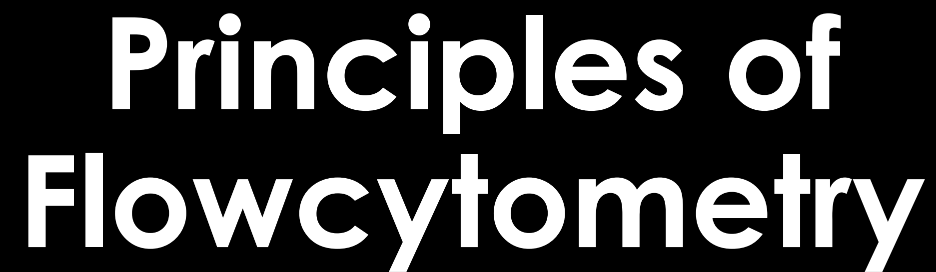
بسم الله الرحمن الرحيم



Dr. Samadi

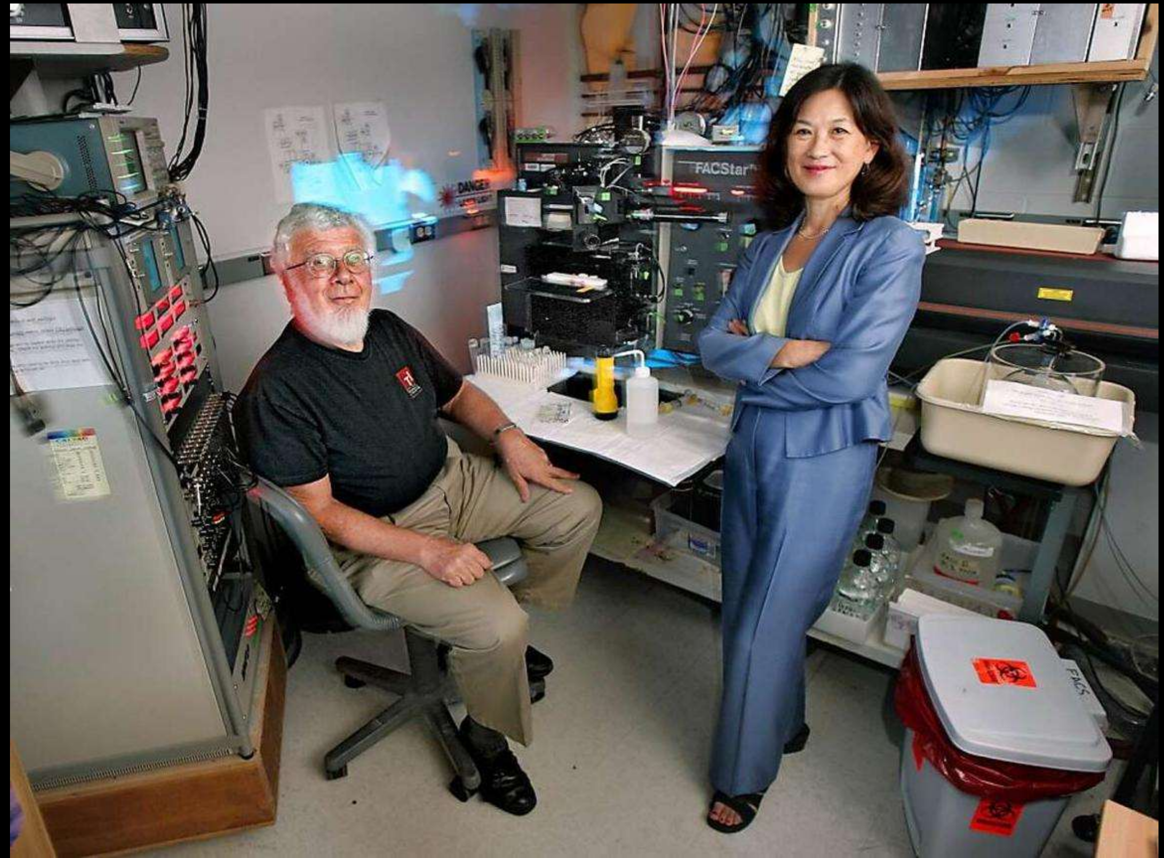
Immunology Department of

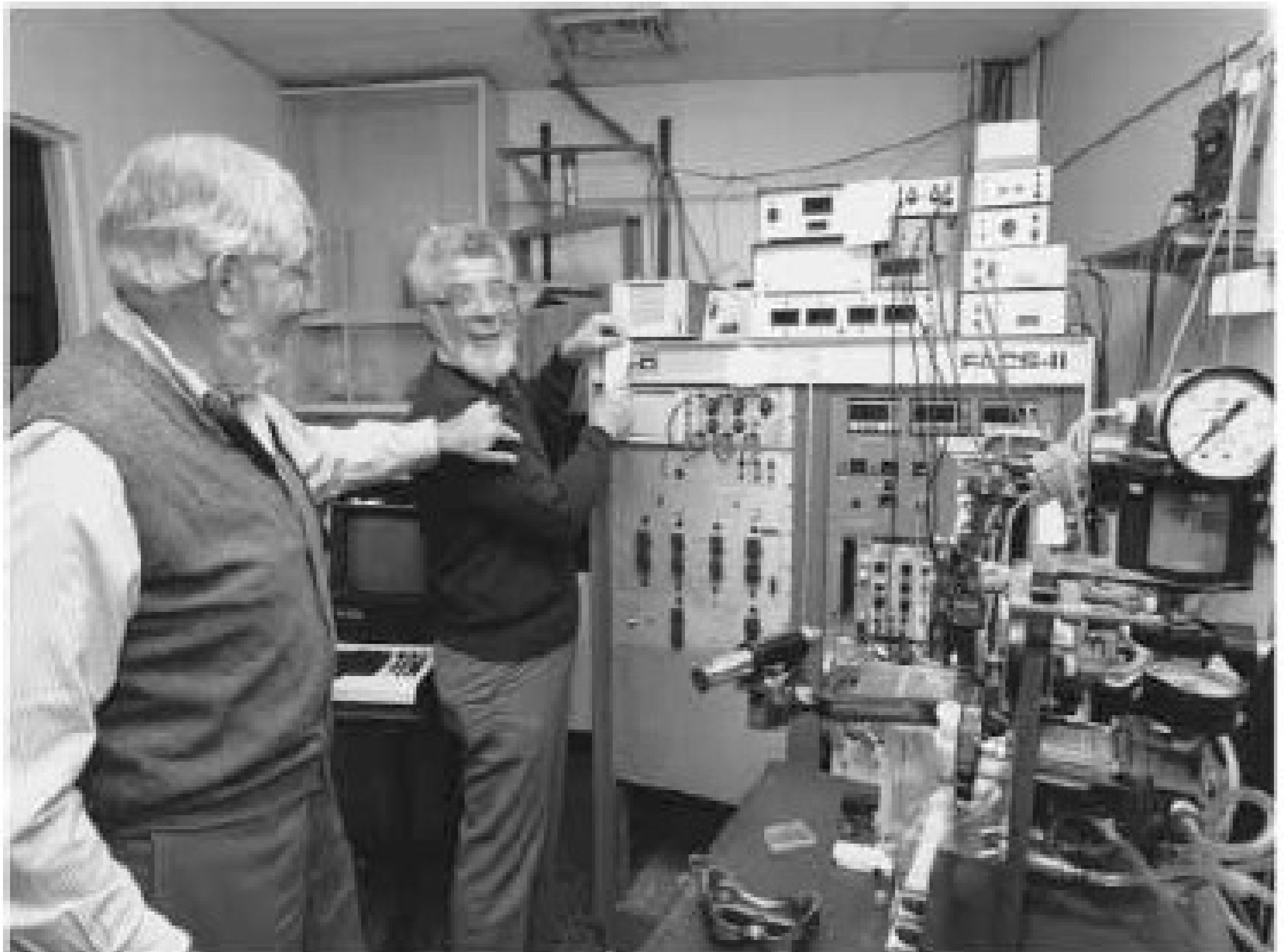
Isfahan University of Medical Sciences

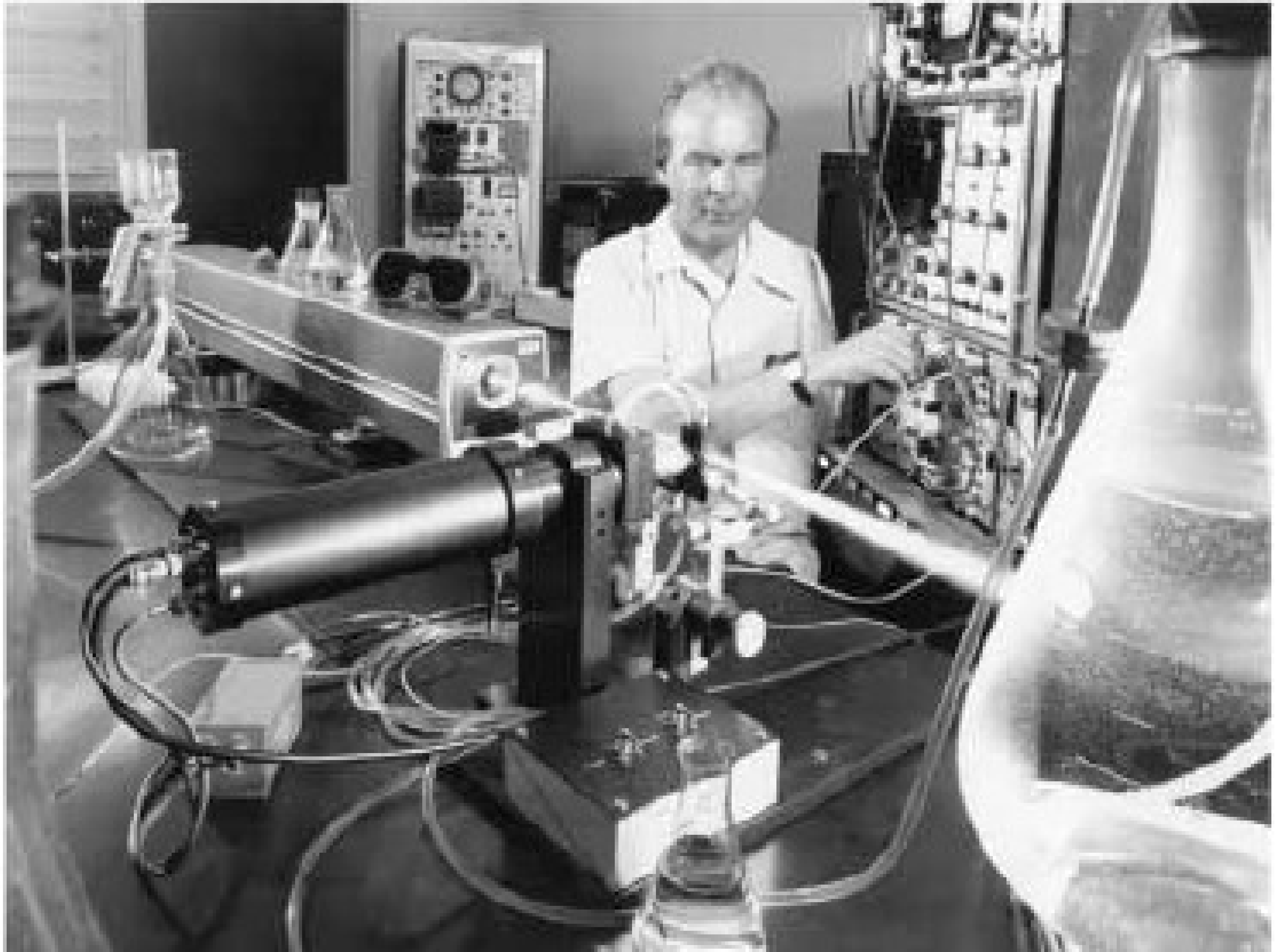
تاریخچه

- **لئونارد هرزنبرگ** Leonard Herzenberg(۱۹۳۱–۲۰۱۳)
- یک ایمونولوژیست آمریکایی در Stanford Universityبود که تأثیر عمیقی بر زیست‌شناسی سلولی و ایمونولوژی گذاشت.
- پیش از کار هرزنبرگ، پژوهشگران می‌توانستند جمعیت سلول‌ها را به‌صورت کلی بررسی کنند، اما جداسازی سلول‌های خاص بسیار دشوار بود.
- مهم‌ترین دستاورد: ابداع FACS
- او همراه همکارانش در دانشگاه استنفورد:
- فناوری **FACS (Fluorescence-Activated Cell Sorting)** را توسعه داد که امکان شناسایی و جداسازی سلول‌ها بر اساس ویژگی‌های فلورسنت را فراهم کرد. این فناوری امروزه یکی از پایه‌های اصلی فلوسایتومتری مدرن است.

