Flow Cytometry: Theory and Application

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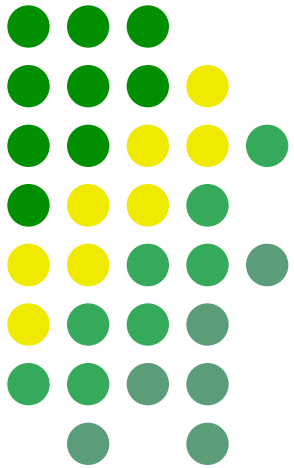
20.109 Lecture

October 7th, 2010

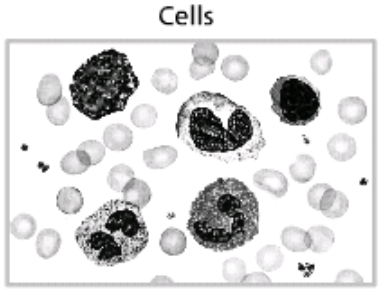


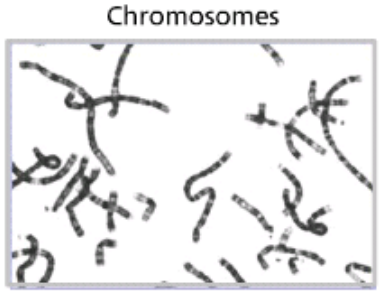
Theory

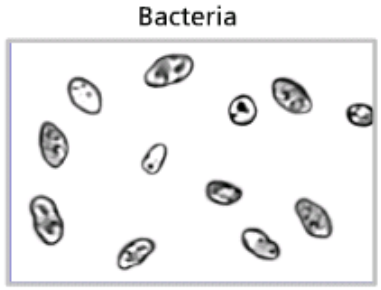
- Flow Cytometry

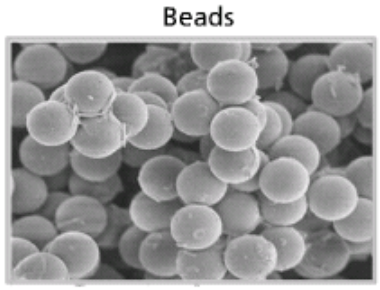
(Dynamic Fluid Single Cell Measurement)

