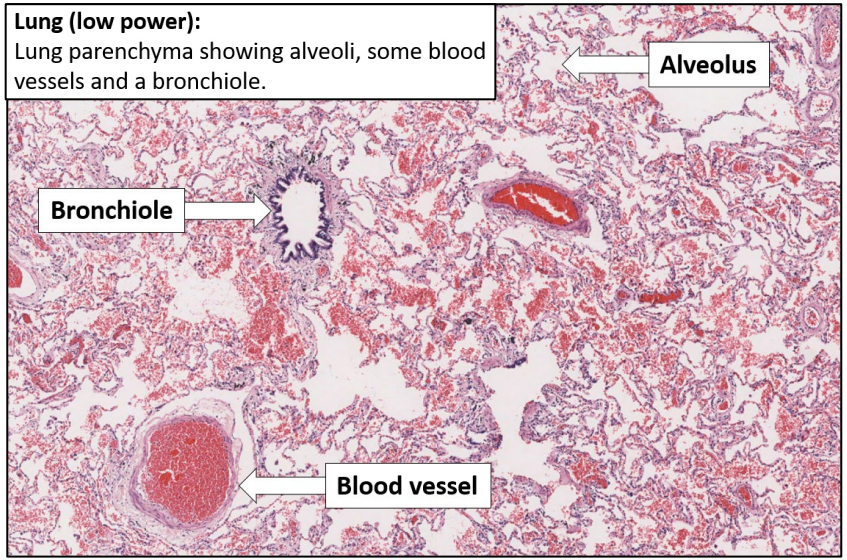


Cells Environment

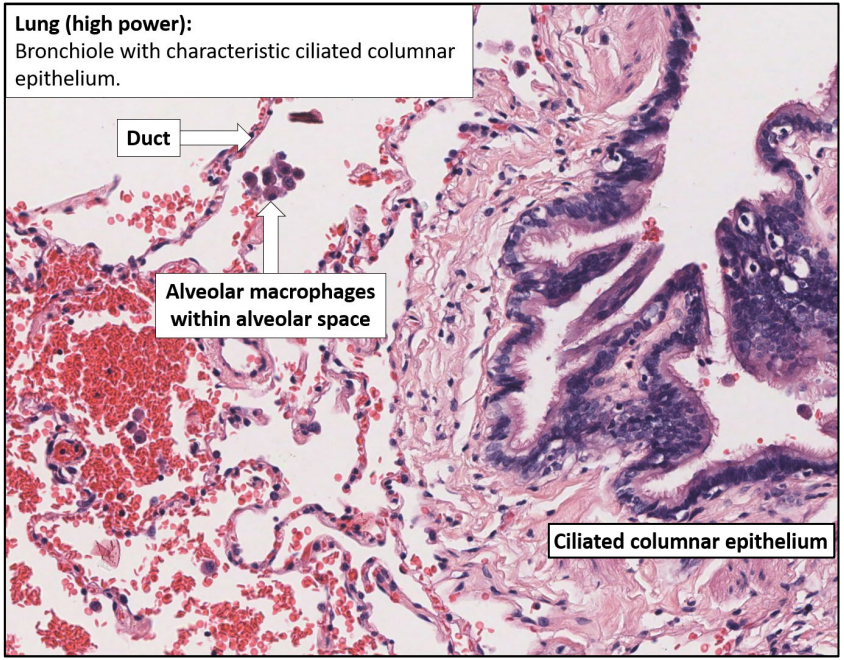


Hagen et al, PLOS Biol, 2021

Abiotic vs Biotic contribution parallels tissue architecture

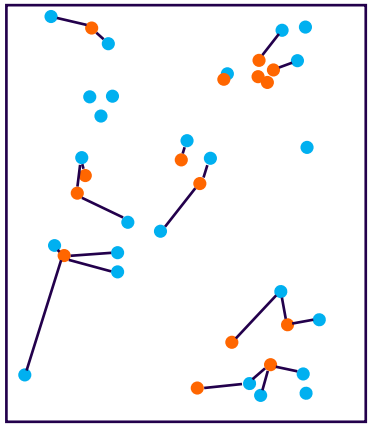


Lung – Normal Histology – NUS Pathweb :: NUS Pathweb



Tissue structure are ubiquitous → **How can we exploit them?**

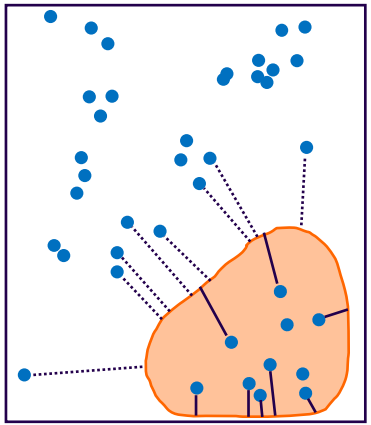
Second order metrics: distance between cells or ROI



Nearest neighbor distance

Type A

Type B



Distance from ROI

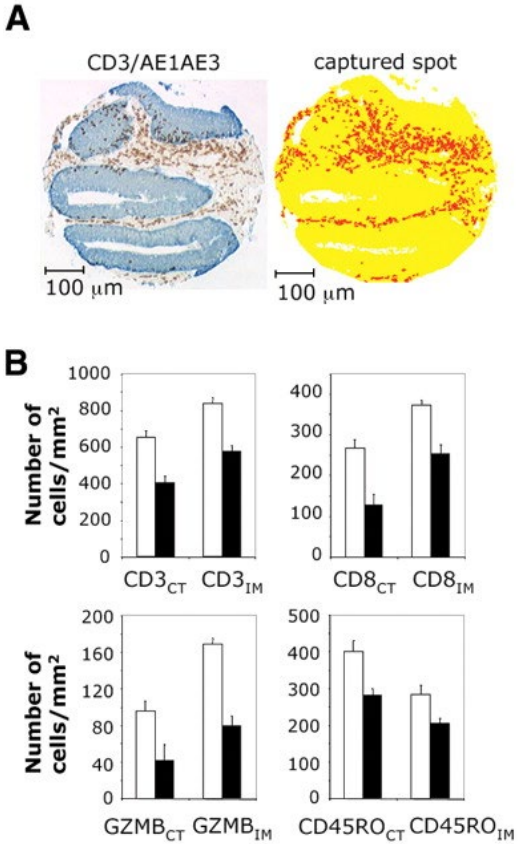
Inside

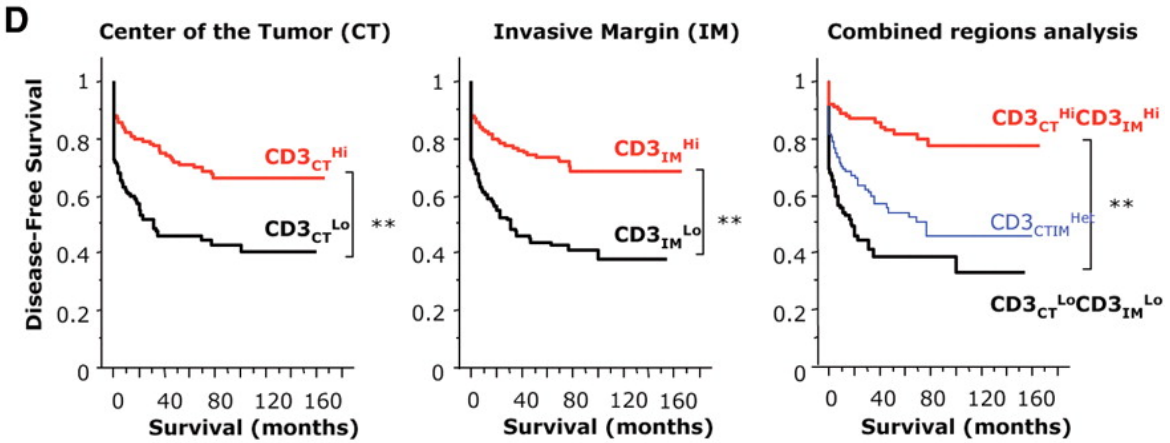
Outside

Agent

Boundary of ROI (Region of Interest)

ImmunoScore – Basic but powerful application of distance



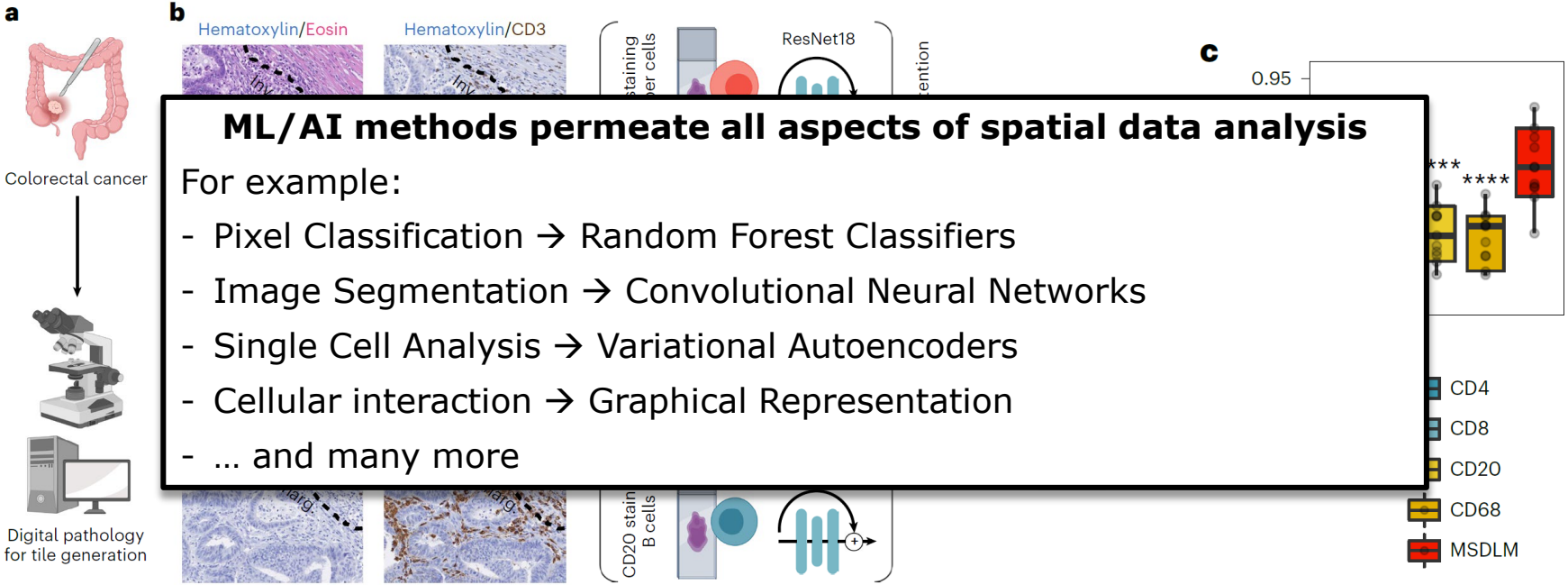


Stratification of patients based on abundance of lymphocytes inside tumors predicts response to therapy

