HESI IMMUNO-SAFETY TECHNICAL COMMITTEE

On-demand Training Course

Systems Biology Applied to Immune Safety

Holden Maecker, Stanford University

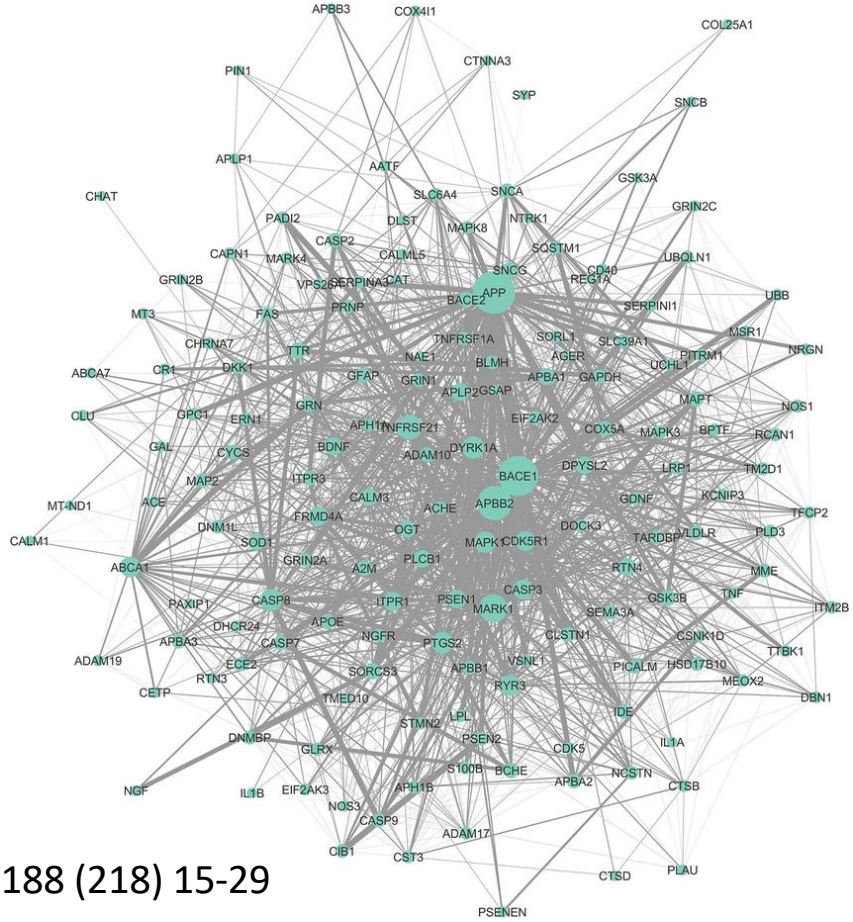
DISCLOSURE

- Scientific Advisory Board: Cytek Biosciences

Outline

- What is systems biology?
- Relevant types of assays (with limitations and examples of their application):
  - Immune cell phenotyping (CyTOF or high-parameter flow cytometry)
  - Multiplexed immunoassays (Luminex, Olink)
  - Gene expression profiling (RNAseq)
- The power of functional assays/in vitro stimulation
- How to analyze high-dimensional data
  - Visualization: dimensionality reduction
  - Clustering algorithms
  - Statistical techniques
- Defining “normal” vs. “abnormal” in the face of biological heterogeneity
  - Getting enough samples
  - Looking at patterns vs. individual readouts
  - Using each person as their own control

Systems biology is an approach in biomedical research to understanding the larger picture—be it at the level of the organism, tissue, or cell—by putting its pieces together. It's in stark contrast to decades of reductionist biology, which involves taking the pieces apart.

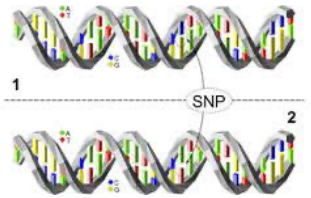


Zachariou et al., Journal of Proteomics 188 (218) 15-29

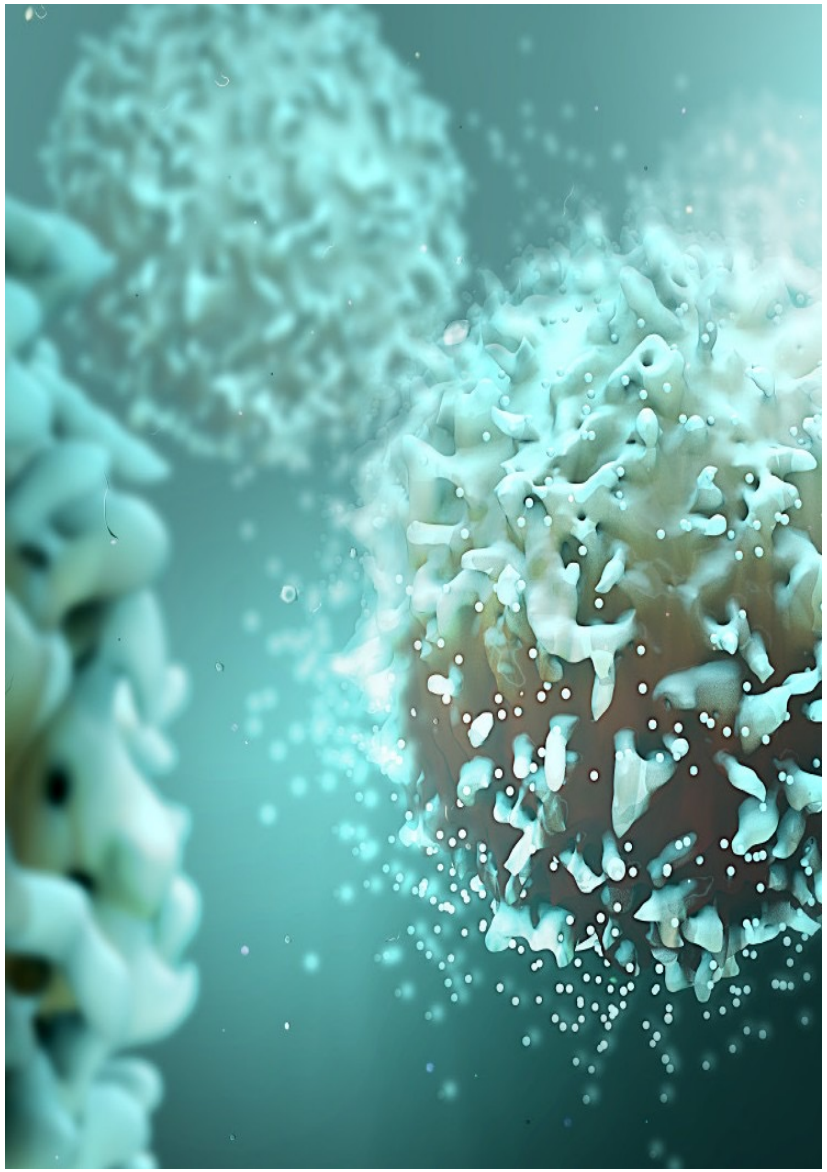
To gain understanding of a system, it's important to perturb the system and then observe the effects on as many of its components as possible.

Disease

Genetic Variant



Vaccine or Drug

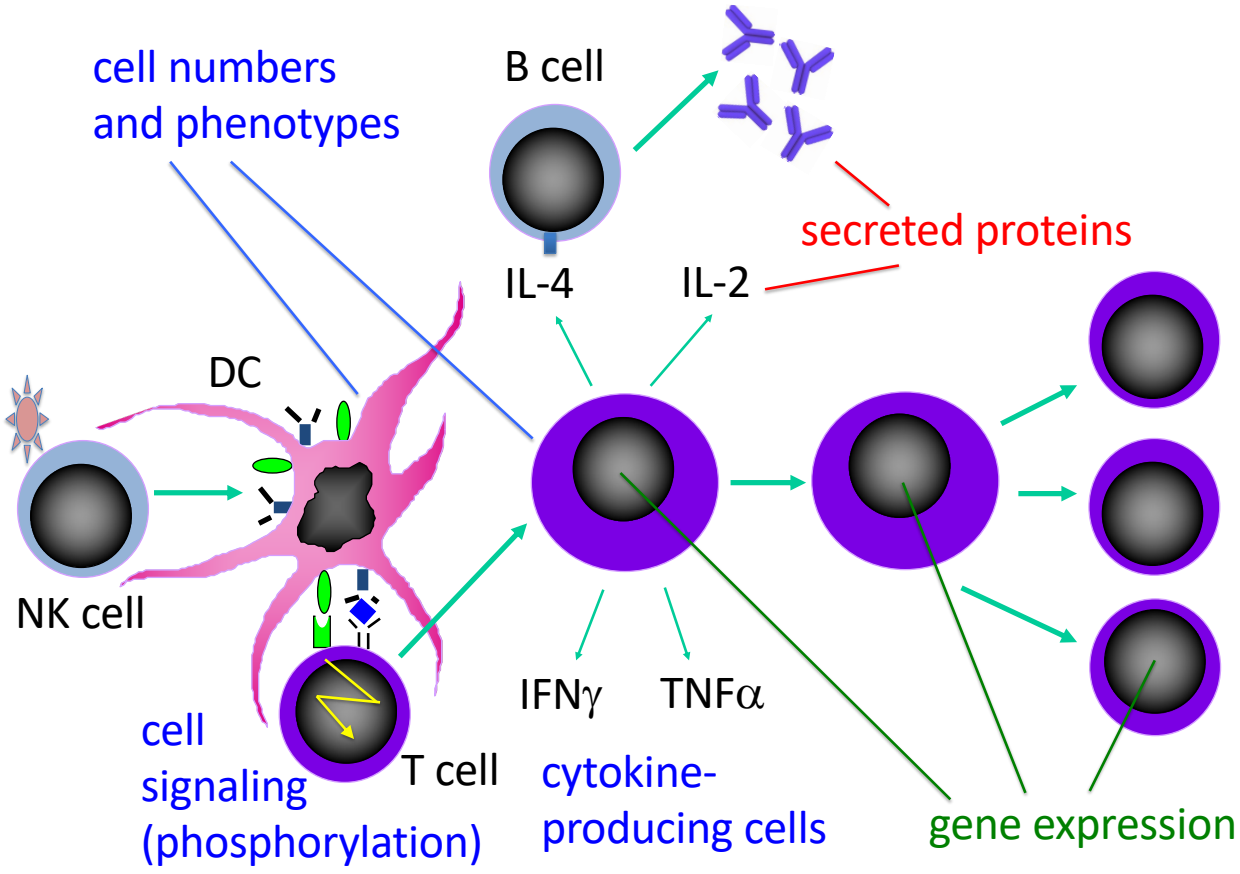


How do we observe comprehensively?