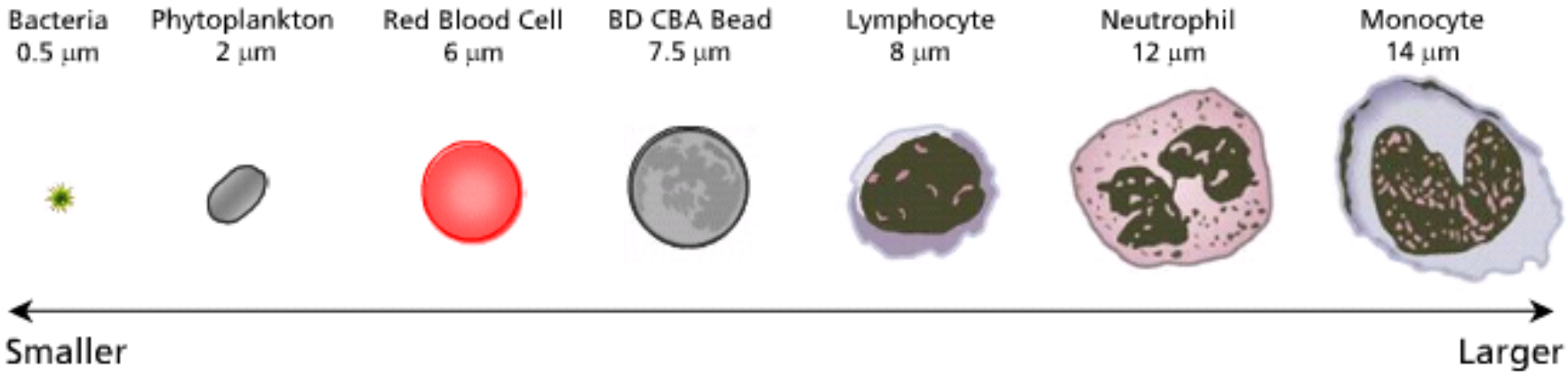
  - Fluidics
  - Optics
  - Electronics



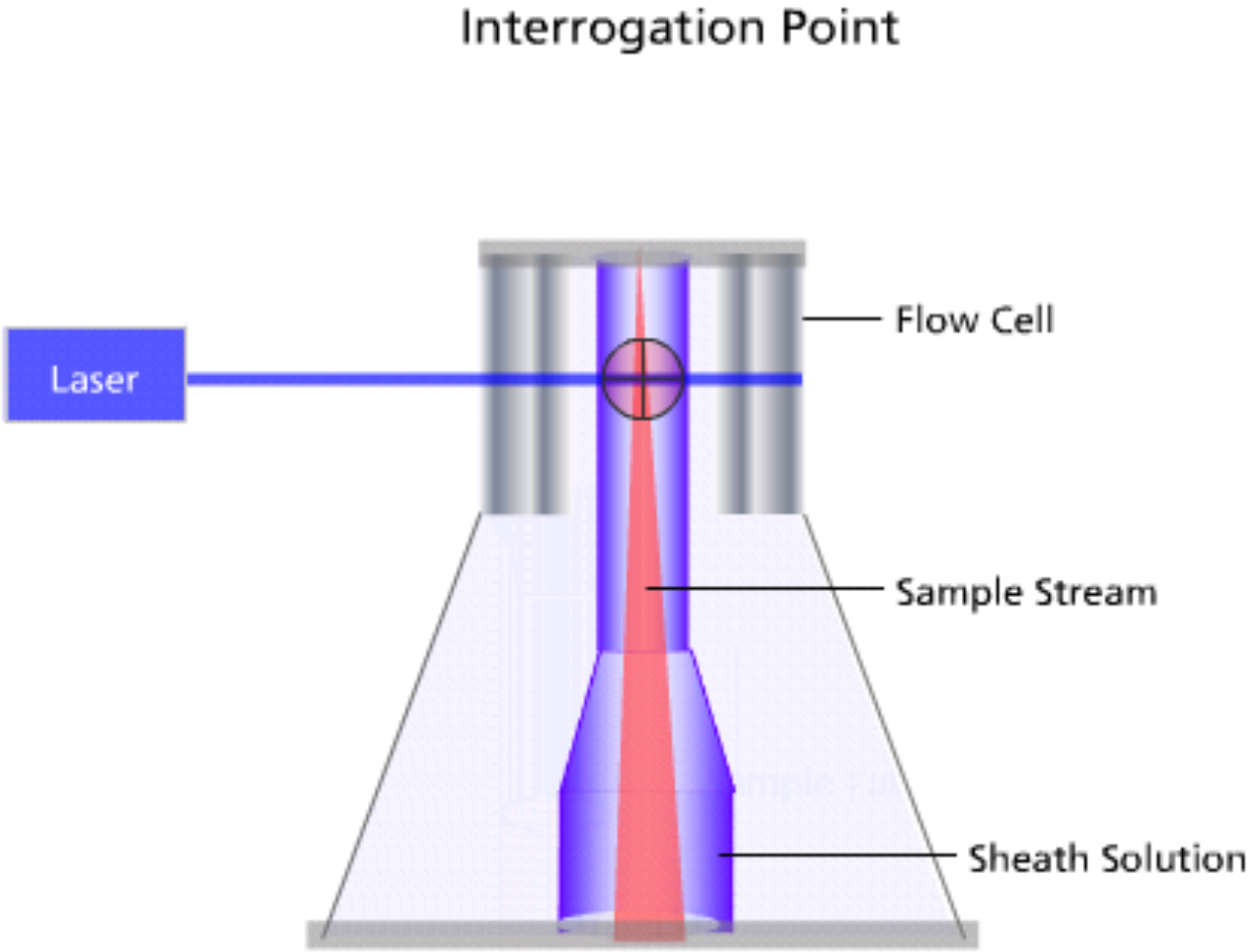




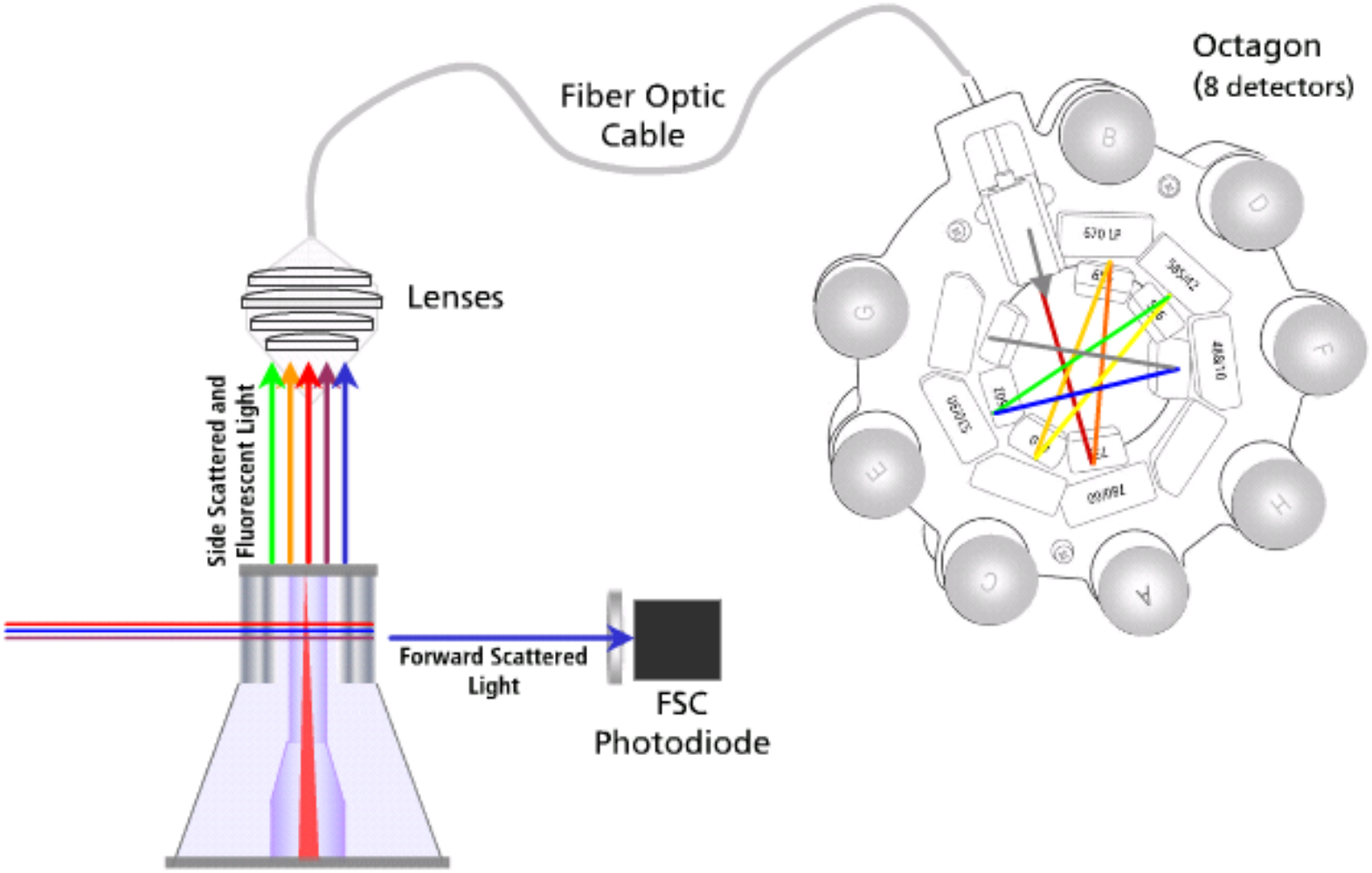




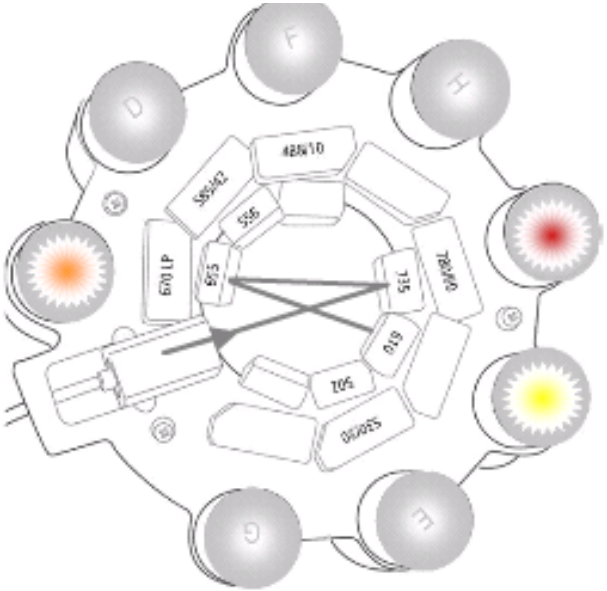
Fluidics


Interrogation Point
Laser
Flow Cell
Sample Stream
Sheath Solution

Optics


Fiber Optic
Cable
Octagon
(8 detectors)
Lenses
Side Scattered and
Fluorescent Light
Forward Scattered
Light
FSC
Photodiode

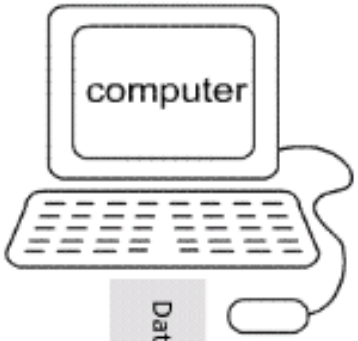
Electronics



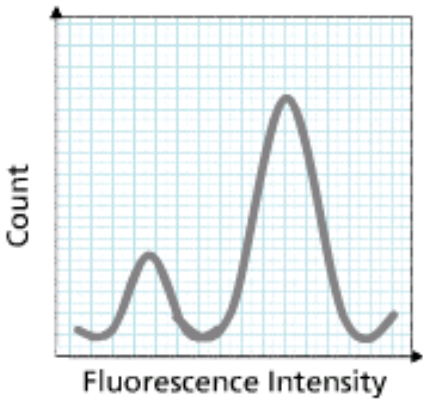
Converts light to electronic signals

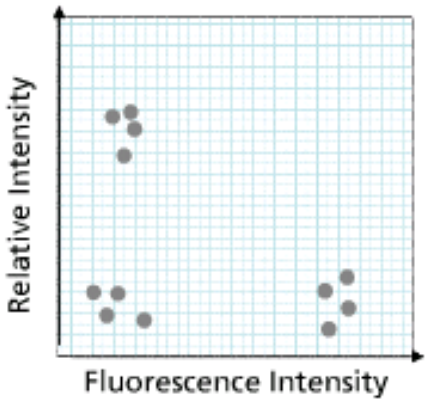
Data		
Event	X	Y
1	33260	120
2	32395	132
3	328	117
4	261853	260226
5	34428	150

