Flow Cytometry

- **What is Flow Cytometry?**
- **How does a Flow Cytometer work?**
- **Dye and Single Color Compensation**
- **Sample Preparation for Flow Cytometry**
- **Applications**

What is Flow Cytometry?

• ***Cytometry*** refers to the measurement of physical/chemical characteristics of cells or other biological particles.

• ***Flow Cytometry*** is the process whereby such measurements are made upon cells/particles as ***they pass through a measuring apparatus*** (hopefully in single file) ***suspended in a fluid stream.***

• ***Flow Sorting (Flow Cytometric Cell Sorting)*** extends flow cytometry with the additional capacity to divert and collect cells exhibiting an identifiable set of characteristics either mechanically or by electrical means ***(Flow Cytometric Analysis)***.

Multi-parameter flow cytometry

A technology that simultaneously measures multiple parameters of single cells at a rapid rate

relative Size : FSC,

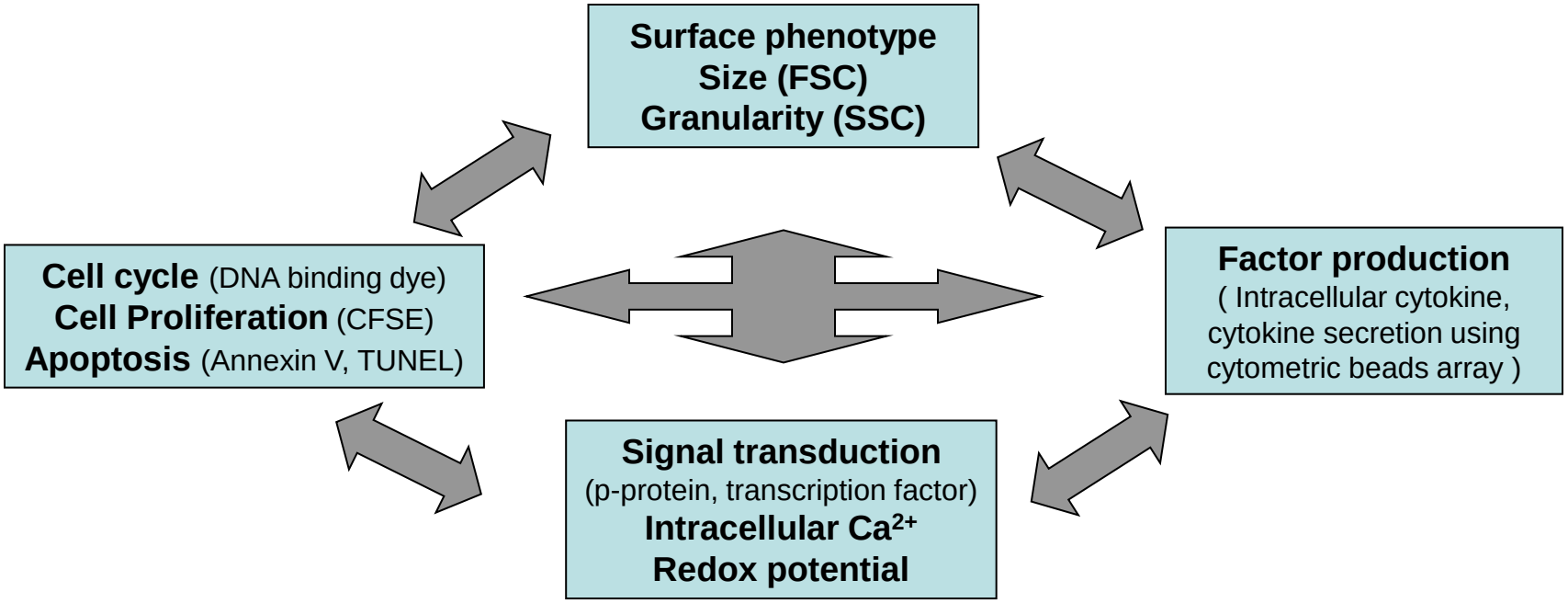
relative Granularity or Internal Complexity : SSC

relative Fluorescence Intensity : FL1, FL2, FL3 ...

