HEMATOLOGY
&LYMPH SYSTEM

Histology sheet

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LAB-2

HEMATOLOGY

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FLOW CYTOMETRY

Flow cytometry is a popular laser-based method to analyze the characteristics of cells or particles.

It is predominantly used to measure fluorescence intensity produced by fluorescent-labeled antibodies detecting proteins, or ligands that

bind to specific cell-associated molecules. Flow cytometry is a method of measuring multiple physical and chemical characteristics of particles by optical means.

Above mentioned technique also used to:

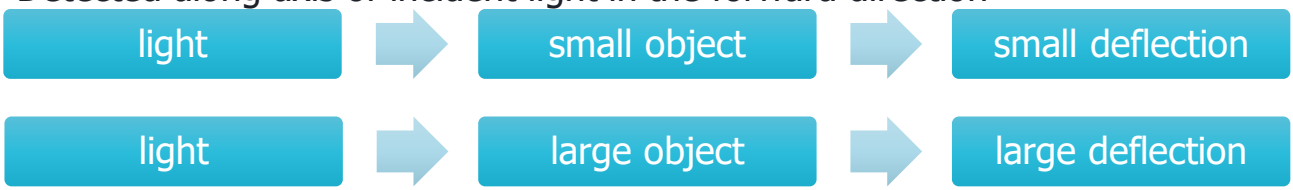
1- identify surface markers
2- during Immunophenotyping processes.

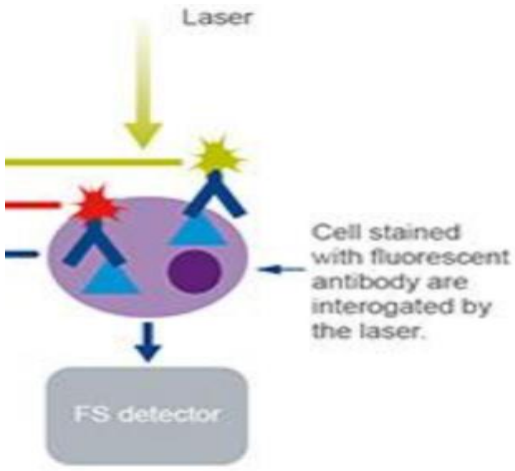
-How does this system work?

We make the cells flow through a nozzle to form a single line of cells then we push them by a fluid that exert hydrostatic pressure. This type of pressure will push the cells one cell at the time. Then we focus a laser (light) on them. This light will change its character so it either will be reflected, deflected or absorbed when it faces an object during its pathway. Therefore, we can measure those changes.

*If we placed the detector at the same path/angel with the laser beam we can assist the **size** of our object, and That's what we called it **"Forward scatter "**

Forward Scatter—diffracted light
-Related to cell surface area
-Detected along axis of incident light in the forward direction

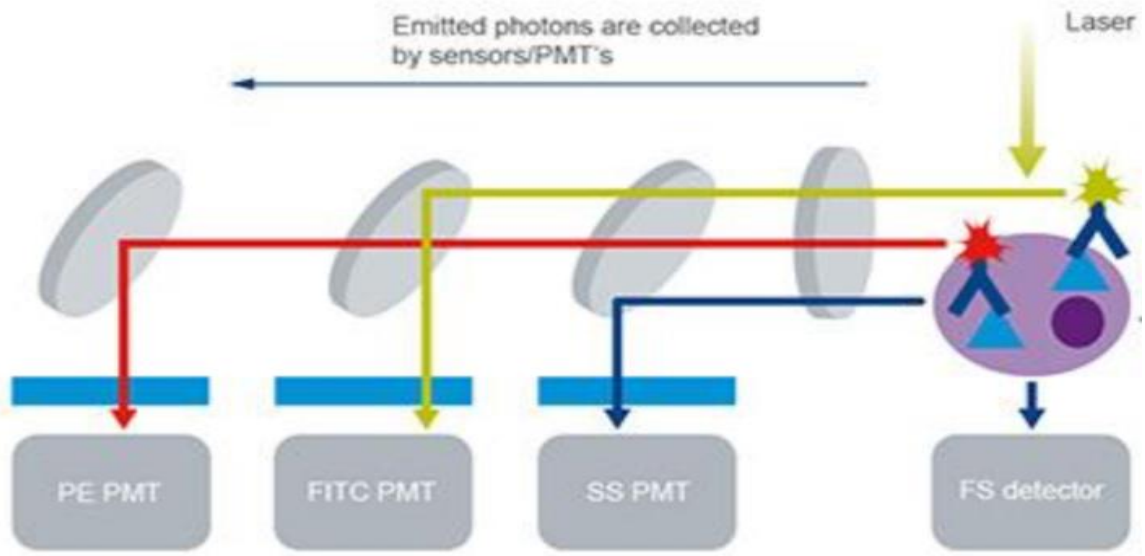




On the other hand, the detectors which is not placed within the laser beam, instead at 90 degrees to the laser beam are the ones to detect **complexity** or **granularity** of said objects. We call this **"lateral or side scatter."** The more complex the object is the wider reflection detection.