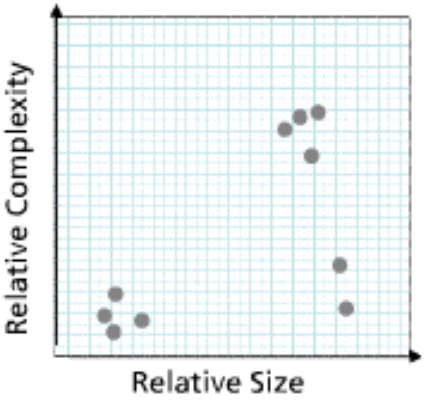
Digitizes data



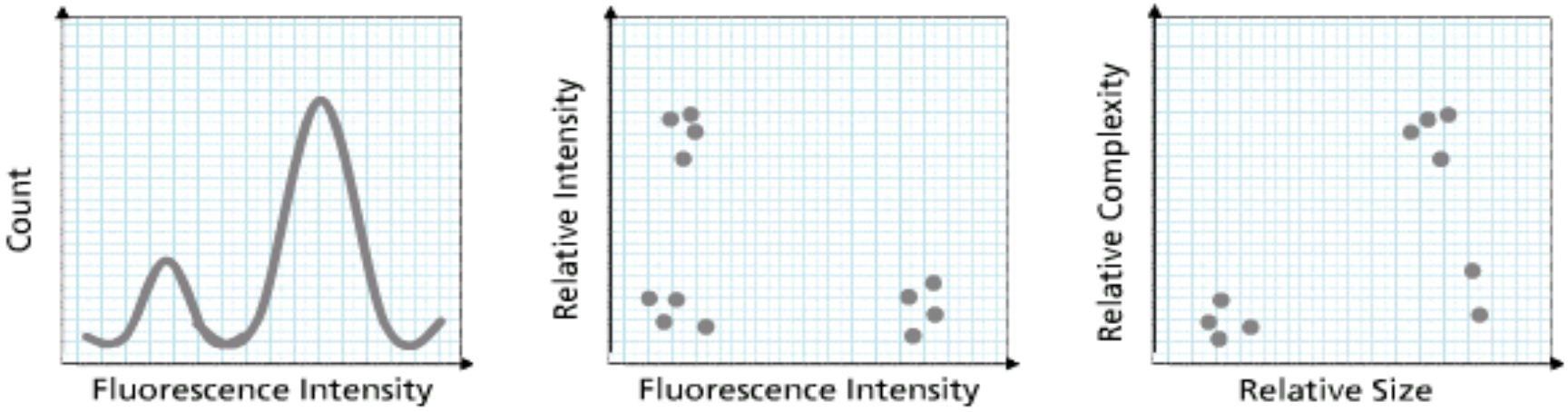
Data Analysis





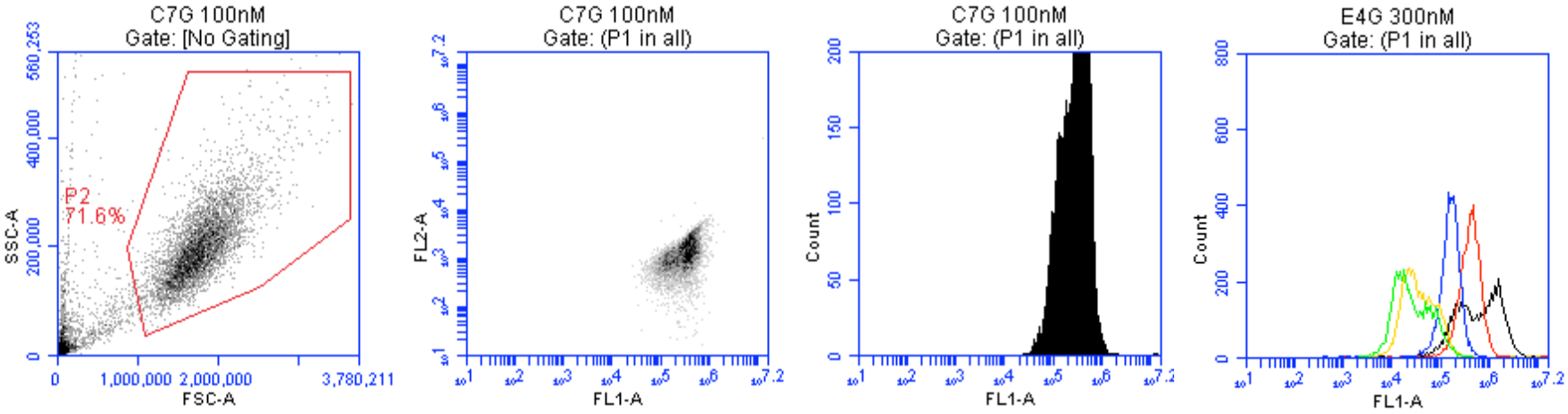


Outputs



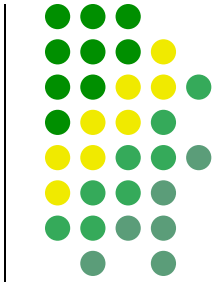
- Forward Scatter → Refraction/Diffraction → Relative Size
- Side Scatter → 90^{o} Reflection → Relative Complexity
- Fluorescence → Relative Signal (1 or 2 colors)
  - + quantitative standard → quantitative signal

Data



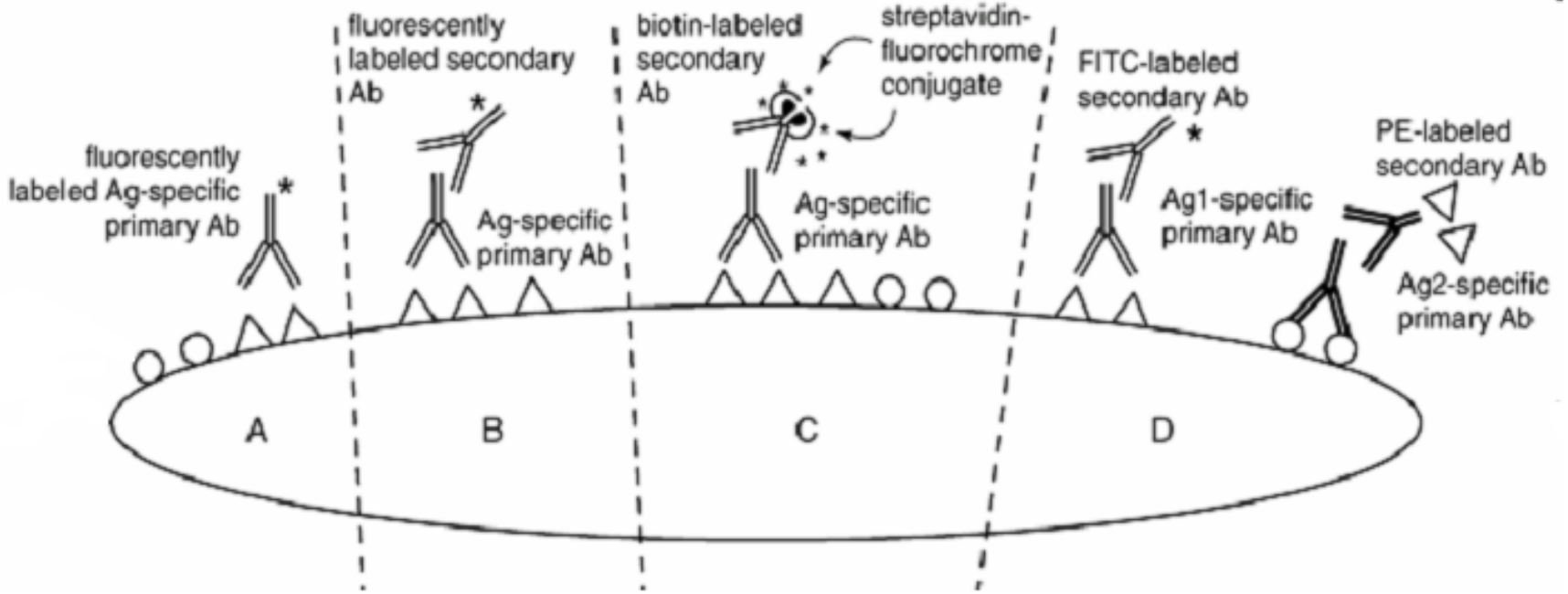
- Isolate cell type
- Observe fluorescence in multiple channels
- Compare populations

Application



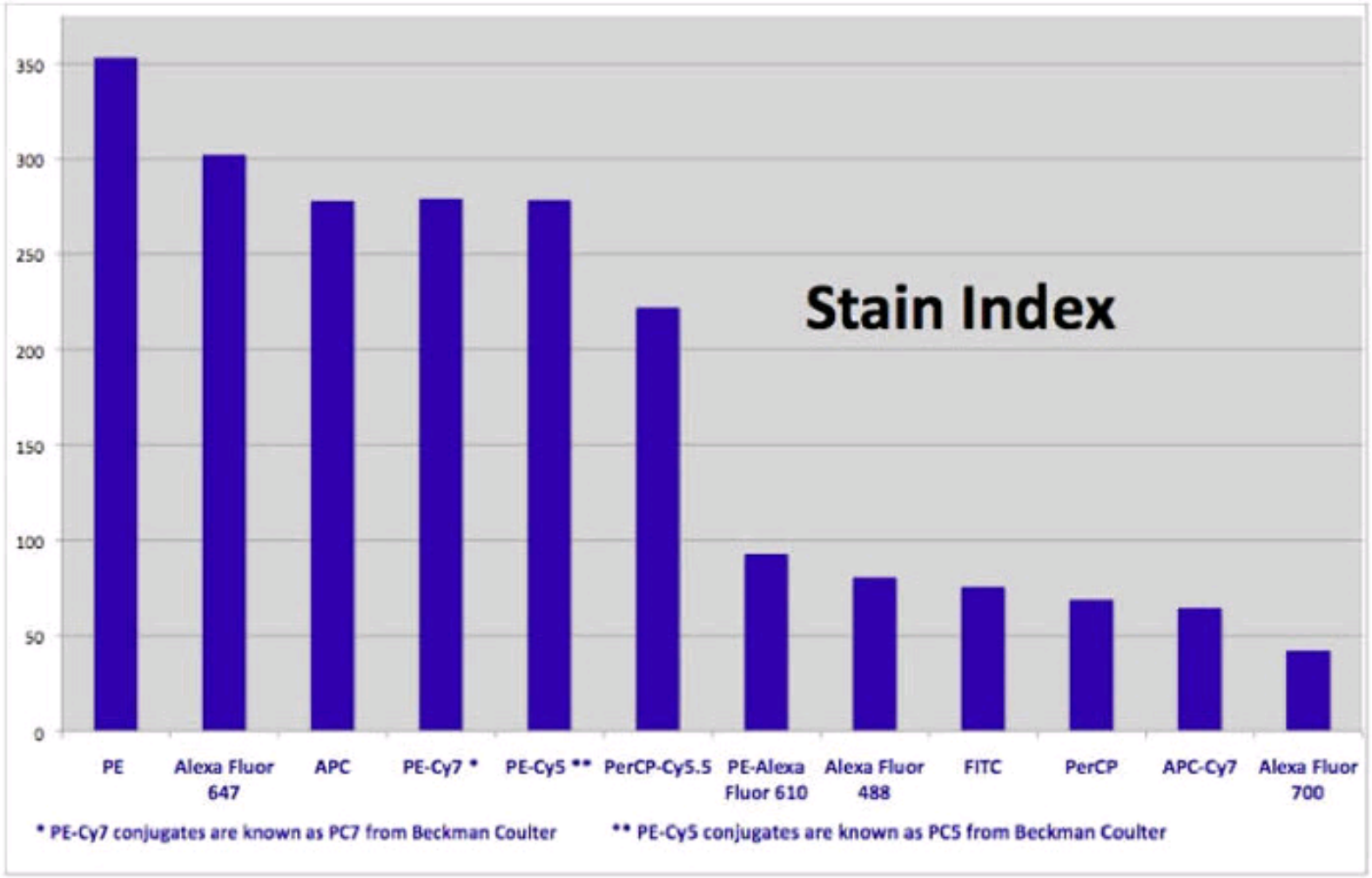
- Analytical
  - Fluorescent labeling (fluorophores)
  - Detectors
  - Compensation
  - Statistics
- Cell Sorting
  - Cell charging
  - Sortable characteristics
- Examples

