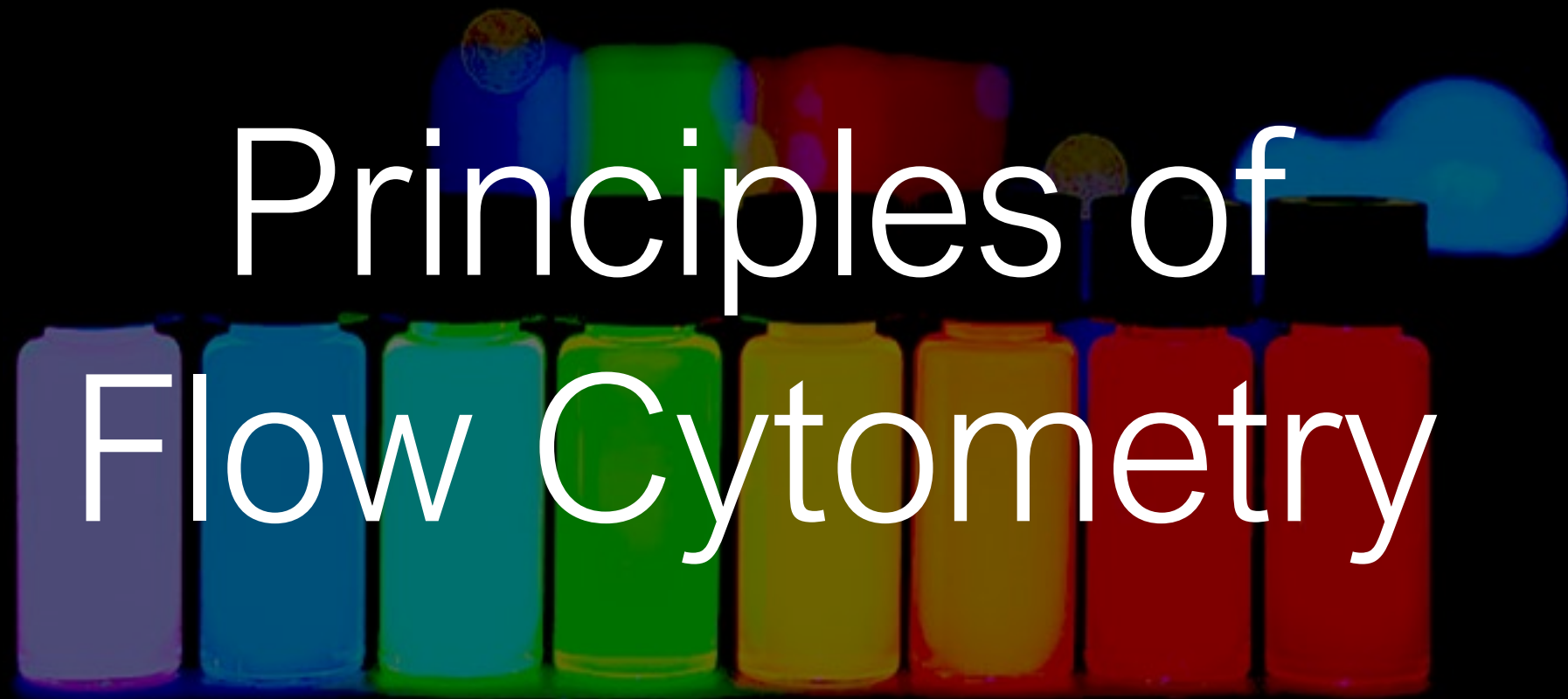




Principles of Flow Cytometry

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COURSE LEARNING OUTCOMES

1. Explain the following aspects of experimental methods used in physiological research: Principles, Methods, Interpretation, Advantages and Disadvantages
2. Apply the knowledge to choose appropriate methods in experimental designs and appraise the choice of methods employed in literatures
3. Participate in discussion of designing or appraising physiological experiments in literatures

OBJECTIVES

The students should be able to

- Explain the principles of flow cytometric analysis
- Read and interpret flow cytometric data
- Assess suitability of flow cytometry application for any given experiment

Flow Cytometry

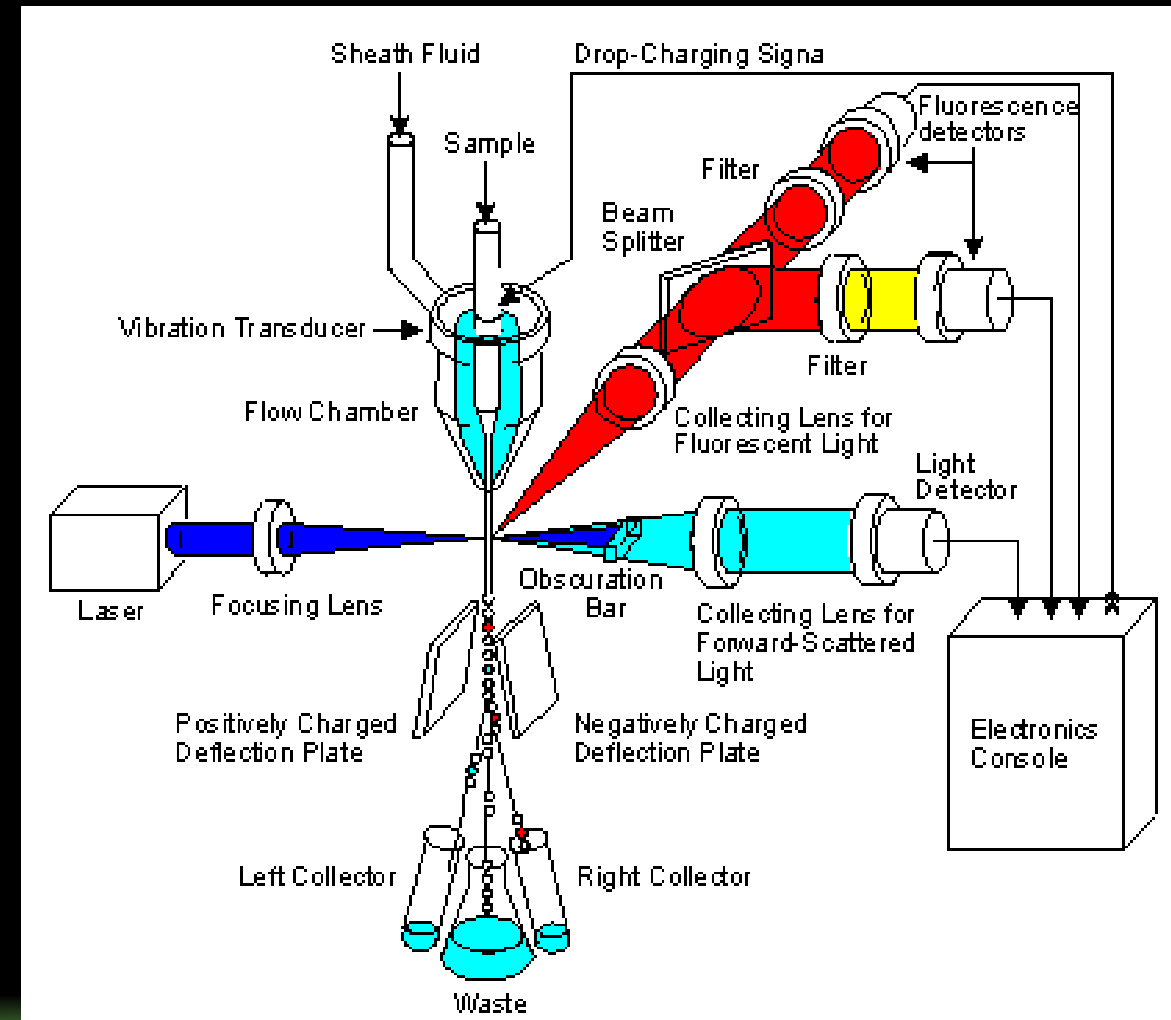
- Simultaneously measures and the analyzes physical characteristics of single particles
 - Relative size
 - Relative granularity or internal complexity
 - Relative fluorescence intensity

Flow Cytometry

- Pros
 - Sensitive (0.2–150 μm)
 - Simultaneous multiple data collection from each and every particle
 - Subpopulation analysis
- Cons
 - Particles have to be separated and in suspension
 - Cannot directly examine morphology or structure
 - Expensive
 - Time consuming (sort)

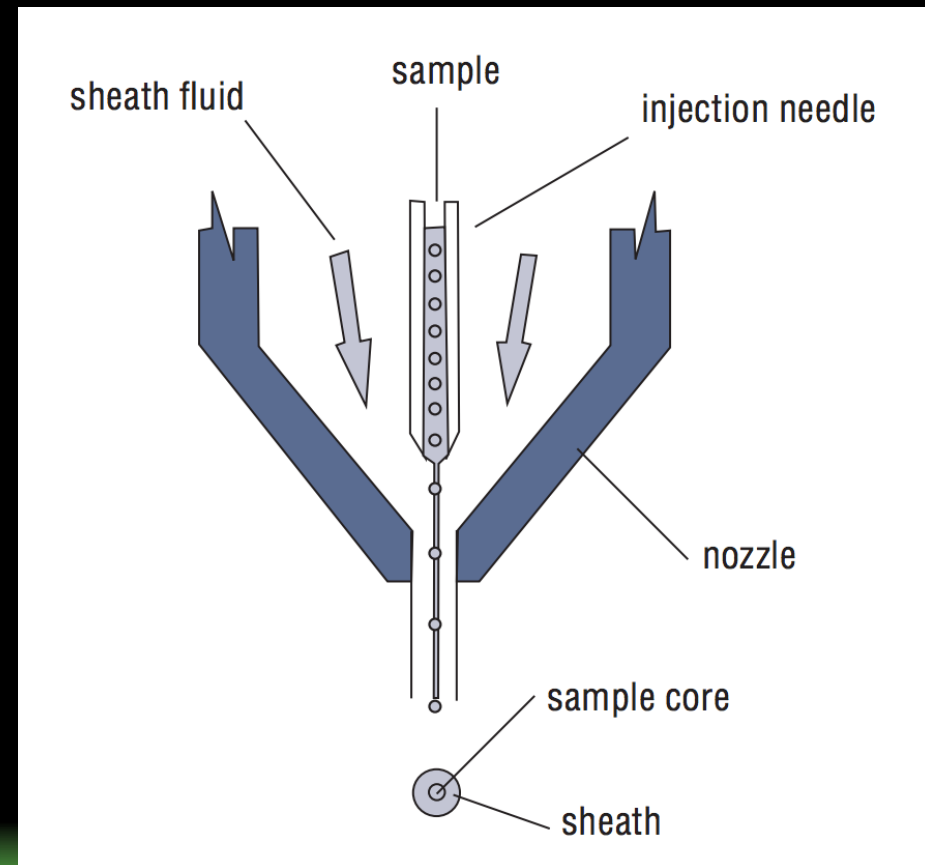
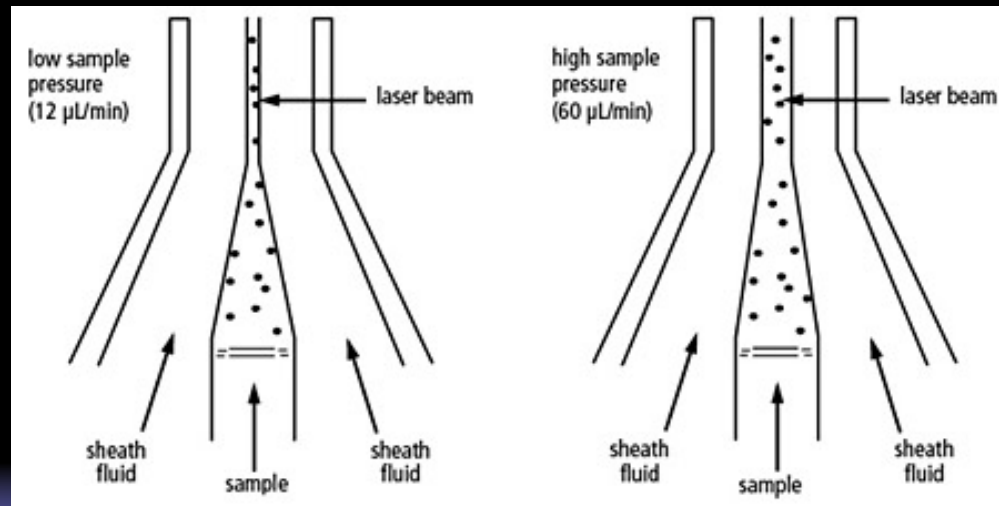
Flow Cytometry