Galon et al, Science 2006

Artificial intelligence extends ImmunoScore

Serial section staining for multiple markers + AI model == greatly improved accuracy



ML/AI methods permeate all aspects of spatial data analysis

For example:

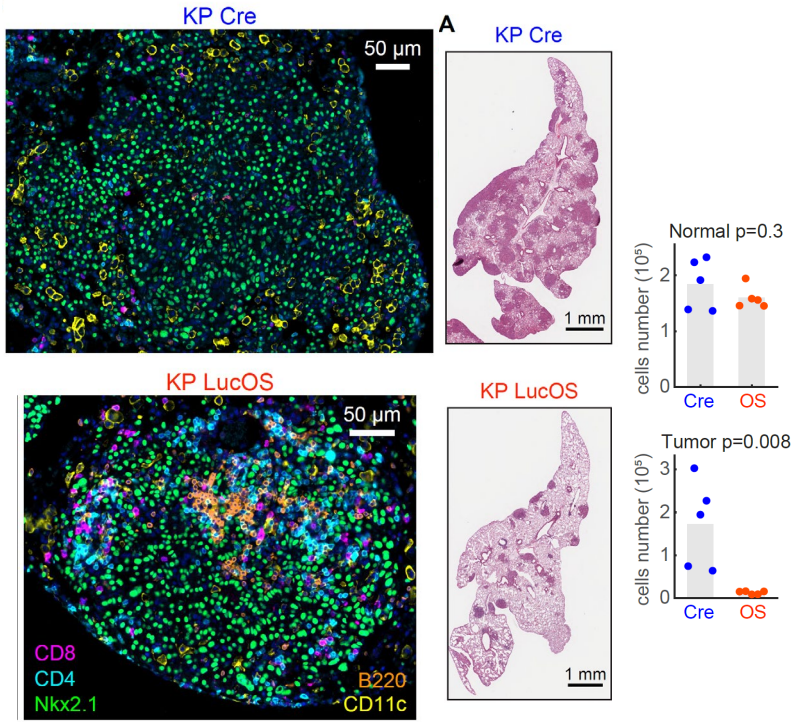
- Pixel Classification → Random Forest Classifiers
- Image Segmentation → Convolutional Neural Networks
- Single Cell Analysis → Variational Autoencoders
- Cellular interaction → Graphical Representation
- … and many more

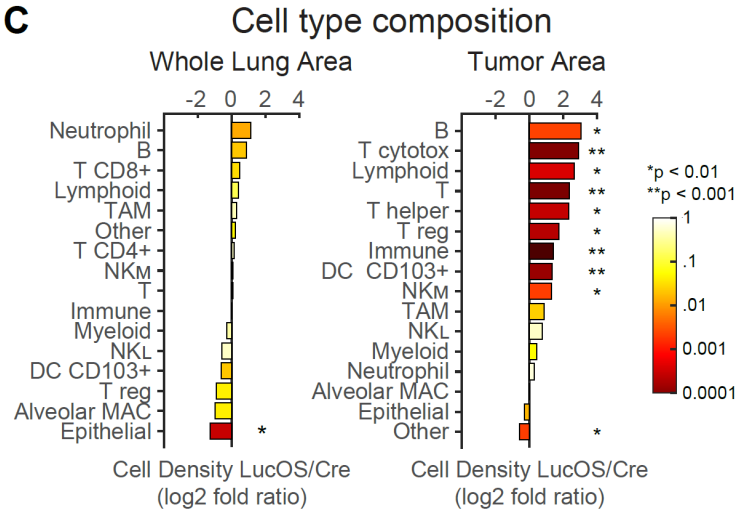
Foersch et al, Nat Medicine 2023

Antigen expression *locally* reshapes the lung immune landscape

FLASHBACK

KP = Kras G12D/p53-/-, LucOS = CD8 T neo-antigen

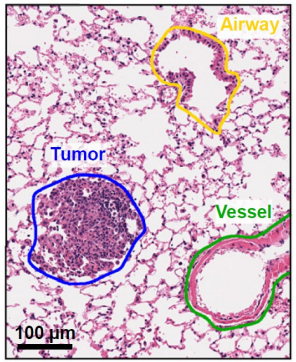


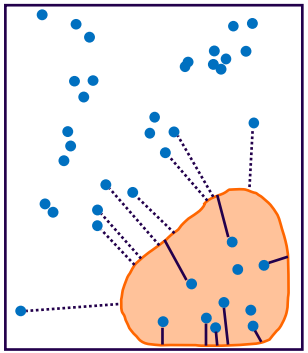


Neo-antigen presentation does not alter <u>whole lung</u> immune landscape

→ **Spatial resolution needed!**

Distance measurements show immune cell infiltration



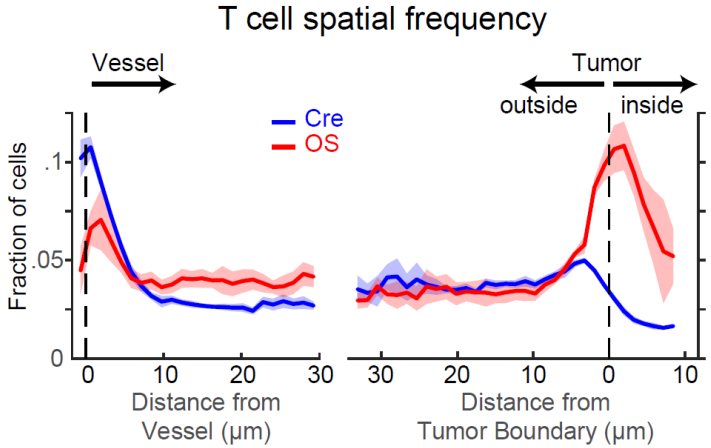


Distance from ROI

Agent

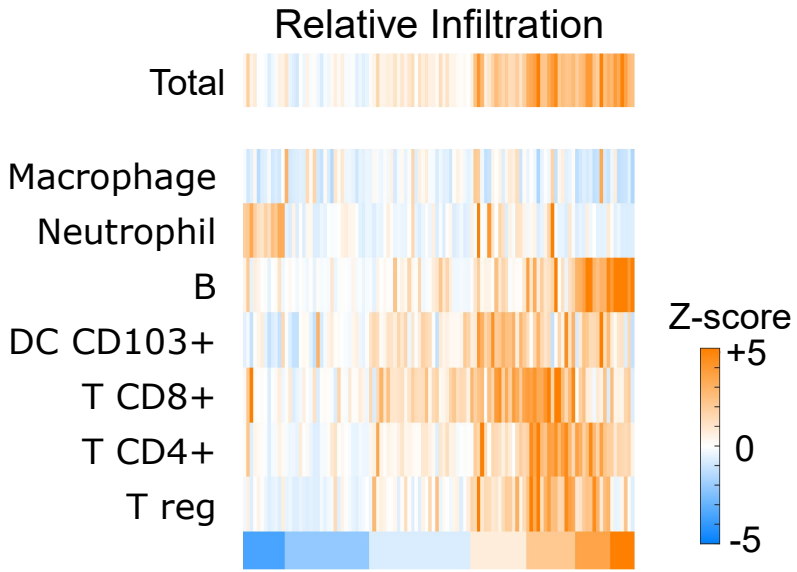
Boundary

Object = single cells!

