Clinical applications of single cell analyses

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Gustave Roussy

DISCLOSURE SLIDE

- Research grant and/or consultant/speaker compensated to the hospital: Novartis, Astra, Pfizer, Daiichi Sankyo, Lilly, Roche

- Founder: Pegacsy

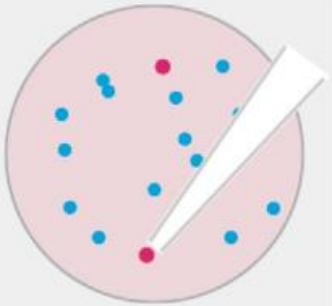
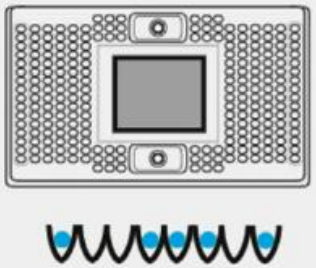
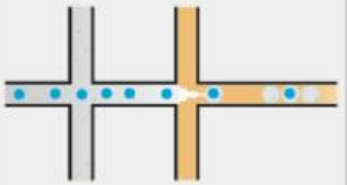
Outline

- **Single cell sequencing: isolation & analyses**
- Single cell proteomics & epigenetics
- Spatial distribution
- Computational analyses
- Conclusion

Flow

Isolation → lysis → DNA amplification → Sequencing → Bioinformatics

Overview methods of single cell isolation

	Micro-manipulation / Automated Pipetting	FACS	Microwell encapsulation	Droplet encapsulation
				
Cell Stress	Low	Moderate	Moderate	Moderate
Selection	Yes	Yes	No* / Yes++	No*
Doublet	Low	Low	Low-High	Moderate
Throughput	Low	Moderate	Moderate	High
Capture efficency	Low	Moderate	Moderate	Low-Moderate
Academic / Commerical scRNA workflow	- CellenONE (Cellenion)‡ - Smart-Seq2 (42)	- MARS-Seq (39) - Smart-Seq2 (42)	- C1 (Fluidigm) - ddSeq (Biorad / Illumina) - ICell8 (Clontech)++ - Rhapsody (BD)	- InDrop (1CellBio) - DropSeq (Dolomite-bio) - 10X (Chromium)
Example of use	Fragile rare cells	Rare cells based on phenotype or marking	Large cell numbers	Large cell numbers

Overview methods of single cell isolation

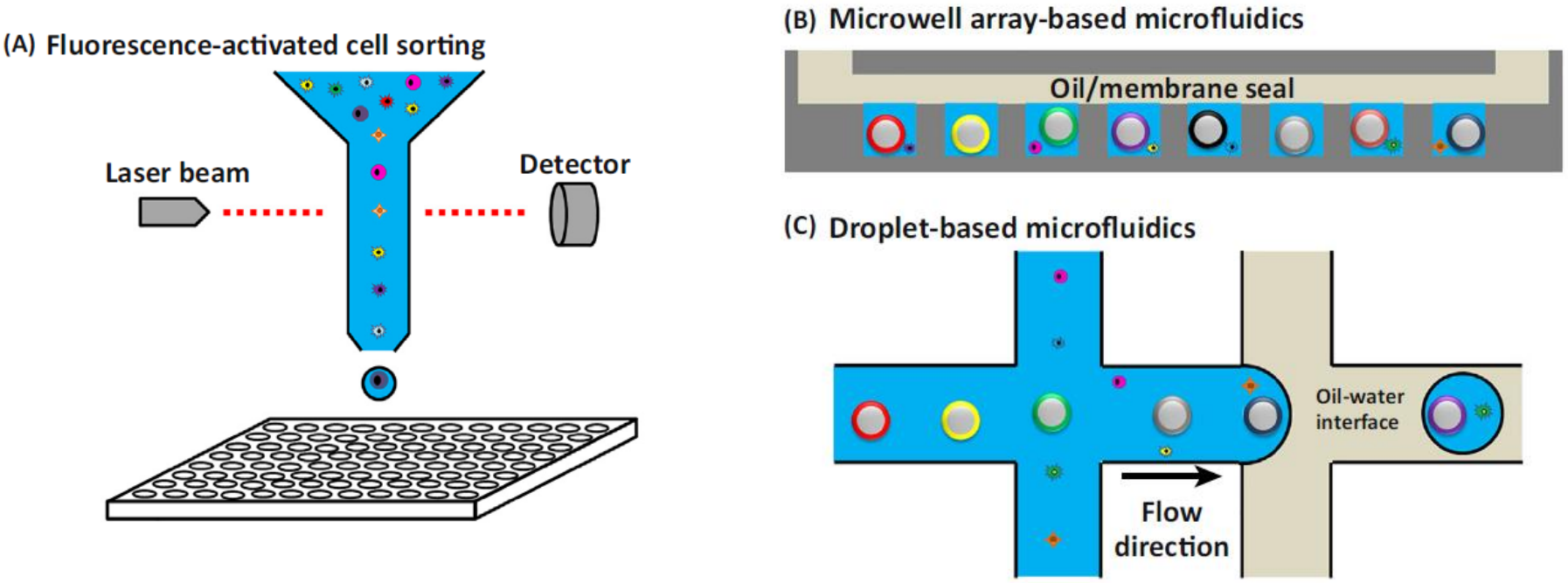
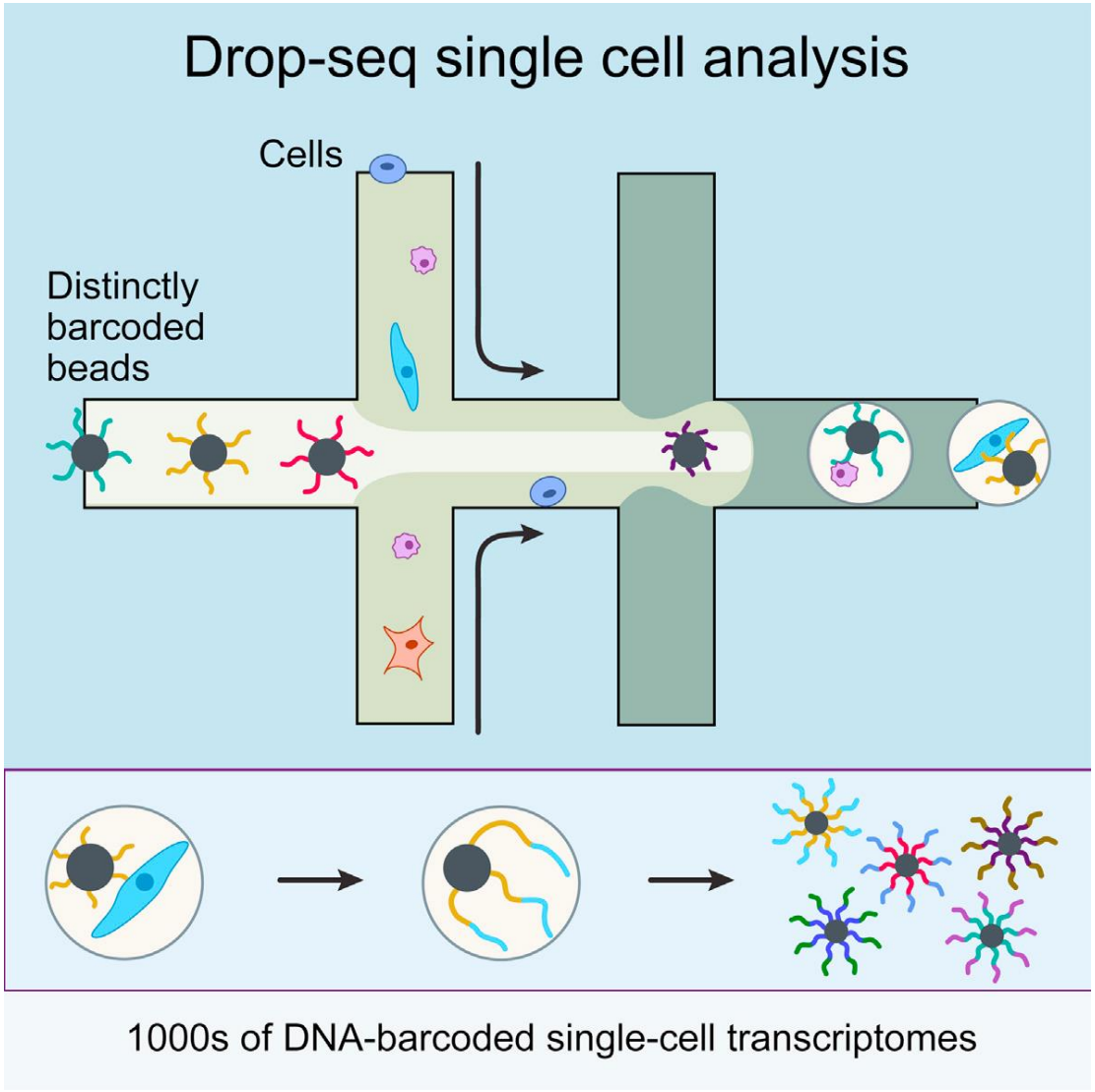


