

One by one cellular analysis; Flow Cytometry

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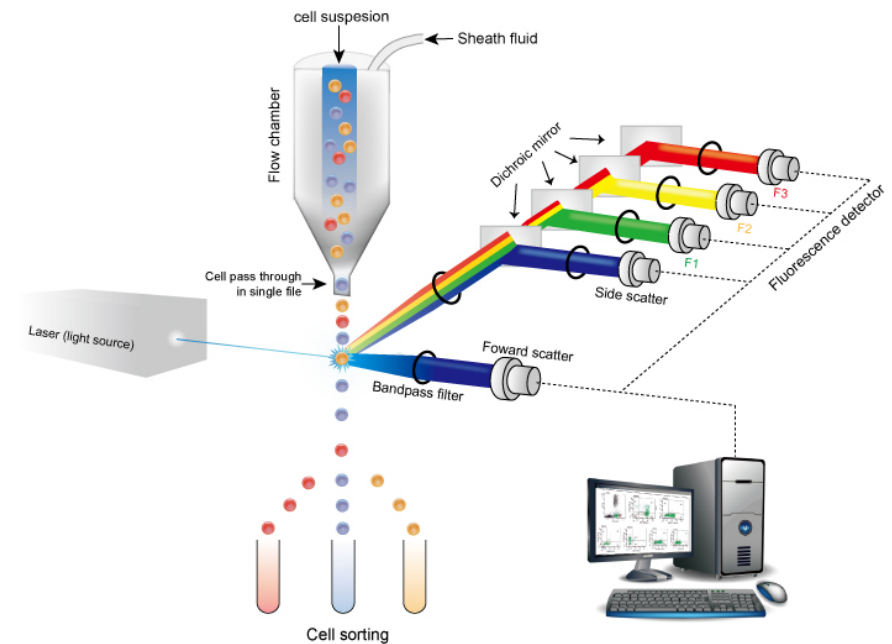
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Content

- What is flow cytometry?
- Conventional methods vs Flow cytometry
- History
- Instrumentation
- Use of Flow
- Example of Flow Cytometry Clinical Application
- New era



What is flow cyotmetry?

- To count and analyze the size, shape and properties of individual cells within a heterogeneous population of cells.
- One by one, but in large numbers!
- FACS – Fluorescence activated cell sorter

Conventional methods vs Flow Cytometry



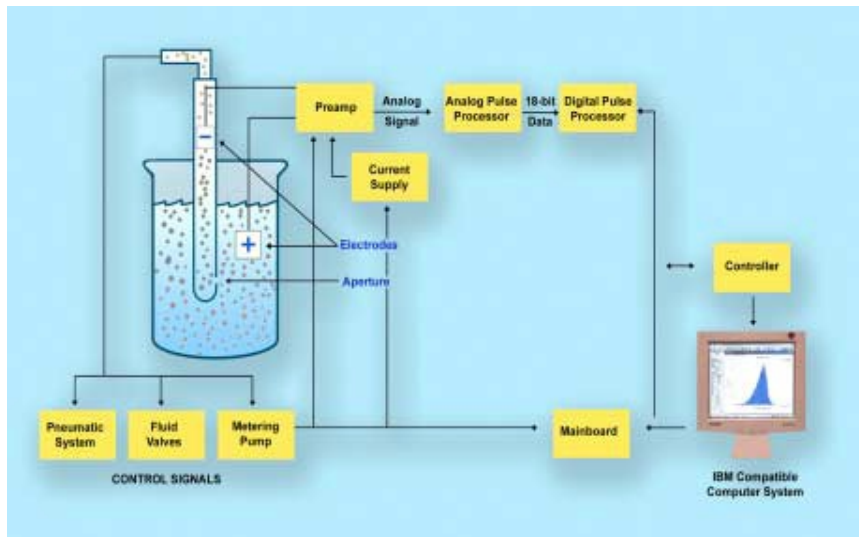
- Visualize cells
- Morphology
- Staining characteristics

Conventional methods vs Flow Cytometry



- Quantitative
- Qualitative

History



- In the 1950's, the Coulter counter automated cell counting based on size.

History



Len Herzenberg, circa 1990, with one of the development flow cytometers in the lab.

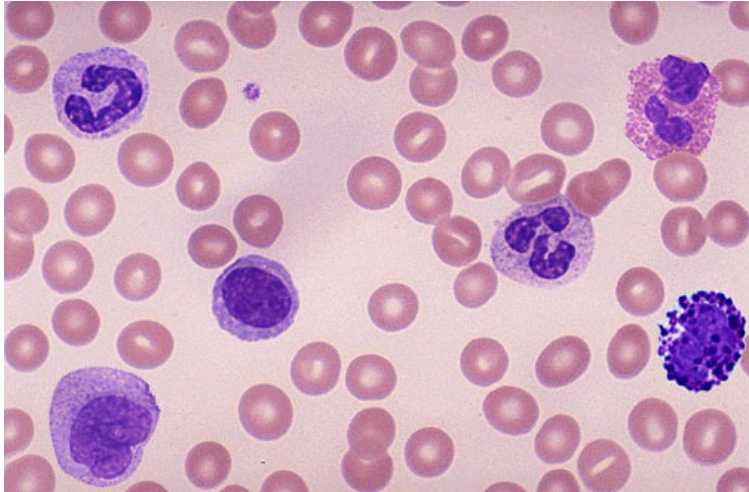
- In 1960, in collaboration with IBM, an automated optical scanner.
- In 1974, Dr. Leonard Herzenberg of Stanford.
 - The first commercial flow cytometers capable of measuring a single fluorescence parameter were introduced.

History

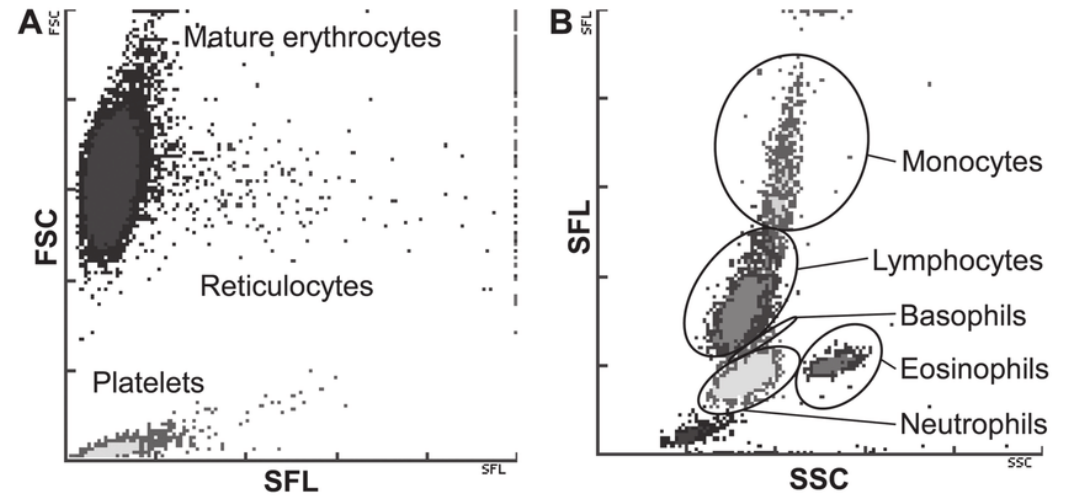


- Late 1970s, instruments configured with two lasers.
- Increased quantity of lasers + detectors → Increased parameters
- 5 lasers – 28 fluorescent parameters