Fluorescent labeling

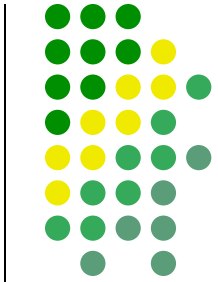


- Many different ways to fluorescently label particles
  - Direct labeling
  - Secondary (tertiary) detection
  - Pre-loading
- Need not be antibody based

Fluorophores

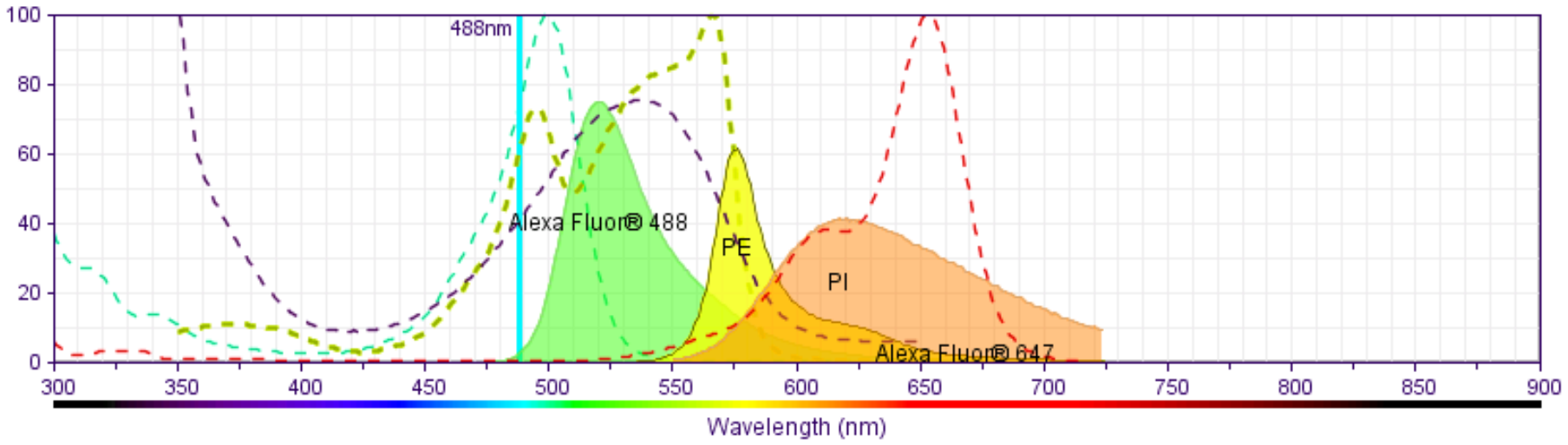


Detectors (ie PMT)



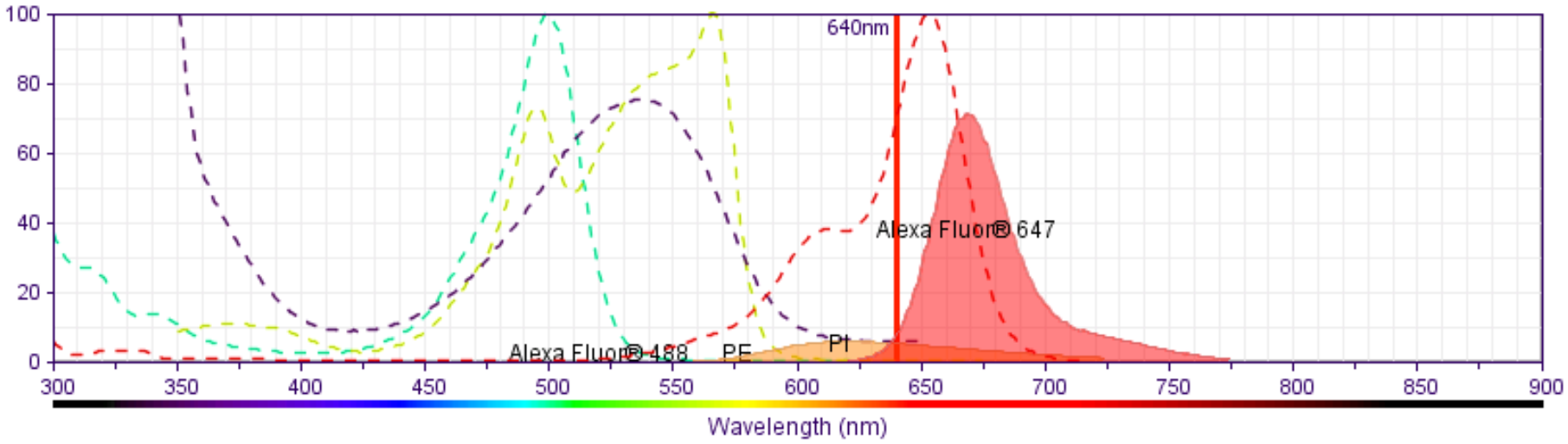
Detector	Wavelength	Color	Fluorophore
FI_1	530/30nm	~green	FITC/GFP/ A488
FI_2	585/42nm	~yellow	PE
FI_3	>650nm	~red	PI/Tandems
FI_4	660/20nm	~red	APC/Cy5

Ex/Em of Common Fluorophores



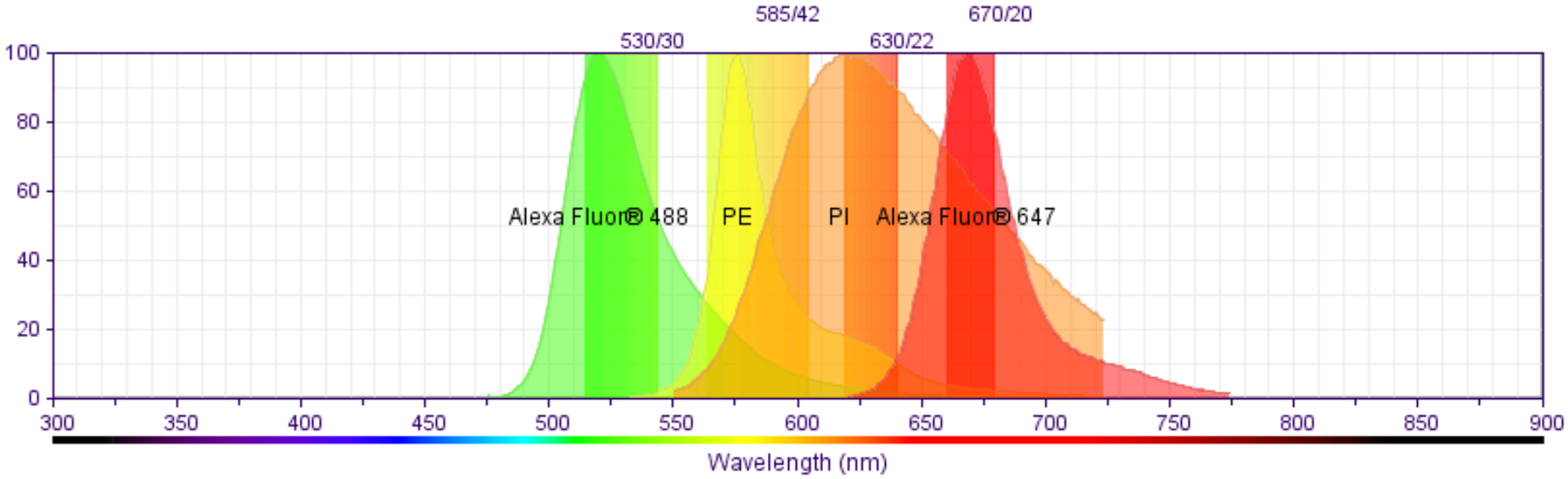
Fluorochrome	%	Ex	Em	Filters	Alexa Flu...	PE	PI	Alexa Flu...
Alexa Fluor® 488	75	☑	☑	530/30	--	--	--	--
PE	62	☑	☑	585/42	--	--	--	--
PI	41	☑	☑	630/22	--	--	--	--
Alexa Fluor® 647	0	☑	☑	670/20	--	--	--	--

