BD Biosciences

2350 Qume Drive

San Jose, CA

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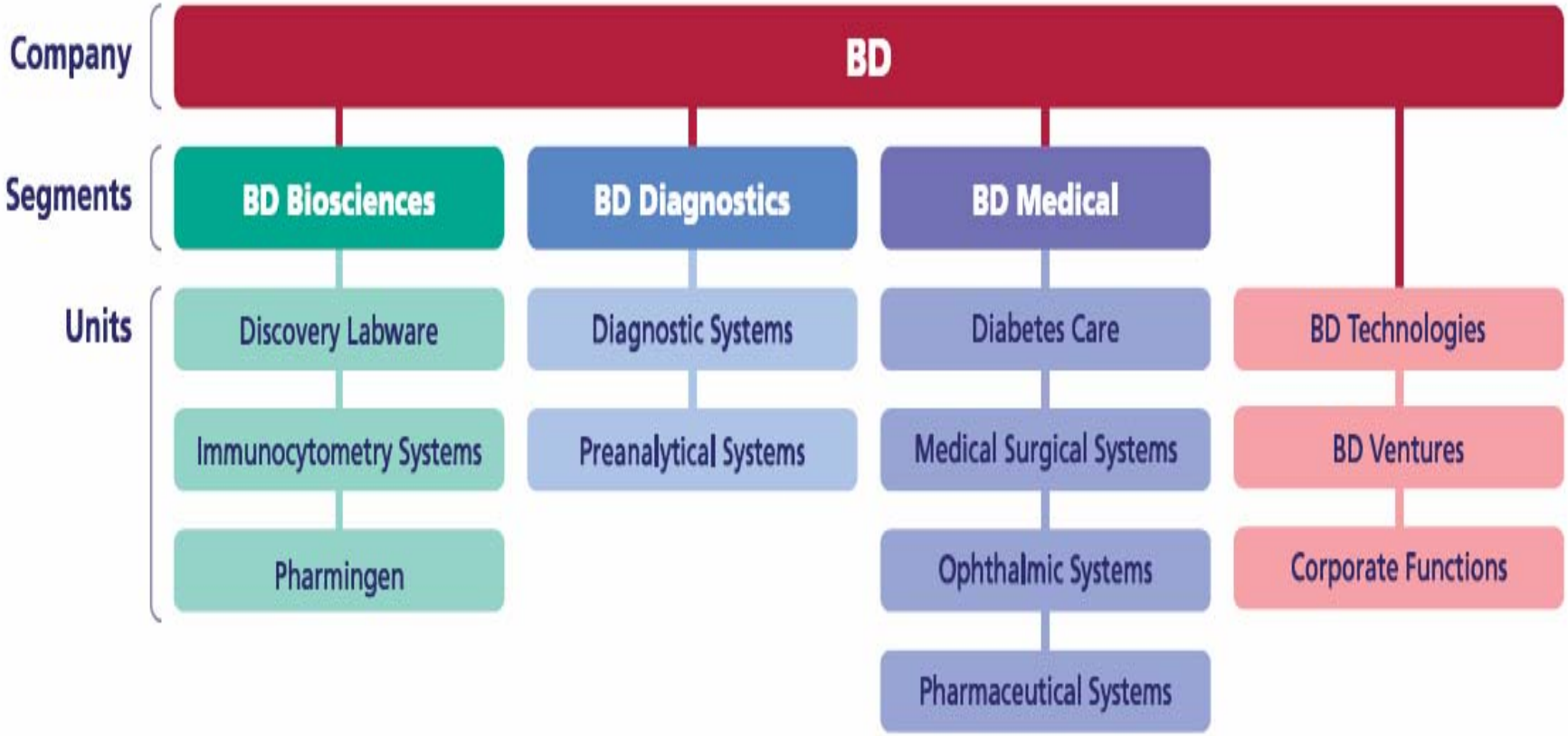
Helping all people live healthy lives

BD – Becton, Dickinson and Company

- Founded in 1897 and headquartered in Franklin Lakes, New Jersey
- Employs approximately 28,000 people in approximately 50 countries throughout the world.
- Is a leading global medical technology company that manufactures and sells medical devices, instrument systems and reagents
- Is focused on improving drug therapy, enhancing the quality and speed of diagnosing infectious diseases, and advancing research and discovery of new drugs and vaccines.



- BD Biosciences (~3,000 associates)
  - Immunocytometry Systems – San Jose
  - Pharmingen – San Diego
  - Discovery Labware – Bedford, MA

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What is Flow Cytometry and FACS?

- Cytometry refers to the measurement of physical and chemical characteristics of cells or other biological particles.
- Flow cytometry is the process whereby such measurements are made from cells or particles as they pass through a measuring apparatus (usually in single file) when suspended in a fluid stream.
- FACS (*Fluorescence Activated Cell Sorting*) - a trademark of Becton Dickinson Immunocytometry Systems (BDIS). All FACS instruments are BD Biosciences systems, but not all cytometers are FACS.
- Sorting extends flow cytometry with the additional ability to divert and collect cells exhibiting an identifiable set of characteristics either mechanically or by electrical means.

Why is it useful?

- It can simultaneously measures multiple physical characteristics of single particles, usually blood cells

- Applications
  - Evaluate immunodeficiency states
  - Classify leukemias/lymphomas
  - Study stem cells
  - Monitor graft recipients

The Analyzers and Sorters

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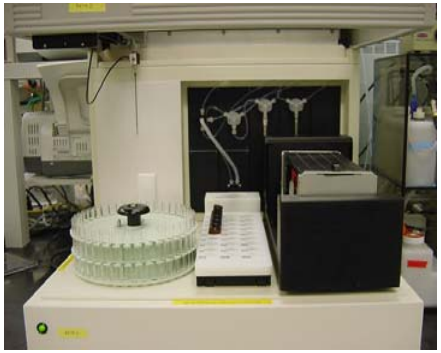
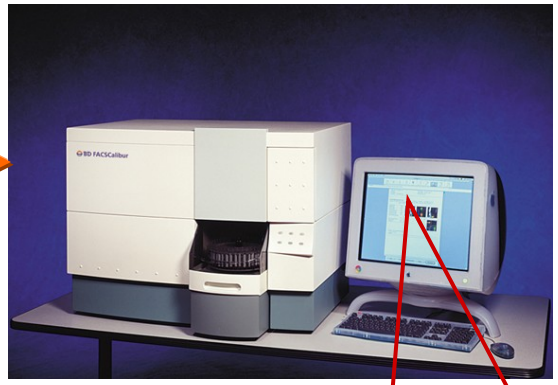
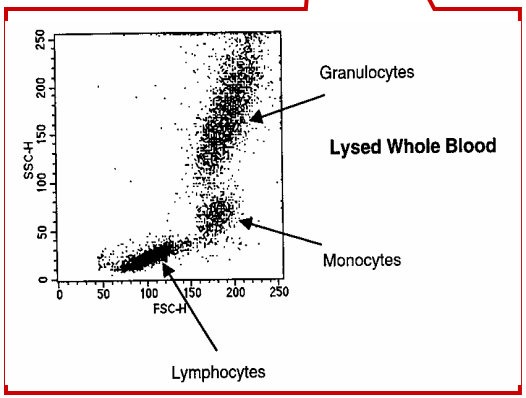




1 Sample Preparation

- Reagent antibodies added to blood sample and incubated
- Lyse to burst RBCs
- Lyse/wash OR Lyse/no wash option



2 Flow Cytometry and Sorting

- Fluidics: Focus sample in a stream, deflect and collect desired cells
- Optics: Lasers, lenses, and prisms to focus light on sample
- Electronics: Collect information for analysis on computer

3 Analysis

- Acquisition and analysis software
- Cell counts, relative fluorescence intensity (FL1-6), cell size (FSC), and granularity (SSC)

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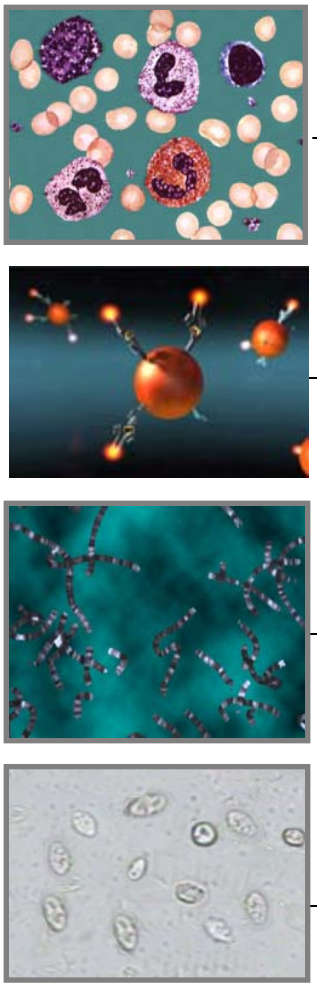
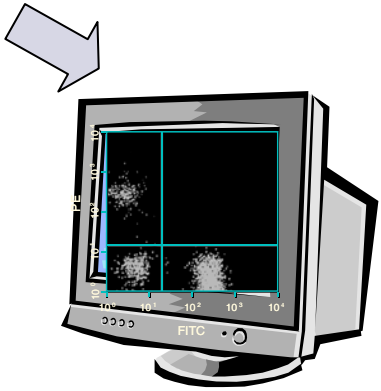
Flow cytometry and cell sorting are powerful tools for characterizing, analyzing, and separating cells. They simultaneously measure and analyze multiple physical characteristics of single particles, usually cells, as they move in a fluid stream through a beam of light. A cell with particular characteristics can then be captured and concentrated for further scientific purposes.

Any suspended particle or cell, from 0.2–50 micrometers in size, is suitable for analysis.