PAST



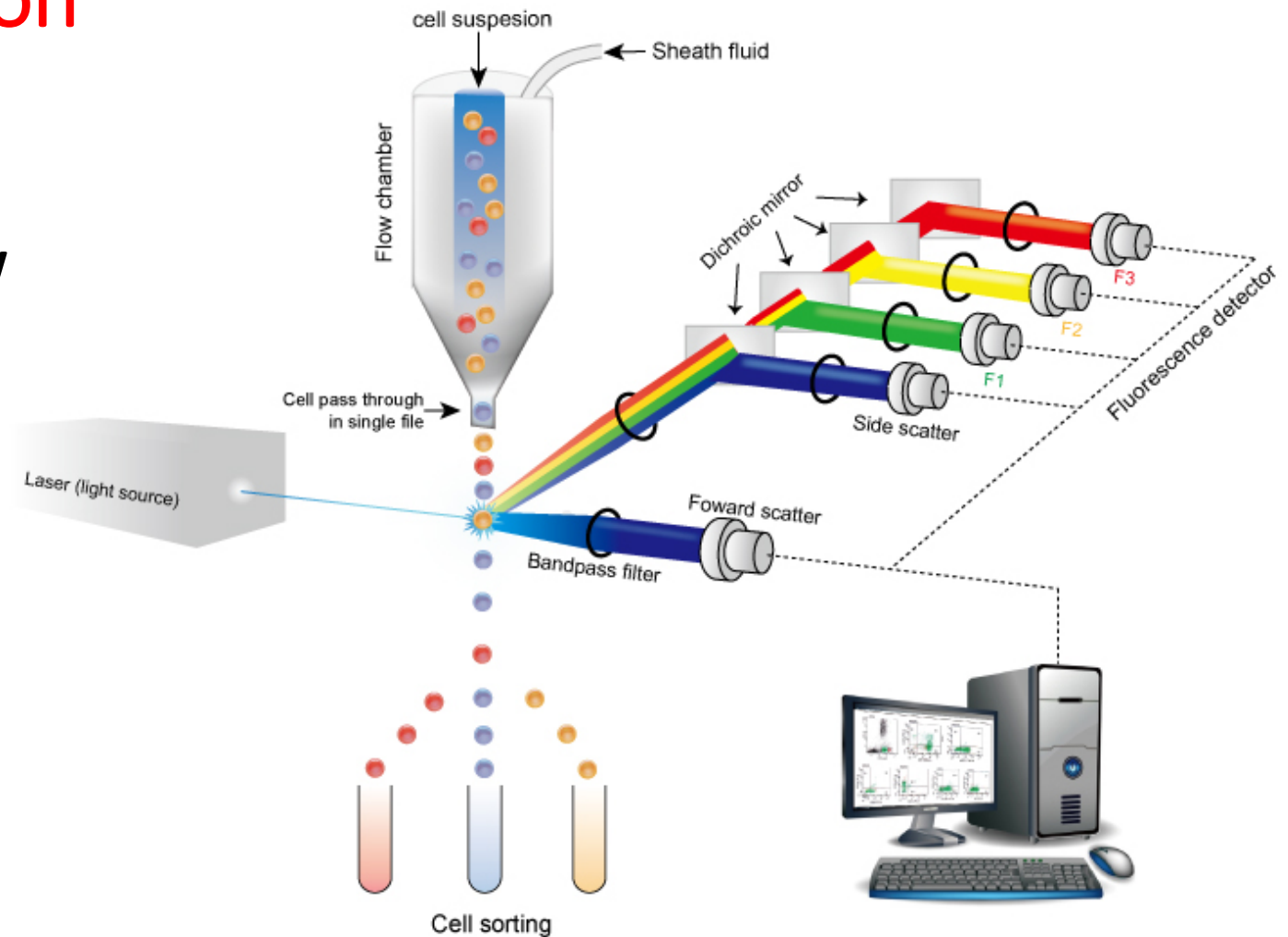
PRESENT



Instrumentation

Components of Flow

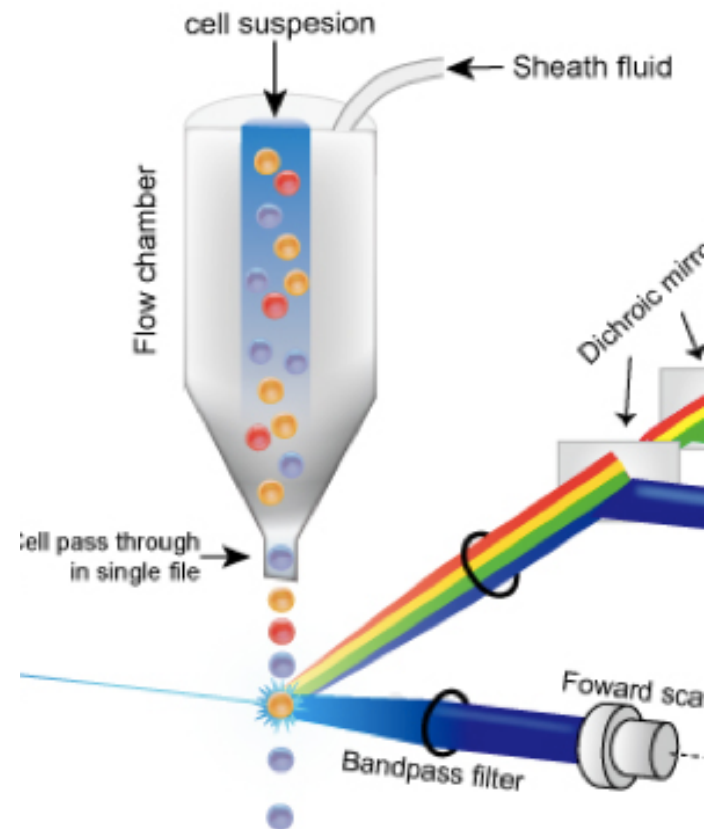
- Fluidics
- Lasers
- Optics
- Detectors
- Electronics



