FLOW CYTOMETRY

FCM

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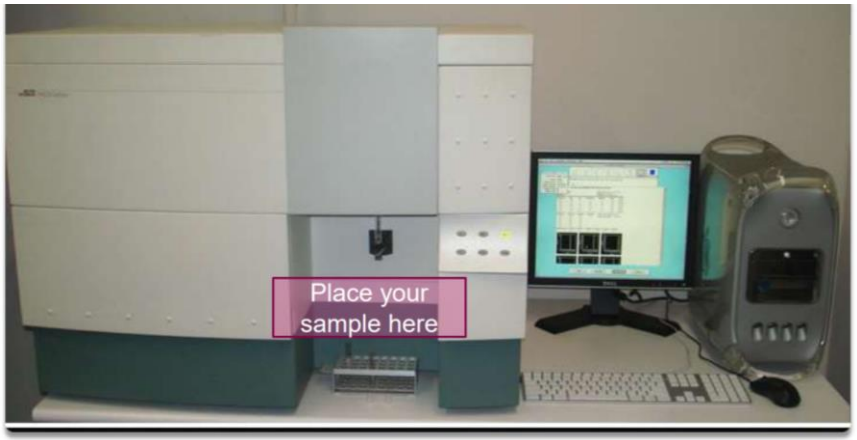
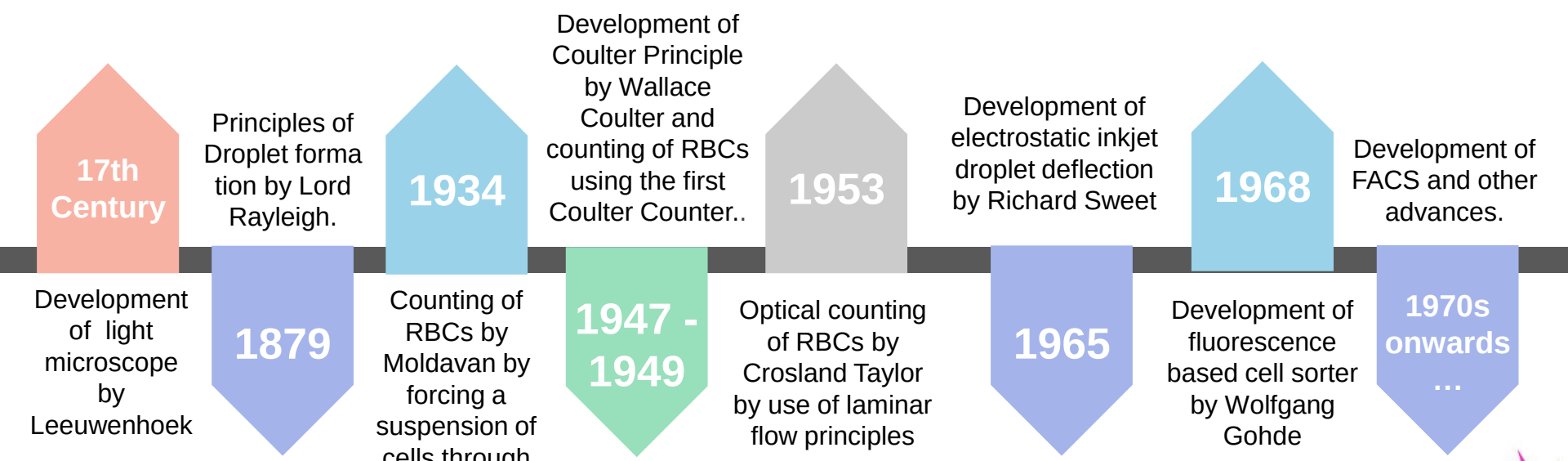
FLOW CYTOMETRY

- ‘Flow Cytometry’ as the name suggests is a technique for cell counting and measurement of different properties of the cell (‘cyto’= cell; ‘metry’=count/measurement).
- It is a laser based technology that measures and analyses different physical and chemical properties of the cells/particles flowing in a stream of fluid through a beam of light.