1. Permits the detailed analysis of markers of cellular differentiation

2. Permits the simultaneous evaluation of cell phenotype and function

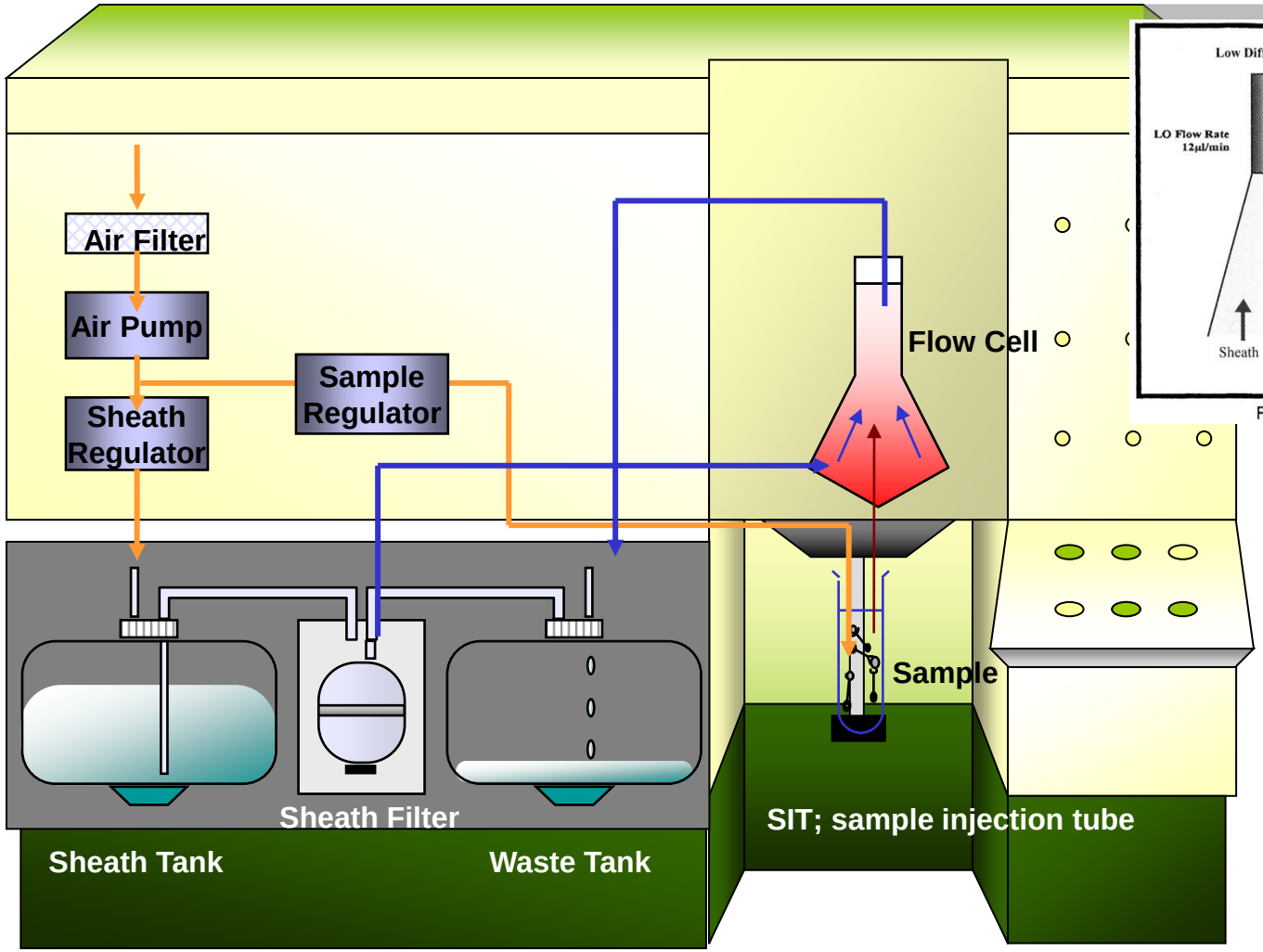
3. Permits cell sorting


Surface phenotype
Size (FSC)
Granularity (SSC)
Cell cycle (DNA binding dye)
Cell Proliferation (CFSE)
Apoptosis (Annexin V, TUNEL)
Factor production
(Intracellular cytokine, cytokine secretion using cytometric beads array)
Signal transduction
(p-protein, transcription factor)
Intracellular Ca2+
Redox potential

How does a Flow Cytometer work?

(Fluidics and Optics)