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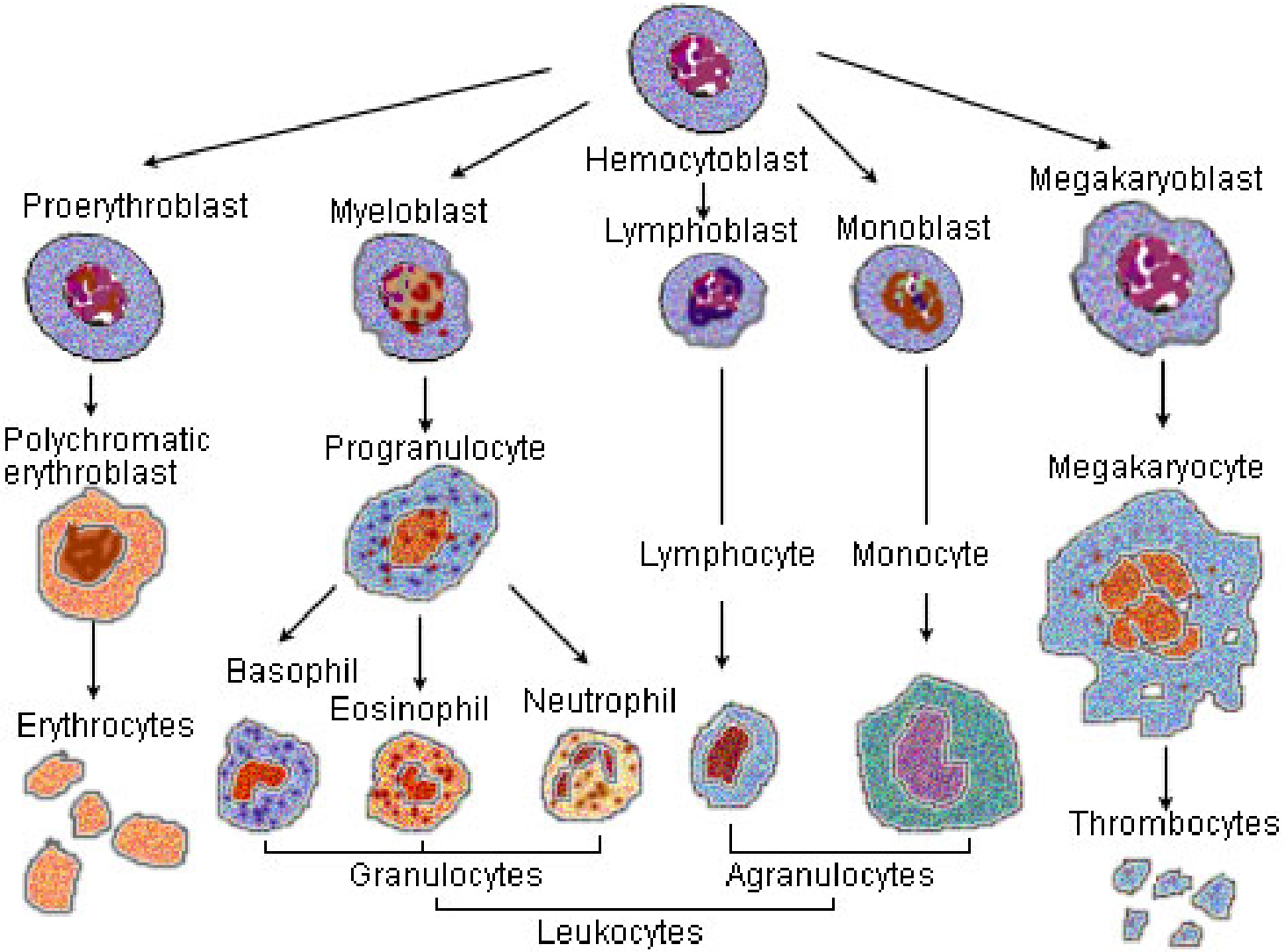
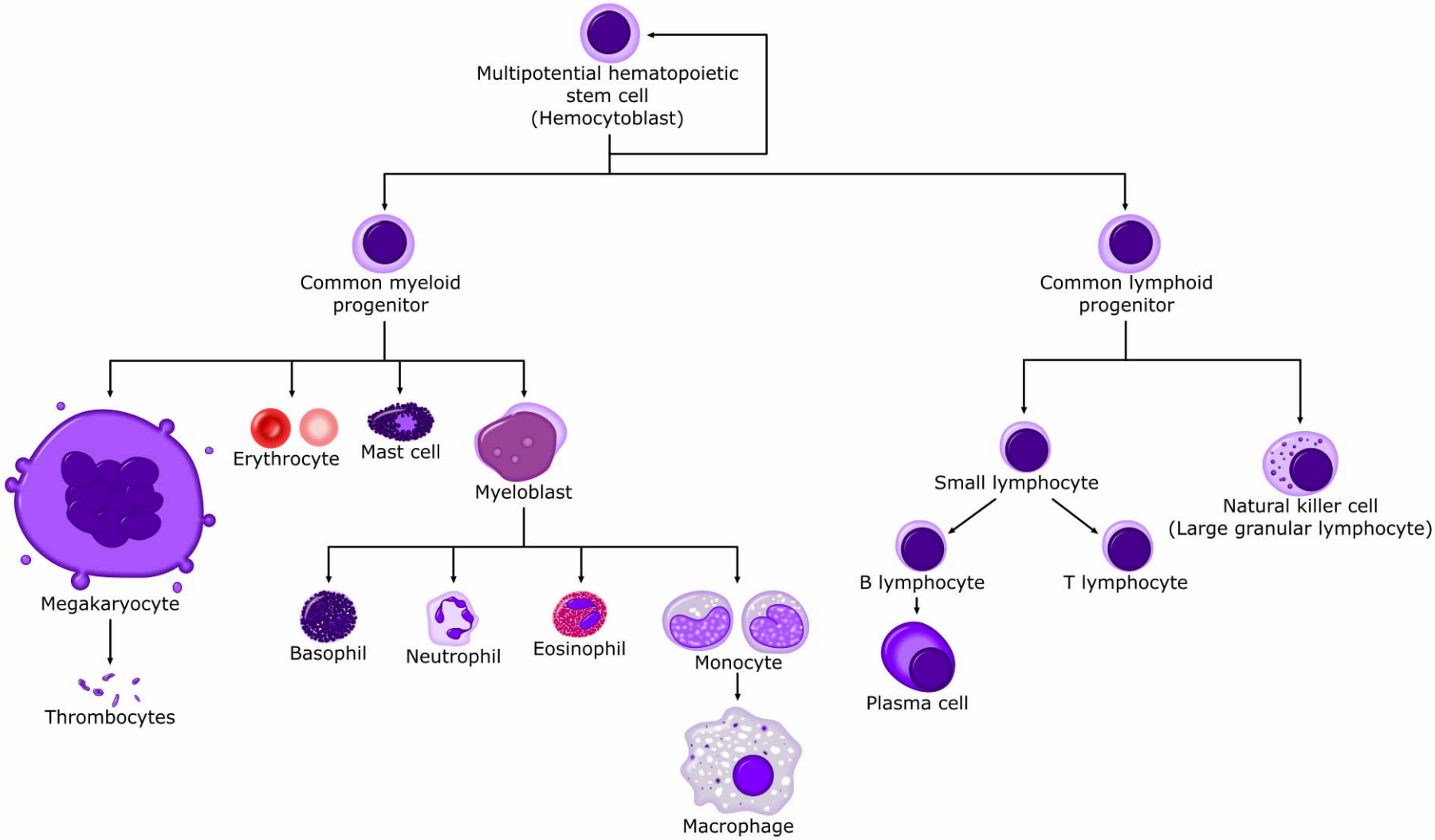
Blood Cells

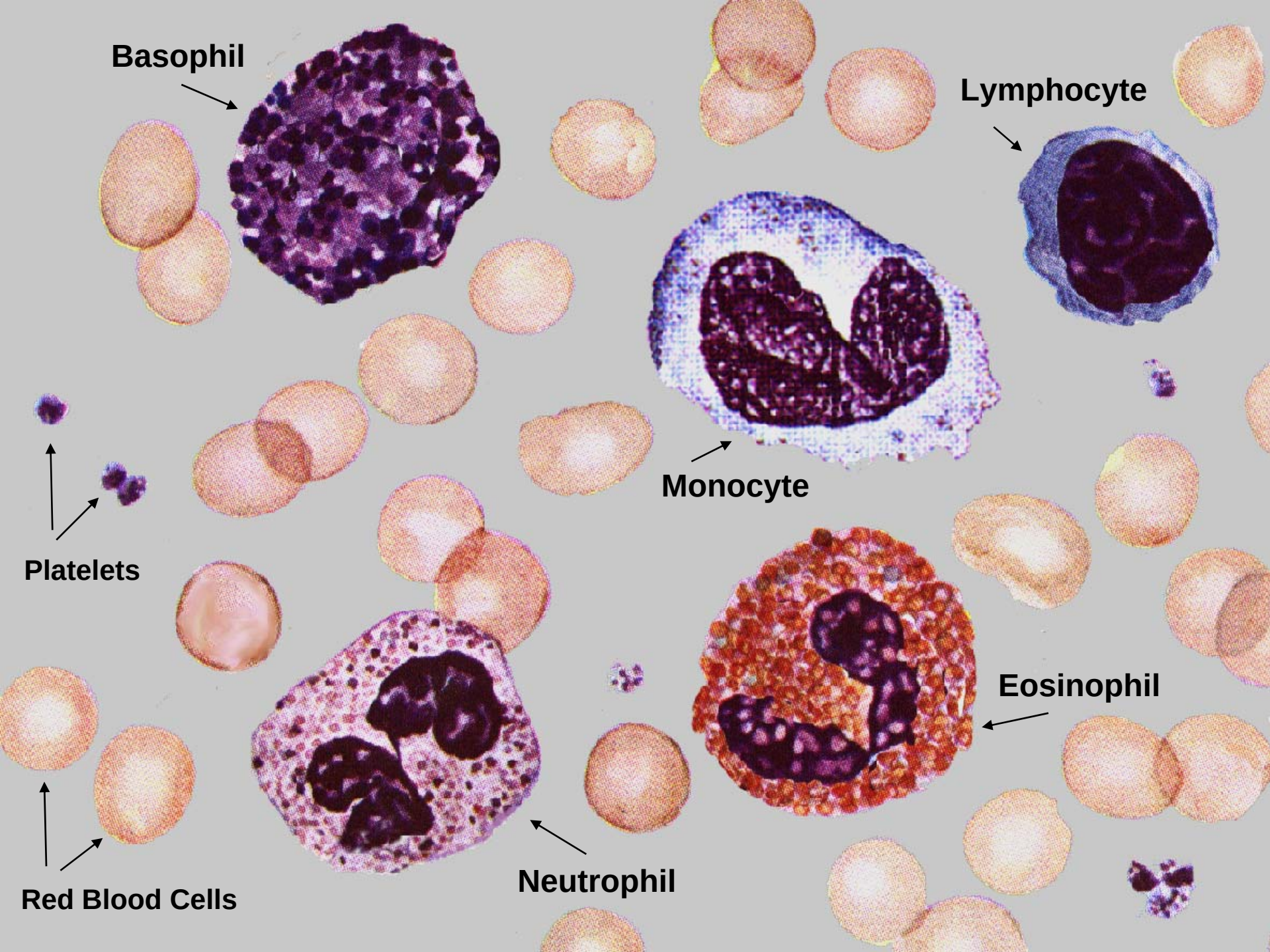
- Erythrocytes (RBCs)
  - Carry oxygen
- Platelets
  - Clot blood
- Plasma
  - Liquid part of blood
- Leucocytes (WBCs)
  - Immune response
  - Includes lymphocytes (B, T, and NK cells)

BLOOD CELL LINEAGE



BLOOD CELL LINEAGE




Basophil
Lymphocyte
Monocyte
Platelets
Eosinophil
Neutrophil
Red Blood Cells

Subsystems

Fluidics

To introduce and focus the cells for interrogation and create a stable breakoff for sorting.