Exciting with alternative laser



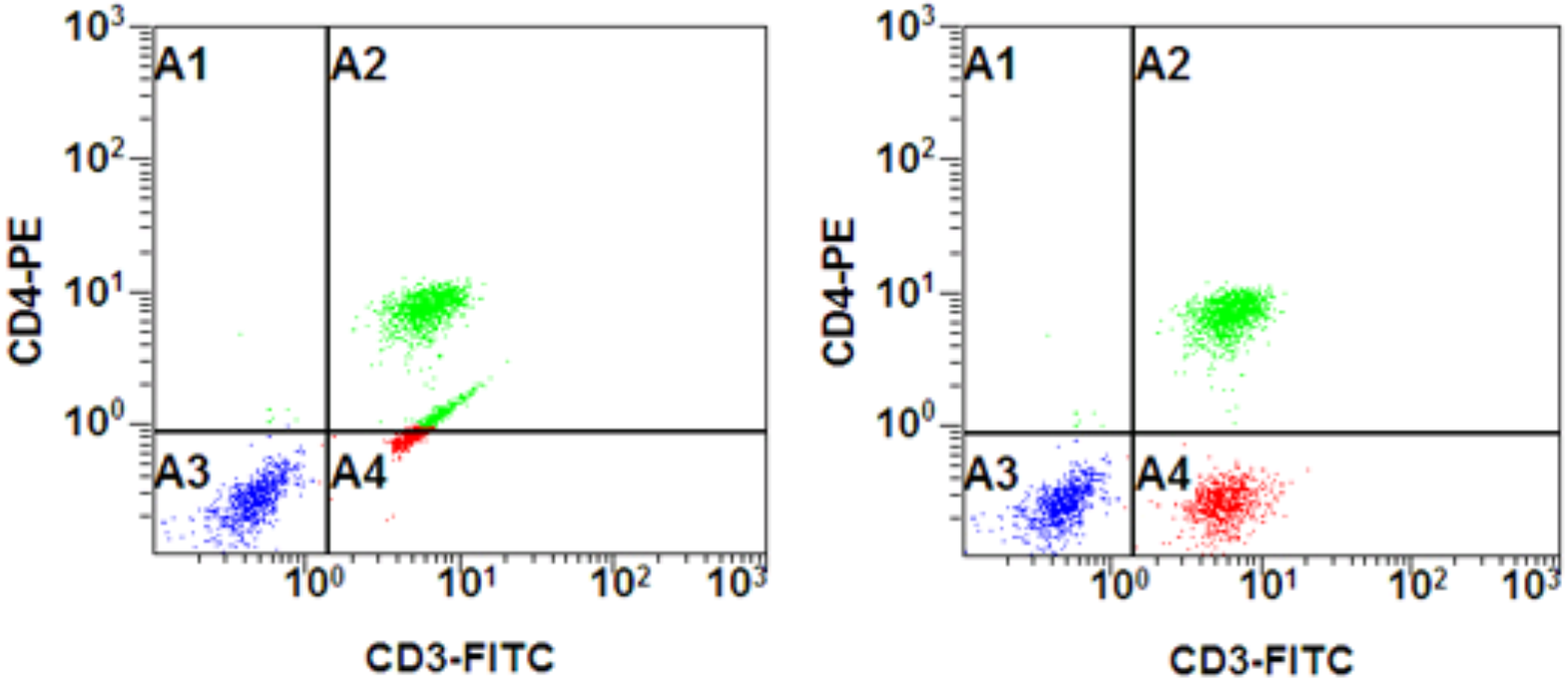
Fluorochrome	%	Ex	Em	Filters	Alexa Flu...	PE	PI	Alexa Flu...
Alexa Fluor® 488	0	☑	☑	530/30	--	--	--	--
PE	0	☑	☑	585/42	--	--	--	--
PI	6	☑	☑	630/22	--	--	--	--
Alexa Fluor® 647	71	☑	☑	670/20	--	--	--	--

Bleeding



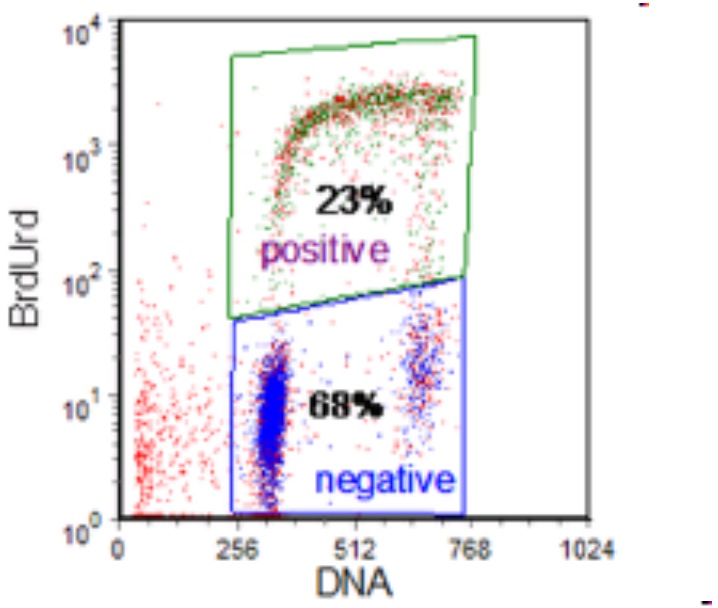
Fluorochrome	%	Ex	Em	Filters		Alexa Flu...	PE	PI	Alexa Flu...
Alexa Fluor® 488	75		☑	530/30	☑	--	--	--	--
PE	62			585/42	☑	--	--	--	--
PI	41			630/22	☑	--	--	--	--
Alexa Fluor® 647	0			670/20	☑	--	--	--	--