Side Scattered — reflected and refracted light
-Related to cell granularity and complexity
- Detected at 90 ° to the laser beam

light → ⬆ more granular & ⬆ complex object → ⬆ more side scatter



fluorescence:

Each electron has certain level of energy so when we direct a laser beam toward certain electrons on the first shell, the said electron will gain more energy and jump to highly energized shell which consider an unstable state then it has to go back to the same shell again. The difference between the amount of energy that lost by that electron during this movement is not equal and that difference of energy loss will be emitted as photons (detectable light)

- If we want to detect certain epitope. We need to label an antibody, which binds to the said epitope, with a Fluorochrome or excitable material then we direct the light against it. If expected photons was appeared = epitope exist
- We need certain detectors that specific to a specific wavelength. Each one represents certain color. As well as, a single detector can only sense one color.

Each system consists of:

1-Fluidics

•To introduce and focus the cells for interrogation.
• The liquid that hydrostatically pushes the cells through the nozzle.

2-Optics: PMT "photomultiplier tubes" or Detectors

• To generate and collect the light signals

3-Electronics

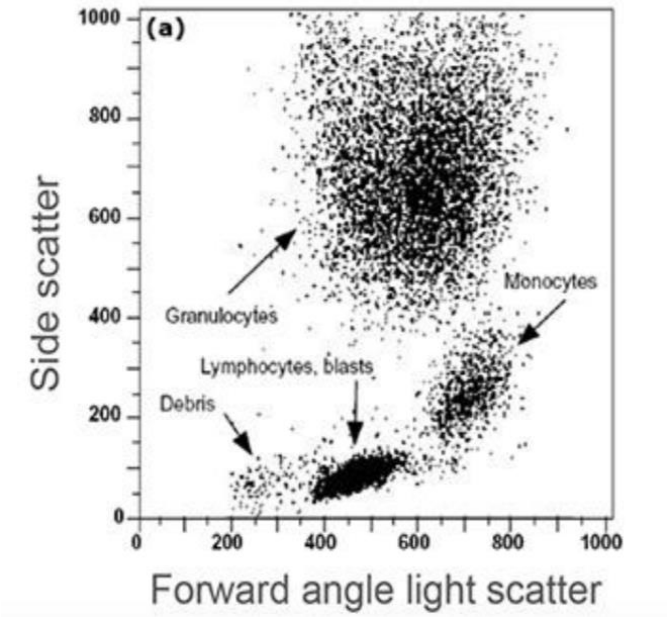
•To convert the optical signals to proportional electronic signals and digitize them for computer analysis.
• Things to translate wavelength into a color we can understand.

Dot Plot:

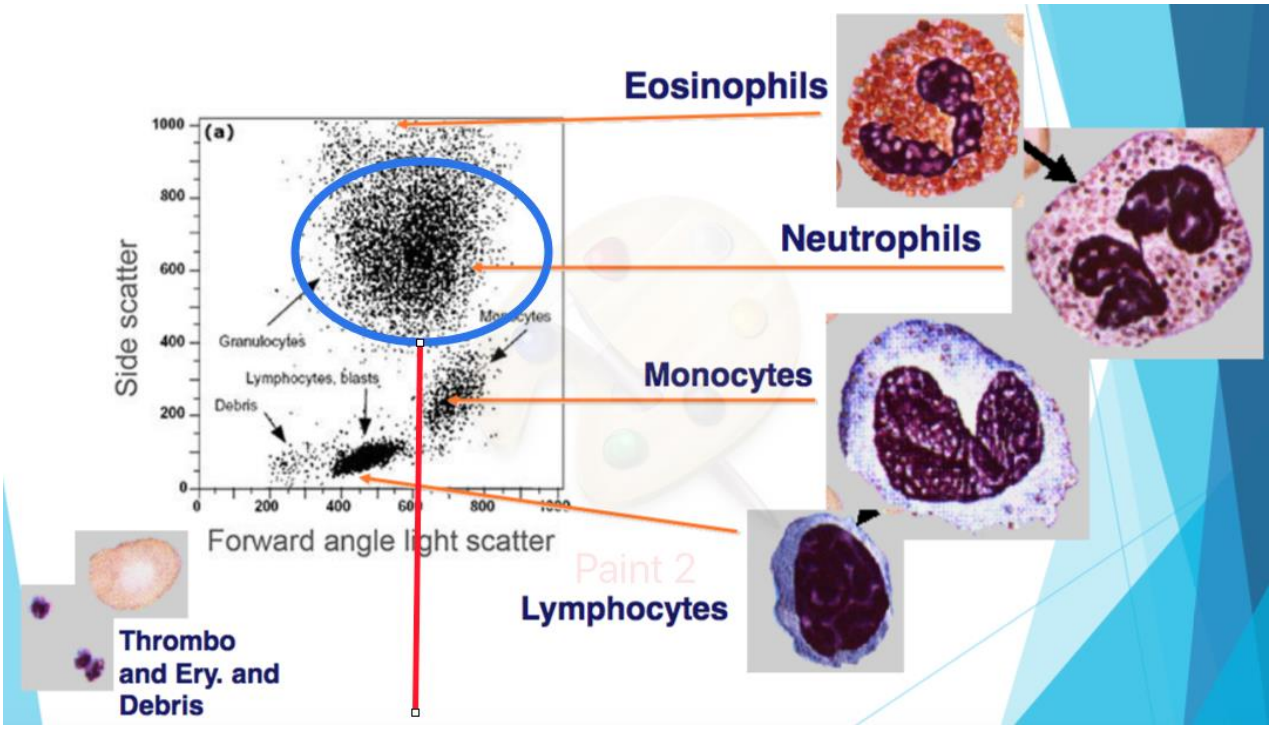
We are plotting dots; each dot represents an event which each cell got hit by laser beam.

(-The most useful when you need to compare multiparametric data (e.g. Intensity of side-scatter vs forward-scatter channels).

- Each distinct event is represented at a single dot)



X-axis represent **Forward Scatter (FS)**: more FS = larger cells. **Y-axis** represent **Side Scatter (SS)**: more SS = more granular or complex cells



Granulocytes: has large FS, and high SS. Since the most numerous type of granulocytes is Neutrophils so the most probably this population is consisting of neutrophils.

Eosinophils: has a larger size than Neutrophils with the highest granularity (SS) among all types of granulocytes.

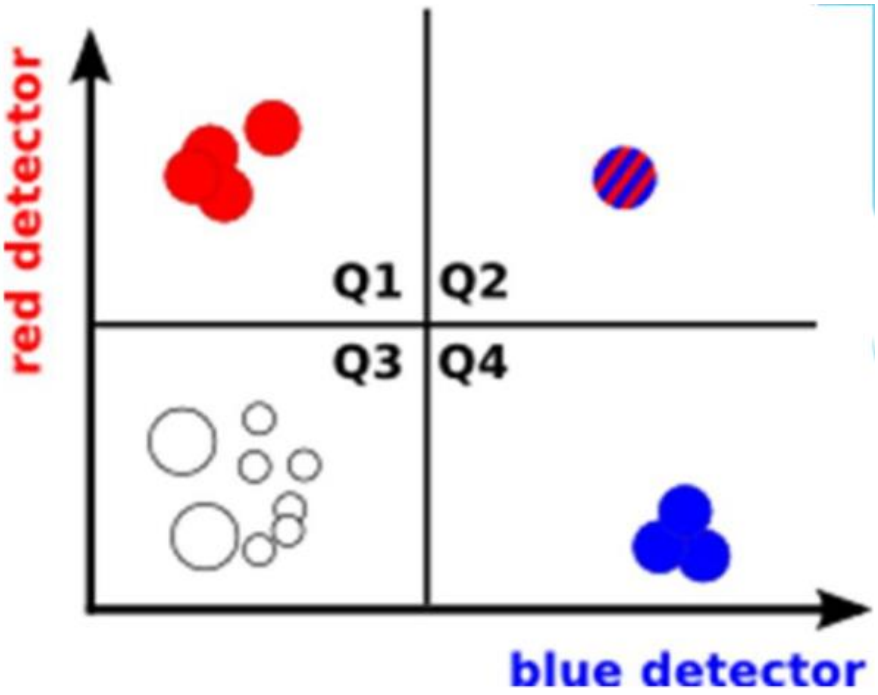
Monocytes: has large size (FS) with low granularity (SS)

Lymphocytes: has an medium size (FS) with low granularity (SS) "Agranular"

Debris: has low size (FS) with low granularity (SS) it may be thrombocytes, platelets, or RBCS.