Instrumentation

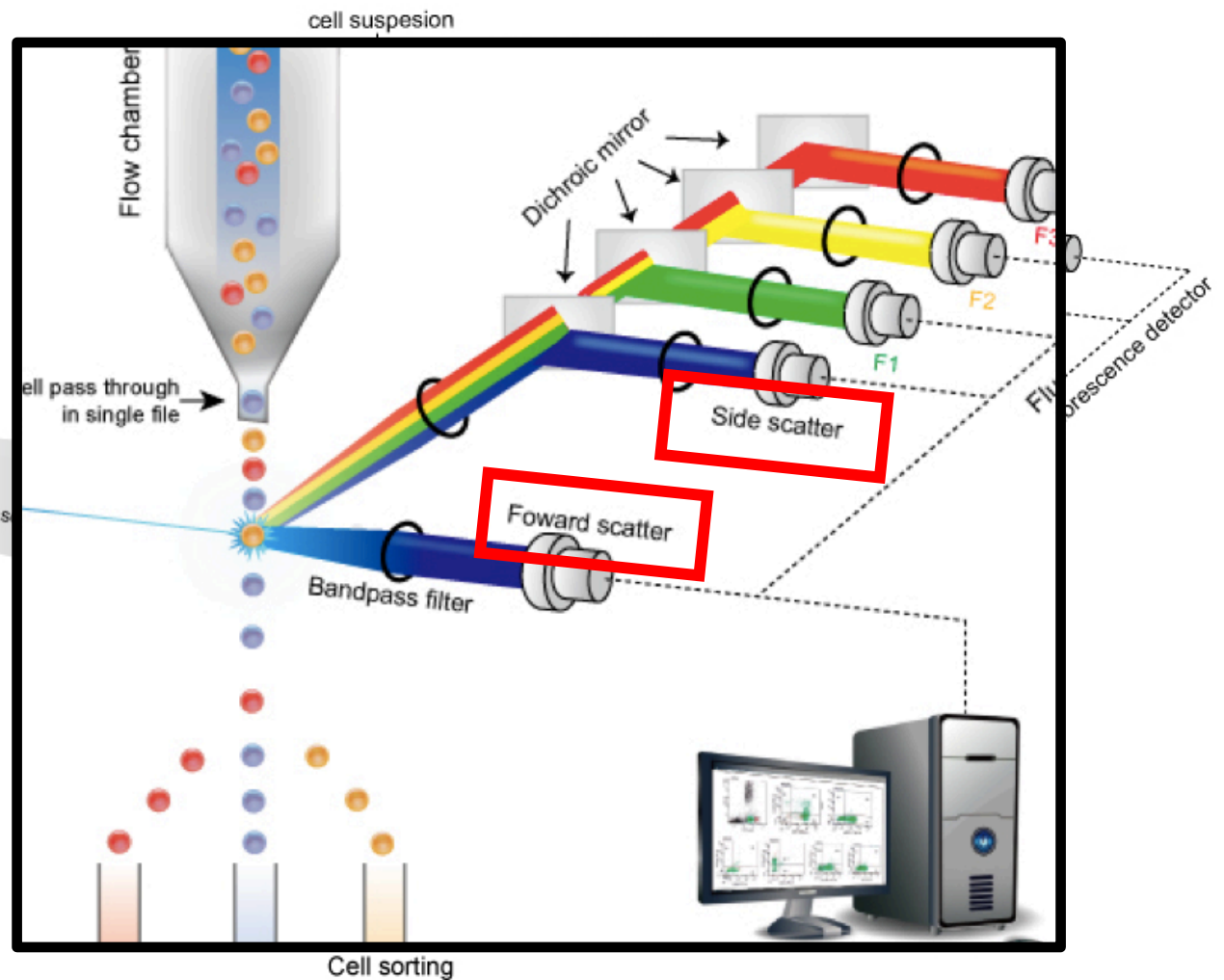
Components of Flow

- Fluidics
- Lasers
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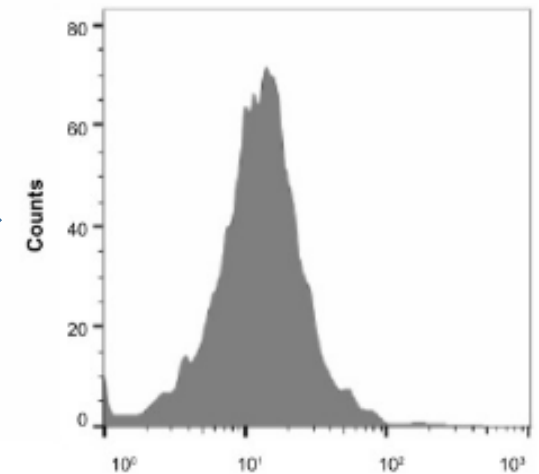
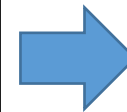
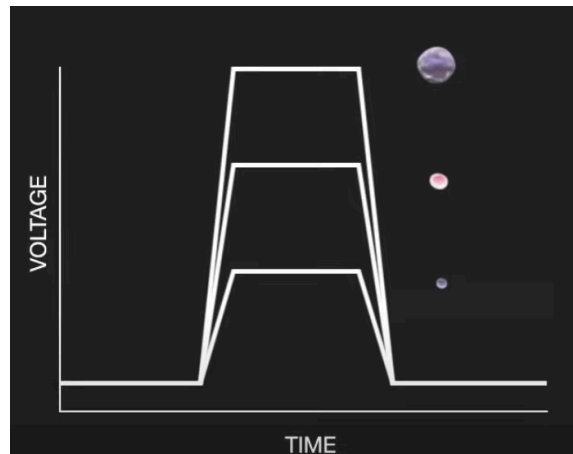
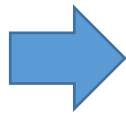
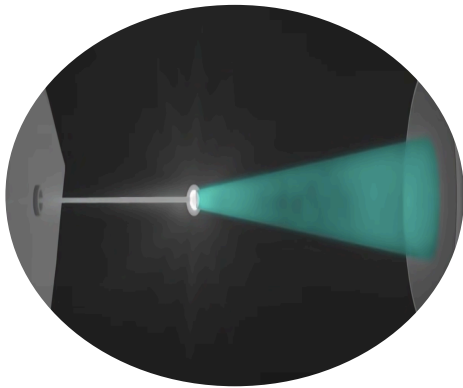
Instrumentation

- Reflection
- **Forward-scattered light (FSC)**
 - **surface area** or size of surface area or size of a cell
- **Side-scattered light (SSC)**
 - **granularity** or internal complexity of a cell



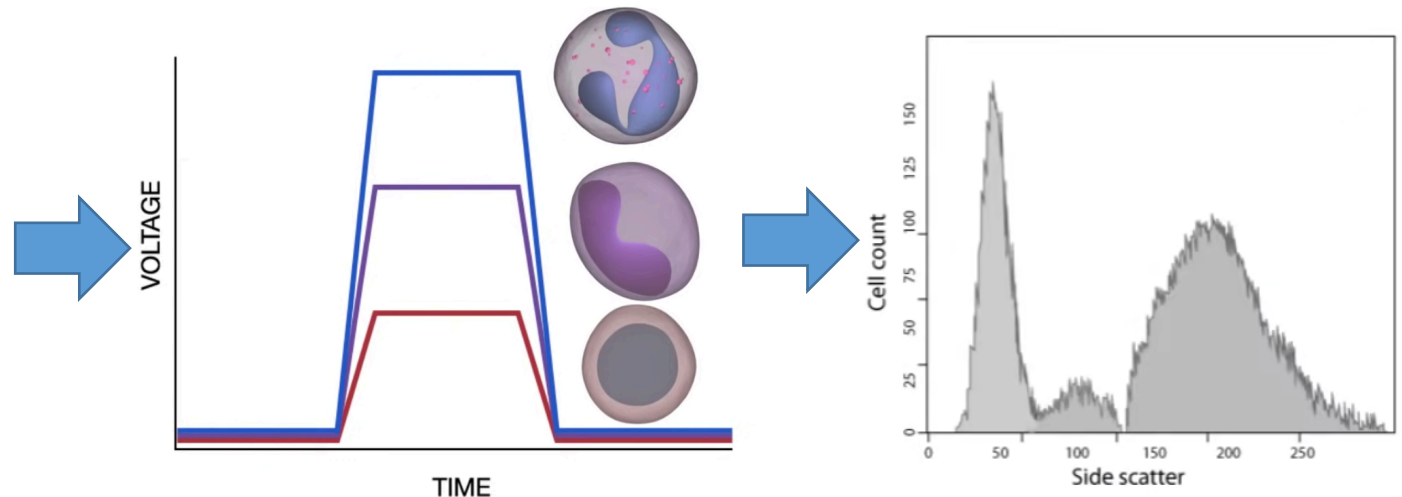
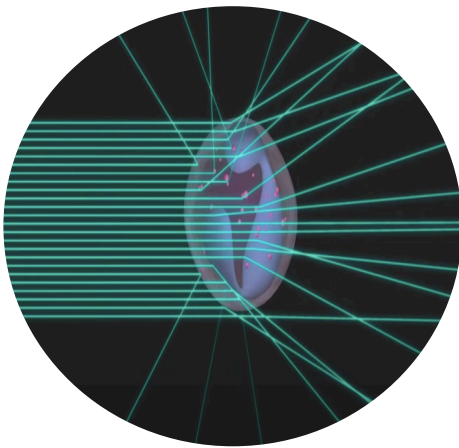
Instrumentation