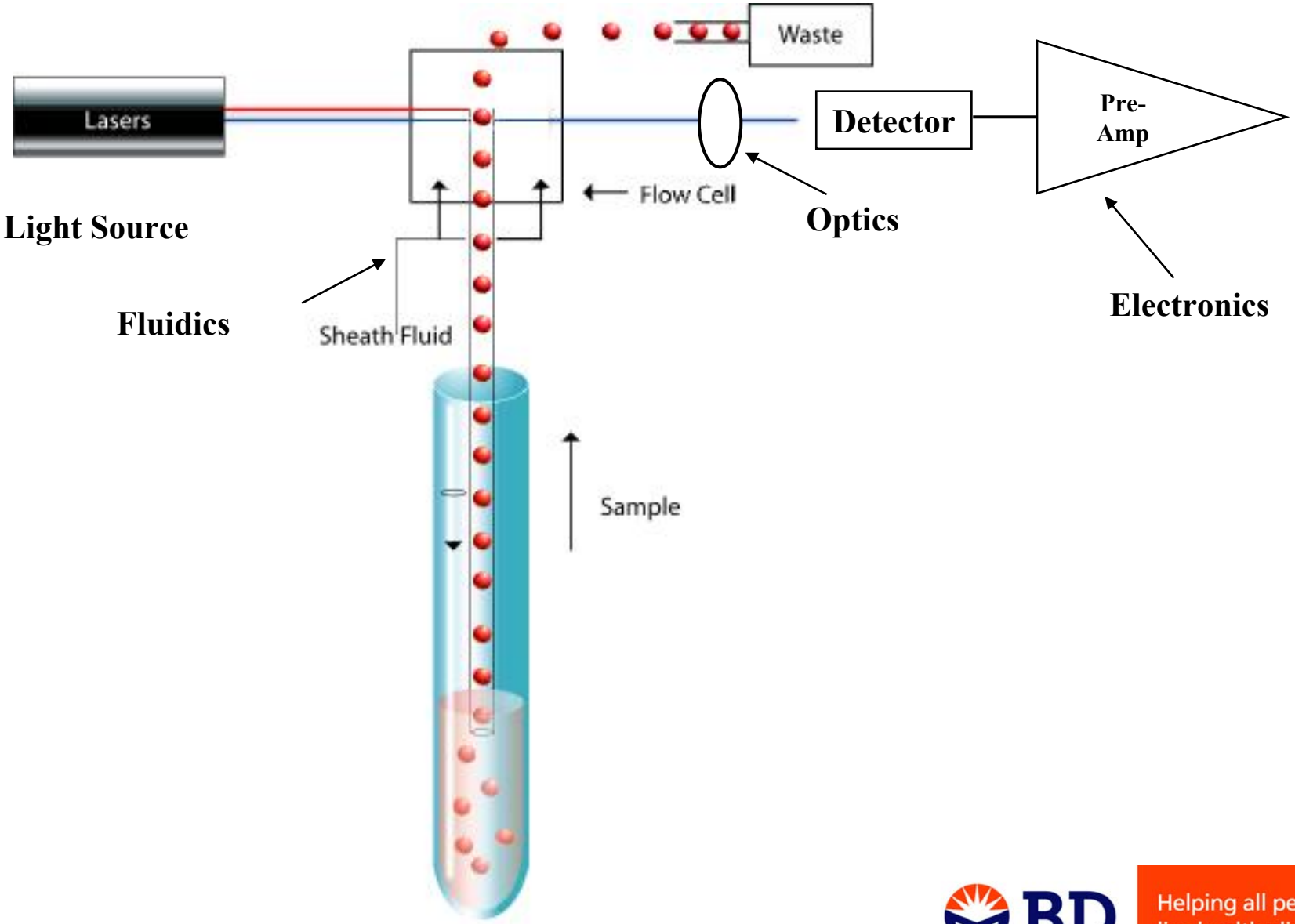
Optics

To generate and collect the light signals.

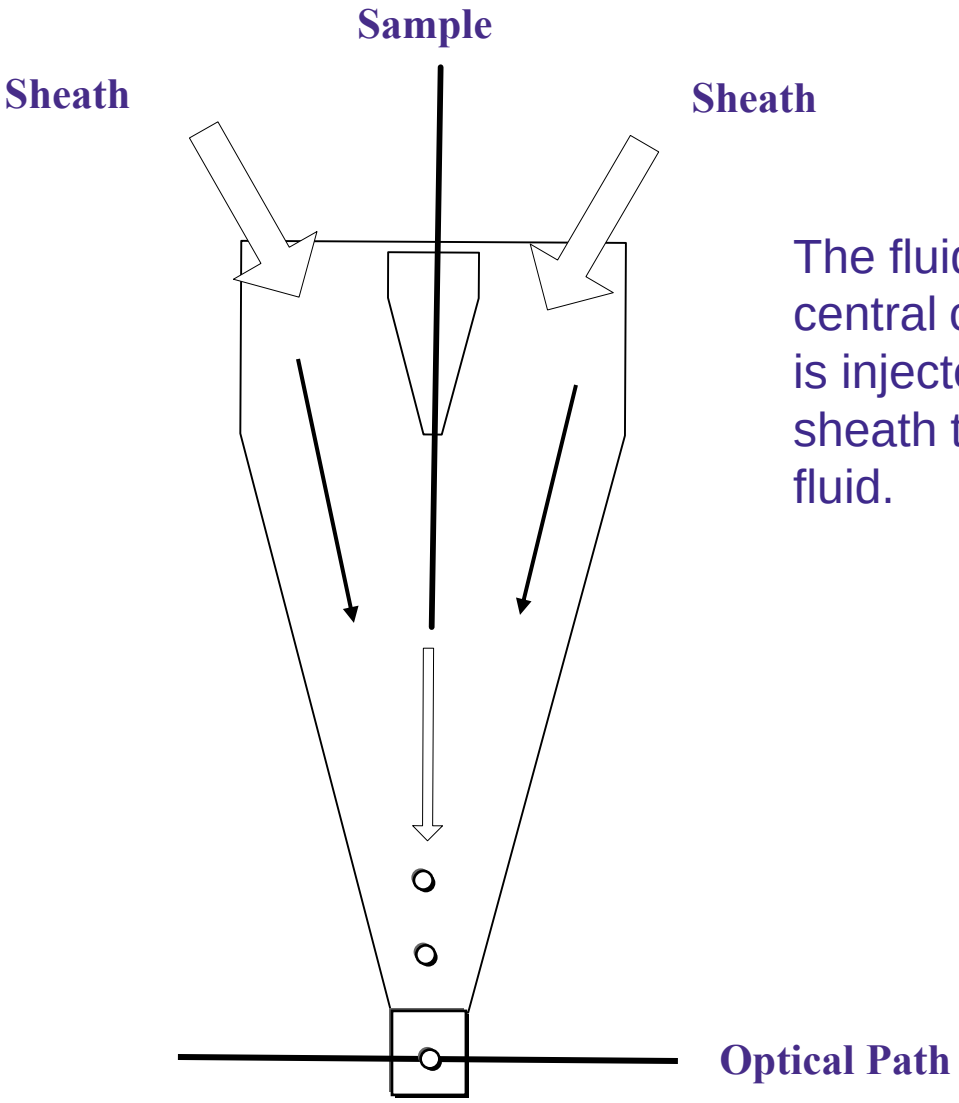
Electronics

To convert the optical signals to proportional digital signals, process the signals, and communicate with the computer.

The Simplified System

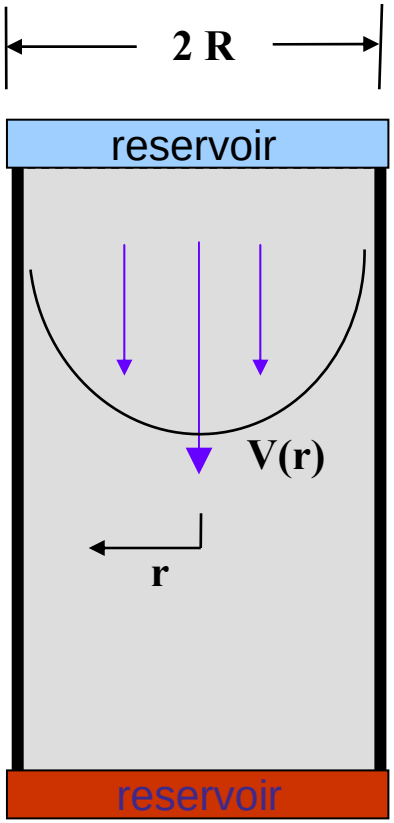


Hydrodynamic focusing produces a single stream of cells



The fluidics system consists of a central core through which the sample is injected, enclosed by an outer sheath that contains faster flowing fluid.