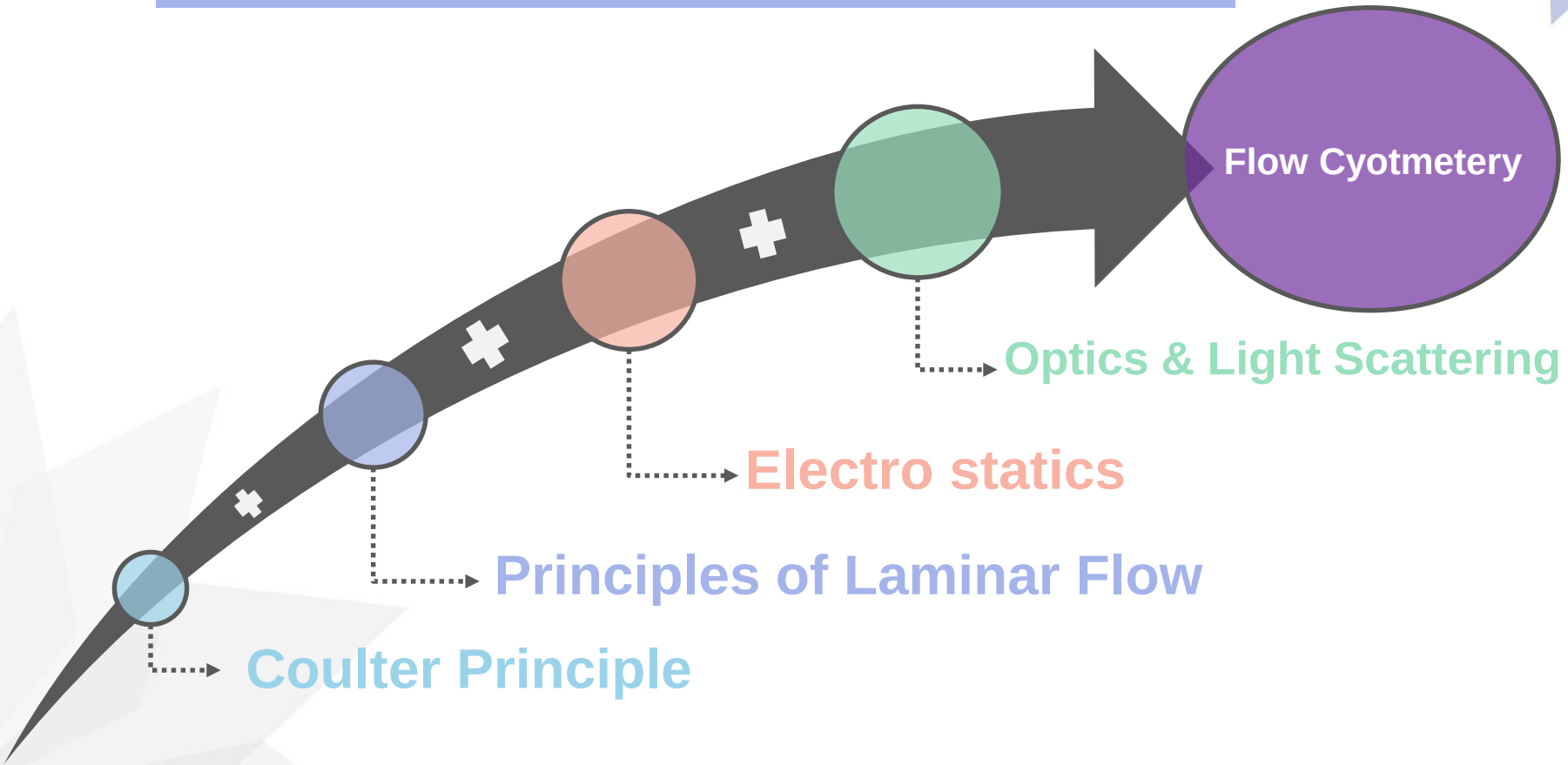


Historical Perspective

Evolution of Flow Cytometry



Principles of working of Flow Cytometer



Components of a Flow Cytometer

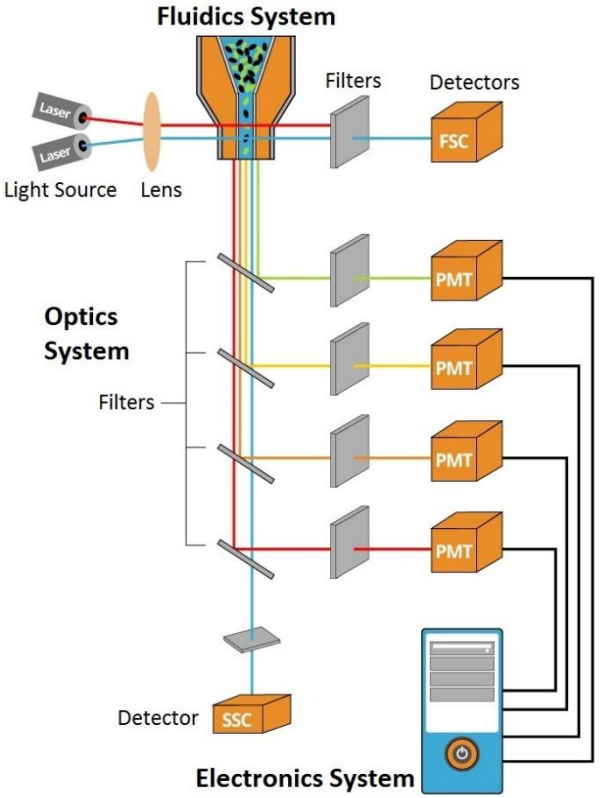
A flow cytometer is made up of three main systems: fluidics, optics, and electronics.

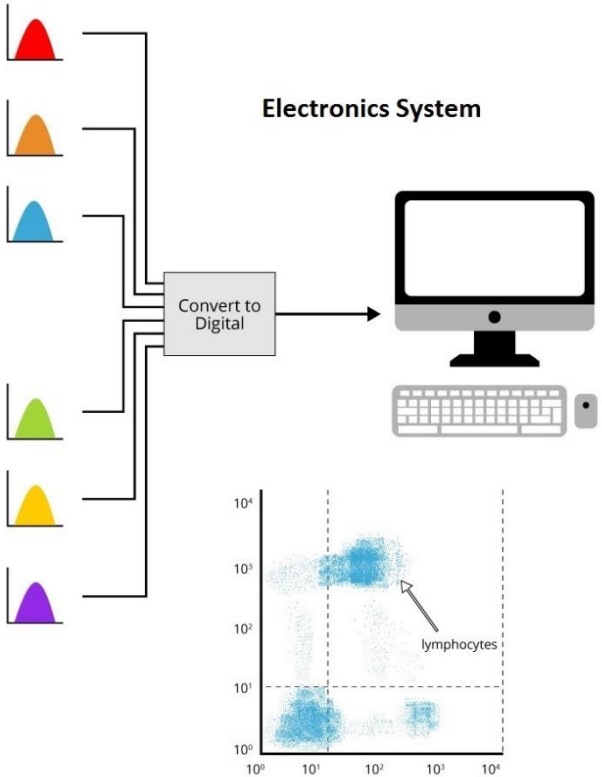
The fluidics system transports particles in a stream to the laser beam for interrogation. .

The optics system consists of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors.

The electronics system converts the detected light signals into electronic signals that can be processed by the computer . For some instruments equipped with a sorting feature, the electronics system is also capable of initiating sorting decisio ns to charge and deflect particles.