Forwards-Scatter (FSC)

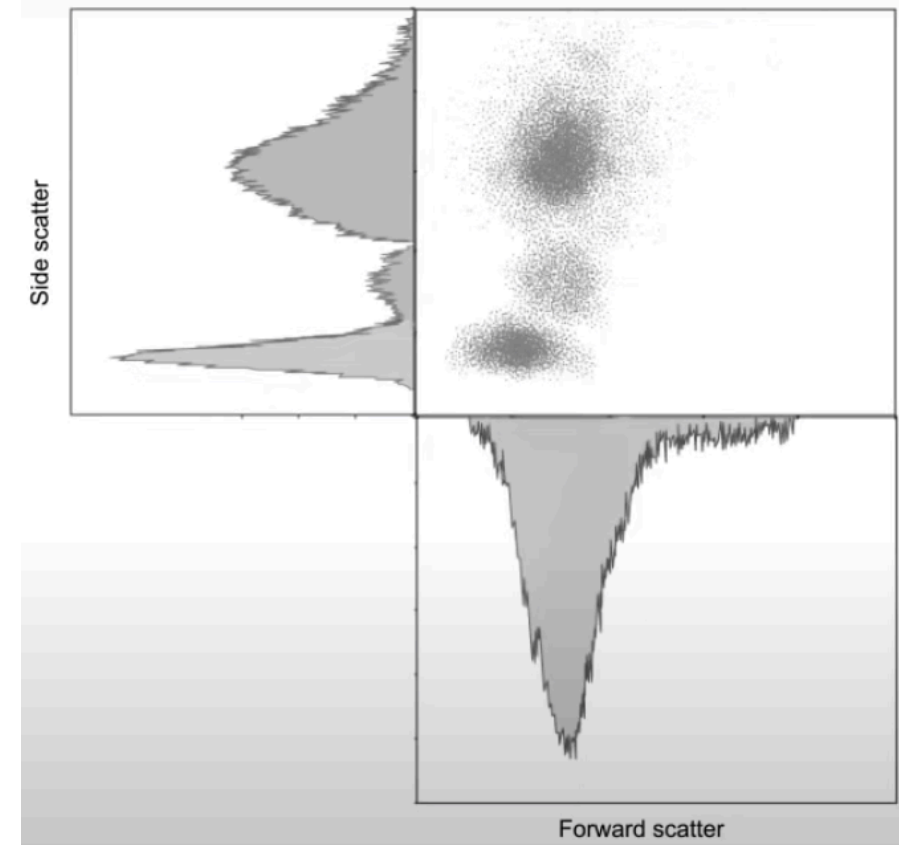
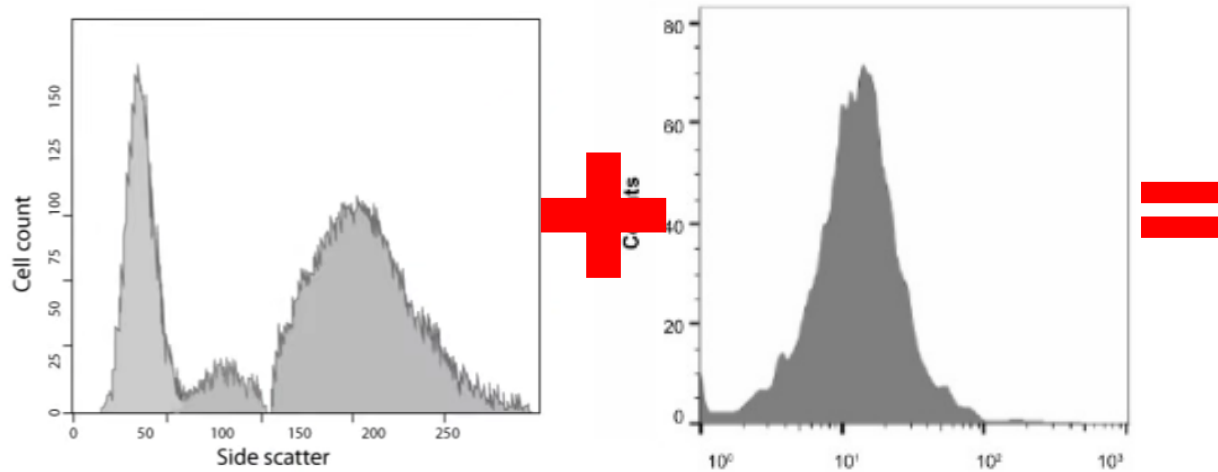


Instrumentation

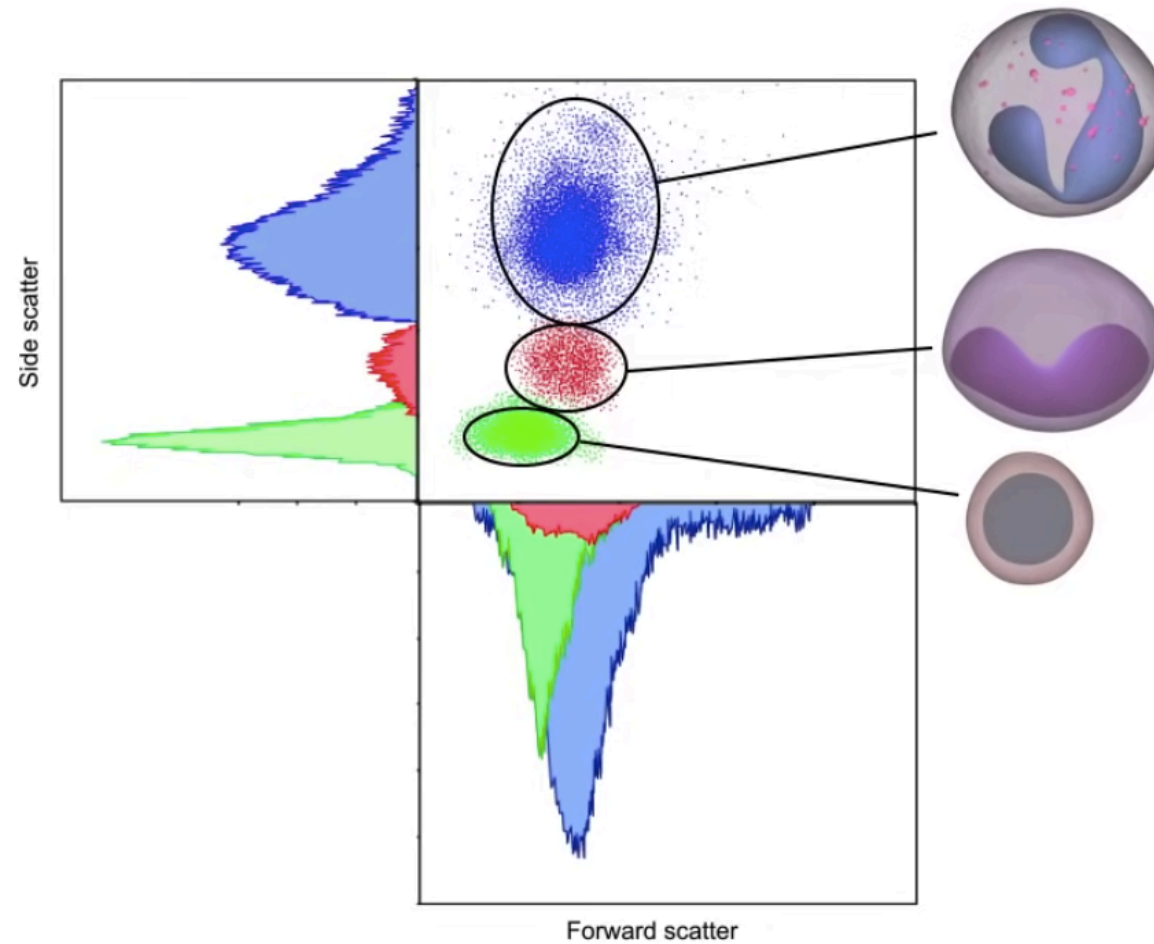
Side- Scatter (SSC)