Fluidic System



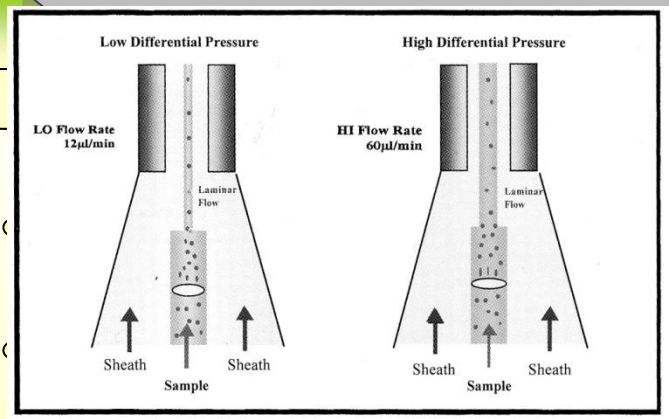
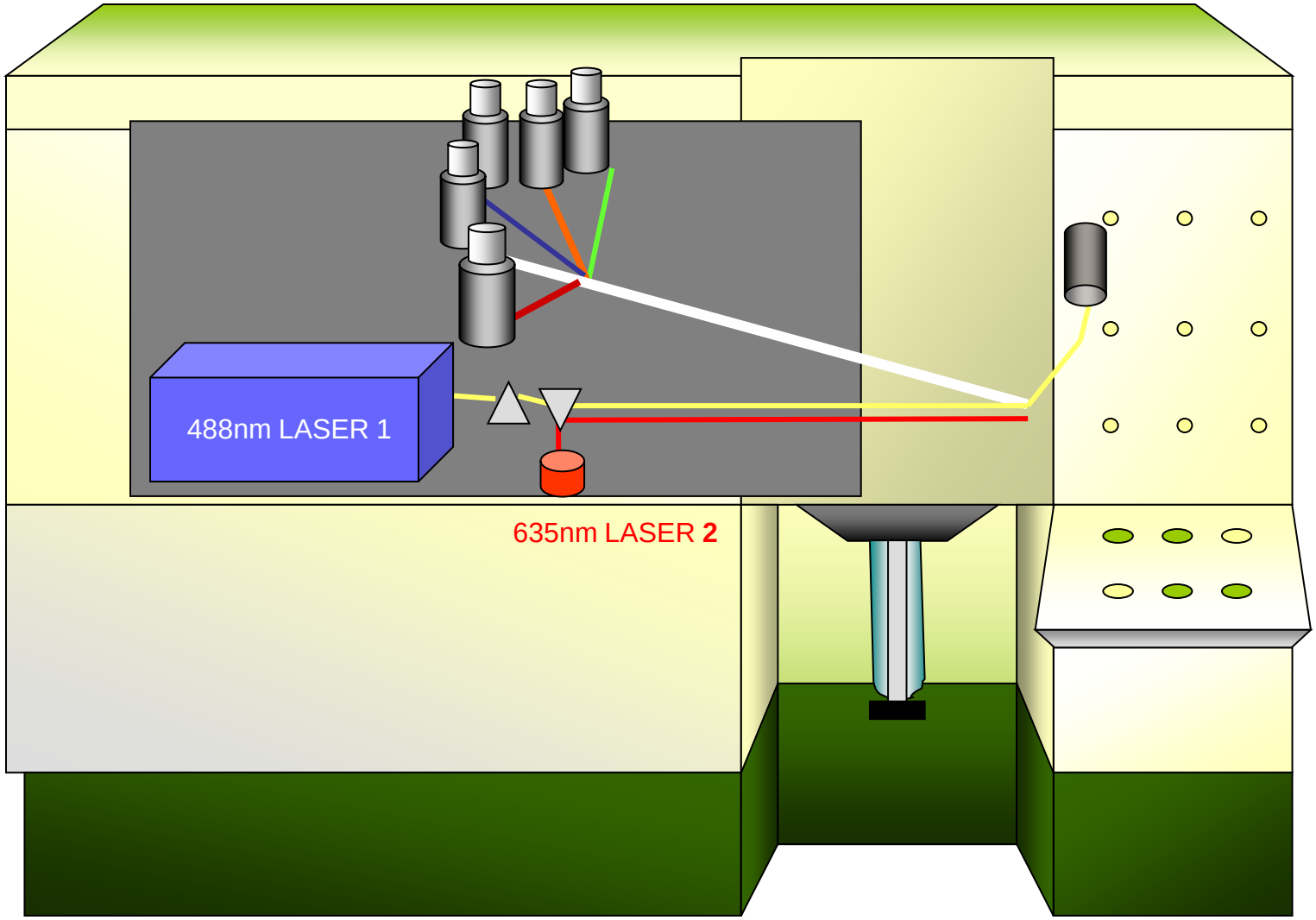


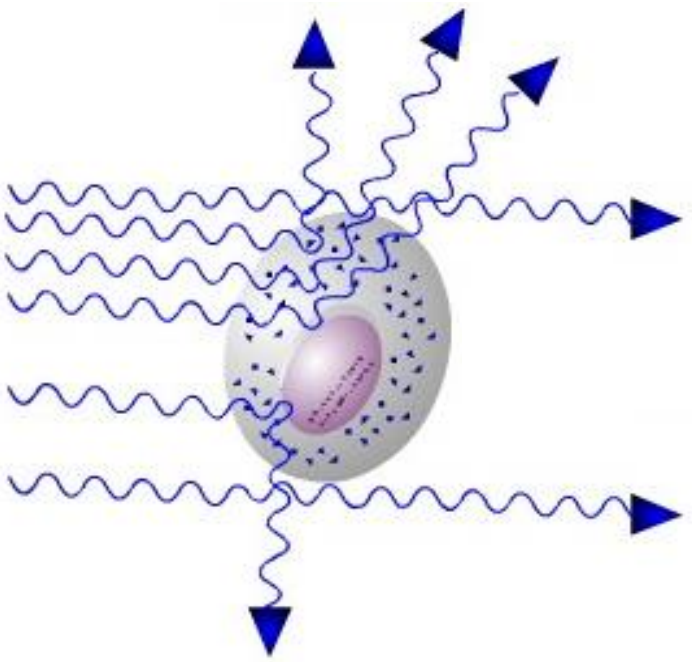
Figure 2 : Hydrodynamic focussing and FACSCalibur flow area