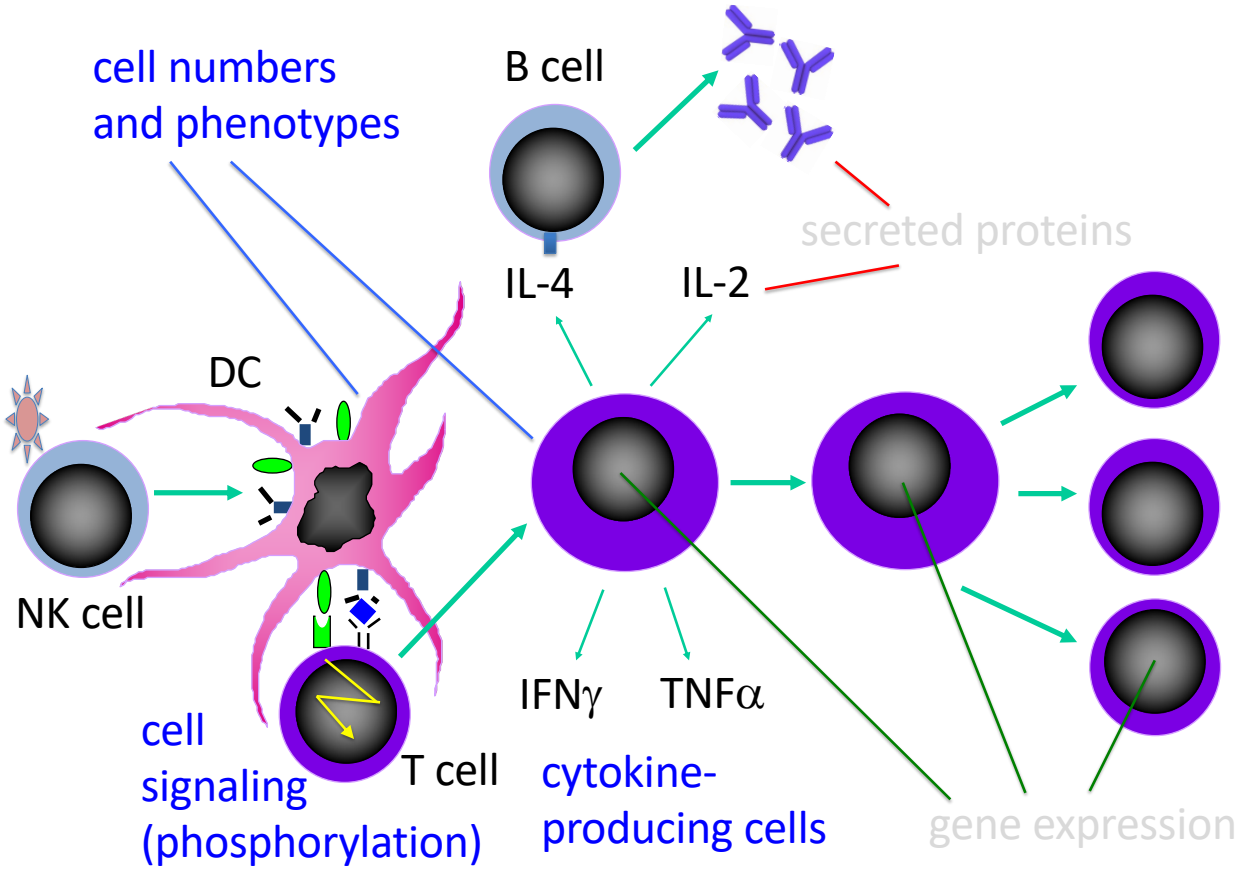
- Cellular Proteins:
  - CyTOF or high-parameter flow cytometry
- Secreted Proteins:
  - Multiplexed immunoassays (Luminex, Olink)
- Gene Expression:
  - RNAseq

"Cells are the quanta of biology." -Mark Davis

What to measure in the immune system

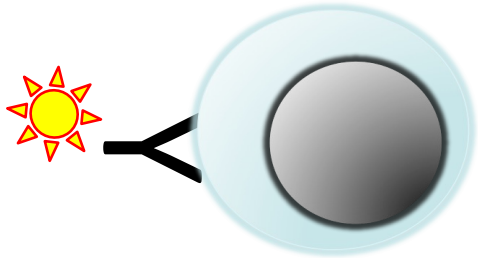


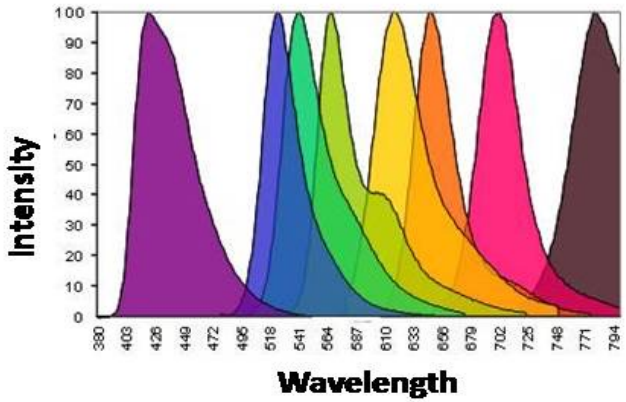
What to measure in the immune system



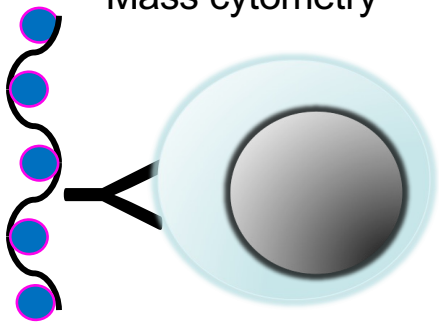
Fluorescence vs. Mass Cytometry (CyTOF)

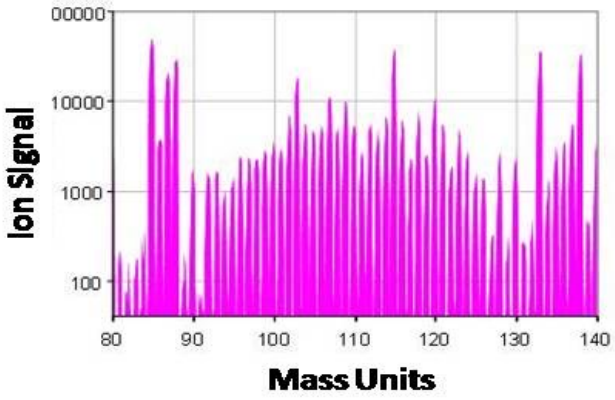
Fluorescence cytometry





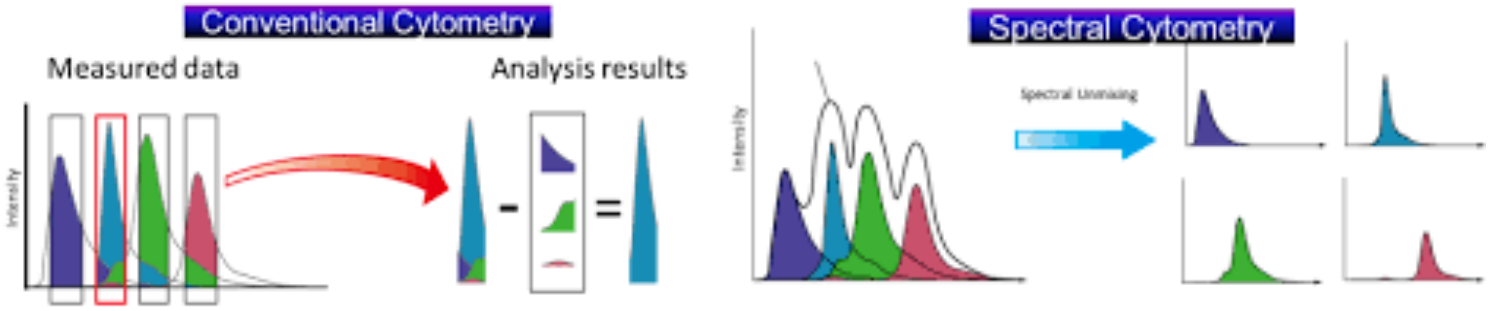
Mass cytometry





- Many more labels (antibodies)
- Little or no spillover

Spectral vs. Conventional Flow Cytometry

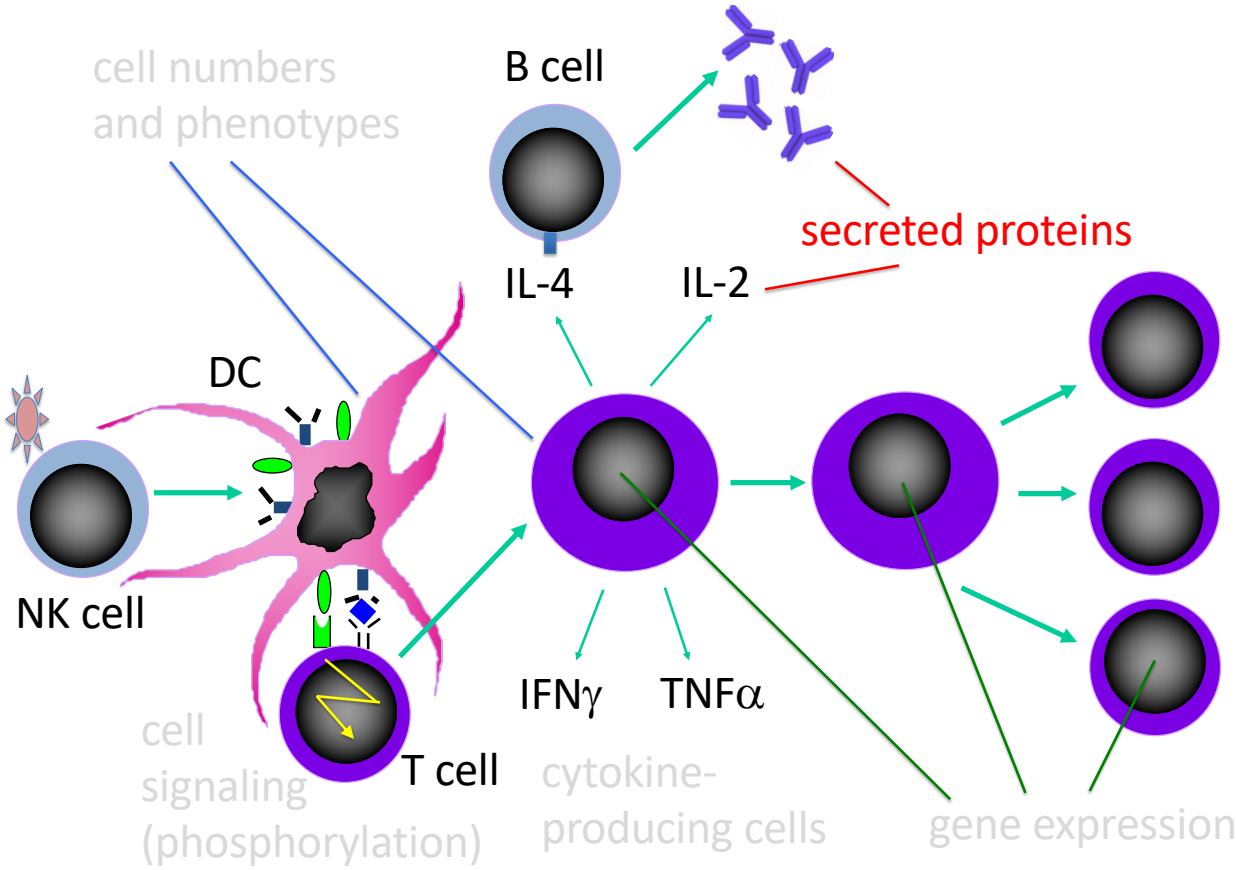


› Cytometry A. 2020 Oct;97(10):1044-1051. doi: 10.1002/cyto.a.24213. Epub 2020 Aug 31.