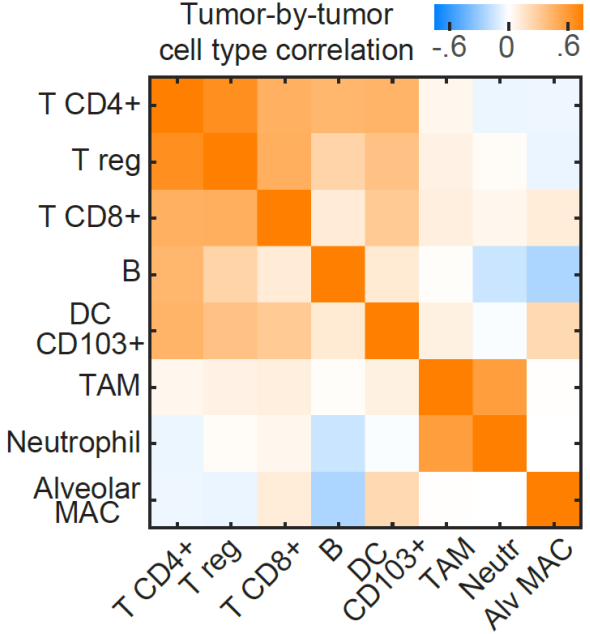


Neo-antigen presentation leads to tumor infiltration

B and T cell infiltration responses are coordinated



Object = single tumors



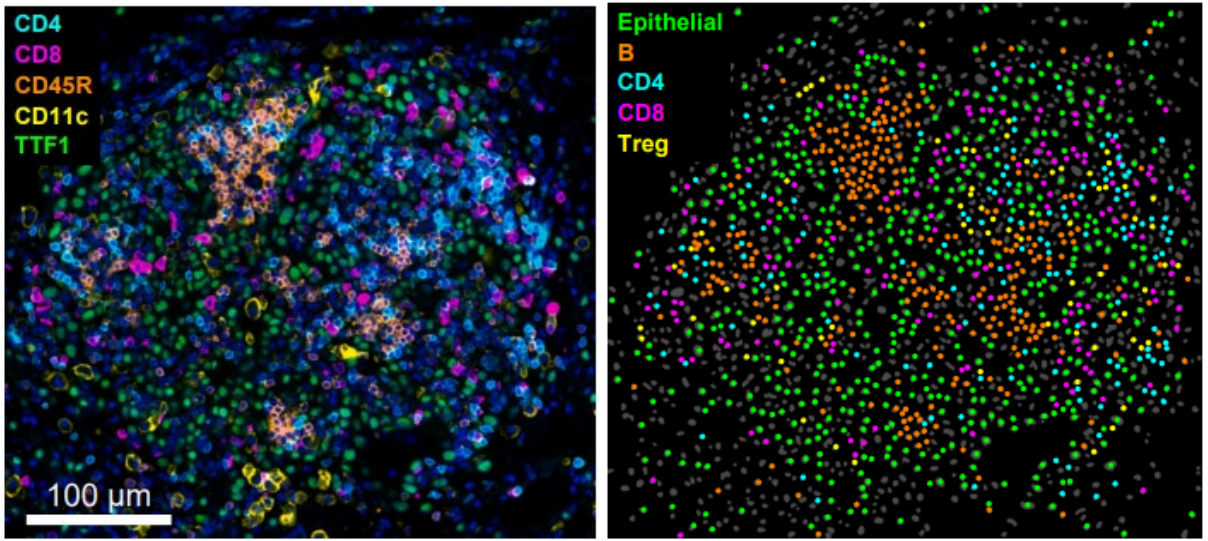
n = 112 individual KP LucOS tumors

Immune cells aggregate into groups

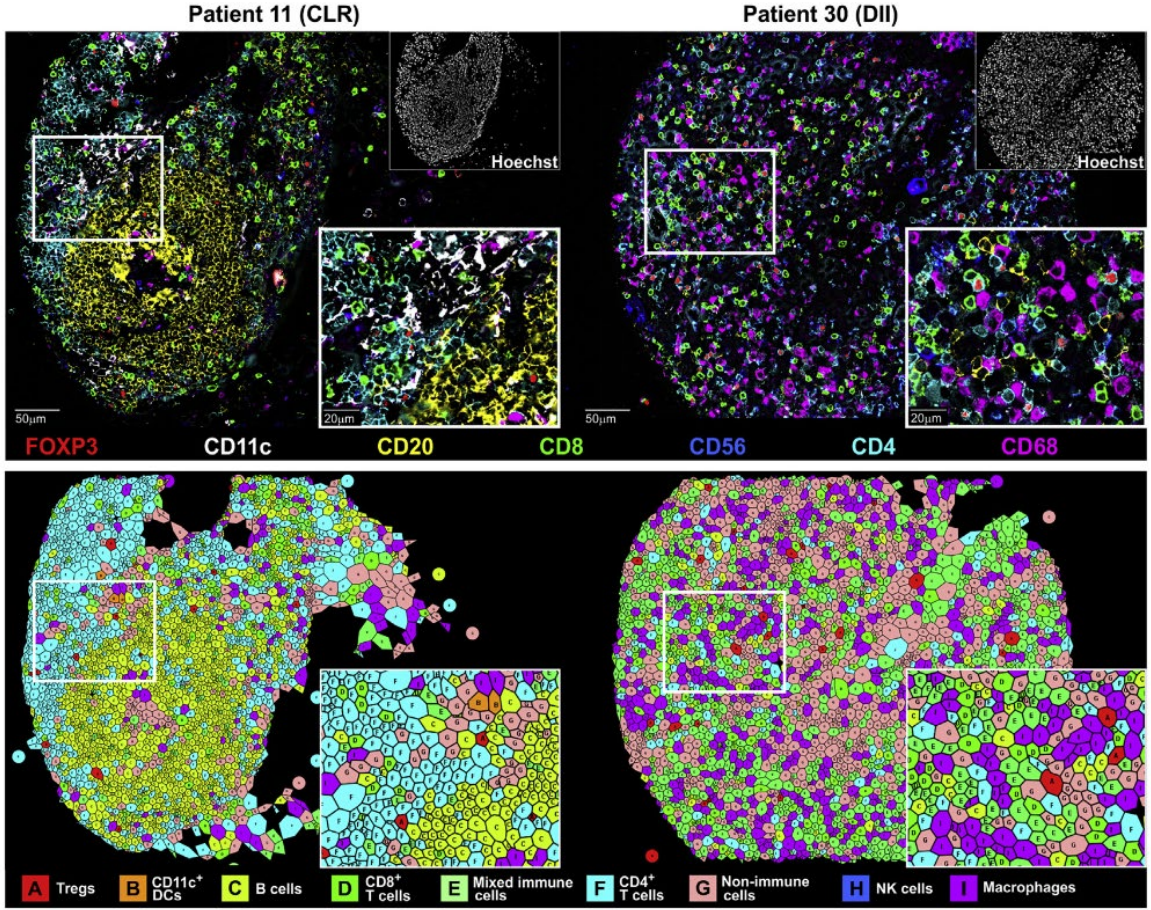
So far:

- First-order metrics
  → clustering patterns

- Second-order metrics
  → distance between objects



Higher order metrics: community detection

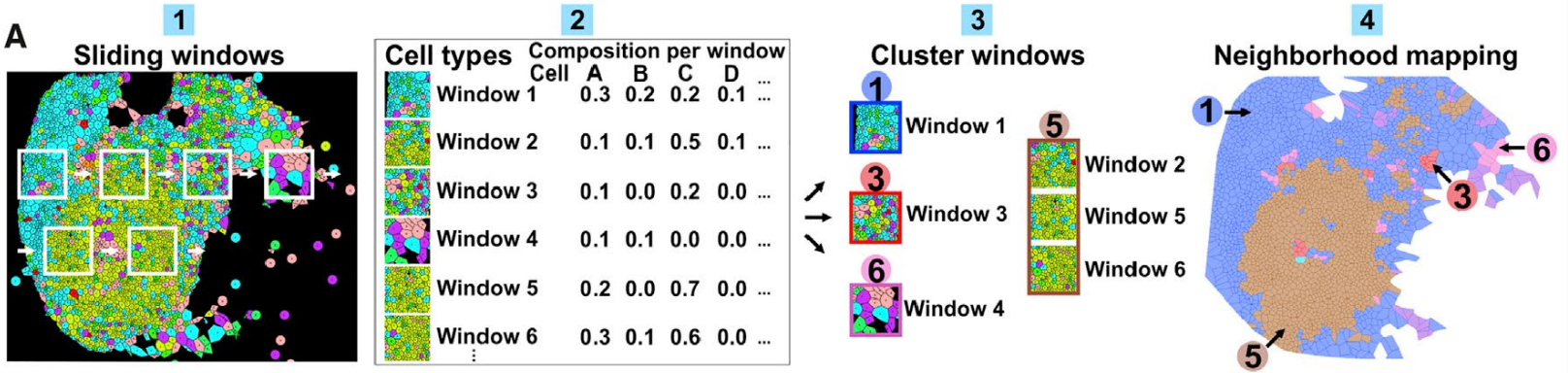


Schurch et al
Cell 2020

Higher order metrics: community detection

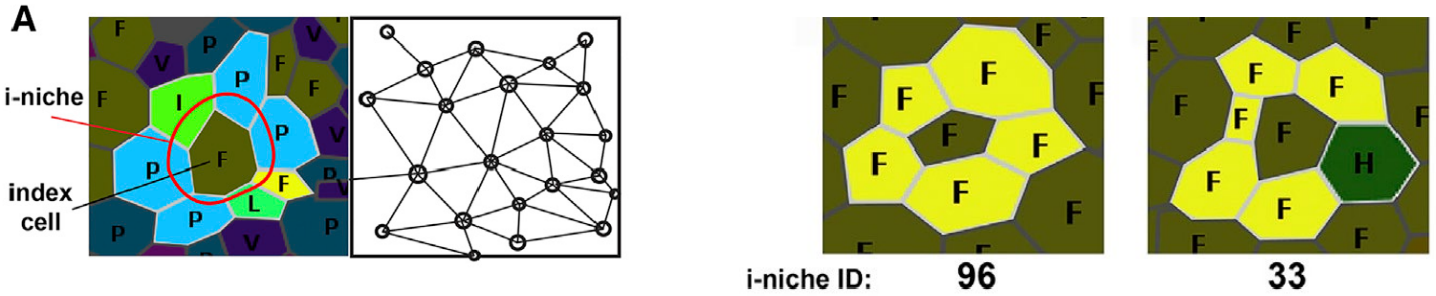
Neighborhoods

Schurch et al
Cell 2020

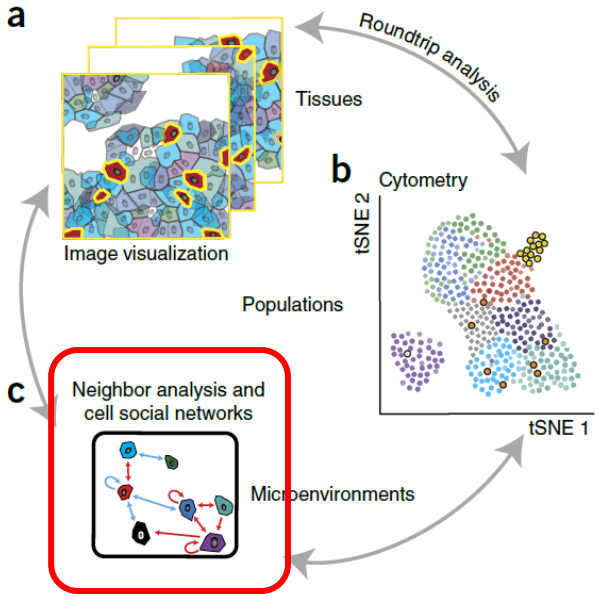


i-niches

Goltsev et al
Cell 2018

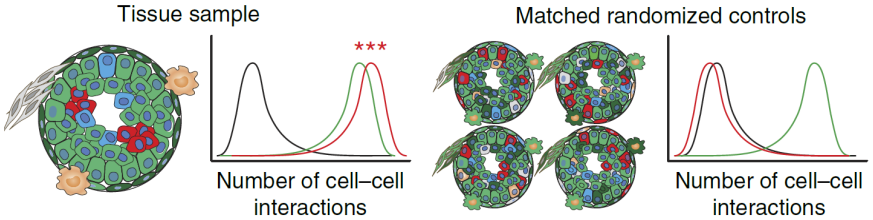


Bootstrapping as a statistical test for spatial significance



How do we statistically test spatial hypotheses?