Figure 1. Panel 1: Technologies. (A) In conventional single-cell RNA sequencing (scRNA-seq), individual cells are flow sorted into multiwell plates. RNA-seq libraries of individual cells are prepared, barcoded in separate wells (per-cell reaction volume: ~10 μl) and pooled together for next-generation sequencing. (B) In high-throughput scRNA-seq with microfabricated microwell arrays, individual cells are co-encapsulated with individual, uniquely barcoded mRNA-capture beads in physically isolated microwells (per-cell reaction volume: ~100 pl). Reverse transcription of bead-captured mRNA molecules results in the incorporation of a bead-specific barcode into each cDNA molecule. The barcoded cDNA molecules from all cells are then pooled together and converted into a single RNA-seq library. (C) High-throughput scRNA-seq with droplet-based microfluidics is similar to (B) except that the co-encapsulation occurs in droplets (per-cell reaction volume: ~1 nl). (D) In conventional RNA

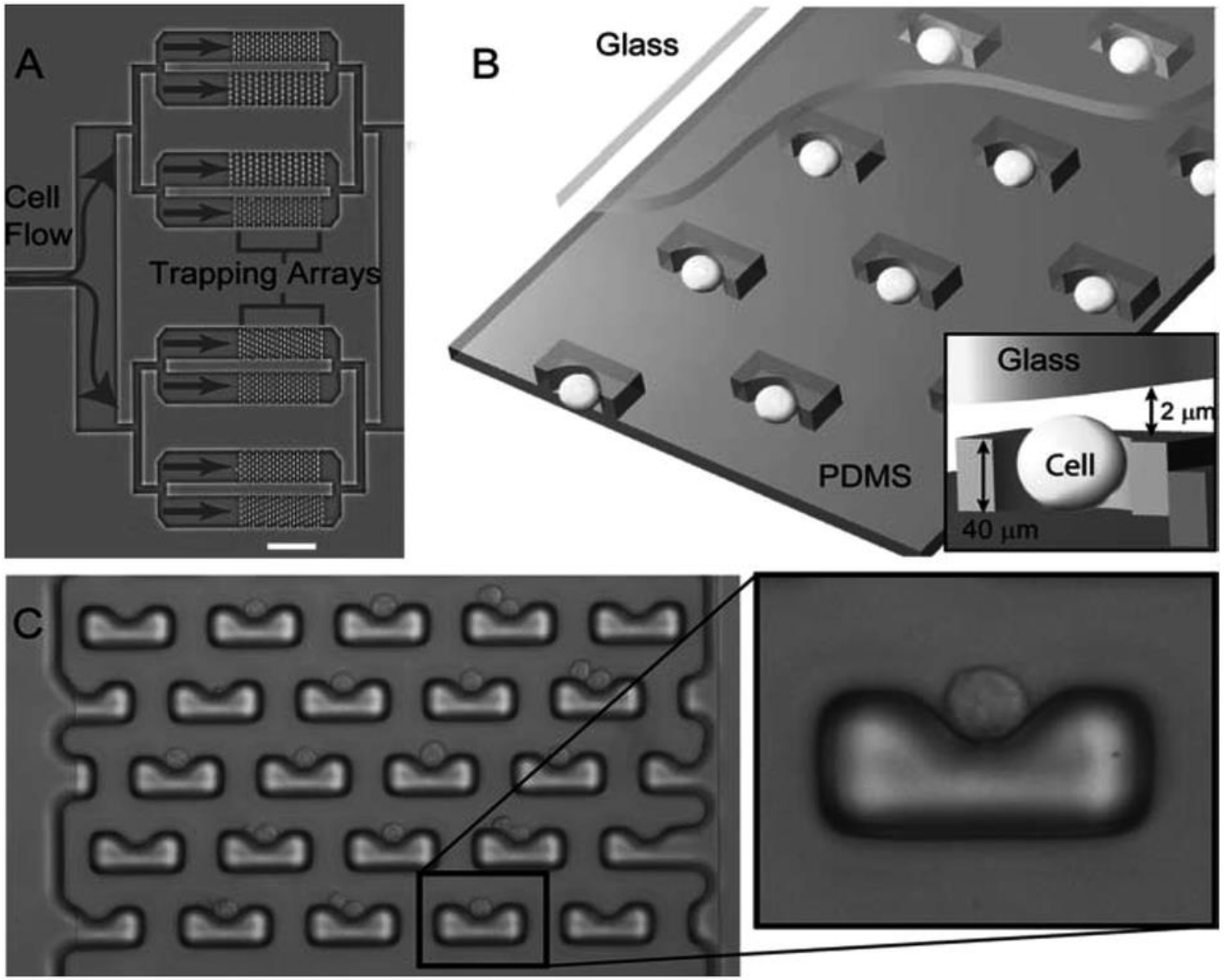
Levitin, Trends in Cancer, 2018

Droplet



Macosko, Cell, 2015

Hydrodynamics trap



Narayanamurthy, Analytical Methods, 2017

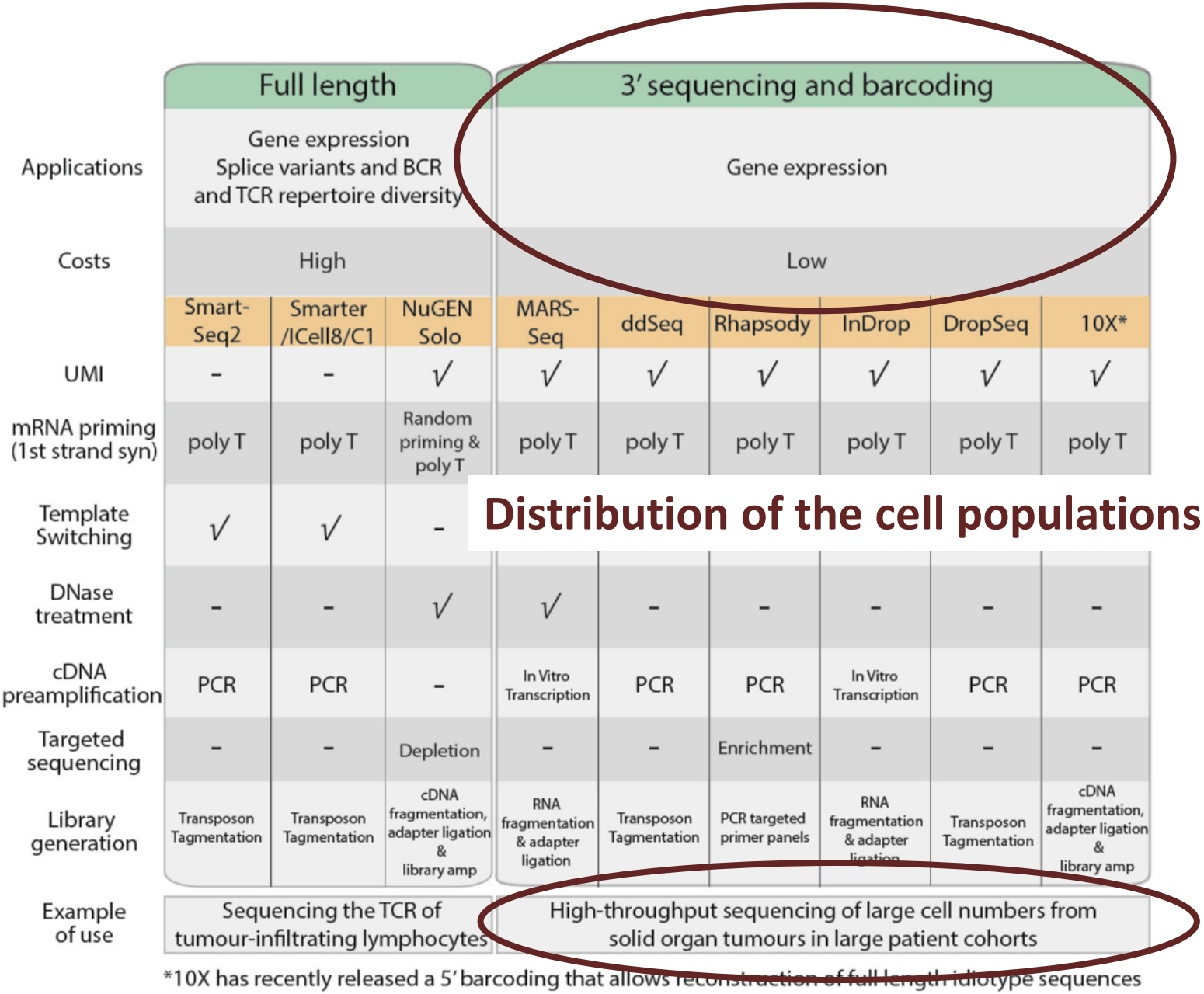
Flow

Isolation → lysis → DNA amplification → **Sequencing** → Bioinformatics

	Full length			3′ sequencing and barcoding					
Applications	Gene expression Splice variants and BCR and TCR repertoire diversity			Gene expression					
Costs	High			Low					