The Flow Cytometer

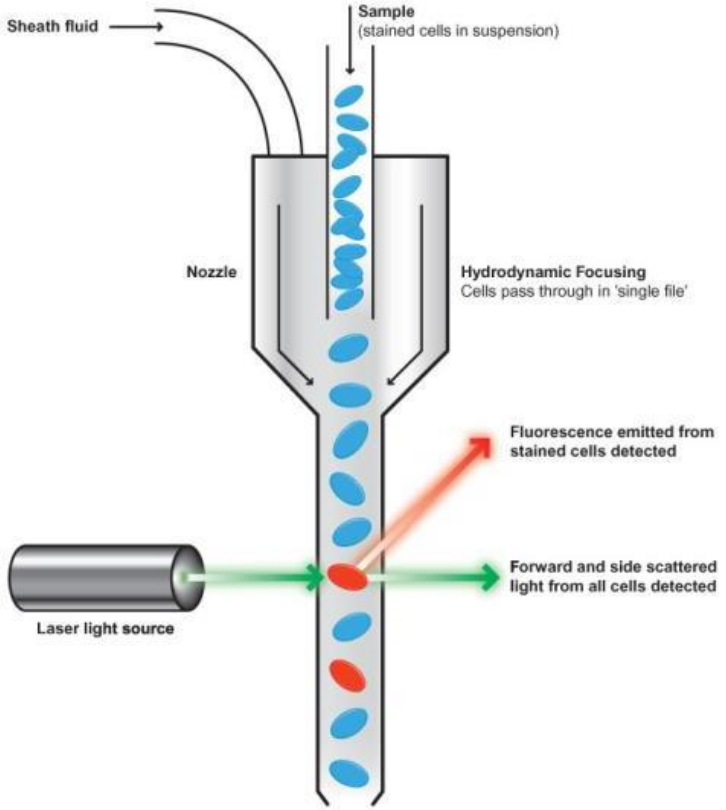




The fluidics system

Working of a Flow Cytometer

- In the flow cytometer, particles are carried to the laser intercept in a fluid stream. Any suspended particle or cell from 0.2–150 micrometers in size is suitable for analysis.
- The inner column of the flow cell consists of the liquid sample.
- The outer column of sheath fluid controls the diameter of the sample column so that it narrows and isolates single particles (**hydrodynamic focusing**).
- The single particles pass through a laser beam.
- The light scatter is detected by a photodetector.



The optics system

Types of Light Scatter

01

❖ Forward scatter (FSC):

As the cell passes through the **laser** beam, light is scattered in all directions and that scattered in the **forward** direction is proportional to the **square** of the **radius** of a sphere, and so to the **size** of the cell or particle.

- Large cells such as monocytes and neutrophils produce more forward scatter than nRBCs, and normal lymph's.

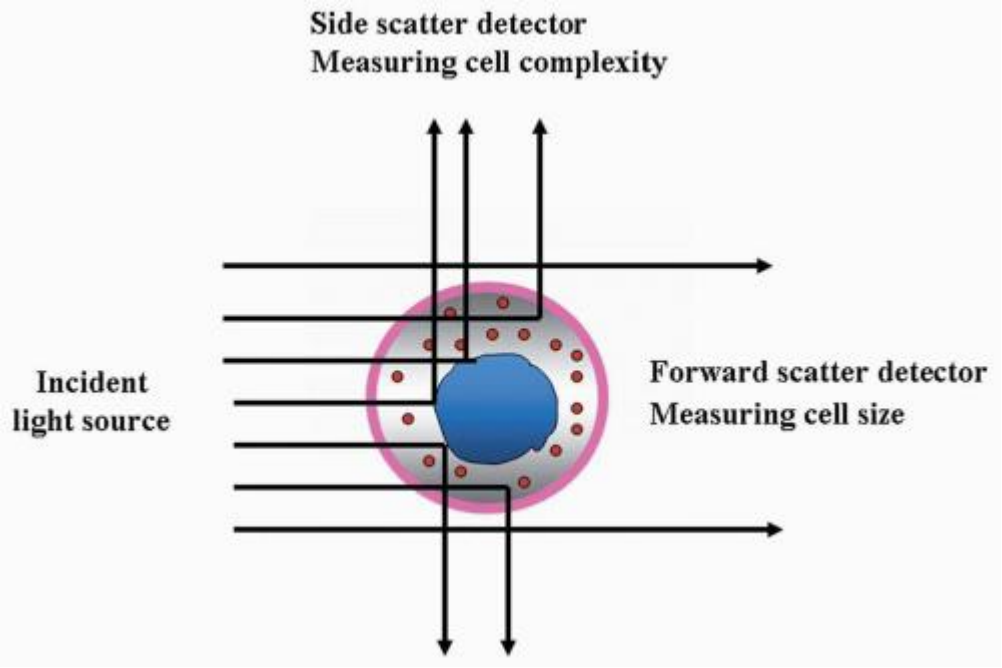
02

❖ Side light scatter (SSC) :

The amount of light scattered at right angle to the incident light beam depends on the **internal complexity** of the particl e, this known as wide angle or **Side Scatter** (SSC) , side sca tter detected at 90, to the laser beam.

