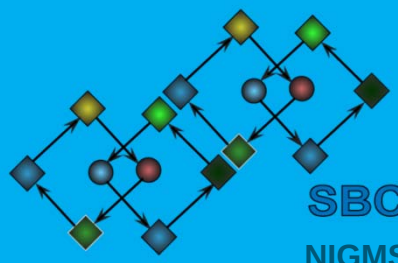


NIGMS funded Center

Experimental Methods in Systems Biology

Part of the Coursera Certificate in Systems Biology

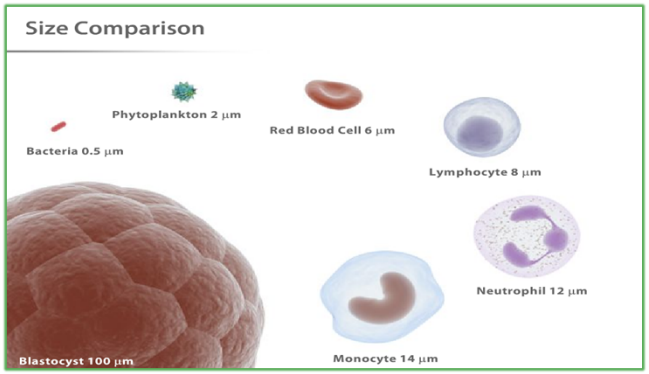
Marc Birtwistle, PhD
Department of Pharmacology & Systems Therapeutics
Fall 2014, Week 5 Flow and Mass Cytometry

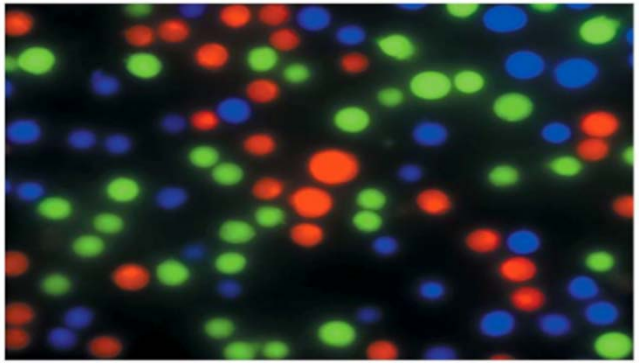
Icahn School of Medicine at Mount Sinai

Outline

- Flow Cytometry
  - How does it work?
  - What can you measure?
- Mass Cytometry
  - How does it work?
  - What can you measure?
- Analysis
  - Gating strategies
    - Debris vs. cells
    - Singlets vs. doublets
    - Automated gating strategies
  - Compensation—what to do when signals overlap
  - Quantification
    - DNA stains
    - Antibody stains

Flow Cytometry





- Flow cytometry is a technology for characterizing and sorting cells as they move in a fluid stream through laser beams
- Allows simultaneous quantitative measurements of multiple parameters on a single cell level in a heterogeneous cell population
- Allows quantitative measurements of cell frequencies