	Smart-Seq2	Smarter /ICell8/C1	NuGEN Solo	MARS-Seq	ddSeq	Rhapsody	InDrop	DropSeq	10X*
UMI	-	-	√	√	√	√	√	√	√
mRNA priming (1st strand syn)	poly T	poly T	Random priming & poly T	poly T	poly T	poly T	poly T	poly T	poly T
Template Switching	√	√	-	-	-	-	-	√	√
DNase treatment	-	-	√	√	-	-	-	-	-
cDNA preamplification	PCR	PCR	-	In Vitro Transcription	PCR	PCR	In Vitro Transcription	PCR	PCR
Targeted sequencing	-	-	Depletion	-	-	Enrichment	-	-	-
Library generation	Transposon Tagmentation	Transposon Tagmentation	cDNA fragmentation, adapter ligation & library amp	RNA fragmentation & adapter ligation	Transposon Tagmentation	PCR targeted primer panels	RNA fragmentation & adapter ligation	Transposon Tagmentation	cDNA fragmentation, adapter ligation & library amp
Example of use	Sequencing the TCR of tumour-infiltrating lymphocytes			High-throughput sequencing of large cell numbers from solid organ tumours in large patient cohorts					

*10X has recently released a 5′ barcoding that allows reconstruction of full length idiotype sequences

Nguyen, Front Immunol, 2018



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Template Switching	√	√	-						
DNase treatment	-	-	√	√	-	-	-	-	-
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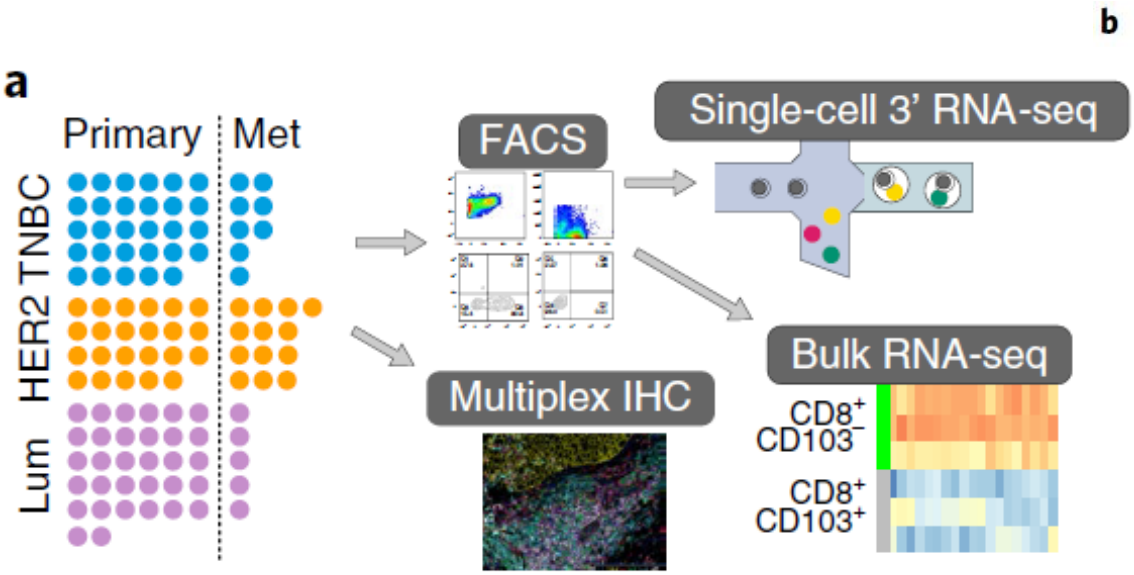
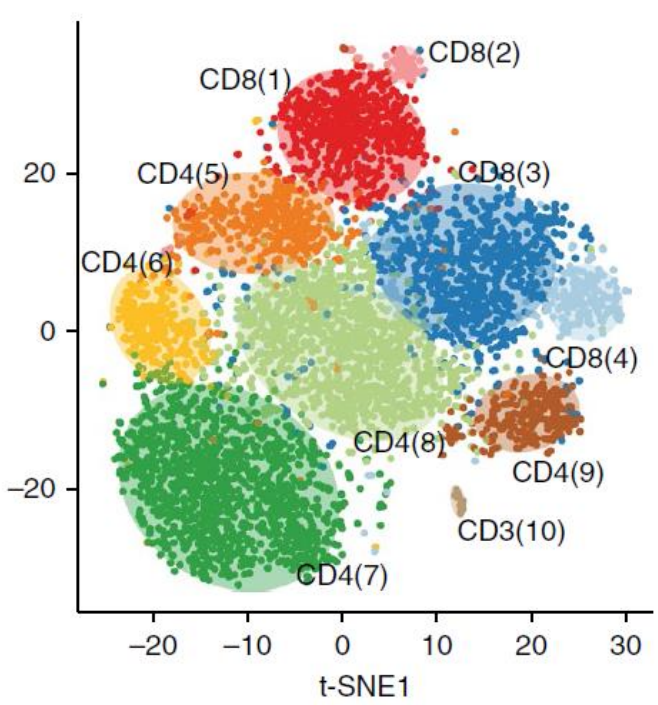
Distribution of the cell populations

Nguyen, Front Immunol, 2018

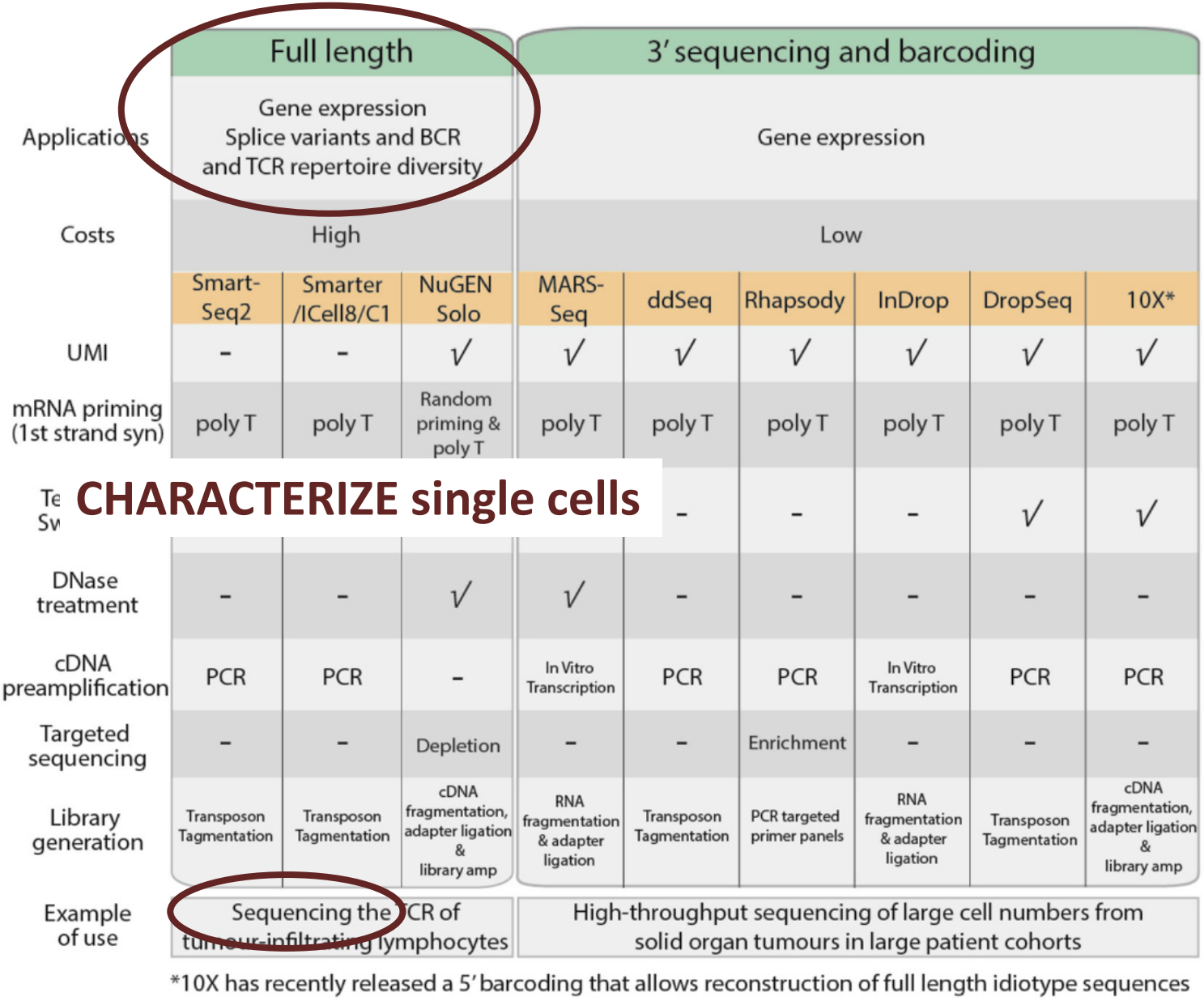
Single cell seq to analyze the distribution of cell populations