- Neutrophils and eosinophils produce a great deal of side scatter due to their cytoplasmic granules

OPTICS
PROPERTIES OF FSC& SSC



Types of Light Scatter

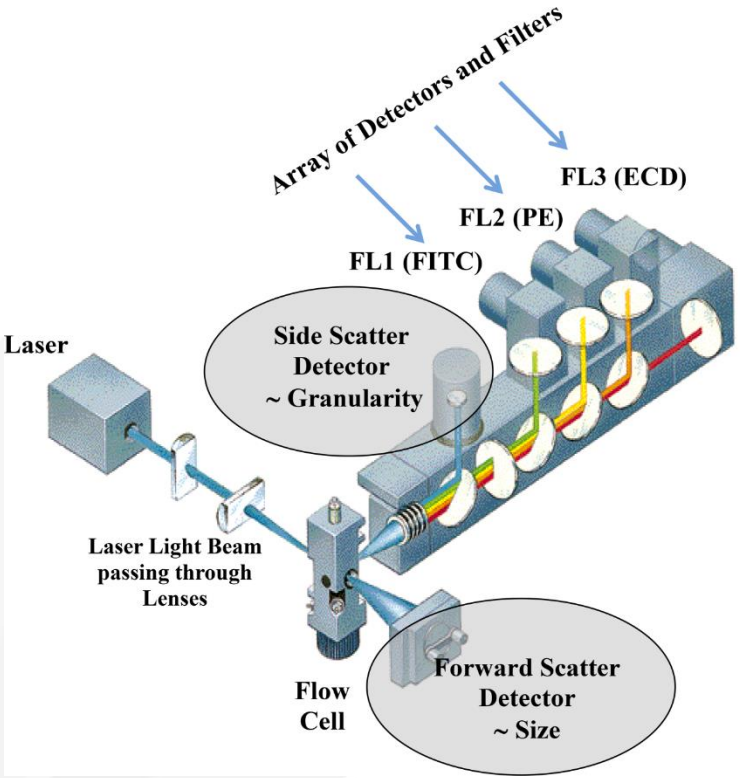
03

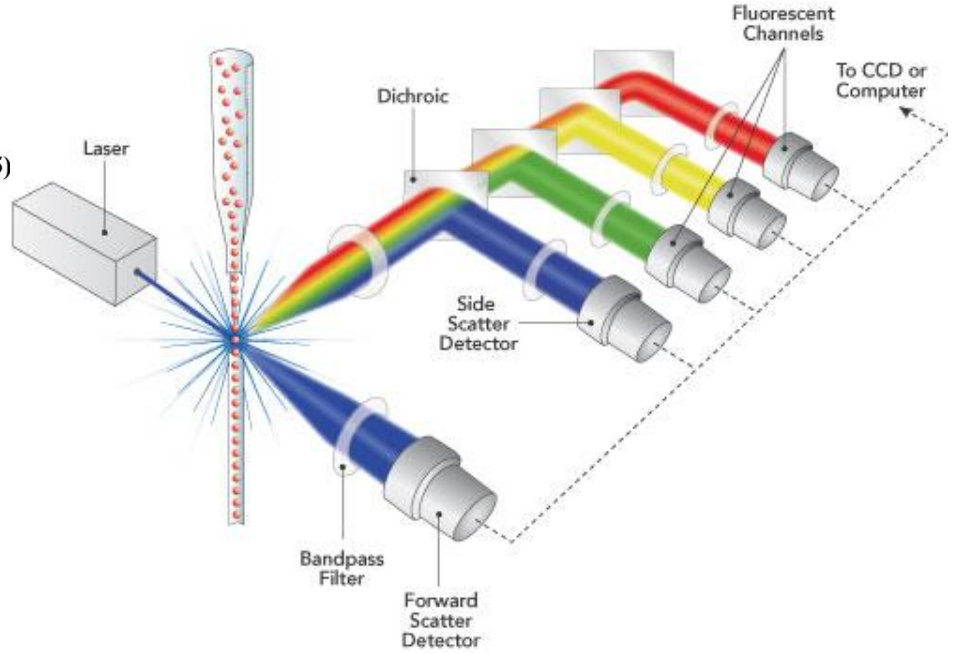
Fluorescence Channels:

❖The cells may be labeled with fluorochrome-linked antibodies or stained with fluorescent membrane, cytoplasmic or nuclear dye.

❖Any fluorescent molecule present in or on the particle will absorb energy from the laser light and release the absorbed energy at longer wave length, the emitted light is collected by lenses and detectors, emitted fluorescence intensity is proportional to the amount of fluorescent compound on the particle.

