Johns Hopkins Engineering

Immunoengineering

Immunoengineering - Immunoprofiling

Microfluidics and Single Cell Analysis

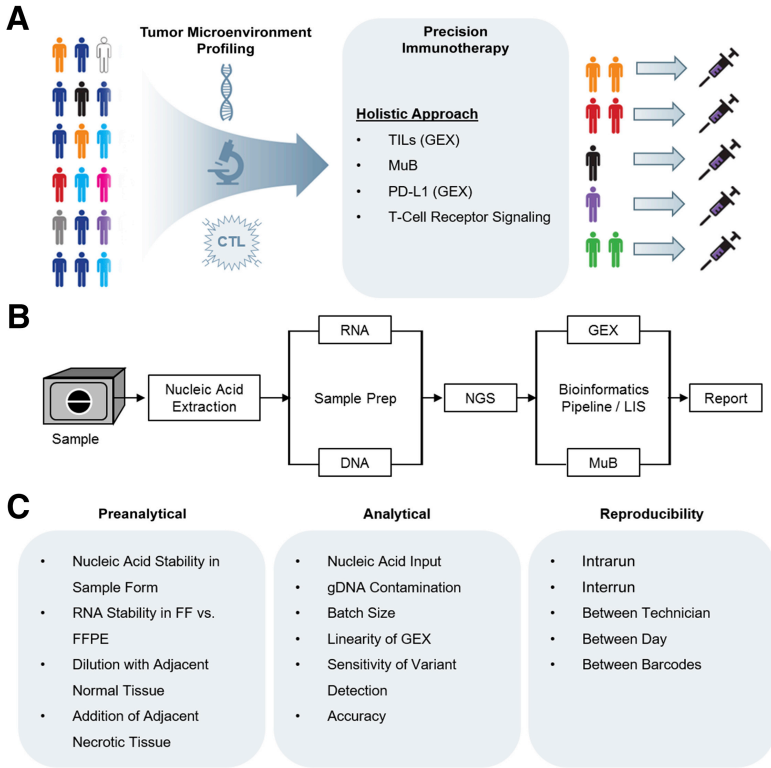
JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

Outline

- Microfluidics introduction
- Applications in immunoprofiling
- Single cell sequencing

Similar to diagnostics in developing new assay

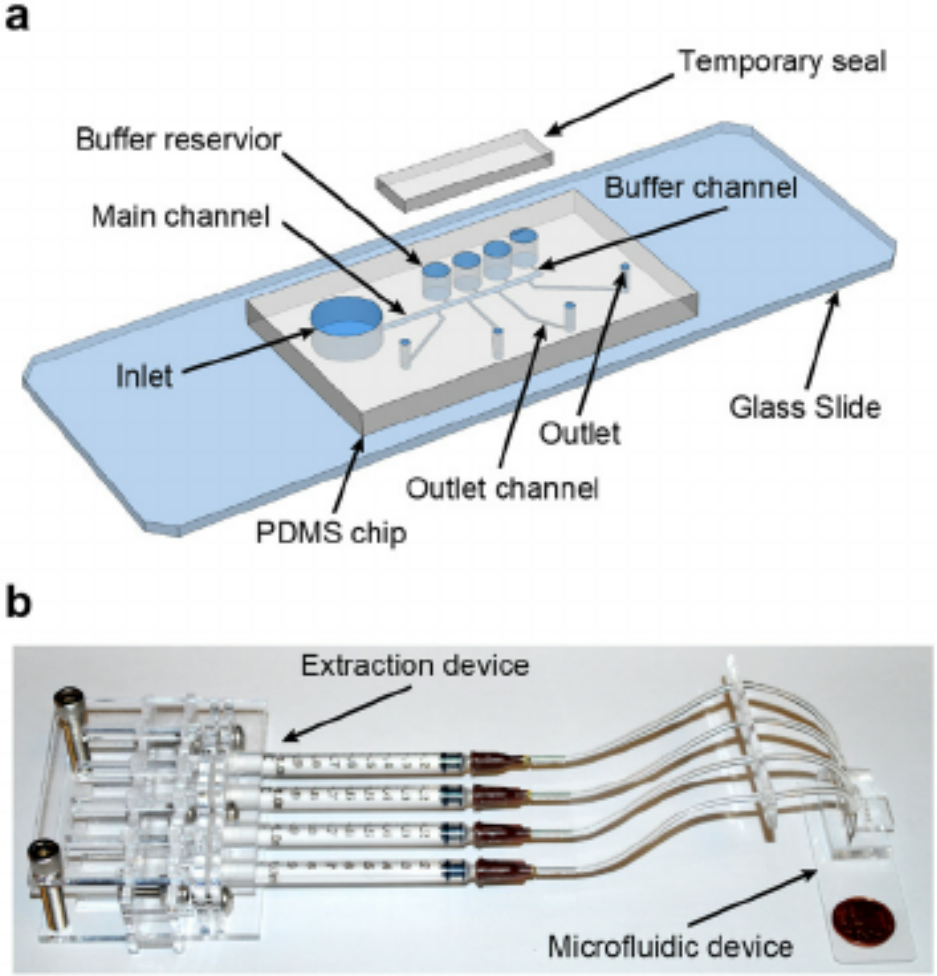
- Isolate RNA out of formalin fixed tissue samples
- Measure 54 anti-cancer immune response genes
  - Generate anti-tumor immune signature
- Test state of patient ready for cancer immunotherapy



Conroy, Jeffrey M., et al. "Analytical Validation of a Next-Generation Sequencing Assay to Monitor Immune Responses in Solid Tumors." *The Journal of Molecular Diagnostics* 20.1 (2018): 95-109.

Microfluidics Intro.

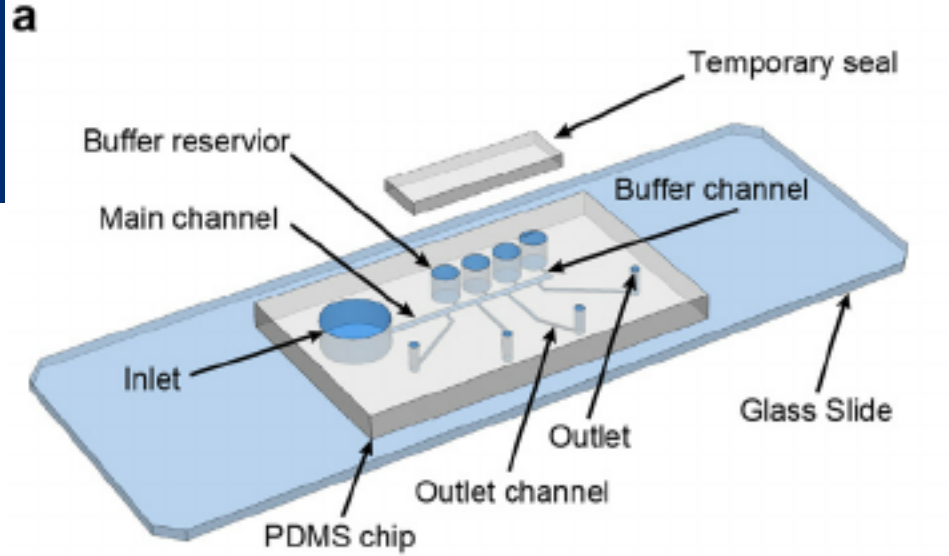
- Decrease necessary reagent and sample input
- Increase sensitivity due to low volume
- Integration of multiple processing steps
- Decreased processing time

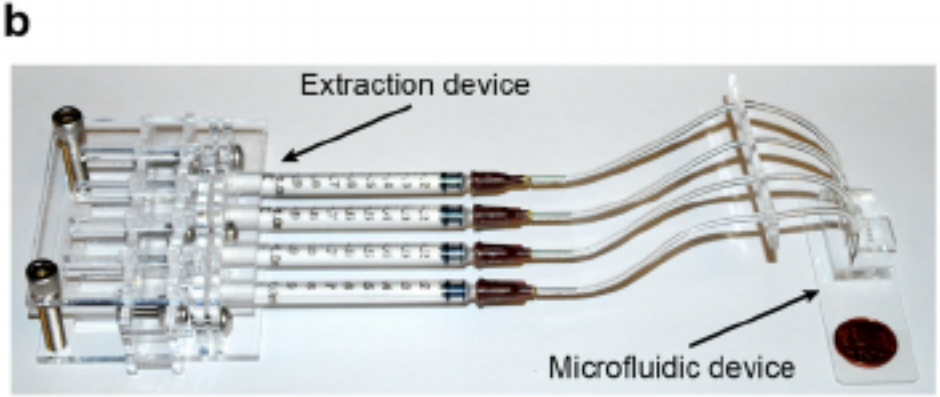


Eamer, Lise, et al. "Microfluidic assessment of swimming media for motility-based sperm selection." *Biomicrofluidics* 9.4 (2015): 044113.