Instrumentation



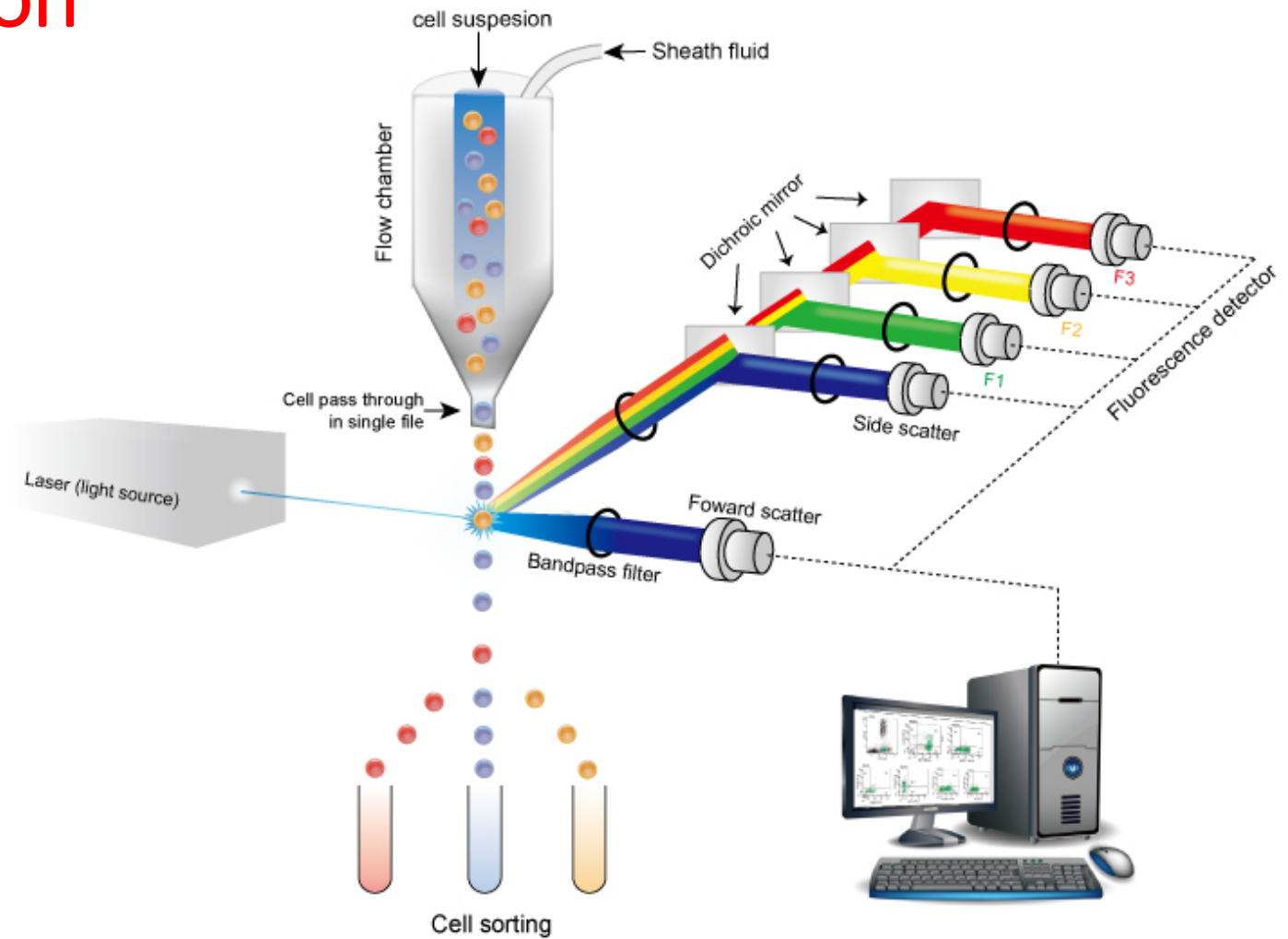
Instrumentation



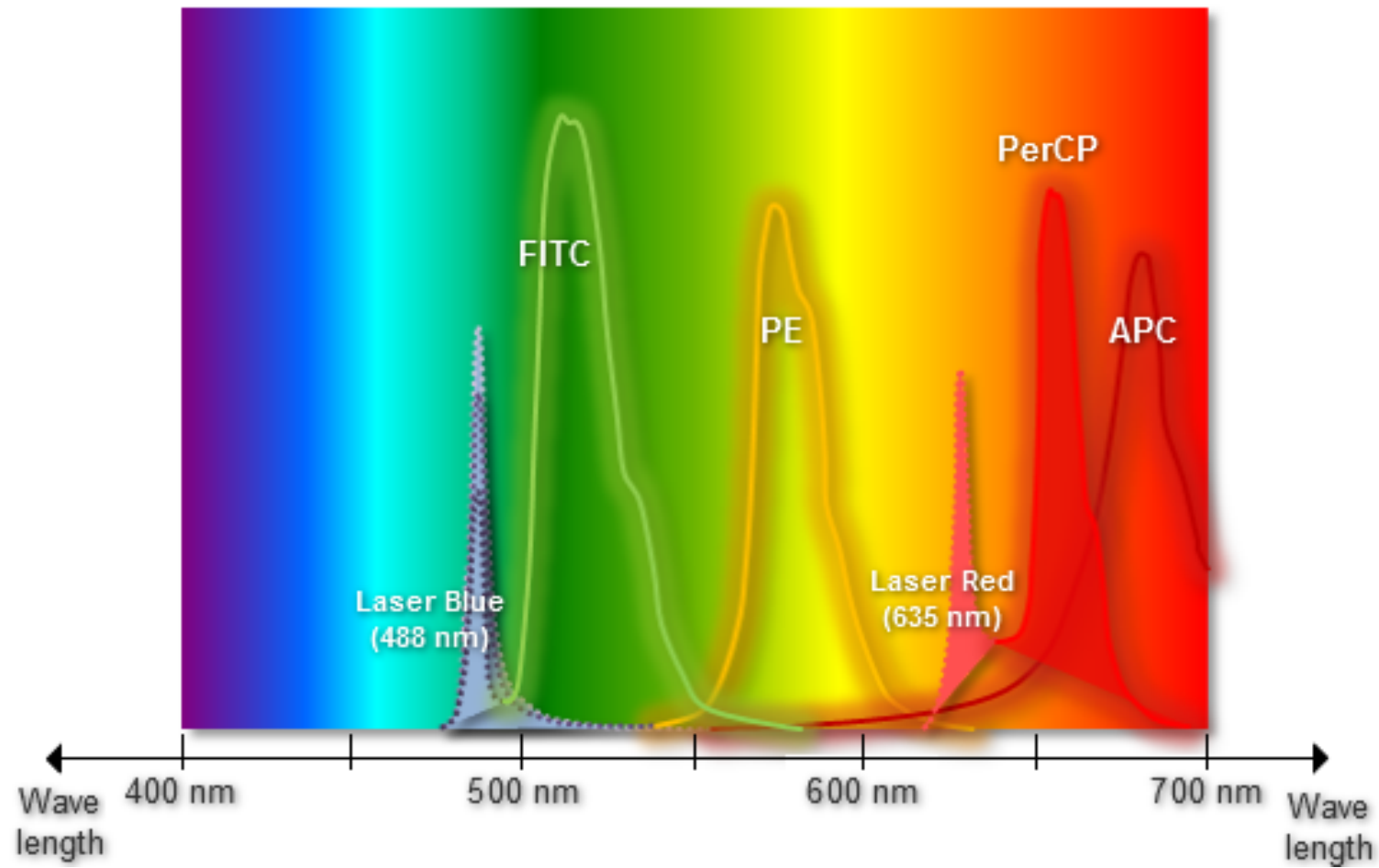
Instrumentation

4 Components

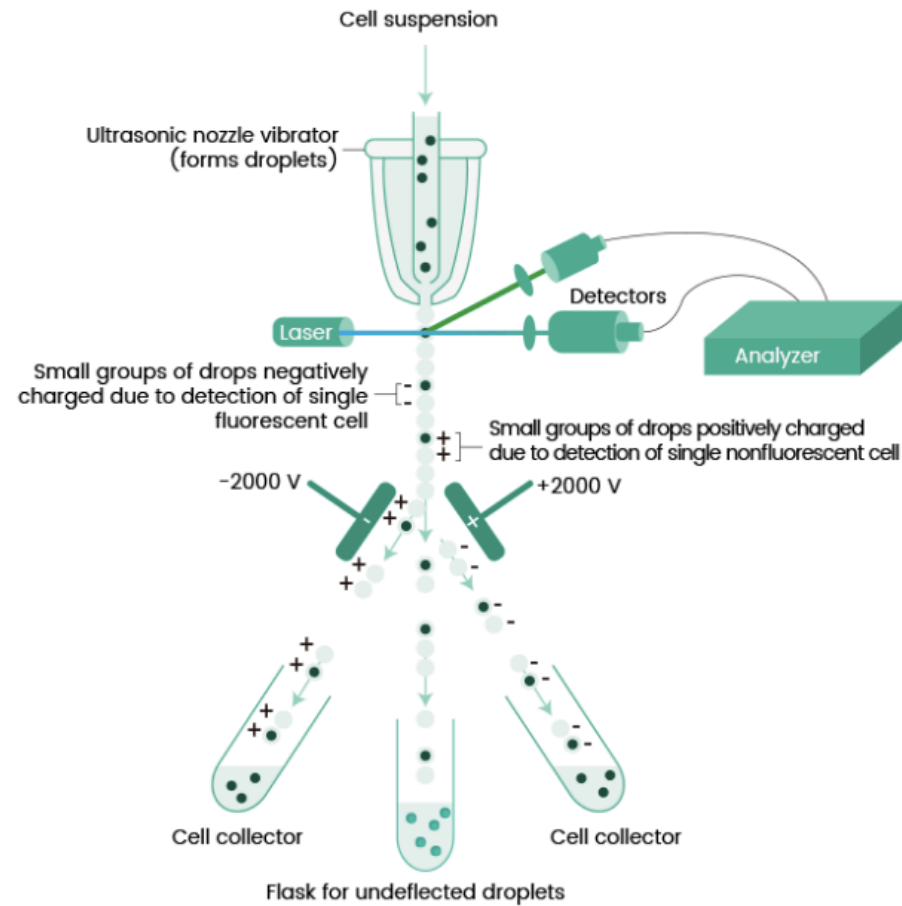
- Fluidics
- Lasers
- Optics
- Detectors
- Electronics