Optics - Fluorescence Channels





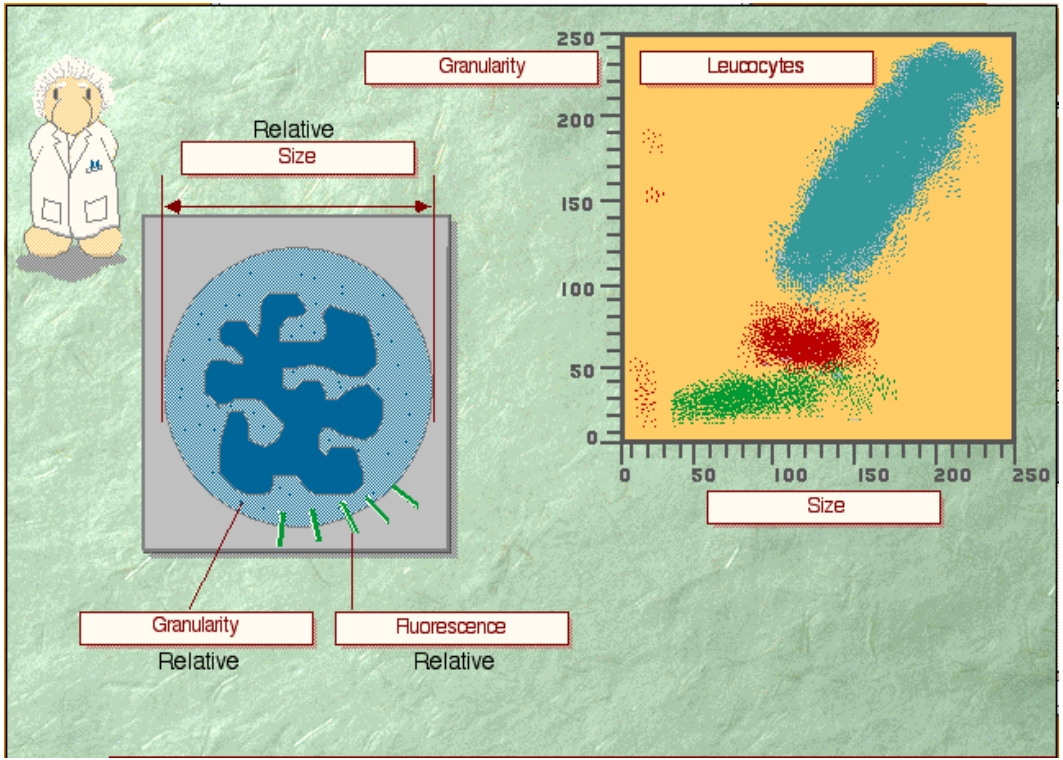
What can FCM tell us about a cell?

- Its relative size (Forward Scatter—FSC)
- Its relative granularity or internal complexity (Side Scatter—SSC)
- Its relative fluorescence intensity (FL1, FL2, FL3, FL4)