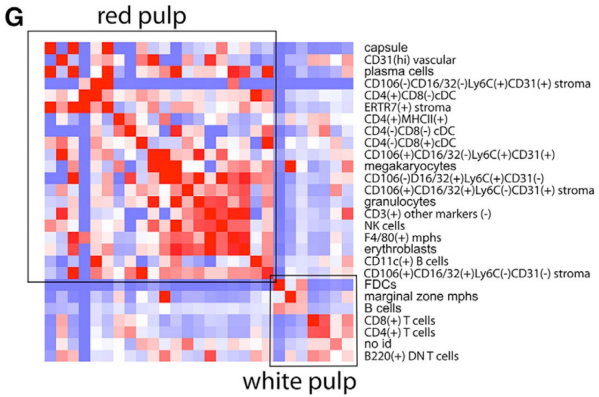
Bootstrapping testing procedure

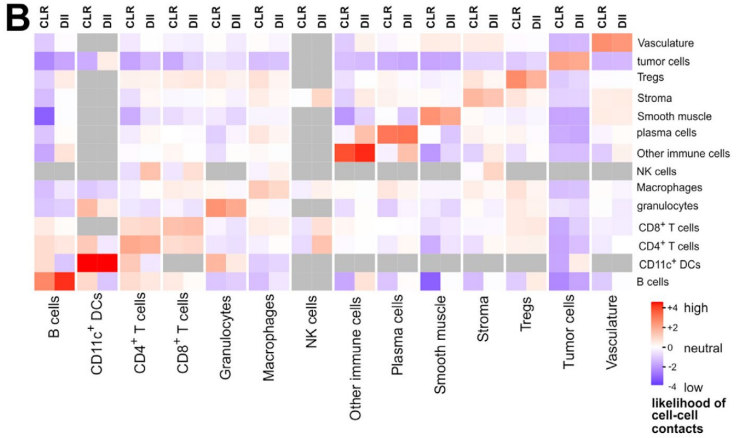


1. Define quantitative phenotype & calculate it
   → e.g. # cell-cell contacts between cells labelled "A" and "B"
2. Randomly shuffle cell labels
   → preserves (1) tissue structure, (2) label proportions
3. Recalculate # cell interactions
4. Repeat step 2 and 3 for 1000 times (or more)
5. The 1000 shuffles create a "**null distribution**"
6. Establish how many times the real measure exceeds the randomly shuffled null distribution → **p-value**!

Most interactions are between cell of the same type!

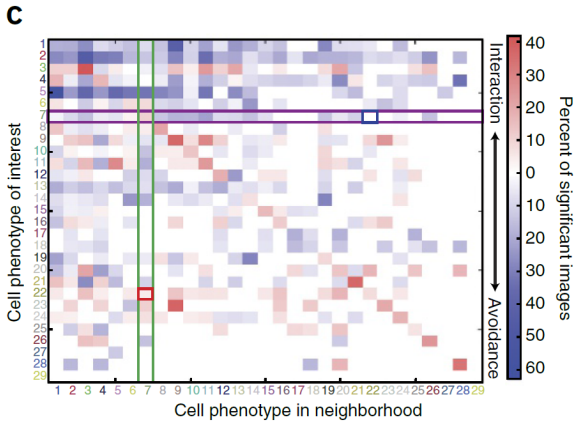
Spleen i-niches
Goltsev et al
Cell 2018





Colorectal Cancer Neighborhoods
Schurch et al, Cell 2020

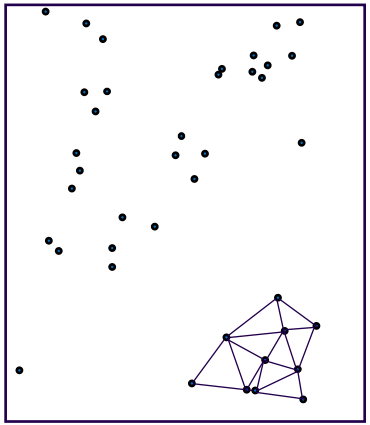
Breast Cancer Interactions
Shapiro et al,
Nature Methods 2017



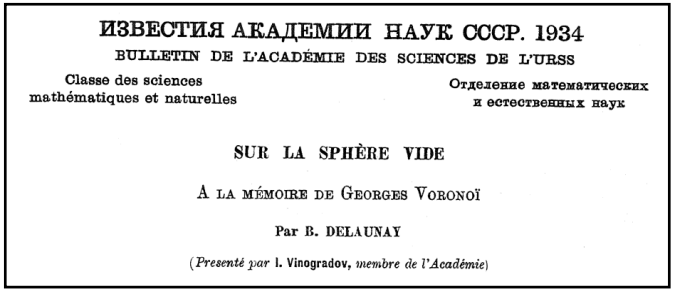
How do you calculate this?

Back to math - triangulation

Point process



Network?



ИЗВЕСТИЯ АКАДЕМИИ НАУК СССР. 1934

BULLETIN DE L'ACADÉMIE DES SCIENCES DE L'URSS

Classe des sciences mathématiques et naturelles

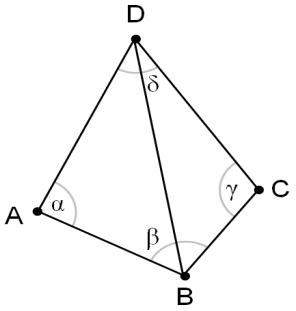
Отделение математических и естественных наук

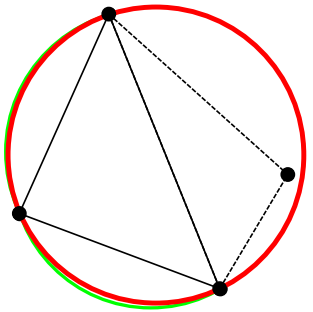
SUR LA SPHÈRE VIDE

A la mémoire de Georges Voronoï

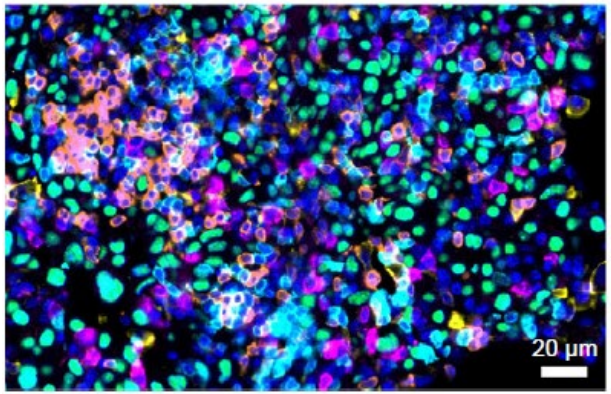
Par B. DELAUNAY

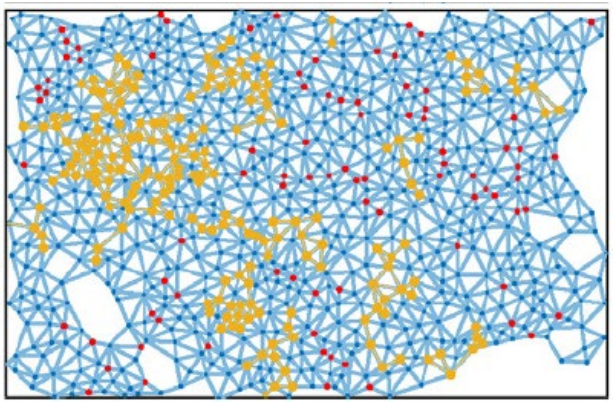
(*Presenté par* I. Vinogradov, *membre de l'Académie*)





How to select connection?
Ie defining neighboring points/cells



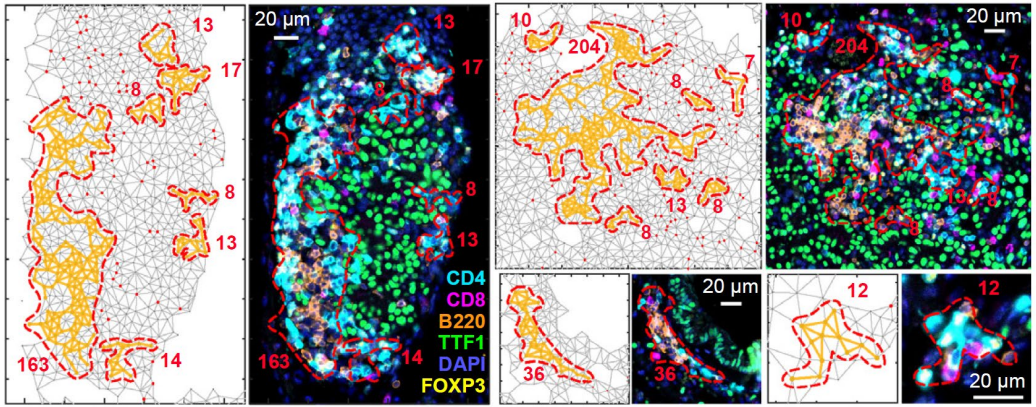


Delaunay Triangulation

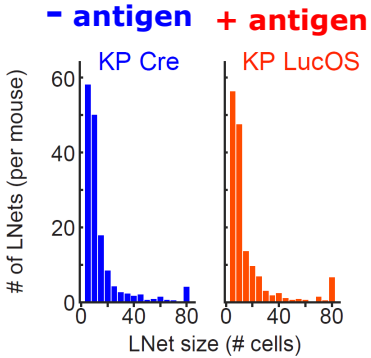
Lymphonets – fully connected networks of lymphocytes

- Non-lymphocyte cells
- Networked Lymphocytes
- Free Lymphocytes

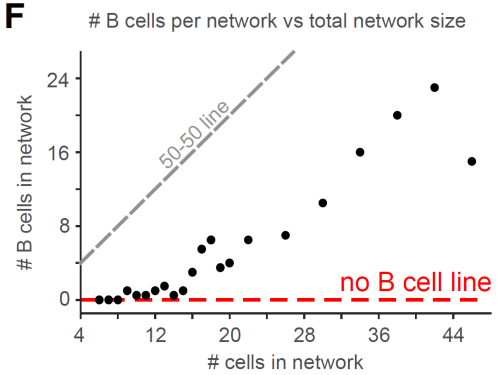
- All cell-cell contacts
- Lymphocyte contacts



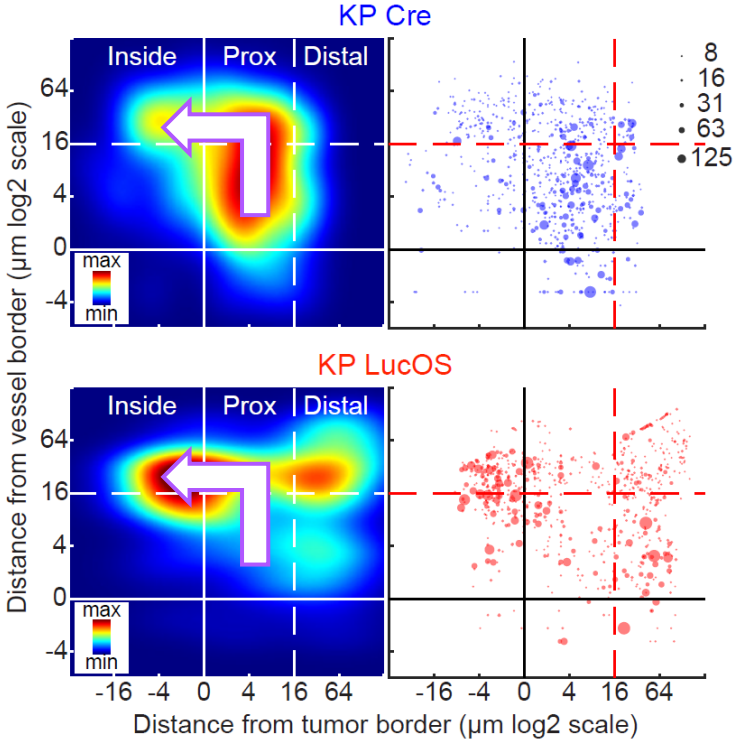
Total number and size is not dependent on neo-antigen