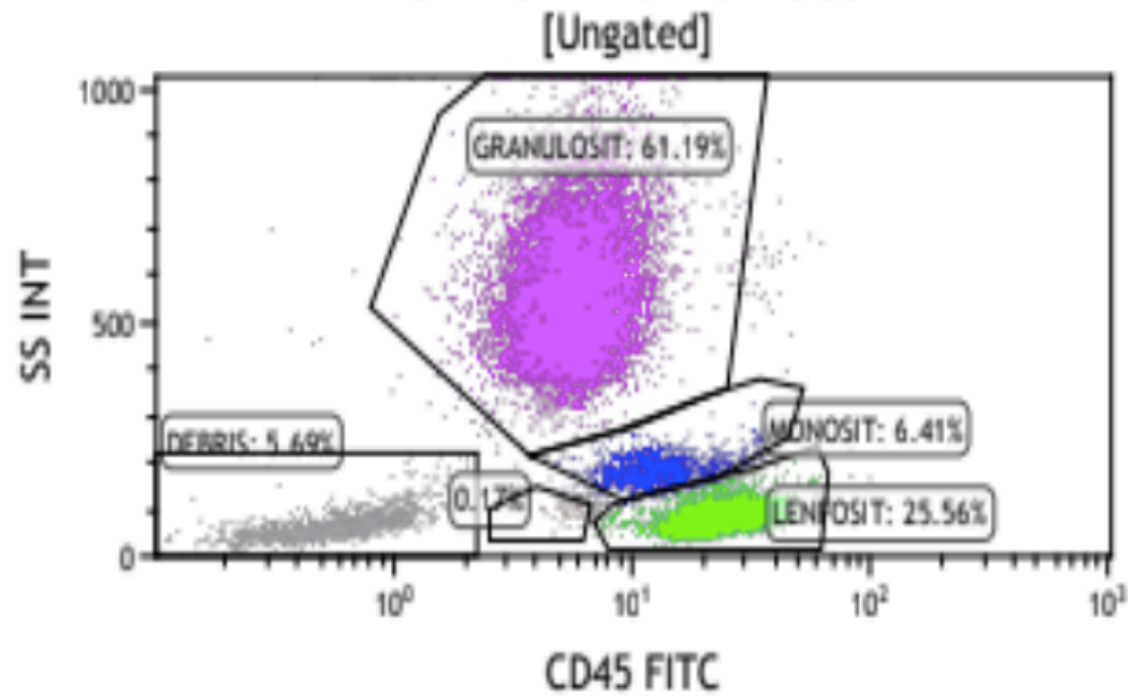
Fluorochromes



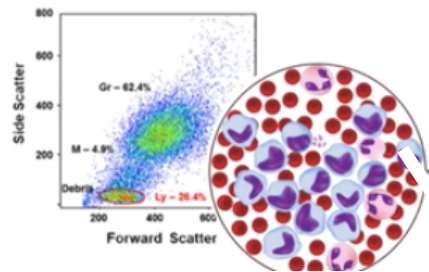
Cell Sorting



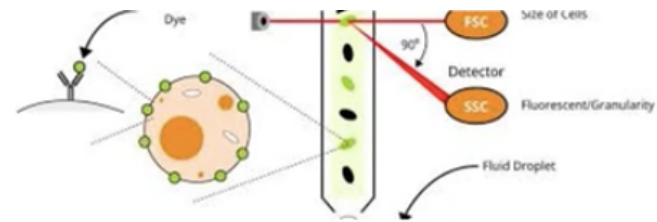
Gating



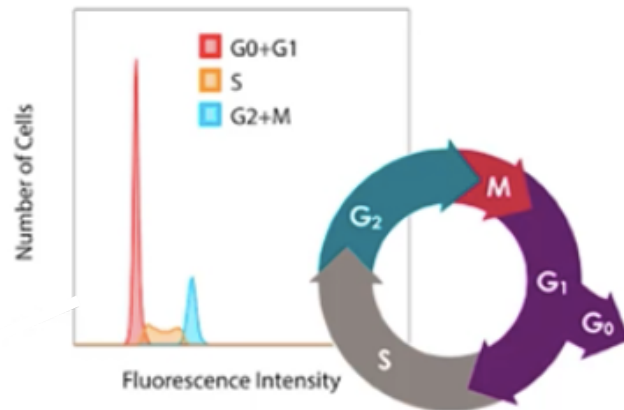
Uses of Flow cytometry



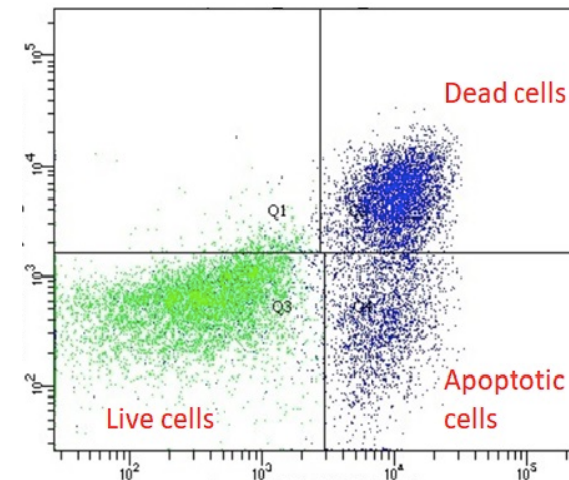
Cell sorting by size and complexity



Sorting cells based on fluorescence



Cell cycle analysis



Cell viability analysis

Example of Flow Cytometry Clinical Application

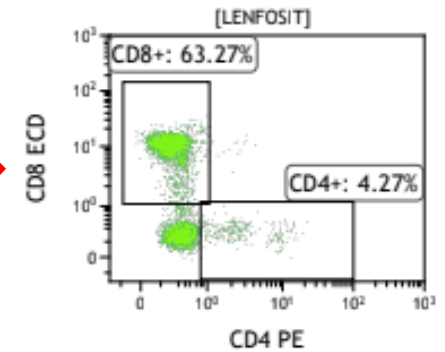