Optics System



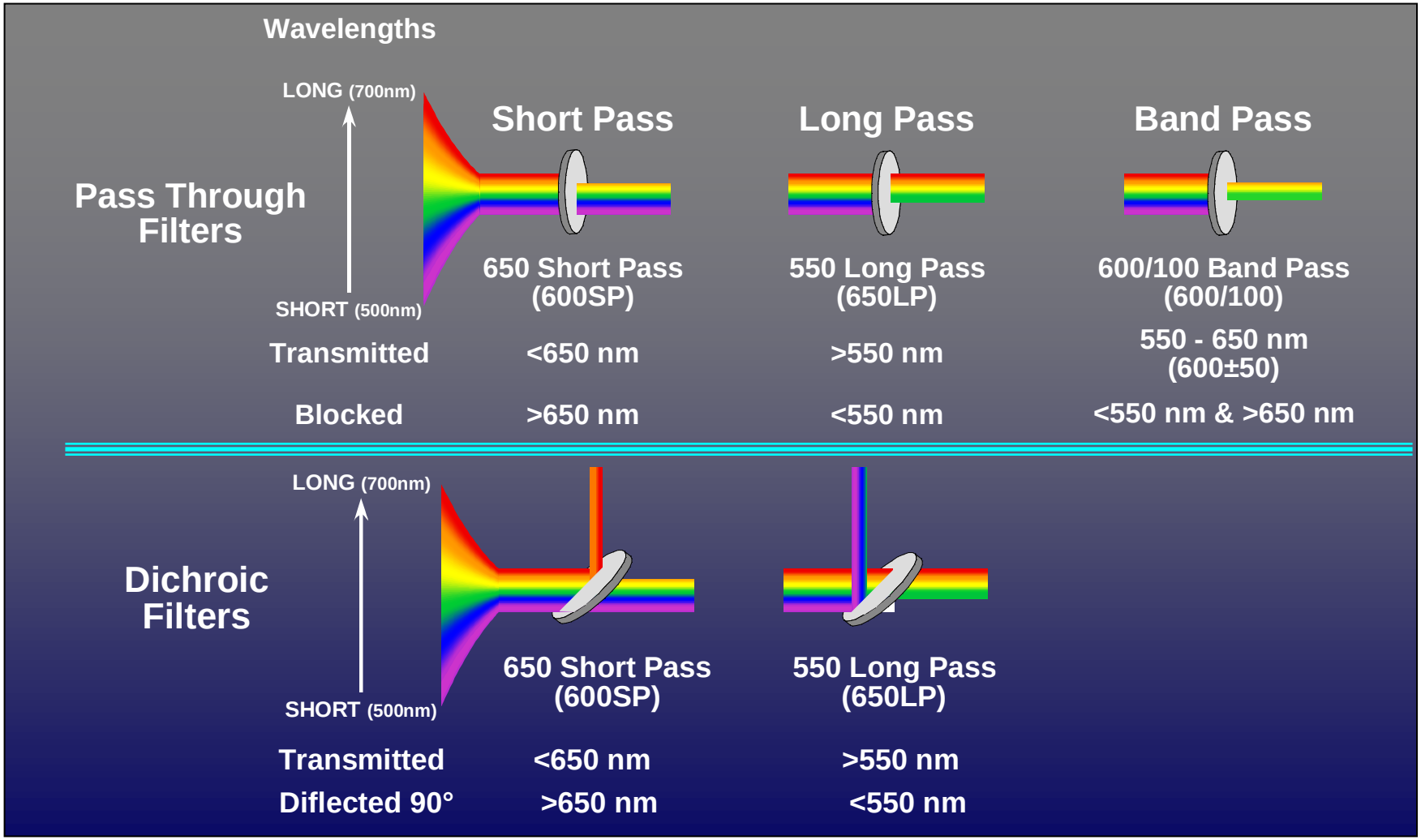
Properties of FSC and SSC

•Side Scatter (SSC)