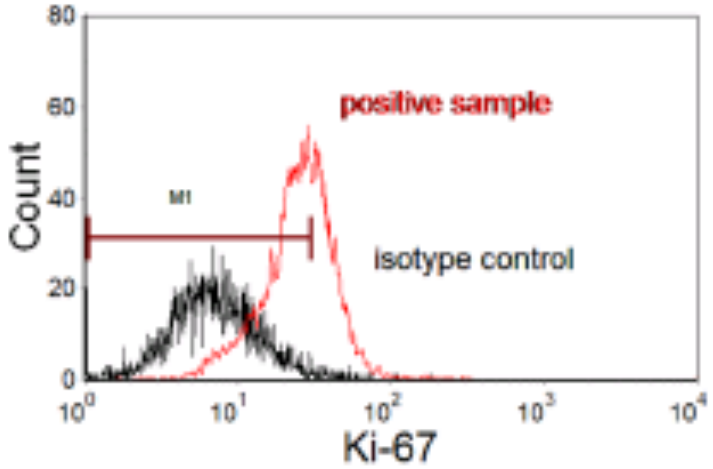
Compensation



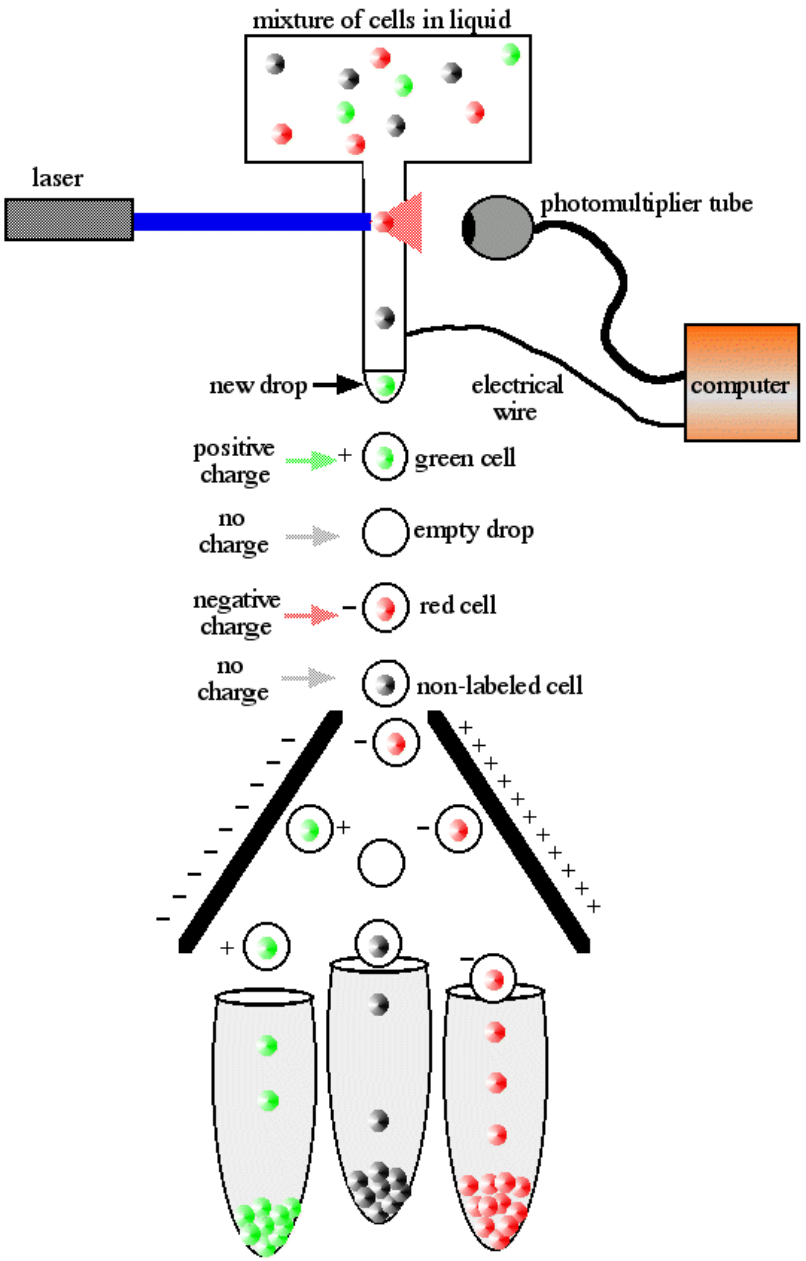
- Uncompensated (left) cells appear to be partially double positive.
- After compensation (right) single positive cells are displayed as such.
- Careful! It's possible to over-compensate.

Statistics in Flow Cytometry





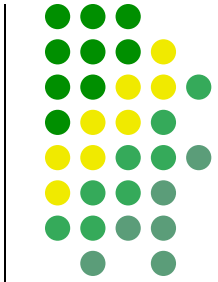
- Median better than mean
- Coefficient of variance better than standard deviation
- Gating improves statistics but can also mislead



FACS

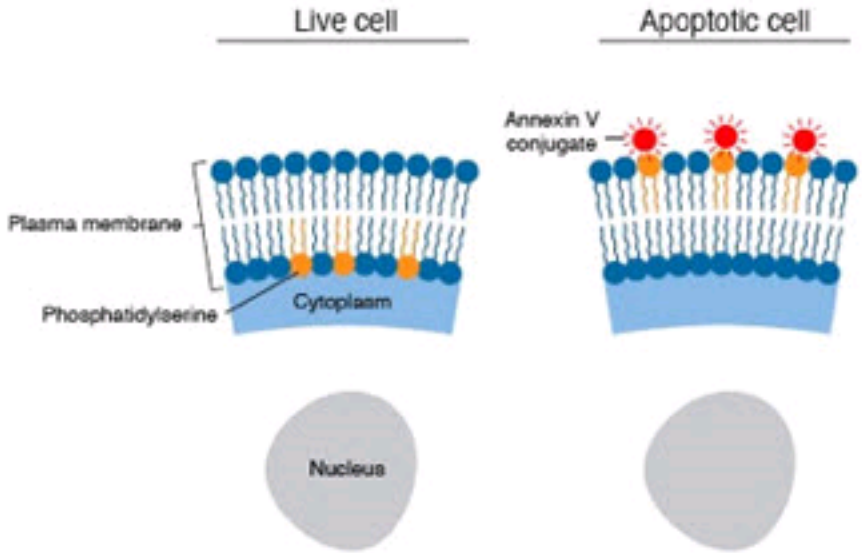
- Nozzle vibrates to form droplets for each event
- Charge on nozzle adjusted for the appropriate event
- Electrostatic plates deflect charged particles into various collection tubes

Examples

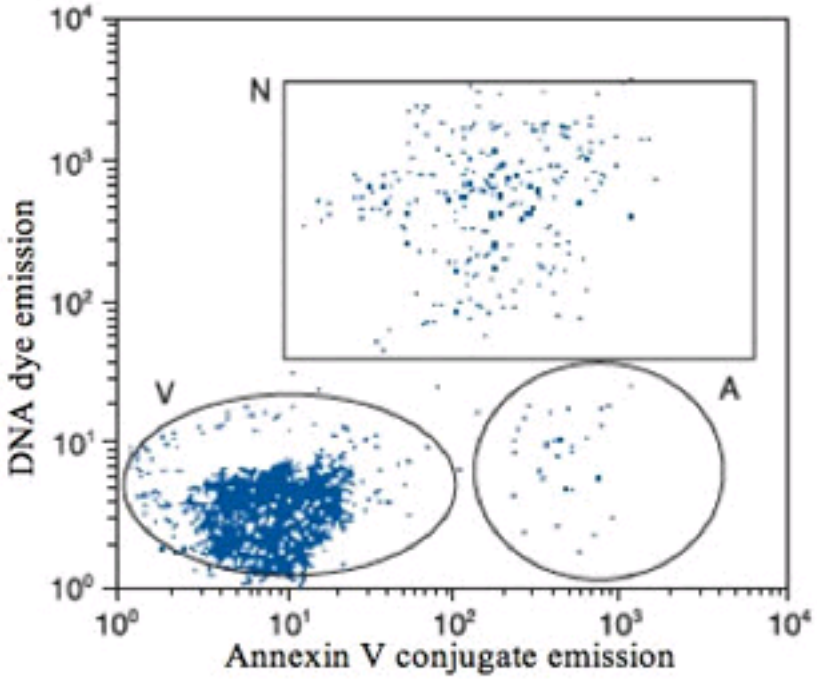


- total DNA content (cell cycle analysis, cell kinetics, proliferation, etc.)
- total RNA content
- DNA copy number variation (by Flow-FISH)
- chromosome analysis and sorting (library construction, chromosome paint)
- protein expression and localization
- Protein modifications, phospho-proteins
- **transgenic products *in vivo*, particularly the Green fluorescent protein or related fluorescent * cell surface antigens (Cluster of differentiation (CD) markers)**
- intracellular antigens (various cytokines, secondary mediators, etc.)
- nuclear antigens
- **enzymatic activity**
- pH, intracellular ionized calcium, magnesium, membrane potential
- membrane fluidity
- **apoptosis** (quantification, measurement of DNA degradation, mitochondrial membrane potential, permeability changes, caspase activity)
- cell viability
- monitoring electropermeabilization of cells
- oxidative burst
- characterising multidrug resistance (MDR) in cancer cells
- glutathione
- various combinations (DNA/surface antigens, etc.)
- cell adherence (for instance pathogen-host cell adherence)
- **Directed evolution of surface displayed proteins**

Apoptosis Detection



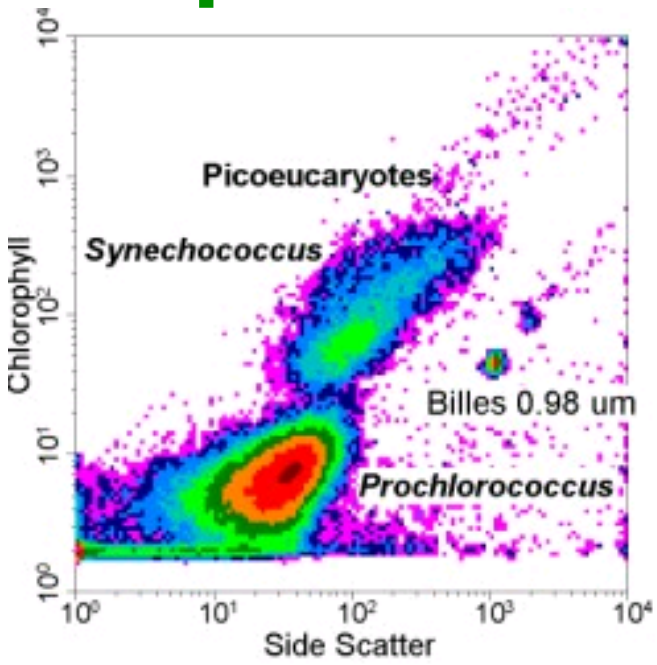
Early apoptosis identified using an annexin V conjugate. In late-stage apoptosis (not shown), the plasma membrane becomes compromised and nucleic acid dyes are permitted to enter the cell and bind with the cell's DNA, which provides and additional marker of apoptosis.