DR. HERZENBERG

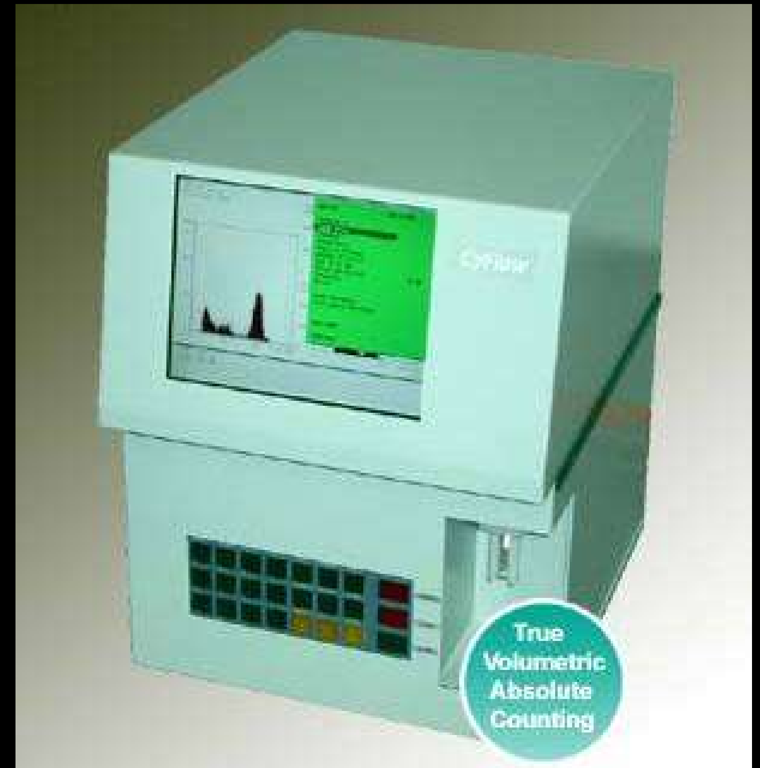






CyFlow Counter

EUR 20,000)



MOBILE FLOW CYTOMETRY LAB

WHAT IS FLOW CYTOMETRY?

- Flow ~ cells in motion
- Cyto ~ cell
- Metry ~ measure
- Measuring properties of cells while in a fluid stream

Flow Cytometer Subsystems

- **Fluidics: To introduce and focus the cells for interrogation.**
- Optics: To generate and collect the light signals.
- Electronics: To convert the optical signals to proportional digital signals, process the signals, and communicate with the computer.
- Interpretation: specialized software

FLUIDICS SCHEMATIC

Sample Tube

Sample Pressure (Variable)

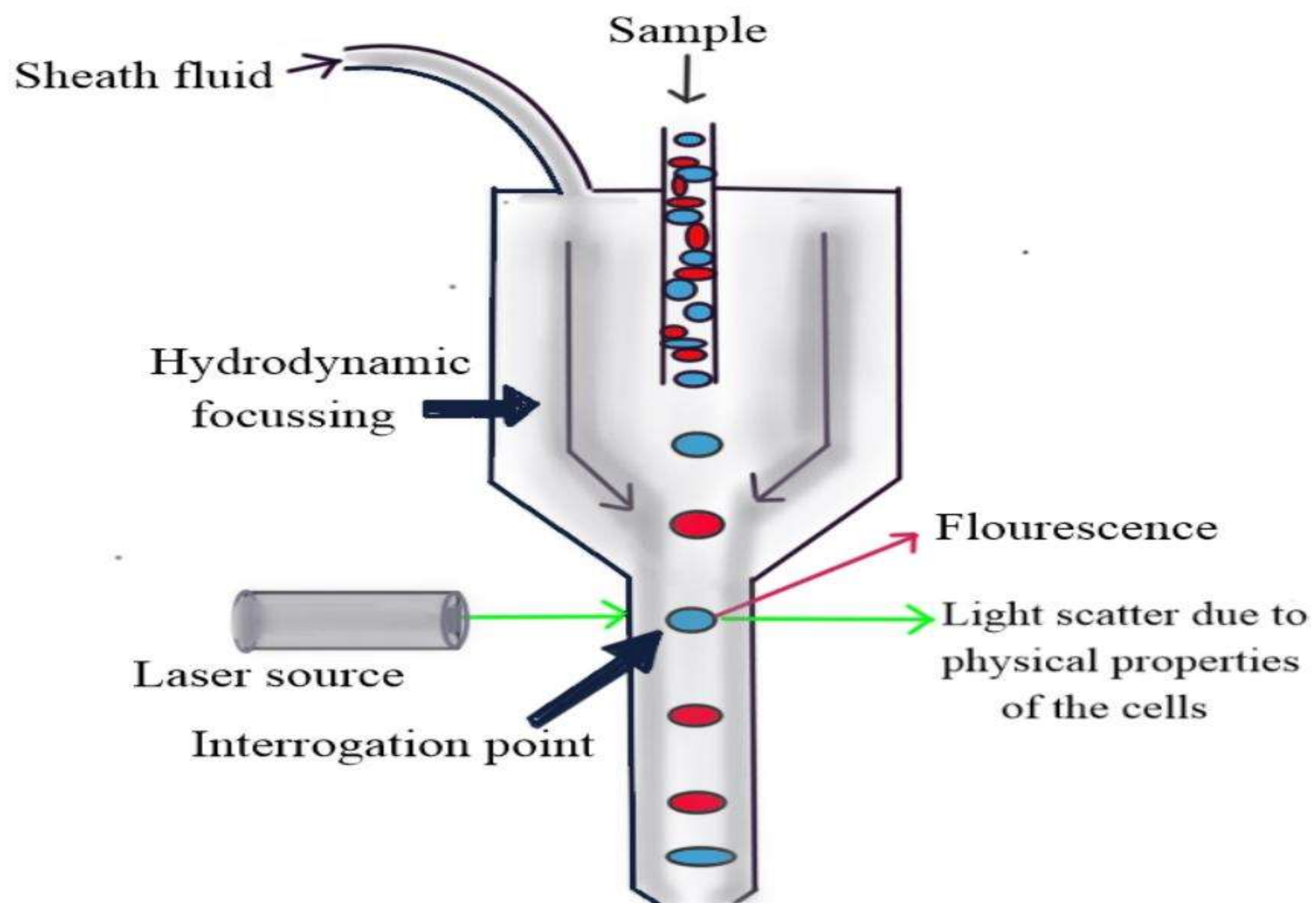
Sheath Pressure (Constant)

Waste Tank

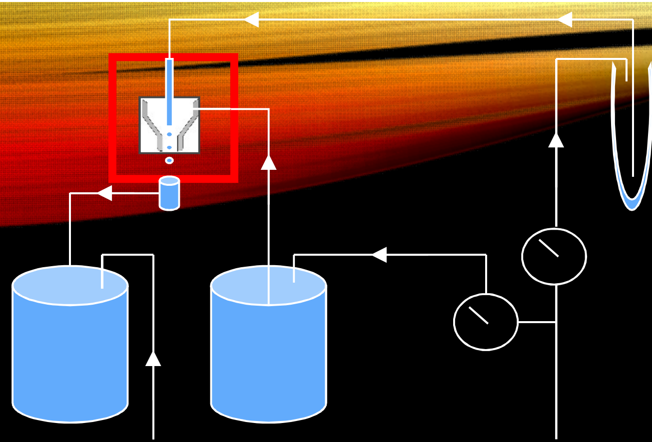
Sheath Tank

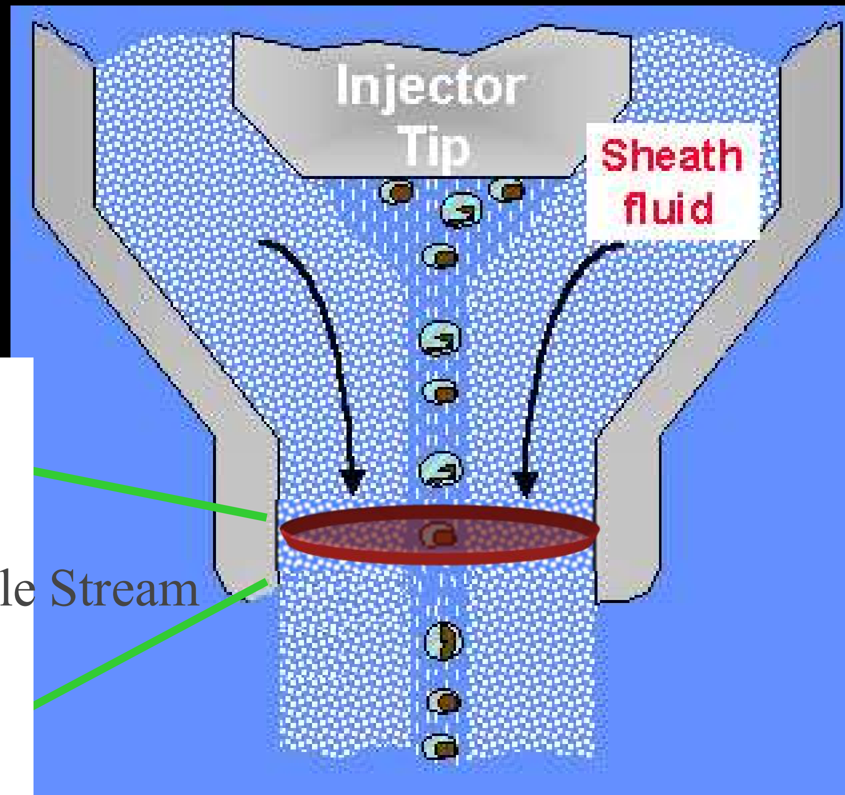
Vacuum

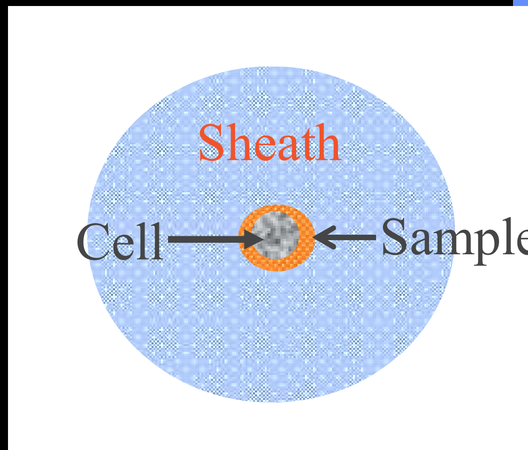
Line Pressure


Sample
Sheath fluid
Hydrodynamic
focussing
Flourescence
Light scatter due to
physical properties
of the cells
Laser source
Interrogation point