Flow Cytometers: Analyzers

BD FACS Calibur
2 lasers; 4 colors

BD FACS Canto
2 lasers; 6 colors
3 lasers; 8 colors

BD LSRII
5 lasers;
18 colors

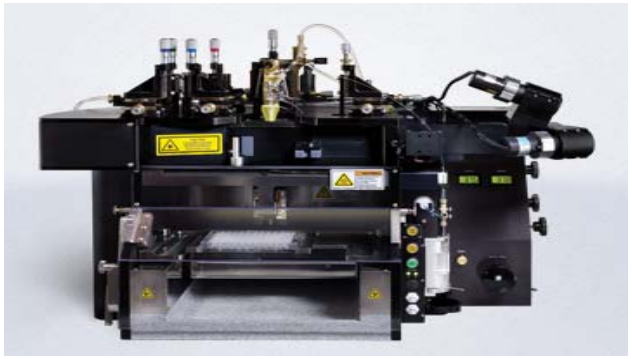
BD LSR
Fortessa
5 lasers;
18 colors

Mass Cytometer

DVS CyTOF2
No lasers; > 40 colors

Flow Cytometers: Cell sorters

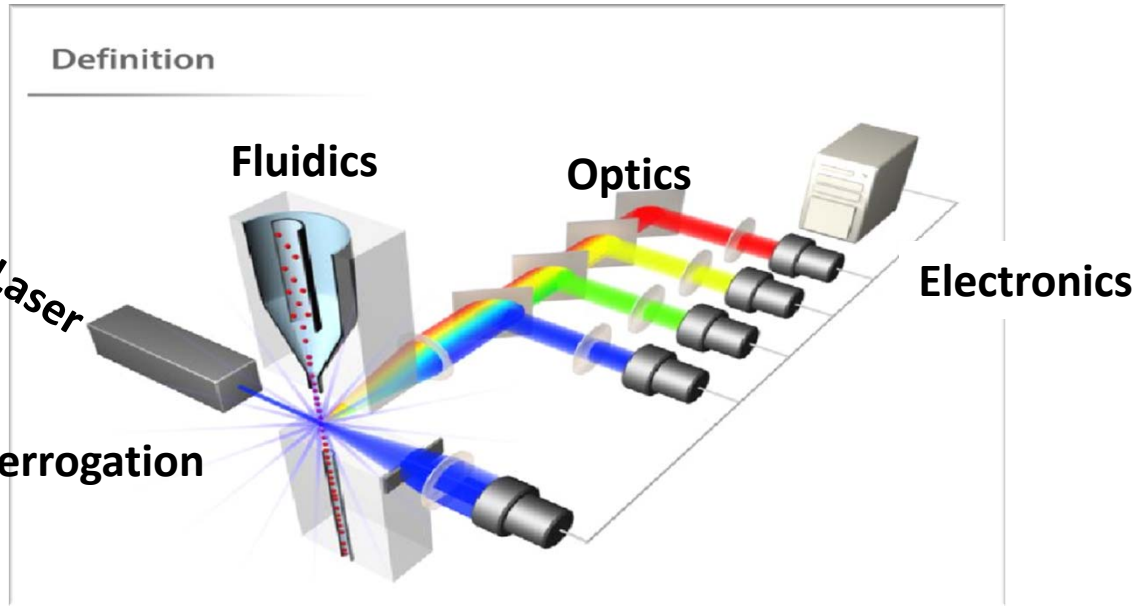
BD FACSAria II
3L, 4L & 5L
Up to 4-way sorting
Non-BSL and BSL2+