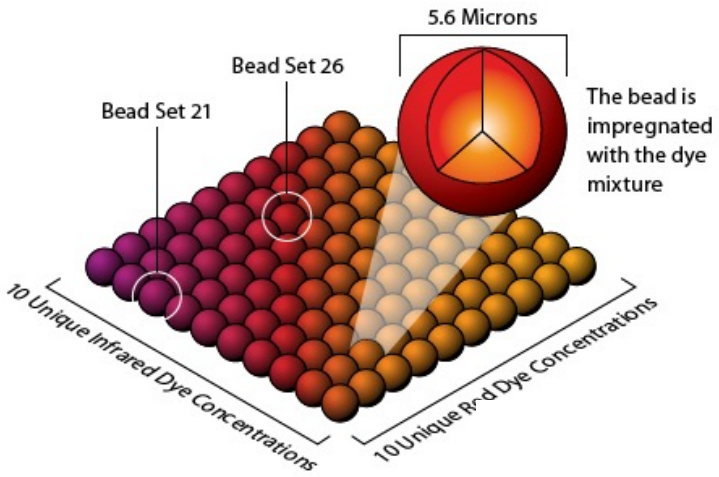
OMIP-069: Forty-Color Full Spectrum Flow Cytometry Panel for Deep Immunophenotyping of Major Cell Subsets in Human Peripheral Blood

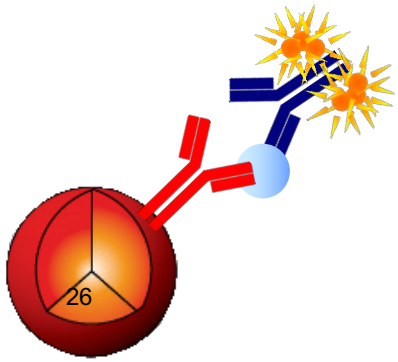
Lily M Park [1], Joanne Lannigan [2], Maria C Jaimes [1]

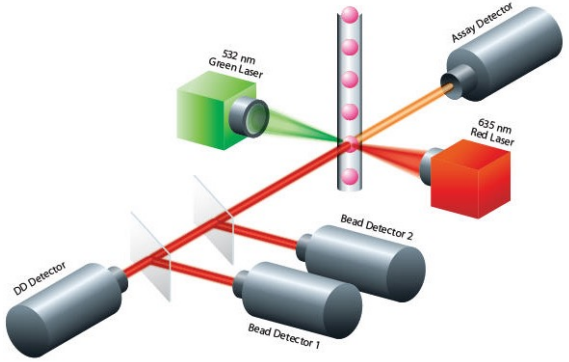
What to measure in the immune system



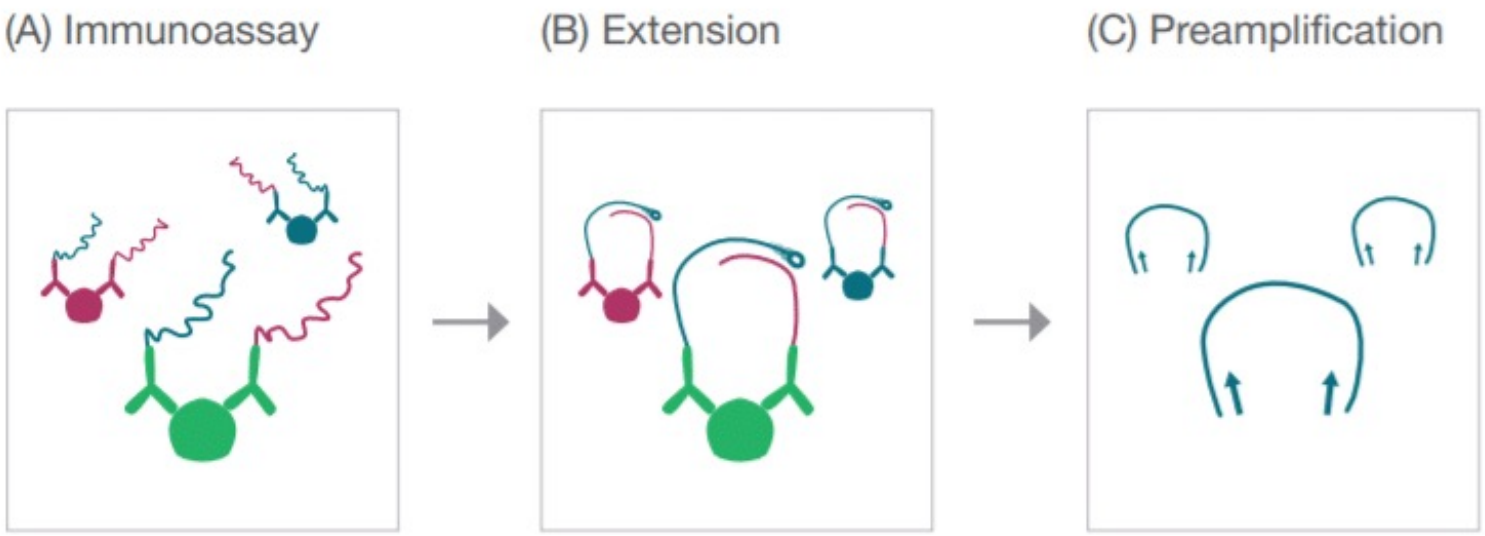
Luminex: Bead-Based Immunoassay



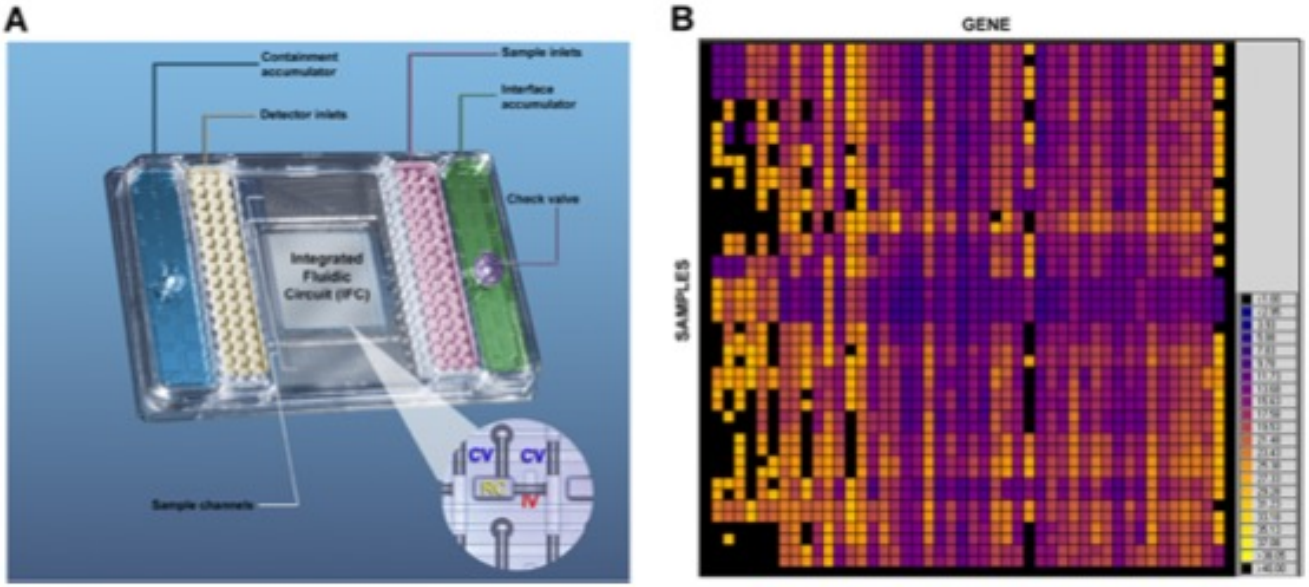




Olink: Multiplexed proximity extension assay (PEA)



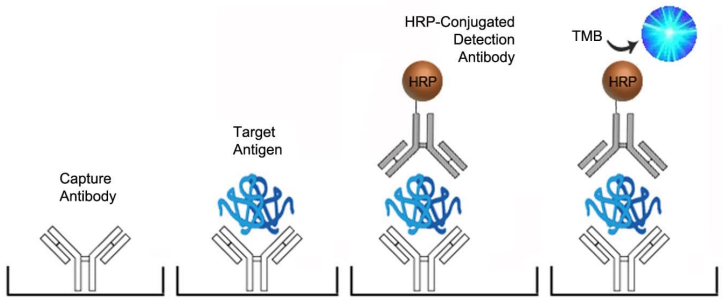
Fluidigm Biomark microfluidic qPCR readout

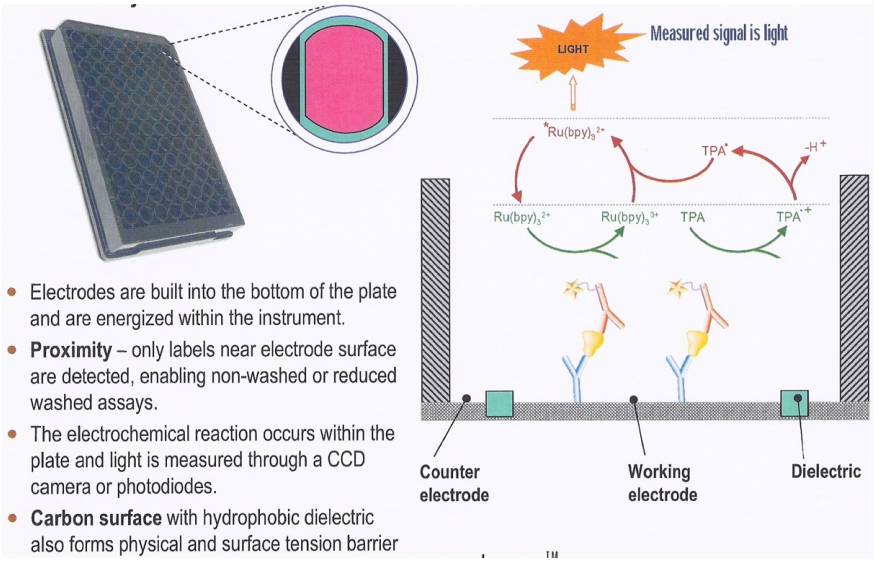


- Load samples on one side, reaction mix on the other
- All combinational assays are carried out in nanofluidic chambers (48x48 or 96x96)
- Minimizes reagent use and pipetting