- Consists of 3 components
 - Fluidics
 - Optics
 - Electronics



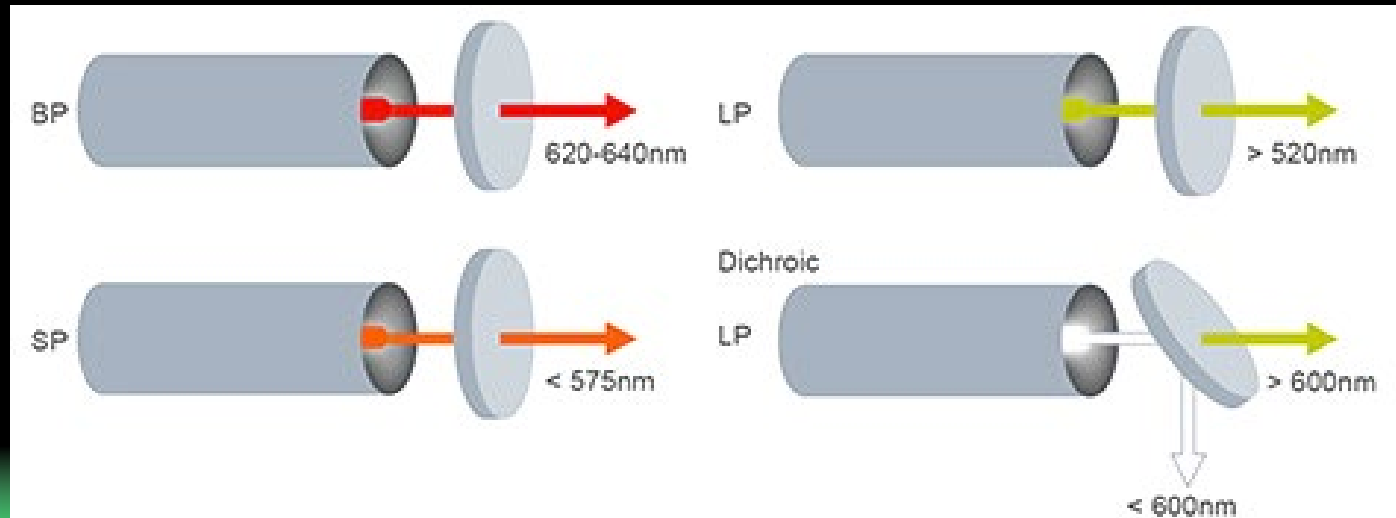
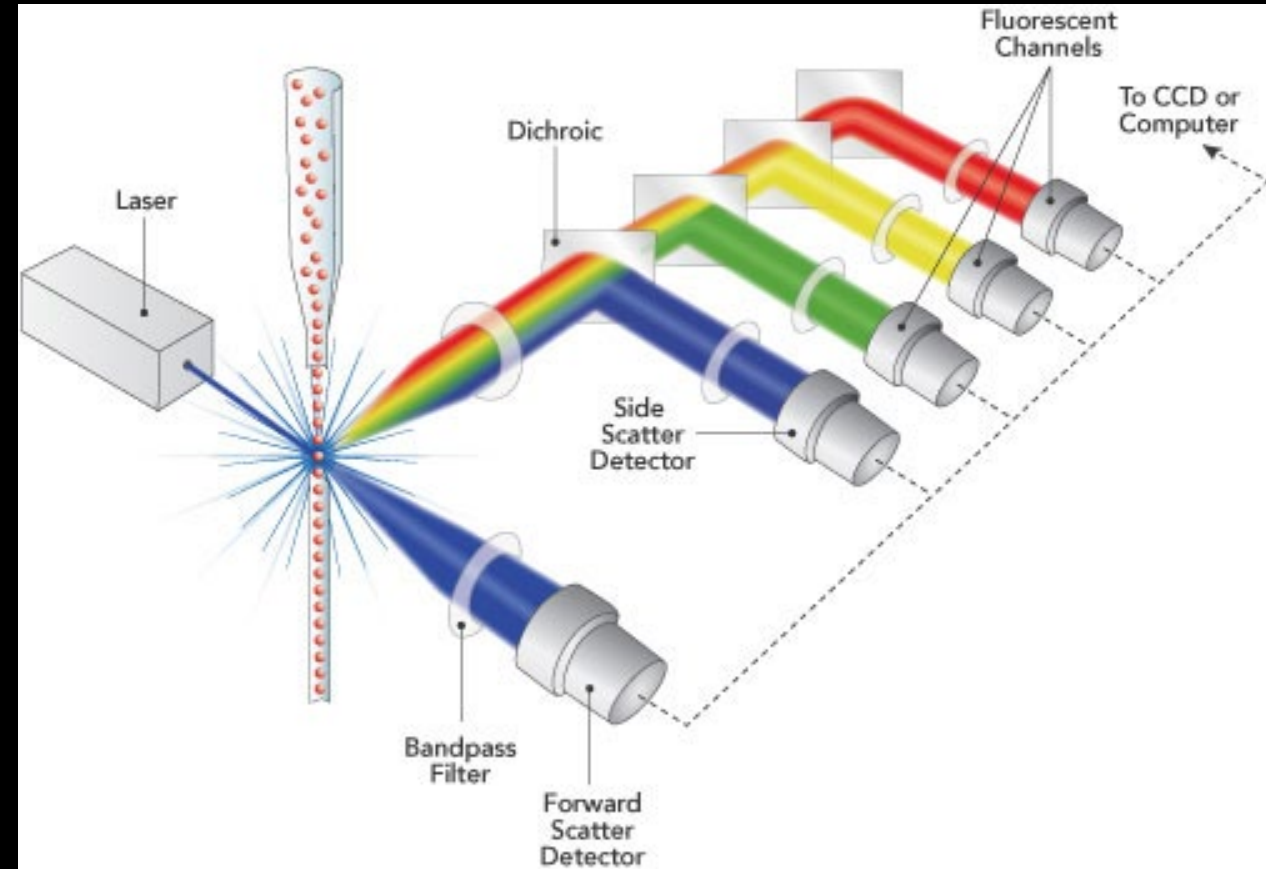
Fluidics

- Transports particles to the laser beam for interrogation
- Hydrodynamic focusing