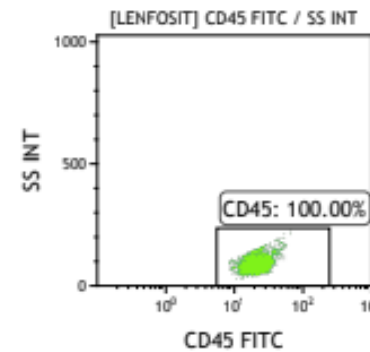
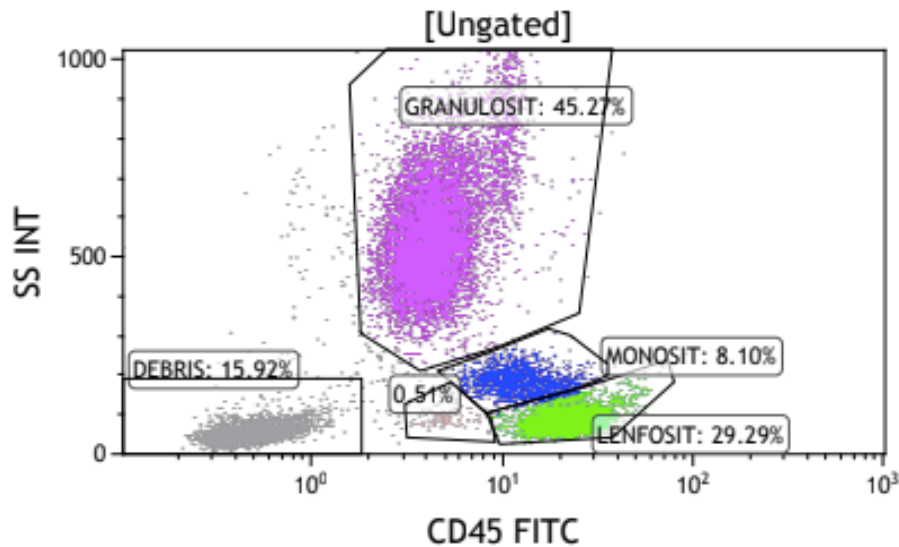
Calculating CD4 number of HIV positive patients

$$\text{Lymphocyte number} \times \%CD4 / 100$$

Normal CD4 number: 600-1200

Patient having closer to normal range of CD4 cells upon treatment means
IMMUNOLOGICAL healing

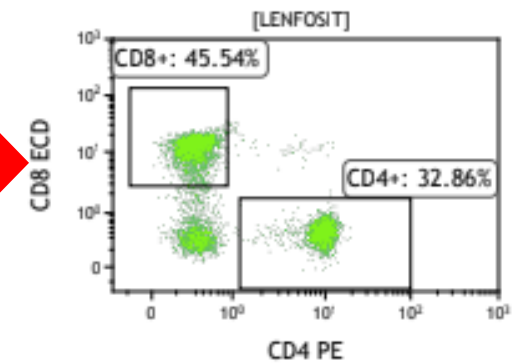
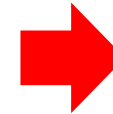
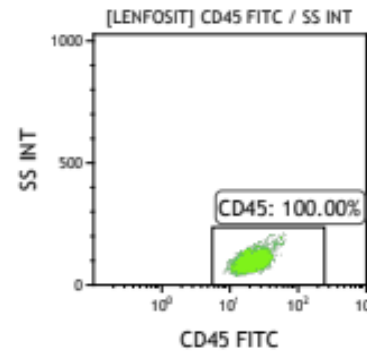
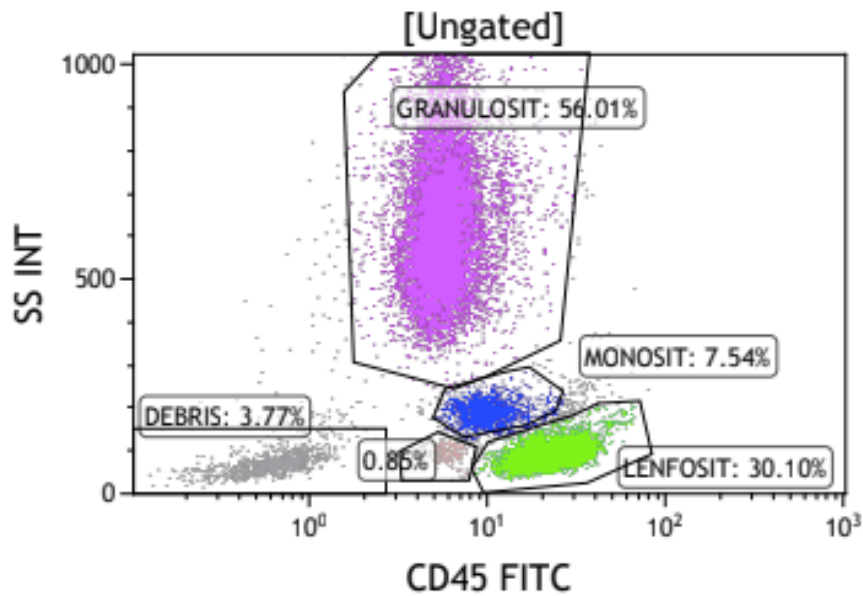
Patient A



