

# Johns Hopkins Engineering

## Immunoengineering

**Immunoengineering - Immunoprofiling**

**Current tools**



JOHNS HOPKINS  
WHITING SCHOOL  
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# Learning Objectives

- Describe major methods and tools currently used primarily by immunologists
- Explain why single cell biology can transform mechanistic understanding of immunology
- Design microfluidic immune organ models
- Describe how new imaging techniques are overcoming limitations in current imaging systems
- Apply immunological assays to understand key features of the immune response
- Explain the importance of systems approach to immunology
- Compare different data analytic and display techniques
- Apply principle component analysis to a simple multivariate data set

# What might an Immunologist want to know?

- How cells communicate
- How cells are functioning, responding, or changing
- Where cells are going
- How cells are different

# What might an Immunologist want to know?

- How cells communicate
- How cells are functioning, responding, or changing
- Where cells are going
- How cells are different
- Cytokines
- Surface Ligands
- Protein Expression
- Signaling pathways
- mRNA expression

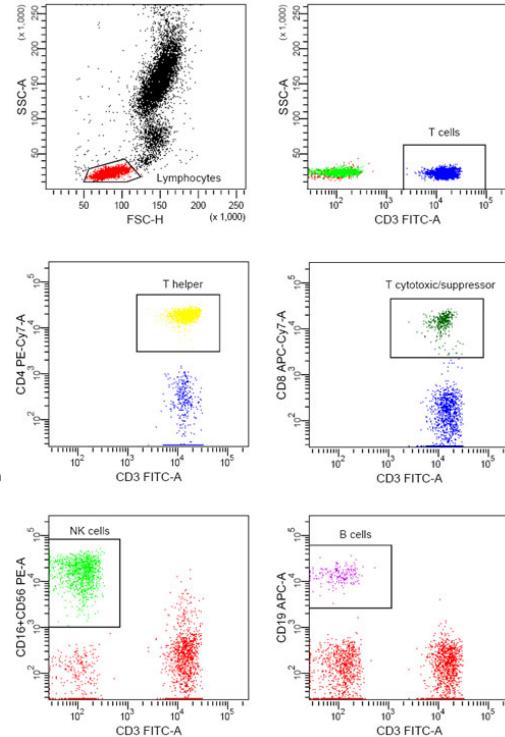
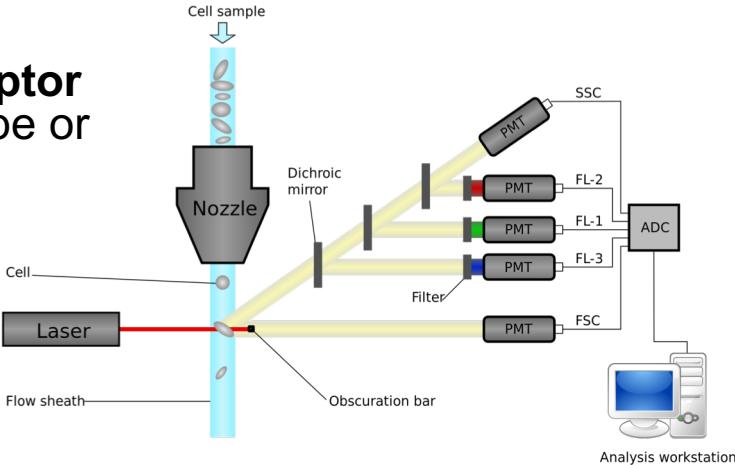
# How does an immunologist find this information?

- Flow/Mass Cytometry
- Microscopy
- ELISA/ELISPOT
- Western Blots
- Immunoprecipitation
- Sequencing

- How it works
- Advantages & questions you can ask
- Disadvantages
- Examples of engineering principles into development