Distribution of lymphocytes in breast cancers





Savas, Nat Med, 2017



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Te Sv					-	-	-	√	√
DNase treatment	-	-	√	√	-	-	-	-	-
cDNA preamplification	PCR	PCR	-	In Vitro Transcription	PCR	PCR	In Vitro Transcription	PCR	PCR
Targeted sequencing	-	-	Depletion	-	-	Enrichment	-	-	-
Library generation	Transposon Tagmentation	Transposon Tagmentation	cDNA fragmentation, adapter ligation & library amp	RNA fragmentation & adapter ligation	Transposon Tagmentation	PCR targeted primer panels	RNA fragmentation & adapter ligation	Transposon Tagmentation	cDNA fragmentation, adapter ligation & library amp
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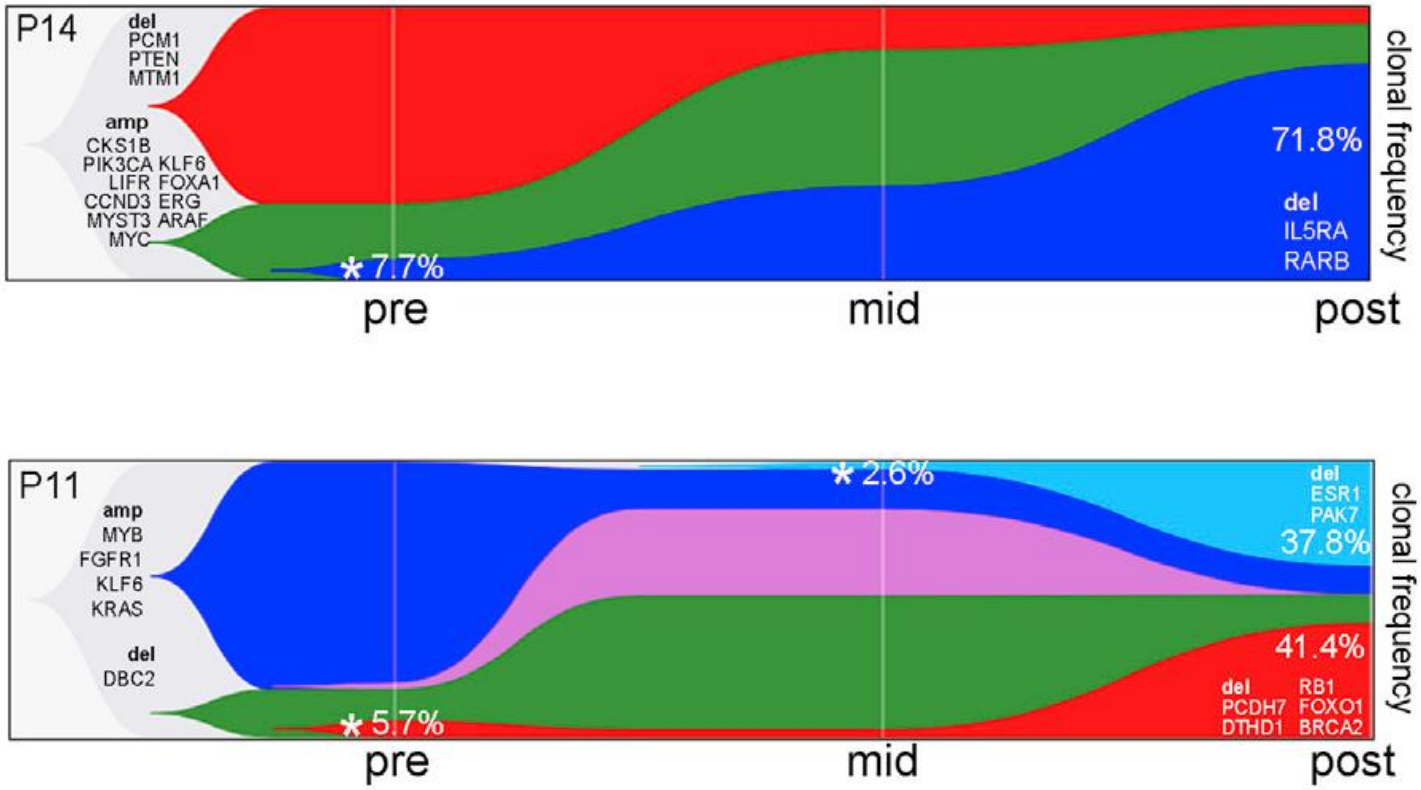
CHARACTERIZE single cells

Nguyen, Front Immunol, 2018

Single cell seq to characterize subclones

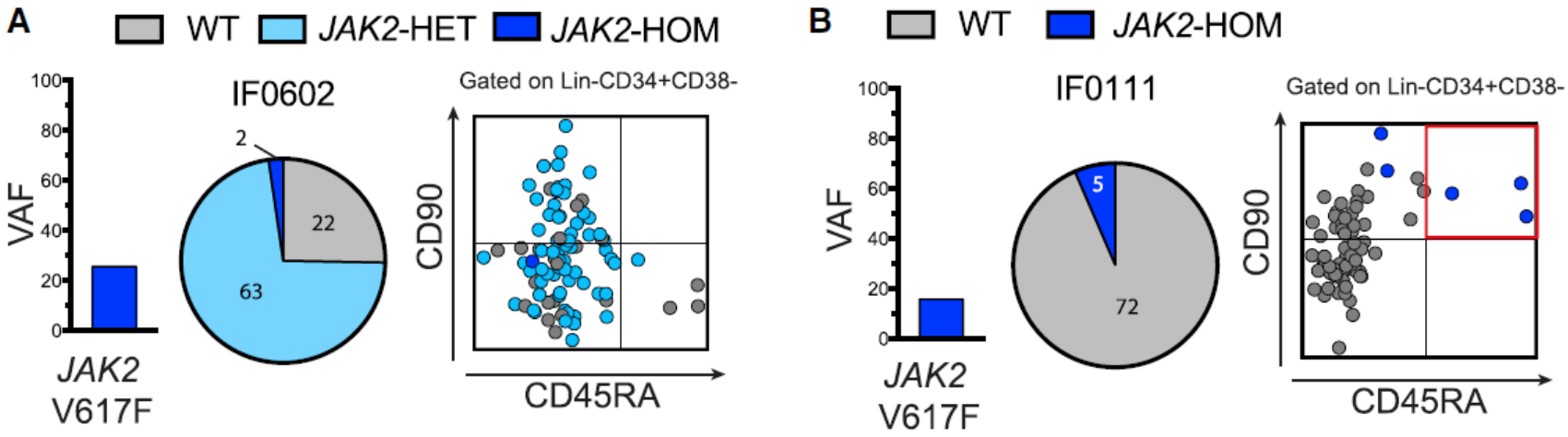
Subclone dynamics



(copy number analyses, not sequencing)