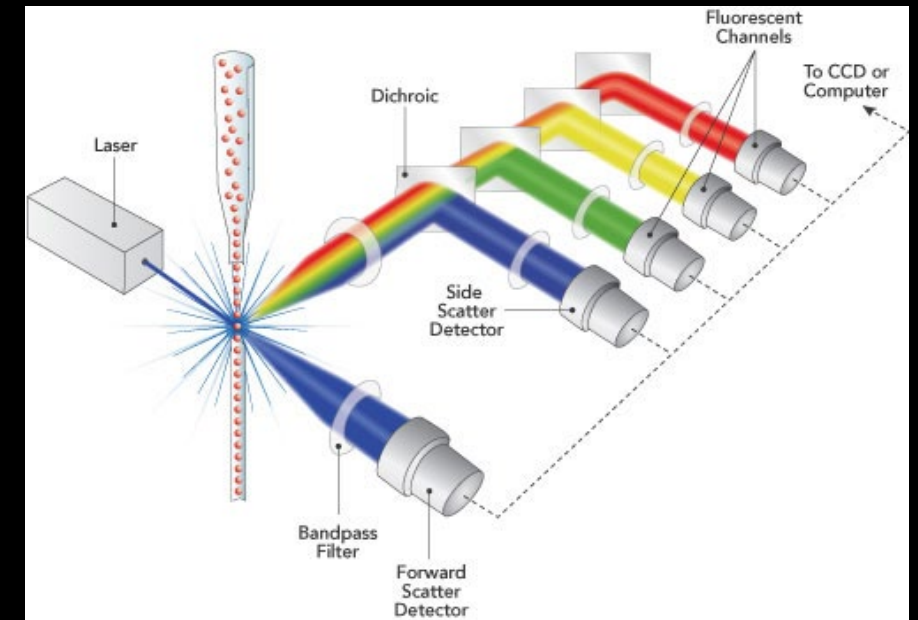
Optics

- Lasers
- Optical filters
- Dichroic vs bandpass

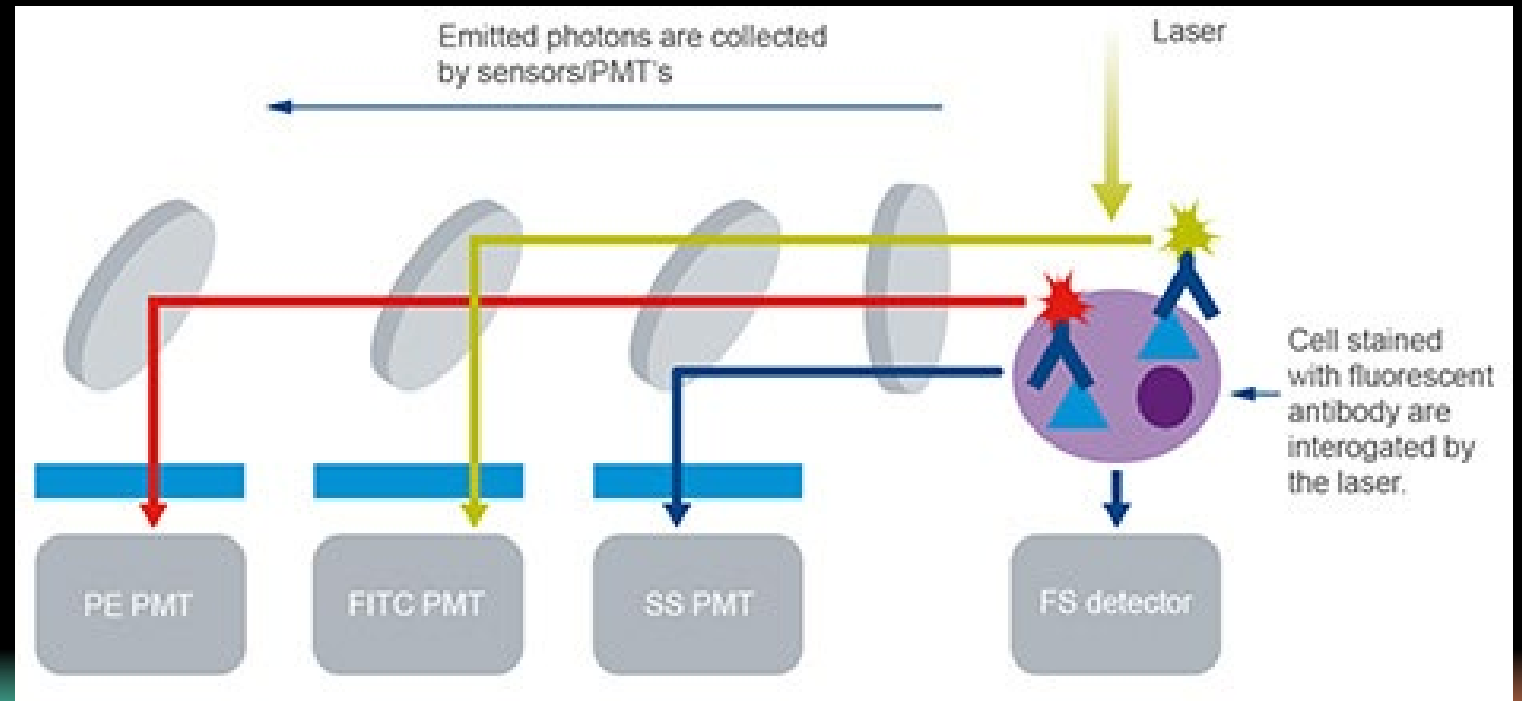


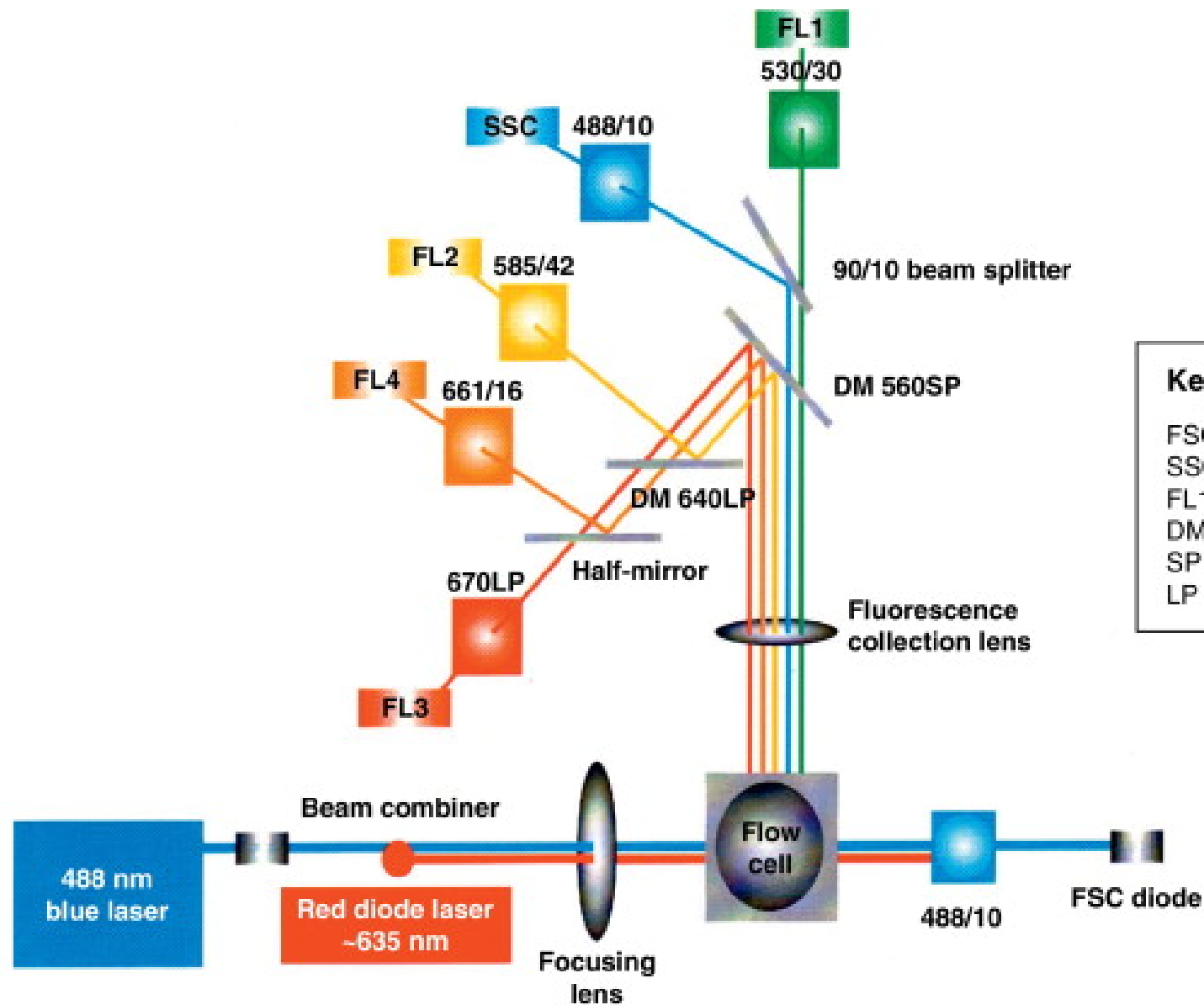
Electronics

- Detectors:
PMT (photomultiplier tube)



- Sorting system



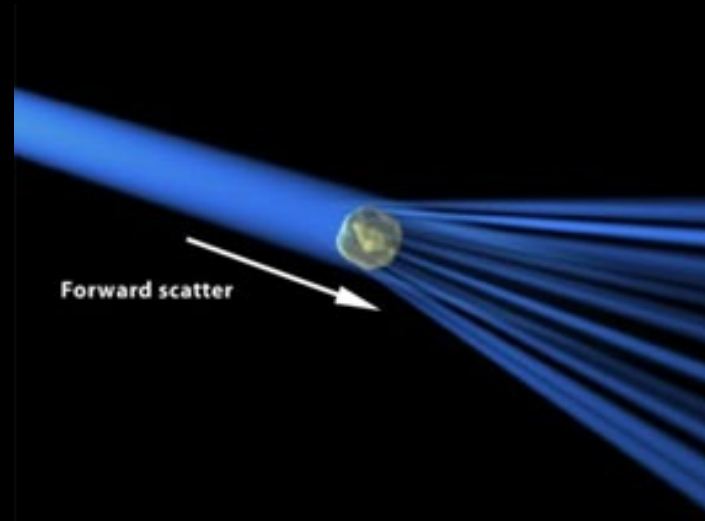


Key:

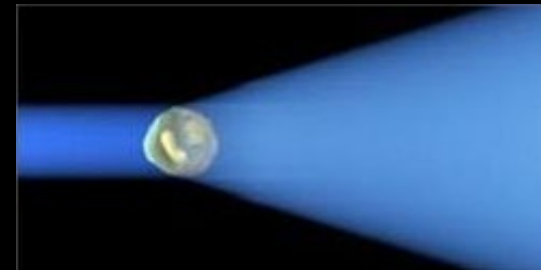
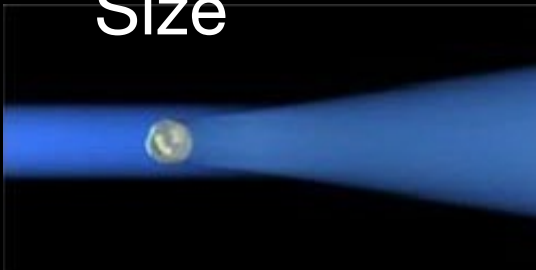
FSC – forward scatter detector
 SSC – side scatter detector
 FL1, FL2, FL3, FL4 – fluorescence detectors
 DM – Dichroic mirror
 SP – shortpass filter
 LP – longpass filter

Data Collection

- Light scatter
 - Forward-scattered light (FSC)

