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Flow Cytometry Technique

BY

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Definition

Measuring properties of cell as they flow in a fluid suspension across an illuminated light path.

simultaneously measures and analyzes multiple physical characteristics of single particles, usually cells, as they move in a fluid stream through a beam of light.

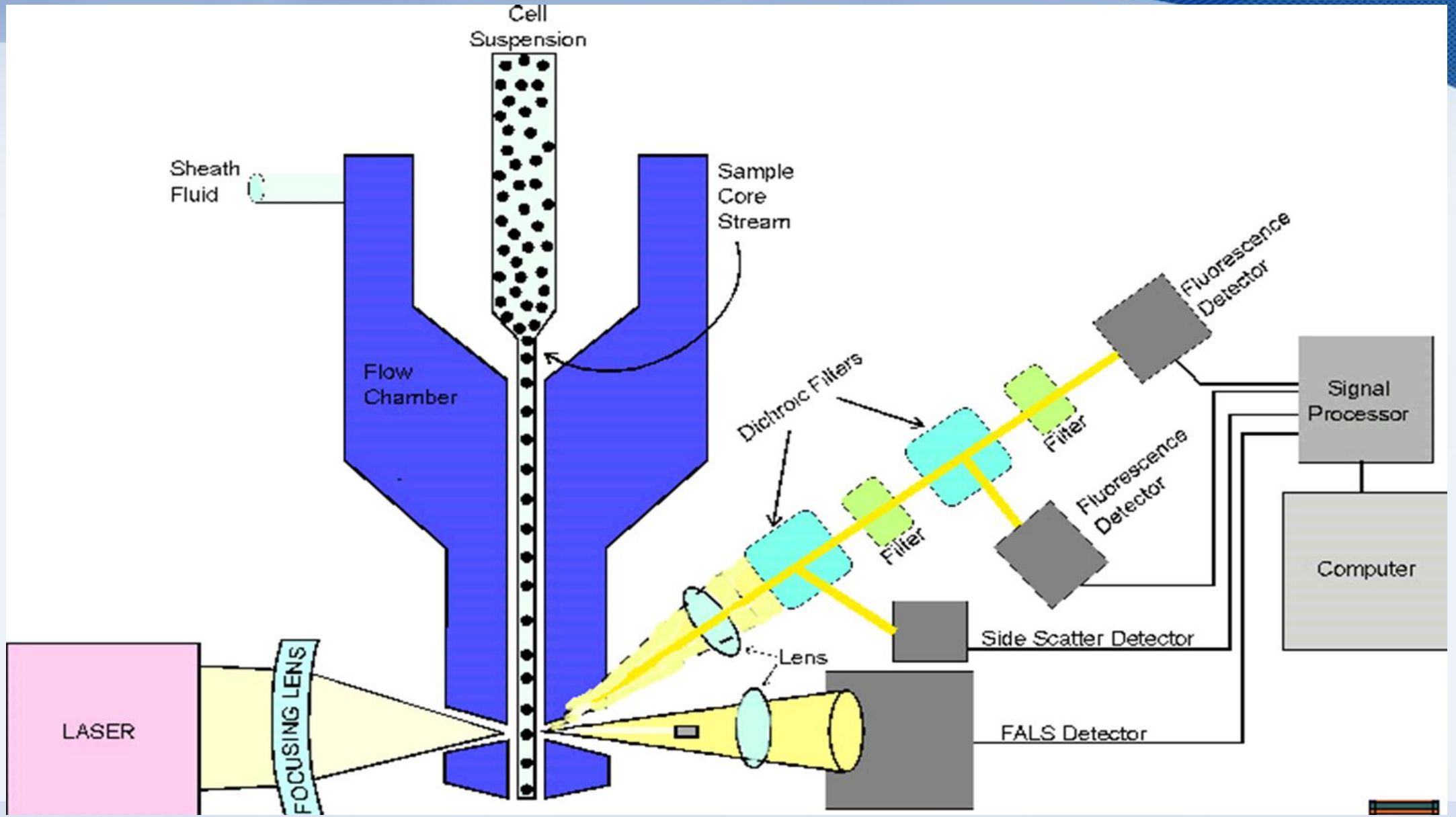
- **Any suspended particle between 0.2 and 50µM is suitable.**