# Flow/Mass Cytometry

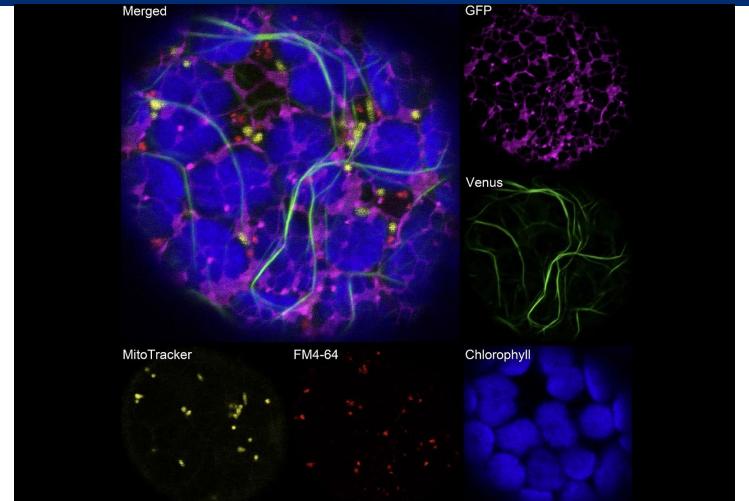
- Fluorescent/metal isotope antibody to **protein** or **receptor**
  - Cells are “stained” for type or state
- Advantages:
  - $10^3$ - $10^8$  single cell
  - Up to  $10^5$  cell/sec
  - Up to 16-40 parameters
  - Sortable
- Challenges:
  - $10^3$ - $10^8$  single cell with 40 parameters
  - Cross-talk in channels or antibodies
  - Expensive



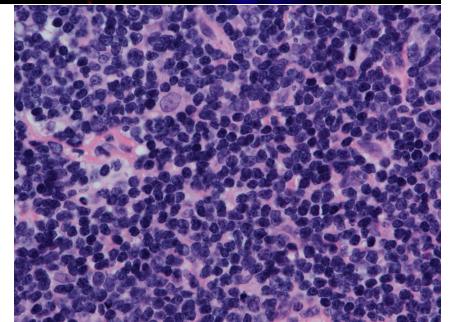
Example: Sort  
“activated” T cells from  
tumor to identify  
tumor-specific T cells

# Microscopy

- Fluorescent antibody to **protein** or **receptor** or genetic incorporation
  - Cells are “stained” for type or state
- Advantages:
  - Spatial information – 2D/3D
  - Structural information
- Challenges:
  - Not quantitative
  - Not multiparametric – 4
  - Photobleaching



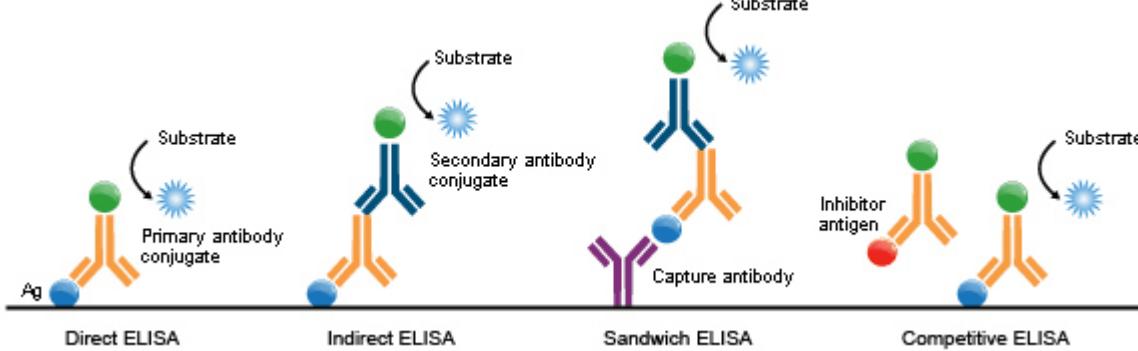
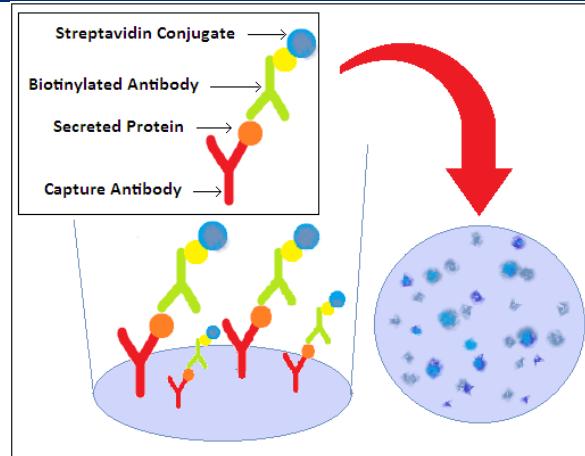
Example: Stain T cells to see how signaling proteins are organized at different stages