Other multiplexed immunoassay platforms

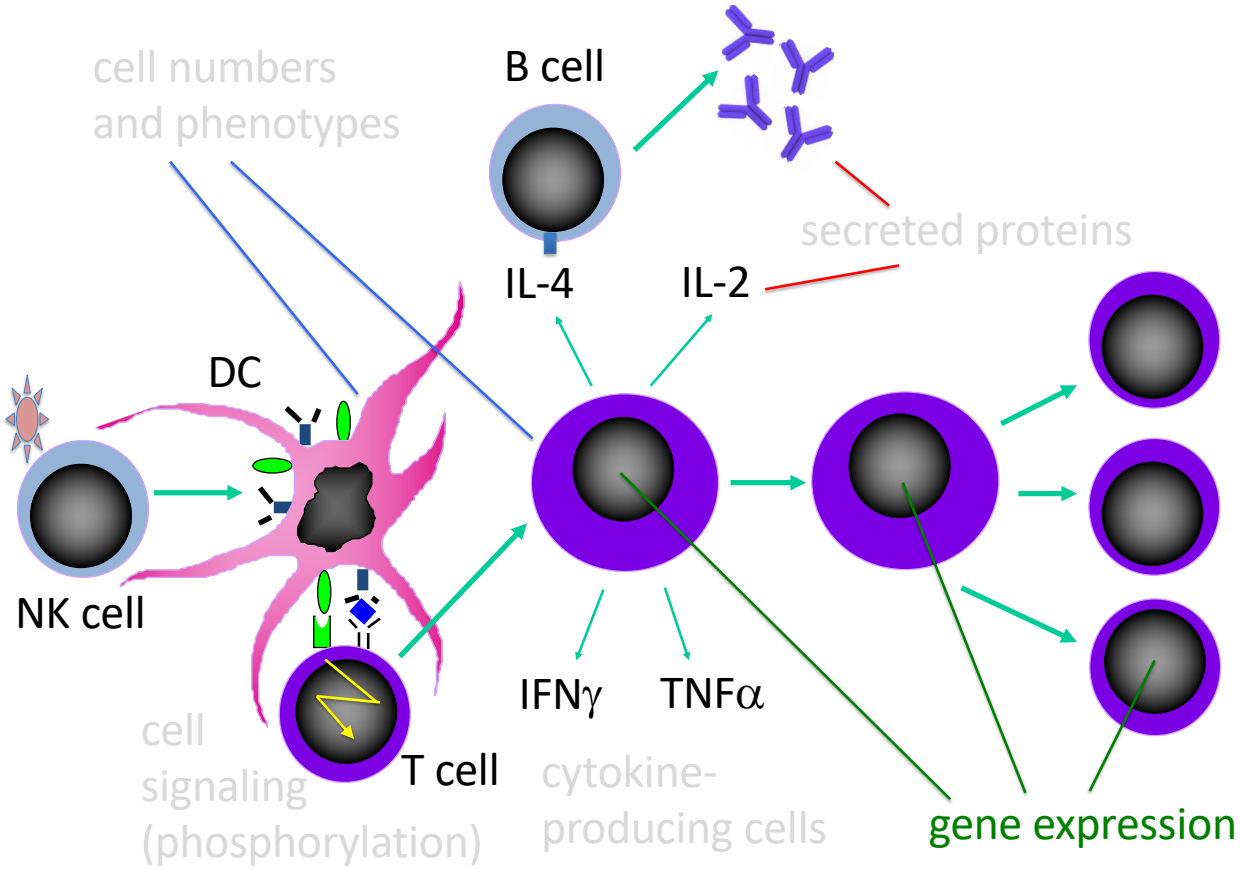
- Olink Explore: NGS readout of 3072 proteins
- SomaLogic SomaScan: Aptamer-based array for up to 7000 proteins
- MesoScale Discovery: Electrochemiluminescence detection of up to 10 protein spots/well
- Quanterix Simoa: Single-molecule detection of 1-10 proteins
- Others on the horizon





- Electrodes are built into the bottom of the plate and are energized within the instrument.
- **Proximity** – only labels near electrode surface are detected, enabling non-washed or reduced washed assays.
- The electrochemical reaction occurs within the plate and light is measured through a CCD camera or photodiodes.
- **Carbon surface** with hydrophobic dielectric also forms physical and surface tension barrier

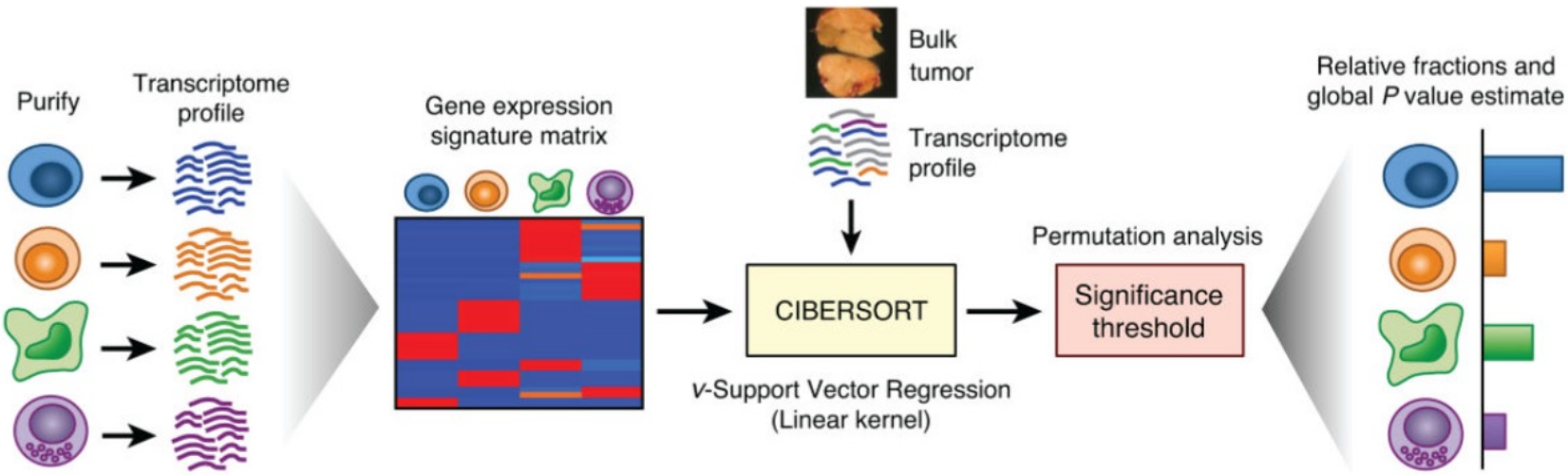
What to measure in the immune system



Single-cell vs. bulk transcriptomics

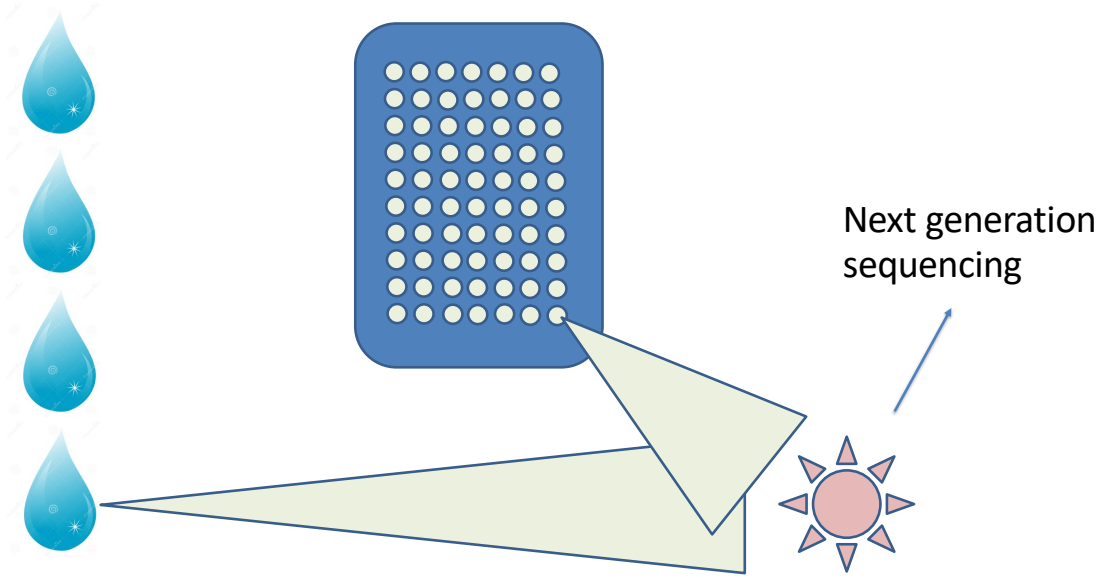
Bulk transcriptome data can be deconvoluted by cell type

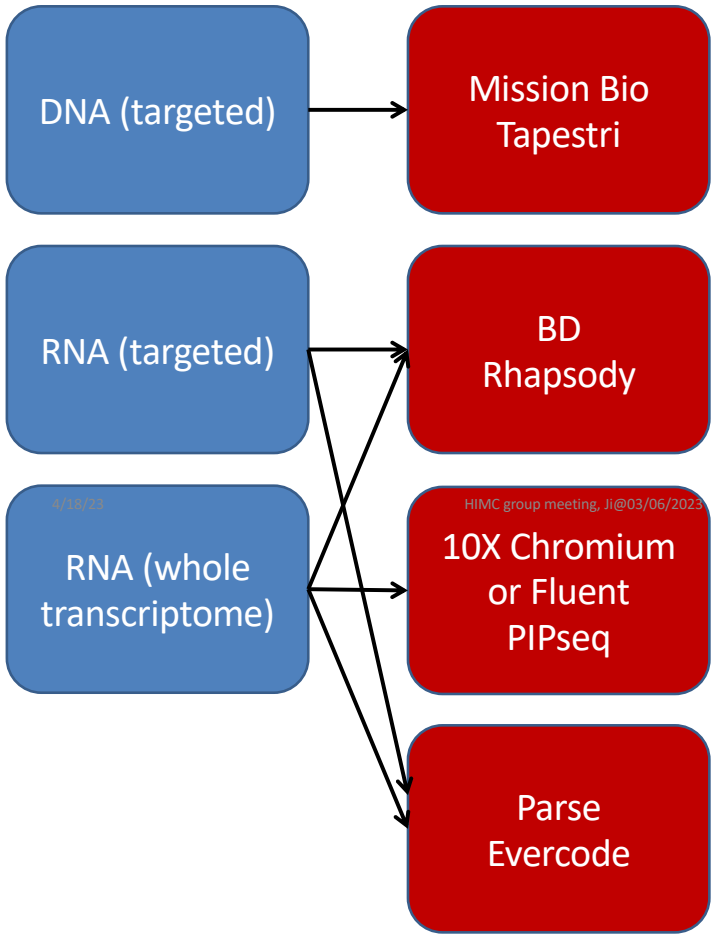
Cibersort (Newman et al., Nat Methods 2015)



Single-cell RNAseq methods

May use droplets (10X) or microwells (BD Rhapsody) to isolate single cells
Use oligo-dT beads to capture mRNA transcripts from each cell

