Flow cytometry

- ***Flow cytometry (FC) is a technique used to detect and measure physical and chemical characteristics of a population of cells or particles.***

- **1953** : The first impedance-based flow cytometry device, using the Coulter principle, **Wallace H. Coulter**.
- **1965** : **Mack Fulwyler** was the inventor of the forerunner to today's flow cytometers - particularly the cell sorter.
- **1968** : The first fluorescence-based flow cytometry device (ICP 11), **Wolfgang Göhde.**

- **A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers high-throughput, automated quantification of specified optical parameters on a cell-by-cell basis.**

Applications:

- Cell counting
- Cell sorting
- Determining cell characteristics and function
- Detecting microorganisms
- Biomarker detection
- Protein engineering detection
- Diagnosis of health disorders such as blood cancers

Flow cytometry

- In flow cytometry, a sample containing cells or particles is suspended in a fluid and injected into the flow cytometer instrument.
- The sample is focused to ideally flow one cell at a time through a laser beam, where the light scattered is characteristic to the cells and their components.
- Cells are often labeled with fluorescent markers so light is absorbed and then emitted in a band of wavelengths.
- Tens of thousands of cells can be quickly examined and the data gathered are processed by a computer.
- A flow cytometry analyzer is an instrument that provides quantifiable data from a sample.

- Other instruments using flow cytometry include cell sorters which physically separate and thereby purify cells of interest based on their optical properties.

Flow cytometry

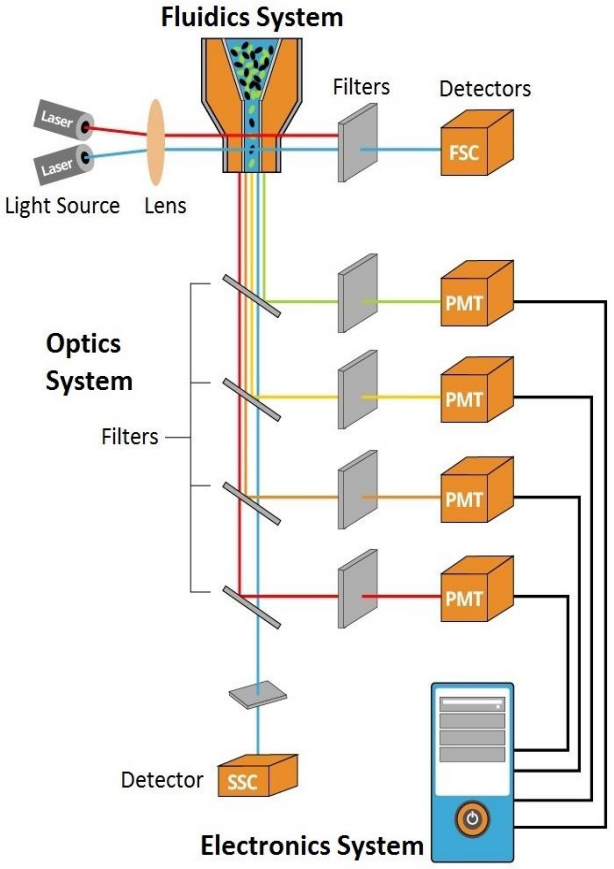
- A flow cytometer has five main components: **a flow cell, a measuring system, a detector, an amplification system, and a computer** for analysis of the signals.