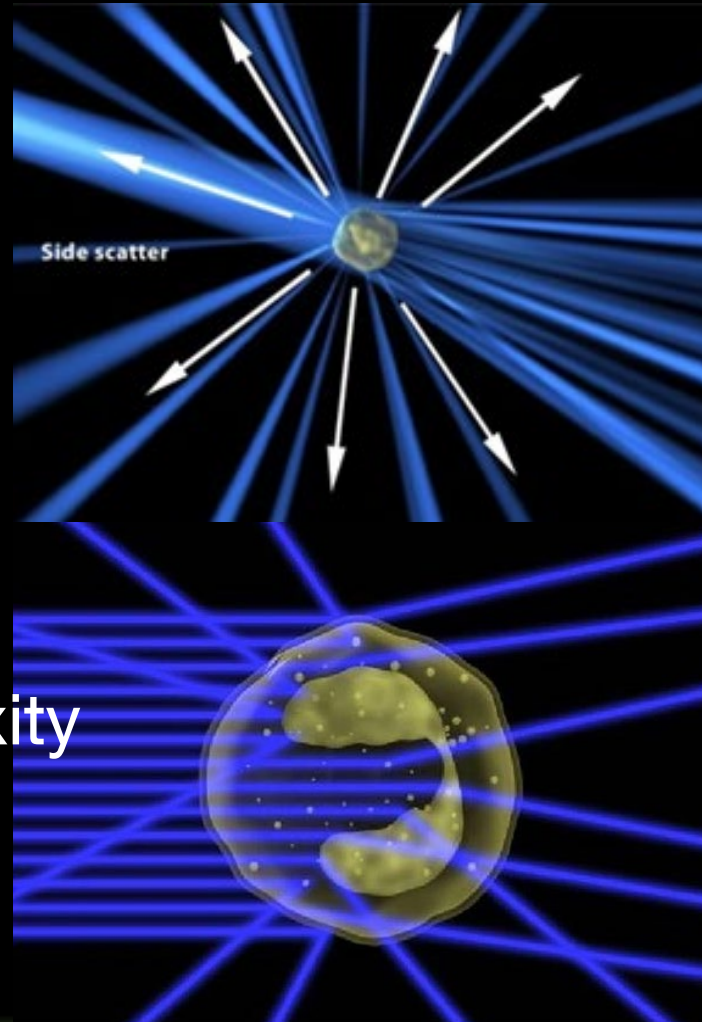


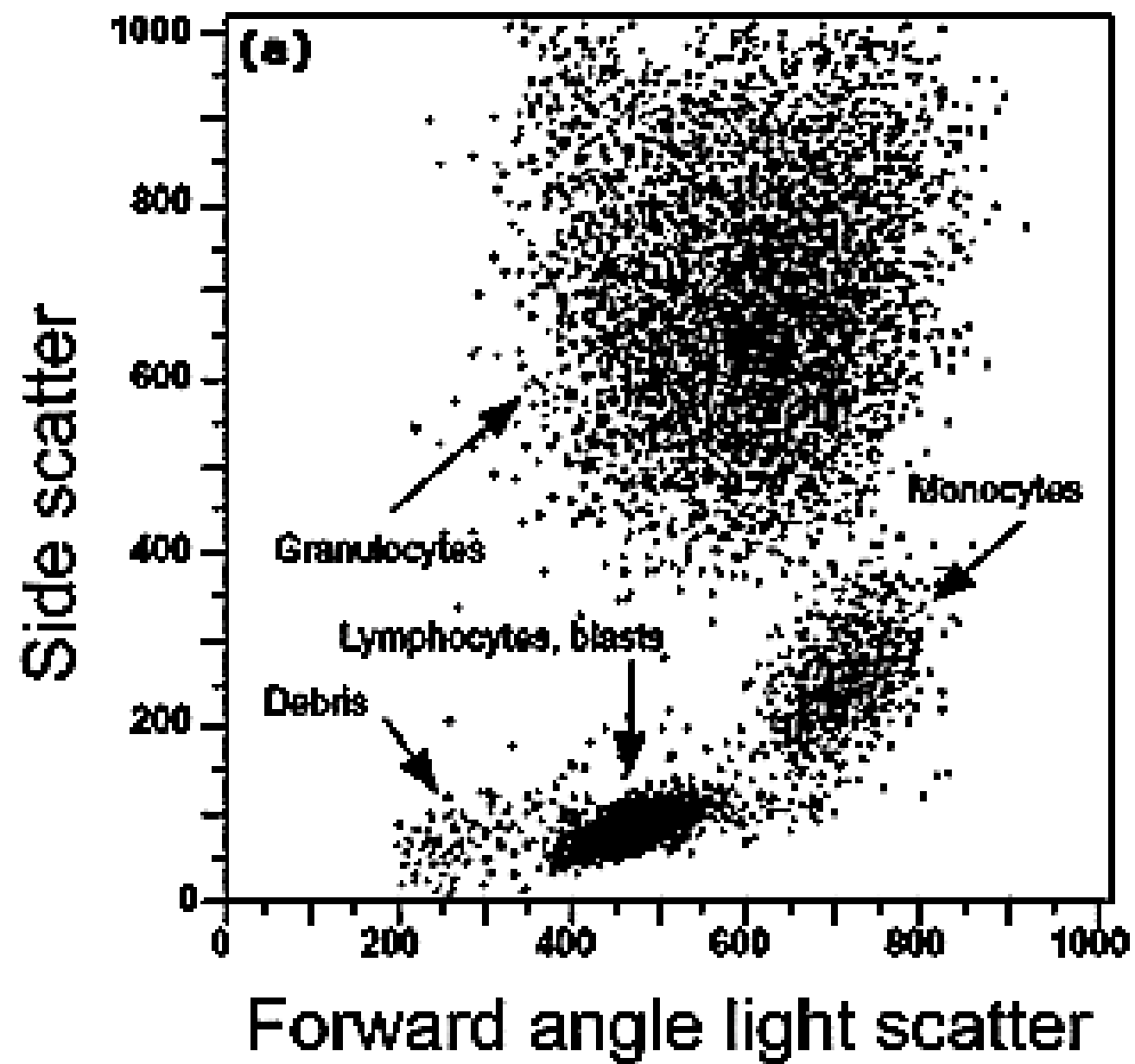
■ Size



Data Collection

- Light scatter
 - Side-scattered light (SSC)
 - Granularity / internal complexity





Data Collection

- Fluorescence
 - Protein expression
 - Fluorescent-conjugated antibody
 - Possible to use more than one antibodies