Pressure-driven Hagen-Poiseuille Flow



Pressure Driven

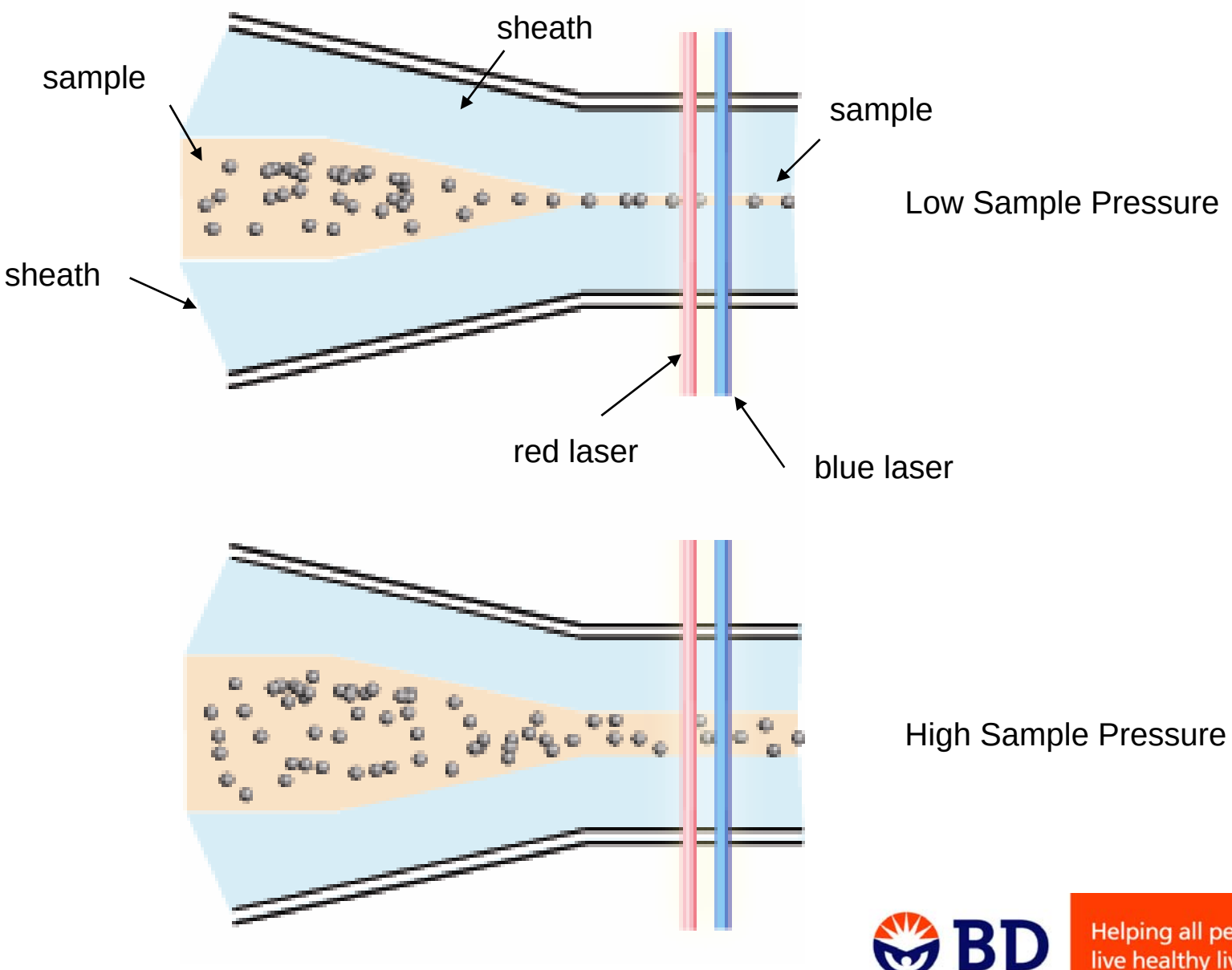
∇P

Volumetric flow rate: Q_{Total}

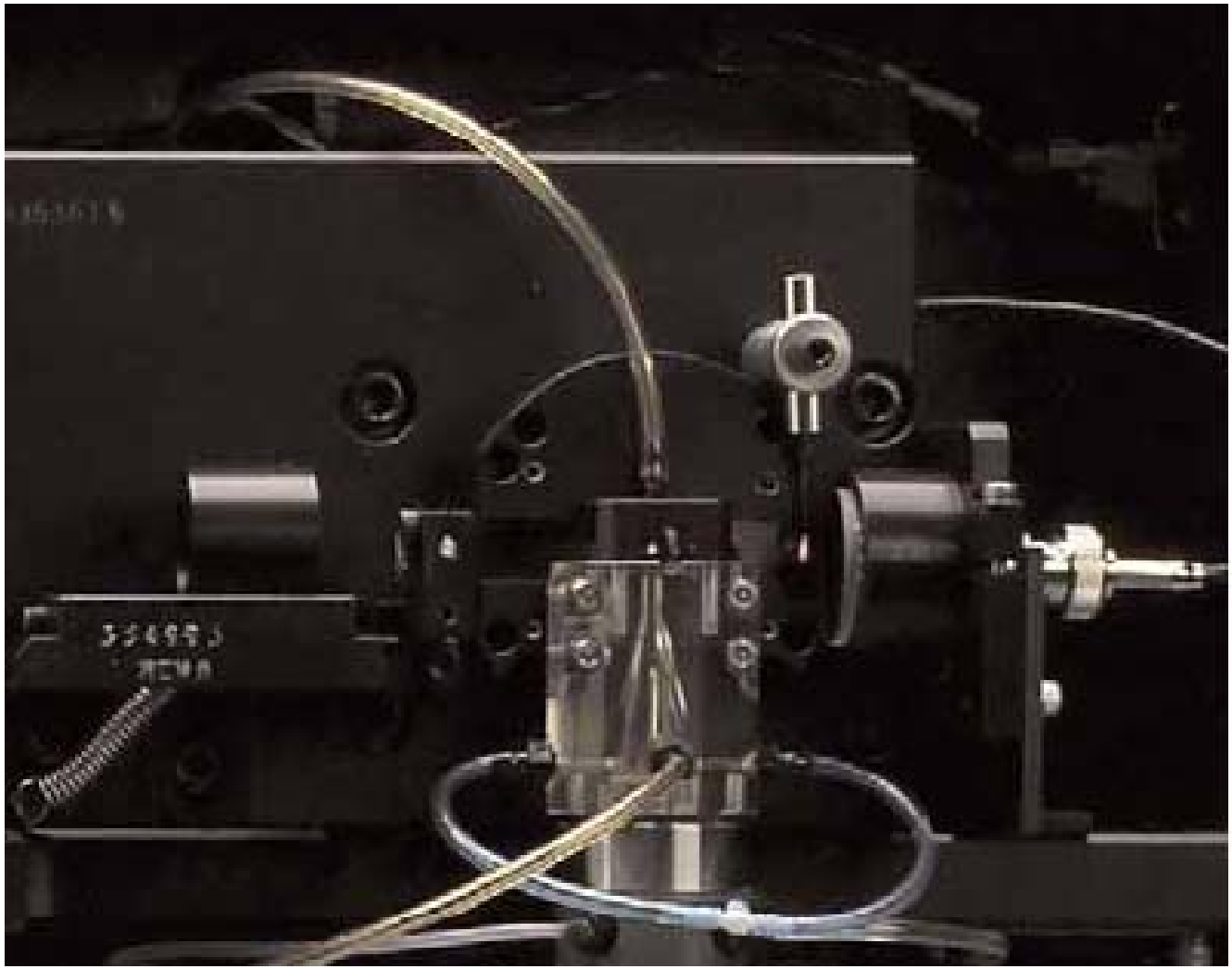
$$Q_{Total} = \Pi R^2 V_{AVG}$$

$$V(r) = V_o (1 - r^2 / R^2)$$

Intersection of Beam and Stream



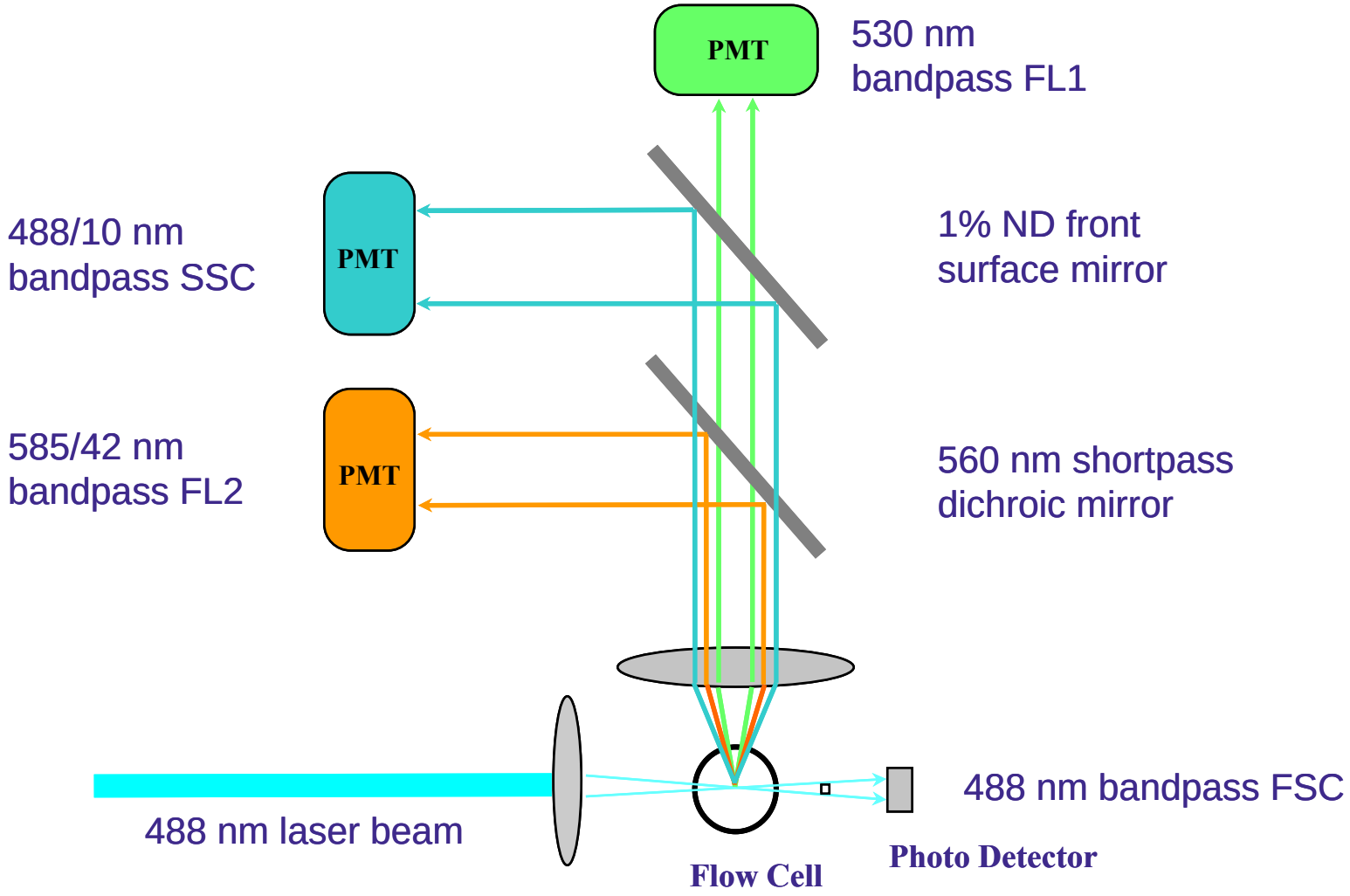
Flow Cell in Optical Path



Optics

- Excitation optics consist of:
  - Lasers
  - Fiber optic cables and prisms that route the laser light to the fluidic stream
- Collection optics consist of:
  - Fiber optic cables that direct the emitted light to the appropriate emission block
  - Filters that direct the signals in the emission block to the appropriate photomultiplier tube (PMT)

