ADVANCED LABORATORY TECHNIQUES

FLOW CYTOMETRY

جامعـــــة ســـــاوة الاهلية — Sawa University

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المرحلـــــــــة الثالثة — third Stage .

الجانب النظري

Theoretical

محاضرة رقم 6

Lecture No. 6

تدريسي المادة : م.م طيبة شاكر

(INTRODUCTION)

- **Flow cytometry** is a **standard laser-based technology that is used in the detection and measurement of physical and chemical characteristics of cells or particles in a heterogeneous fluid mixture.**

- The use of flow cytometry has increased over the years as it provides a rapid analysis of multiple characteristics (both qualitative and quantitative) of the cells.

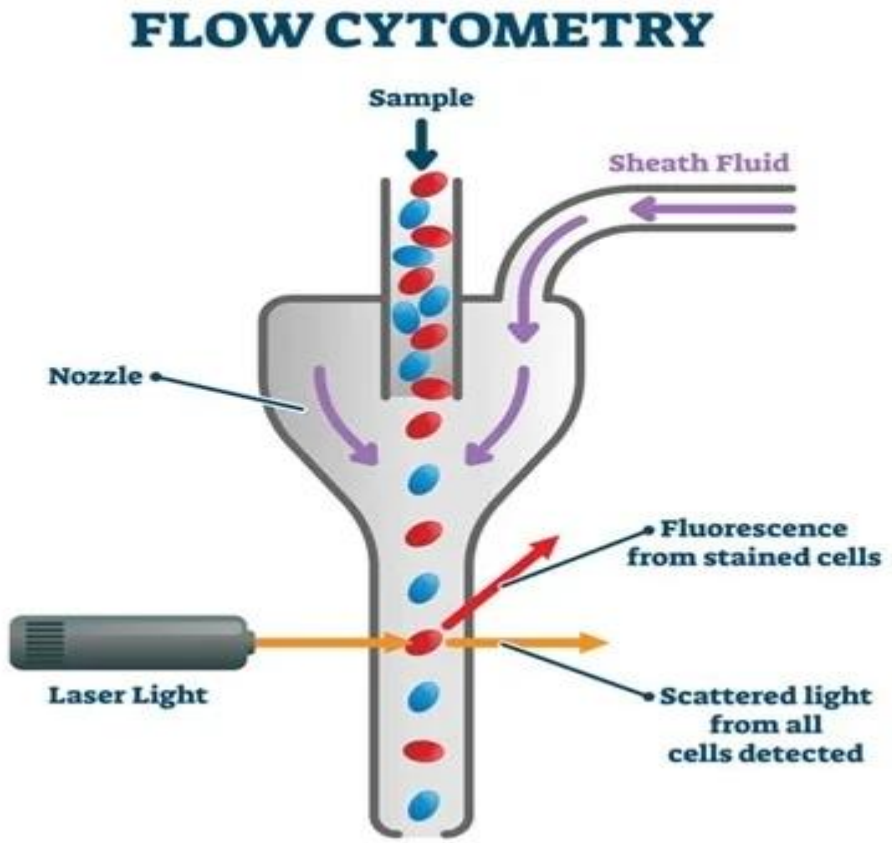
- The properties that can be measured by this process include a **particle's size, granularity or internal complexity, and fluorescence intensity.**

- These characteristics are determined using an optical-to-electronic coupling system that detects the cells based on laser scattered by the cells.

- A flow cytometer, despite its name, does not necessarily deal with cells; it deals with cells quite often, but it can also deal with chromosomes or molecules or many other particles that can be suspended in a fluid.

Flow cytometry principle

The **basic principle of flow cytometry is based on the measurement of light scattered by particles, and the fluorescence observed when these particles are passed in a stream through a laser beam**



Flow cytometry is a powerful analytical technique used to measure **physical and chemical characteristics** of cells or particles suspended in a fluid as they pass individually through a **laser beam**.

It relies on the emission and detection of **light scattering** and **fluorescence** from labeled cells.

- When a cell passes through the **laser beam (usually 488 nm blue light)**, it scatters light and may emit fluorescence if stained with **fluorochrome-conjugated antibodies** (e.G., FITC, PE, percp).
- Each signal is converted into an electrical pulse and then analyzed by a **computer system**.

Components of a flow cytometer

1. **Fluidics system** – directs cells in a narrow stream so they pass one by one through the laser beam.