Start from T cell exclusive networks up to ~12-15 cells



Antigen exposure re-localizes lymphonets to inside tumors



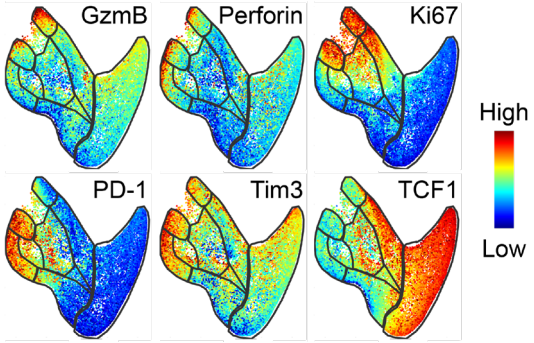
Lymphonets in regular KP mice are located near tumors

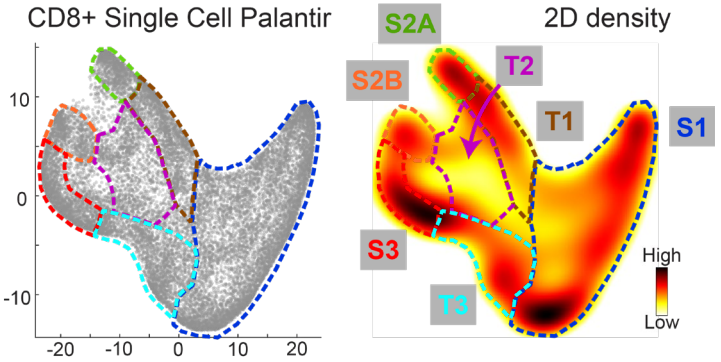
Lymphonets in KP mice expressing neoantigen are located inside tumors

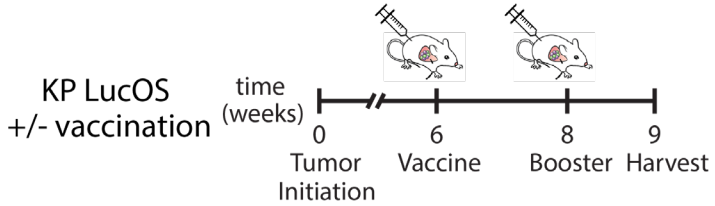
CD8 T cells exhibit multidimensional phenotypes

CD8 T cells
Six phenotypic markers

Palantir algorithm
Setty M et al, Nature Biotechnology, 2019





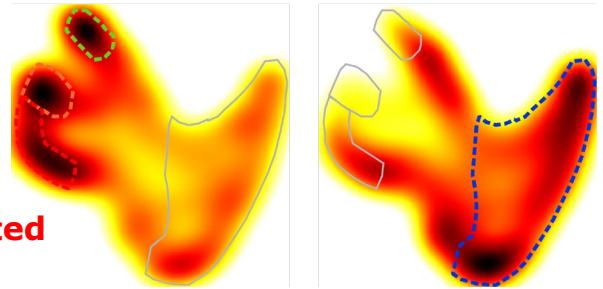


Vaccination causes CD8 T cell to switch from TCF1+ state to cytotoxic

S2A - Cytotoxic

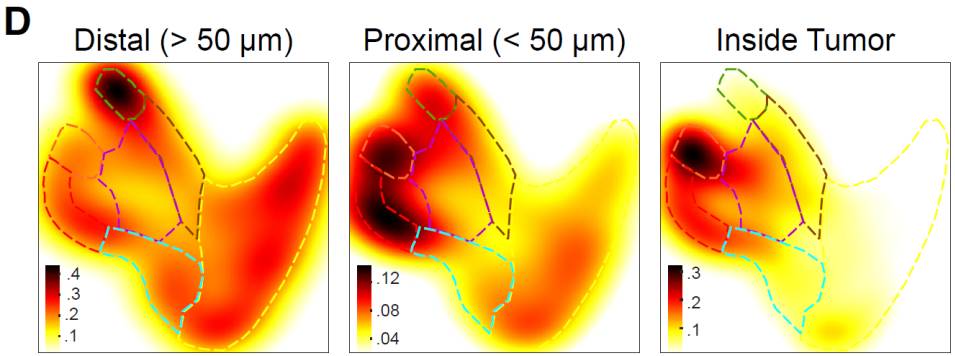
S2B - Antigen Experienced

S3 - Exhausted

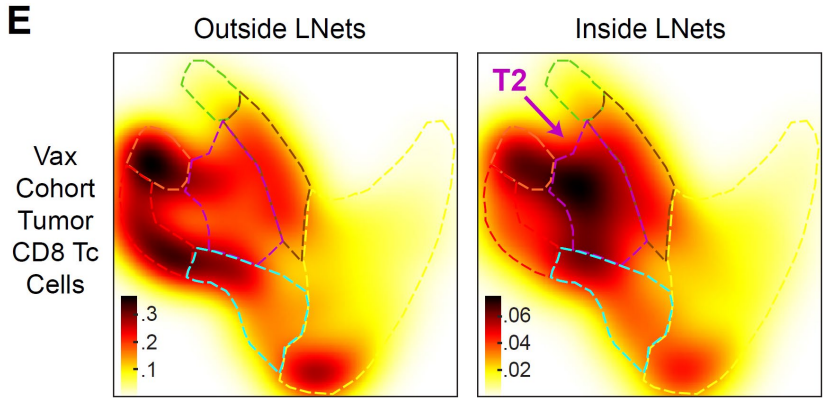


S1 Naive

Mapping phenotypic landscape and physical space



Binning cells by distance from tumor



Binning cell by location relative to lymphonets

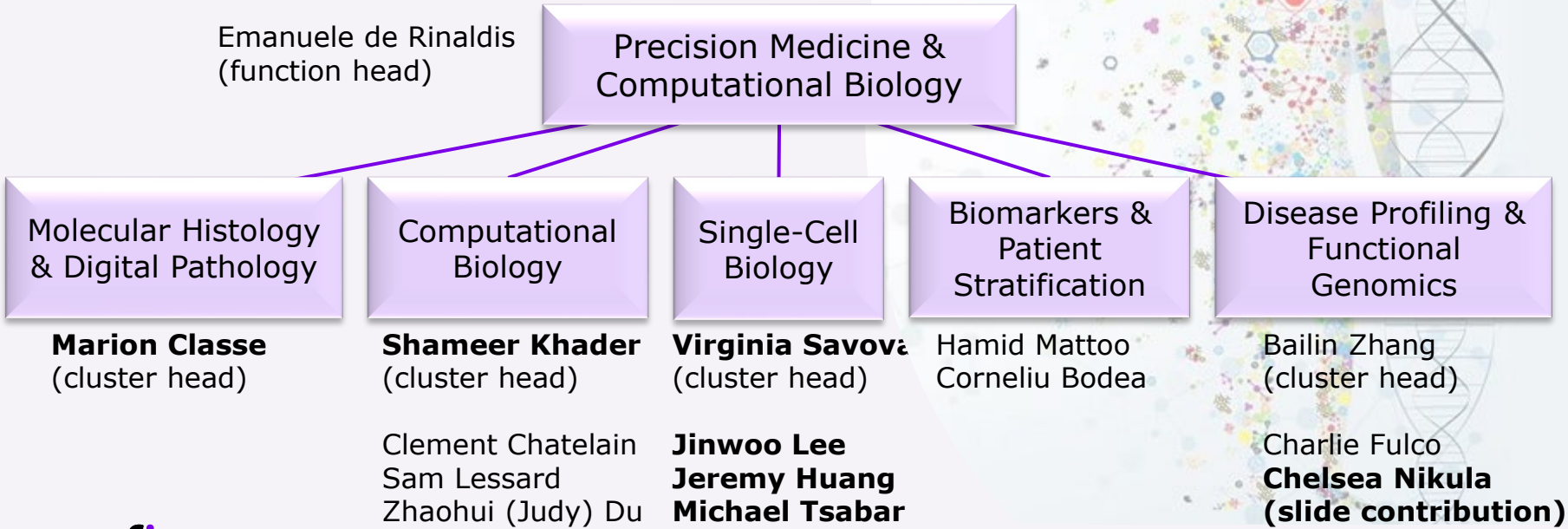
→ Lymphonets harbor progenitor TCF1+/PD1+ CD8 T cells (**T2**)

Summary and main take homes

- Spatial technologies and analytics are in flux with new methods and algorithms coming out every month
- Methods that do not reach single cell resolution can leverage bulk RNA-seq algorithm BUT have limited use in spatial analysis
- Spatial analysis can be classified in three orders
  - First-order – Aggregation indices
  - Second-order – Distance between cells or to regions of interest
  - Higher-order – Neighborhoods/Communities
- Statistical tests are somewhat limited and need further development
- The field is shifting from atlas creation to biological discovery

Thank you!

FOCIS Systems Biology Course – 2023 Faculty
Emanuele de Rinaldis, PhD – Sanofi
Magnus Fontes, PhD – Merck
Shameer Khader, PhD MBA – Sanofi



sanofi

Spatial Biology for Immunologists Additional Materials

Giorgio Gaglia, PhD
Single Cell Discovery and Validation Lead
Precision Medicine and Computational Biology
Sanofi US R&D

First-order global metric: spatial variability with Moran's I

Weight Matrix

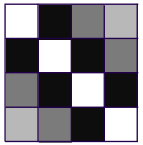
Deviations from mean for axis "i" and "j"

$$I = \frac{N}{W} \frac{\sum_{i=1}^{N} \sum_{j=1}^{N} w_{ij}(x_i - \bar{x})(x_j - \bar{x})}{\sum_{i=1}^{N} (x_i - \bar{x})^2}$$

where

- N is the number of spatial units indexed by i and j;
- x is the variable of interest;
- $\bar{x}$ is the mean of x;
- w_{ij} are the elements of a matrix of spatial weights with zeroes on the diagonal (i.e., $w_{ii} = 0$);
- and W is the sum of all w_{ij} (i.e. $W = \sum_{i=1}^{N} \sum_{j=1}^{N} w_{ij}$).