Kim, Cell, 2018

Assessing intratumor heterogeneity



Rodriguez, Mol Cell

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- **Single cell proteomics & epigenetics**
- Spatial distribution
- Computational analyses
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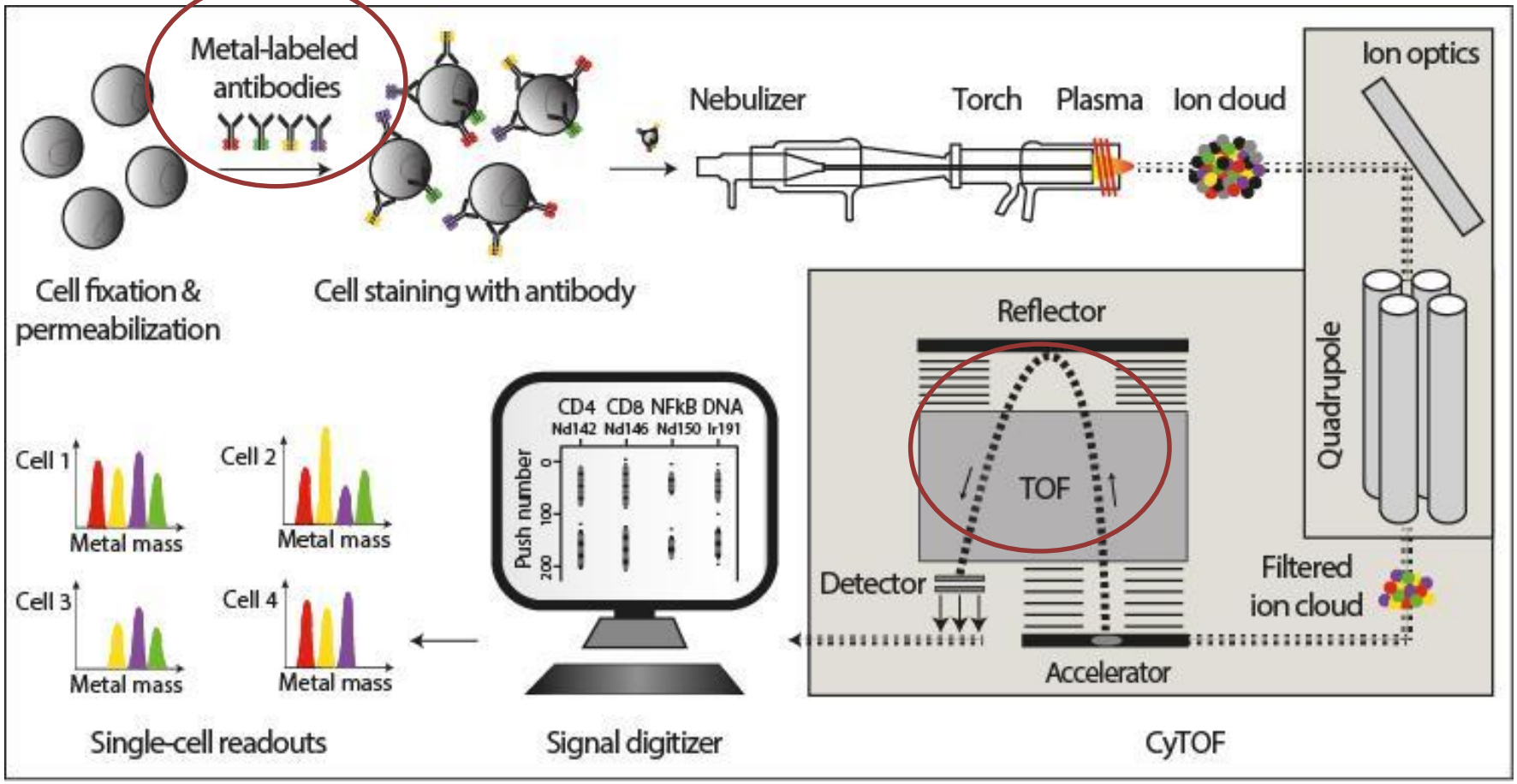
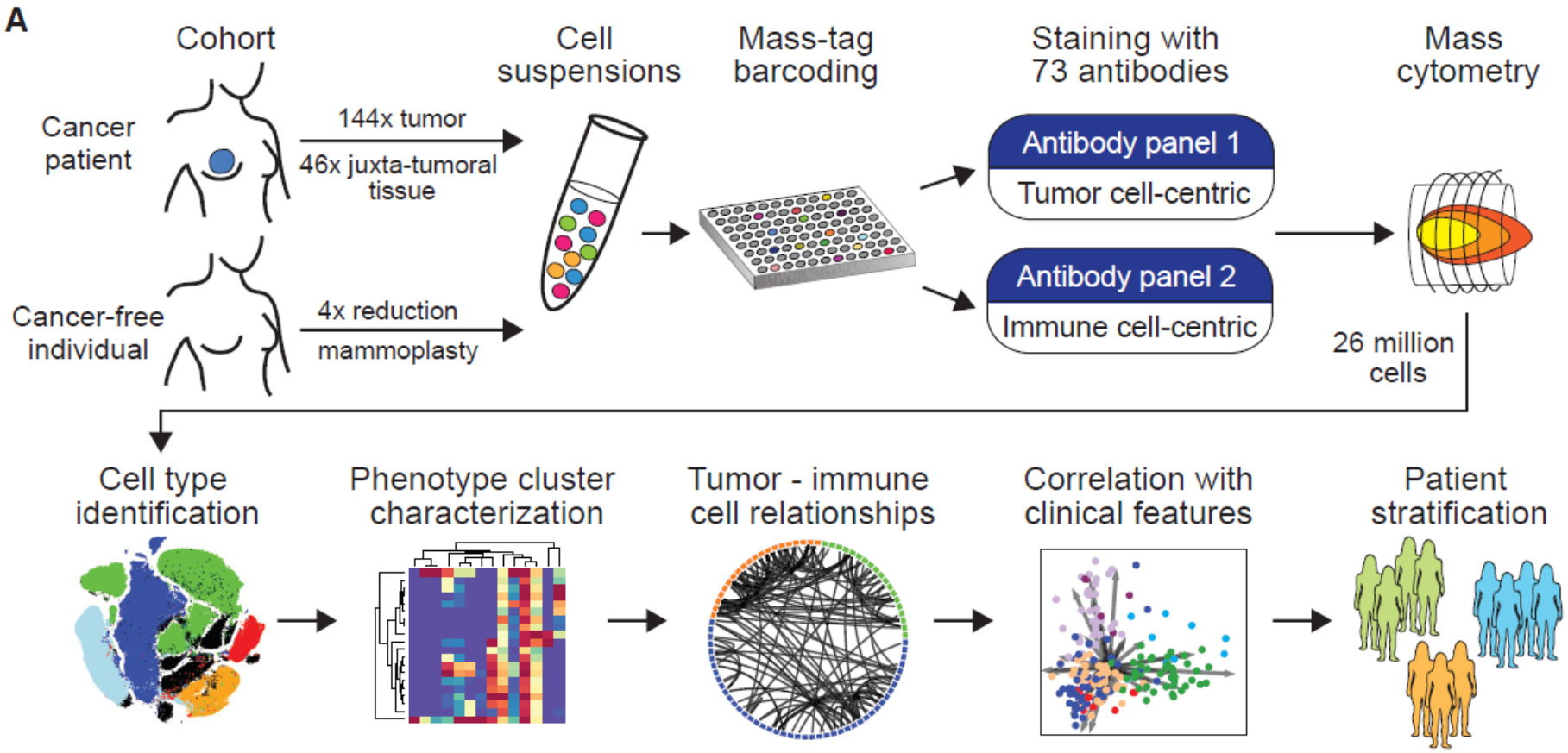
Mass Cytometry