THE FLOW CELL







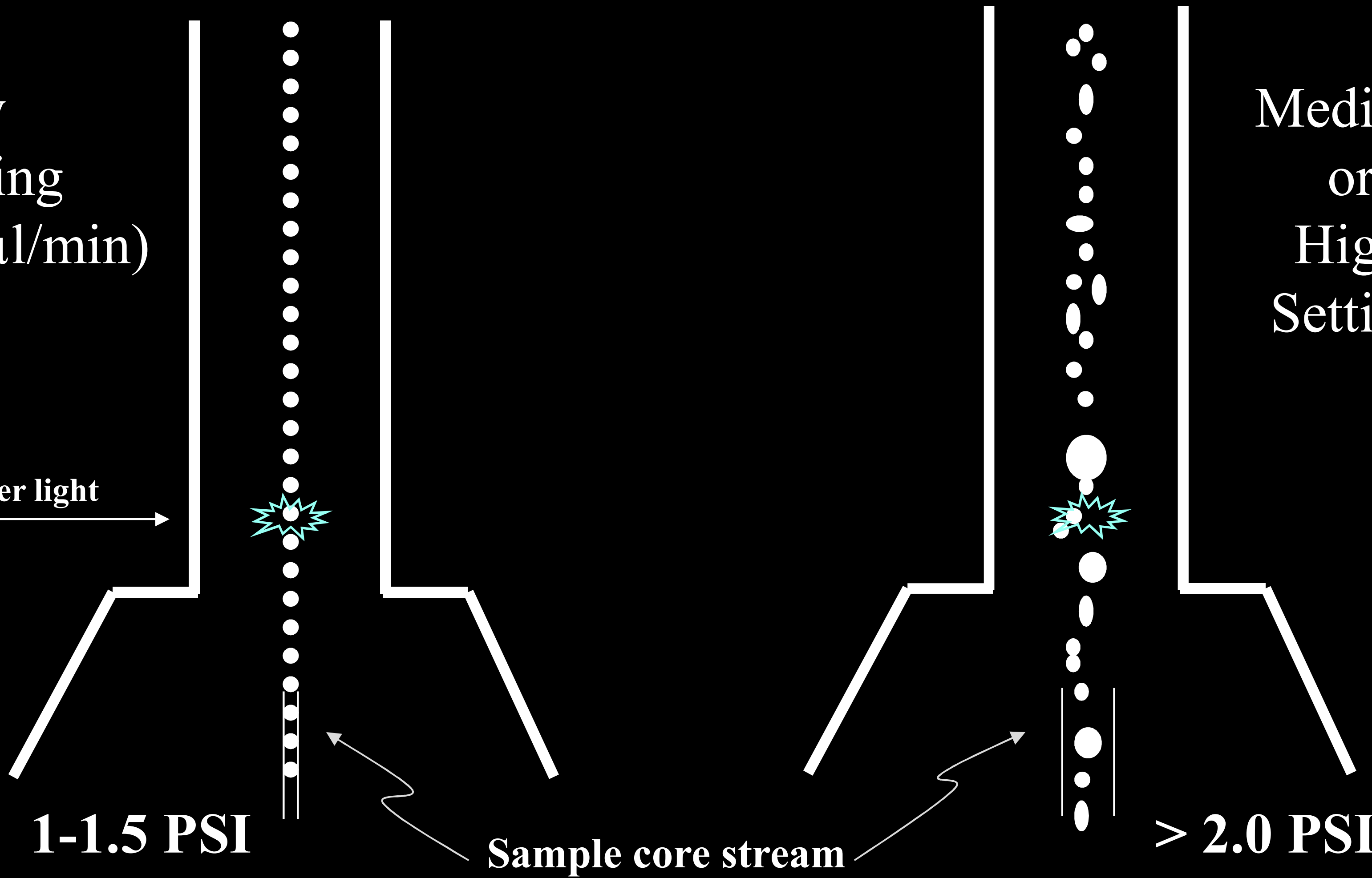
The introduction of a large volume into a small volume in such a way that it becomes "focused" along an axis is called Hydrodynamic Focusing.

Original from Purdue University Cytometry Laboratories, Modified by James Marvin

Low differential pressure

- Better resolution
- Better doublet discrimination (CV)
- Better cell cycle fittings

High differential pressure



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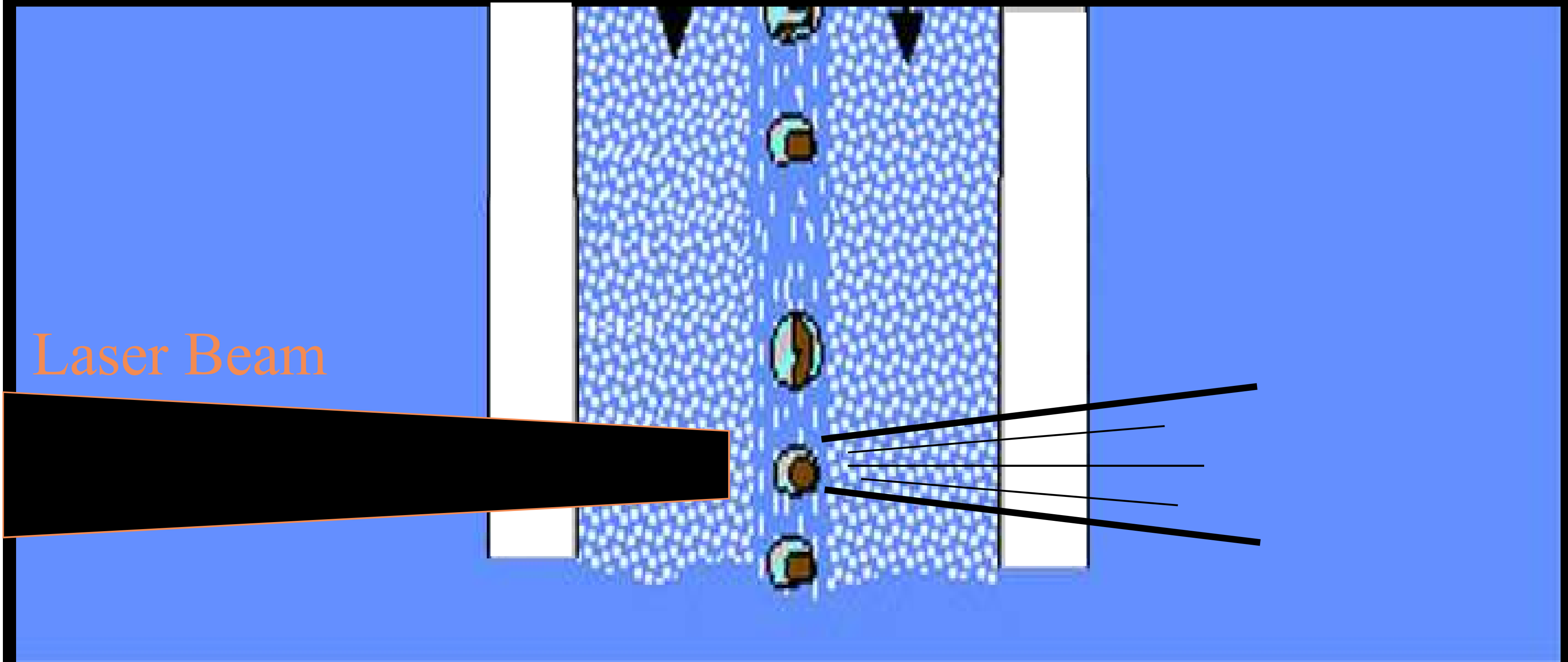
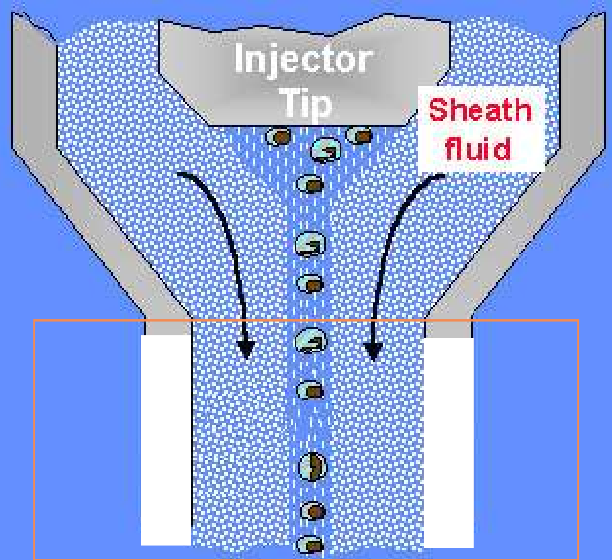
INTERROGATION

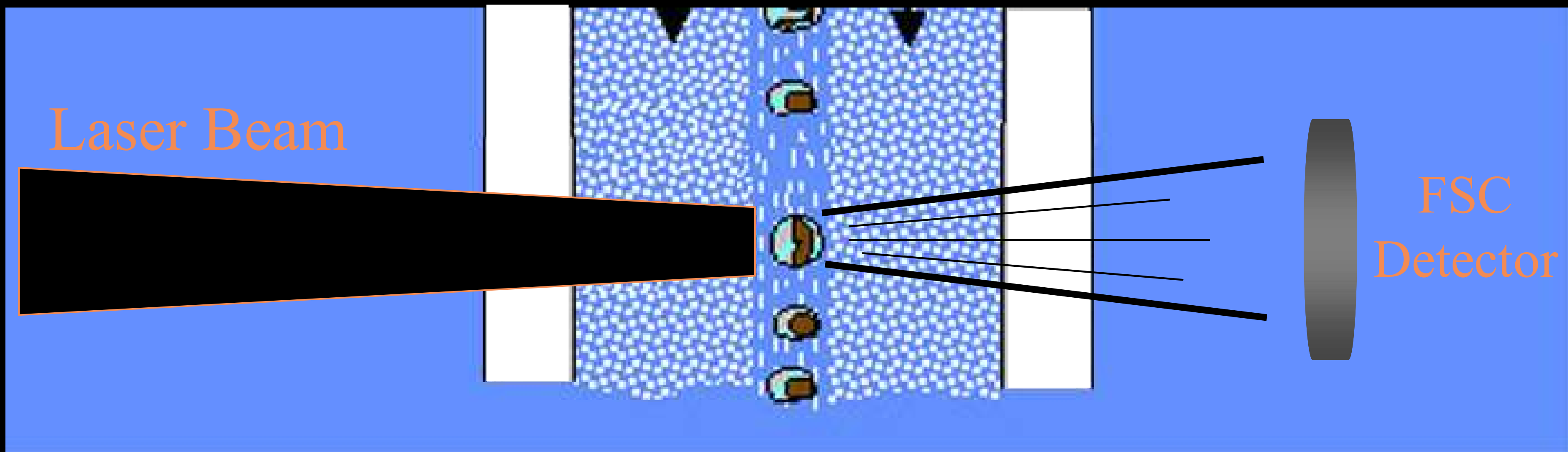


What Can a Flow Cytometer Tell Us About a Cell?