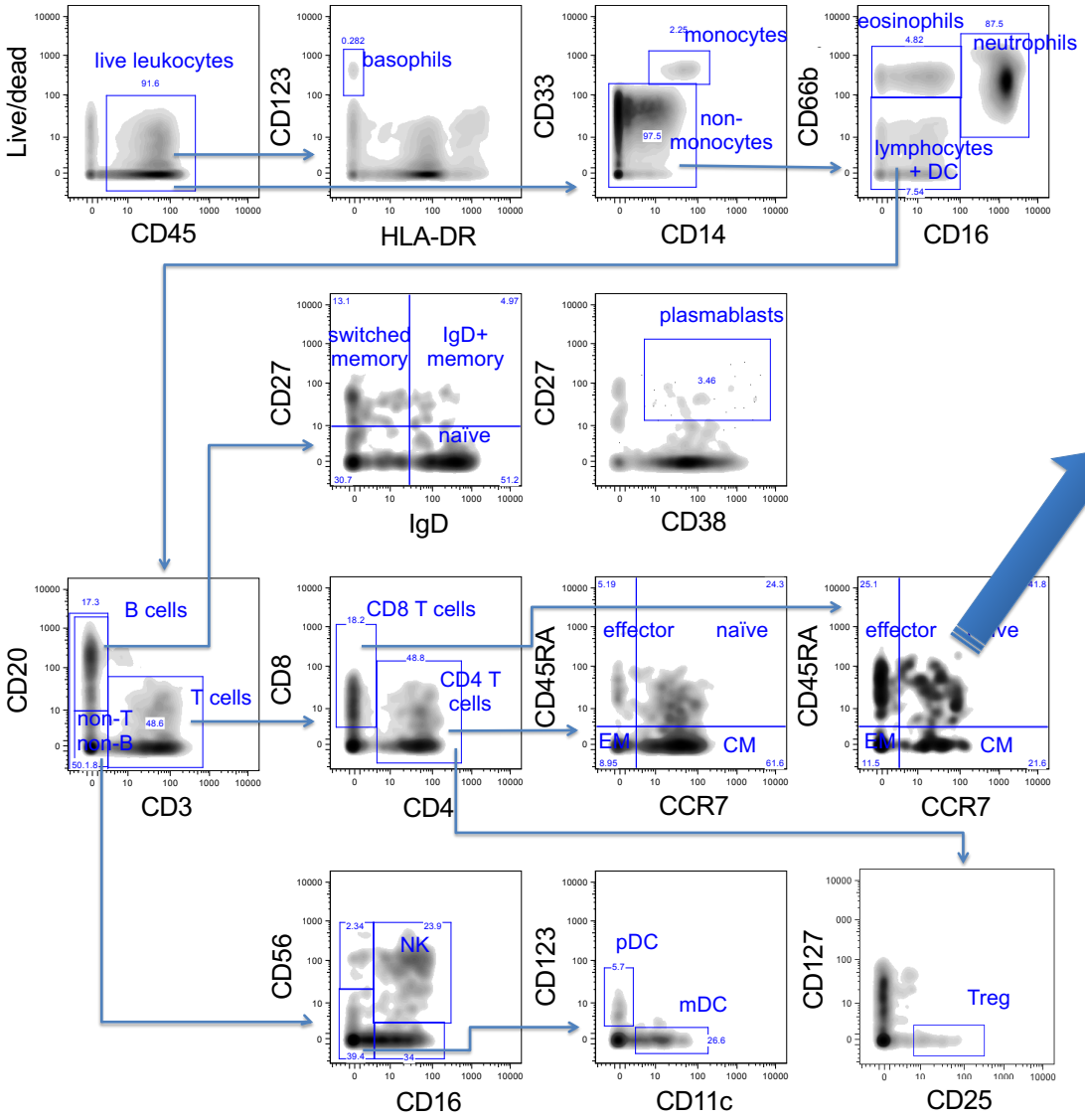


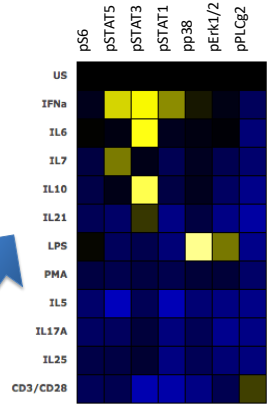


Primary analyte	Platform	AbSeq/ CITEseq add-on	BCR/TCR targeting add-on	Sample multiplexing	Cell input # (capture #)	Other features
DNA (targeted)	Mission Bio Tapestri	yes	no	no	~1M cells (~10K cells)	Droplet gel beads, good for assessing genetic heterogeneity
RNA (targeted)	BD Rhapsody	yes	TCR+BCR	yes	50K-500K cells (40K cells)	Magnetic beads+microwell cartridge, good for multiple small samples
RNA (whole transcriptome)	10X Chromium or Fluent PIPseq	yes	yes	yes	10K-500K cells (2K-200K cells)	Droplet gel beads, scalable cell #'s
	Parse Evercode	no	TCR	yes	200K-10M cells (2K-1M cells)	Can fix cells for later capture, cells barcoded by sample splitting, good for high cell #s

In vitro stimulation readouts

- CyTOF or high-parameter flow cytometry
  - Intracellular cytokines
  - Phospho-proteins
- Multiplexed immunoassays (Luminex, Olink)
  - Stimulated cytokines
- Gene expression profiling (RNAseq)
  - Gene expression changes from baseline

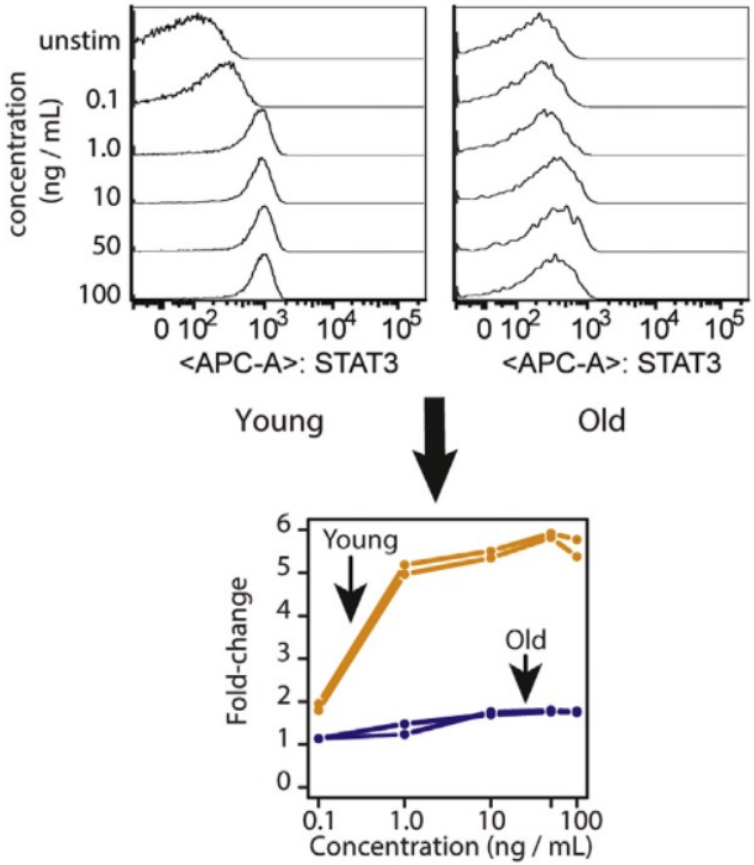


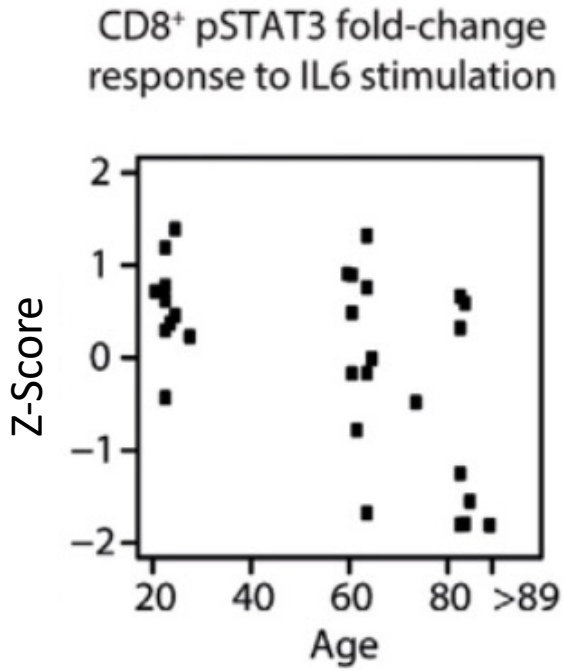


32 cell subsets
x 12 stimuli
x 8 phosphoepitopes

= 3072 data points

Use of phospho-signaling assay in aging study

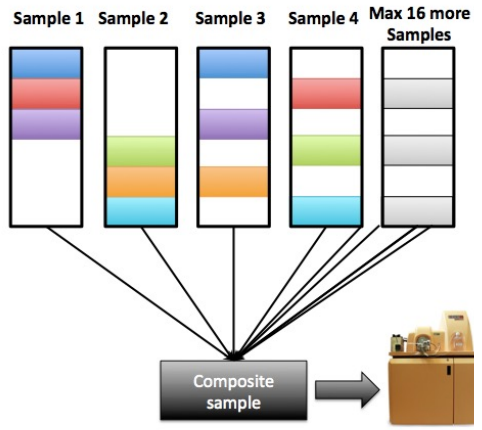




Shen-Orr et al., Cell Systems 2016

How to analyze high-dimensional data: First steps

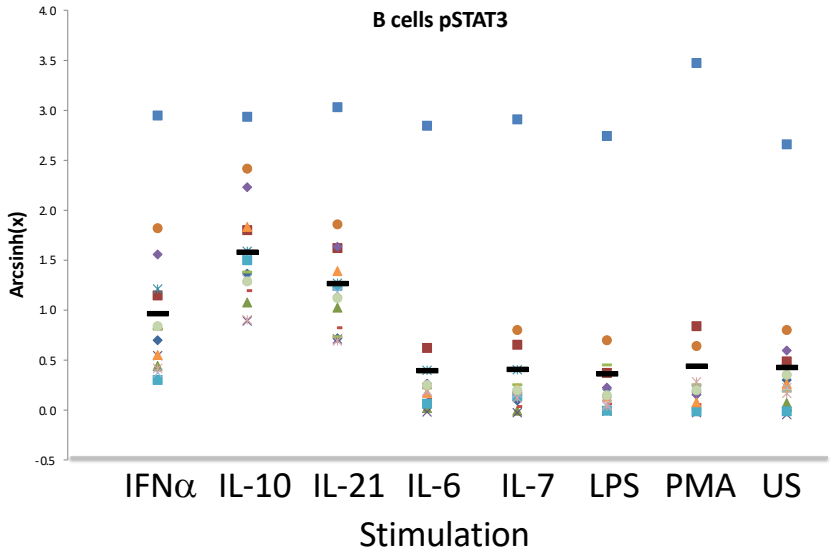
- Data pre-processing:
  - Intensity normalization via beads (CyTOF)
  - Sample de-multiplexing (for barcoded samples)
  - Initial gating to live, intact, single cells
- Data quality control:
  - Manual gates to check marker performance
  - Time gating
  - Check for adequate number of cells collected
  - Comparison of batch controls, batch correction if needed



Batch controls and batch correction

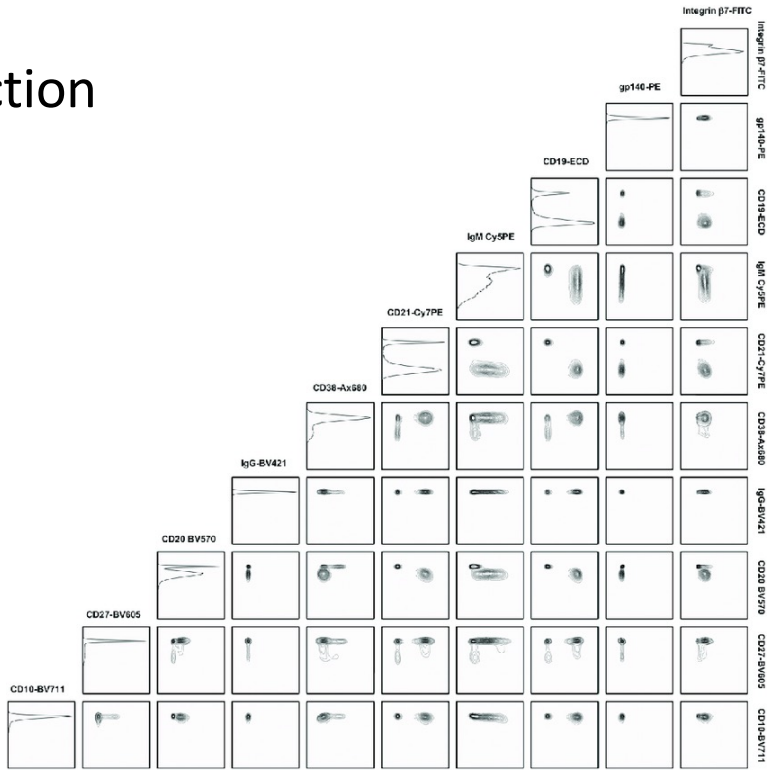
- Comparing batches:
  - Shared control (subject to artifacts)
  - All samples (need large, balanced batches)
- Dealing with outlier batches:
  - Discard or repeat outlier batch(es)
  - Perform batch correction, e.g., CyCombine:

https://biosurf.org/cyCombine_CyTOF_1panel.html



How to analyze high-dimensional data

- Visualization: dimensionality reduction
- Clustering algorithms
- Statistical techniques