: Granularity or Internal Complexity



•Forward Scatter (FSC) **: Cell Size**

Types of Optical Filters



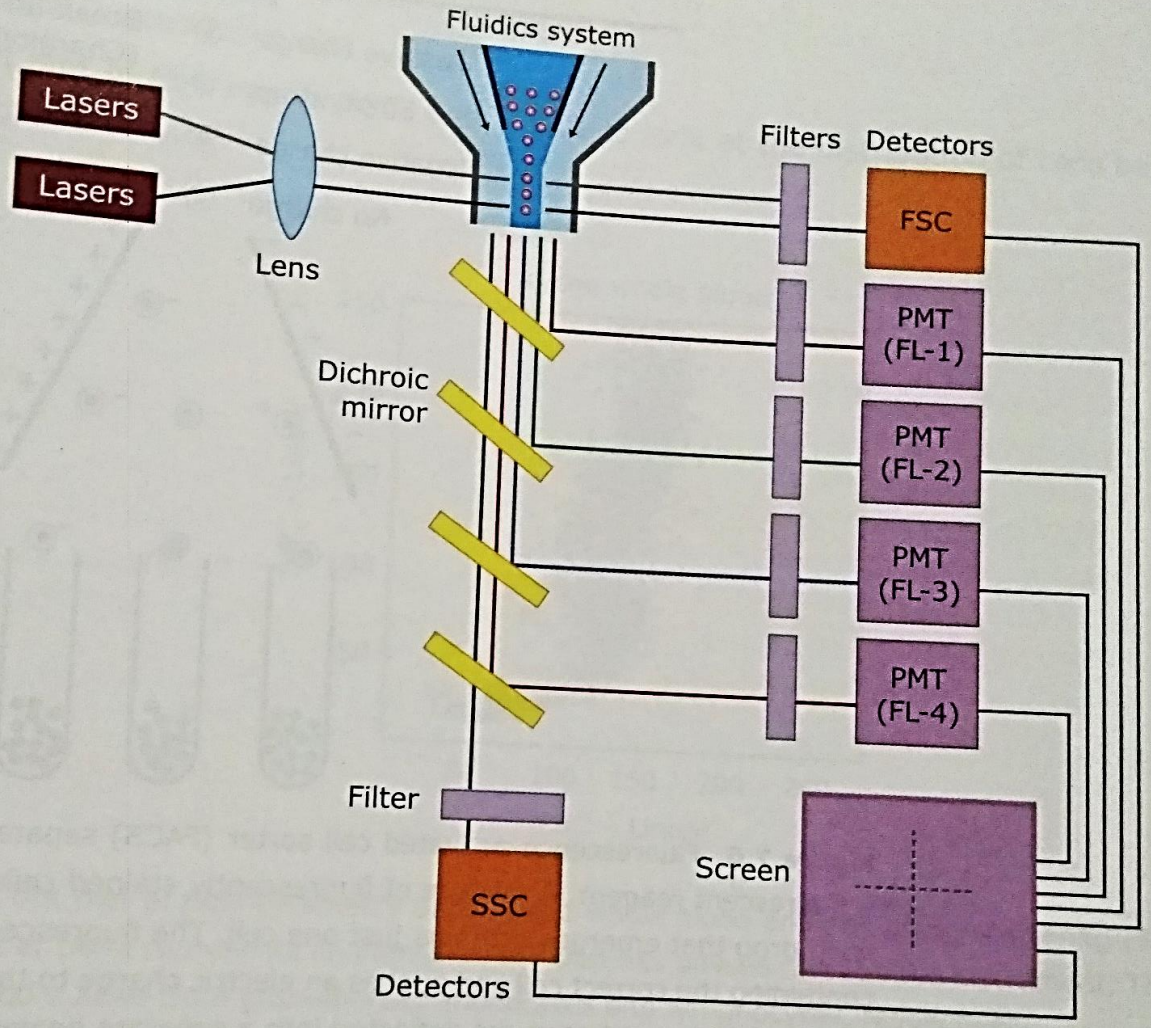
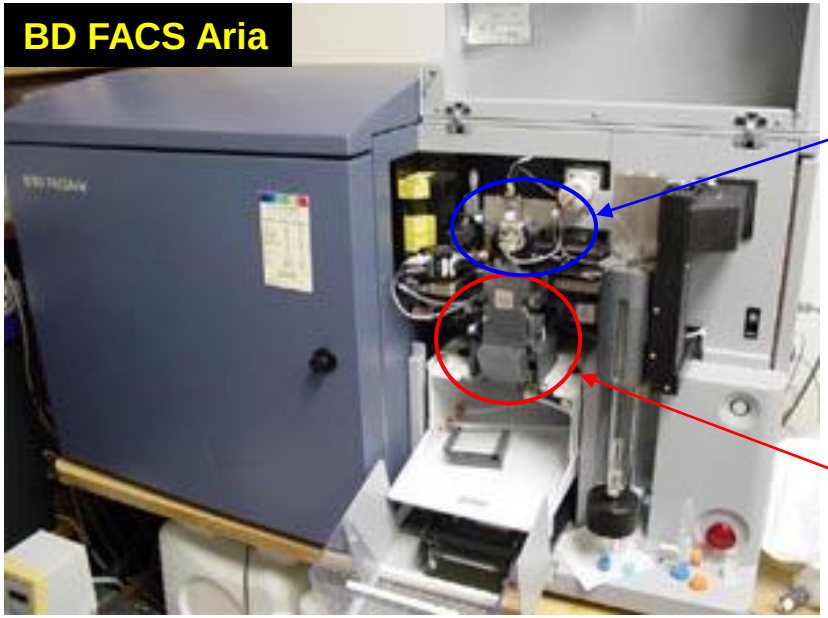
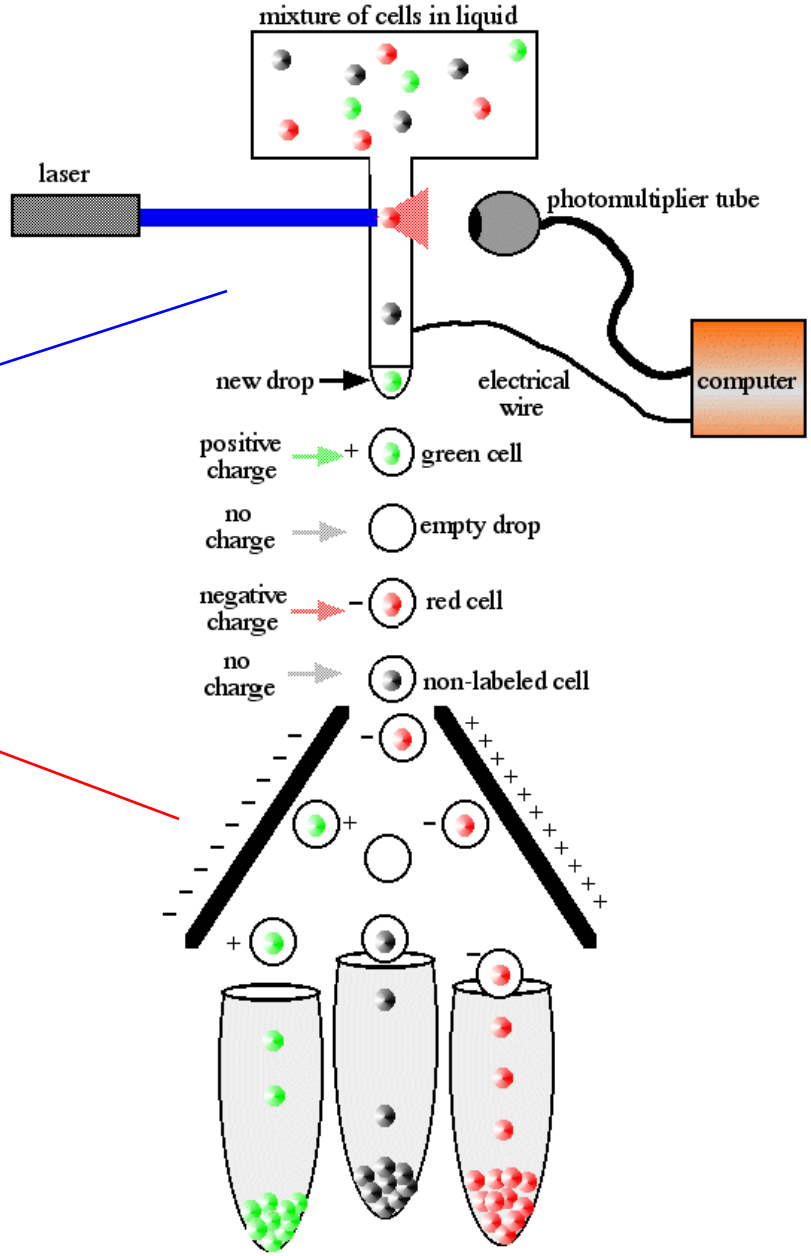