- Its relative size (Forward Scatter—FSC)
- Its relative granularity or internal complexity or cytosolic structure (Side Scatter—SSC)
- Its relative fluorescence intensity

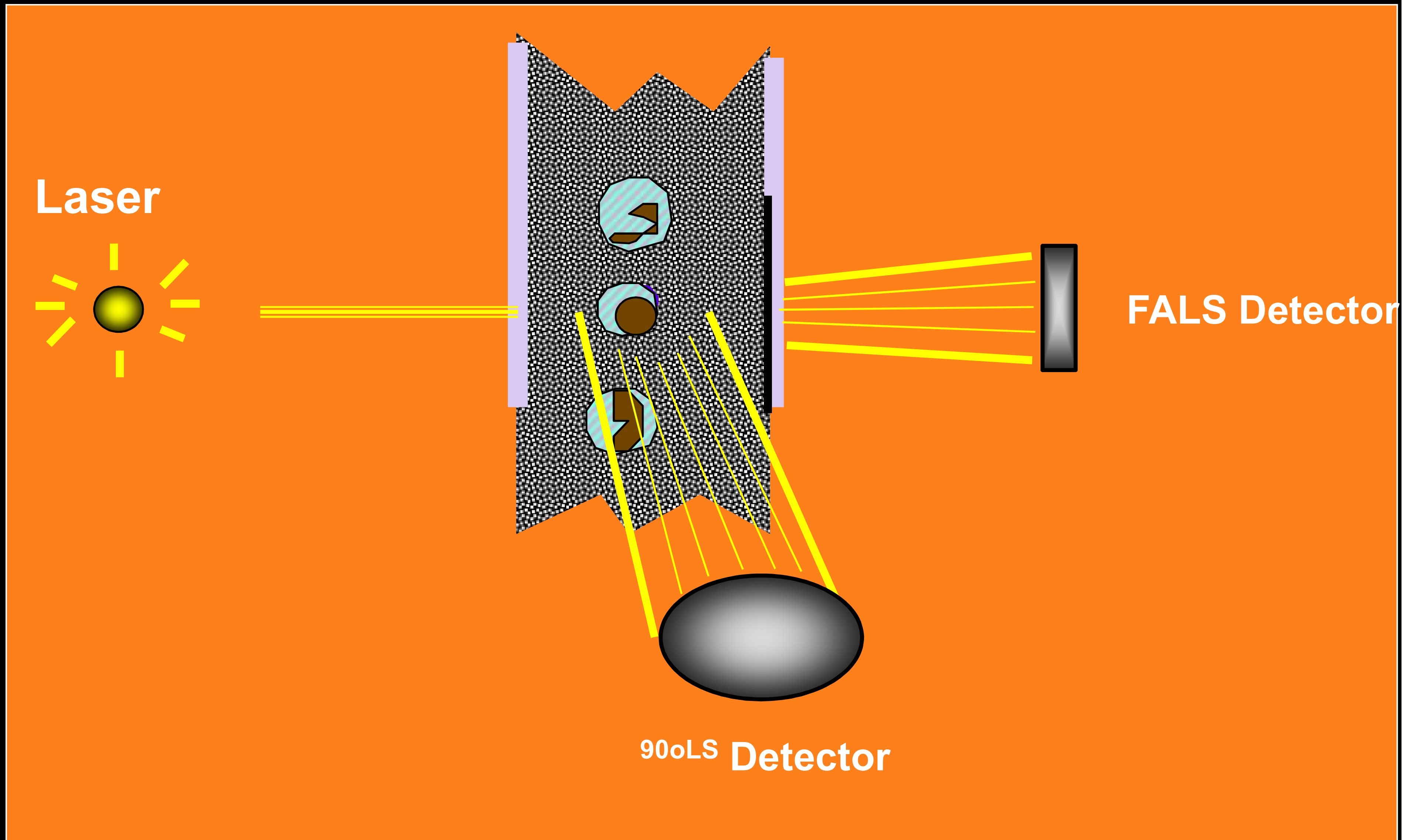
FORWARD SCATTER



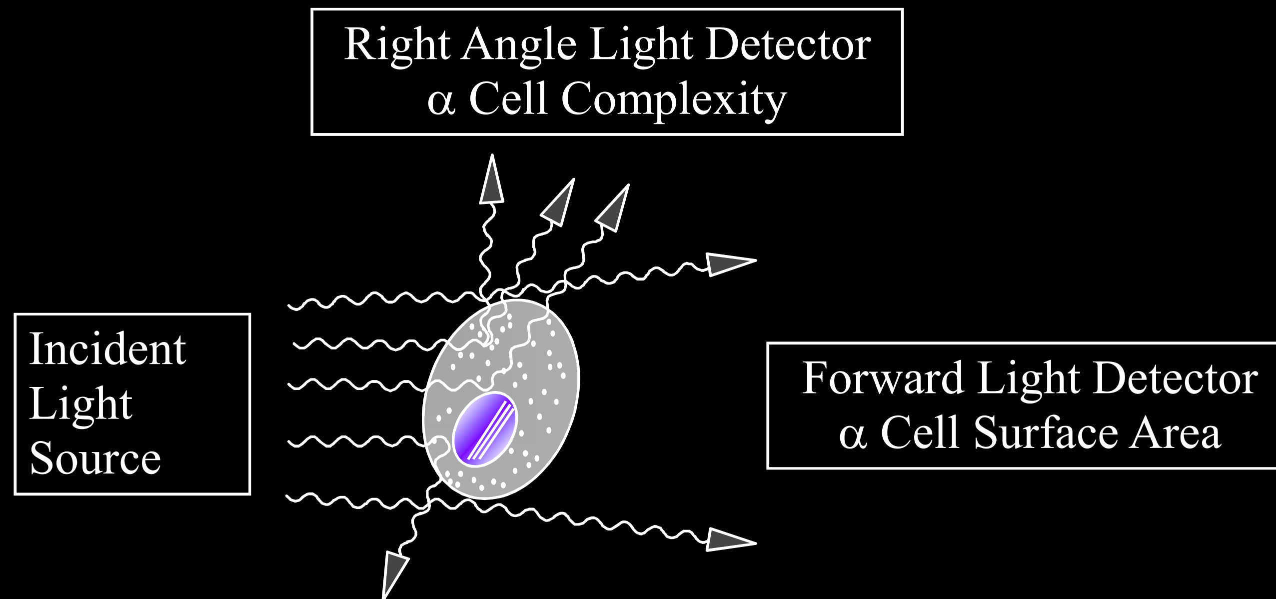


Original from Purdue University Cytometry Laboratories

SIDE (90°) SCATTER

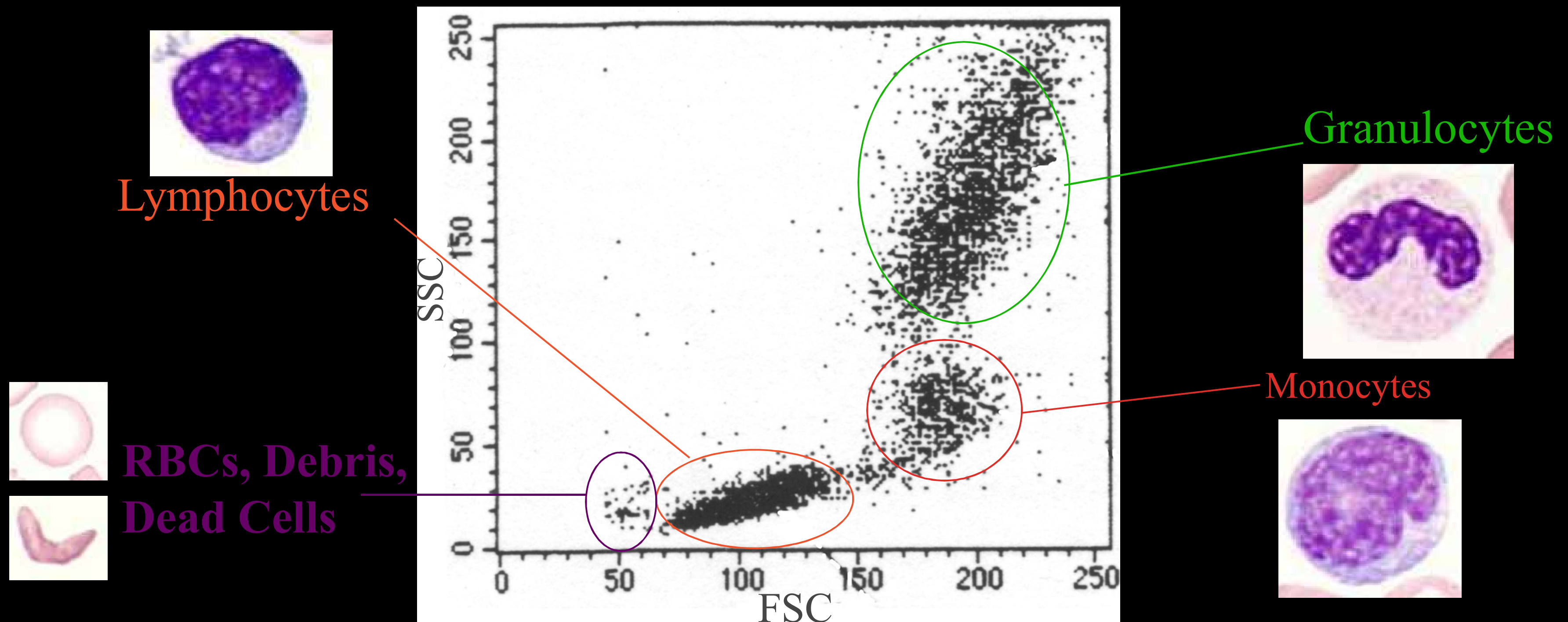


Light Scattering properties of cells

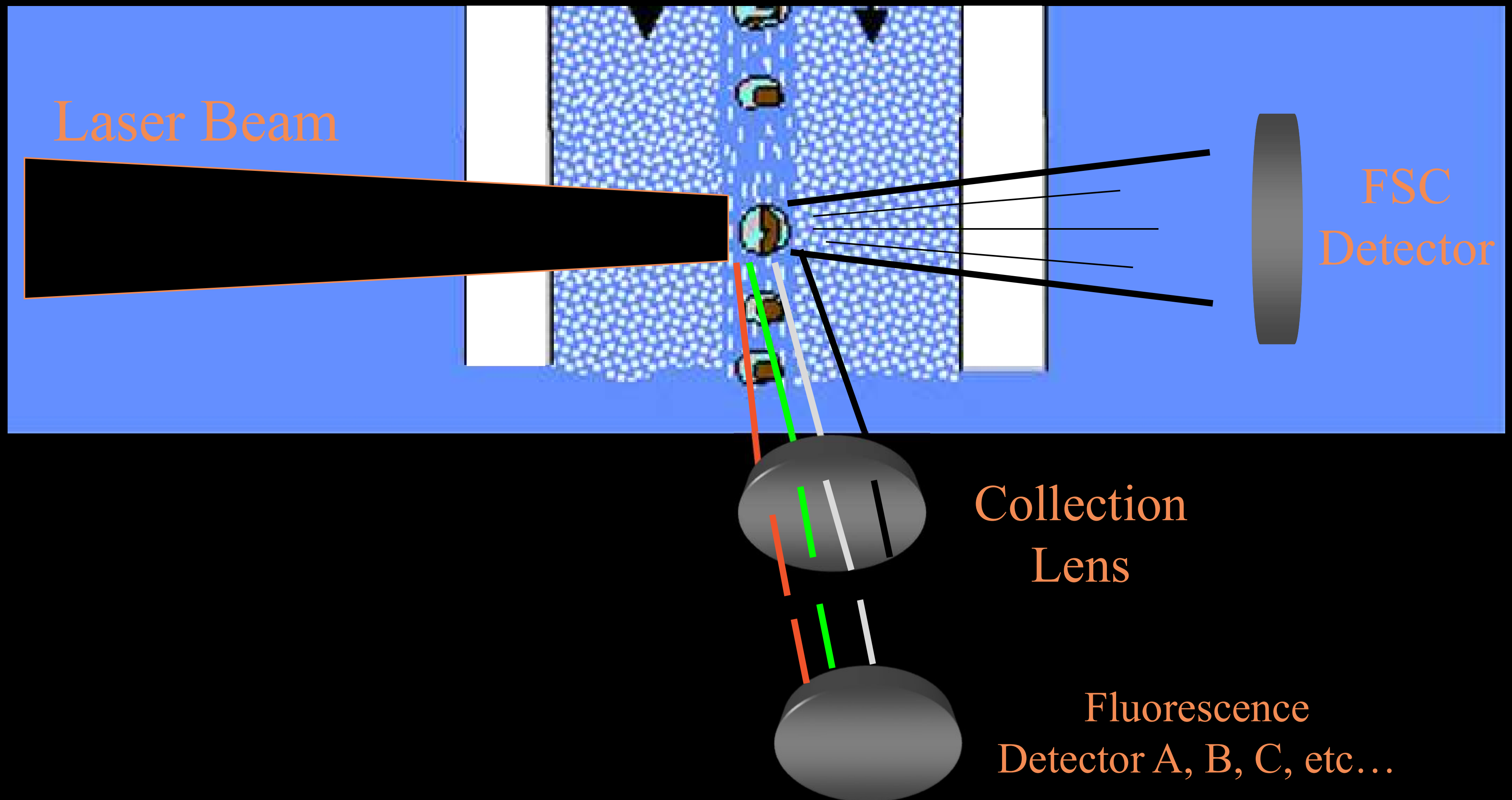


WHY LOOK AT FSC V. SSC

- Since FSC ~ size and SSC ~ internal structure, a correlated measurement between them can allow for differentiation of cell types in a heterogenous cell population

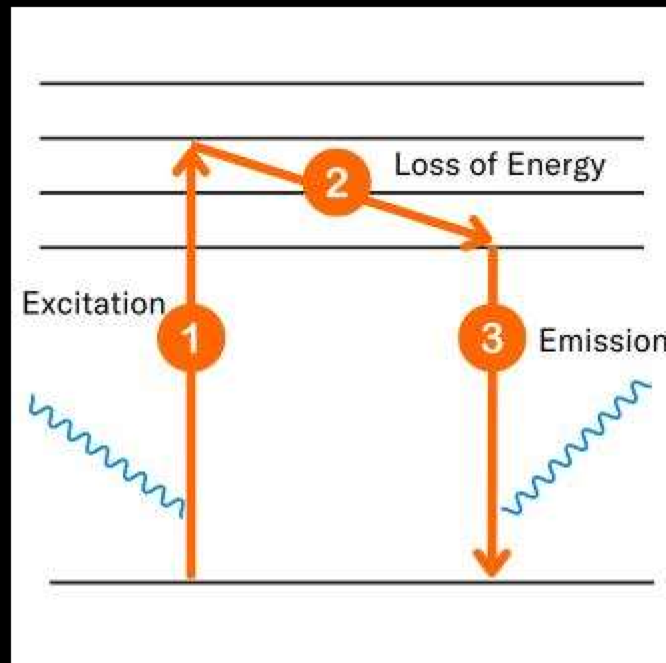