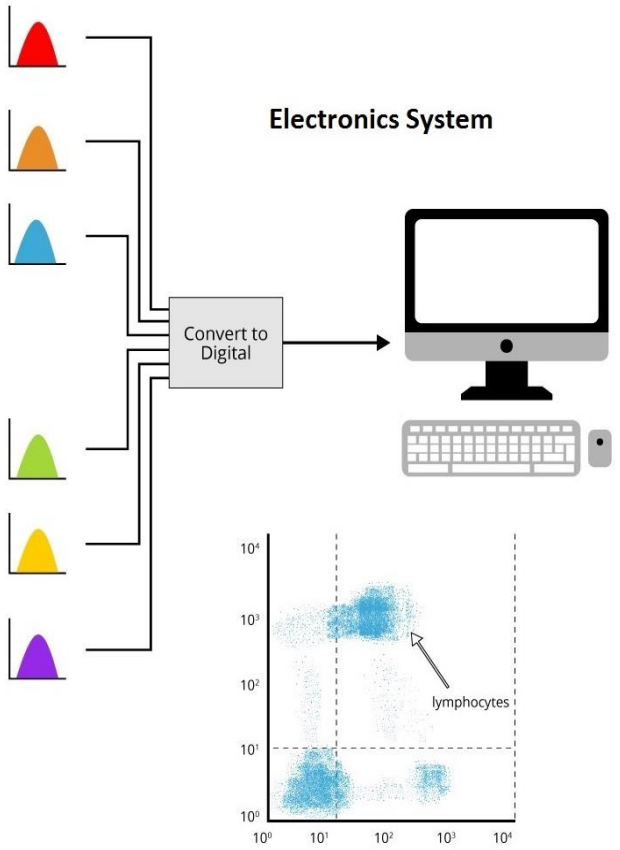
- The **flow cell** has a liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing.
- The **measuring system** commonly uses measurement of impedance (or conductivity) and optical systems - lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals.
- The **detector and analog-to-digital conversion (ADC) system** converts analog measurements of forward-scattered light (FSC) and side-scattered light (SSC) as well as dye-specific fluorescence signals into digital signals that can be processed by a computer.
- The **amplification system** can be linear or logarithmic.
- The process of collecting data from samples using the flow cytometer is termed "acquisition". Acquisition is mediated by a **computer** physically connected to the flow cytometer, and the software which handles the digital interface with the cytometer.

Flow cytometry

The flow cytometer instrument consists of three core systems: fluidics, optics, and electronics.

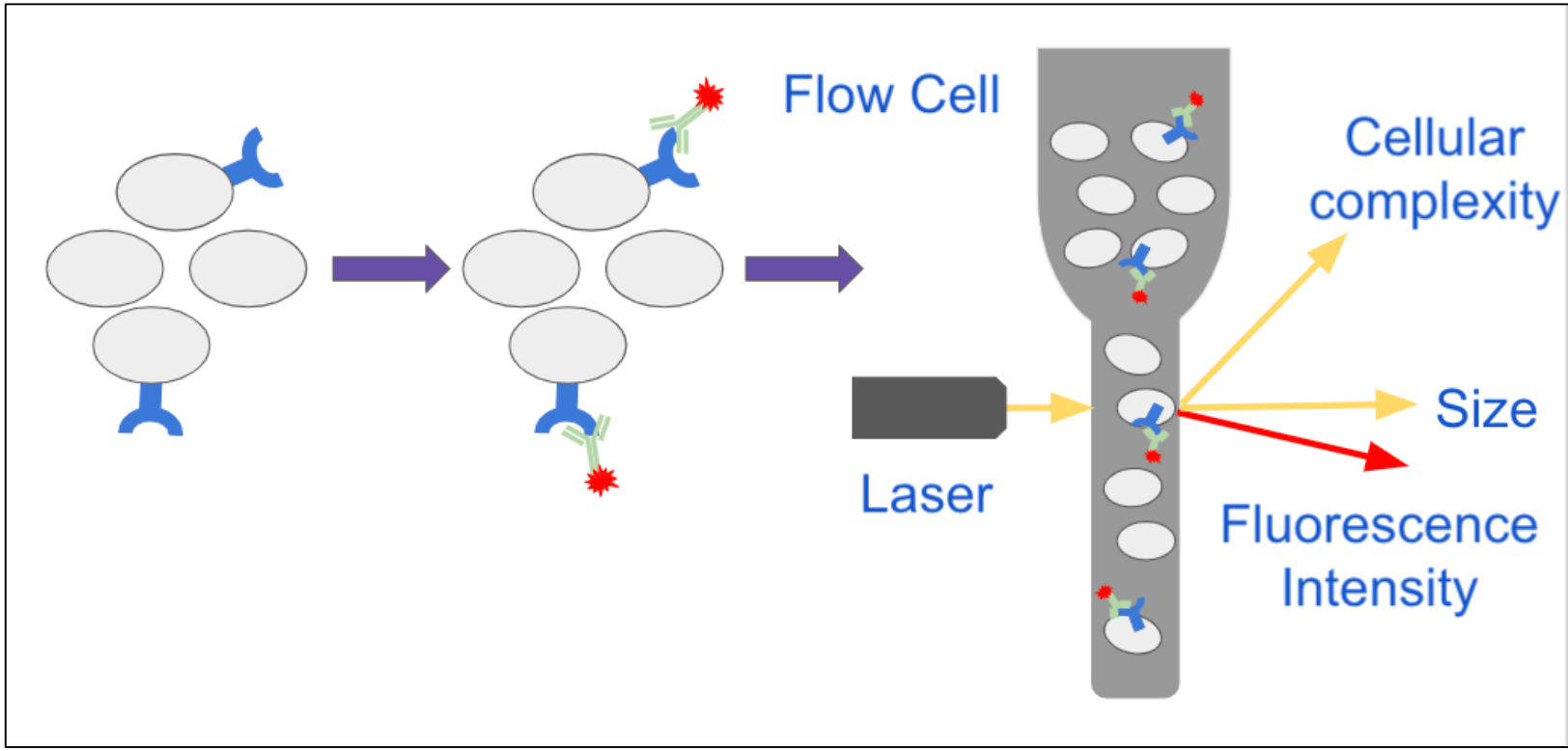
- **The fluidics system:** transporting sample from the sample tube to the flow cell. Once through the flow cell (and past the laser), the sample is either sorted or transported to waste.
- **The optical system:** include excitation light sources, lenses, and filters used to collect and move light around the instrument and the detection system that generates the photocurrent.
- **The electronics:** the brains of the flow cytometer. Here, the photocurrent from the detector is digitized and processed to be saved for subsequent analysis.