Microfluidics Intro.

- Reliability
- Manufacturability
- Necessity for external syringe pumps
- Require sample preparation





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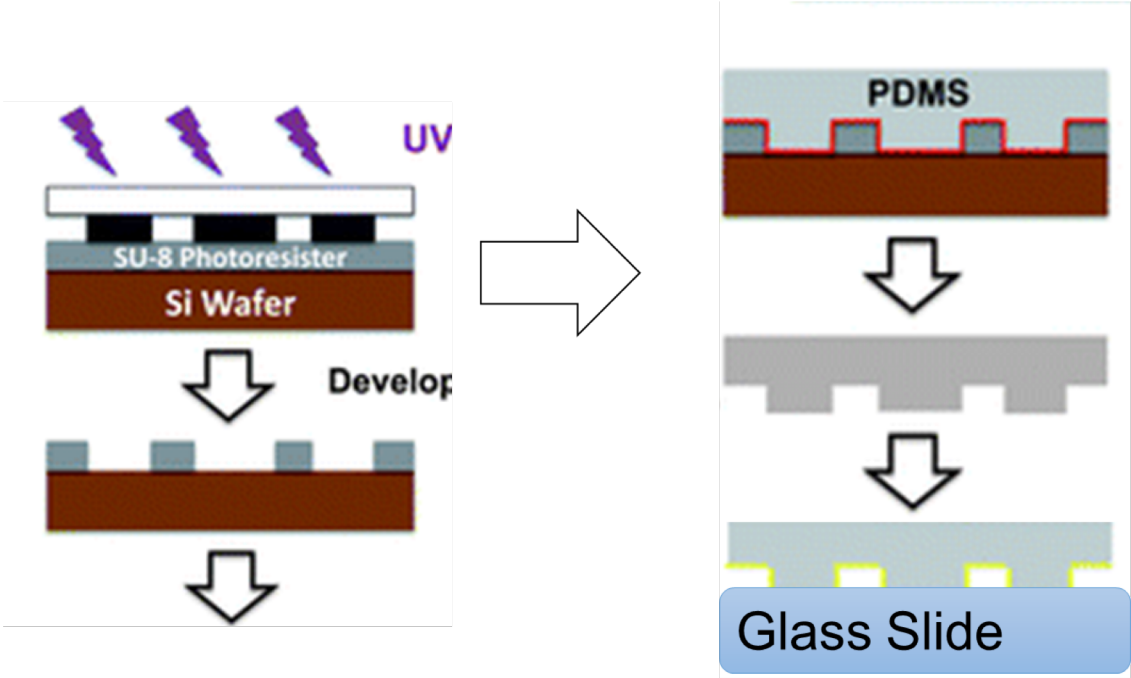
Microfluidics Intro.

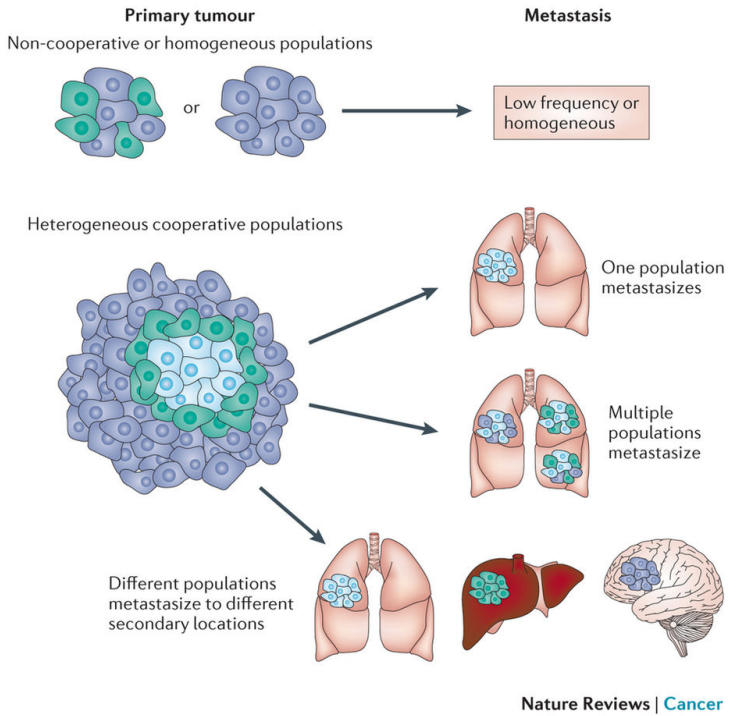
- Traditionally produced by lithographic techniques
- PDMS bonded to glass coverslip



Tang, Xin, M. Yakut Ali, and M. Taher A. Saif. "A novel technique for micro-patterning proteins and cells on polyacrylamide gels." *Soft matter* 8.27 (2012): 7197-7206.

Single Cell Analysis – Cell Heterogeneity

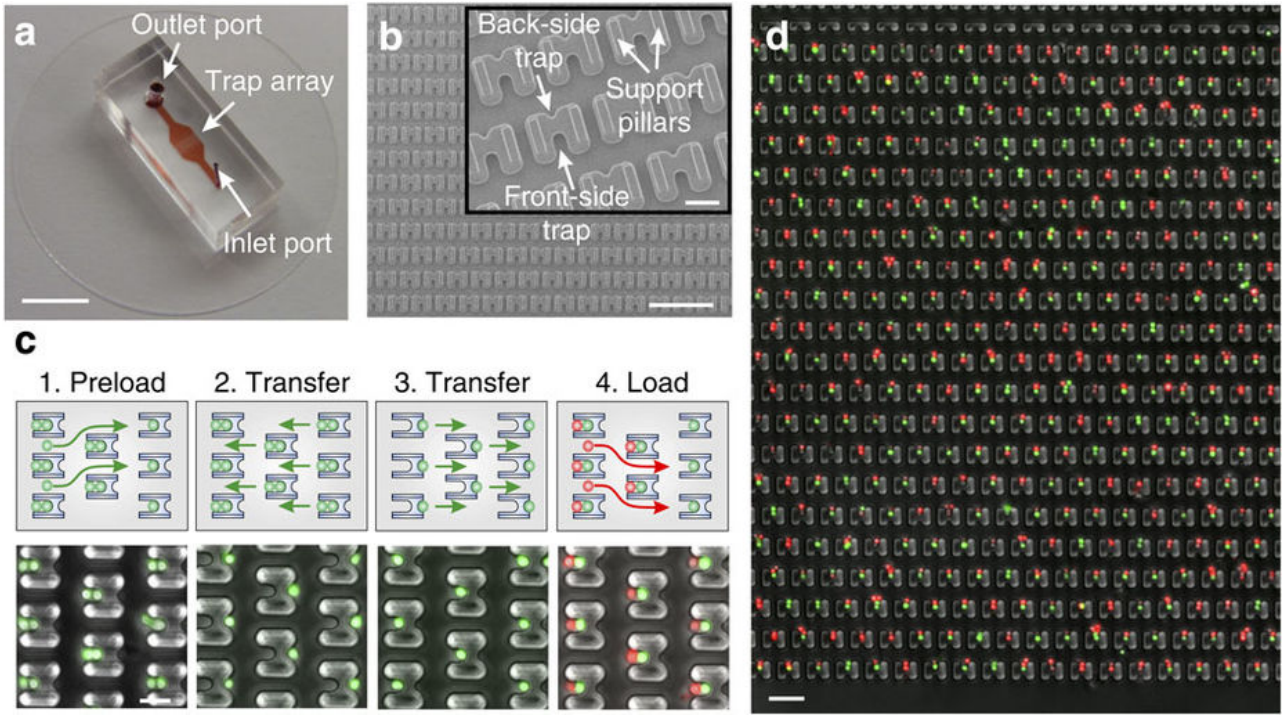
- Bulk analyses could miss cellular heterogeneity
- Understanding heterogeneity leads to better understanding of biology and better therapies



Tabassum, Doris P., and Kornelia Polyak. "Tumorigenesis: it takes a village." *Nature Reviews Cancer* 15.8 (2015): 473.