FLUORESCENCE DETECTORS

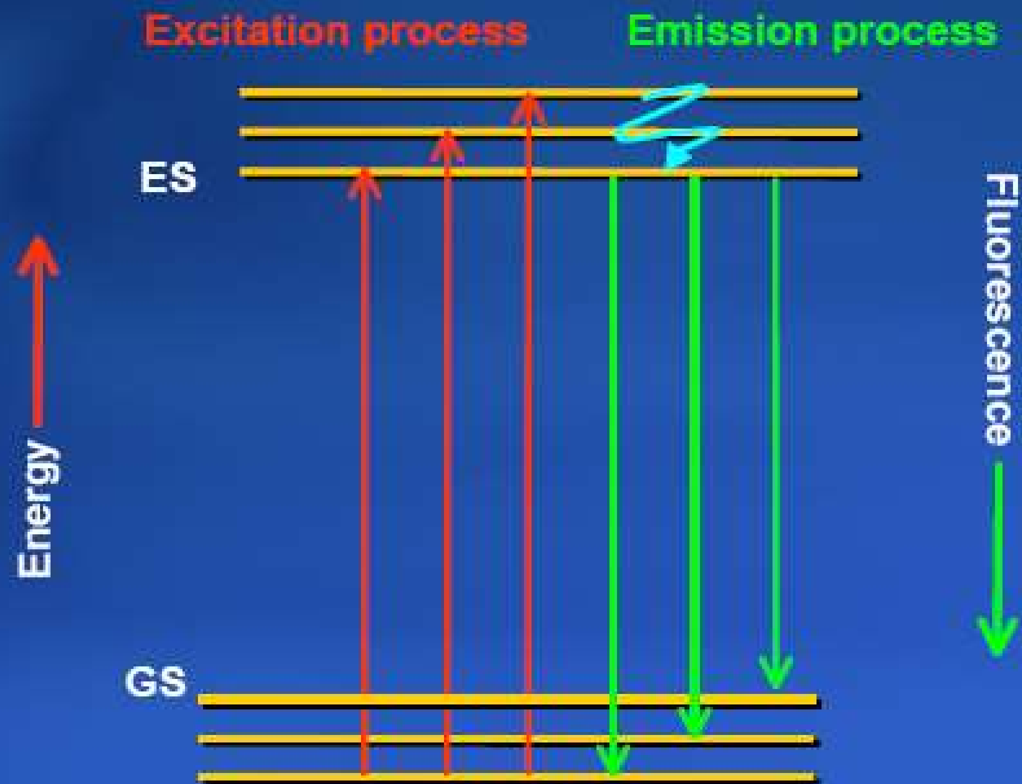


Original from Purdue University Cytometry Laboratories. Modified by James Marvin

فلورسانس
FLUORESCENCE

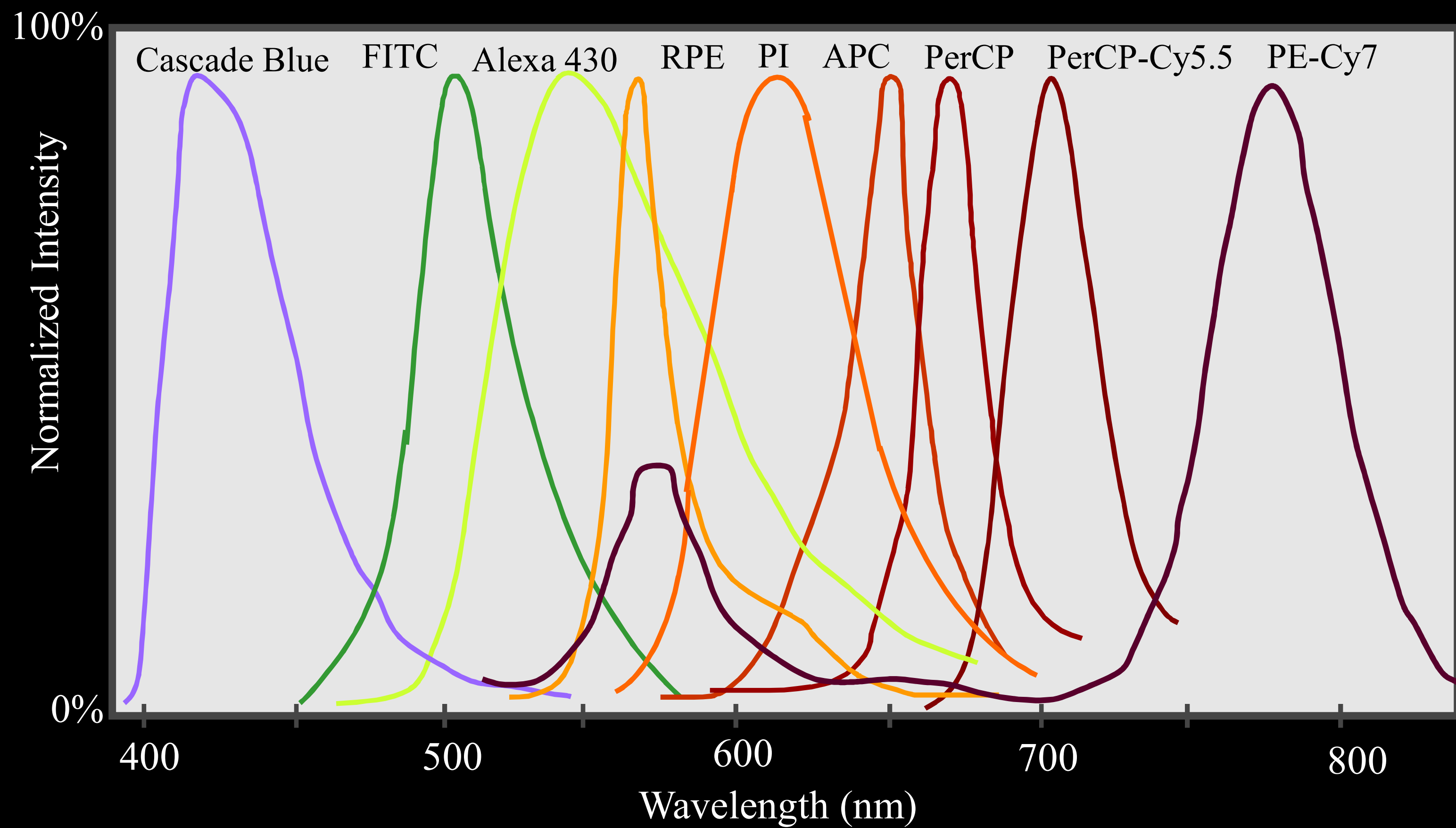


Fluorescence: Definition



BECKMAN COULTER

Emission Spectra



EIGHT COLOR

488 nM laser

- FITC
- PE
- PE-TR
- PE-CY5

605 nM laser

- TR
- APC
- APC-CY7

UV laser

- cascade blue

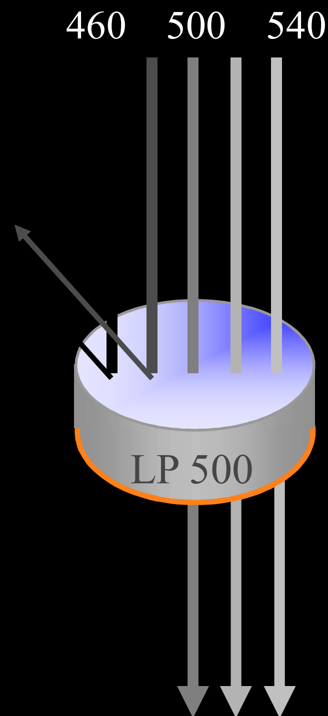
LASERS & FLUOROCHROMES BY APPLICATION

Substrate	Probe	Ex_{max}/ Em_{max}	" Best" Laser
Plasmid Marker:	EGFP…………………….	488/510	488
Conjugated to antibodies:	FITC……………………...	494/517	488
	PE………………………...	480/578	488
	PerCP…………………….	475/675	488
	APC………………………	650/660	633