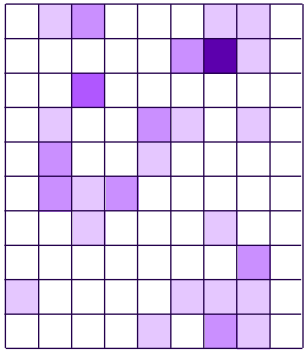
W_{ij}



Weight Matrix

Matrix from grid

i



j

Count

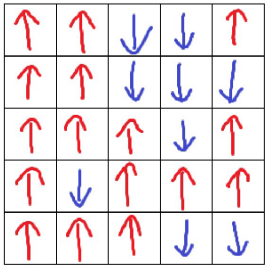
1 2 3 4

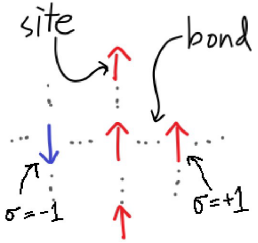
Historical perspective

Other major academic scientific fields have investigated spatial organization:

- Statistical Physics

Ising model for "spin" of magnetic particles





- Lattice structure is closer to biological systems yet too regular & homogeneous
- Still computationally intractable
- Variable state space (n = 2) is far from biologic reality

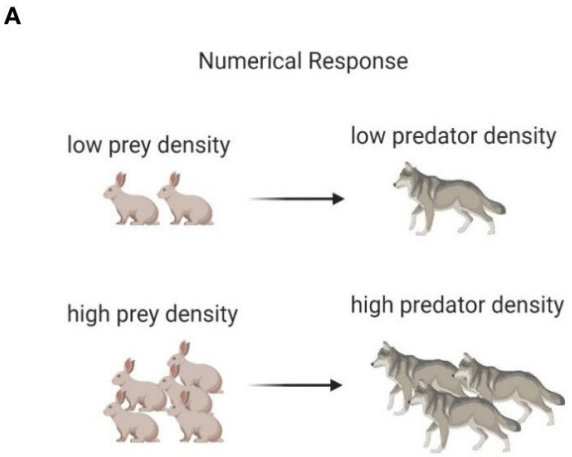
SCALING LAWS FOR ISING MODELS NEAR T_c*

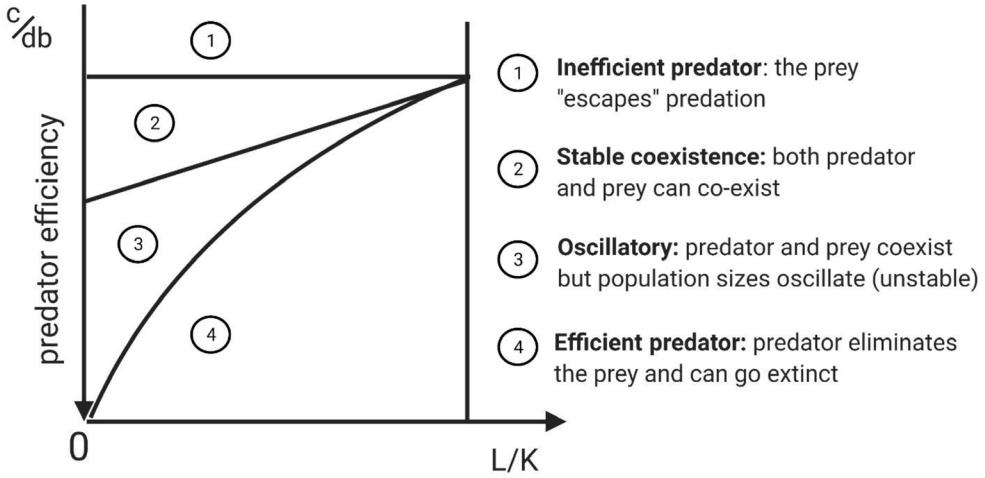
Abstract

A model for describing the behavior of Ising models very near T_c is introduced. The description is based upon dividing the Ising model into cells which are microscopically large but much smaller than the coherence length and then using the total magnetization within each cell as a collective variable. The resulting calculation serves as a partial justification for Widom's conjecture about the homogeneity of the free energy and at the same time gives his result $sv' = \gamma' + 2\beta$.

Kadanoff LP, Physics 1966

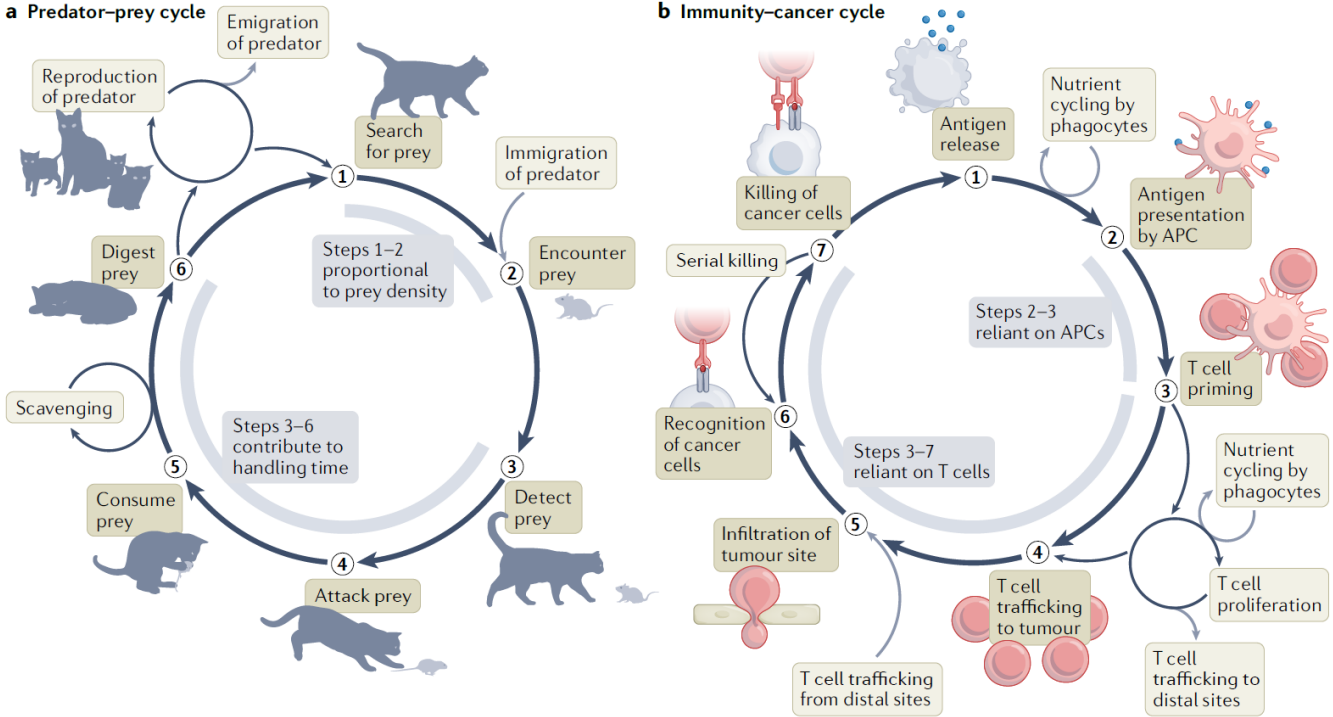
Ecology parallels: Predator-prey model





Kareva et al, Front. Immunol., 2021

Ecology parallels: Predator-prey model



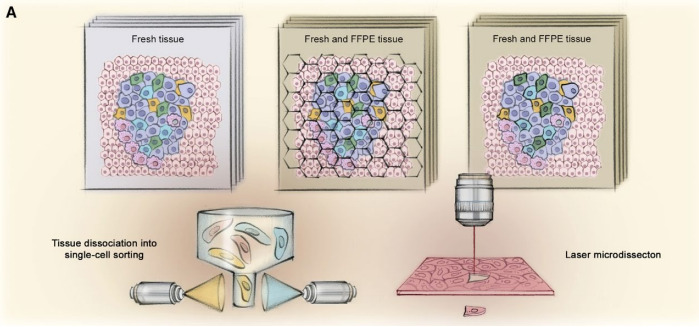
Tumour immunotherapy: lessons from predator–prey theory
Hamilton et al, Nat Rev Immunology, 2022

What can we measure?

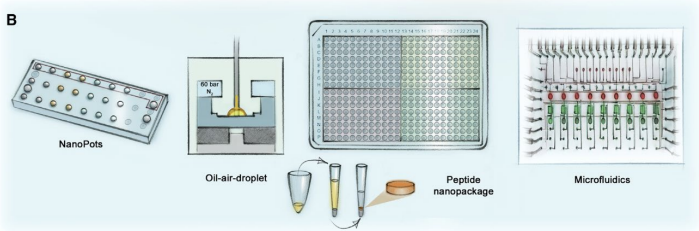
- Genes
- Proteins

Unbiased Proteomics (non-spatial)

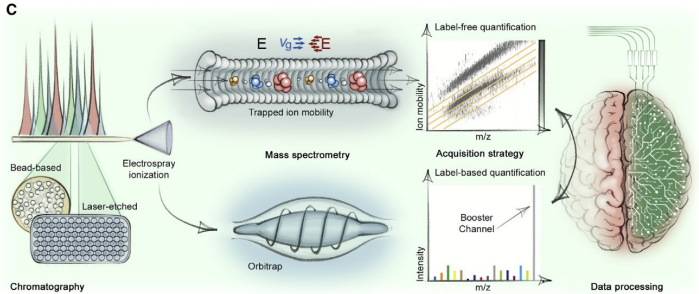
A) Tissue collection and dissociation



B) Single cell separation



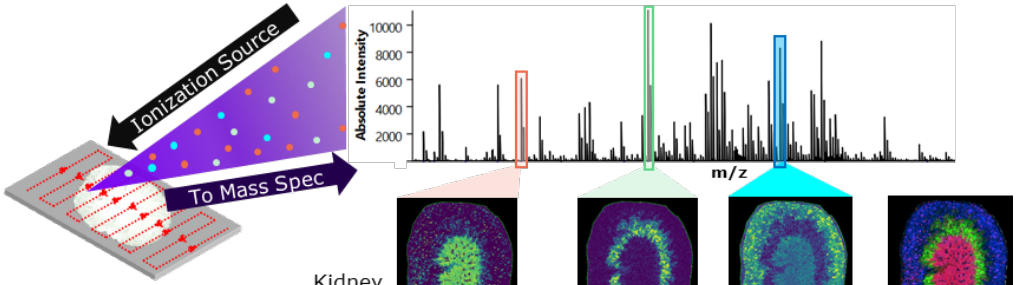
C) Mass spectrometry



Mund et al, Mol Cell 2022

Spatial Omics with Mass Spectrometry Imaging

Mapping metabolites, lipids, proteins, and drugs



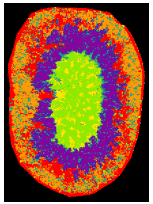
Kidney
30 µm x 30 µm pixel size
Positive Polarity

Where can MSI be helpful?