2. **Optical system** – includes:
    1. **Laser light source**
    2. **Lenses** to focus light
    3. **Filters and dichroic mirrors** to separate emitted fluorescence

3. **Detectors (photomultiplier tubes, pmts)** – capture forward and side scatter plus fluorescence.

4. **Electronics and computer system** – convert light signals to digital data for analysis

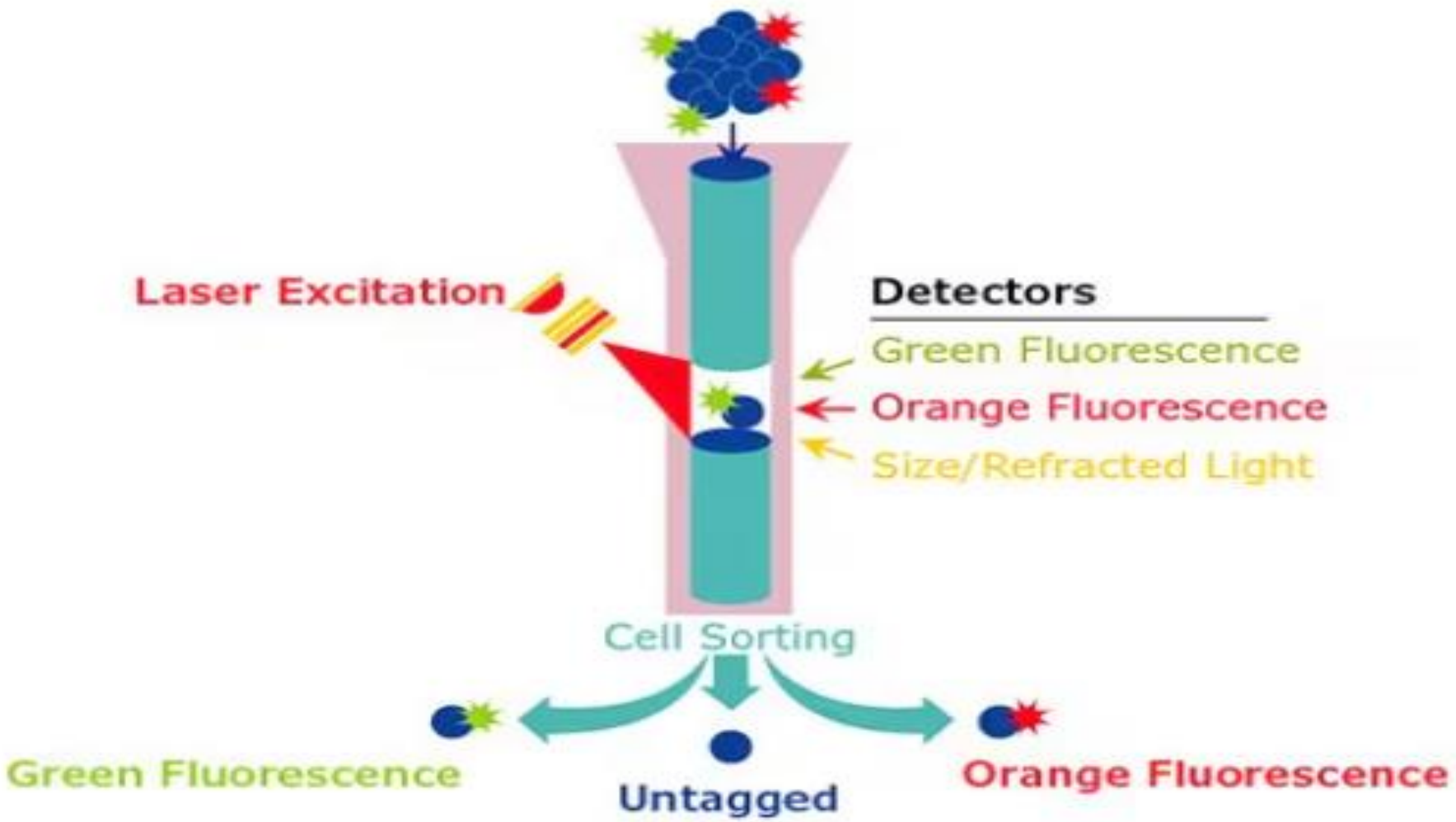
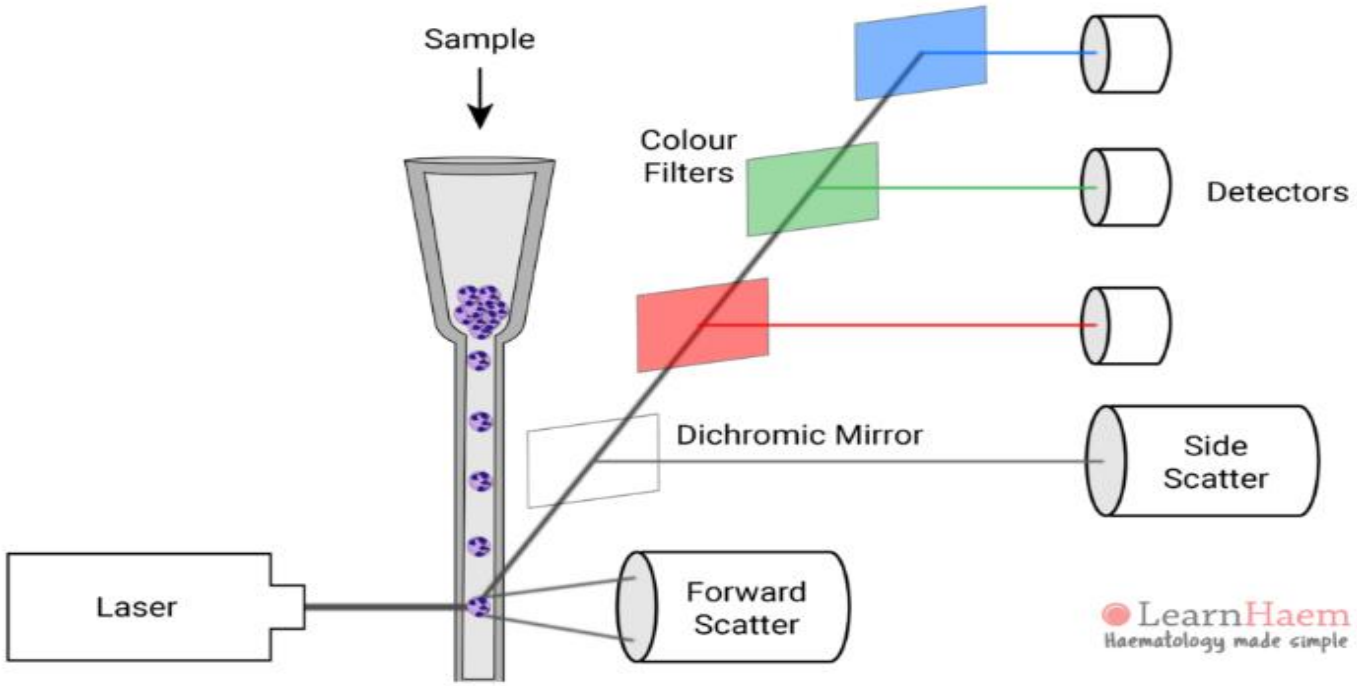