- **Larger particles, solid tissue or clumps of cells must be disaggregated to be analyzed.**

Examples: **lymphocytes, protozoa, micron beads, chromosomes**

- **The particles in the fluid stream scatter incident light , which reveals internal properties, size and granularity.**

- **The particles also fluoresce; they emit laser light at the interrogation point; this light is picked up by detectors arrayed at a different angle to detectors of scattered light.**


Cell
Suspension
Sheath
Fluid
Sample
Core
Stream
Flow
Chamber
Dichroic Filters
Filter
Fluorescence
Detector
Signal
Processor
Filter
Fluorescence
Detector
Computer
Side Scatter Detector
Lens
LASER
FOCUSING LENS
FALS Detector

Principle of Flow Cytometry

Biological sample

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Label it with a fluorescent marker

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Cells move in a linear stream through a focused light source (laser beam)

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Fluorescent molecule gets activated and emits light that is filtered and detected by sensitive light detectors (usually a photomultiplier tube)

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Conversion of analog fluorescent signals to digital signals

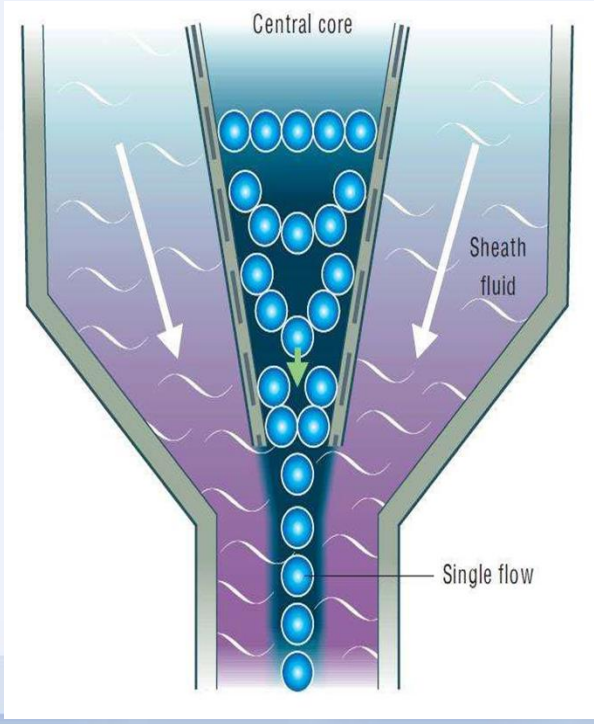
The Flow System

- One of the fundamentals of flow cytometry is the ability to measure the properties of individual particles, which is managed by the fluidics system.
- When a sample is injected into a flow cytometer, it is ordered into a stream of single particles.
- The fluidic system consists of a **FLOW CELL** (Quartz Chamber):
  - Central channel/ core - through which the sample is injected.
  - Outer sheath - contains faster flowing fluid k/a Sheath fluid (0.9% Saline / PBS) , enclosing the central core.

Hydrodynamic Focusing

Once the sample is injected into a stream of sheath fluid within the flow chamber, they are forced into the center of the stream forming a single file by the PRINCIPLE OF HYDRODYNAMIC FOCUSING

'Only one cell or particle can pass through the laser beam at a given moment.'



Fluidics

Concerns:

1.Shear rates for cells: check after you complete a run to ensure that the cells are intact.

2.Larger tips are needed for cell sorting.

Optics

Filters resolve overlapping wavelengths of emitted light.

- **Longpass Filter:** transmits light of longer than or equal to a specific wavelength.
- **Shortpass filter:** transmits light of shorter than or equal to a specific wavelength.
- **Bandpass filter:** transmits light only within a narrow range of wavelengths.

Electronics

The electronic system

1. Quantifies the voltage pulse.
2. Converts analog signals to digital values.
3. Performs compensation.
4. Transfers data to the computer for analysis.


THank
you