****Require negative control for accurate data interpretation**

Case study

Fruit Cytometry

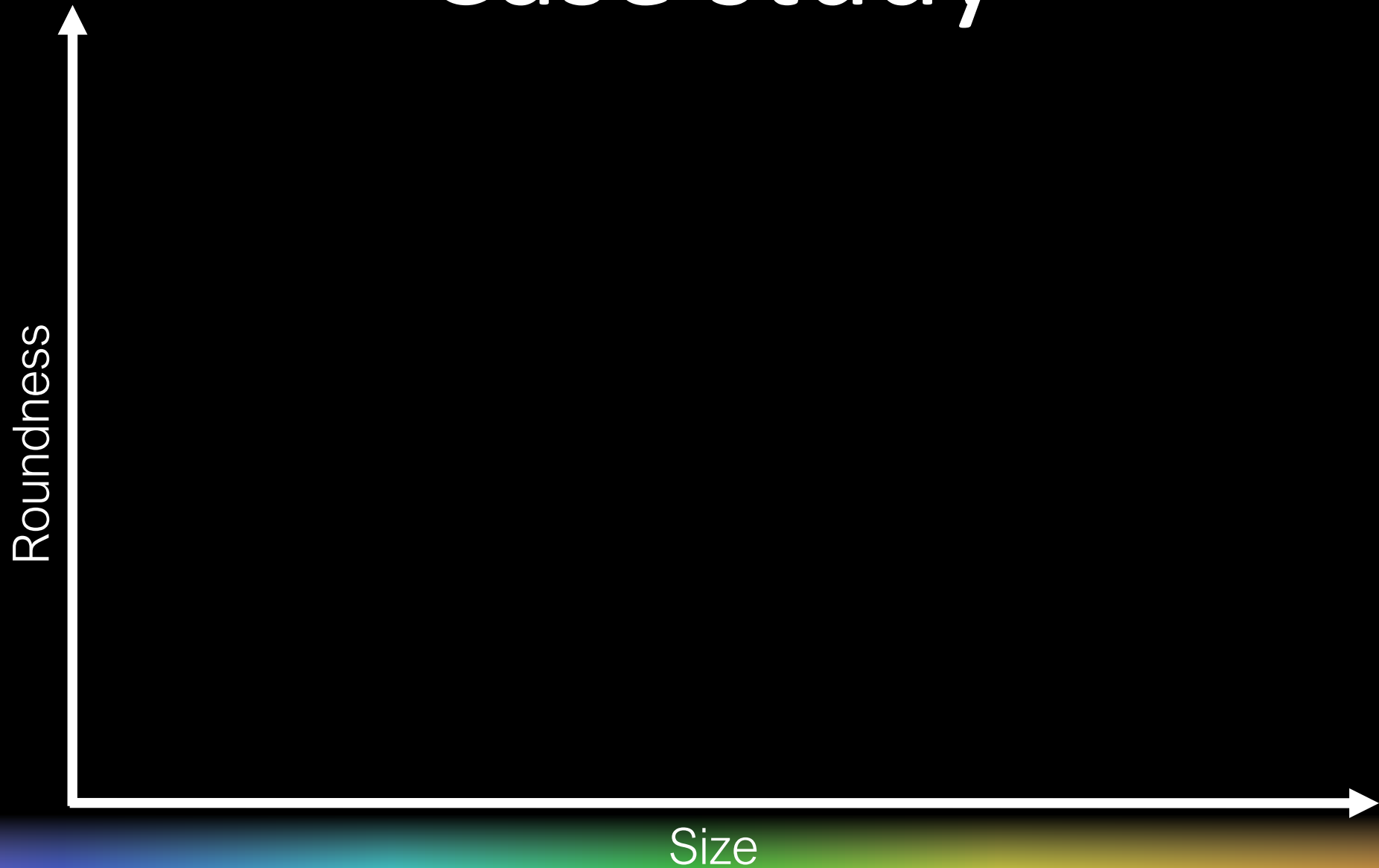


Case study

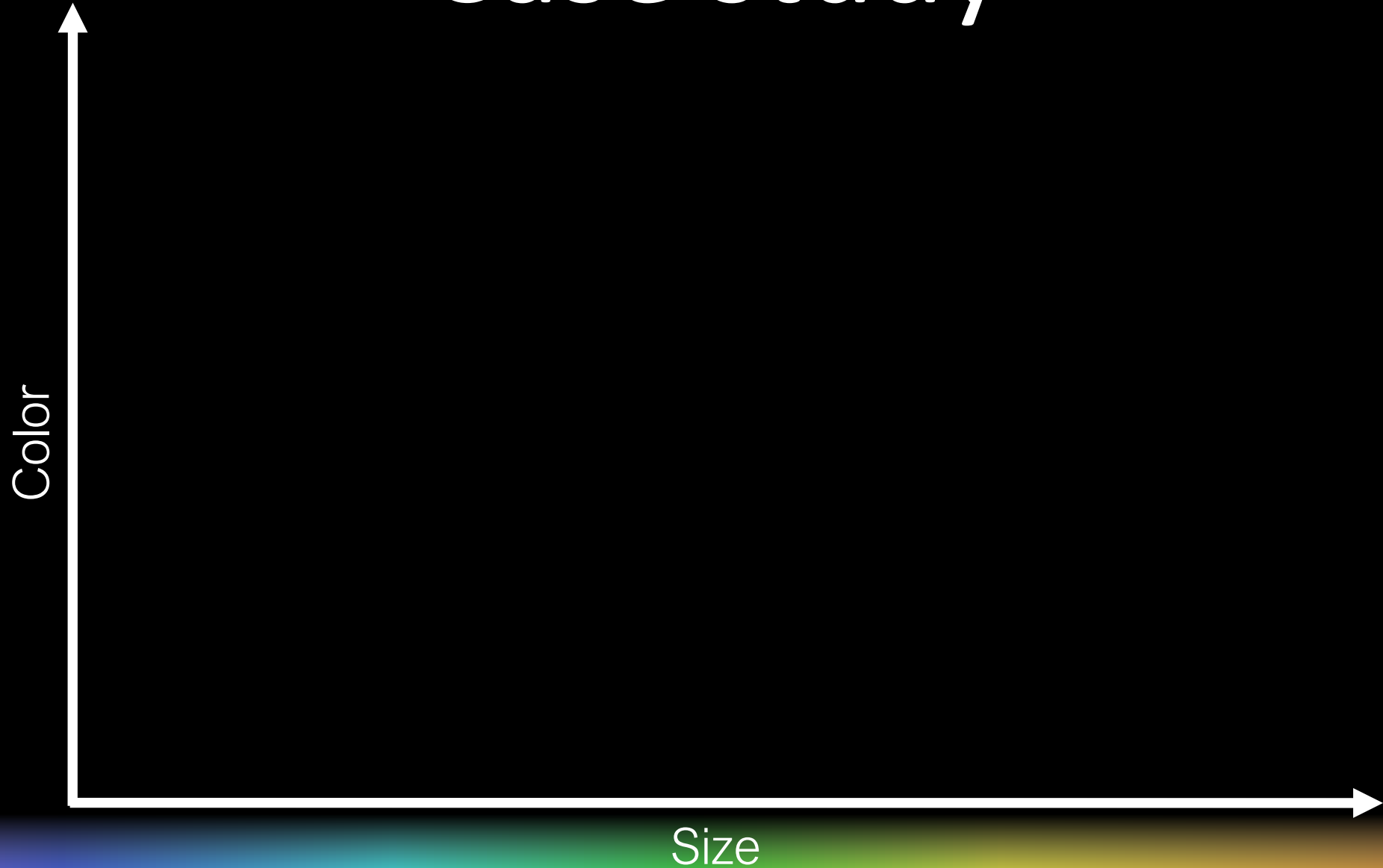


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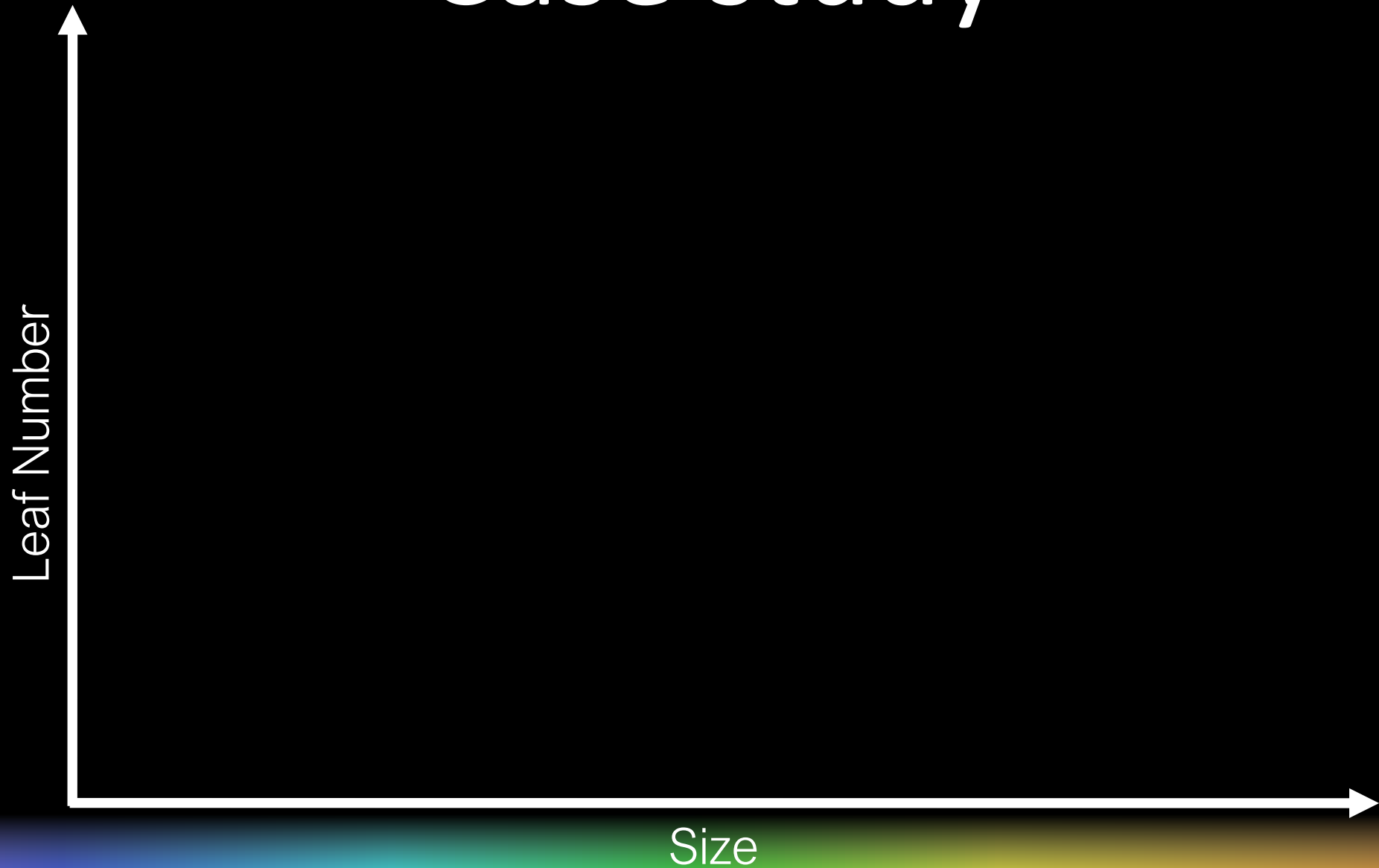
Case study



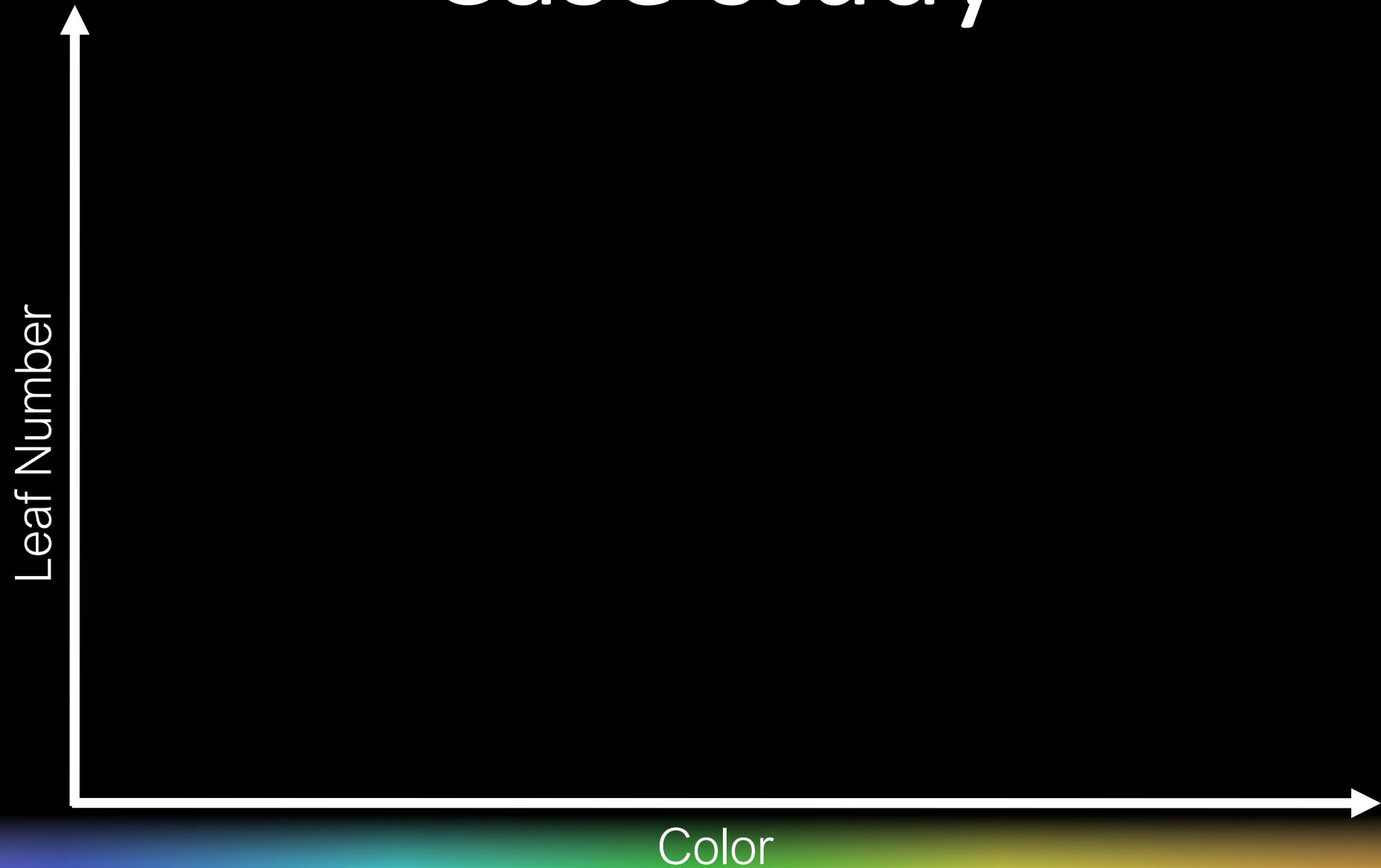
Case study



Case study

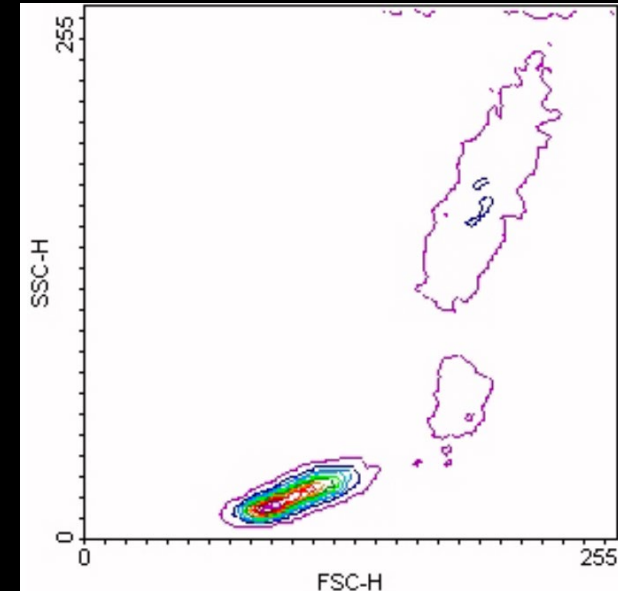
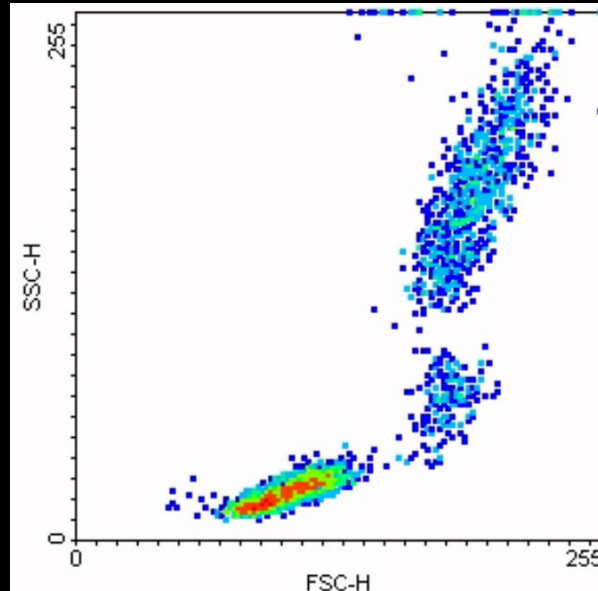
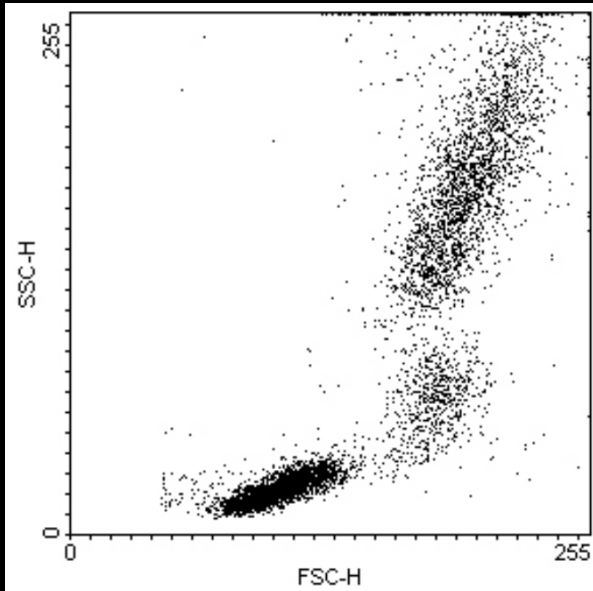