Figure 7.5 *Optical system of a typical flow cytometer: The optical system consists of excitation optics and collection optics. The excitation optics consist of the laser and lenses that are used to shape and focus the laser beam. The collection optics consist of a collection lens to collect light emitted from the particle–laser beam interaction and a system of optical mirrors and filters to route specified wavelengths of the collected light to designated optical detectors.*

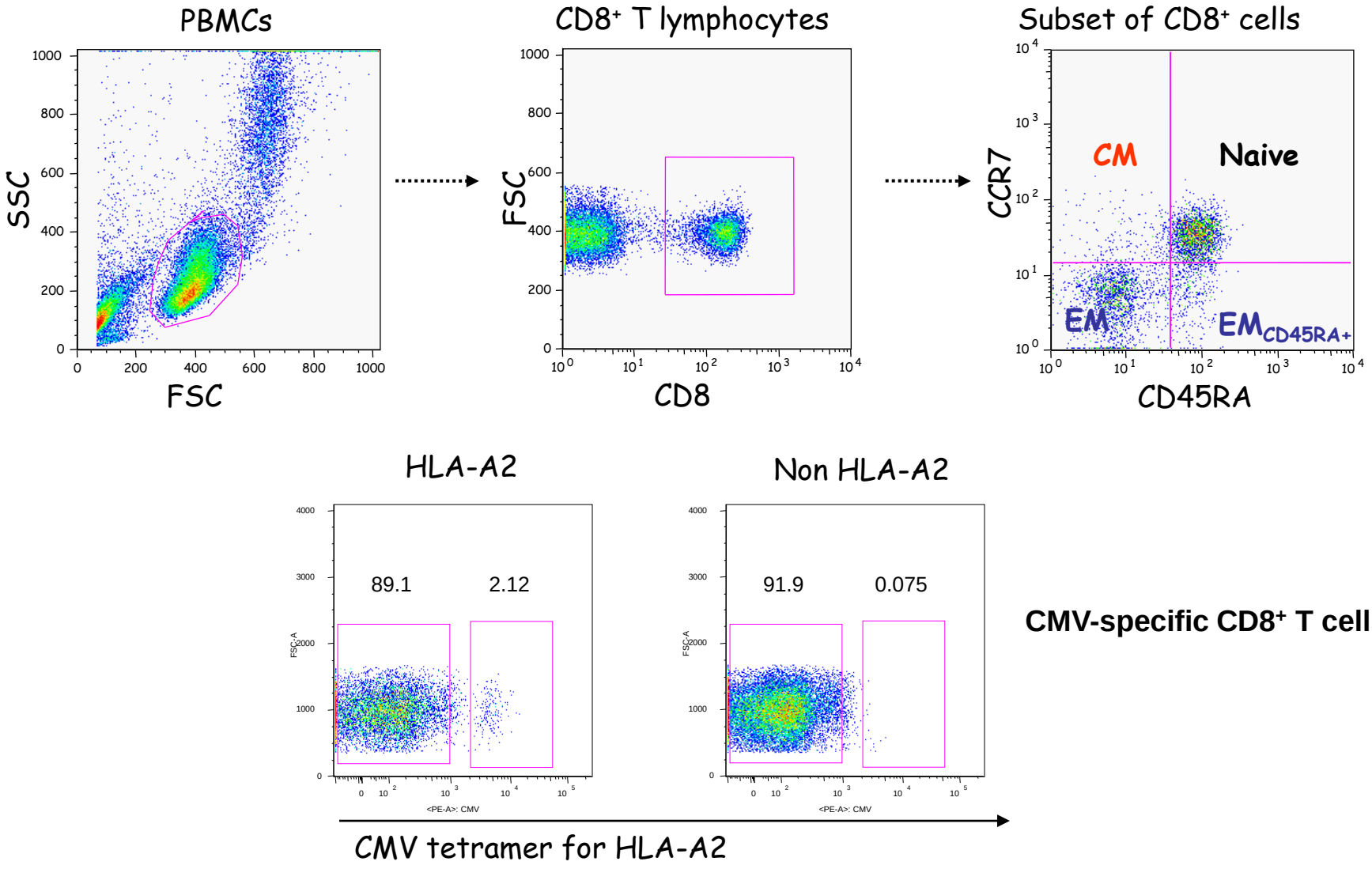
FACS sorting





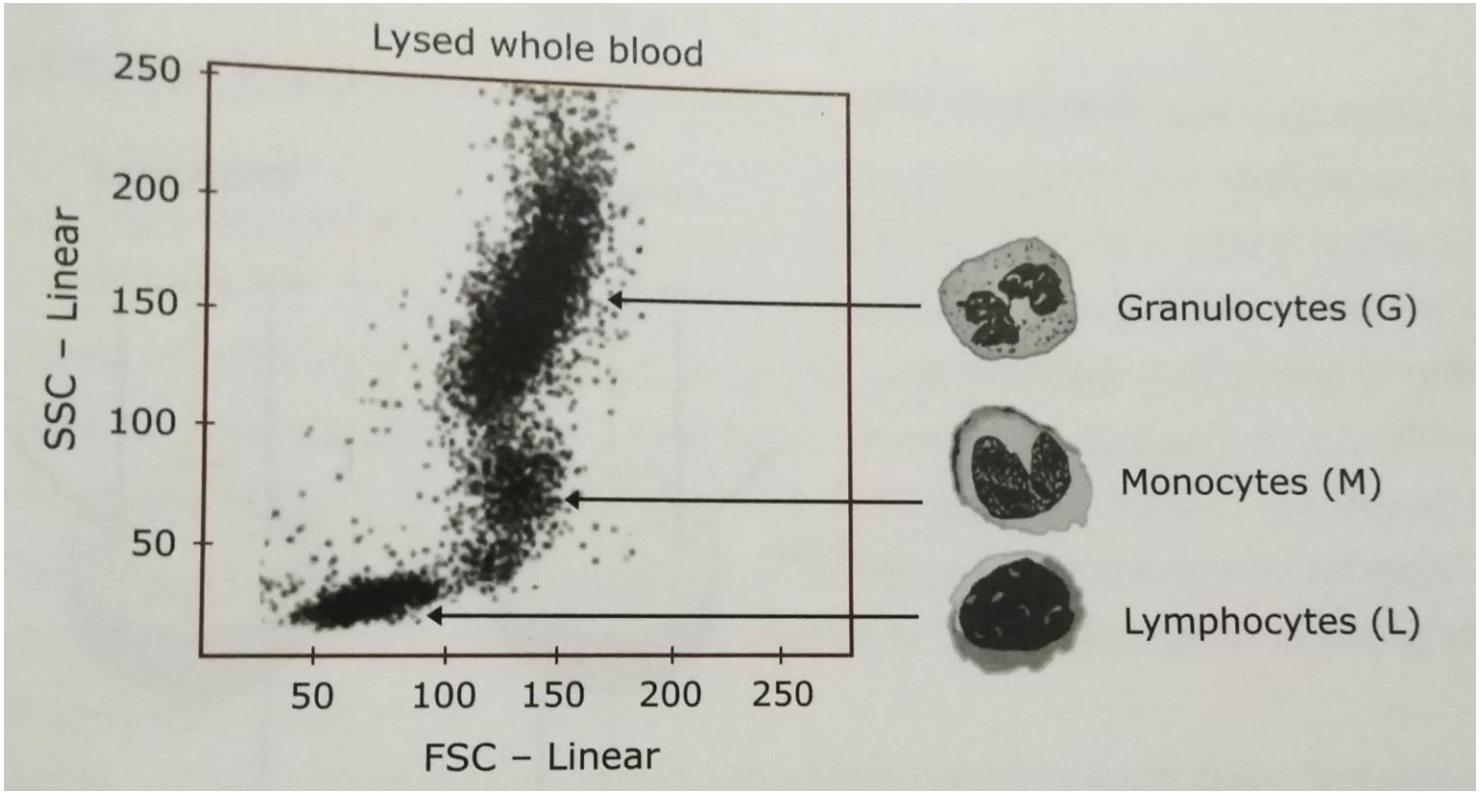
Applications

Surface phenotype, Ag-specific T cells



CMV-specific CD8⁺ T cells