Flow cytometry

Cells are first resuspended in a pressurized buffer called sheath fluid and transported through tubes or capillaries to a laser. As the cells move through the fluidics system they pass through a flow cell which restricts the size of the stream and forces the cells to line up in single file in a process termed hydrodynamic focusing. This allows each cell to pass through the path of the laser in single-file where it is interrogated by the optical system (Figure).



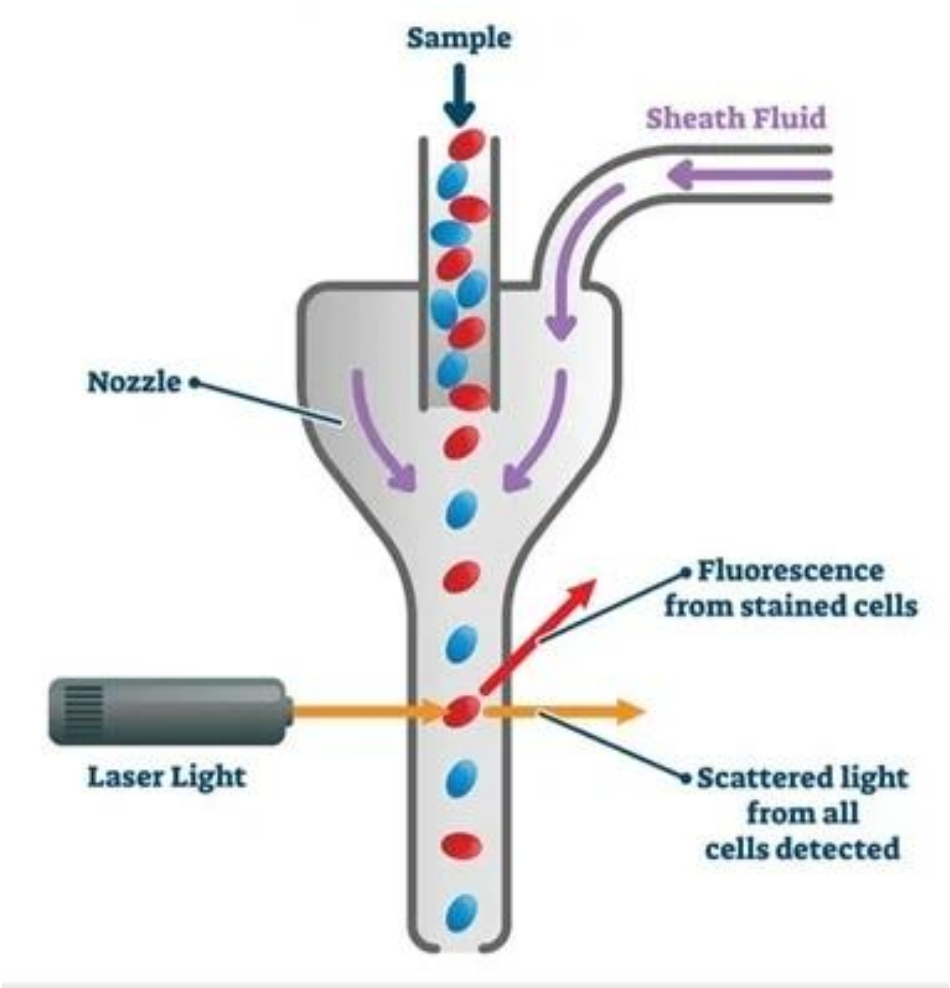
Cells expressing a particular surface receptor are stained with a fluorescent antibody against the receptor and analyzed on a flow cytometer. As cells pass through the flow cell the size of the sample stream is reduced forcing the cells to line up in a single file. Single cells pass through the path of a laser and the instrument collects information about the cell's size, complexity and fluorescence intensity.