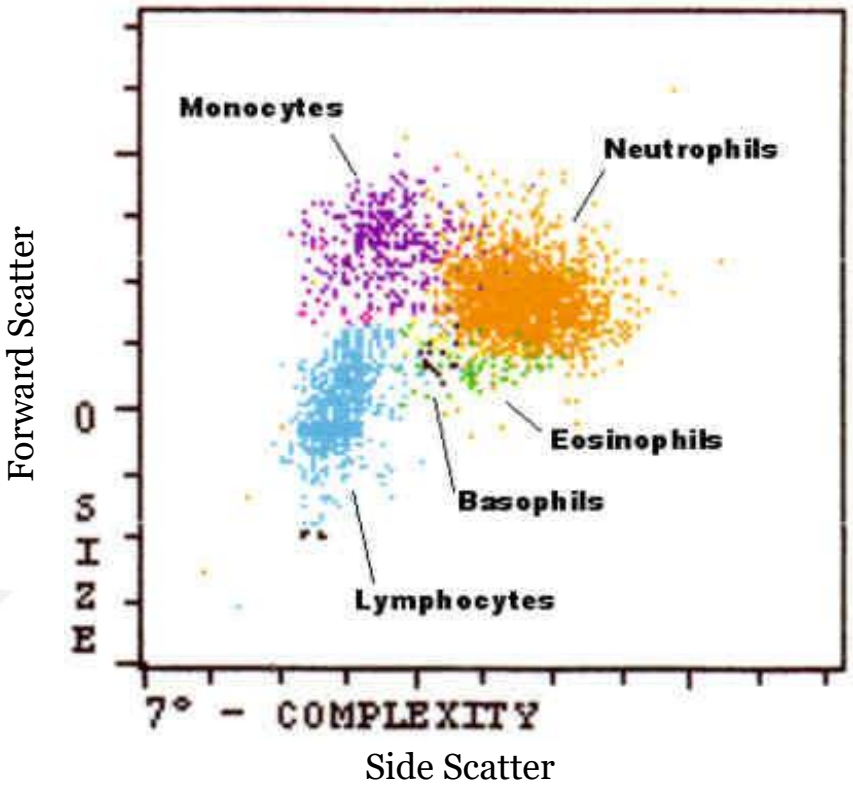
The electronics system

Electronics System

The scattered light from particl es passing the laser light is to digital values that stored in the computer for analysis.



Electronics System



Applications of FCM

Immunophenotyping

Cell subsets are measured by labeling population-specific proteins with a fluorescent tag on the cell surface. In clinical labs, immunophenotyping is useful in diagnosing hematological malignancies such as lymphomas and leukemia.

Cell Sorting

The cell sorter is a specialized flow cytometer with the ability to physically isolate cells of interest into separate collection tubes. The sorter uses sophisticated electronics and fluidics to identify and "kick" the cells of interest out of the fluidic stream into a test tube.

DNA Content Analysis

The measurement of cellular DNA content by flow cytometry uses fluorescent dyes, such as propidium iodide, that intercalate into the DNA helical structure. The fluorescent signal is directly proportional to the amount of DNA in the nucleus and can identify gross gains or losses in DNA.

Cell Cycle Analysis

Flow cytometry can analyze replication states using fluorescent dyes to measure the four distinct phases of the cell cycle. Along with determining cell cycle replication states, the assay can measure cell aneuploidy associated with chromosomal abnormalities.

Apoptosis

The two distinct types of cell death, apoptosis and necrosis, can be distinguished by flow cytometry on the basis of differences in morphological, biochemical and molecular changes occurring in the dying cells.

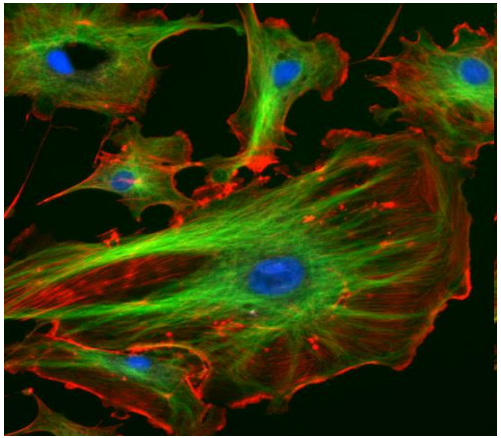
Cell Proliferation Assays

The flow cytometer can measure proliferation by labeling resting cells with a cell membrane fluorescent dye, carboxyfluorescein succinimidyl ester (CFSE). When the cells are activated, they begin to proliferate and undergo mitosis. As the cells divide, half of the original dye is passed on to each daughter cell. By measuring the reduction of the fluorescence signal, researchers can calculate cellular activation and proliferation.

Fluorochromes

Fluorochromes are fluorescent compounds that can be bound by antibodies to the particles in the sample.