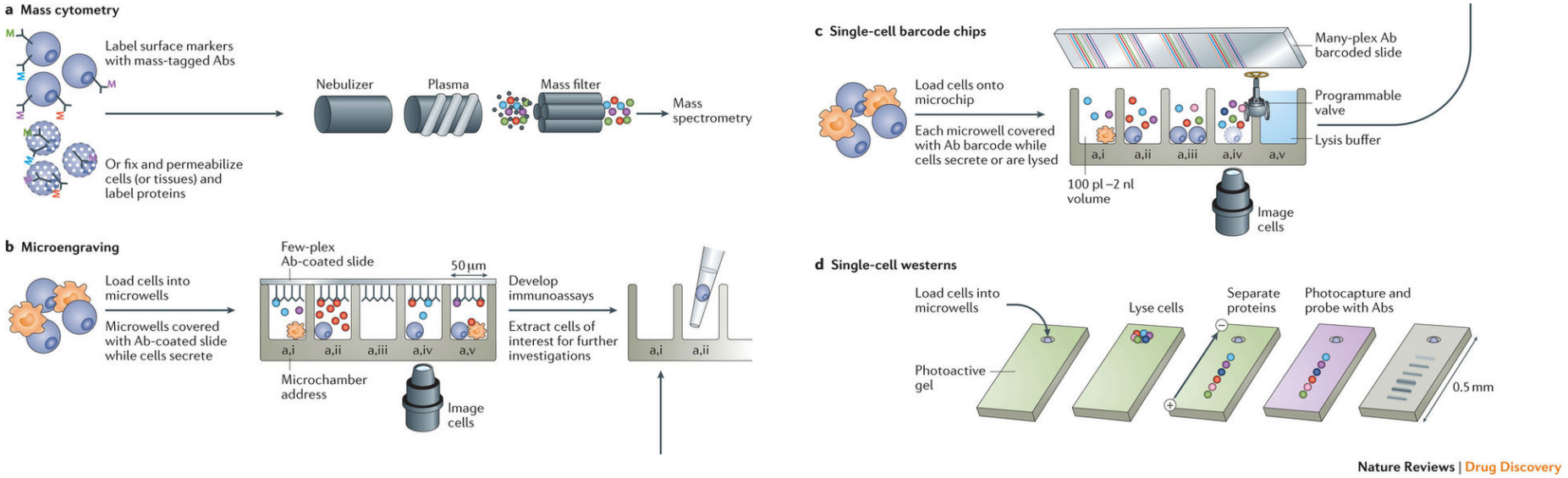
Profiling Thousands of Lymphocyte Interactions

- Lack single-cell interacting devices
- Use micro-traps to capture individual cells and utilize fluid flow to pair
- Track APC-T cell interaction dynamics and heterogeneity



Dura, Burak, et al. "Profiling lymphocyte interactions at the single-cell level by microfluidic cell pairing." *Nature communications* 6 (2015): 5940.

Single Cell Proteomics



Heath, James R., Antoni Ribas, and Paul S. Mischel. "Single-cell analysis tools for drug discovery and development." *Nature reviews Drug discovery* 15.3 (2016): 204.

Single Cell Proteomics

Table 1 | **Characteristics and capabilities of single-cell proteomics methods**

Method	Protein detection method	Comments	Refs
Fluorescence activated cell sorting (FACS)	Staining with fluorophore-labelled antibodies	• Standard for cell sorting based on membrane protein cell surface markers • High-throughput tool with excellent statistics • Mature technique • Multiplexing is colourimetric • Typically requires large sample sizes • Sorted and analysed cells are viable for subsequent analysis • Commercial product (many vendors)	4,7
Mass cytometry (CyTOF)	Staining fixed cells with mass-tag labelled antibodies	• Good for cytoplasmic proteins • Excellent statistics • Demonstrated as a drug screening tool • >30 proteins assayed per cell • Multiplexing is via mass spectrometry • Applicable to fixed-tissue analysis • Commercial product (Fluidigm)	9,11,35, 41,104
Single-cell barcode chips (SCBCs)	Spatially encoded antibody array for fluorescent immunoassays of secreted proteins or analytes released from lysed cells	• Permits absolute quantitation • Suitable for small (100–1,000 cells) biospecimen sizes • Demonstration of >40 proteins assayed per cell • Secreted proteins detected from viable cells • Some designs integrate cell lysis to permit cytoplasmic protein assays and integrated protein and metabolite assays • Analysis of cell–cell interactions • Cost-effective • Multiplexing is via spatially encoded arrays • Commercial service (Isoplexis)	8,10,23, 89,91
Microengraving	Fluorescent immunoassays of secreted proteins	• Small numbers of secreted proteins • >10,000 single cells assayed in parallel • Cost-effective • Permits kinetic studies of protein secretion • Recovery of analysed cells for further analysis • Analysis of cell–cell interactions • Suitable for small (100–1,000 cells) biospecimen sizes • Multiplexing is colourimetric	31,76, 85,99
Single-cell western blotting (scWestern)	Miniaturized, automated western blotting on a microchip	• Suitable for small (100–1,000 cells) biospecimen size • 1,000 cells assayed per microchip • Multiplexing to ~12 proteins demonstrated • Permits cytoplasmic proteins from lysed cells • Reasonably fast (4 hours) • Provides protein ladder reference • Relative quantitation	77

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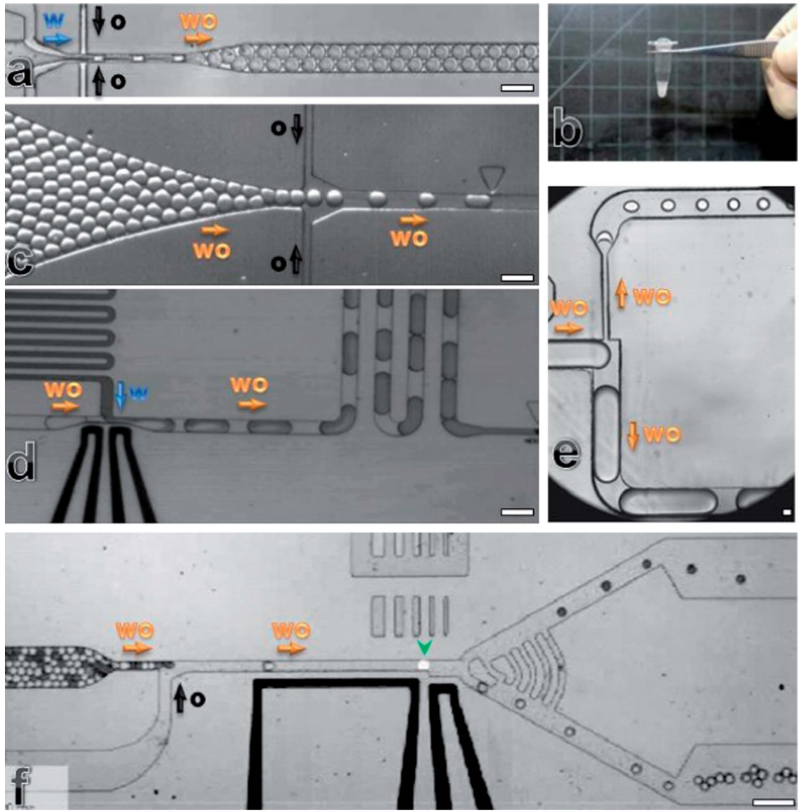
Droplet Microfluidics for Immunoprofiling