BD Influx
5L
Up to 6-way sorting
Non-BSL

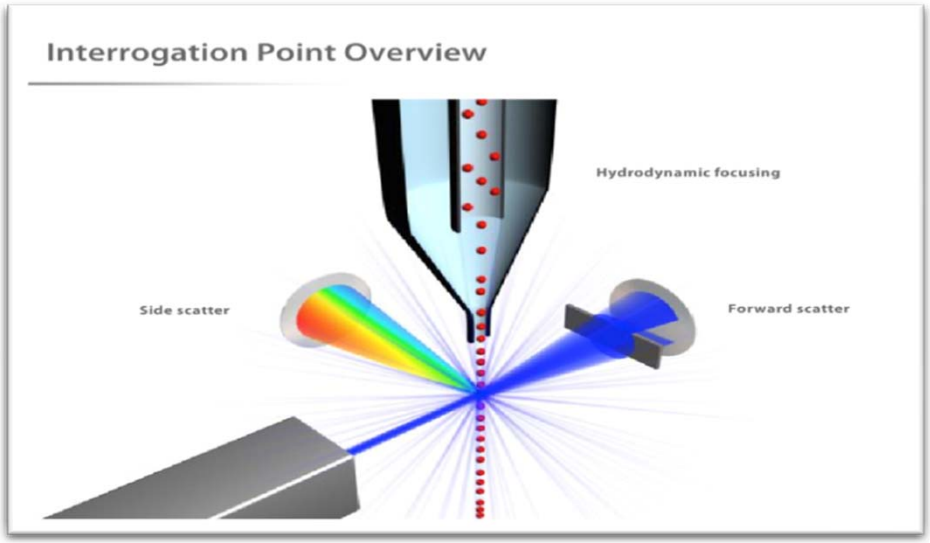
Flow Cytometer

Components & Principles

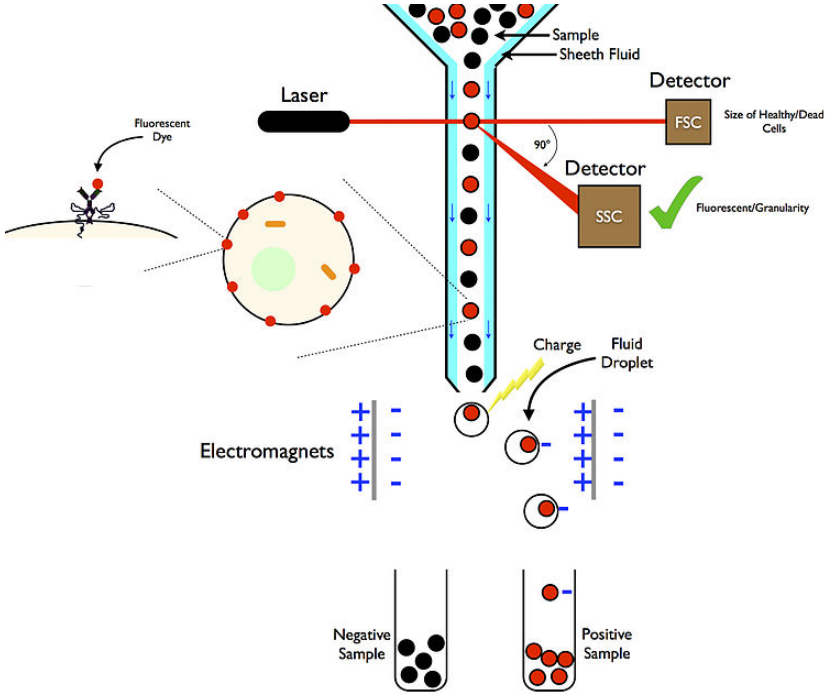


Liquid management: fluidics cart

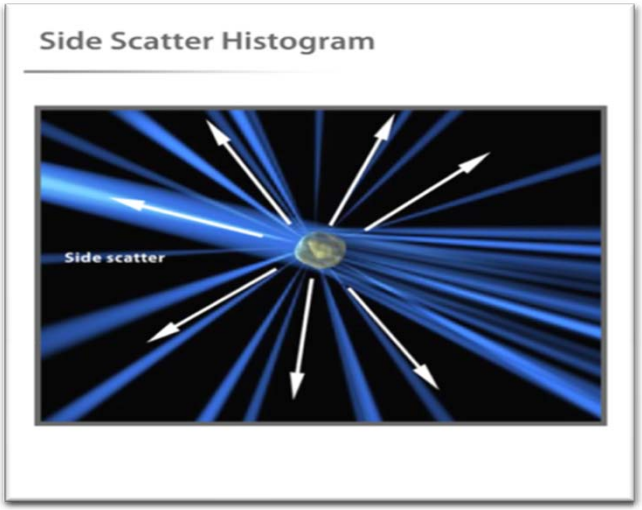
The Flow Cell: hydrodynamic focusing and interrogation

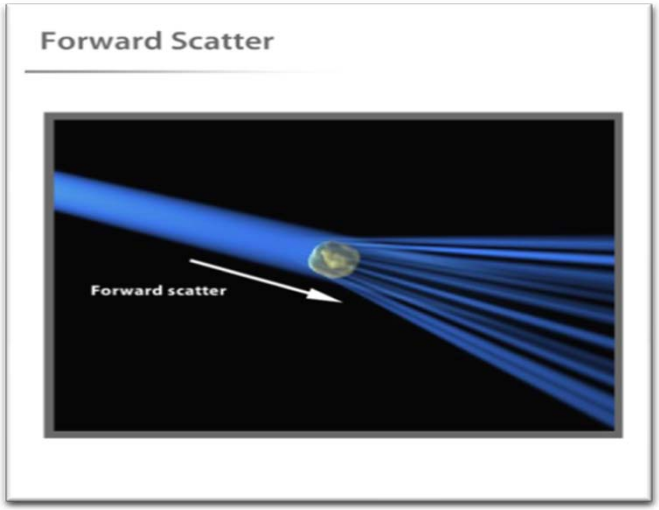


Fluorescence Activated Cell Sorting (FACS)