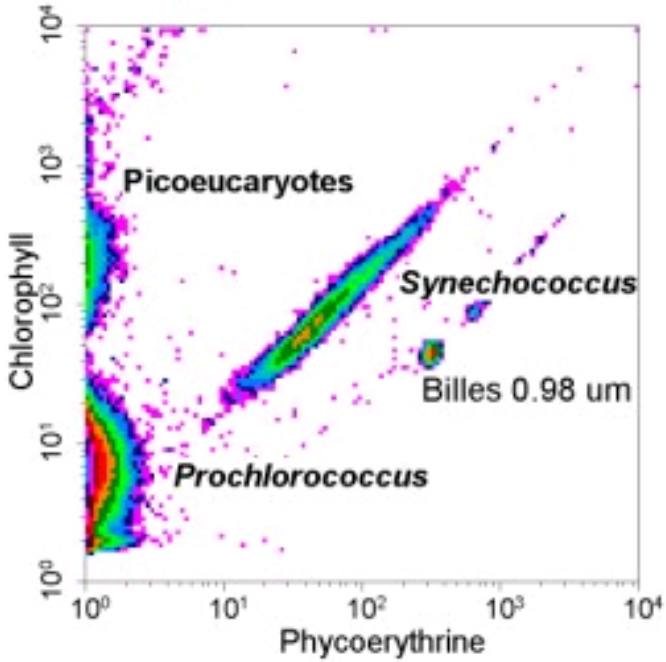


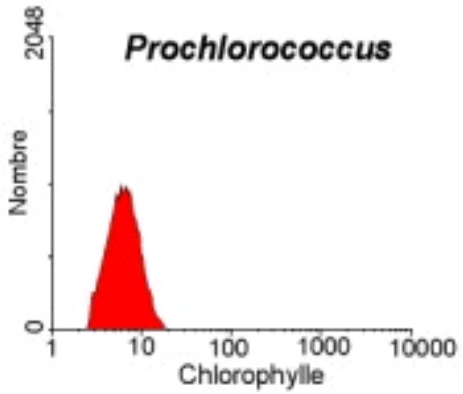
Cells undergoing apoptosis.
V = viable cells
A = apoptotic cells
N = necrotic (late apoptotic) cells