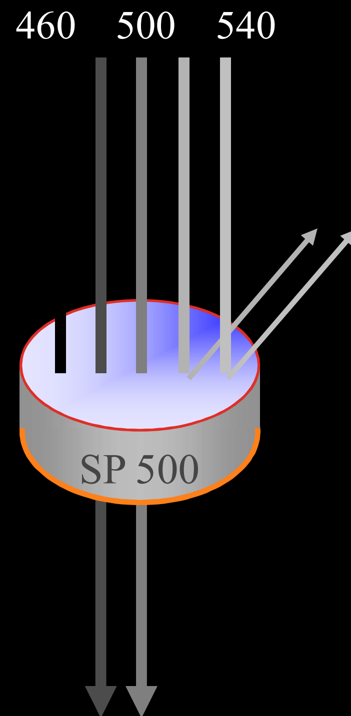
Fluorochrome	Excitation Laser (nm)	Fluorescent Emission Maximum (nm)	FL Channel
Alexa Fluo 488 [1]	488	519	Green
FITC (Fluorescein)	488	525	Green
PE (R-PE, R-Phycoerythrin)	488	575	Yellow
PE-Cy5 [2]	488	670	Red
PE-Cy5.5 [2]	488	690	Red
PE-Cy7 [2]	488	774	Infra-Red
APC (Allophycocyanin)	633, 635	660	Red
Alexa Fluo 647 [1]	633, 635	668	Red
APC-Cy5.5 [2]	633, 635	690	Red
APC-Cy7 [2]	633, 635	774	Infra-Red

Optical Filters

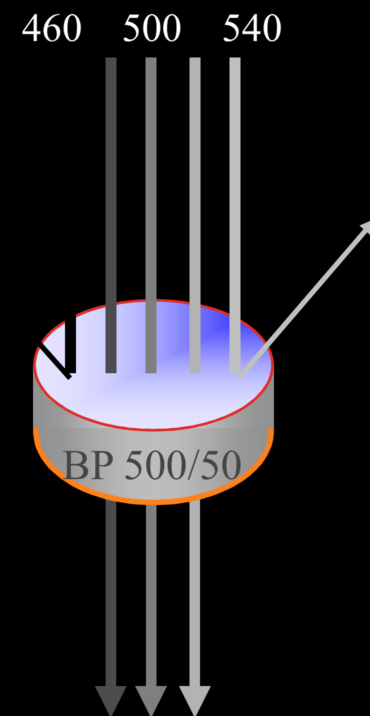
Longpass



Shortpass

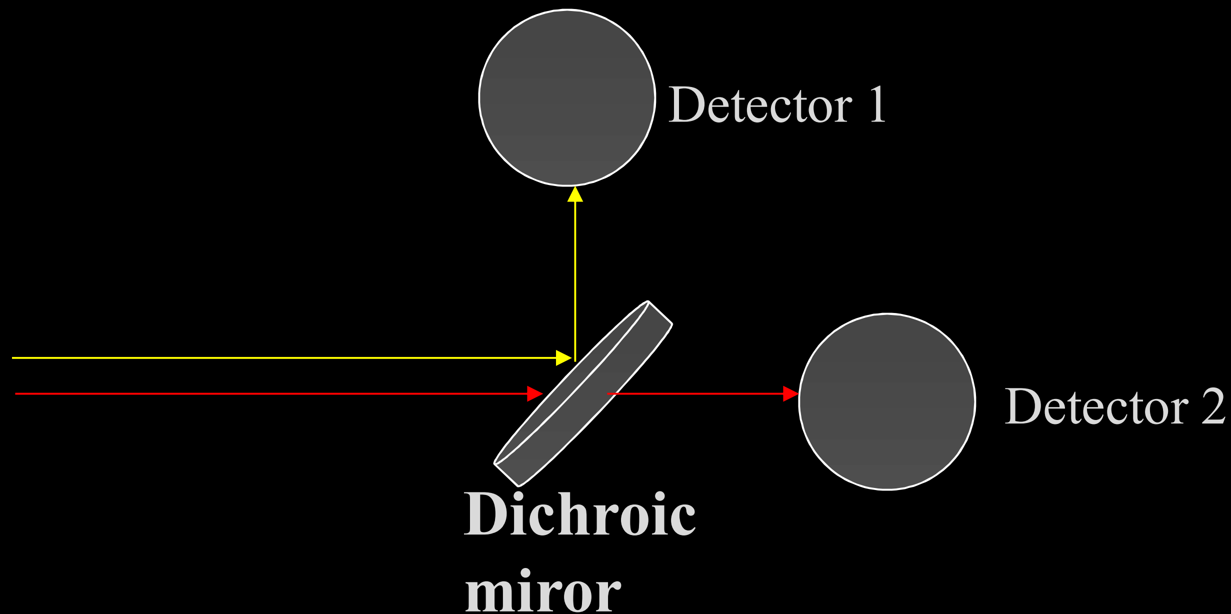


Bandpass

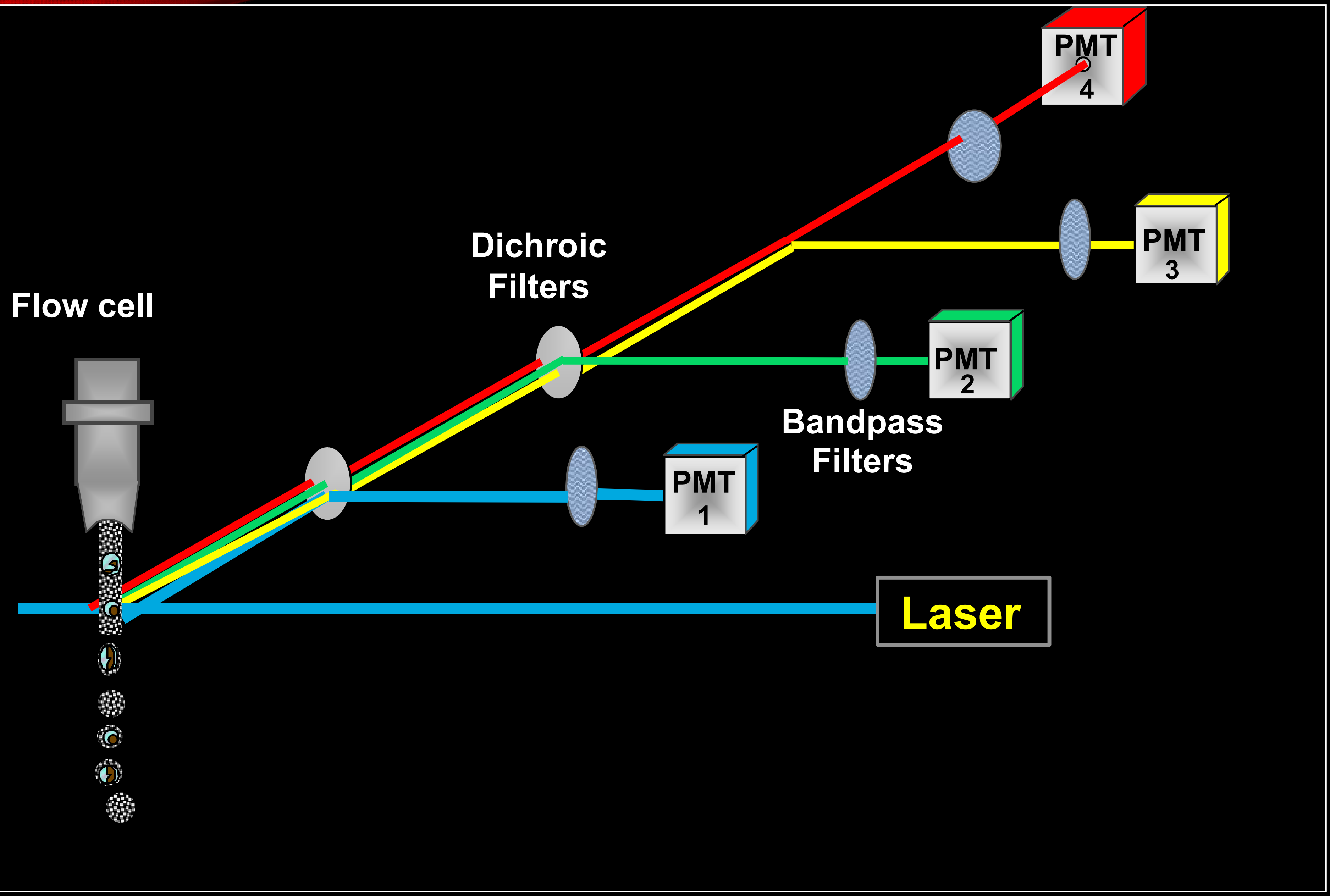


DICHROIC MIRORS

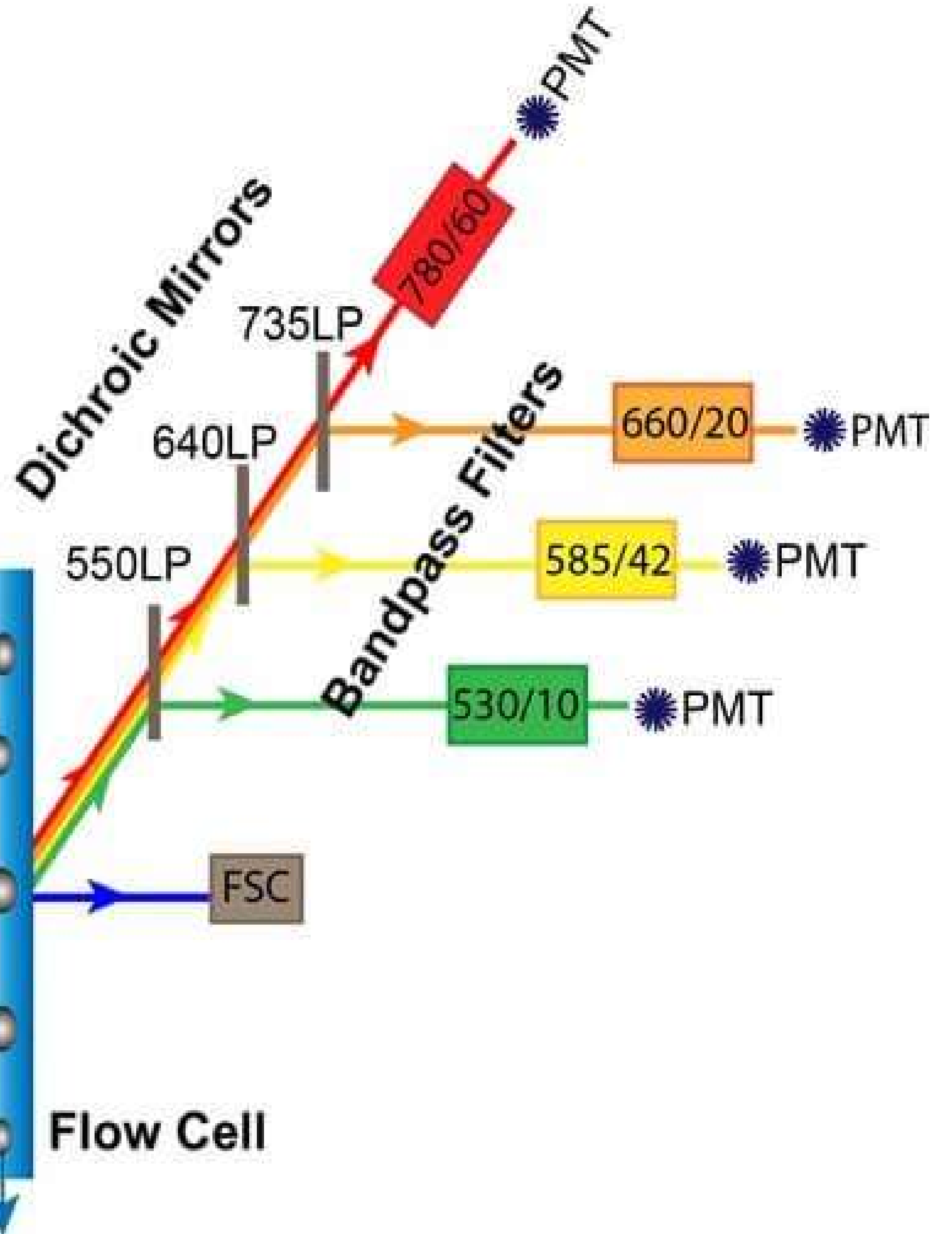
- Filter is placed at a 45° angle to the incident light
- Part of the light is reflected at 90° to the the incident light, and part of the light is transmitted and continues on.