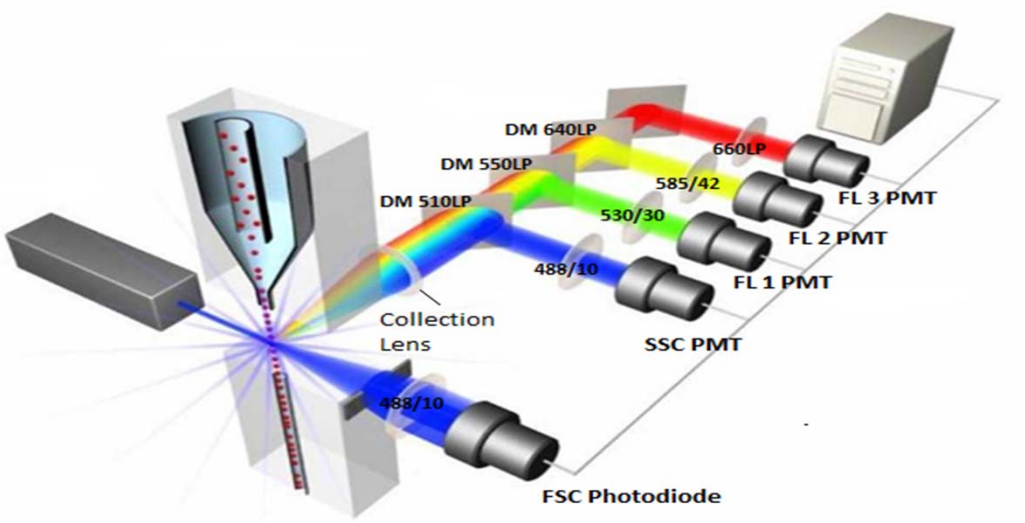


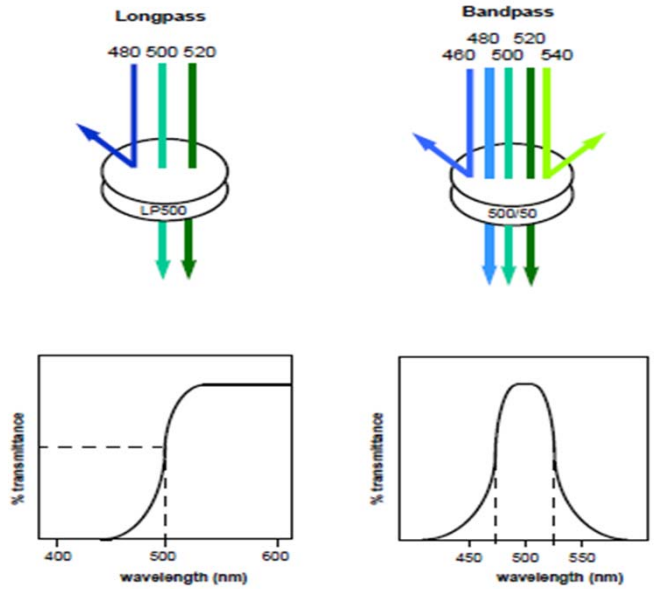
Light Scatter: FSC & SSC





Fluorescence Detection: Filters



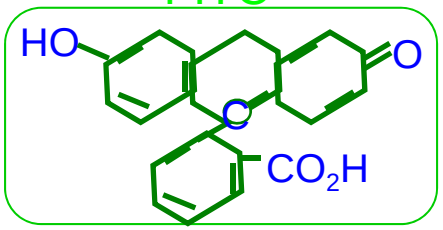


Example: Fluorescein (FITC)