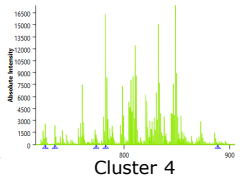
- Biodistribution of drugs/metabolites
- Defining molecular heterogeneity
- Molecular stratification
- Biomarker discovery

★ Label-free ★

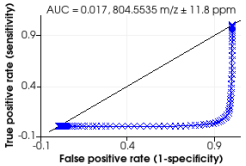
Untargeted Analyses

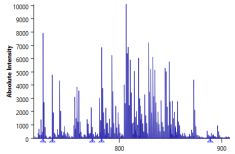


Bisecting k-means
(k=7)

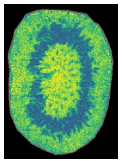


Cluster 4



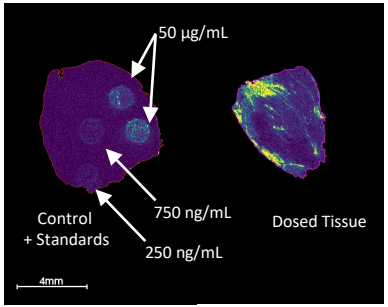


Cluster 6



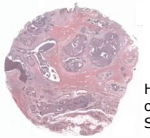
m/z 804.5535
PC (38:6)
ppm 2
AUC 0.0017

Targeted Analyses

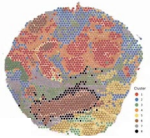


Multimodal Studies

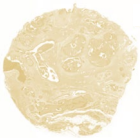
→Much Potential!



H&E, IHC, and other Tissue Staining



Spatial Transcriptomics



Other modalities: Fluorescence, Raman, Elemental imaging + LC-MS

Modified from 10X Genomics

Slide courtesy of Chelsea Nikula, Sanofi US R&D

Examples of Spatial Metabolomics

Mapping metabolites, lipids, proteins, and drugs

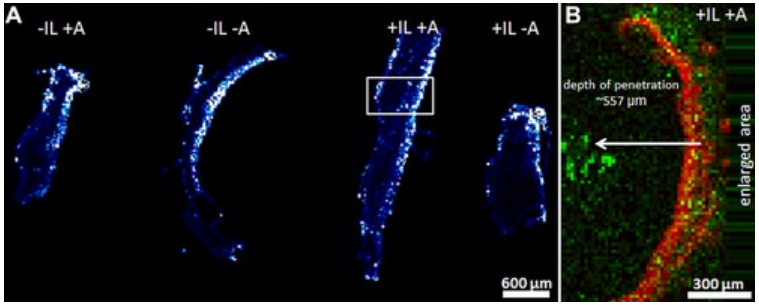
1718 DOI 10.1002/pmic.201600036 *Proteomics* 2016, *16*, 1718–1725

RESEARCH ARTICLE

MALDI-MSI for the analysis of a 3D tissue-engineered psoriatic skin model

Amanda Harvey[1], Laura M. Cole[1], Rebecca Day[1], Maggie Bartlett[2], John Warwick[2], Richard Bojar[2], David Smith[1], Neil Cross[1] and Malcolm R. Clench[1]

[1] Centre for Mass Spectrometry Imaging, Biomolecular Sciences Research Centre, Sheffield Hallam University, Sheffield, UK
[2] Innovenn, Sand Hutton Innovation Campus, York, UK



→Drug and endogenous species distribution in psoriatic skin models show penetration depth of drug

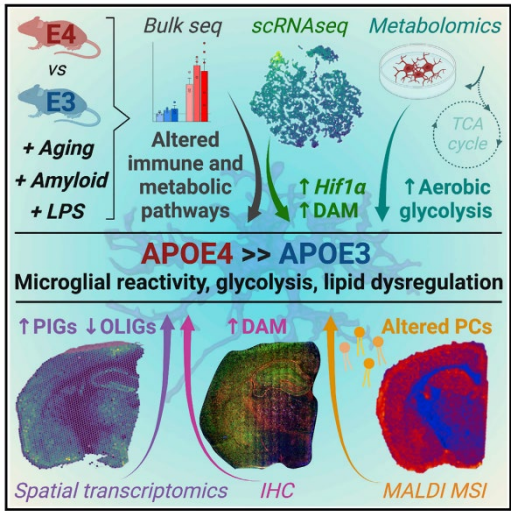
Cell Reports

CellPress
OPEN ACCESS

Resource

APOE modulates microglial immunometabolism in response to age, amyloid pathology, and inflammatory challenge

Sangderk Lee,[2,7] Nicholas A. Devanney,[1,2,7] Lesley R. Golden,[1] Cathryn T. Smith,[1] James L. Schwartz,[2] Adeline E. Walsh,[1] Harrison A. Clarke,[3,5,6] Danielle S. Goulding,[2] Elizabeth J. Allenger,[1] Gabriella Morillo-Segovia,[1] Cassi M. Friday,[1] Amy A. Gorman,[2] Tara R. Hawkinson,[3,5,6] Steven M. MacLean,[1] Holden C. Williams,[1] Ramon C. Sun,[2,3,4,5,6] Josh M. Morganti,[2,3,8,*] and Lance A. Johnson[1,2,8,9,*]

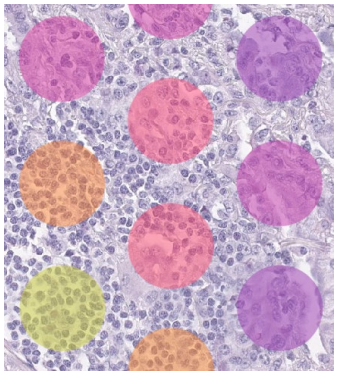
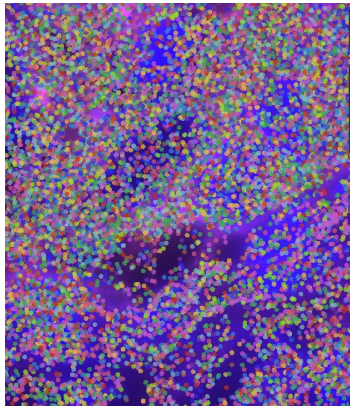
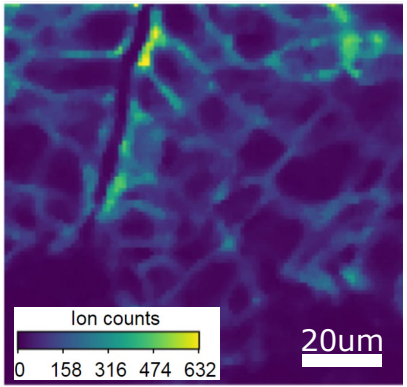
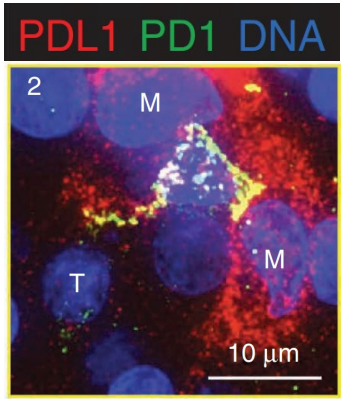


→ Spatial and non-spatial multi-omicscombined to understand immunometabolism of amyloid pathology

Slide courtesy of Chelsea Nikula, Sanofi US R&D

What variables are important in spatial biology

- Spatial resolution Ref: Cell size (diameter) range = 5um – 20um

Multicellular	Cellular	Cellular	Subcellular
			
RNA Sequencing	RNA In Situ Hybridization	Imaging Mass Spec or Cytometry	Multiplexed Immunofluorescence

What variables are important in spatial biology

- Spatial resolution
- Number of features Ref: 20K genes, 20-100K proteins

Unbaised	**Probe panel**	**Antibody panel**	**Antibody panel**
~2-4K genes (average)	Range: 4-1k mRNA	40-100 proteins	40-60 proteins
	ACD: ~4 mRNA Vizgen: ~1k mRNA GeoMX: ~1k mRNA Xenium: ~100 mRNA	IMC: ~40 proteins MIBI: ~100 proteins	CODEX: ~40 proteins cycIF: ~60 proteins
RNA Sequencing	RNA In Situ Hybridization	Imaging Mass Spec or Cytometry	Multiplexed Immunofluorescence

What variables are important in spatial biology

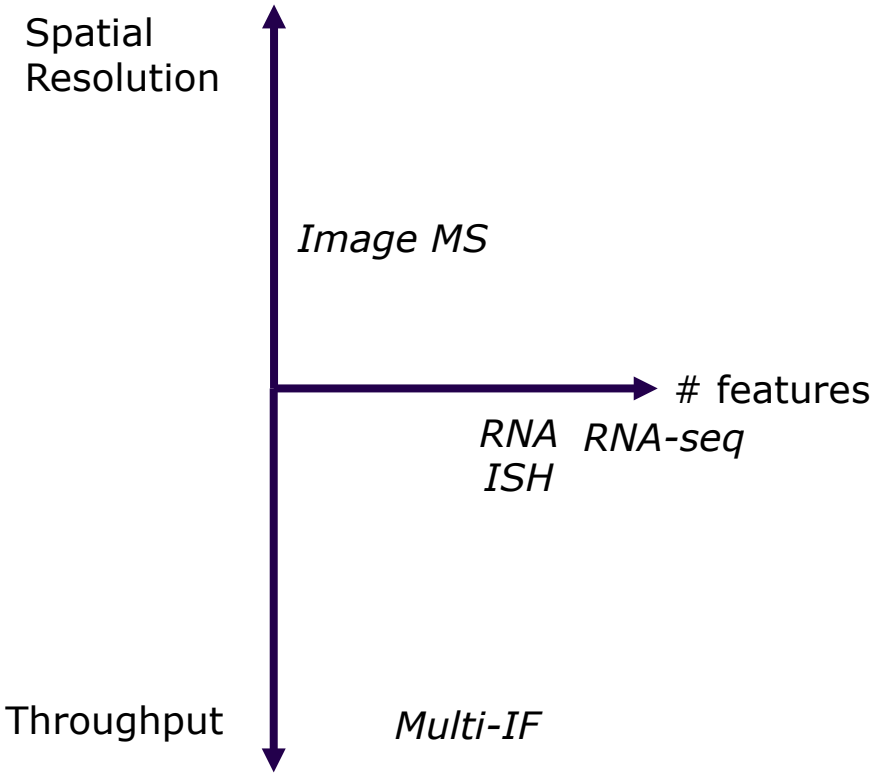
- Spatial resolution
- Number of features
- Throughput (area x time)