-How to distinguish between T-cells and B-cells?

Since both of them have the same size and complexity. We need to do immunophenotyping by using Cluster of Differentiation (CD). This can be accomplished by adding a labeled antibody which binds to a specific CD marker. As we know that B-cell have CD19, CD20 and T-cell has CD3 marker. Therefore, by adding a labeled anti-CD3 antibody with green light and incubated it with our cells will allow us to identify all T-cells which will posses the labeled light or stain attached to it whereas The remaining cells with no light must be B-cells.



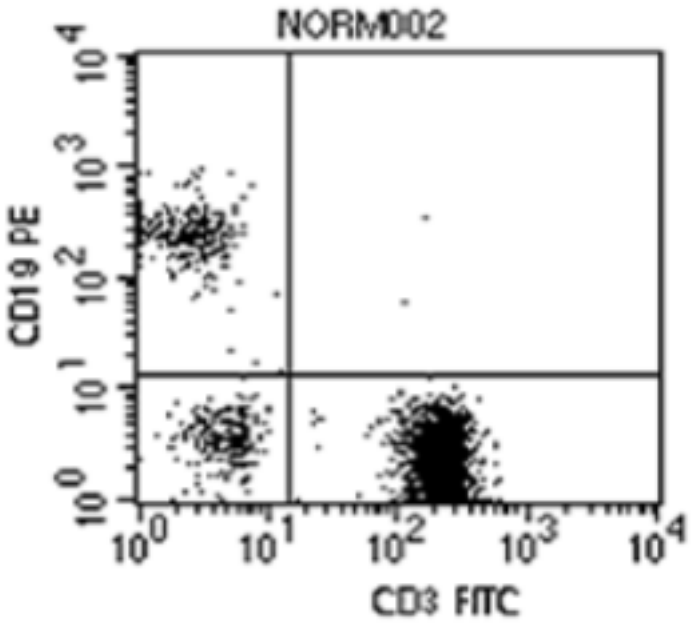
Q1: Single positive for the red detector +

Q2: Double positive for red and blue ++

Q3:Double Negative for red and blue ++

Q4: Single positive for the blue detector +

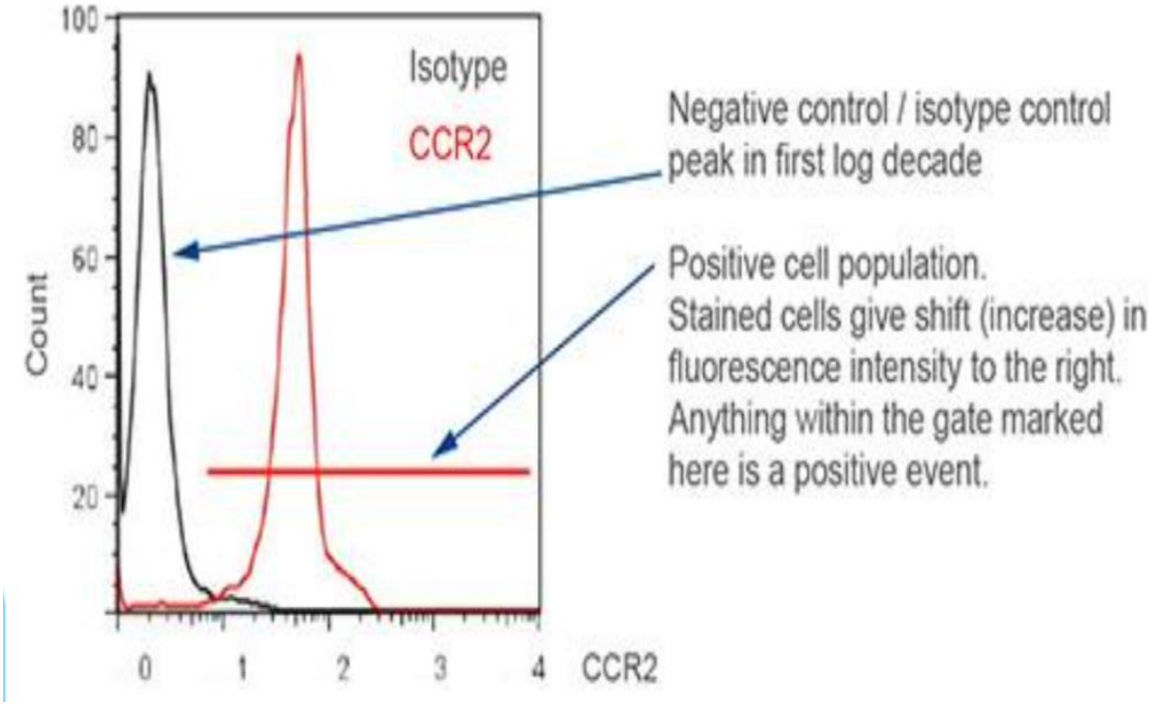
its important to know in the previously mentioned graph that we are talking about an independent detector. A single detector can only see a single color. So let's say we have an RBC sample and we want to phenotype our cells. First of all, we should label our antibodies. For example, anti-A antibody will be labeled with red color and anti-B with blue color. Therefore, the result will appear accordingly to its antigen-antibody matching quadrant, so we will have blood group type A in Q1 quadrant, blood group type B in Q4, blood group type O in Q3, and blood group type AB in Q2.