Case study



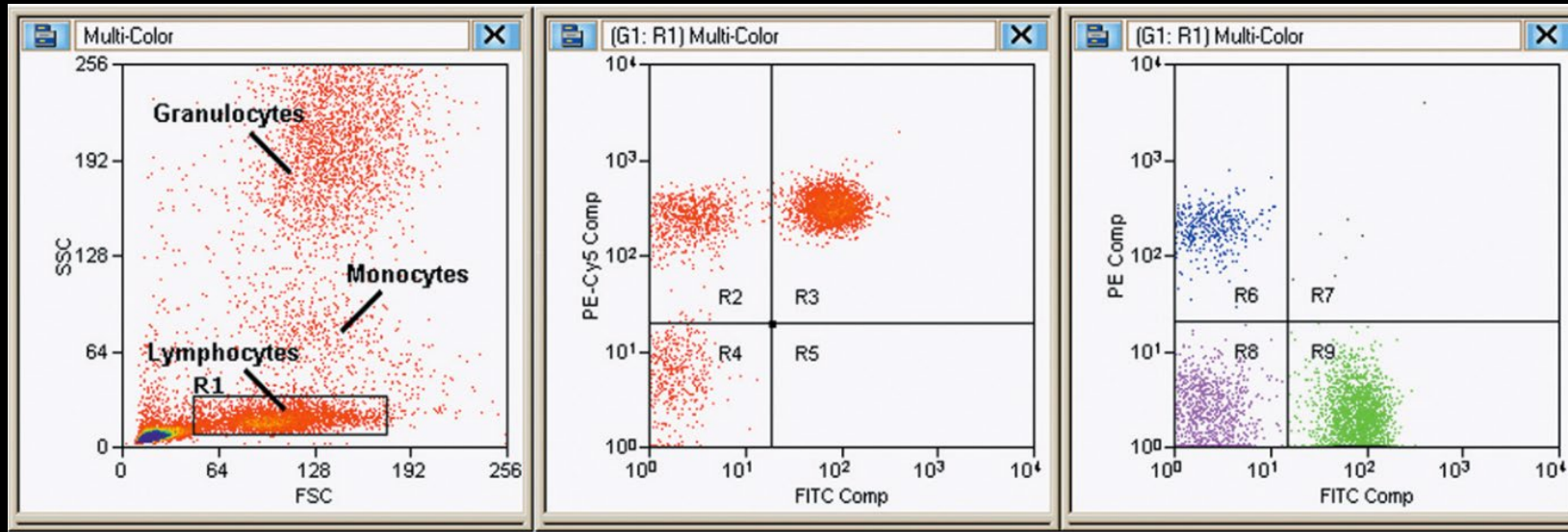
Data Analysis

- Dot / density / contour plot



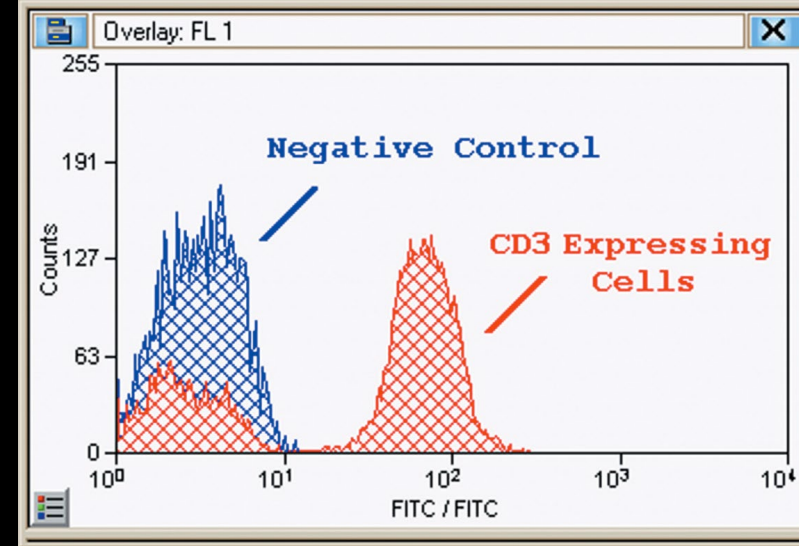
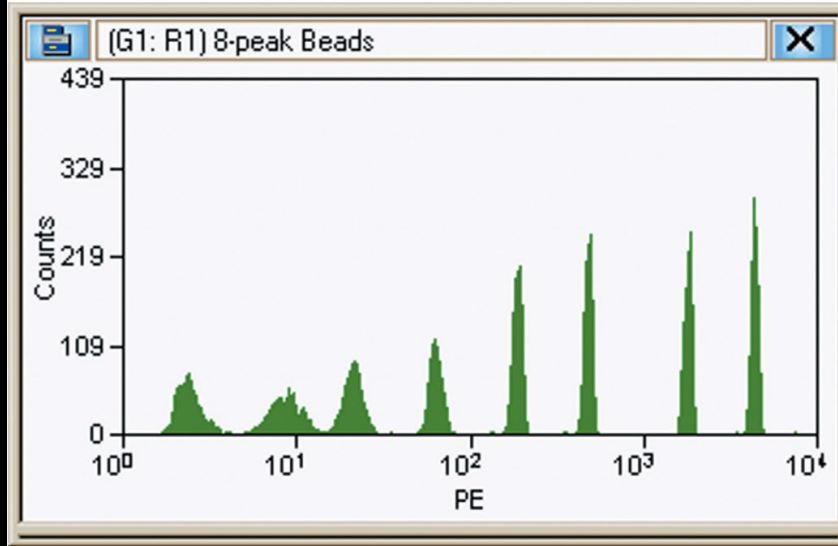
Data Analysis

- Gating



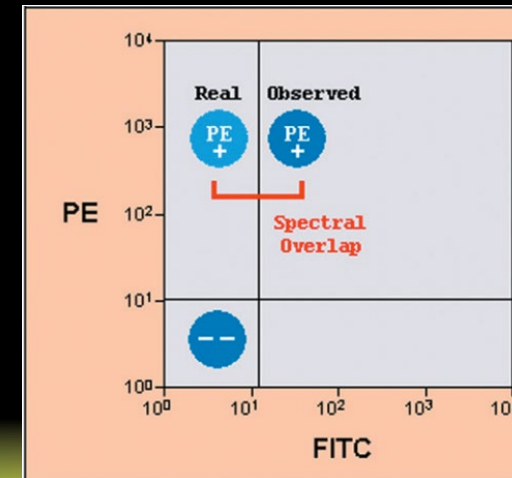
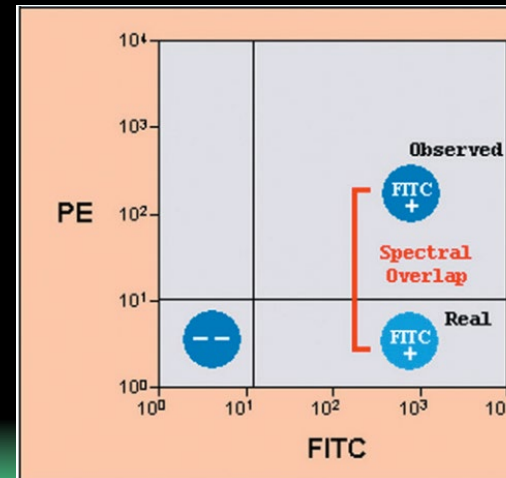
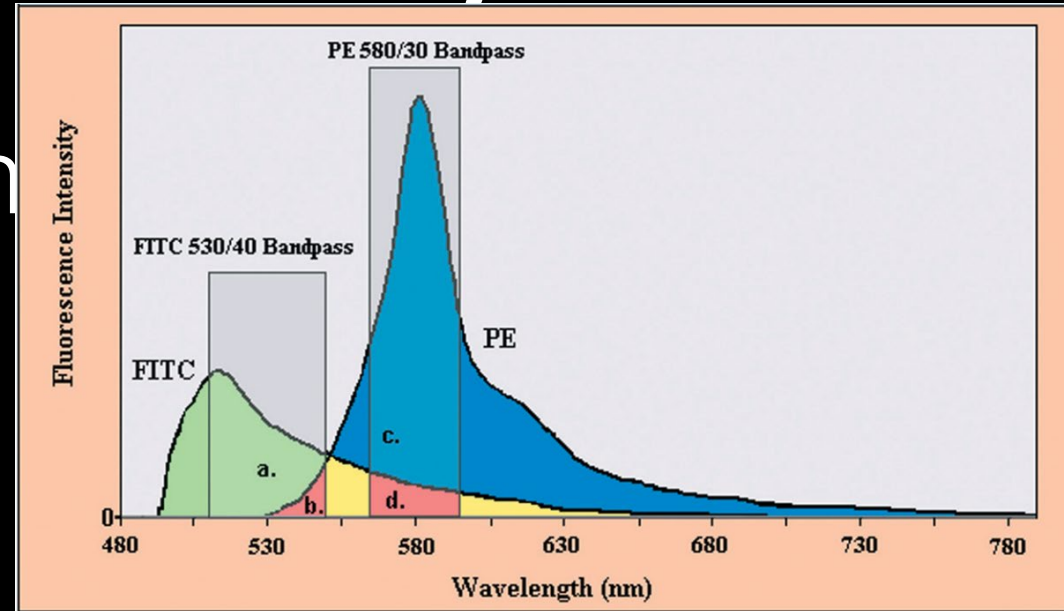
Data Analysis

- Histogram
- Overlay



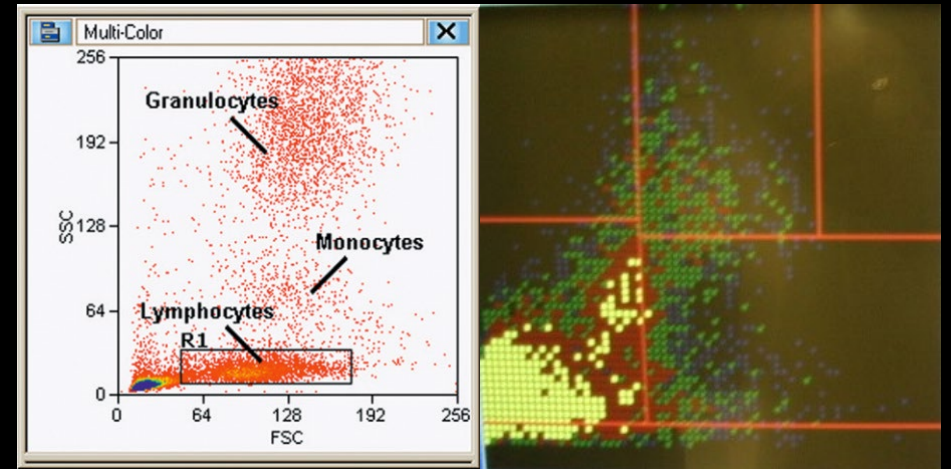
Data Analysis

- Compensation

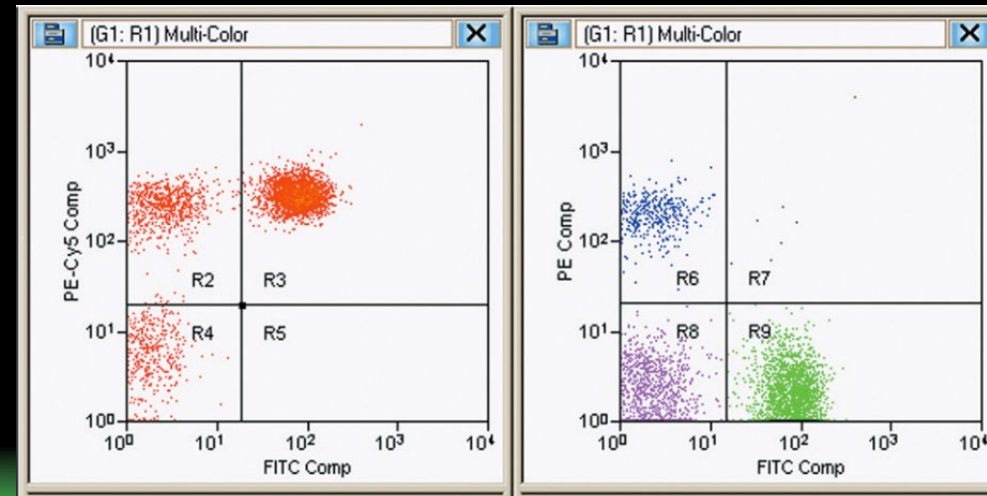


Sample Applications

- Cell type and number analysis



- Antigen detection
 - surface markers
 - cell death/apoptosis
 - etc.