# ELISA/ELISPOT

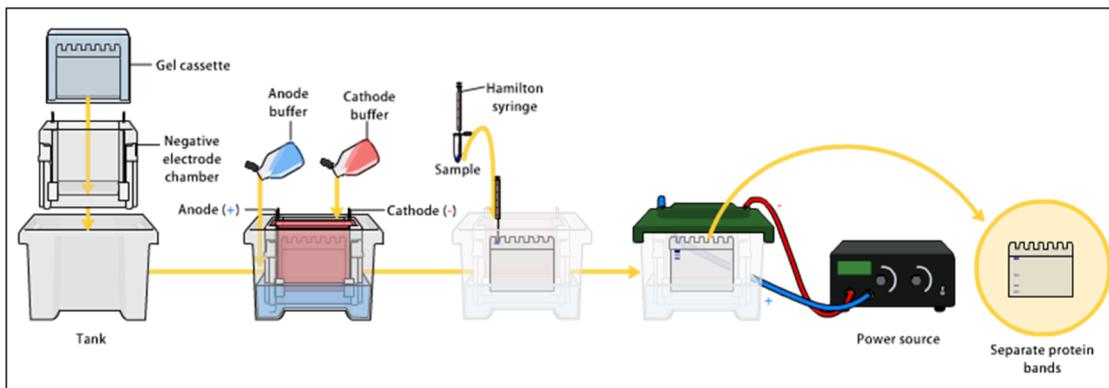
- Enzyme-linked antibody to **protein**
  - Total protein quantified
- Advantages:
  - Low sample requirements
  - Quantitation
  - Useful for binary detection in a mixed soup of proteins
  - High throughput
- Challenges:
  - Not multiparametric
  - Need for multiple reactive antibodies – Expensive
  - May give false positive

Example: Test the amount of cytokines in the blood following new immunotherapy



# Western Blot

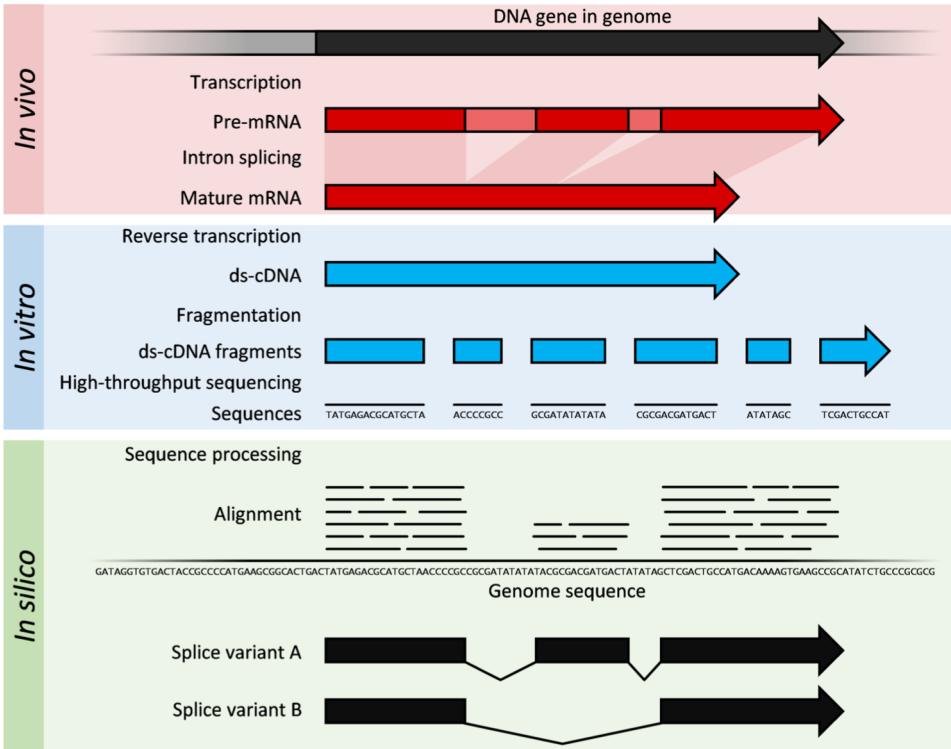
- Antibody to **protein** and charge
  - Move protein down gel
- Advantages:
  - Increased specificity – low signal to noise ratio
  - Provide size of protein - Useful for confirmation or exploratory research
  - Only need one antibody
- Challenges:
  - Not multiparametric
  - Not as quantitative
  - Labor intensive