Lymphocyte number x %CD4 / 100

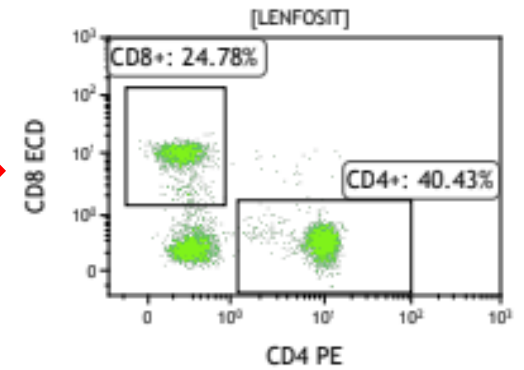
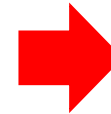
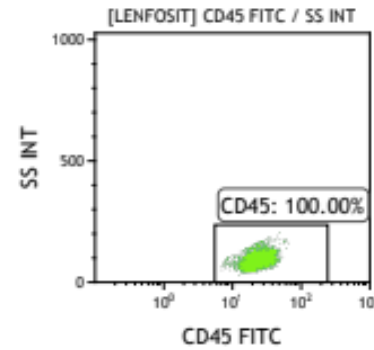
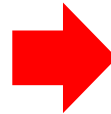
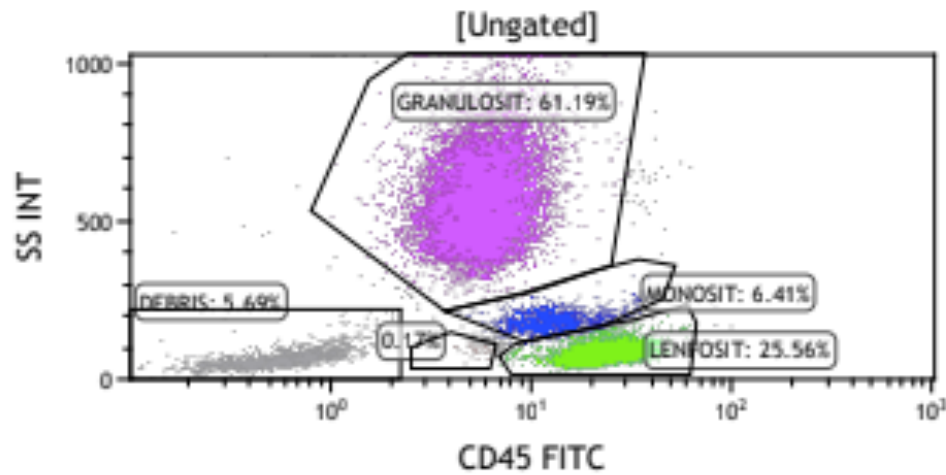
<200 CD4 absolute count – AIDS related
CD4 : CD8 proportion, if <1 = Problem

Patient B



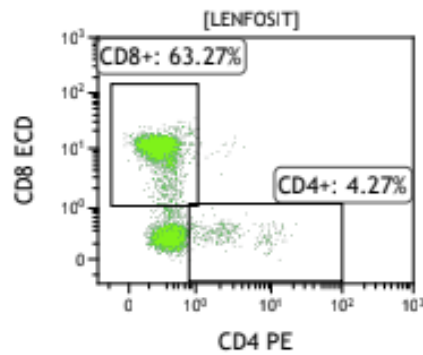
Lymphocyte number x %CD4 / 100

Patient C



Lymphocyte number x %CD4 / 100

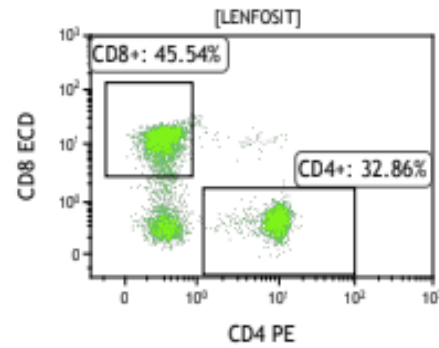
Patient A



CD4 : CD8

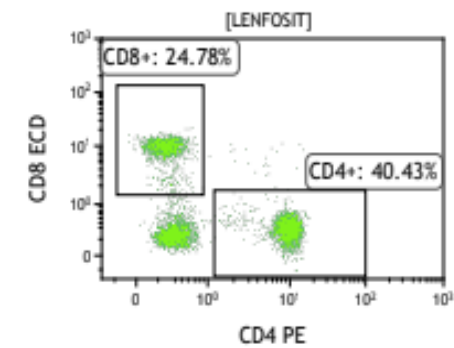
0.07

Patient B



0.72

Patient C



1.63

Flow Cytometer of New Era

Attune Flow Cytometers



- Sample throughput rates of 1,000 $\mu\text{L}/\text{min}$
- 10x faster than traditional cytometers without compromising data integrity.

CytoQuant Flow Cytometer



- Precise counts for each are provided **in 30 seconds**
- No** pre-treatment, incubation, or chemical reagents.

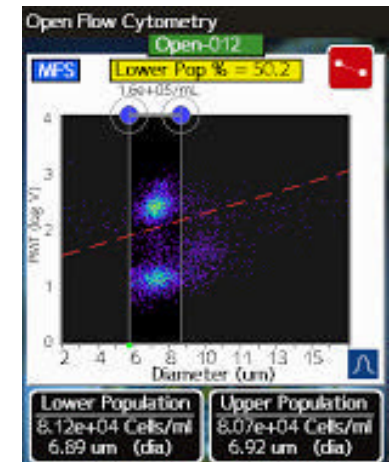
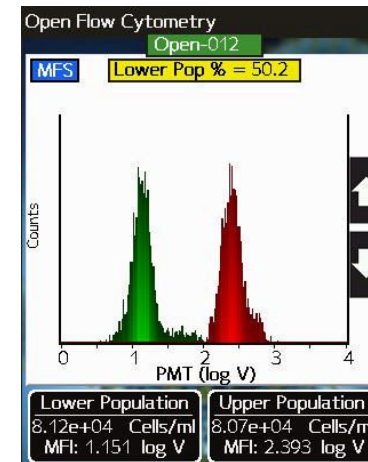
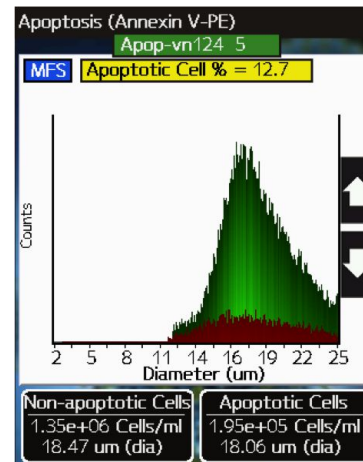
Flow Cytometer of New Era

MaxiFlow



Flow Cytometer of New Era

MaxiFlow





Thank you