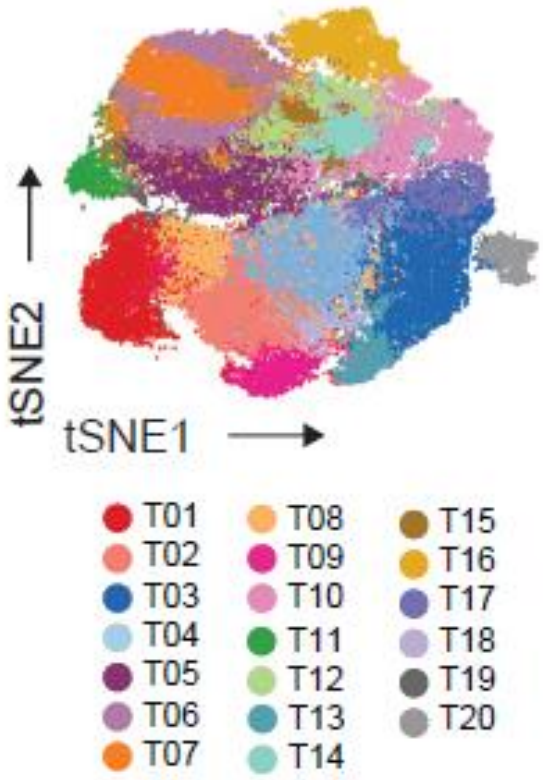
Bodenmiller's lab webpage

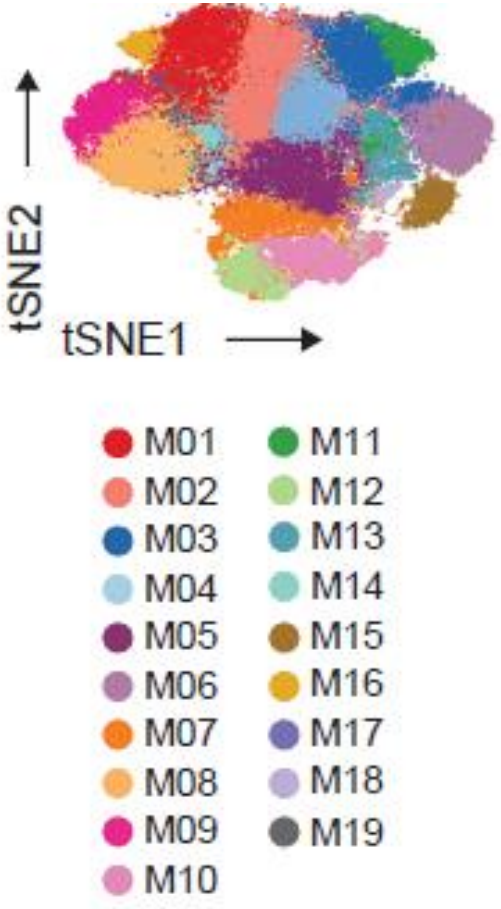
Flow of a research project on mass cytometry



Wagner, Cell 2019

Immune landscape in breast cancer patients





Lymphocytes and myeloid populations in a single patient
NO spatial distribution

Wagner, Cell 2019

Outline

- Single cell sequencing: isolation & analyses
- Single cell proteomics & epigenetics
- **Spatial distribution**
- Computational analyses
- Conclusion

Concept

- Use a spatial guided laser to isolate and localize each cell
- Apply single cell analyses
- Relocate molecular signal according to the initial position of the laser

Imaging mass cytometry

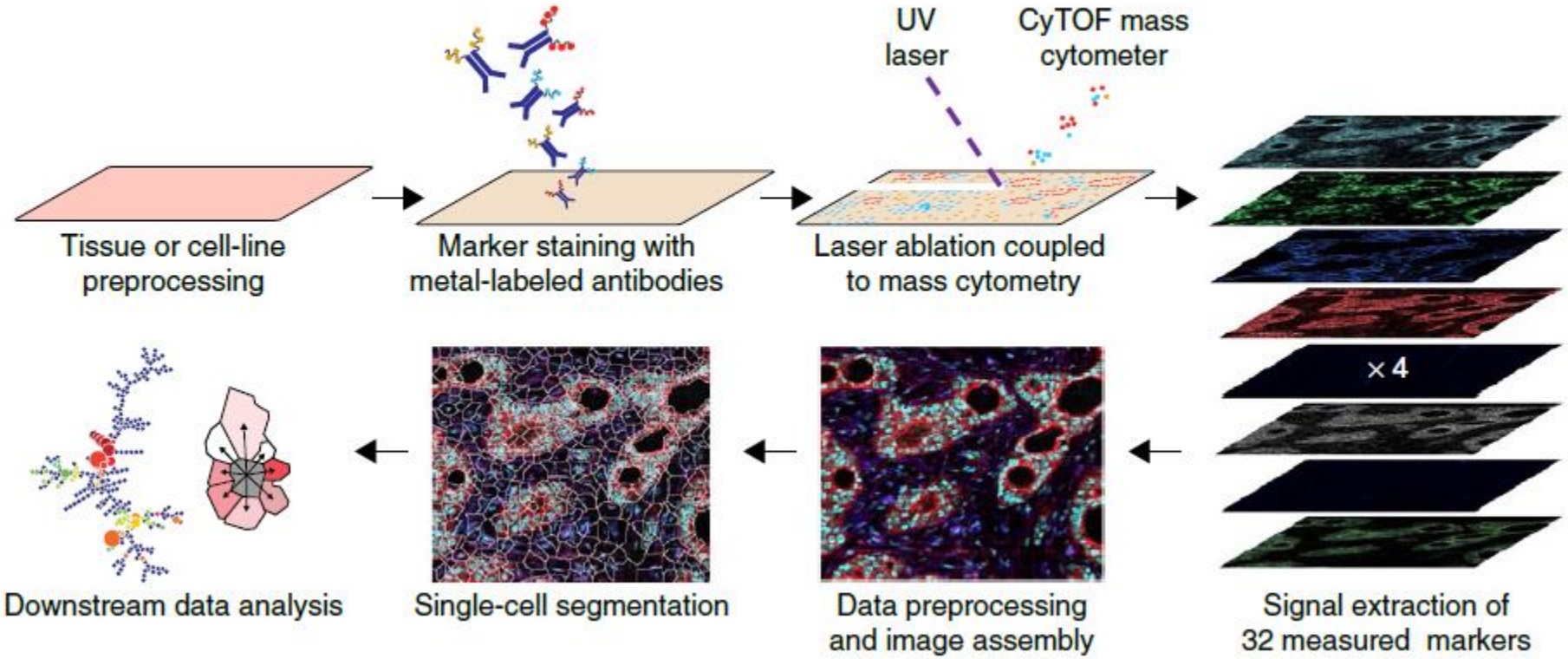
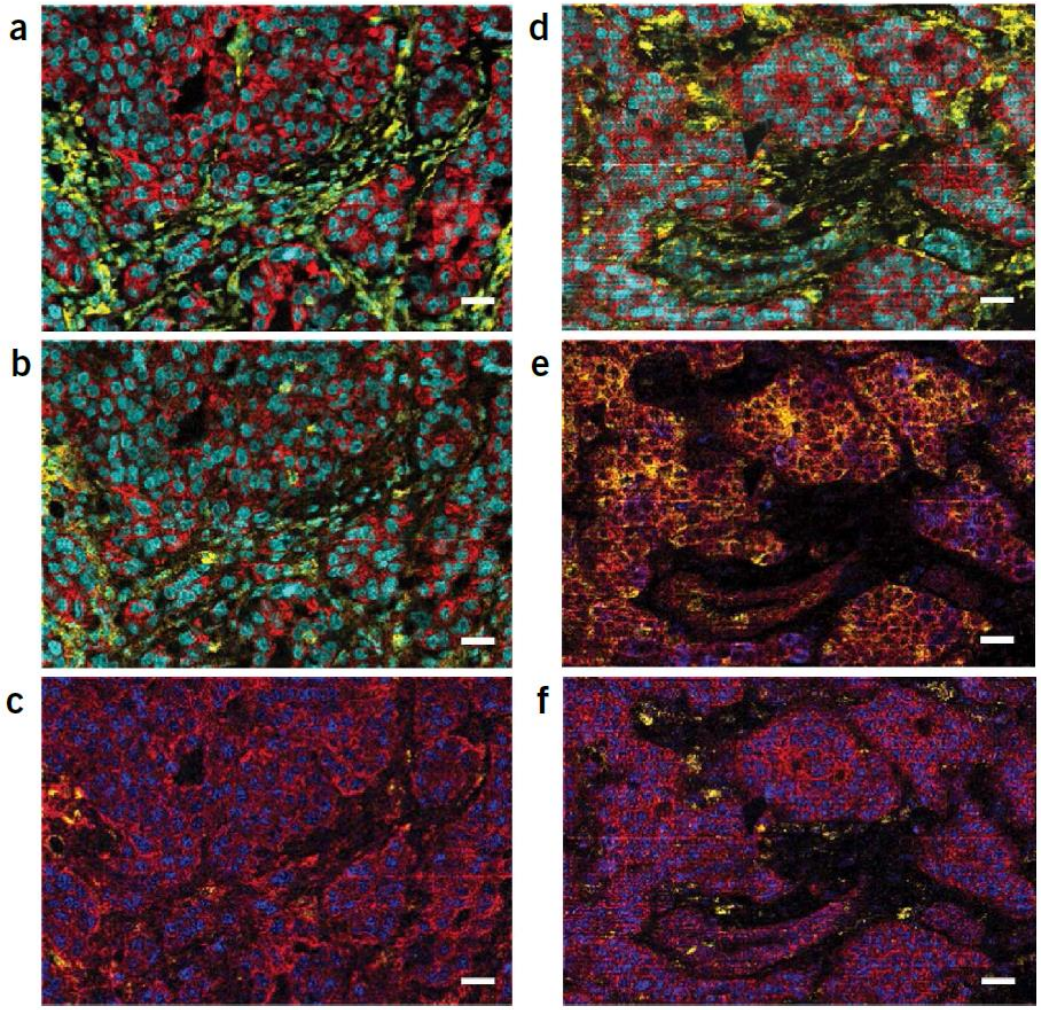


Figure 1 | Workflow of imaging mass cytometry.