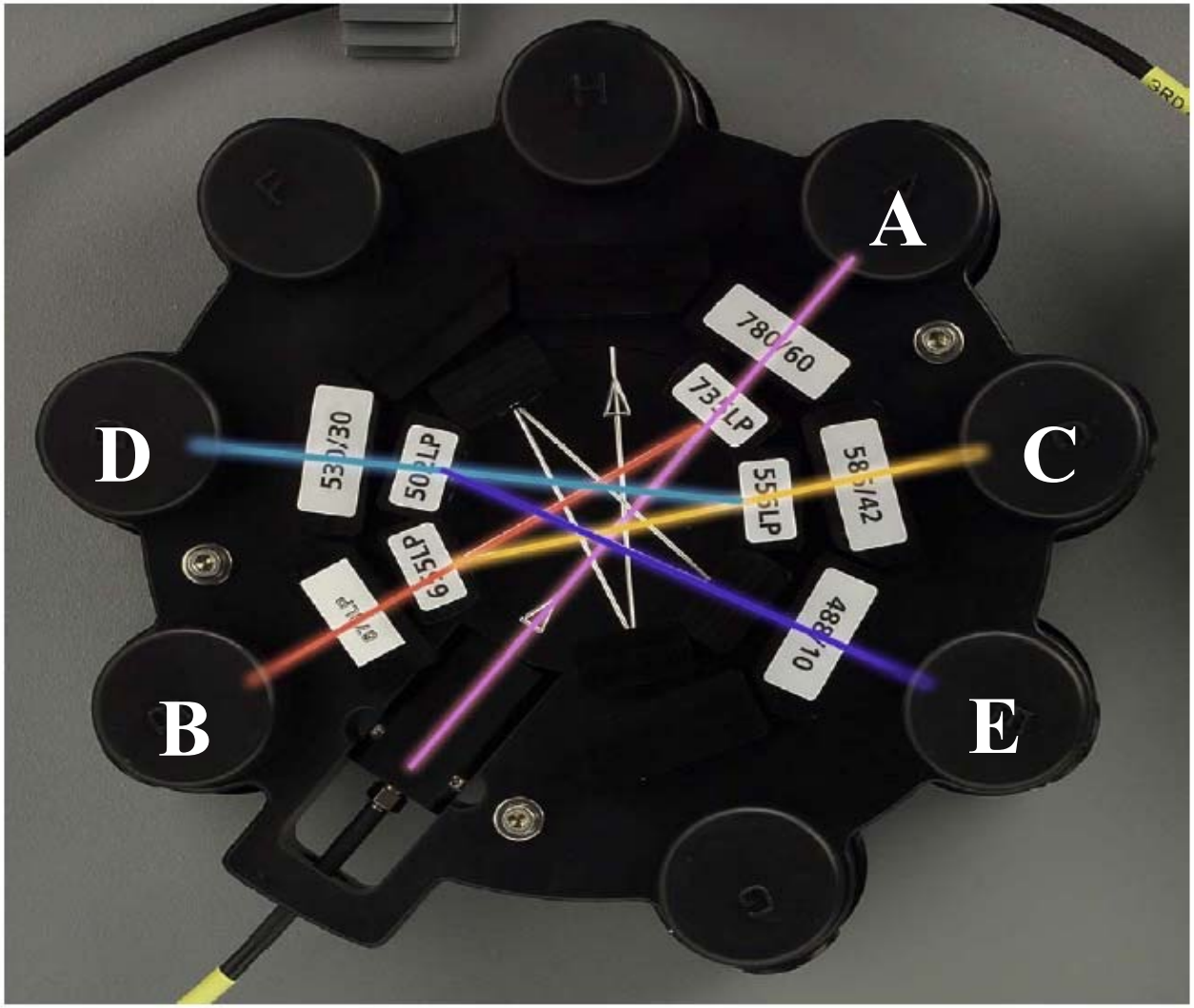
Simplified Traditional Layout



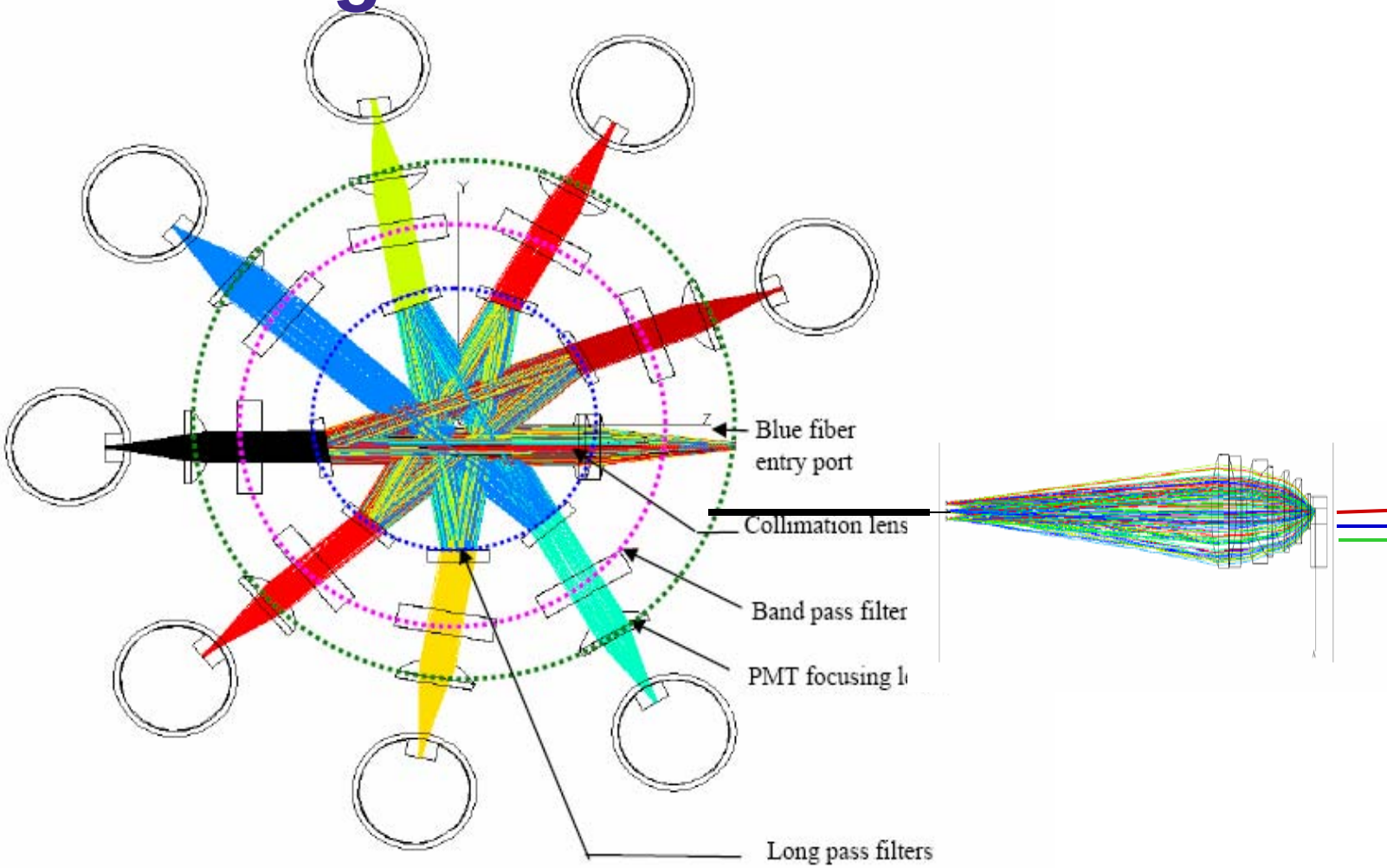
Detection Configuration

Laser	Primary Fluorochrome	PMT	Dichroic Mirror	Bandpass Filter
488 nm (Blue)	Side Scatter	E	None	488/10
	FITC	D	502 LP	530/30
	PE	C	556 LP	585/42
	PerCP or PerCP-Cy 5.5	B	655 LP	670 LP
	PE-Cy7	A	735 LP	780/60