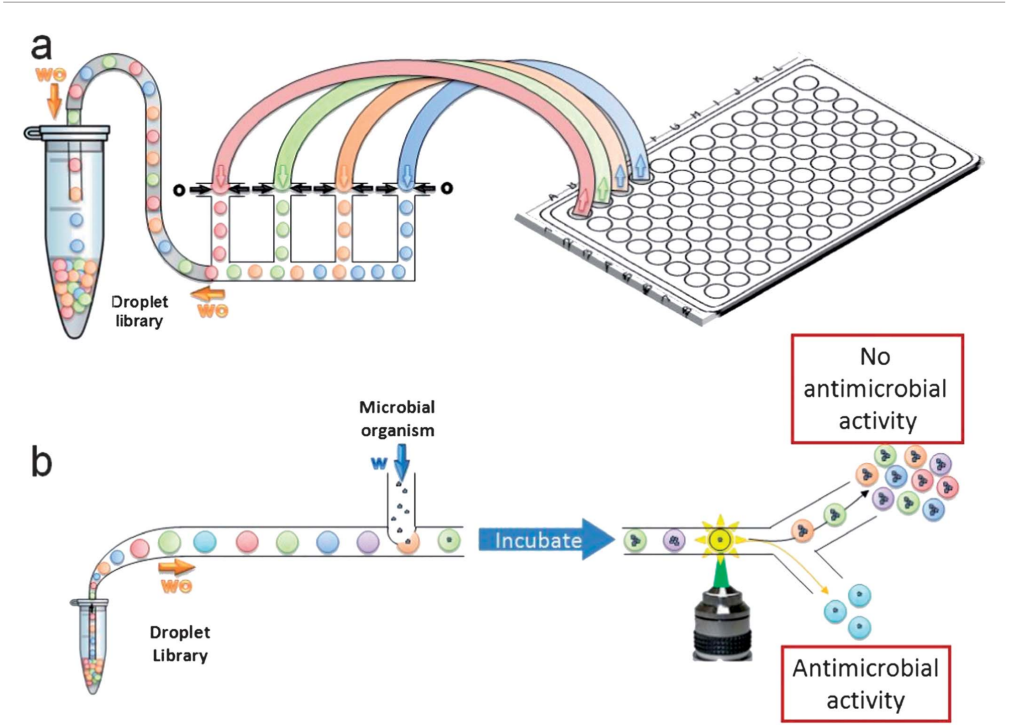
- Use water oil interface to create microdroplets
- 10^8 samples per day
- 0.05 pL to 1 nL volume
- Functionalities
  - Can be sorted and collected
  - Can mix in additional reactions



Guo, Mira T., et al. "Droplet microfluidics for high-throughput biological assays." *Lab on a Chip* 12.12 (2012): 2146-2155.

Droplet Microfluidics for Immunoprofiling

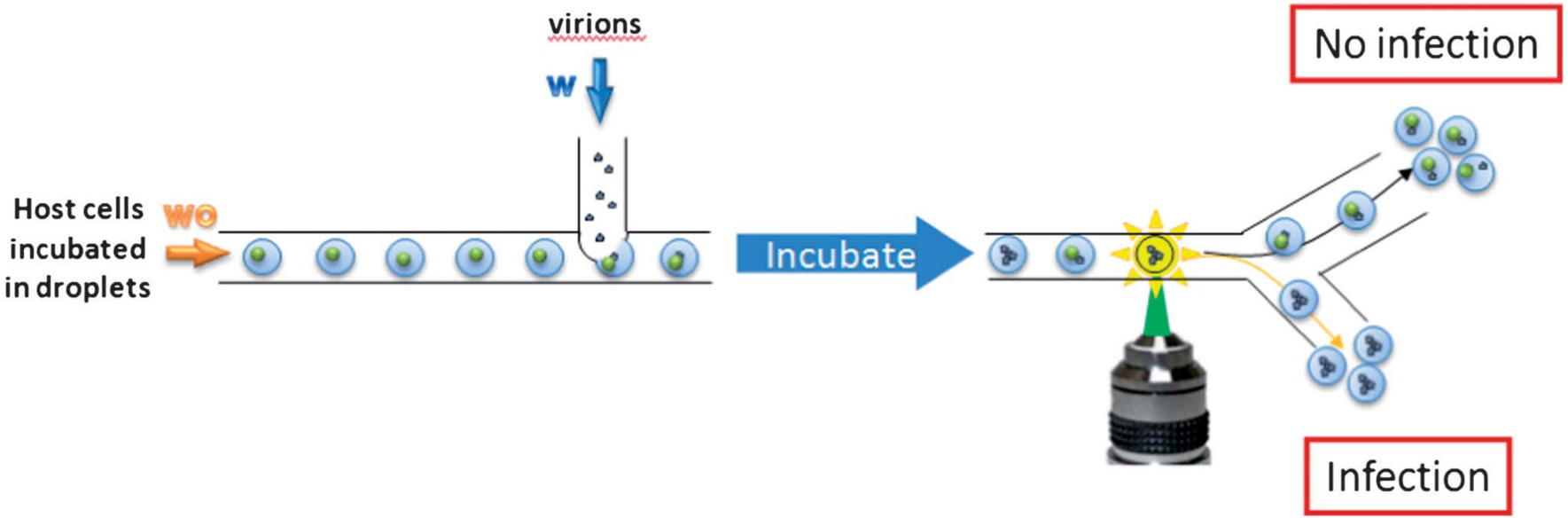
- Can be used to create droplet libraries
- Example: Antibiotic screens



Guo, Mira T., et al. "Droplet microfluidics for high-throughput biological assays." *Lab on a Chip* 12.12 (2012): 2146-2155.

Droplet Microfluidics for Immunoprofiling

- High throughput cell-pathogen interactions



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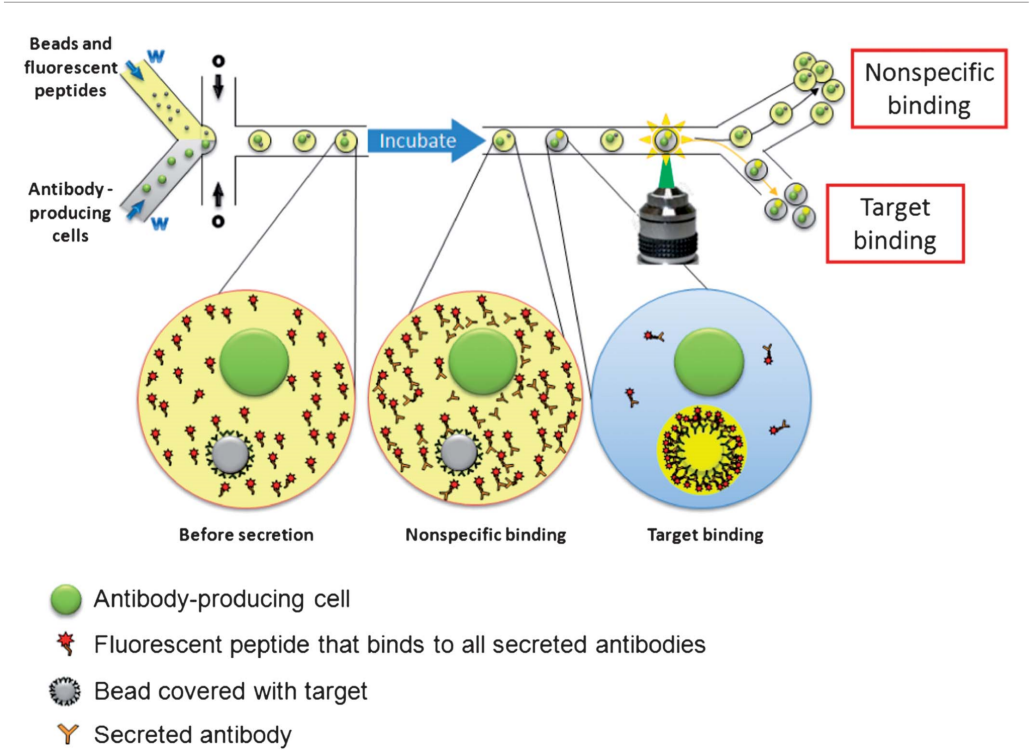
Droplet Microfluidics for Immunoprofiling