Hydrodynamic focusing

Hydrodynamic focusing is a technique for sample focusing and control, characterized as ***the squeezing of the sample fluid of interest utilizing another fluid****, which is also known as the sheath fluid.*

Principle:

- When two streams of fluids with different flow rates are running side-by-side and in the same direction into a flow cell, then a laminar flow is created. The central stream (sample stream) is focused and surrounded by the secondary slower stream (sheath fluid).
- By manipulating the pressure differences between the two fluids, we get the desired cross-sectional area (i.e., the diameter of a cell). Hydrodynamic focusing properly aligns your cells, one by one, at the junction where the analysis by lasers begins.



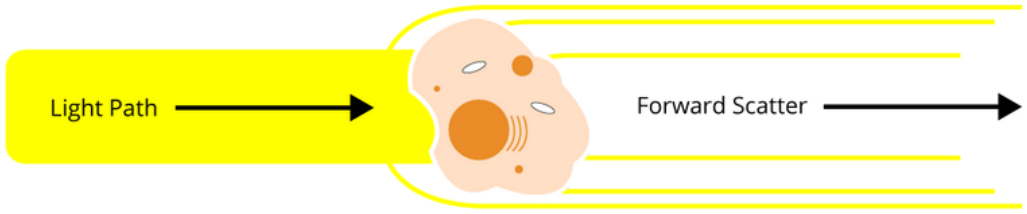
Forward scattered light vs Side scattered light

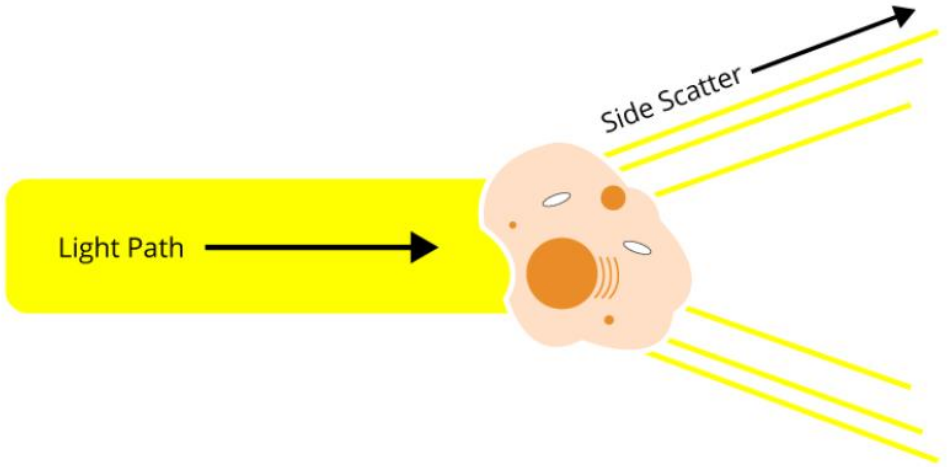
Forward scattered light (FSC) is light that is refracted by a cell in the forward direction, and continues in the same direction that the light was already traveling

- Provides information about the size of the cells.
- Bigger particles will produce more forward scattered light than smaller ones, and larger cells will have a stronger forward scatter signal.

Side scattered light (SSC) is light that is refracted by cells and travels in a different direction than its original path

- It usually provides information about the granularity and complexity of the cells.
- Cells with a low granularity and complexity will produce less side scattered light, while highly granular cells with a high degree of internal complexity (such as neutrophils) will result in a higher side scatter signal.