λ = 488 nm

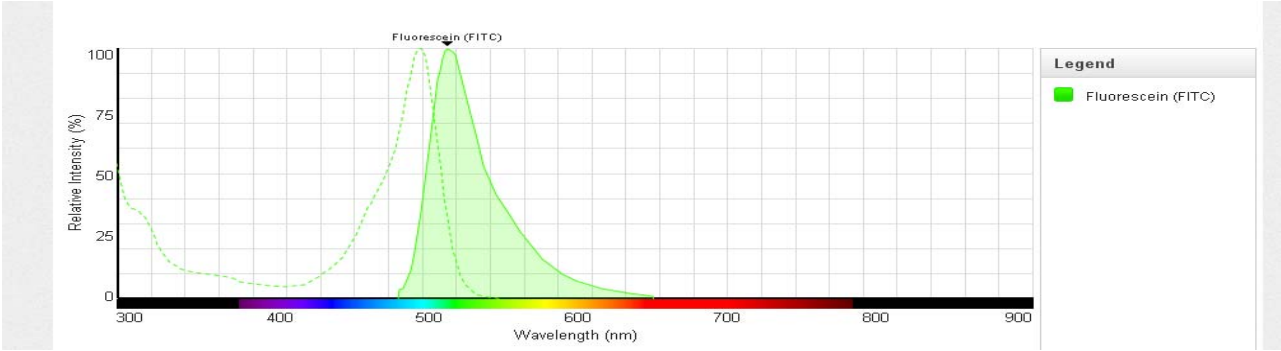
Incident
Light Energy

FITC



λ =530 nm

Emitted
Fluorescent Light



Linear vs. Logarithmic scales

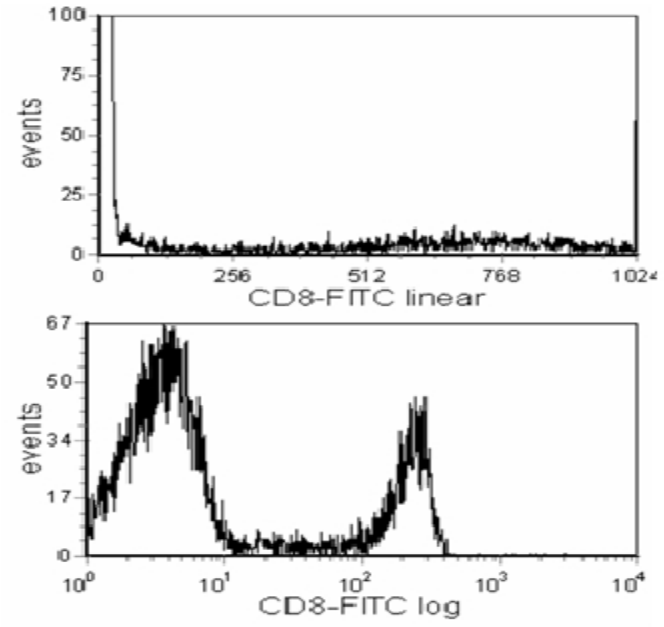
Generally speaking…

- FSC and SSC parameters are displayed on a linear scale
- Fluorescent parameters are displayed on a log scale

Some notable exceptions:

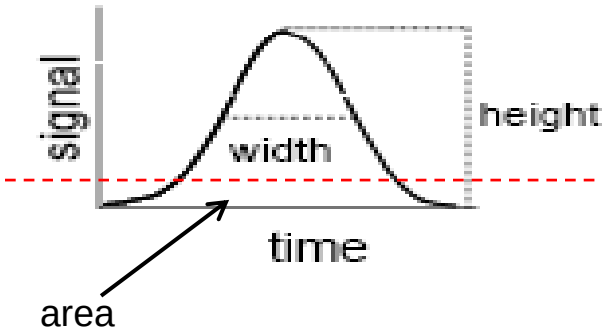
- RBC/platelet analysis is often done using FSC on log scale
- DNA content should be examined on a linear scale

Linear: x= y Log: y = ln(x)



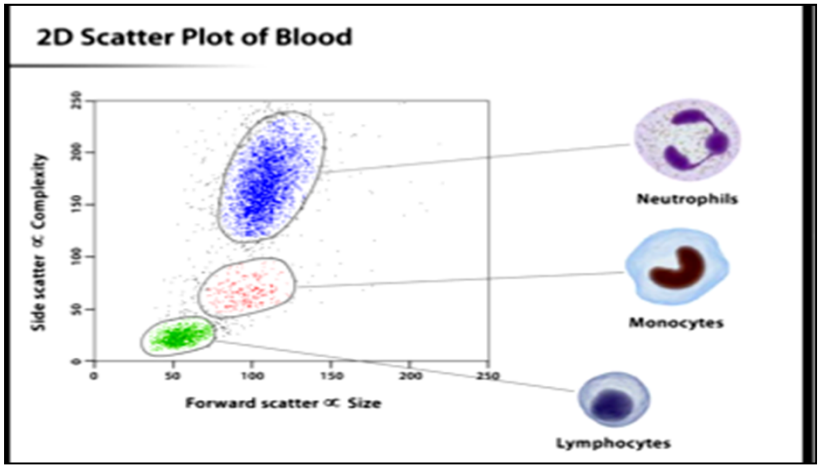
What Can Be Measured with a Flow Cytometer