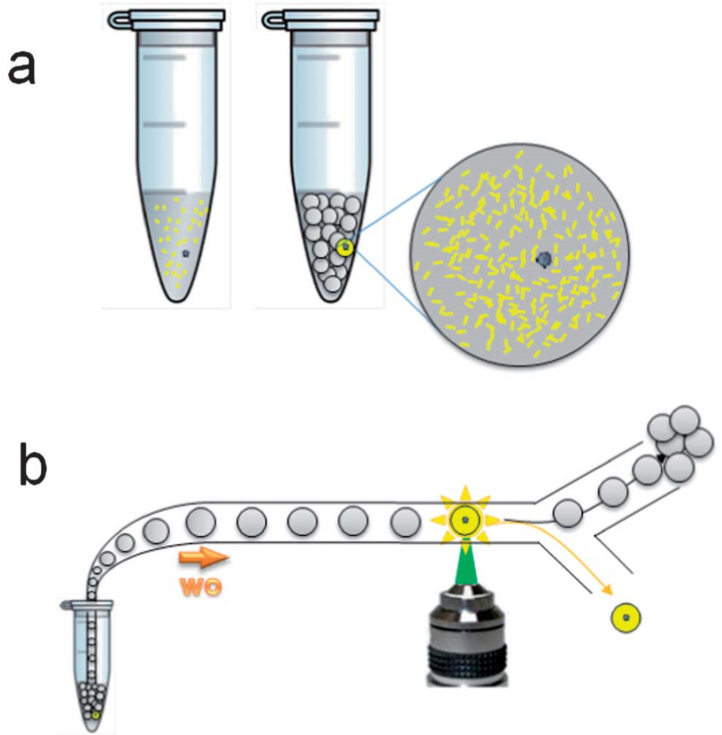
- Antibodies for studying proteins or therapeutic produced in hybridomas
- Hybridomas need to be made from single pure B cells
- High throughput selection



Guo, Mira T., et al. "Droplet microfluidics for high-throughput biological assays." *Lab on a Chip* 12.12 (2012): 2146-2155.

Droplet Microfluidics for Immunoprofiling

- Detection of rare microbes
- Use PCR reactions in concentrated droplets
- Quick turnaround time



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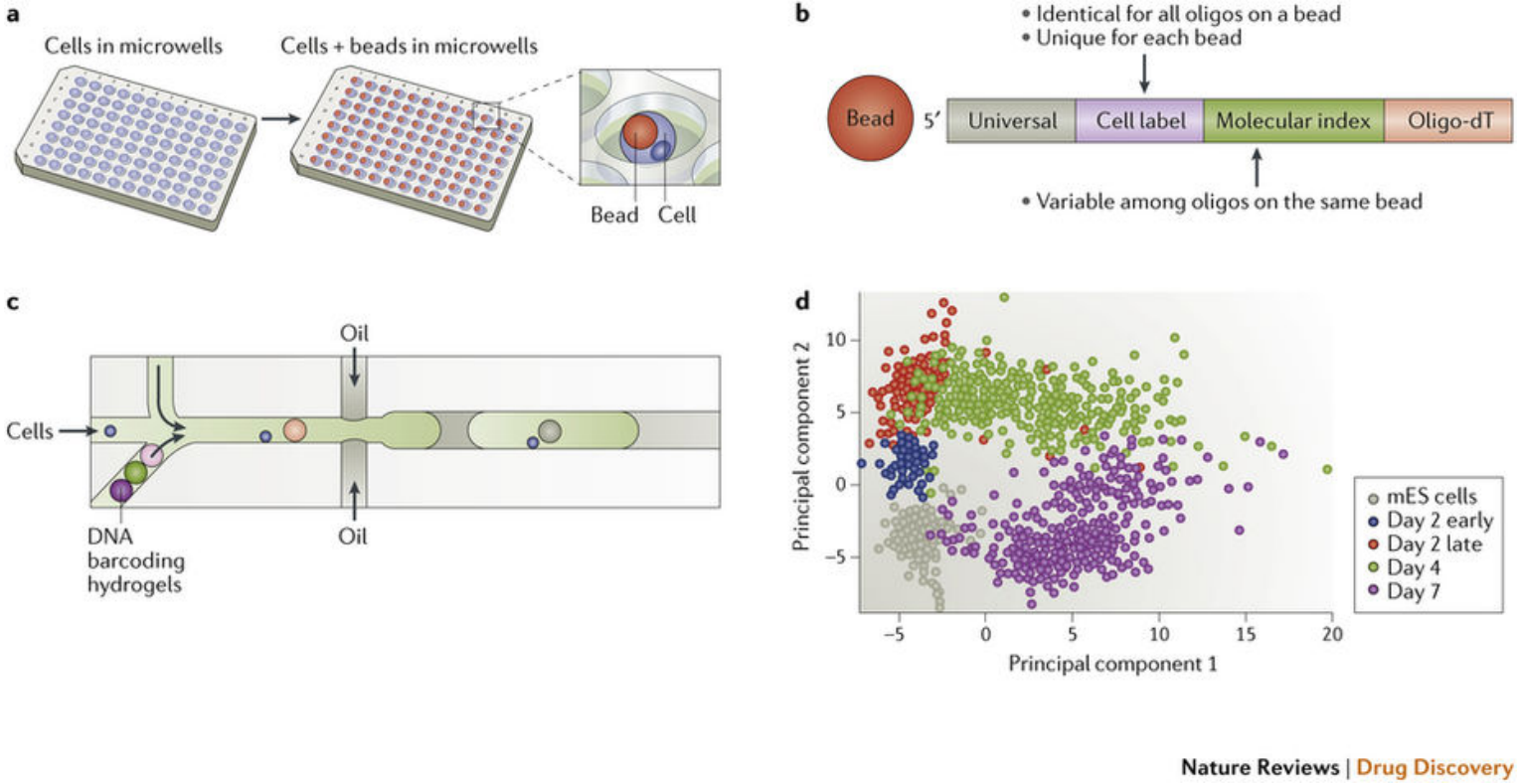
Single Cell Sequencing



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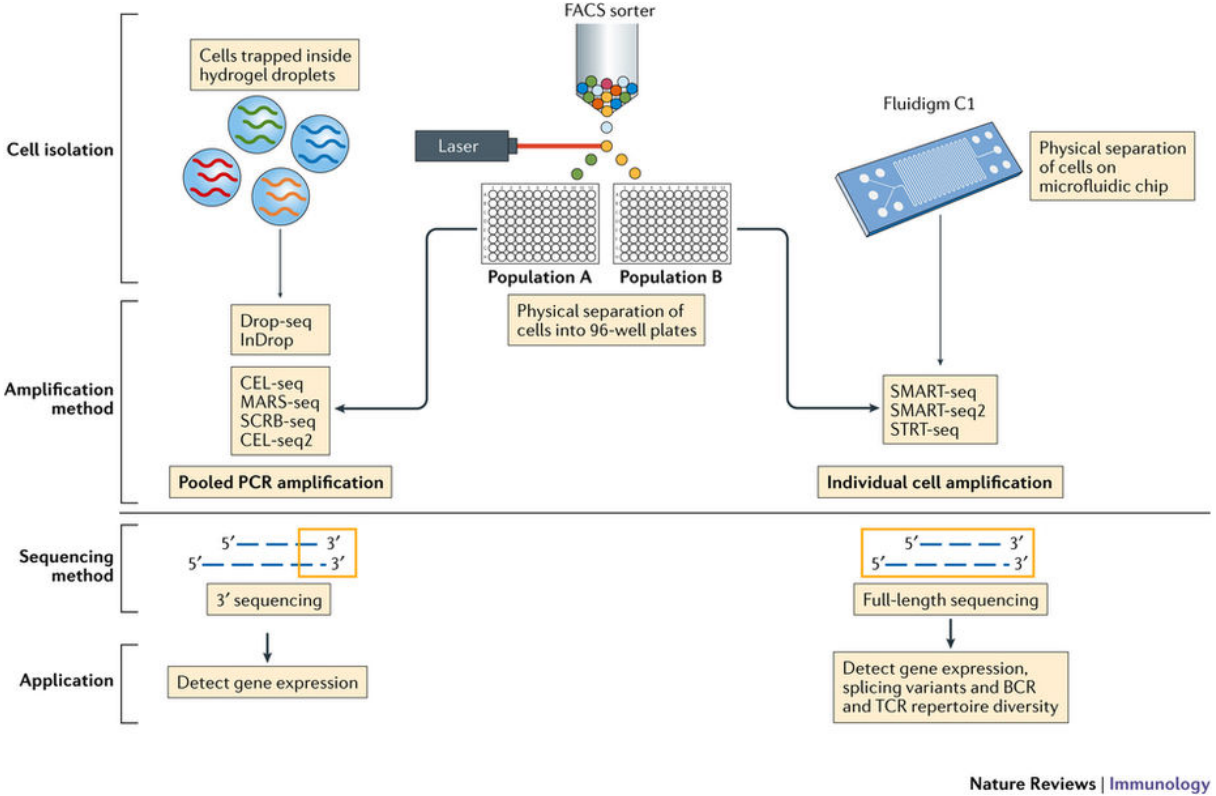
Single Cell Sequencing Techniques Growing



Papalexi, Efthymia, and Rahul Satija. "Single-cell RNA sequencing to explore immune cell heterogeneity." *Nature Reviews Immunology* 18.1 (2018): 35.