Dot plot of Whole blood cells



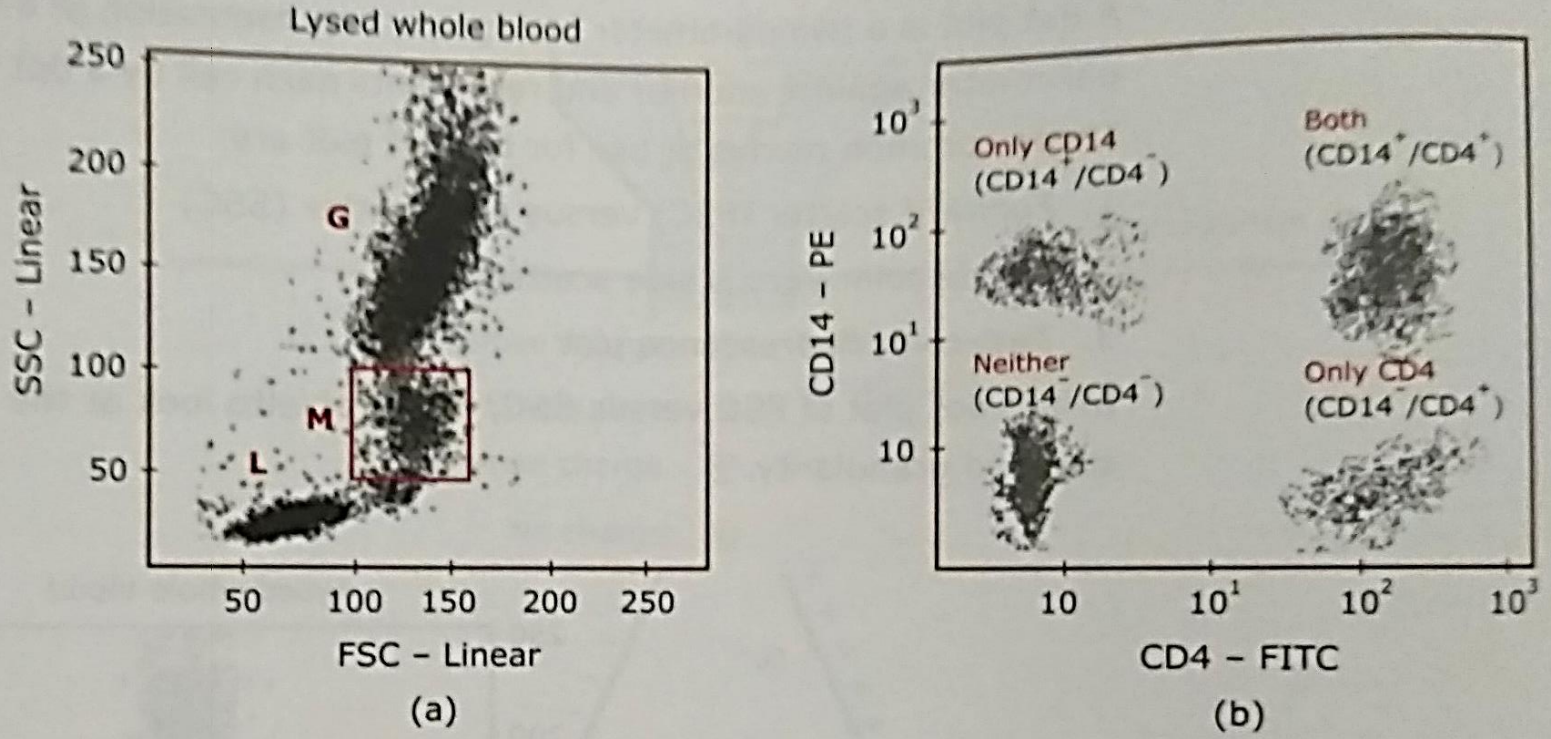


Figure 7.9 (a) Light scatter plot – the clusters labeled G, M and L arise from granulocytes, monocytes and lymphocytes respectively. A gate is applied to the monocyte population of a peripheral blood sample. (b) Fluorescence data from the gated region of monocytes clarifies which cells contain surface markers (CD14 and CD4). The cells are stained with fluorescently labeled antibodies directed against specific cell-surface markers, a FITC-conjugated antibody against CD4 and PE conjugated antibody against CD14.

FITC–Fluorescein isothiocyanate dye and PE–Phycoerythrin dye.