Events

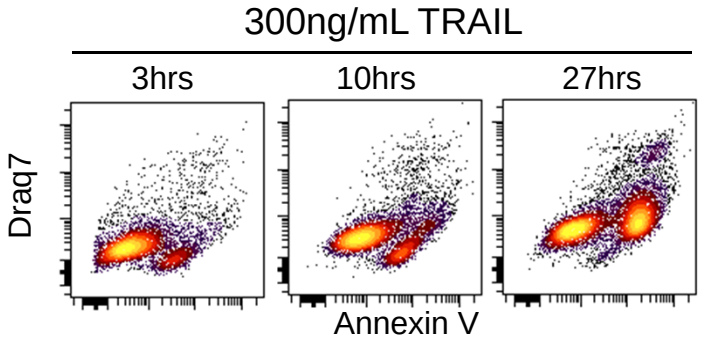


Threshold
(above background)

2D scatter plot of FSC vs SSC

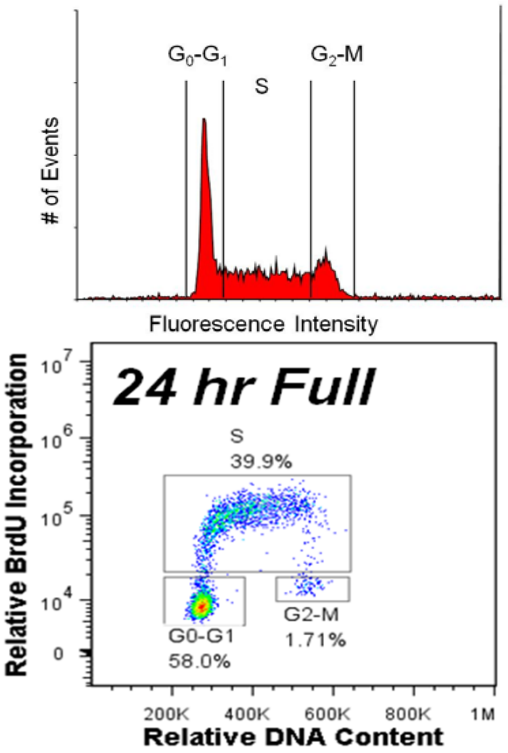


Viability and Apoptosis



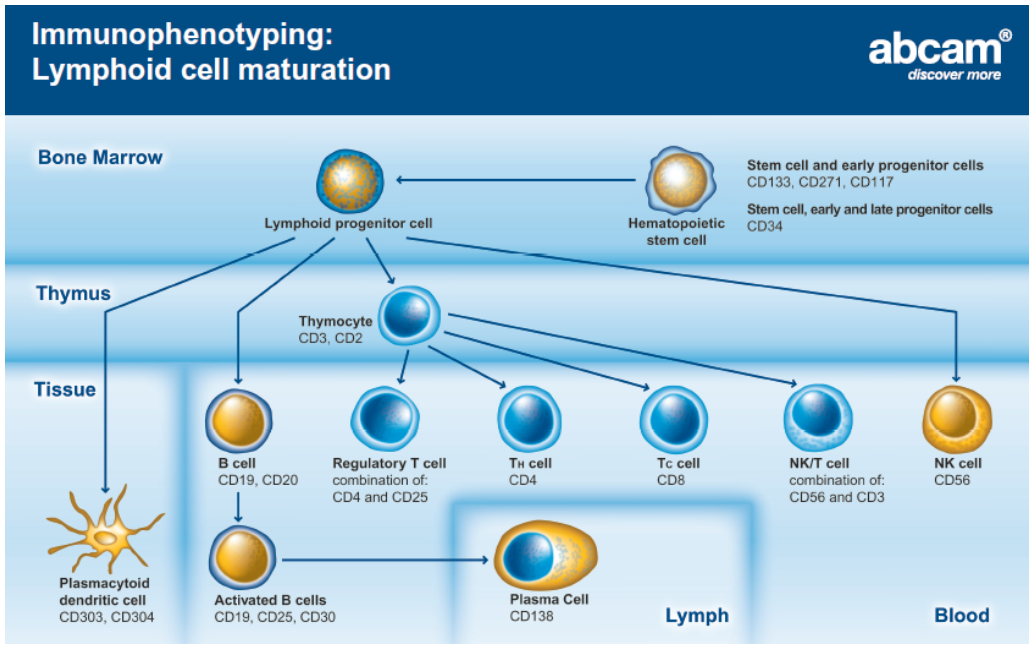
- Many fluorescent dyes are cell impermeant unless the membrane is compromised
  - E.g. propidium iodide, Draq7
- This allows detection of viability
- That can be combined with fluorophore-labeled Annexin-V
  - binds to exposed phosphatidylserine, a marker of apoptosis