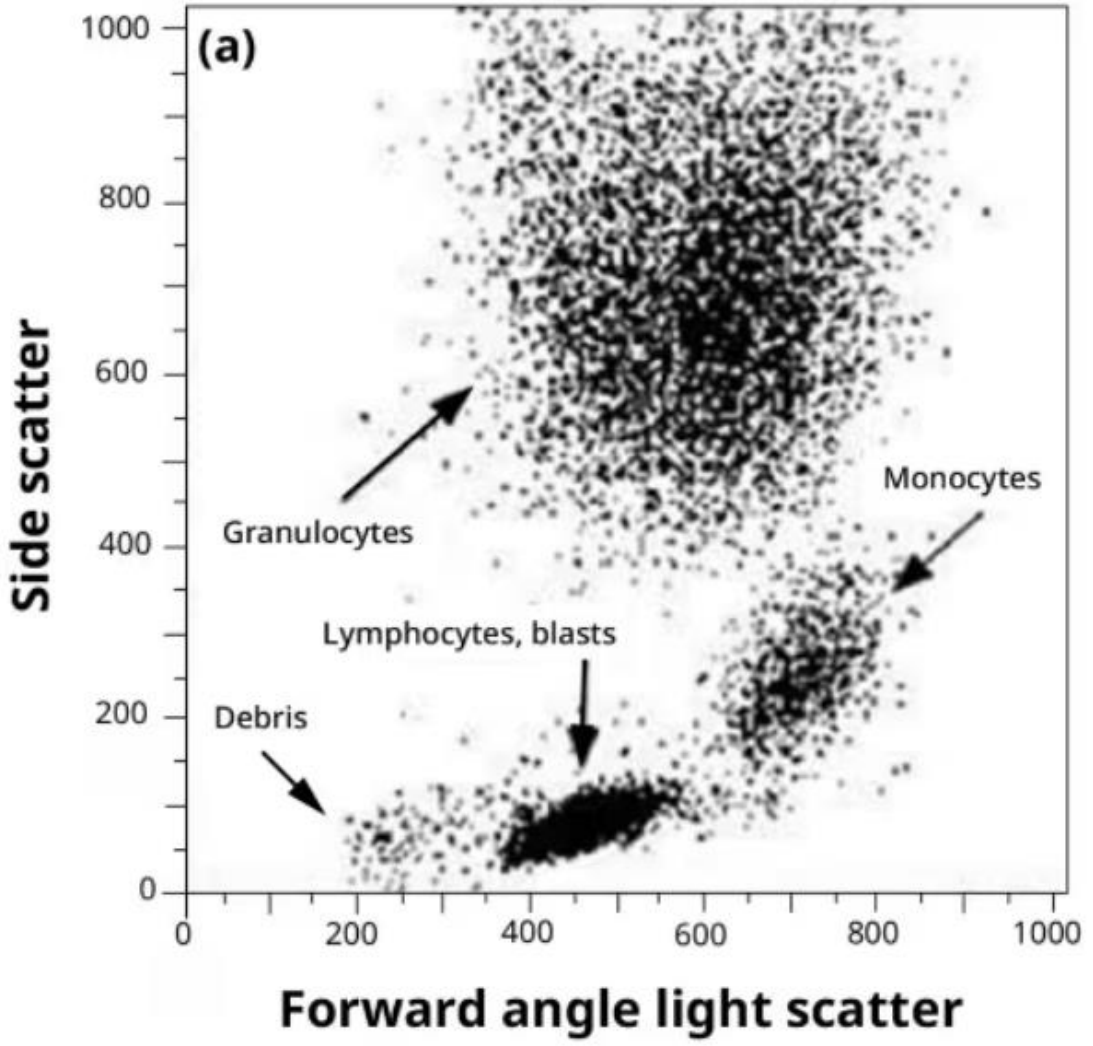
Example of Flow cytometry to distinguish immune cells

By using forward and side scattered light detection, cell populations can often be distinguished based on characteristic differences in cell size and granularity.