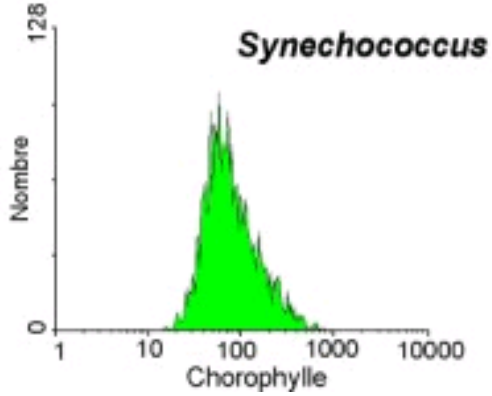
Pictures courtesy of Invitrogen

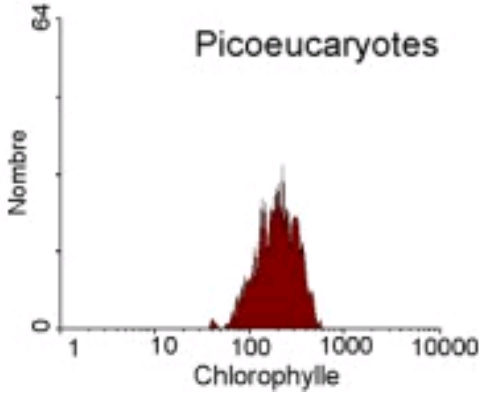
Populations beyond FSC-SSC





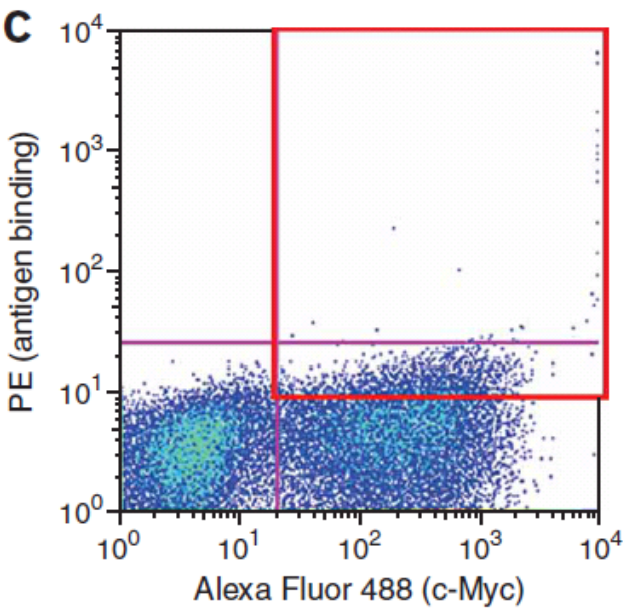


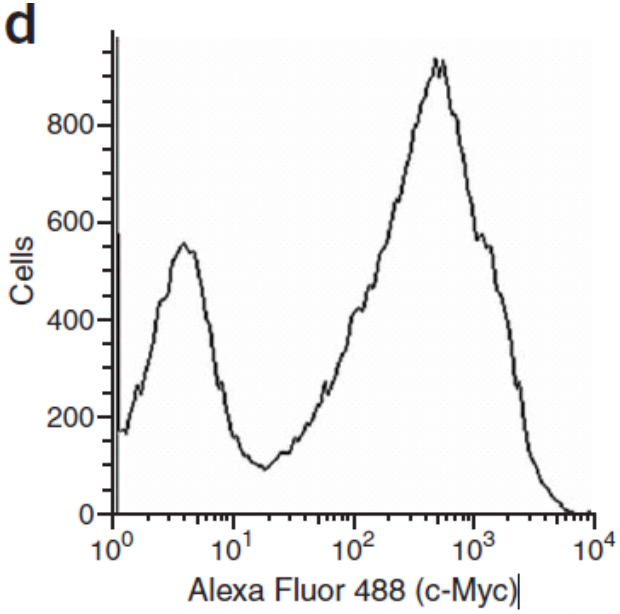


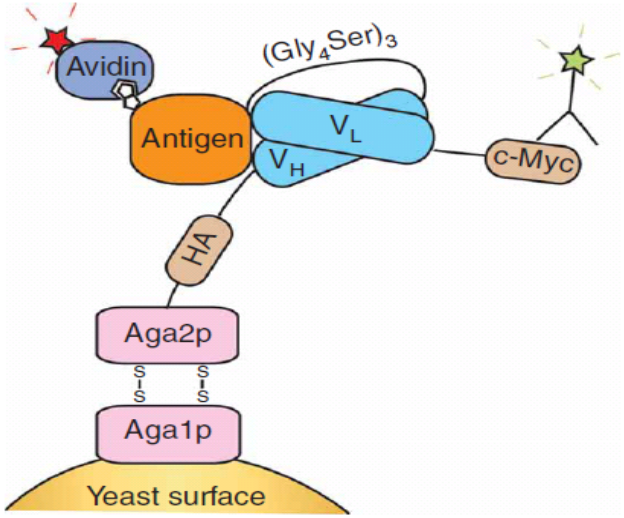


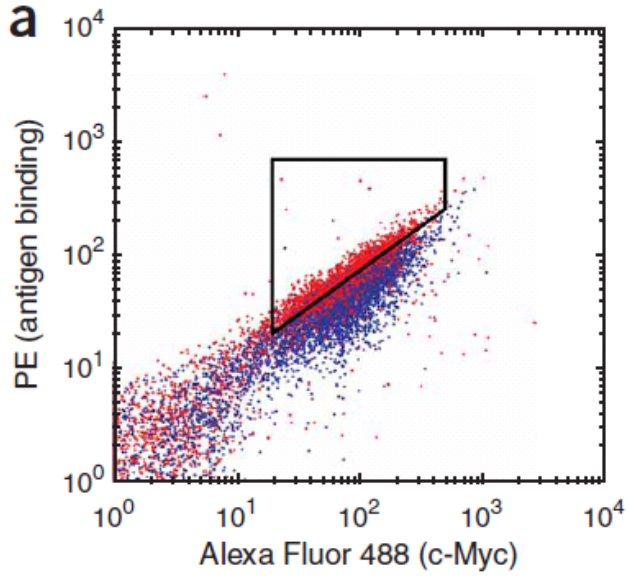
Mari *et at.* FEMS Microbiol Ecol. 2010

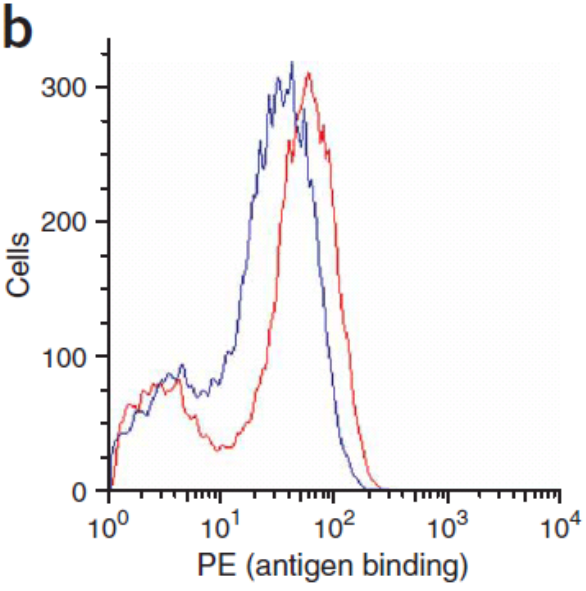
Sorting Surface Display Yeast







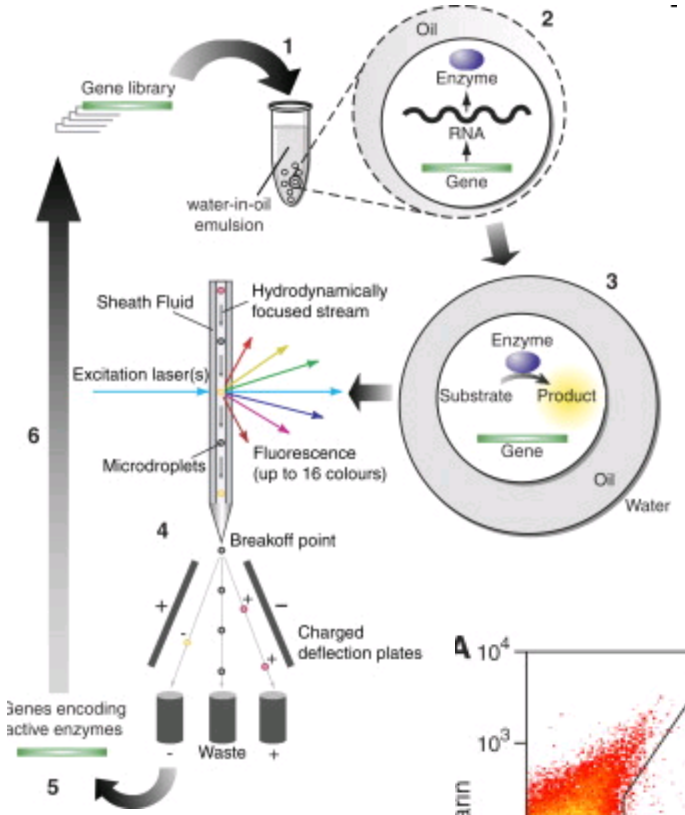


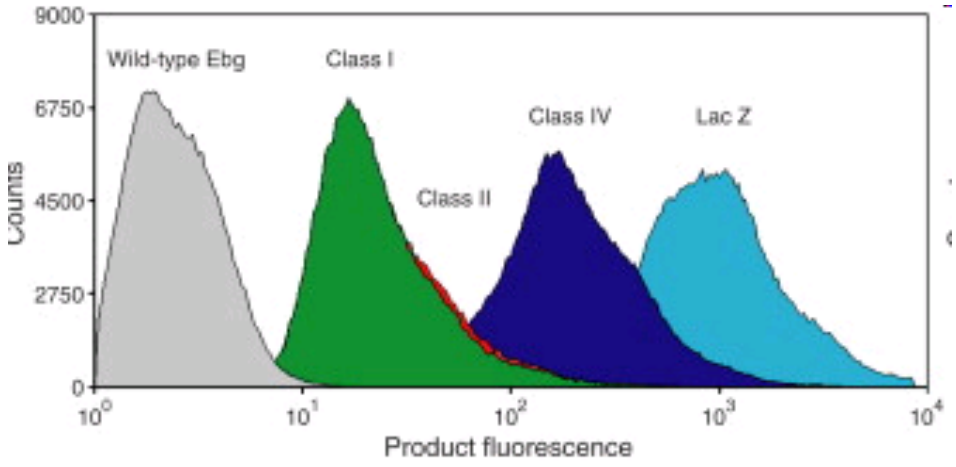


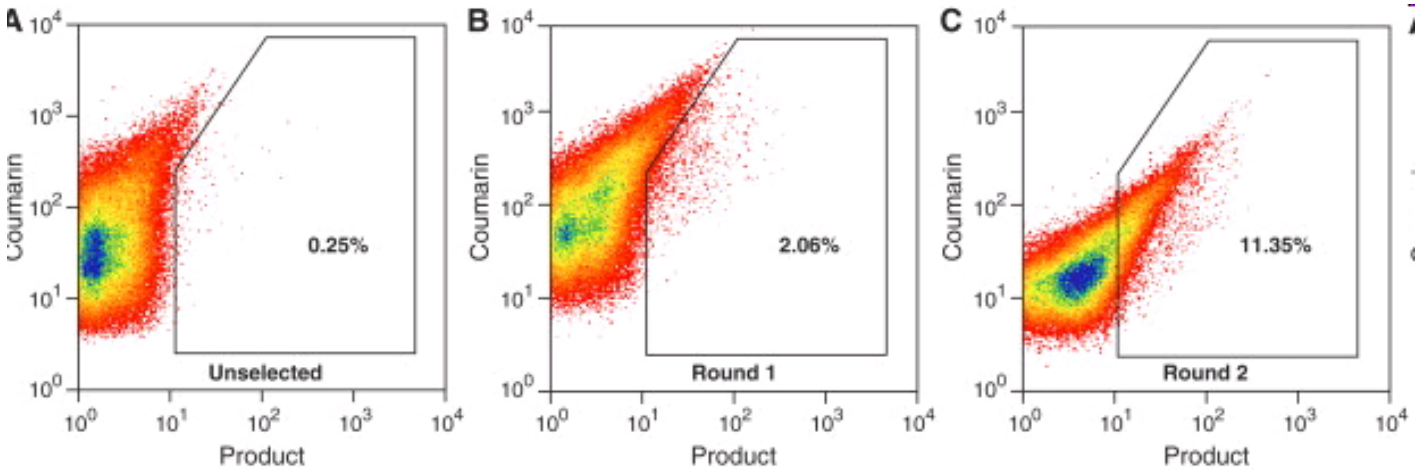
- Label and sort for display and antigen binding.

Chao *et al.* Nature Protocols 2006

Emulsions with enzymes

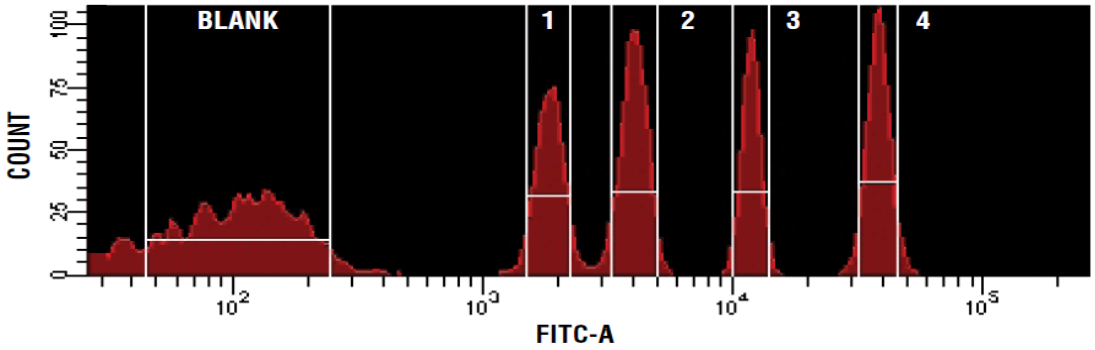






Mastrobattista *et al.* Chemistry & Biology 2005

Quantification of internalization



- Measured MFU/ Fluorophore on beads allows correlation with internalized toxins