RNA-seq

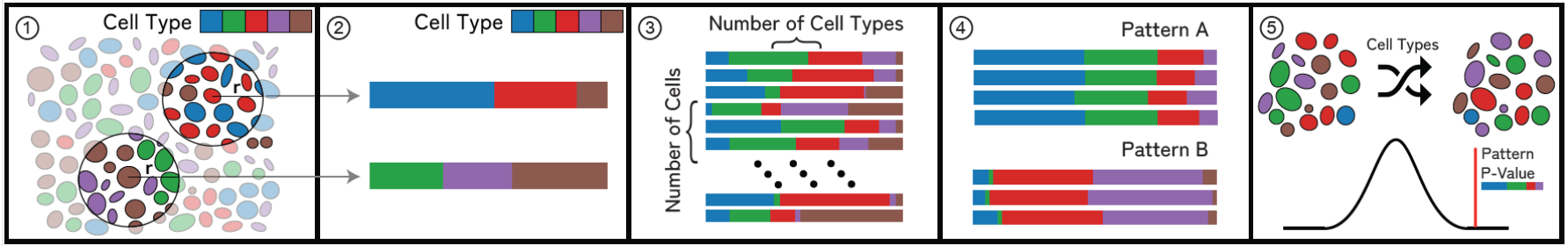
RNA ISH

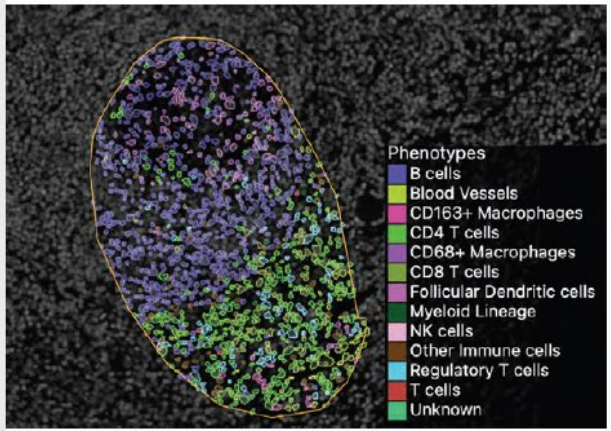
Image MS

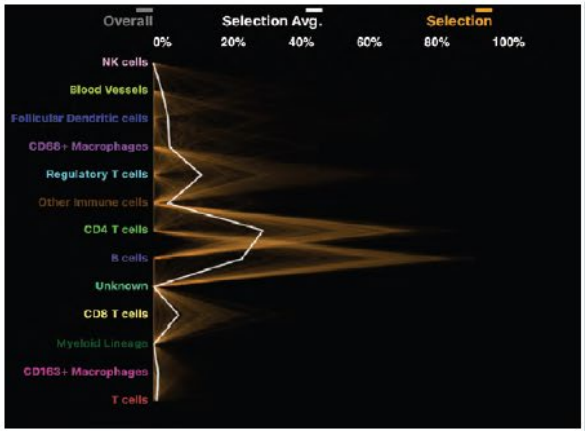
Multi-IF

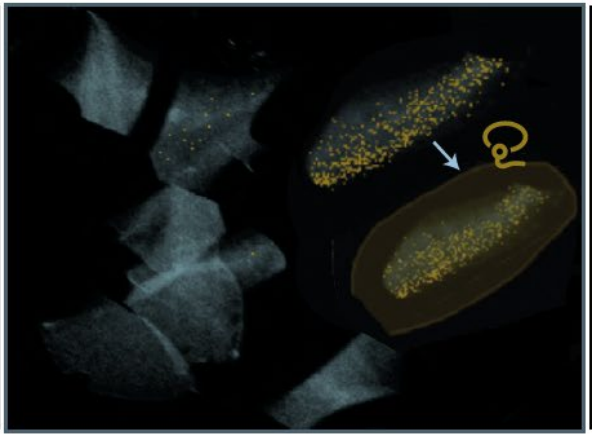


Visinity: Visual Spatial Neighborhood Analysis for Multiplexed Tissue Imaging Data









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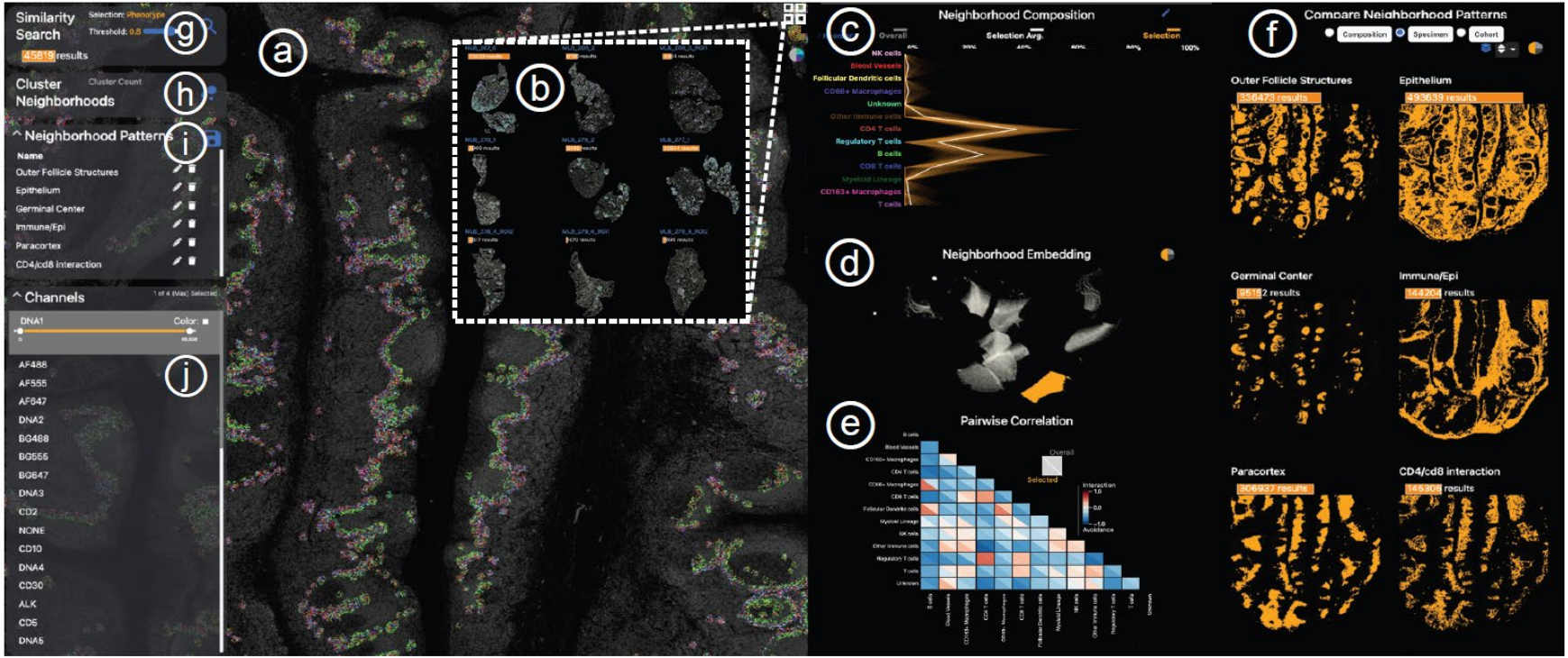
Visinity: Visual Spatial Neighborhood Analysis for Multiplexed Tissue Imaging Data



(1) Visinity: Visual Spatial Neighborhood Analysis for Multiplexed Tissue Imaging Data — Teaser - YouTube

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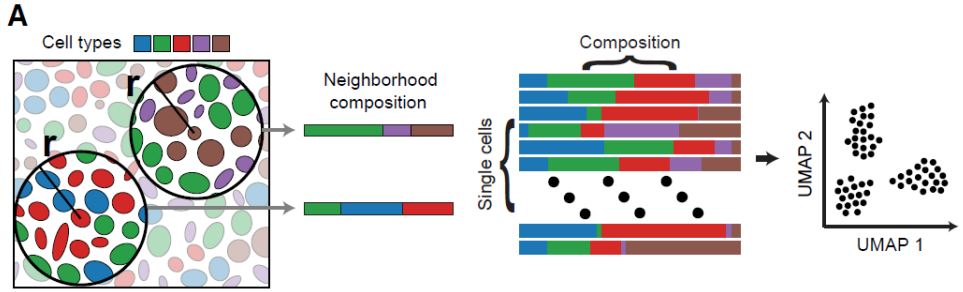
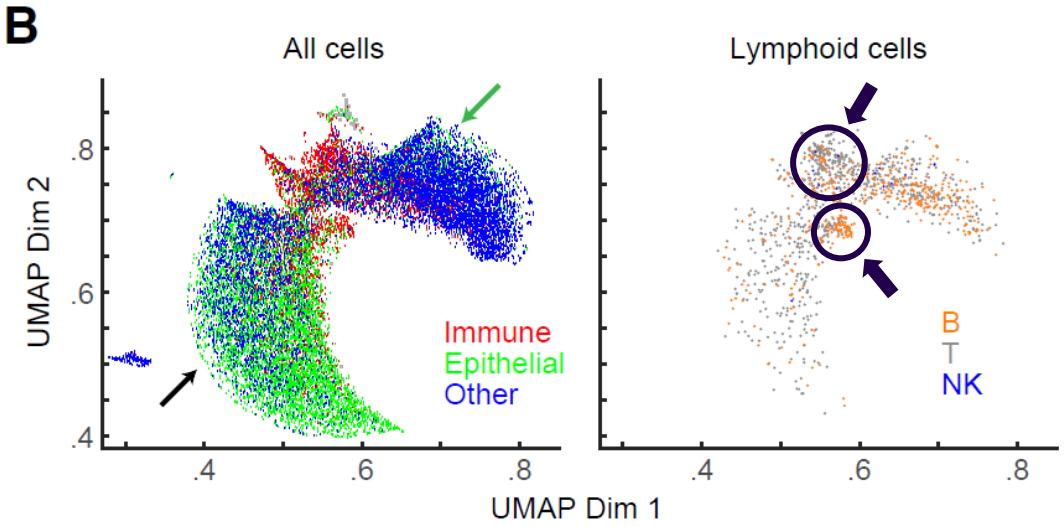
Visinity highlights B & T cell homotypic interactions



Cells closer to each other by their neighborhood composition



Lymphocyte neighborhoods clustering together