Single Cell Sequencing Techniques Growing

	FACS	CyTOF	qPCR	Plate-based protocols (STRT-seq, SMART-seq, SMART-seq2)	Fluidigm C1	Pooled approaches (CEL-seq, MARS-seq, SCRB-seq, CEL-seq2)	Massively parallel approaches (Drop-seq, InDrop)
Cell capture method	Laser	Mass cytometry	Micropipettes	FACS	Microfluidics	FACS	Microdroplets
Number of cells per experiment	Millions	Millions	300–1,000	50–500	48–96	500–2,000	5,000–10,000
Cost	$0.05 per cell	$35 per cell	$1 per cell	$3–6 per well	$35 per cell	$3–6 per well	$0.05 per cell
Sensitivity	Up to 17 markers	Up to 40 markers	10–30 genes per cell	7,000–10,000 genes per cell for cell lines; 2,000–6,000 genes per cell for primary cells	6,000–9,00 genes per cell for cell lines; 1,000–5,000 genes per cell for primary cells	7,000–10,000 genes per cell for cell lines; 2,000–6,000 genes per cell for primary cells	5,000 genes per cell for cell lines; 1,000–3,000 genes per cell for primary cells

CEL-seq, cell expression by linear amplification and sequencing; CyTOF, cytometry by time of flight (mass cytometry); FACS, fluorescence-activated cell sorting; InDrop, indexing droplets sequencing; MARS-seq, massively parallel single-cell RNA sequencing; qPCR, quantitative PCR; SCRB-seq, single-cell RNA barcoding and sequencing; STRT-seq, single-cell tagged reverse transcription sequencing.

Nature Reviews | Immunology

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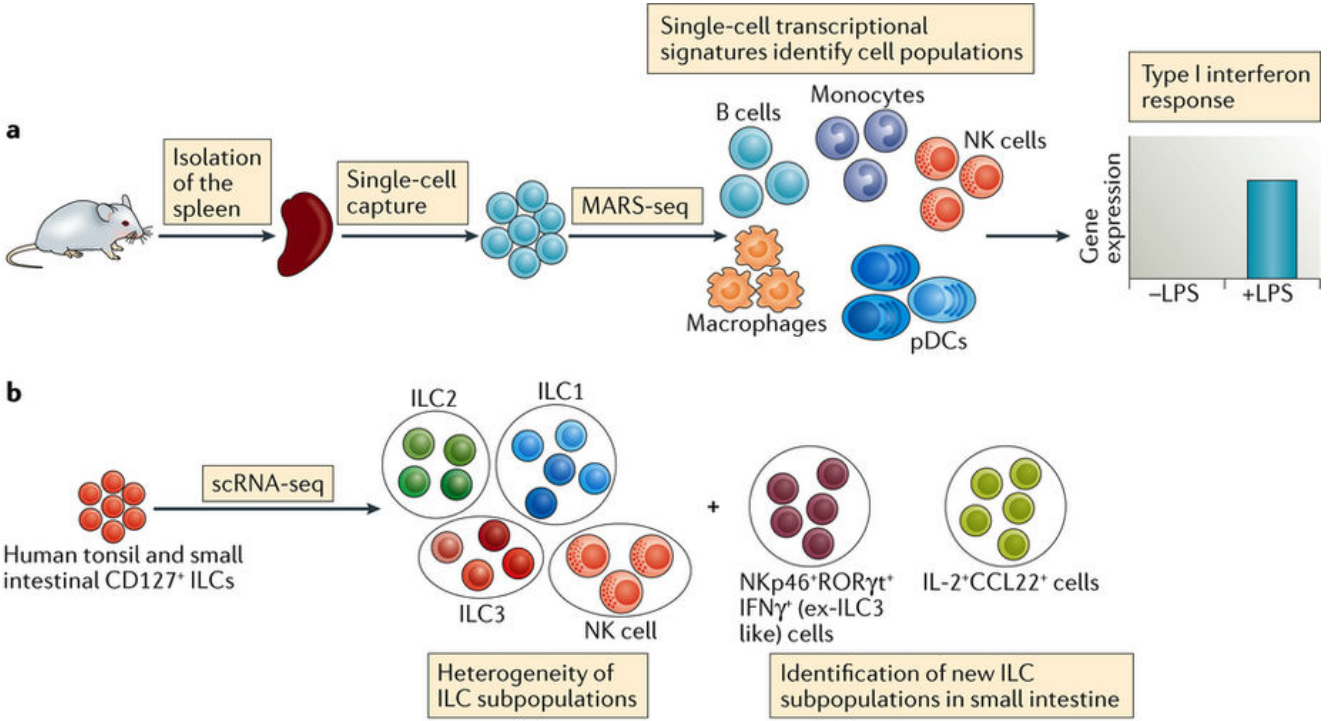
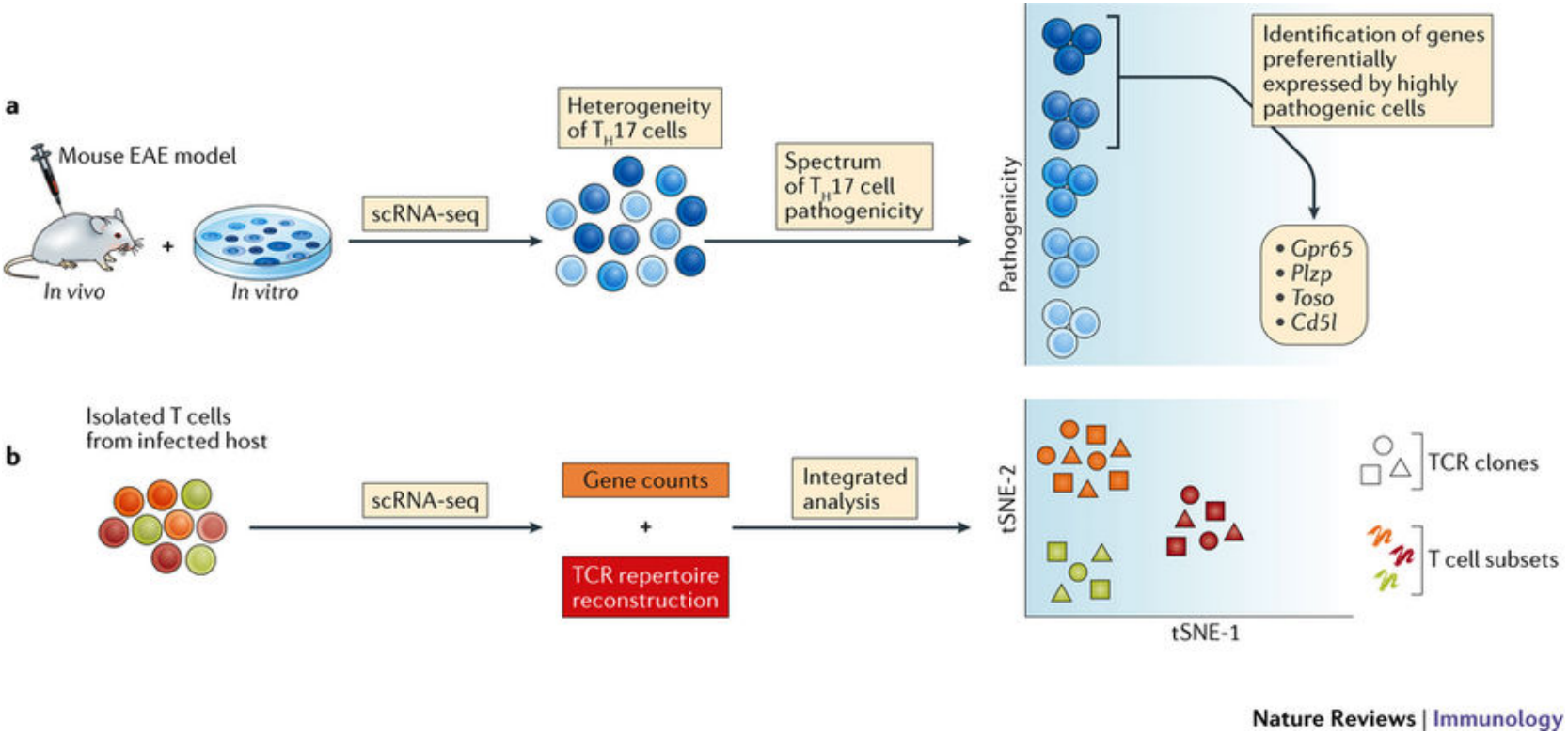
Single Cell Sequencing: Identifying Novel Cell Types