Example: Examine levels of phosphorylation of signaling proteins in regards to new autoimmune B cell activation pathway

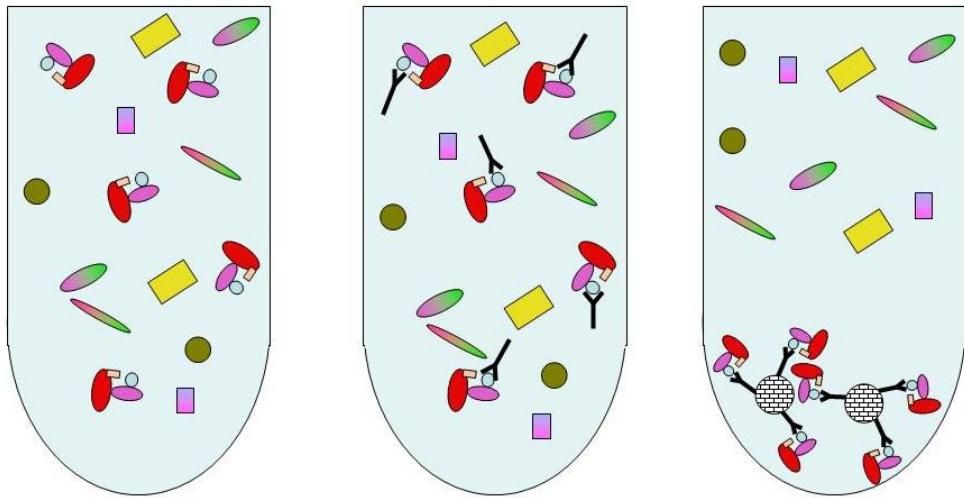
# Sequencing

- mRNA transcription and amplification
  - Advantages:
    - Sensitivity
    - Multiparametric – can do many genes at once
    - Provides clues to function and state of cells
    - Quantitative
  - Challenges:
    - Expensive
    - Not single cell
    - Large data sets
- Example: Sequence macrophages from diabetic wound site to see signature of anti-wound healing response



# Immunoprecipitation

- Antibody tagging **protein, protein complex, RNA or DNA** isolation
- Advantages:
  - Reduce signal to noise
- Challenges:
  - Efficiency



Example: Isolating part of the DNA that a novel transcription factor is effecting in response to HIV infection in CD4+ T cells

# General Challenges With All Assays

- Single time point measurement
- Destructive to sample
- One dimensional in properties assessed

# Rising Immunology Profiling Tools



Products

Diagnostics

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Log In

## Vantage 3D™ Immuno-Oncology Assays

Quantify protein and gene expression in one experiment and gain deeper insight into your immuno-oncology research. Designed with protein content matched to the gene expression 770-plex PanCancer Immune Profiling Panel for characterization of up to 800 total targets, these assays enable more data from less sample.

- Detect 770 mRNA and up to 30 proteins from as few as 20,000 cells
- Content designed to profile immune cell type and functional state

10X GENOMICS

SOLUTIONS & PRODUCTS

RESEARCH & APPLICATIONS

EDUCATION & RESOURCES

SUPPORT

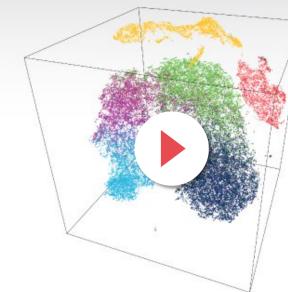
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### SINGLE CELL GENE EXPRESSION

## Massive Single Cell Profiling with an Easy-to-Use Workflow

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