Figure 1. Diagram of typical flow cytometric analysis.

Types of light detected

- **Forward scatter (FSC):** correlates with **cell size**.
- **Side scatter (ssc):** correlates with **cell complexity or granularity**.

These parameters allow **cell population identification** in mixed samples



Fluorescence detection

Cells stained with **fluorescently labeled antibodies** emit light at longer wavelengths after excitation.

- Common fluorochromes:
  - **FITC (green emission)**
  - **PE (orange-red emission)**
  - **Percp (red emission)**
- Multiple dyes can be detected simultaneously using **optical filters** to separate their emissions.

Applications

Clinical uses:

- Immunophenotyping (e.G., CD markers)
- Tumor cell detection and monitoring
- Cell-cycle and chromosomal analysis
- Quantification of apoptosis

Research uses:

- Analysis of receptor distribution and expression
- Intracellular signaling studies
- Measurement of cytokine production

Advantages:

- Rapid analysis (hundreds to thousands of cells per second)
- High sensitivity and specificity
- Multiparametric measurement (size, granularity, multiple antigens)

Limitations:

- Requires single-cell suspension
- Fluorescence overlap between dyes needs compensation
- Expensive instrumentation and technical expertise

QUIZ

H.W:

- If two cell populations show identical forward scatter (FSC) values but very different side scatter (SSC) intensities in a flow cytometry analysis — what could this indicate about their structural or biological differences, and why?

Any questions?