Nature Reviews | Immunology

Papalexi, Efthymia, and Rahul Satija. "Single-cell RNA sequencing to explore immune cell heterogeneity." *Nature Reviews Immunology* 18.1 (2018): 35.

Single Cell Sequencing: Identifying Disease States



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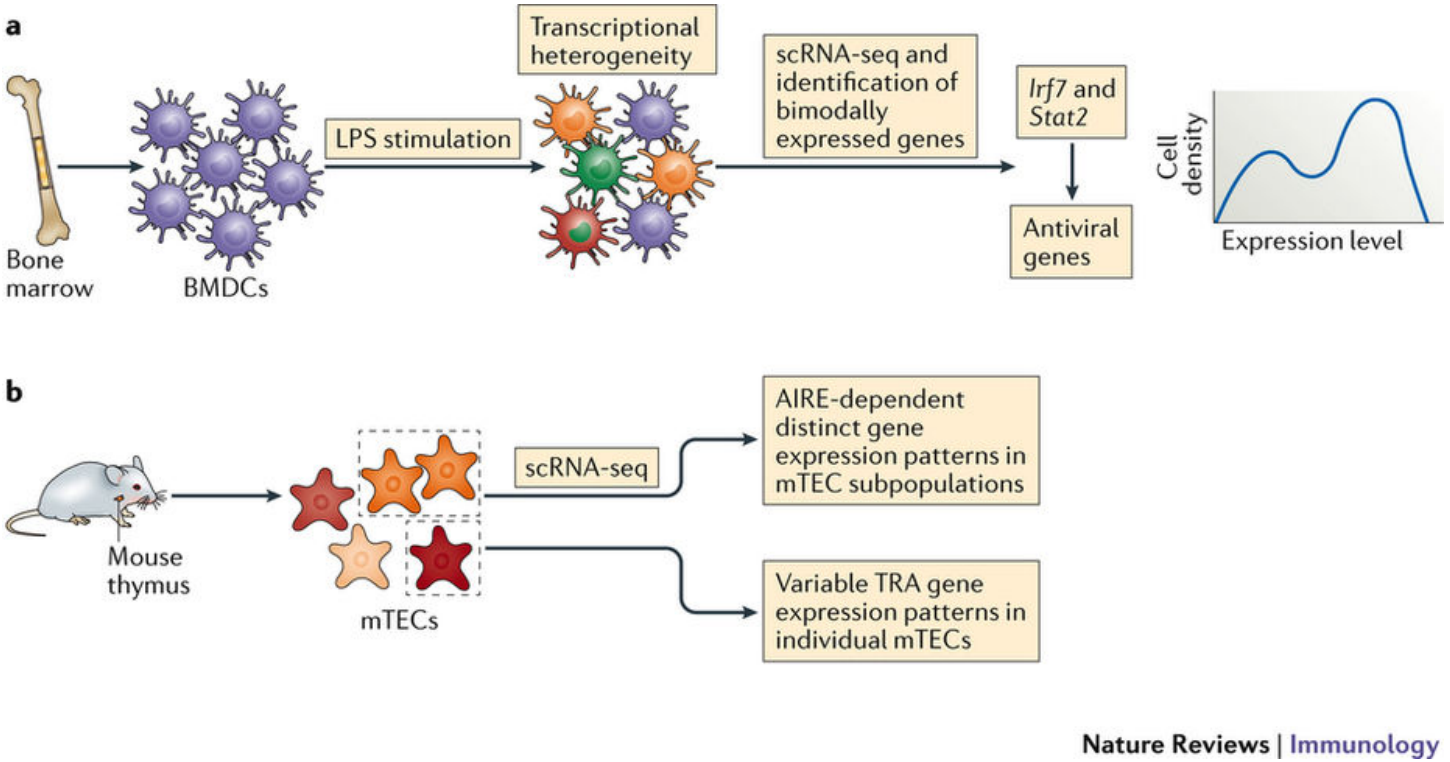
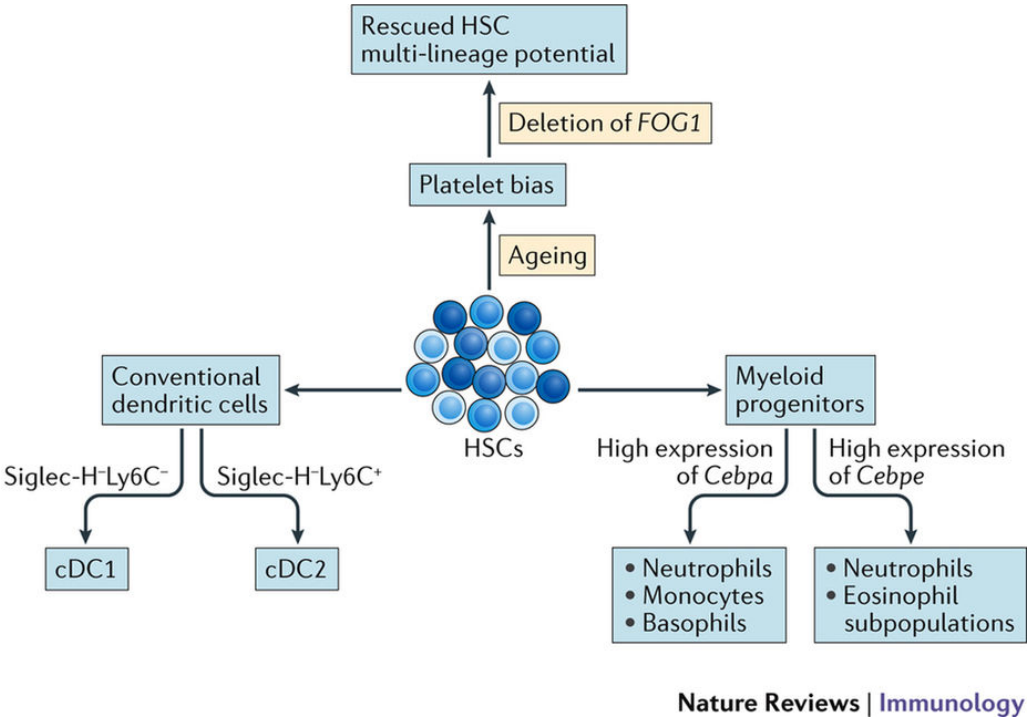
Single Cell Sequencing: Identifying Cell Heterogeneity



Papalexi, Efthymia, and Rahul Satija. "Single-cell RNA sequencing to explore immune cell heterogeneity." *Nature Reviews Immunology* 18.1 (2018): 35.