RESEARCH ARTICLE OPEN ACCESS

Effects of Genistein on Inflammasome Induced Pancreatic β -Cell Apoptosis by Dexamethasone

Nattinee Jitprawet¹ | Jatuporn Sujjitjoon² | Petcharee Maneethorn¹ | Namoiy Semprasert¹ | Suwattanee Kooptiwut¹ 

¹Department Physiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand | ²Division of Molecular Medicine, Research Department, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

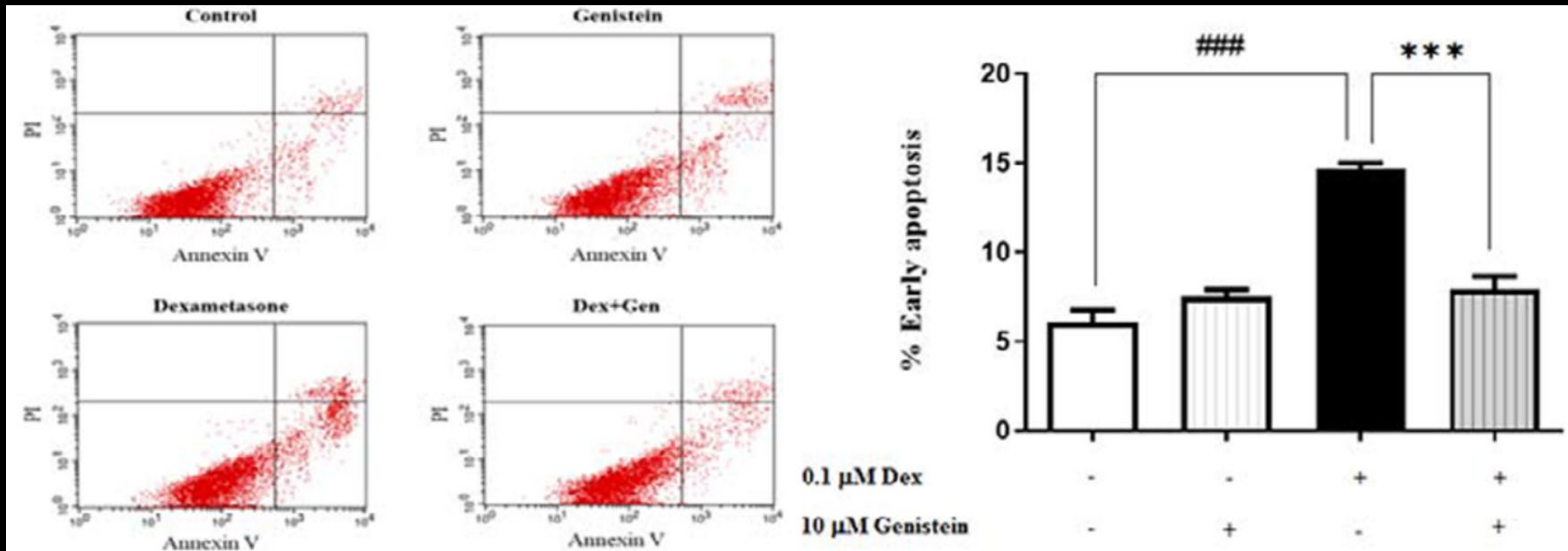
Correspondence: Suwattanee Kooptiwut (suwattanee.koo@mahido.ac.th)

Received: 7 March 2025 | **Revised:** 20 June 2025 | **Accepted:** 12 August 2025

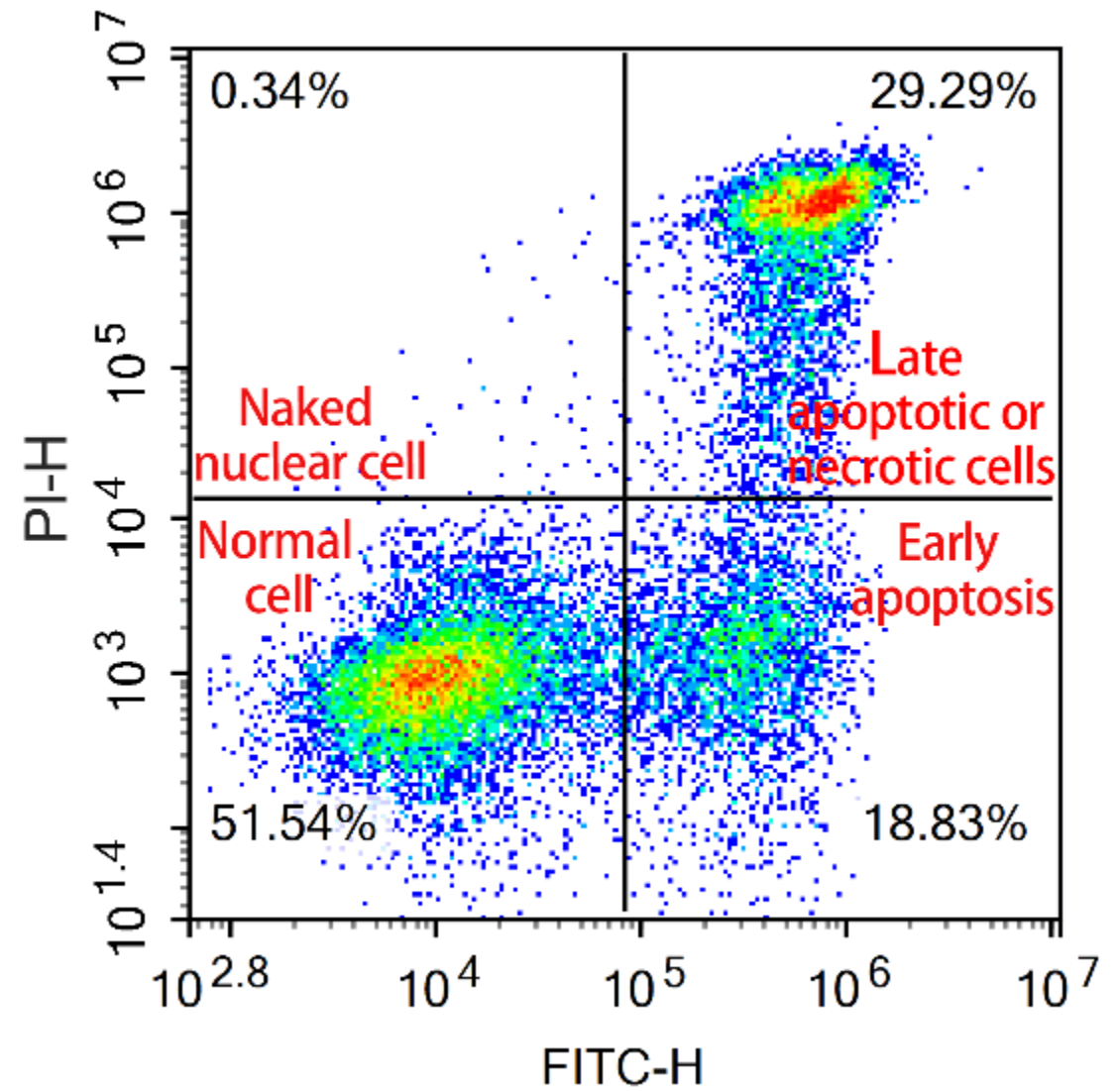
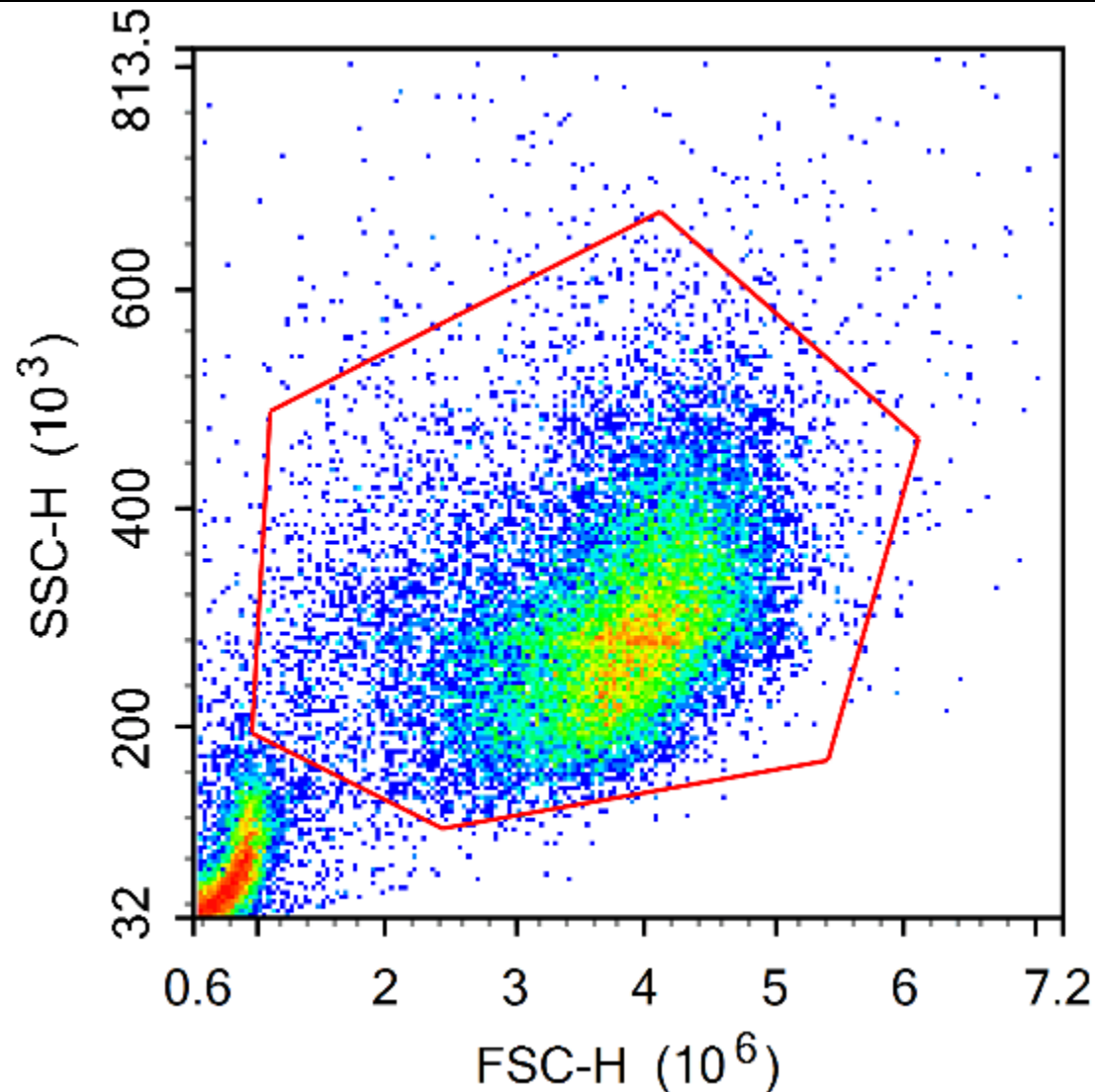
Funding: This research project was supported by the Faculty of Medicine, Siriraj Hospital, Mahidol University, Grant Number (IO) R016533012 (to S.K., J.S., N.S.). S.K. and J.S. were supported by a Chalermphrakiat Grant from the Faculty of Medicine, Siriraj Hospital, Mahidol University.