FLOW CYTOMETER OPTICS



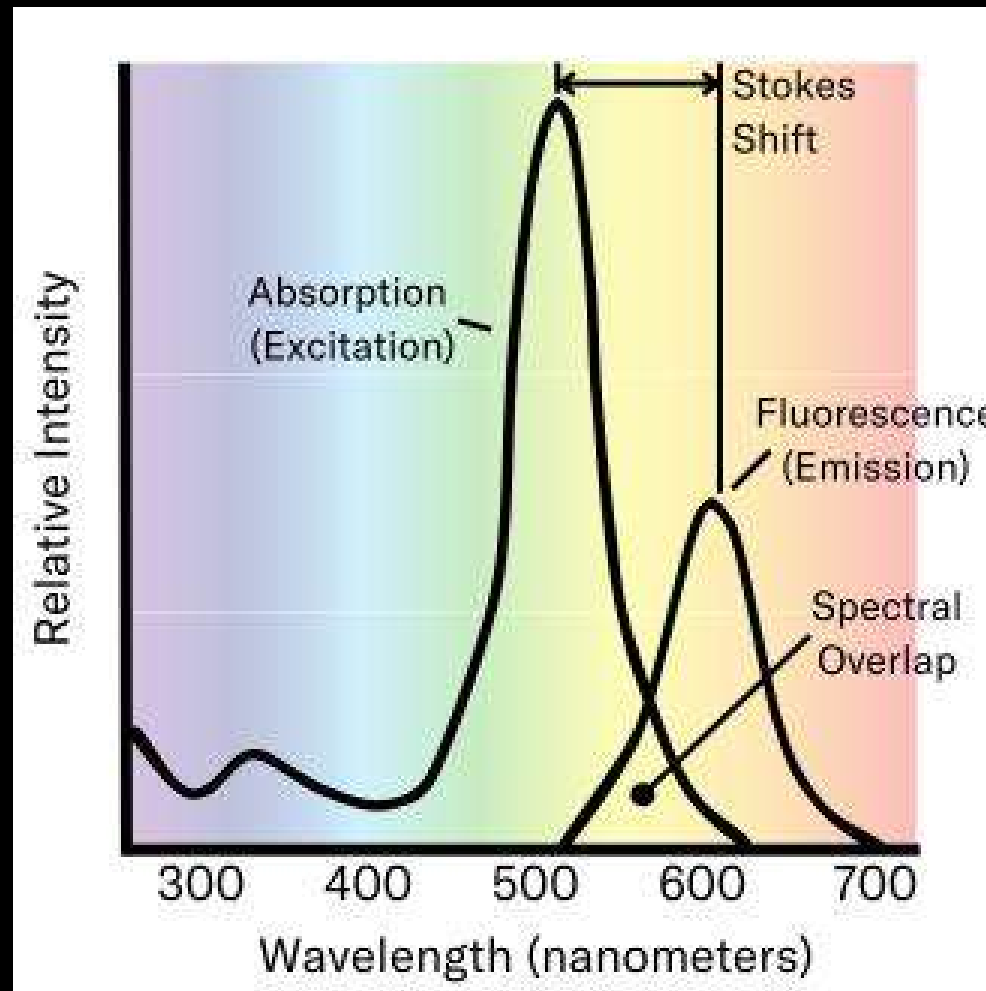
فلوروفور	حداکثر نشر تقریبی
FITC	۵۳۰–۵۱۹nm
PE	۵۸۰–۵۷۵nm
PerCP	۶۷۰nm
APC	۶۷۰–۶۶۰nm



ویژگی	آیینه دایکروئیک	فیلتر نوری
عملکرد اصلی	تقسیم نور به دو مسیر	انتخاب محدوده مشخصی از طول موج
خروجی	هم نور بازتابی و هم عبوری	معمولاً فقط نور عبوری مورد استفاده قرار می‌گیرد
محل استفاده	قبل از آشکارسازها برای جداسازی کانال‌ها	جلوی هر آشکارساز برای دقیق‌تر کردن طول موج

- آیینه‌های دایکروئیک فقط بر اساس **طول موج** عمل می‌کنند و هیچ اطلاعی از نوع فلوروکروم ندارند. بنابراین اگر دو رنگ فلورسانس طول موج‌های نزدیک به هم داشته باشند، مقداری از نور آن‌ها وارد کانال‌های مجاور می‌شود که به آن **Spillover** می‌گویند.

Spillover



Spectral overlap : همپوشانی طیفی

تداخل نور منتشرشده از فلوروکروم‌های مختلف که نیاز به جبران‌سازی (Compensation)دارد.

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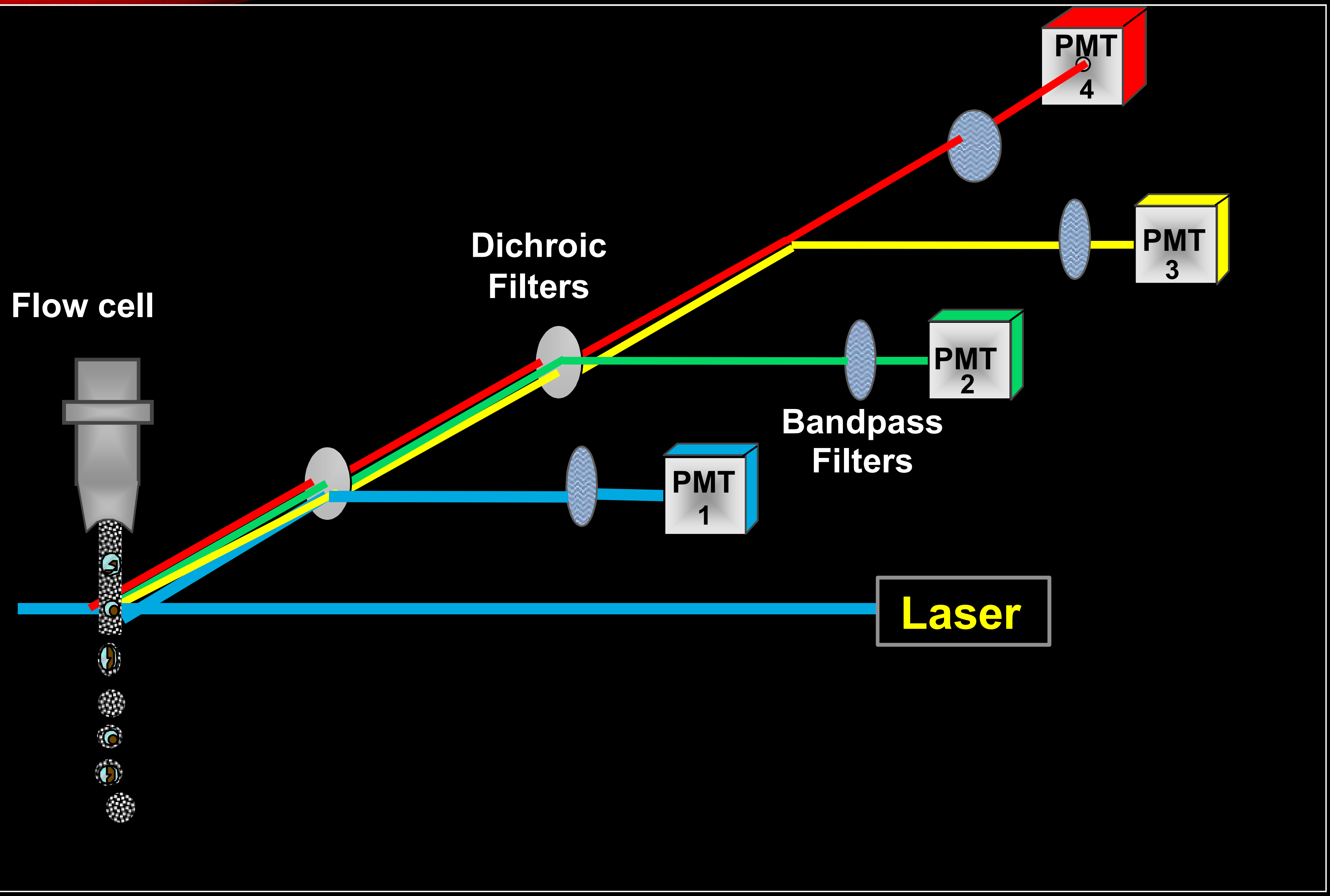
FLOW CYTOMETER OPTICS

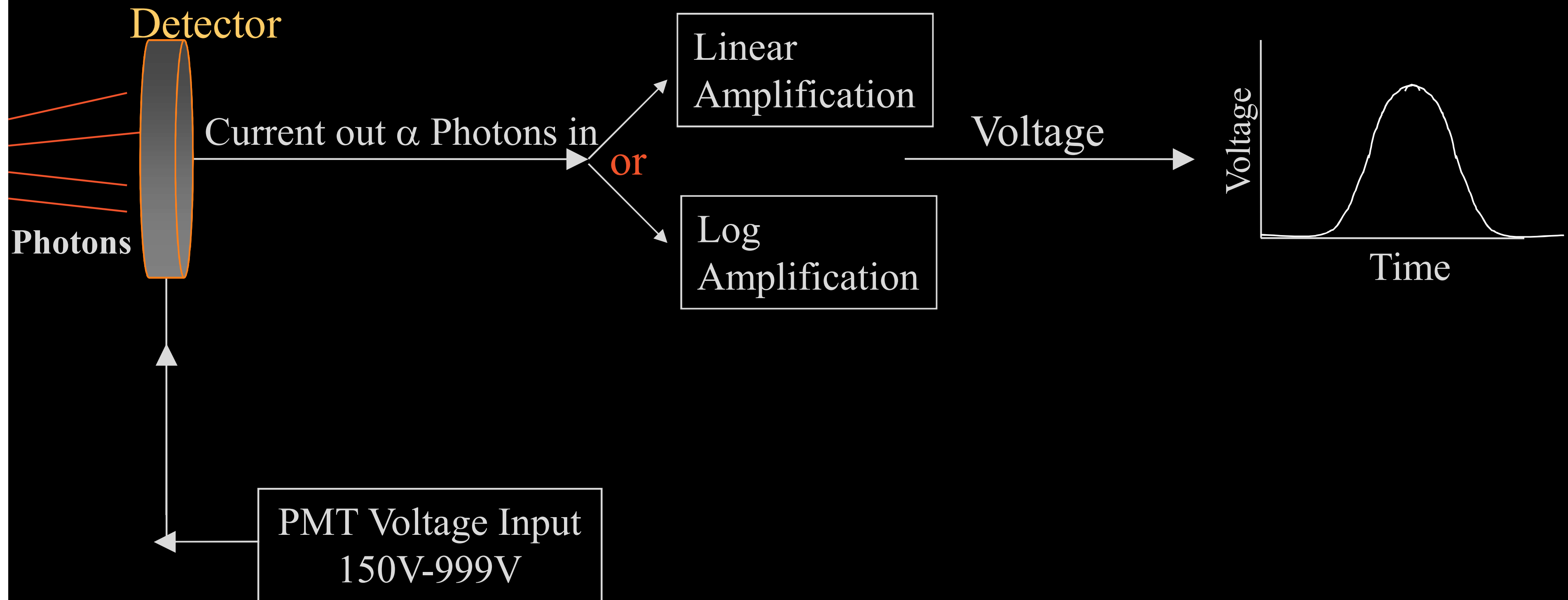


- دتکتورها اجزایی هستند که **نور پراکنده‌شده و نور فلورسانس تولیدشده توسط سلول‌ها را به سیگنال الکتریکی قابل اندازه‌گیری تبدیل می‌کنند**. این سیگنال‌ها سپس تقویت، دیجیتال‌سازی و توسط نرم‌افزار تحلیل می‌شوند.