Cell Cycle Analylsis



- There are many fluorescent dyes that can stoichiometrically bind to DNA
  - E.g. propidium iodide
- DNA dyes can be combined with BrdU staining to separate cells actively replicating their DNA
  - A pulse of BrdU gets incorporated into the DNA, which can then be recognized by an antibody

Immunophenotyping



http://docs.abcam.com/pdf/immunology/lymphoid_cell.pdf

- One can stain cells with labeled antibodies against lineage-specific extracellular markers

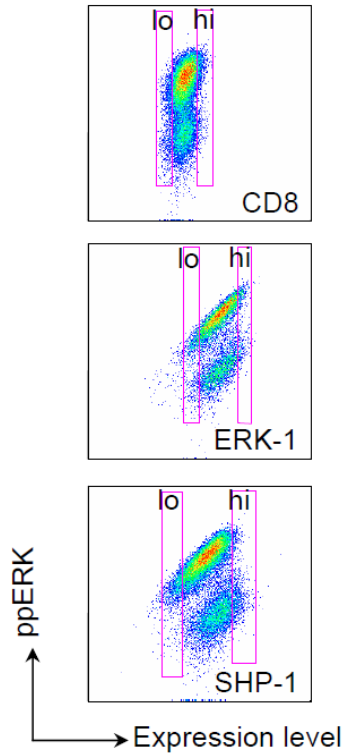
Intracellular Immunostaining

SCIENCE VOL 321 22 AUGUST 2008

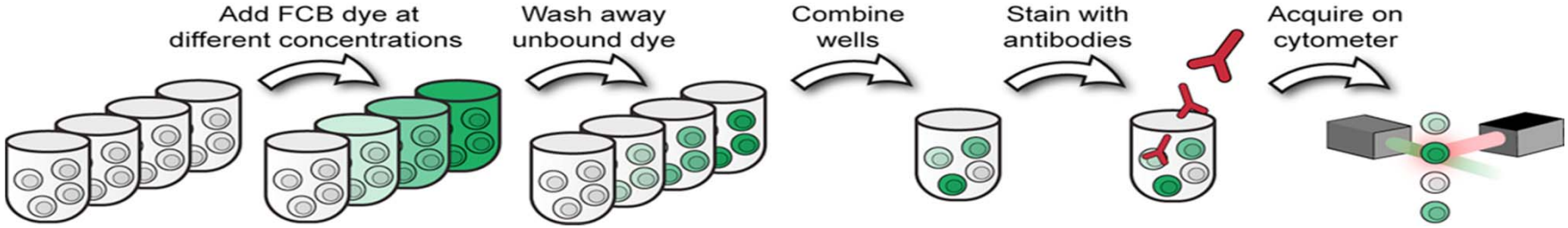
Variability and Robustness in T Cell Activation from Regulated Heterogeneity in Protein Levels