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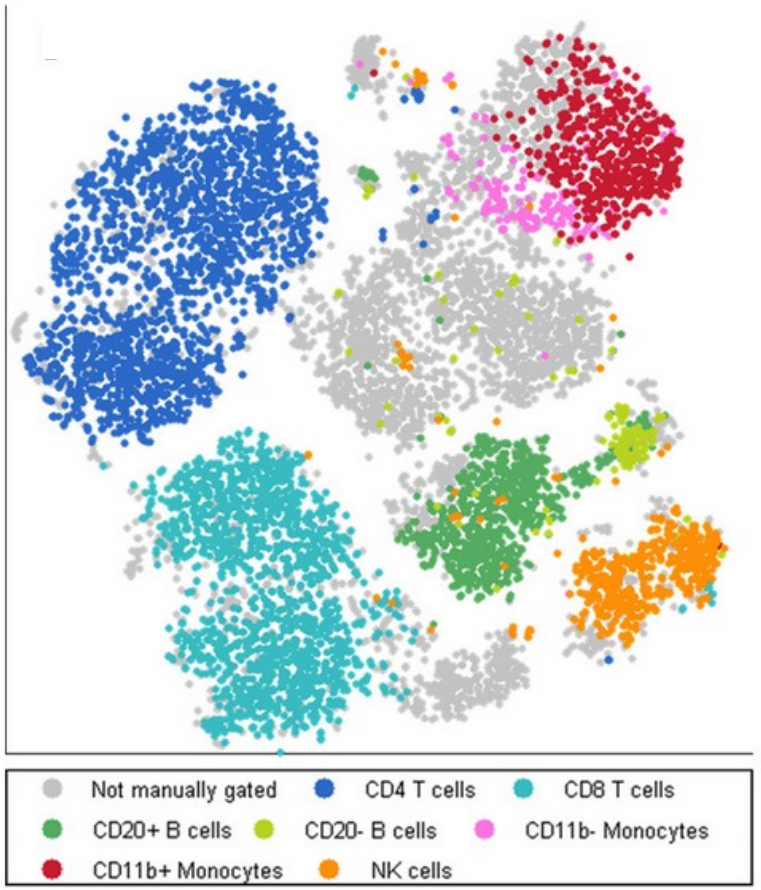
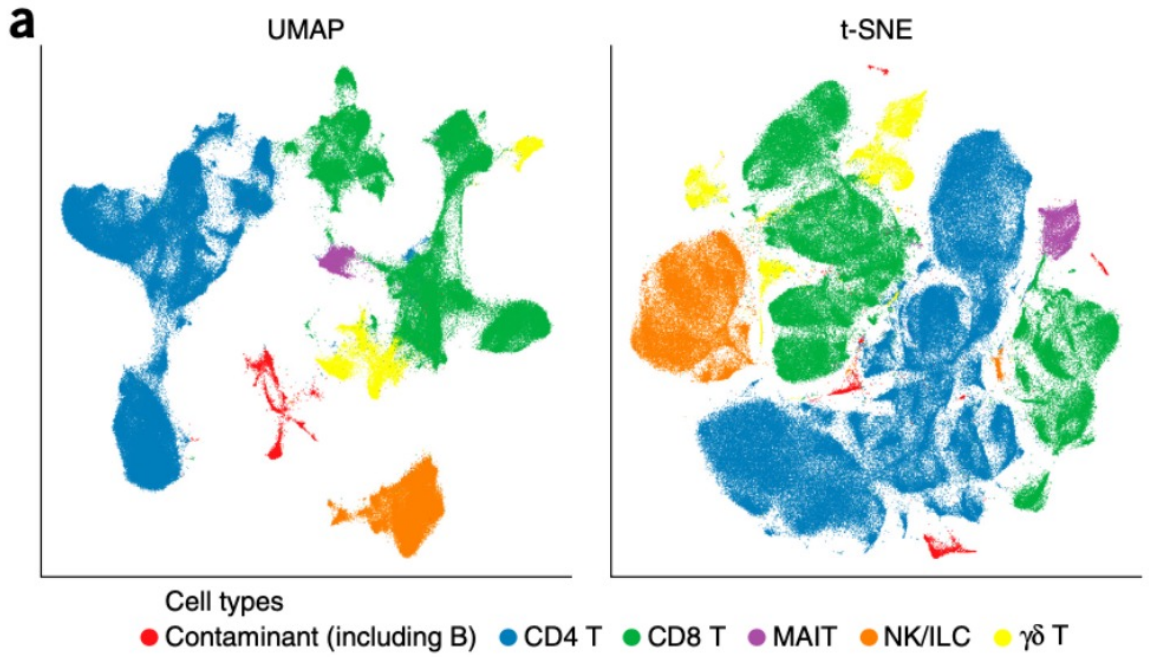
viSNE (tSNE)

Amir et al., Nature Biotechnology, 2013

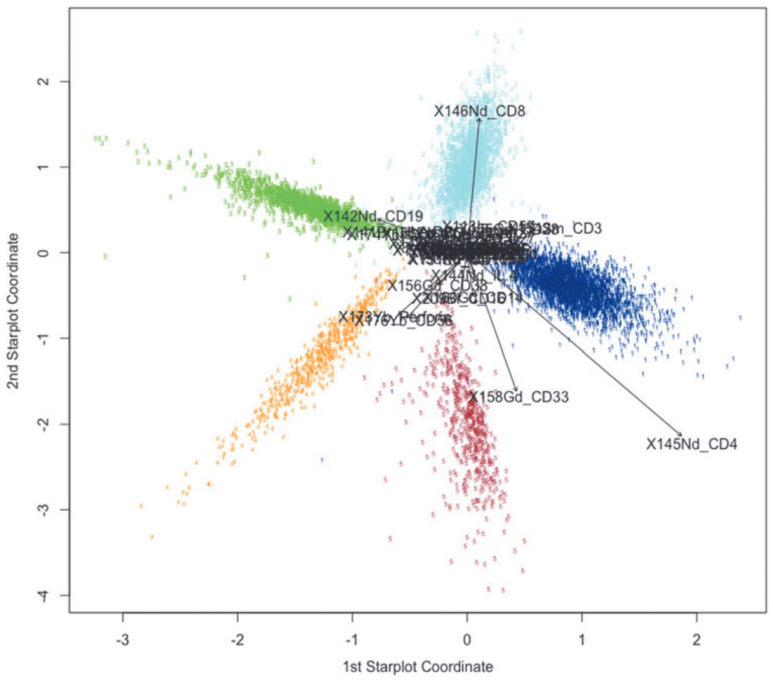


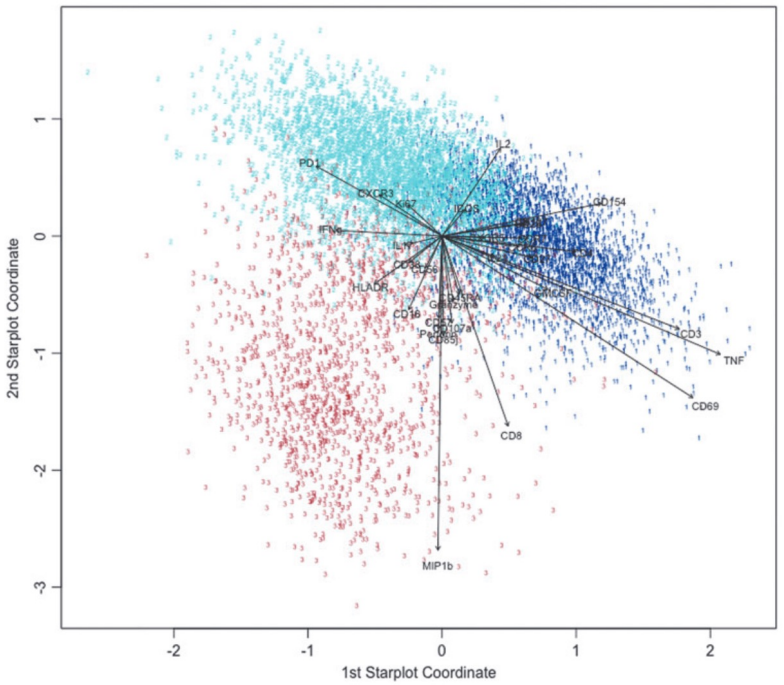
UMAP



Becht et al., Nature Biotechnology, 2019

Starplots separate pre-defined clusters





Holmes et al., Viral Immunology 2019

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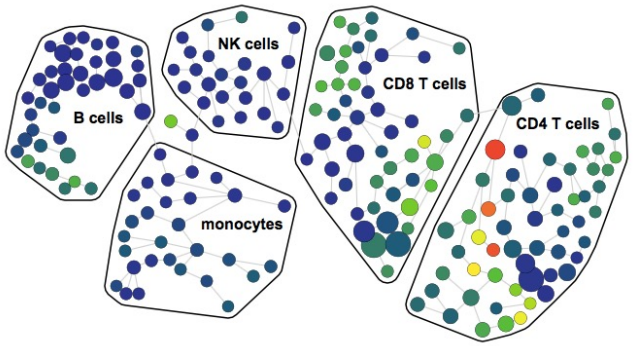
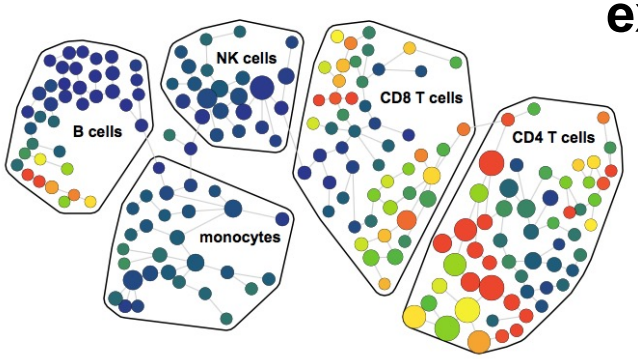
SPADE analysis of melanoma patients prior to immunotherapy