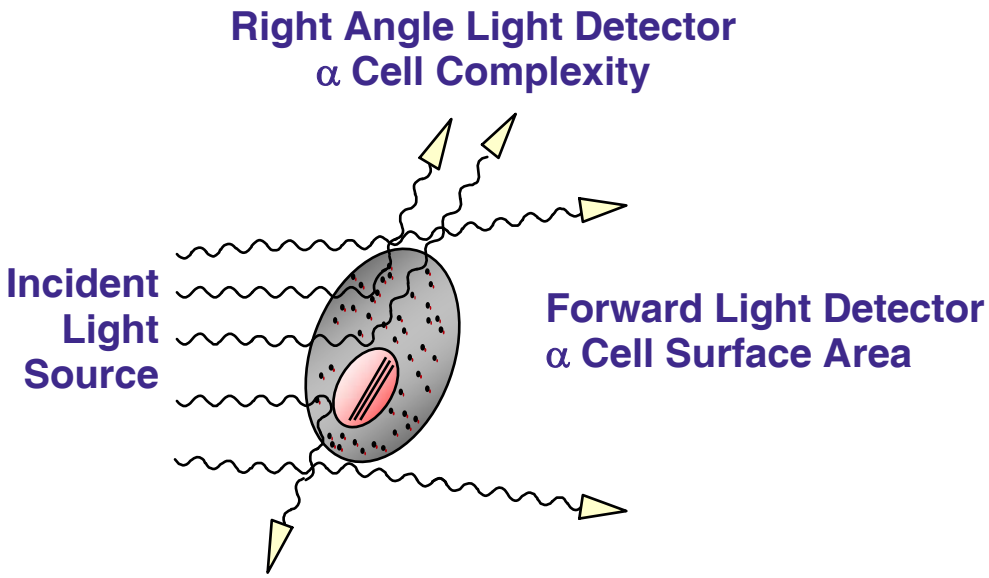
Detector Sub-Assembly



7 Fluorochromes and Side Scatter from Single Excitation Source

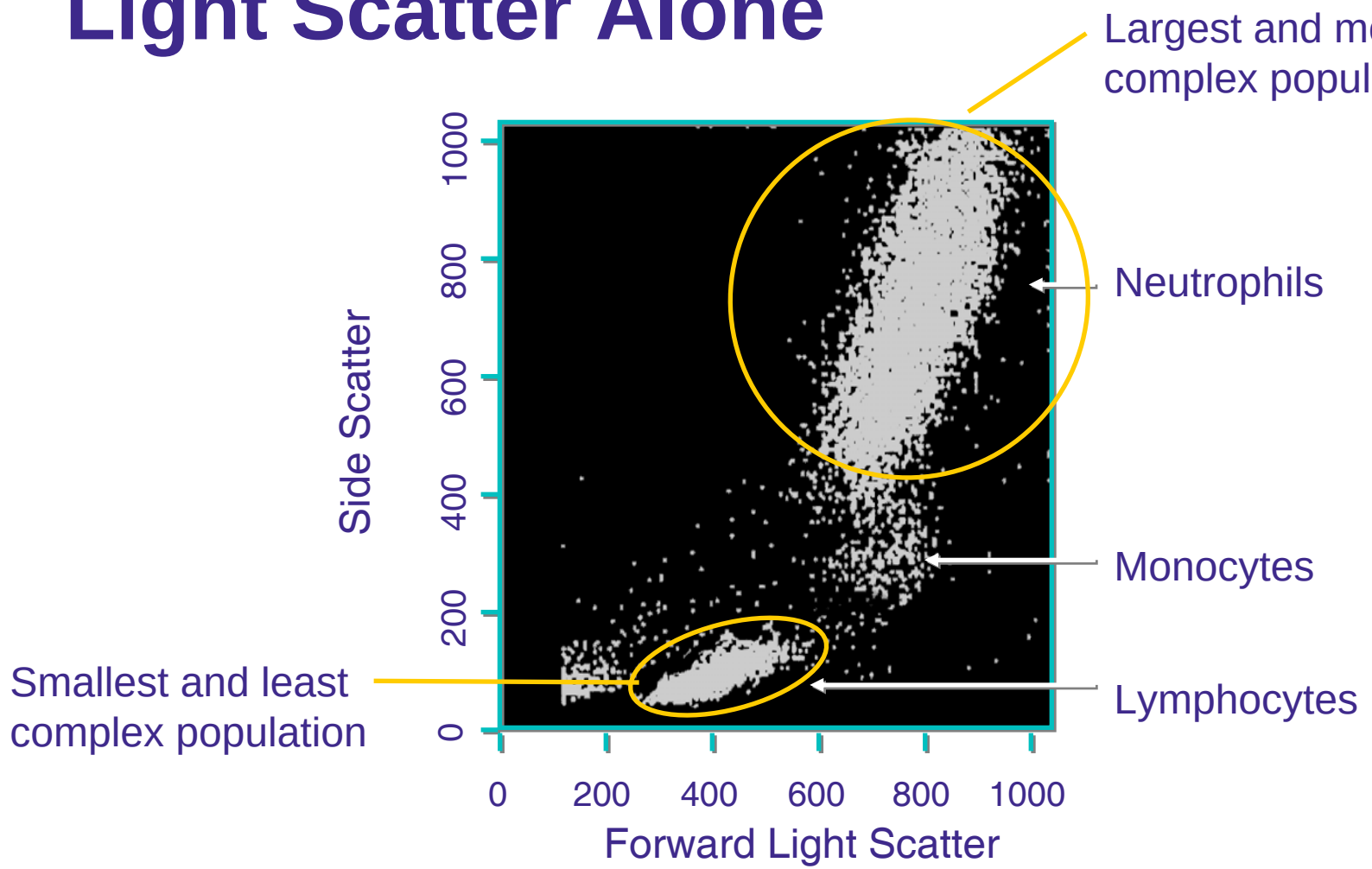


Properties of FSC and SSC

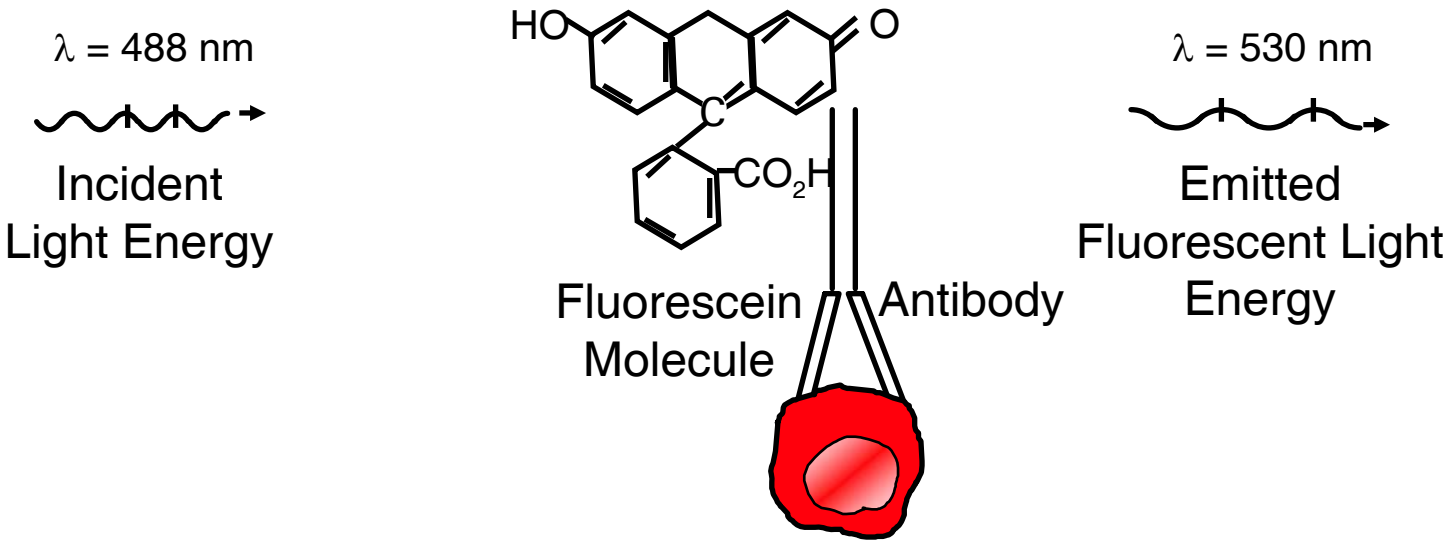


- FSC: Forward Scatter—complex measurement
  - Related to cell surface area and index of refraction (Mie Scattering, Gustav Mie – 1908 spherical particles)
  - Detected along axis of incident light in the forward direction
- SSC: Side Scatter—reflected and refracted light
  - Related to cell granularity and complexity
  - Detected at 90° to the laser beam