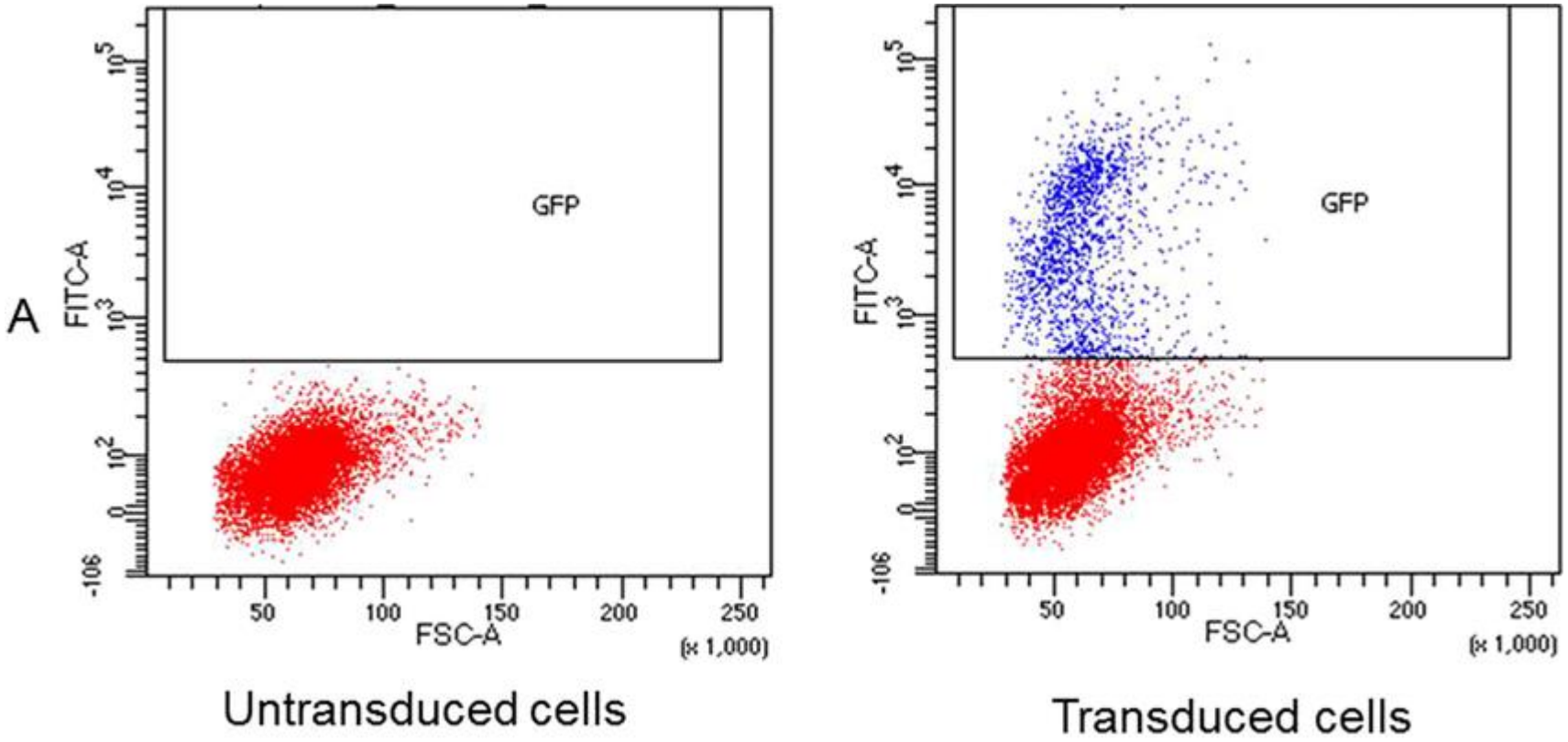
Figure shows a typical plot showing how different immune cell types can be distinguished based on FS and SS data.

- Larger and more granular granulocyte cells are seen as a large population with a high SS and FS.
- Monocytes are large cells but not so granular, so these produce a separate population with a high FS but lower SS.
- Smaller lymphocytes and lymphoblasts form a separate population with less FS, and also have a low SS as they are not granular cells.

Therefore, these cells can be separated into different populations based on their FS and SS alone



Example for Quantification of GFP expression by flow cytometry



Quantification of GFP expression by flow cytometry in HEK 293T cells following lentiviral transduction in order to establish the functional titer of lentiviral particles. The horizontal axis represents the forward scatter size (FSC-A) where an increased signal may indicate an increase in cell size or budding. The vertical axis indicates the GFP fluorescence intensity (FITC-A). Blue dots represent GFP-positive cells. Red dots represent GFP-negative cells. The threshold for GFP positivity was determined according to negative control (non-transduced cells).