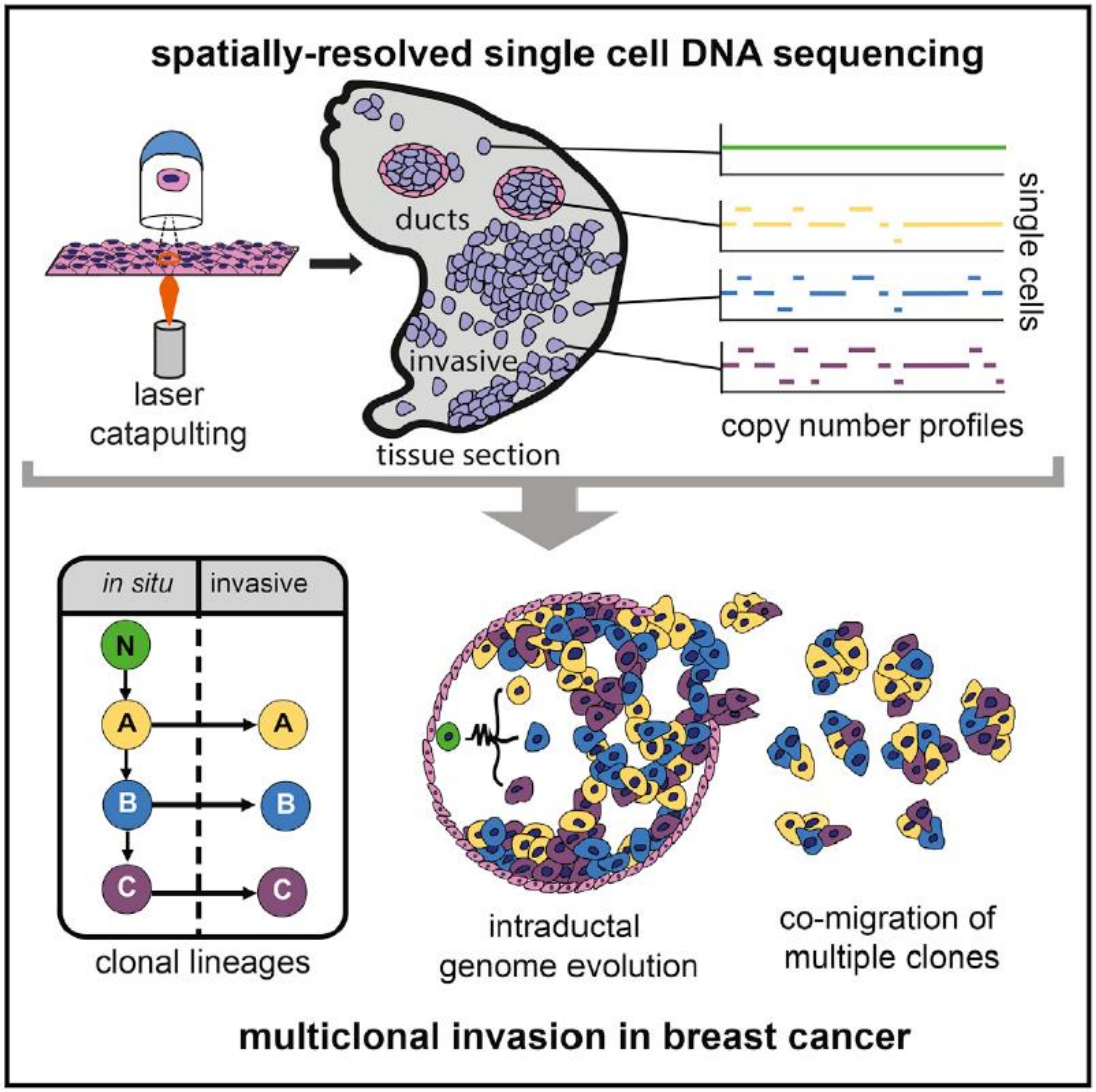
Giesen, Nat Methods, 2014

Imaging mass cytometry



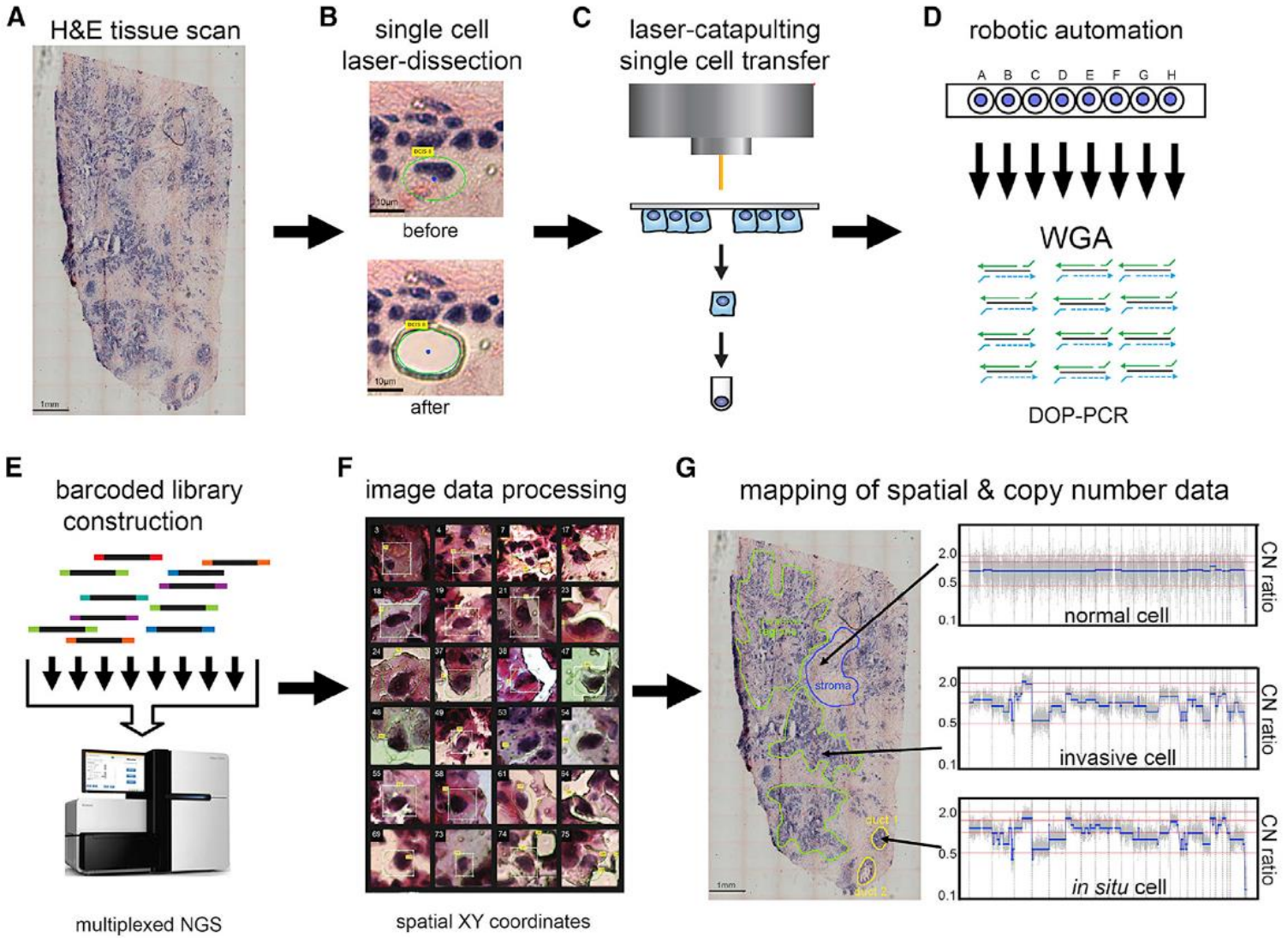
Giesen, Nat Methods, 2014

Spatially resolved single cell DNA seq



Casasent, Cell 2018

Linking DCIS and invasive cancers



Applications

- Whether spatial distribution predicts outcome, drug responses

- Correlate different compartments

Outline

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- **Computational analyses**
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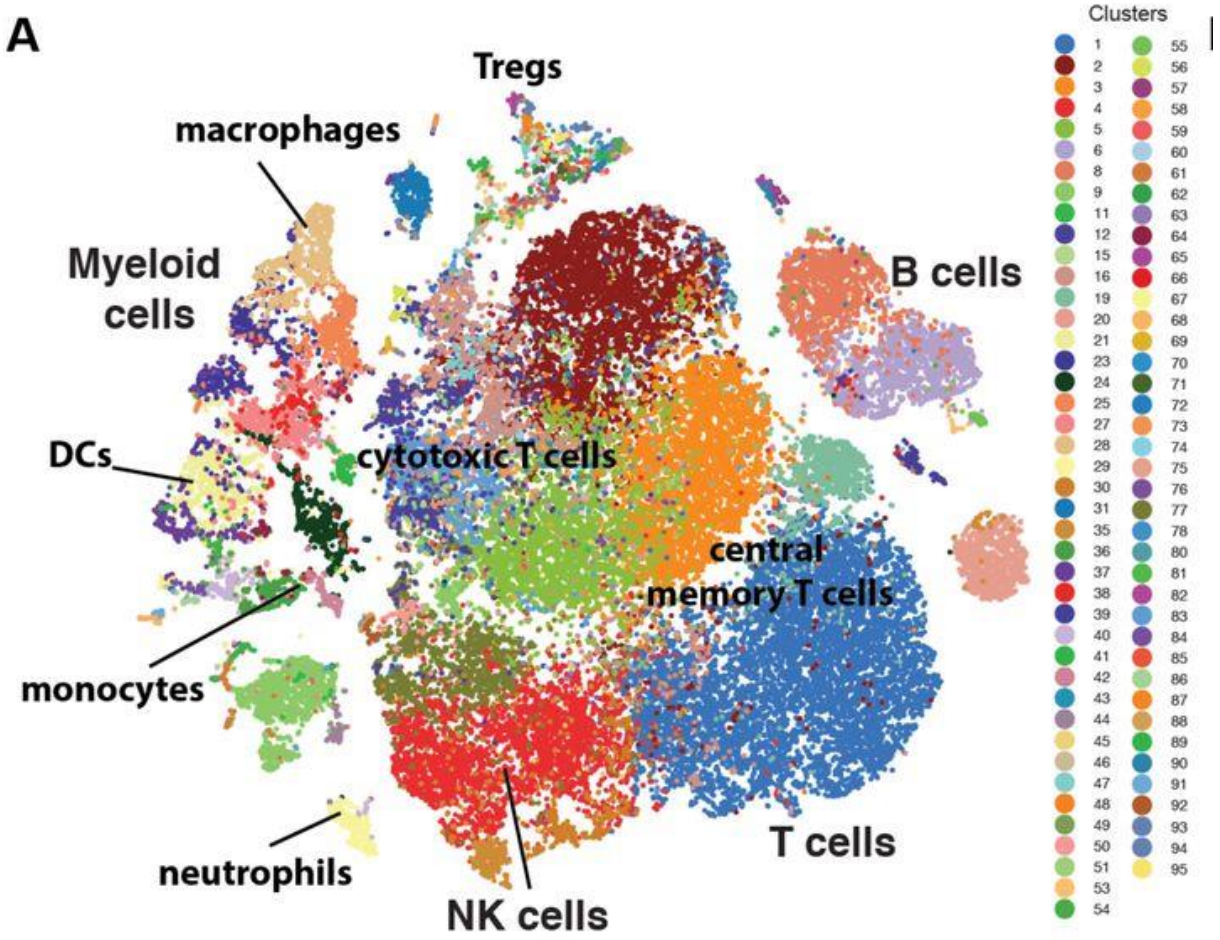
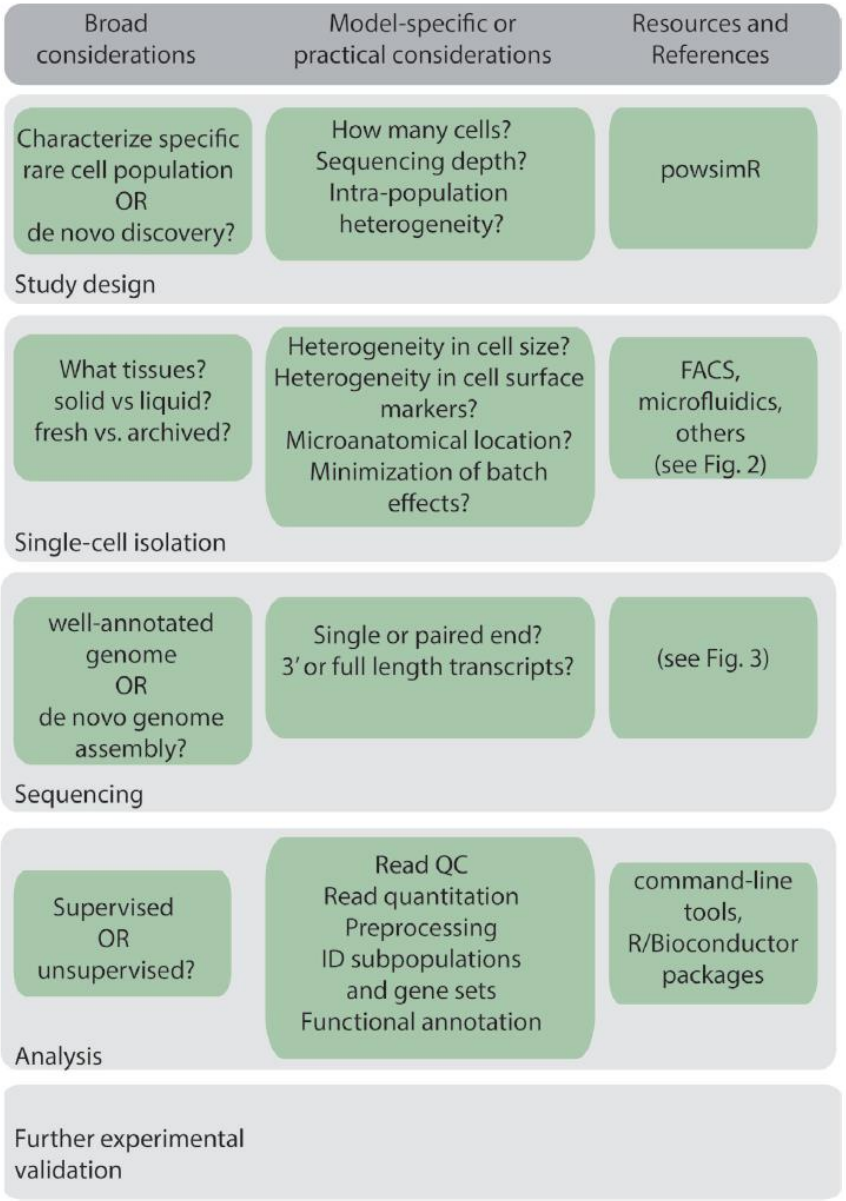
Data vizualization: **tsne** reduction of dimension



Azizi, Cell, 2018

Conclusion


Broad considerations
Model-specific or practical considerations
Resources and References
Characterize specific rare cell population OR de novo discovery?
How many cells? Sequencing depth? Intra-population heterogeneity?
powsimR
Study design
What tissues? solid vs liquid? fresh vs. archived?
Heterogeneity in cell size? Heterogeneity in cell surface markers? Microanatomical location? Minimization of batch effects?
FACS, microfluidics, others (see Fig. 2)
Single-cell isolation
well-annotated genome OR de novo genome assembly?
Single or paired end? 3' or full length transcripts?
(see Fig. 3)
Sequencing
Supervised OR unsupervised?
Read QC Read quantitation Preprocessing ID subpopulations and gene sets Functional annotation
command-line tools, R/Bioconductor packages
Analysis
Further experimental validation