Lysed Whole Blood: Light Scatter Alone

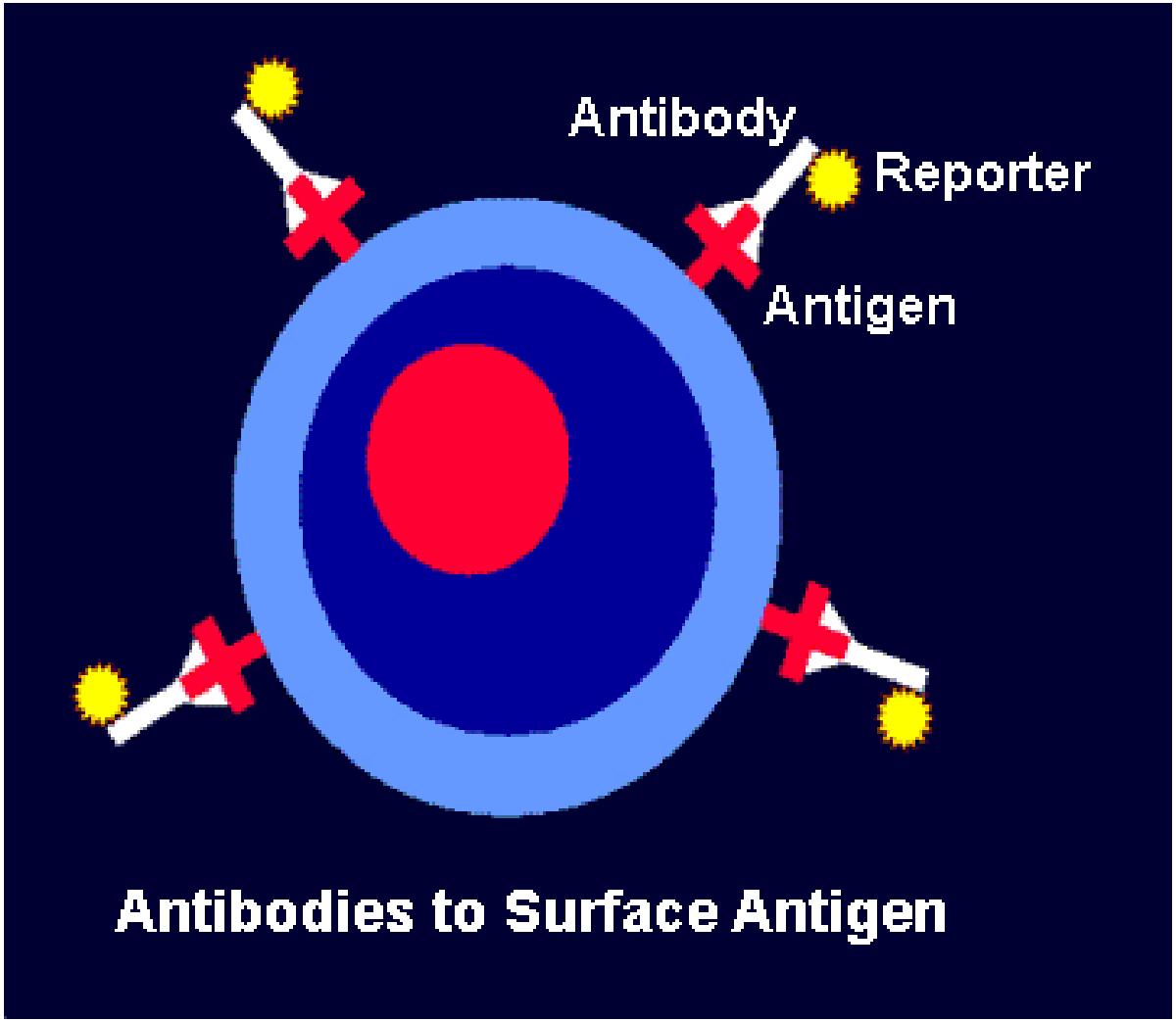


Fluorescence Detection



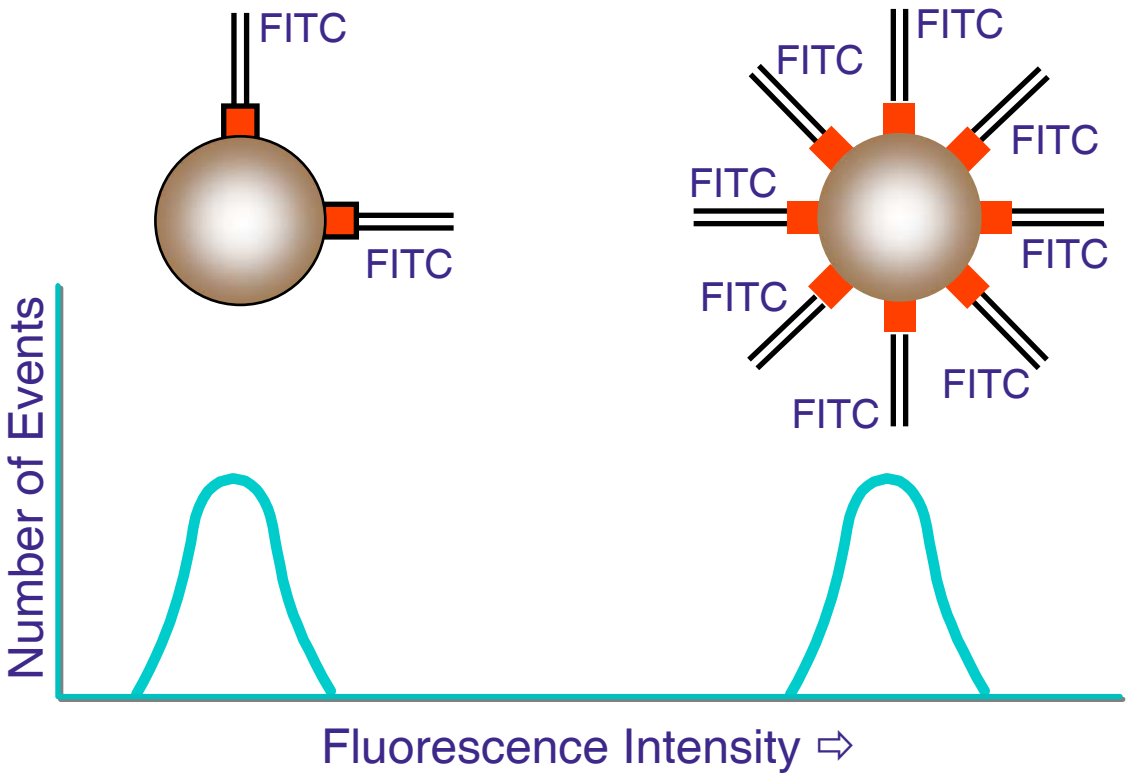
- The fluorochrome absorbs energy from the laser
- The fluorochrome releases the absorbed energy by:
  - Vibration and heat dissipation
  - Emission of photons of a longer wavelength

Antibody and Reporter Fluorochrome attached to cell



Fluorescence

Emitted Fluorescence Intensity $\propto$ Binding Sites



We measure fluorescence with some efficiency (Q) over a background (B)

IMMUNOPHENOTYPING

- Refers to the technique of identifying molecules that are associated with lymphoma cells and that help to characterize them. The molecules are analyzable because in most cases they are expressed on the outer cell surface membrane (CD Marker).
- The molecules are characterized by using special antibodies that bind to them specifically . In this context these molecules are called "antigens," and the specific part of the molecule to which the antibody attaches is called the "epitope".

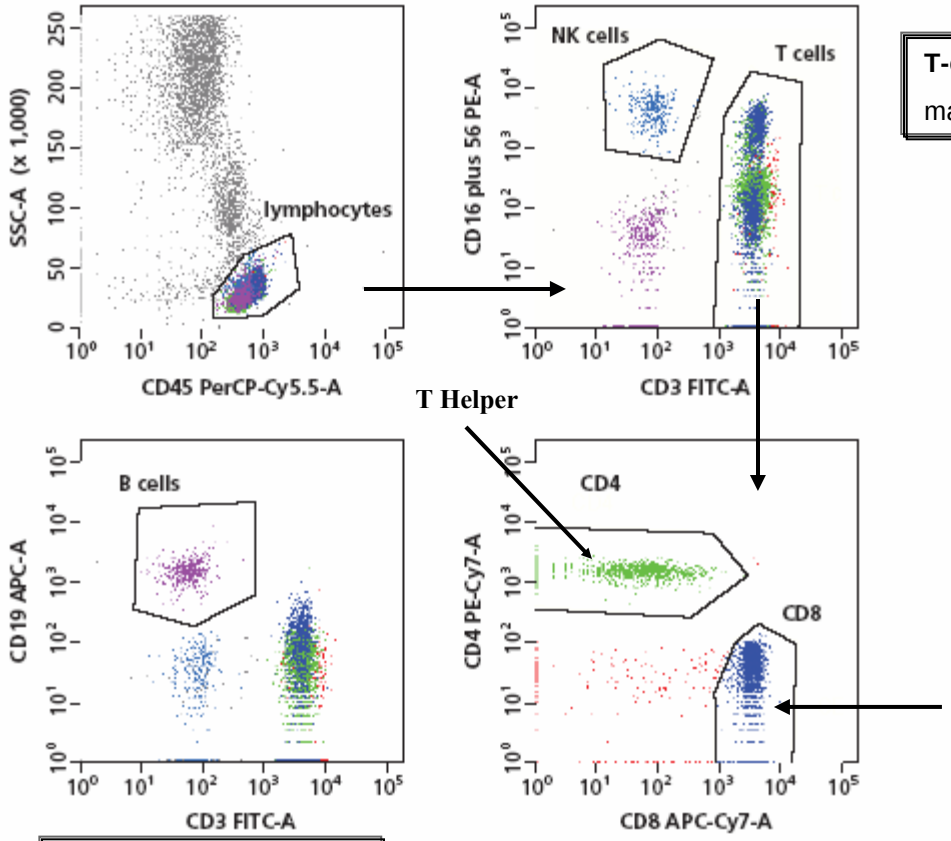
Population Analysis

All Events					**CD Marker**	
	Leukocytes				CD 45 +	
		Lymphocytes			CD 45 + Side Scatter	PerCP – Cy 5.5 and SSC
			T- Cells		CD 3 +	FITC
				Cytotoxic T- Cells	CD 3+ CD 8 +	APC + PerCP – Cy 5.5
				Helper T-Cells	CD 3+ CD 4+	APC + APC - Cy7
			B Cells		CD 3- CD 19+	APC
			NK Cells		CD 3- CD16 + CD 56+	PE

Leucocyte subset analysis showing whole blood stained with six-color reagents

Lymphoid cells are reactive for CD45 leukocyte common antigen

Natural-killer cells: These guys are associated with CD16 and CD56



T-cells: Pan T-cell marker CD3

Tube: 3/16+56/45/4/19/8/

Population	#Events	%Parent	%Total
All Events	10,000		100.0
Lymphocytes	5,223	52.2	52.2
T cells	4,415	84.5	44.2
CD8	1,988	45.0	19.9
CD4	2,249	50.9	22.5
NK cells	322	6.2	3.2
B cells	394	7.5	3.9

B-cells almost all of these are reactive for CD19

Most T-cells mark with either CD4 (helper cells) or CD8 (suppressor cells or cytotoxic cells).

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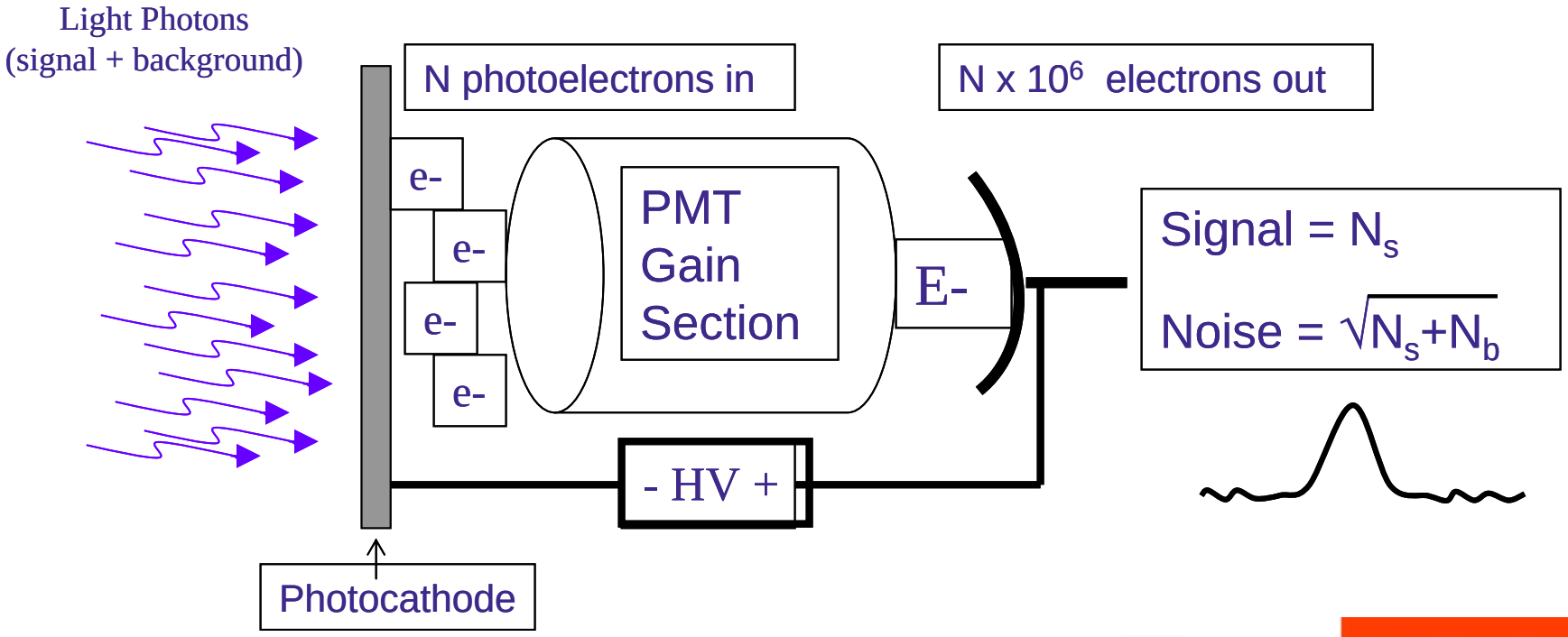
Electronics