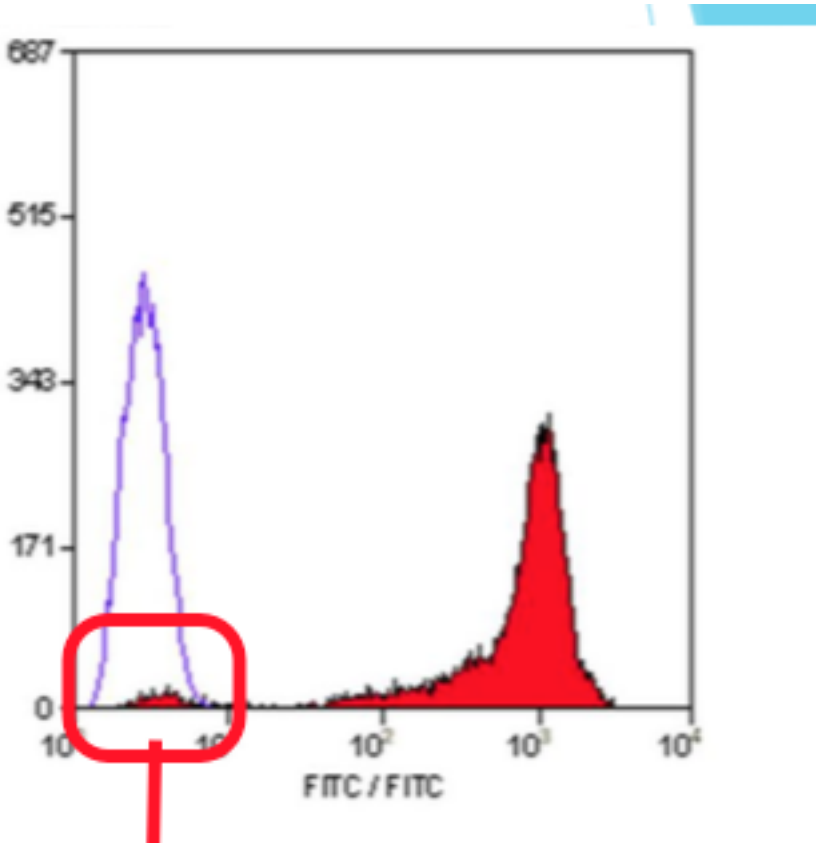
in this diagram we have a sample of mononuclear cells (monocytes and lymphocytes). We added anti CD3, CD19 antibody to phenotype our cells. We represented CD3 (T-cell marker) on the X axis and CD19 (B-cell marker) on the Y axis. Therefore, all cells appeared in Q1 are B-cells, Q4 = T-cells, and Q3 are either Monocytes or Natural Killer cells or both.

Histogram:

-most useful when only one parameter (e.g. intensity from a single fluorescent channel) is important
-Usual representation includes the intensity of a single channel (horizontal axis) vs number of detected events (vertical axis).



used to study a single detector as opposed to Dot Plot. The Y-axis represent the number of event while the X-axis represent the fluorescence intensity = "amount of light."



This slight elevation is either due to background noises or due to auto fluorescence ability of some of the cells in said sample.

We overcome this issue by using control. A control can be either positive or negative.

A **Negative control** is a sample that will not show the effect we are experimenting.