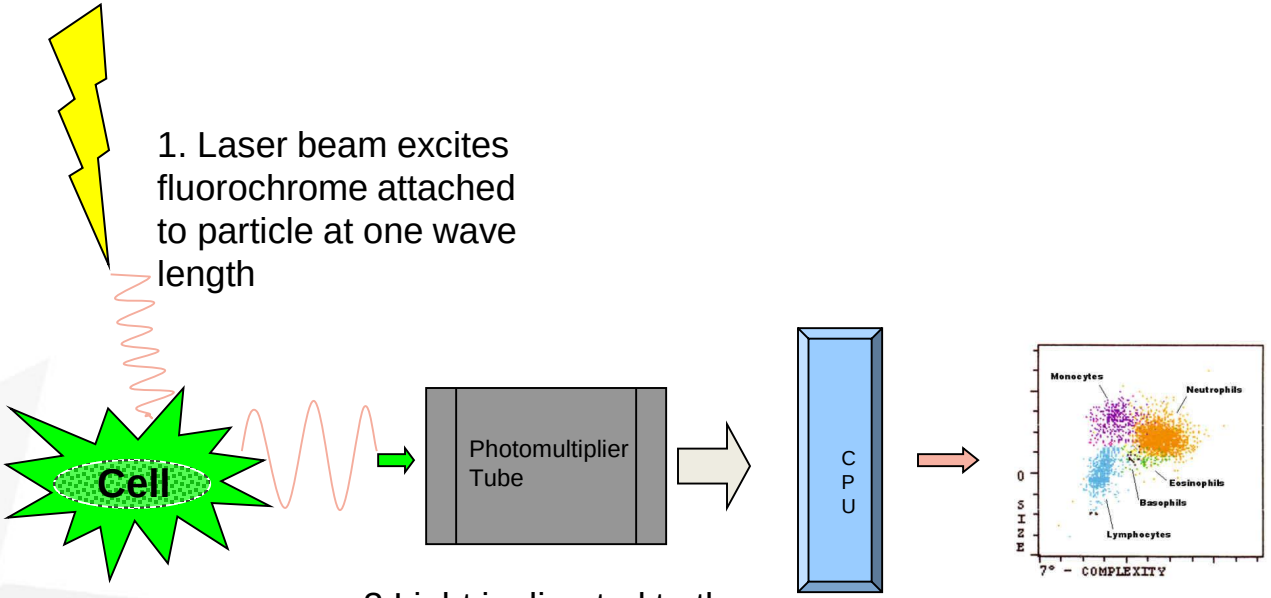
- Examples: FITC, PE, Propidium iodide, Acridine orange



Fluorochromes are used in:

- Immunophenotyping
- Cell differentiation and enumeration
- Reticulocyte counting
- DNA analysis

Detection of Fluorochromes



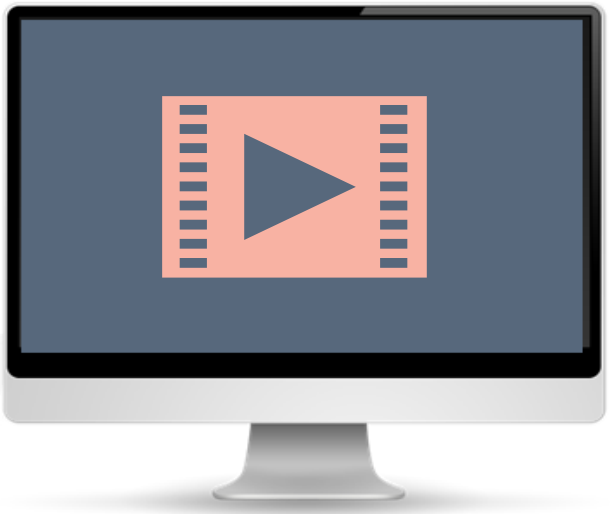
1. Laser beam excites fluorochrome attached to particle at one wave length

2. Fluorochrome em its light atdifferent wave length

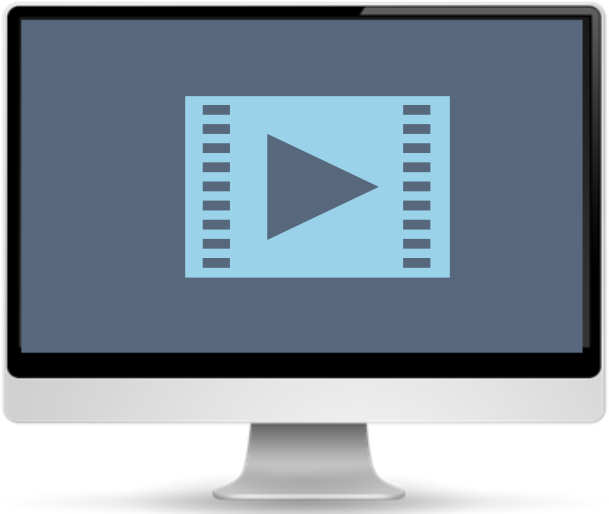
3.Light is directed to the photomultiplier tube that detects and quanitifies the light.

4. Computer software analyzes data and generates scatterplot

Educational videos



Interdiction



Principal

Thank you

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