Non-responder



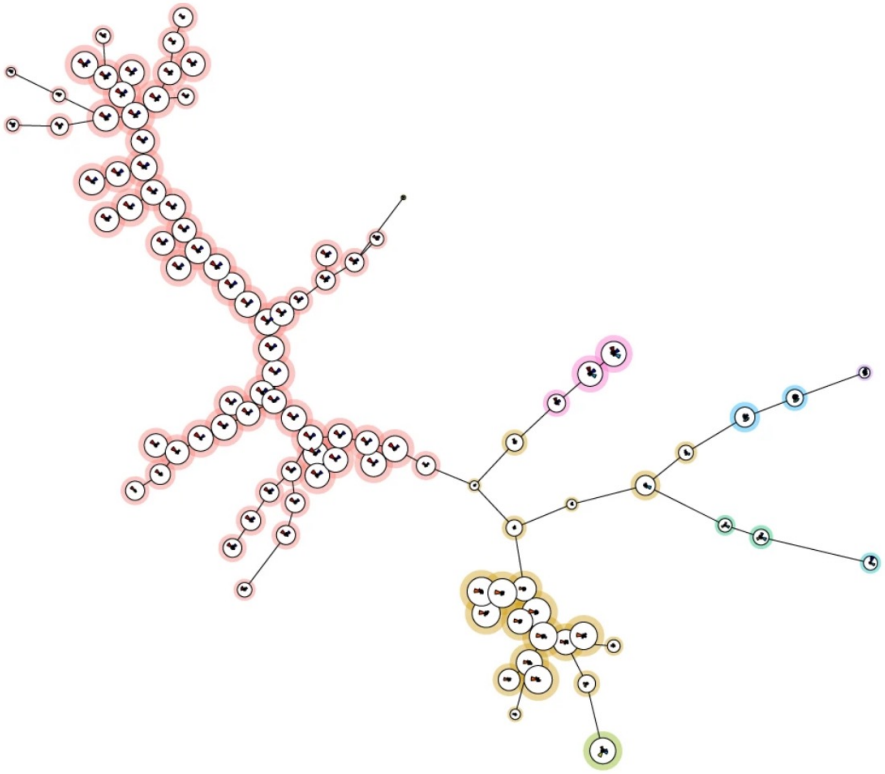
IL-2 expression

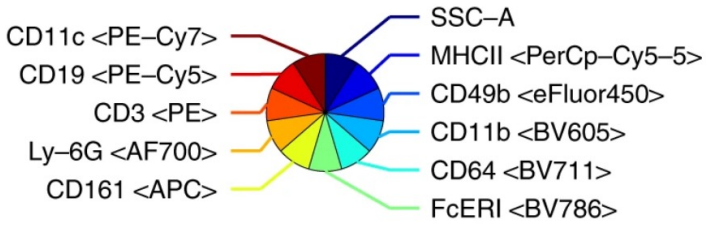
Responder



Hiniker et al.,
Int J Radiat Oncol Biol Phys 2016

FlowSOM

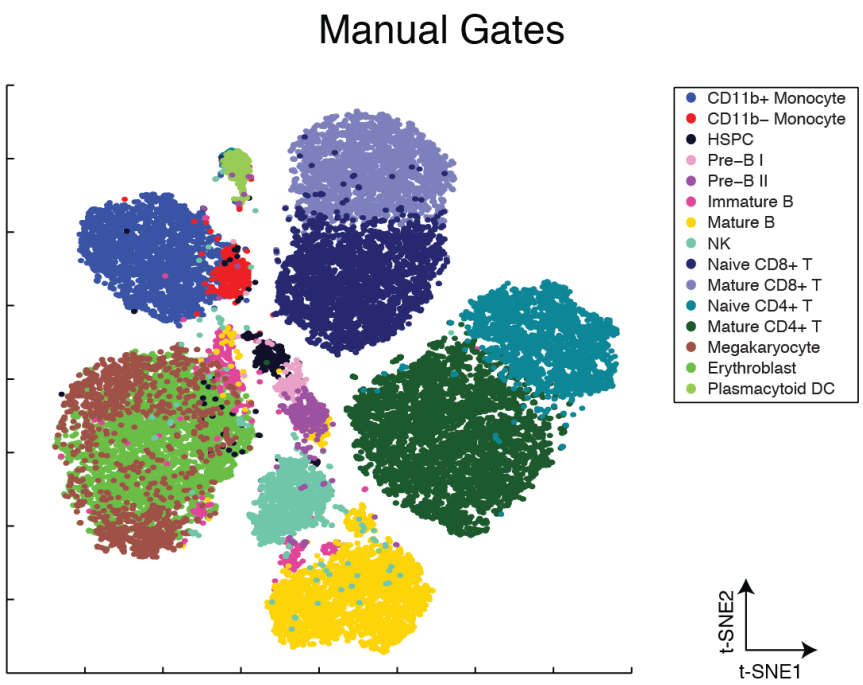




Quintelier et al., Nature Protocols 2021

Clusters are shown as circles with star plots displaying the cluster median marker intensities. The background coloring represents the meta-clustering. Legends of the star plot and meta-clustering are shown on the right side.

Phenograph



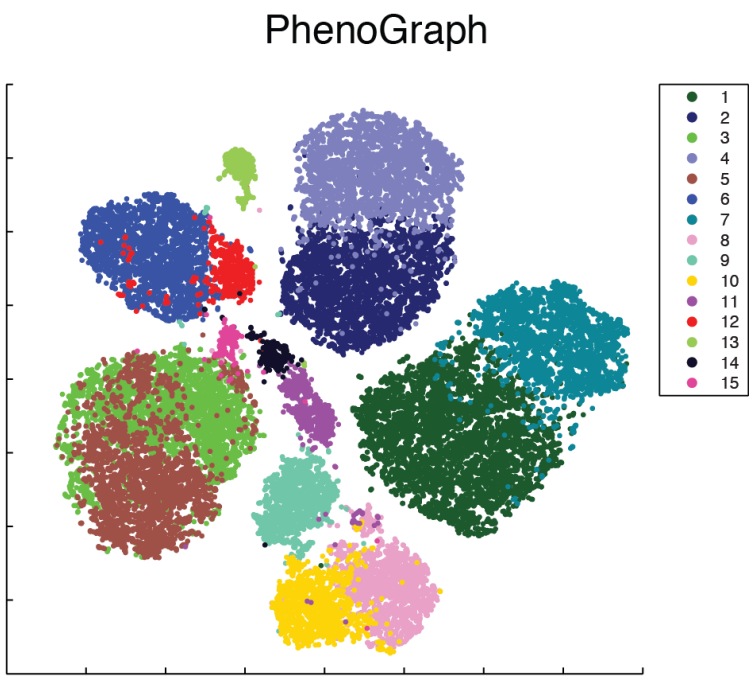


Image from Dana Pe’er lab

https://pypi.org/project/PhenoGraph/

How to analyze high-dimensional data

- Visualization: dimensionality reduction
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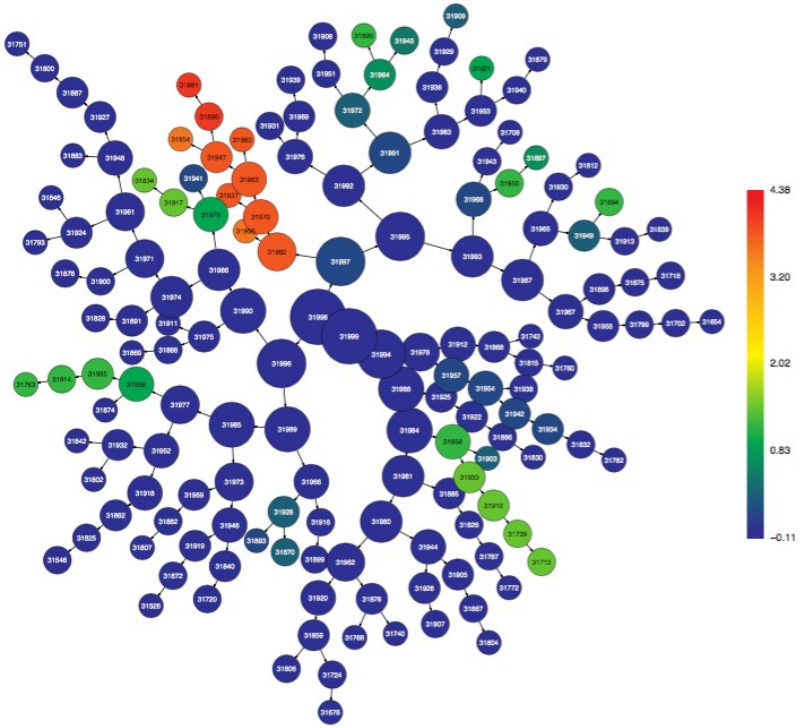
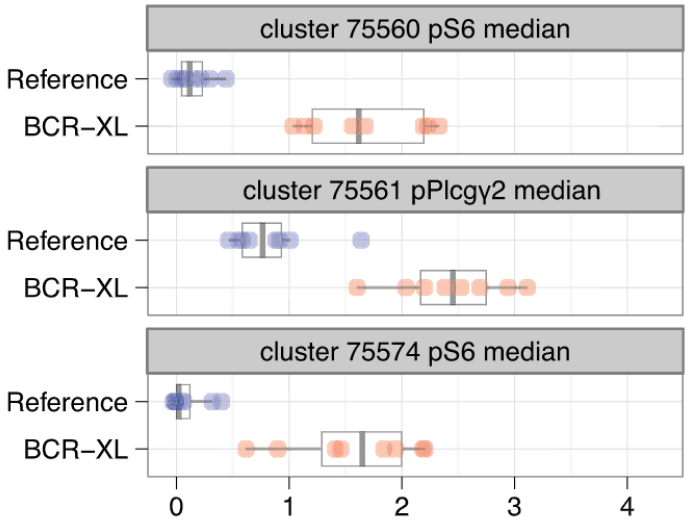
- Statistical techniques

Citrus



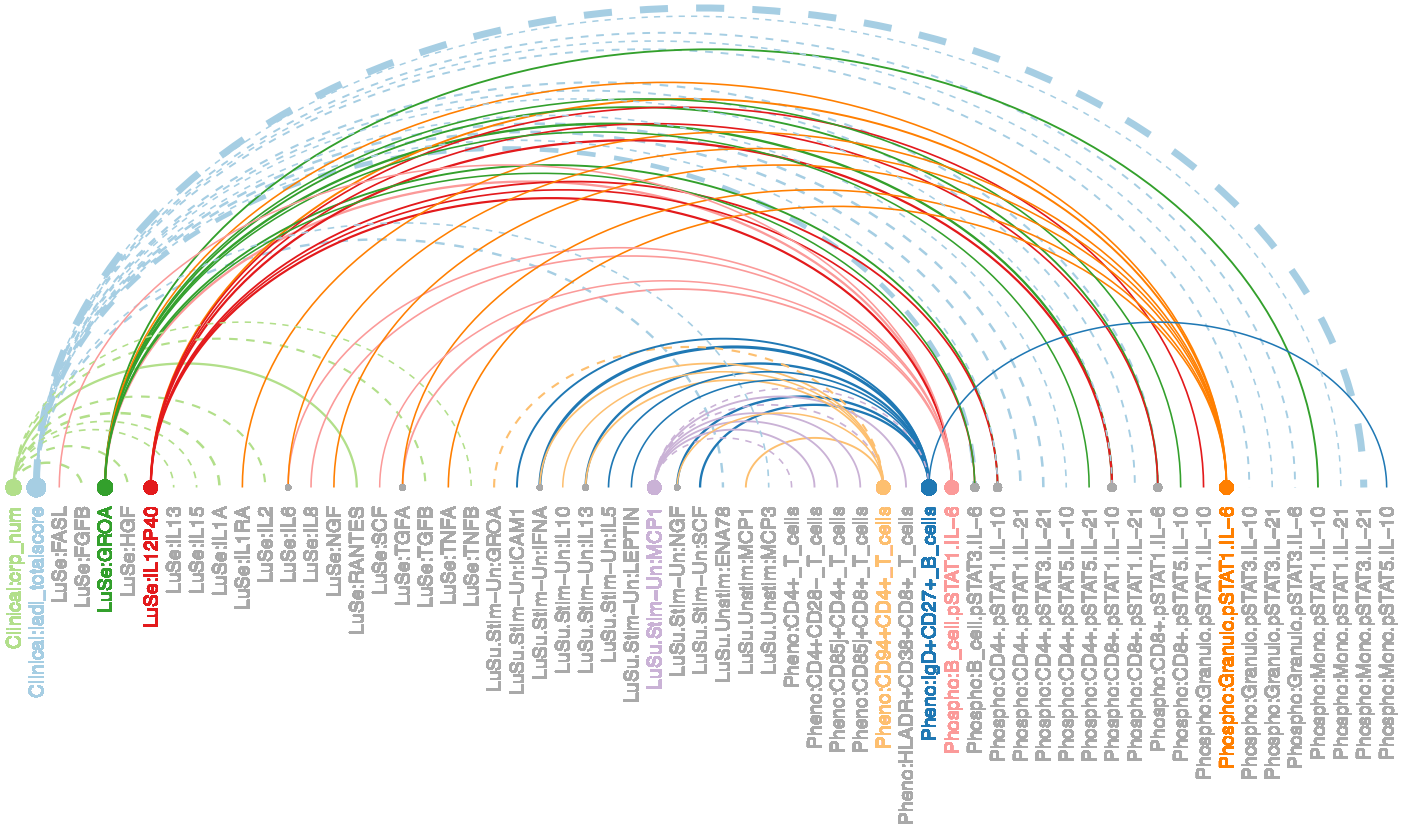
Bruggner et al.,
PNAS 2014



Statistical models:

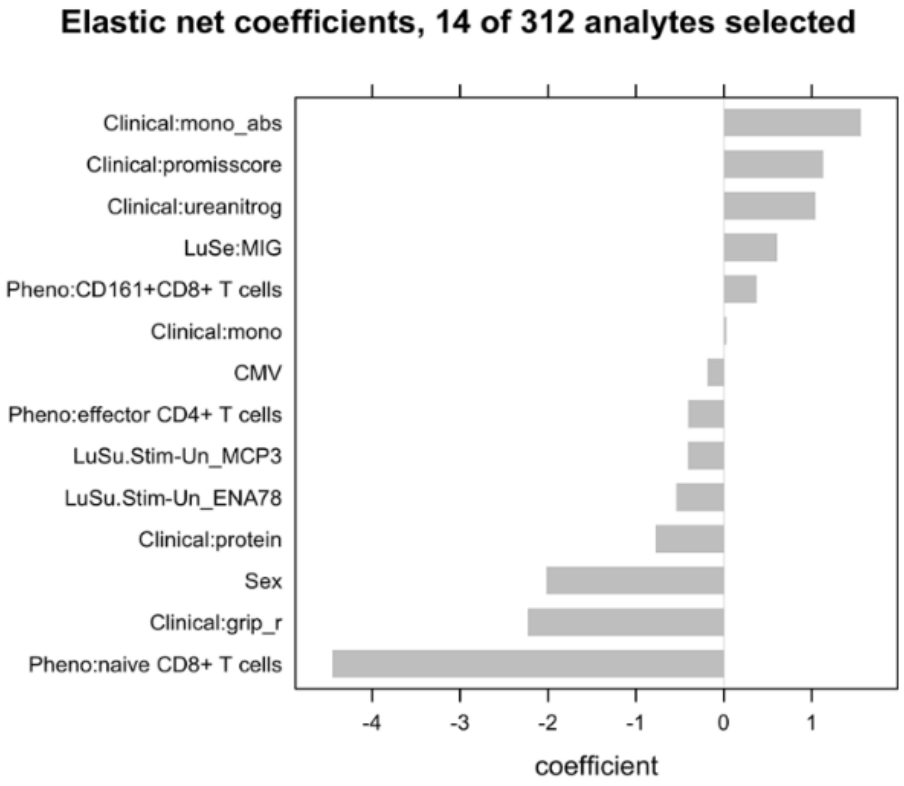
- SAM
- PAMR
- Glmnet

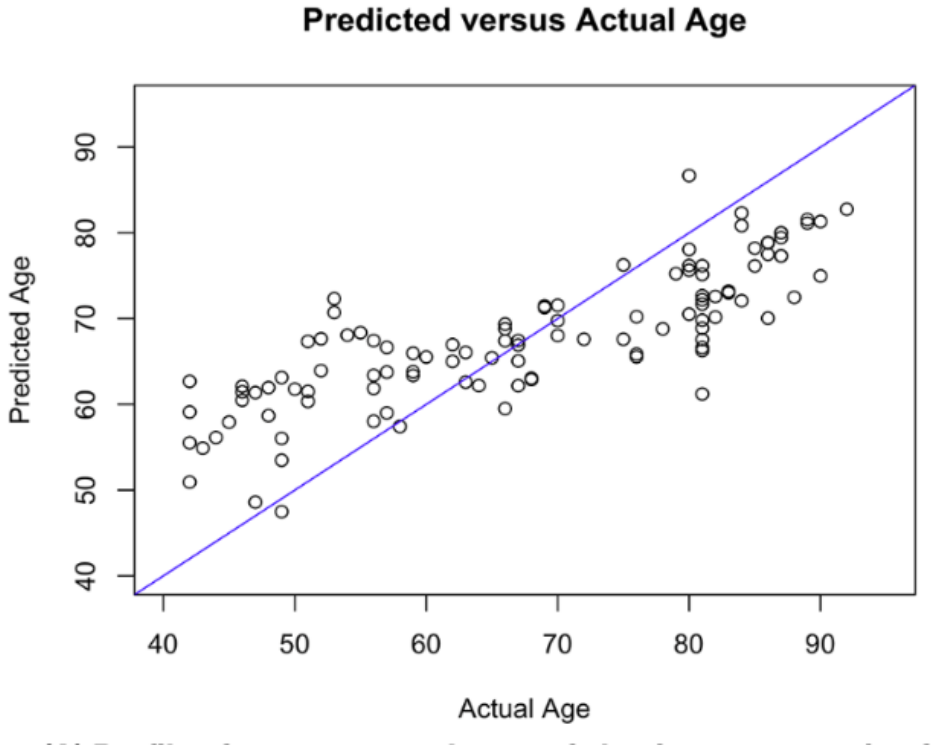
Integrating Multiomic Data via Machine Learning and Visualization via an Arc Diagram



Whiting et al.,
PLoS One 2015

Integrating Multiomic Data via Multivariate Model



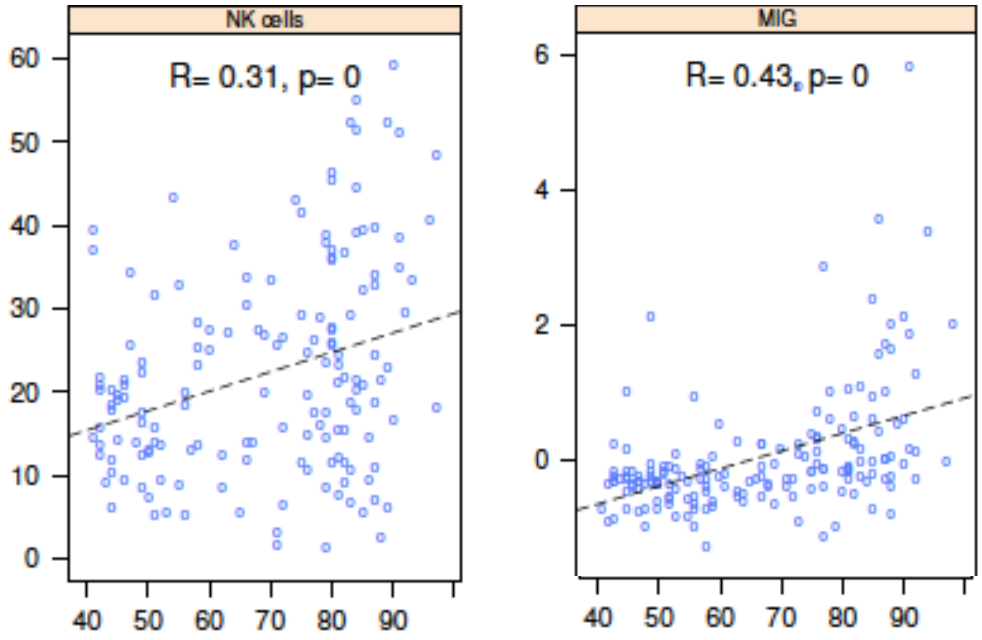


Whiting et al., PLoS One 2015

Defining “Normal” in the face of biological heterogeneity

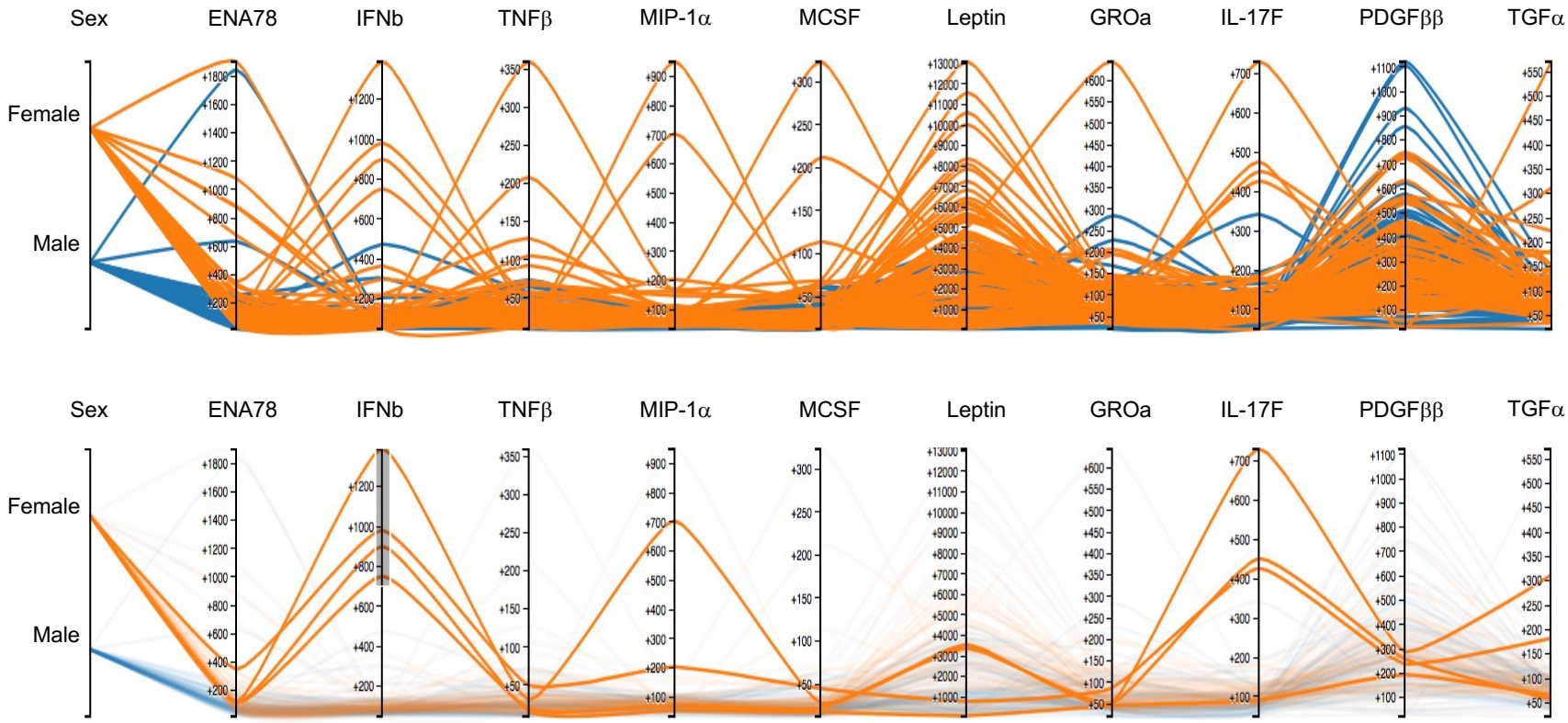
- Getting enough samples
- Looking at patterns vs. individual readouts
- Using each person as their own control

Examples of trends with age



Whiting et al., PLoS One 2015

Patterns in serum cytokine outliers



http://earlybird.cytoanalytics.com

SUMMARY

- Systems Biology is useful to studying immune safety, as it seeks to examine the immune system comprehensively
- Common assays for systems analysis include:
  - Immune phenotyping, e.g., CyTOF or multiparameter flow cytometry
  - Secreted protein analysis, e.g., Luminex or Olink
  - RNAseq, either at the bulk or single-cell level
- Functional readouts can be applied via in vitro stimulation for a more informative output compared to the resting state
- Analysis of high-dimensional data requires pre-processing and quality control, and benefits from algorithms to visualize, cluster, and statistically analyze large data sets
- Integrative analysis across assays can be aided by machine learning, multivariate modeling