Keywords: dexamethasone | ER Stress | genistein | NLRP3 Inflammasome | pancreatic β -cell apoptosis

(One of the) Results

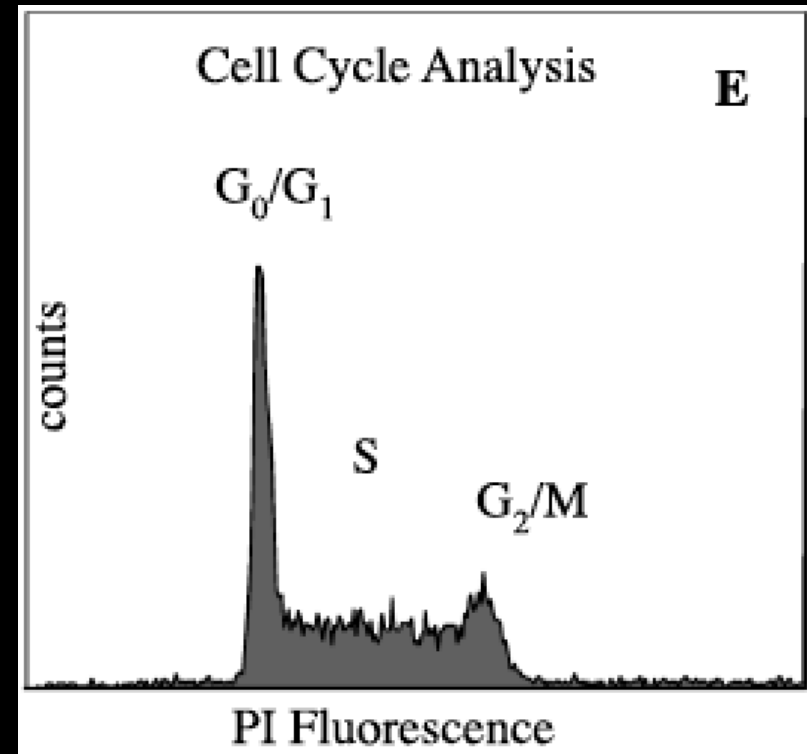
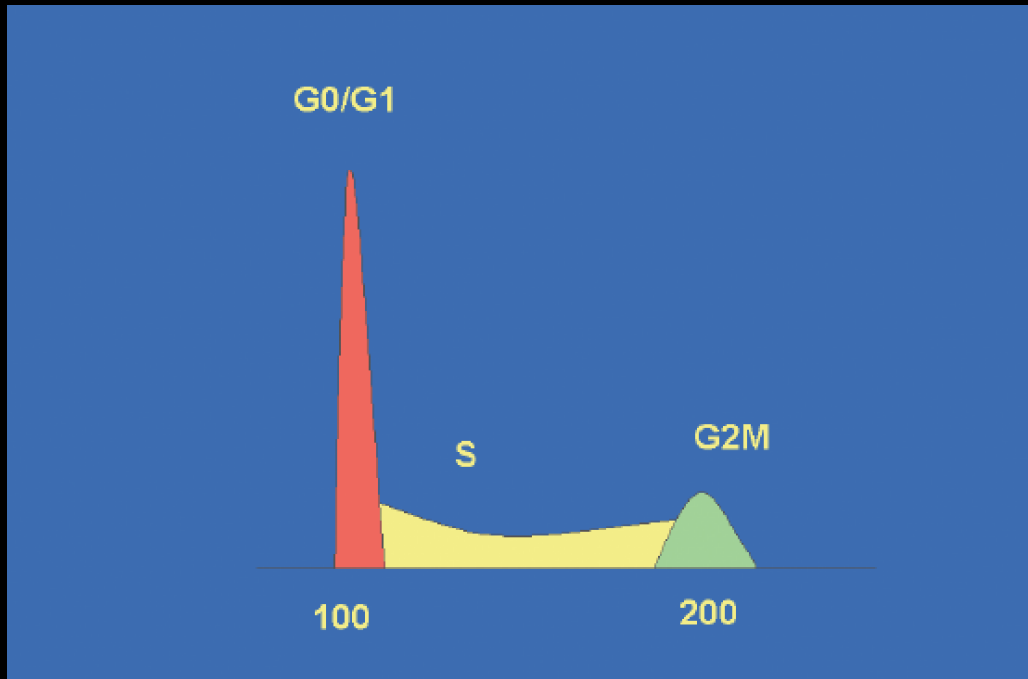


Principle of Annex V-PI staining



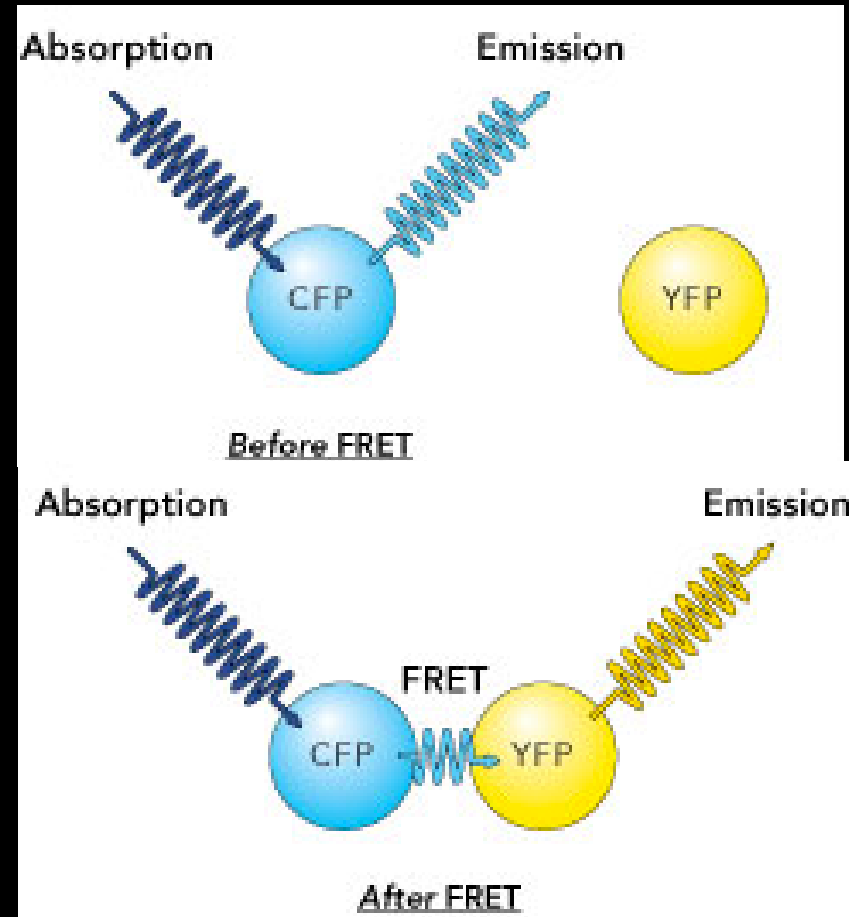
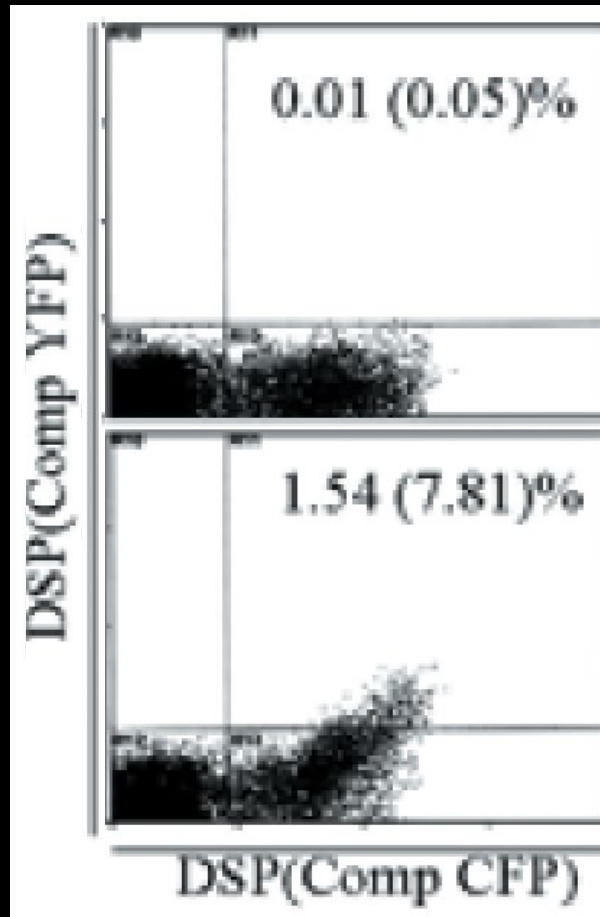
Sample Applications

- Cell cycle analysis

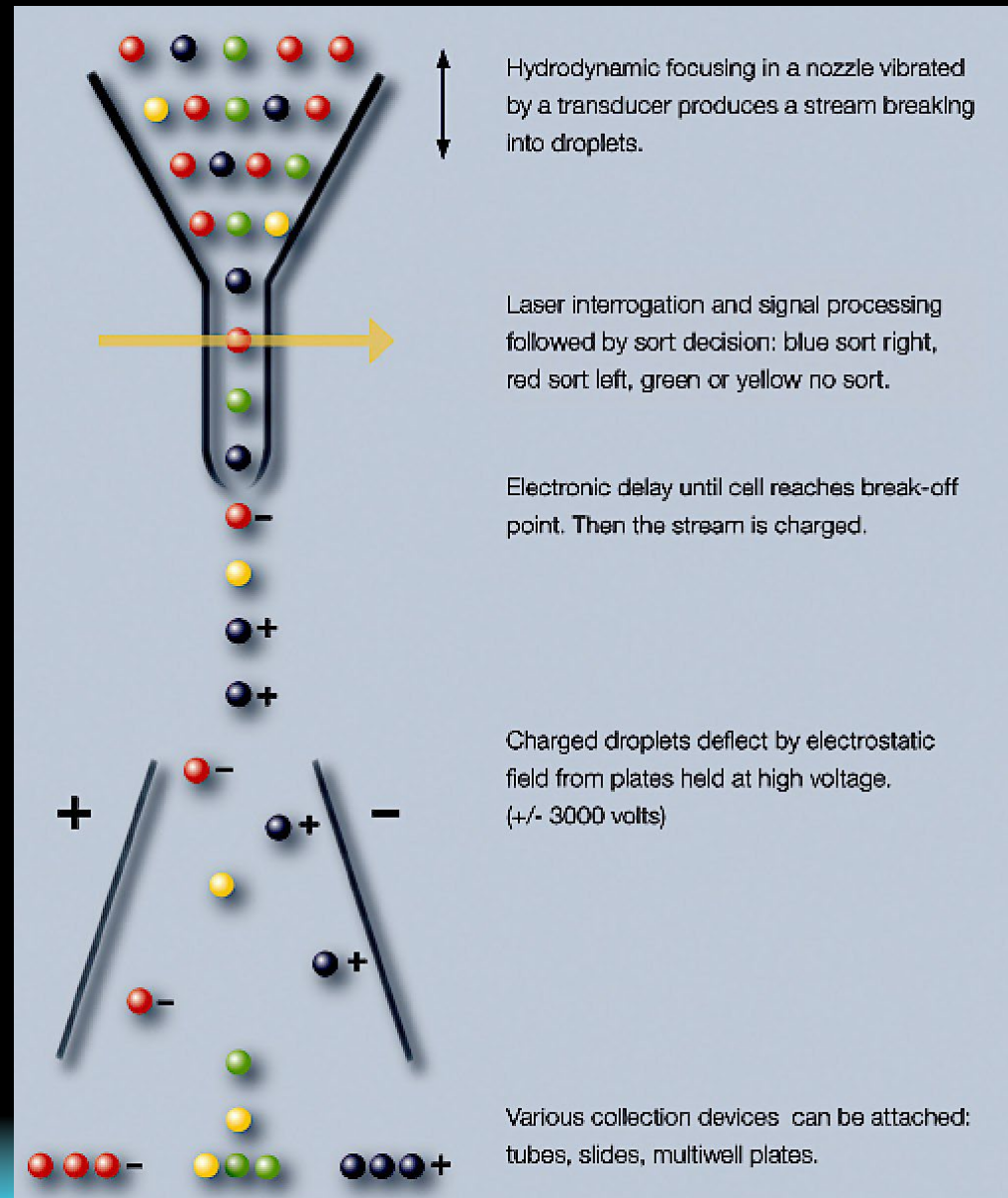


Sample Applications

- Protein-protein interaction using FRET



Fluorescent-Activated Cell Sorting (FACS)



Summary

- Flow cytometry: Fluidics + Optics + Electronics
- Single particle (cell) analysis
 - FSC + SSC + Specific marker(s) of choice
- Data analysis: Dot plot / Histogram
- Application: Cell (population) analysis, FRET, FACS

Question?

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