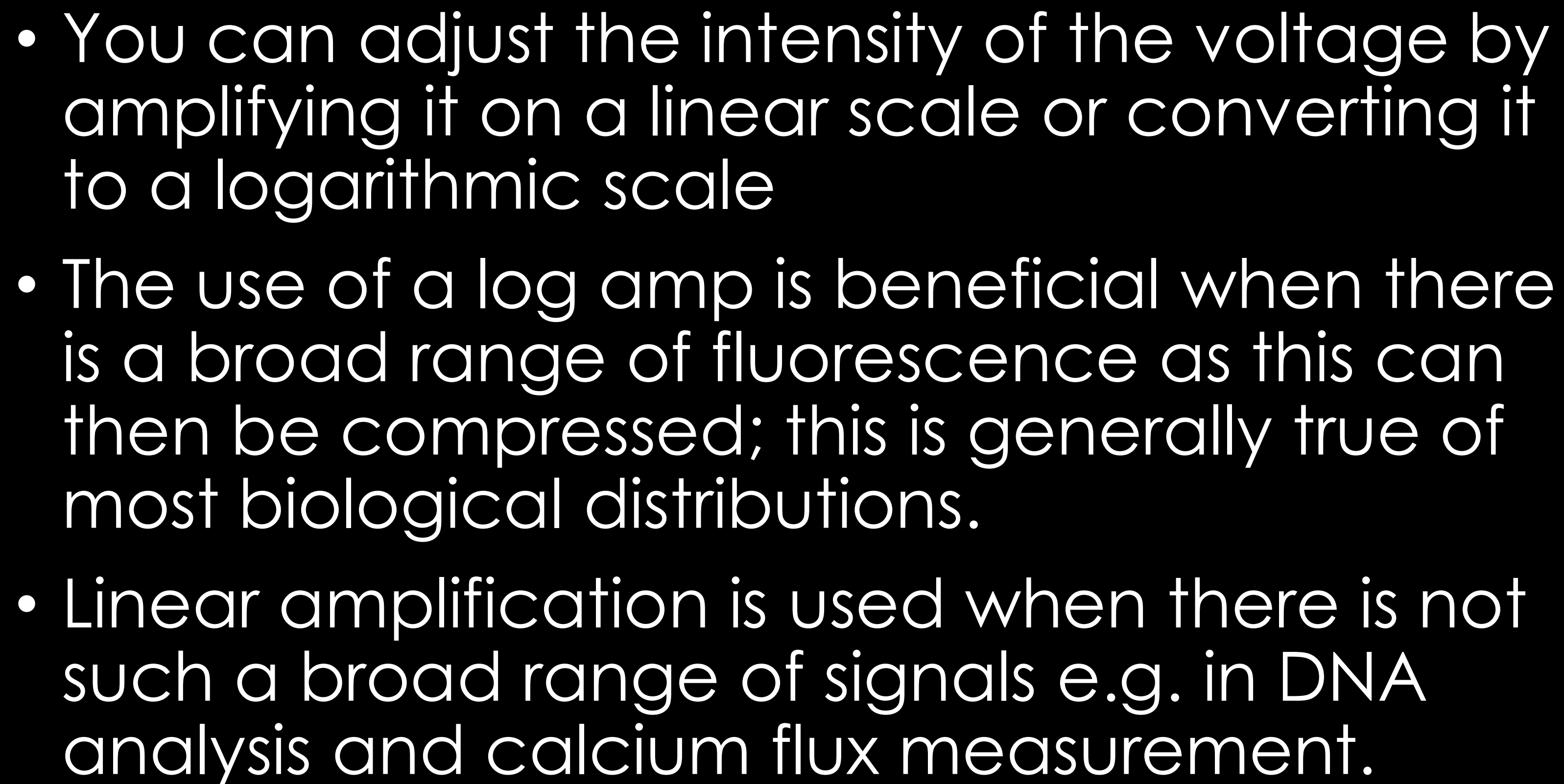
DETECTORS

- There are two main types of photo detectors used in flow cytometry
  - Photodiodes
    - Used for strong signals, when saturation is a potential problem (eg. FSC detector)
  - Photomultiplier tubes (PMT)
    - More sensitive than a Photodiode, a PMT is used for detecting small amounts of fluorescence emitted from fluorochromes.

ELECTRONICS SCHEMATIC

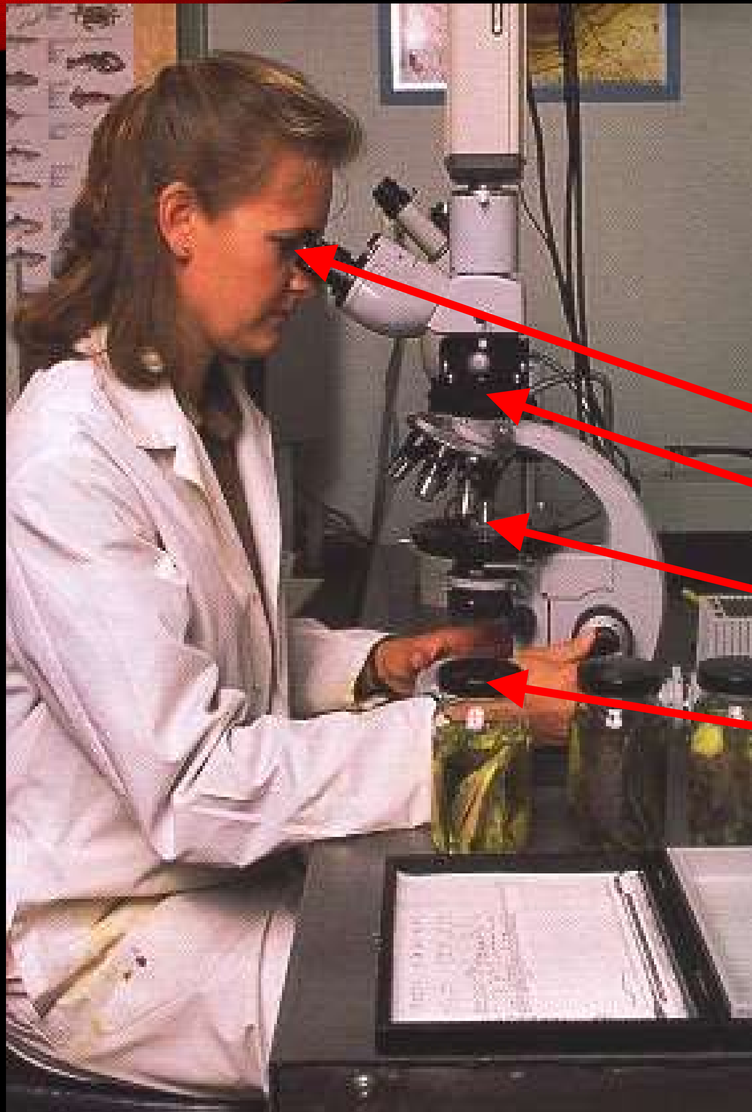


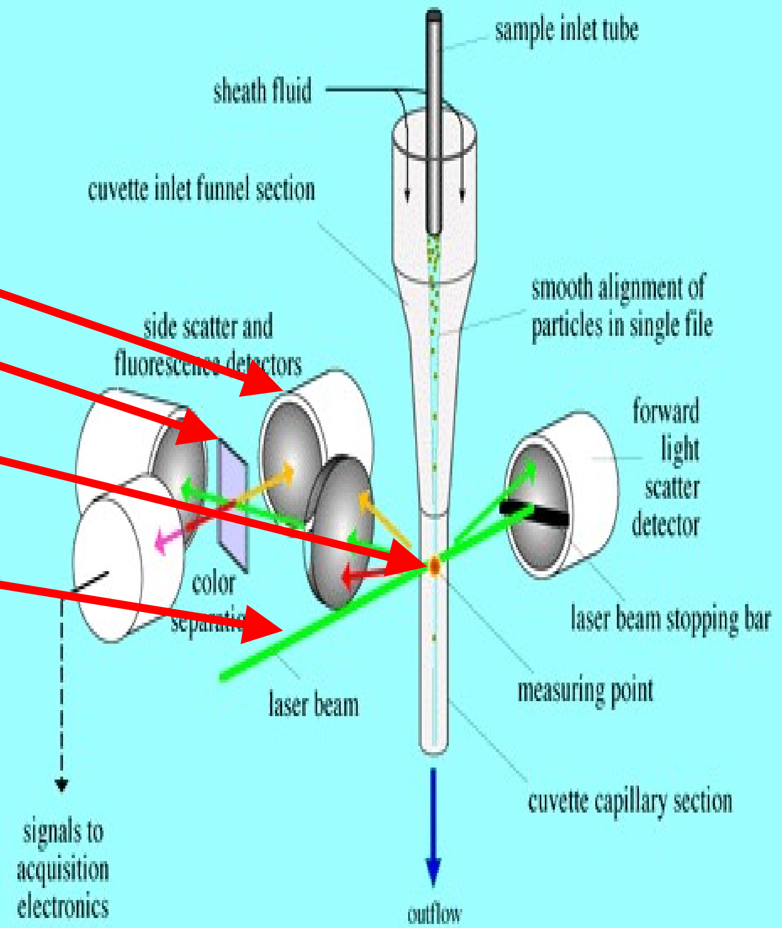
Original from Becton Dickinson Training manual, Modified by James Marvin


• You can adjust the intensity of the voltage by amplifying it on a linear scale or converting it to a logarithmic scale
• The use of a log amp is beneficial when there is a broad range of fluorescence as this can then be compressed; this is generally true of most biological distributions.
• Linear amplification is used when there is not such a broad range of signals e.g. in DNA analysis and calcium flux measurement.

ANALOG TO DIGITAL CONVERTERS

- An ADCs takes the voltage pulse and converts it to discrete binary numbers depending on total resolution
- The binary signal generated is converted to a relative bin number
- Those relative bin numbers are acquired as a list of values from each detector for each event (cell) and are eventually plotted on a graph.





Flow cytometer operating principle
sample inlet tube
sheath fluid
cuvette inlet funnel section
smooth alignment of
particles in single file
side scatter and
fluorescence detectors
forward
light
scatter
detector
color
laser beam stopping bar
measuring point
laser beam
cuvette capillary section
signals to
acquisition
electronics
outflow

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INTERPRETATION

- Once the values for each parameter are in a list mode file, specialized software can graphically represent it.
- The data can be displayed in 1, 2, or 3 dimensional format
- Common programs include…
  - Cell Quest
  - WinMDI