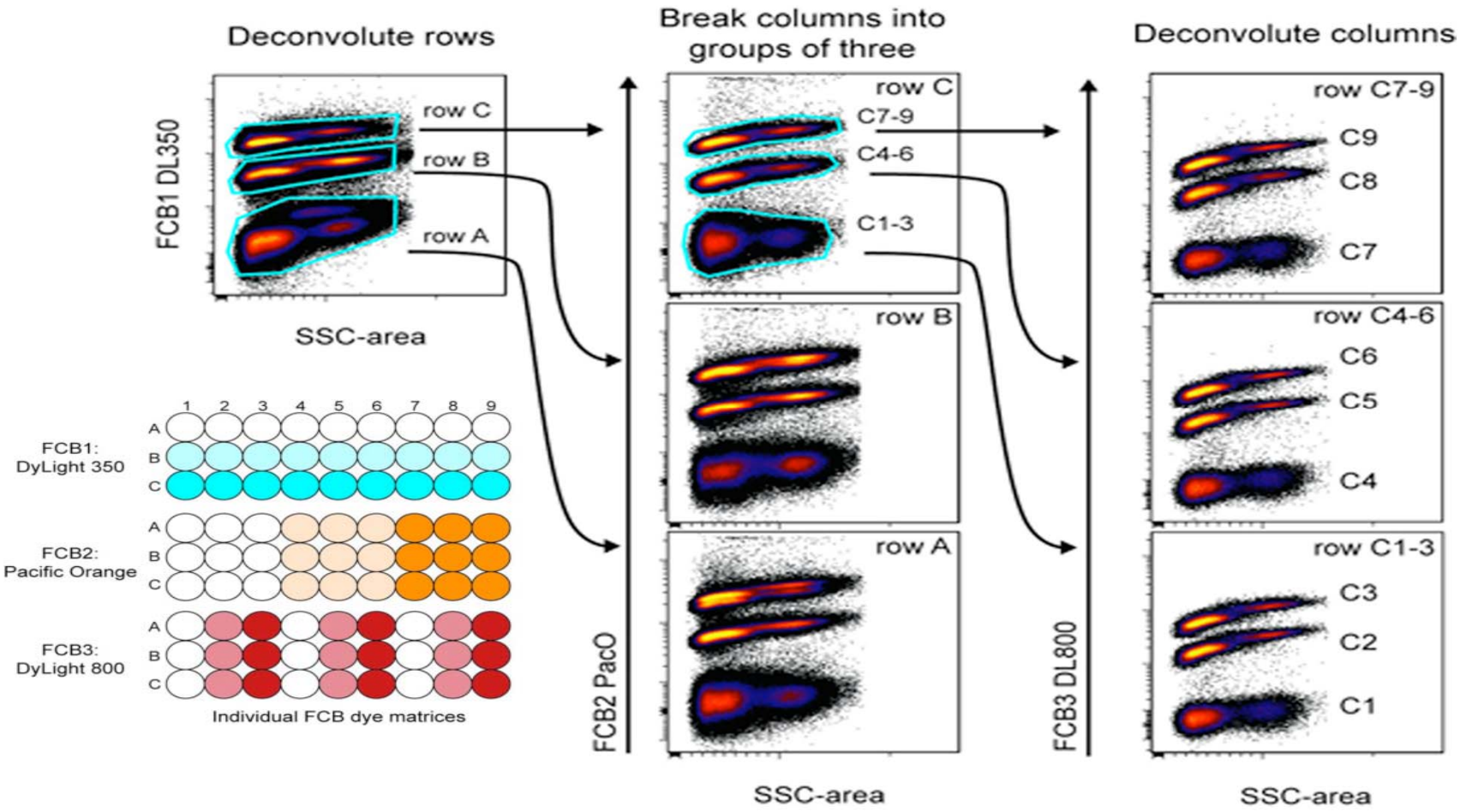
Ofer Feinerman,[1]* Joël Veiga,[1]* Jeffrey R. Dorfman,[1]†
Ronald N. Germain,[2] Grégoire Altan-Bonnet[1]‡

- After cell fixation, one can detect intracellular epitopes
- E.g. panels of phosphorylated proteins



Fluorescent Cell Barcoding (FCB) for Multiplex Flow Cytometry




Deconvolute rows
Break columns into groups of three
Deconvolute columns
FCB1 DL350
row C
row B
row A
SSC-area
row C
C7-9
C4-6
C1-3
row B
row A
FCB2 PacO
SSC-area
row C7-9
C9
C8
C7
row C4-6
C6
C5
C4
row C1-3
C3
C2
C1
FCB3 DL800
SSC-area
1 2 3 4 5 6 7 8 9
A
B
C
FCB1: DyLight 350
FCB2: Pacific Orange
FCB3: DyLight 800
Individual FCB dye matrices