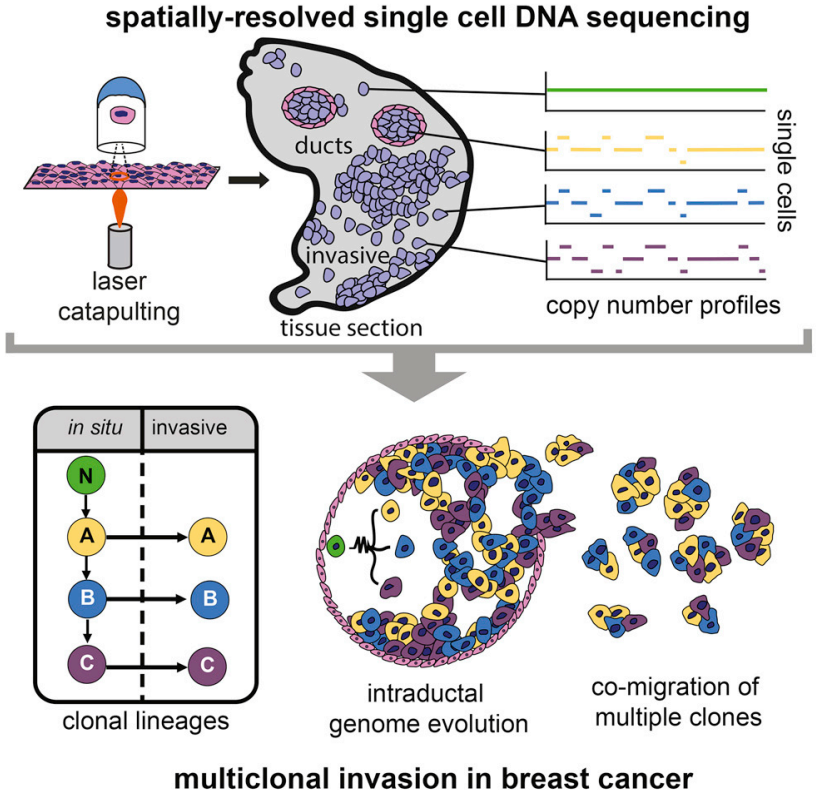
Single Cell Sequencing: Identifying Differentiation Paths



Papalexi, Efthymia, and Rahul Satija. "Single-cell RNA sequencing to explore immune cell heterogeneity." *Nature Reviews Immunology* 18.1 (2018): 35.

Spatial Sequencing Data

- Works with H&E stained slides
- Understand how mutations lead to cancer invasion and where those mutations arose

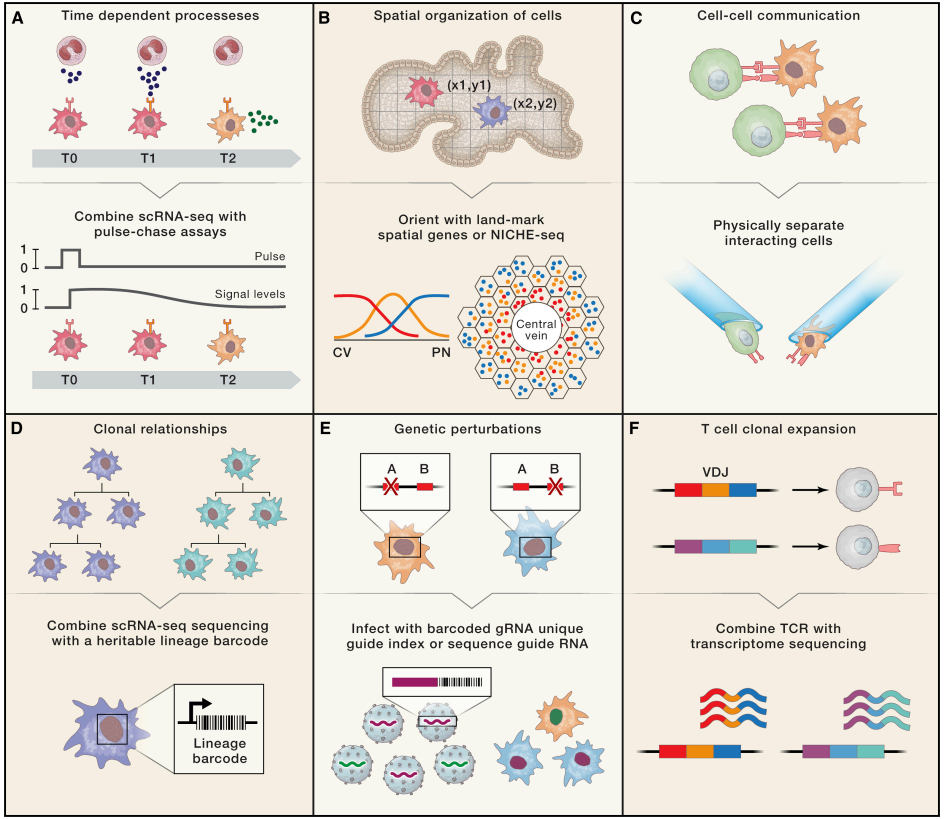


Casasent, Anna K., et al. "Multiclonal Invasion in Breast Tumors Identified by Topographic Single Cell Sequencing." *Cell* (2018).

Challenges Facing Single-Cell Approaches

- Data interpretation
- Multiplexed reagent quality-e.g. antibodies
- Standardization
- Device simplicity
- Definition of cell type vs. state
- Lack integration of multiple output signals – just mRNA

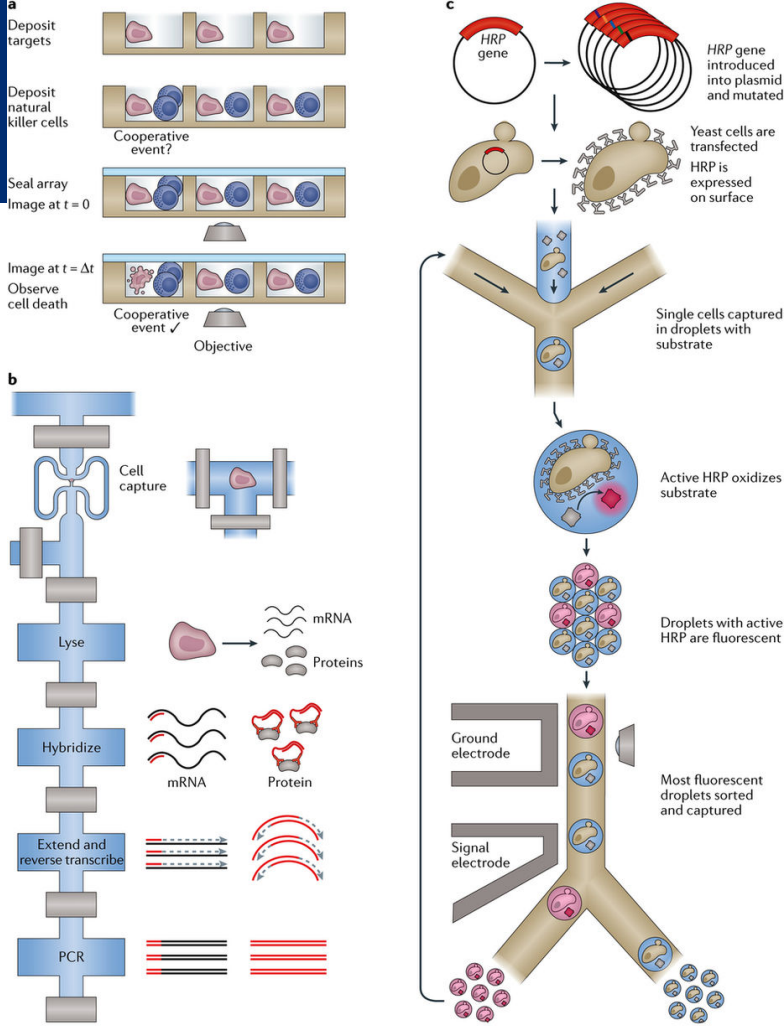
New Technologies Combining Multiple Readouts



Giladi, Amir, and Ido Amit. "Single-Cell Genomics: A Stepping Stone for Future Immunology Discoveries." *Cell* 172.1 (2018): 14-21.

New Approaches with Microfluidics

- Cooperative events vs. single cell interactions
- DNA and protein detection simultaneously
- Evolution of enzymes and immune-based proteins



Prakadan, Sanjay M., Alex K. Shalek, and David A. Weitz. "Scaling by shrinking: empowering single-cell'omics' with microfluidic devices." *Nature Reviews Genetics* 18.6 (2017): 345.