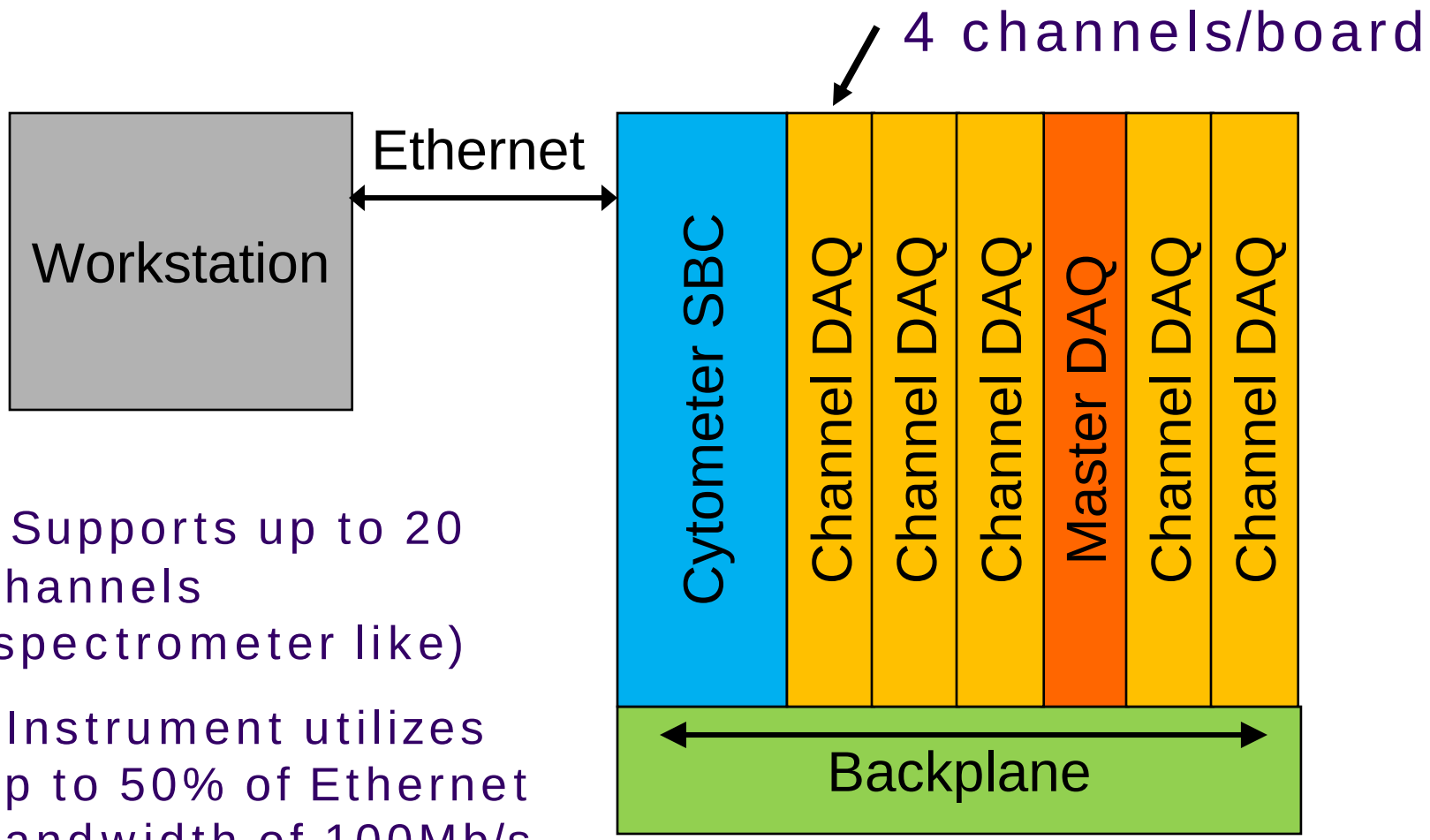
Emitted Fluorescent

Light Energy

Photomultiplier

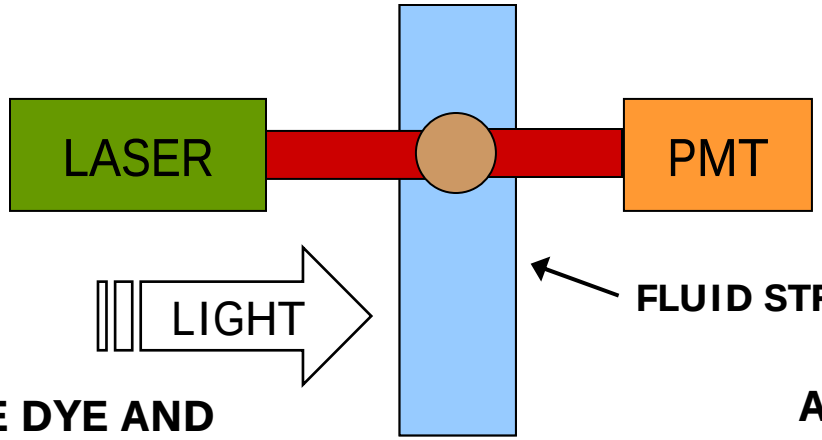


Cytometer Acquisition Electronics

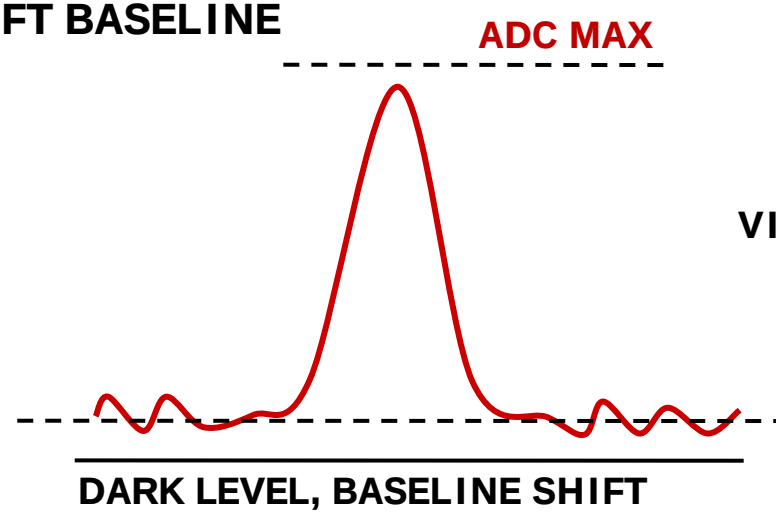


- Supports up to 20 channels (spectrometer like)
- Instrument utilizes up to 50% of Ethernet bandwidth of 100Mb/s, or 50Mb/s, 6.25MB/s

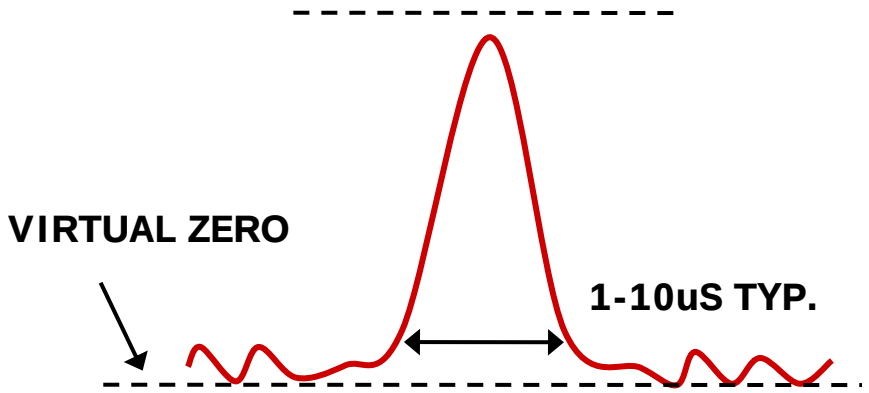
PMT Current to Voltage and Analog Baseline Restoration



FREE DYE AND BACKGROUND LIGHT CAN SHIFT BASELINE

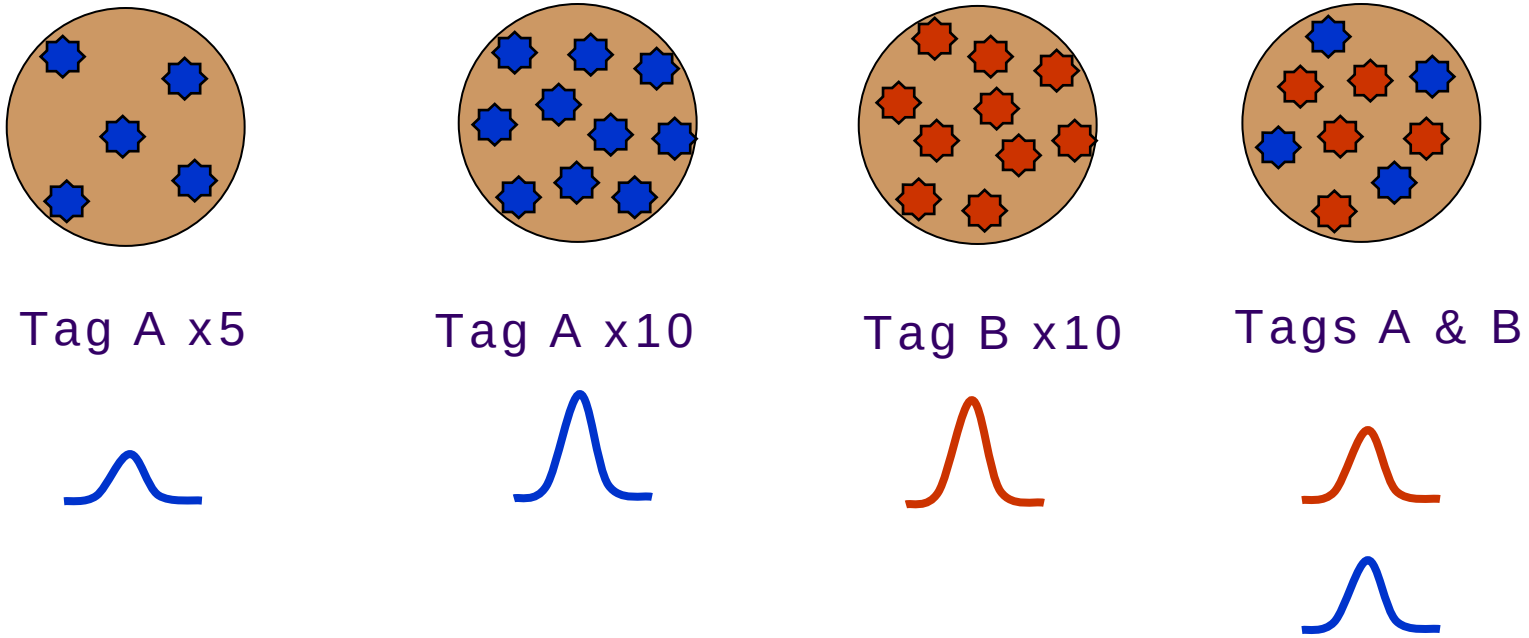


ANALOG BASELINE RESTORATION CIRCUIT REMOVES DC AND LOW-FREQ. TO MAINTAIN RESOLUTION OF ADC

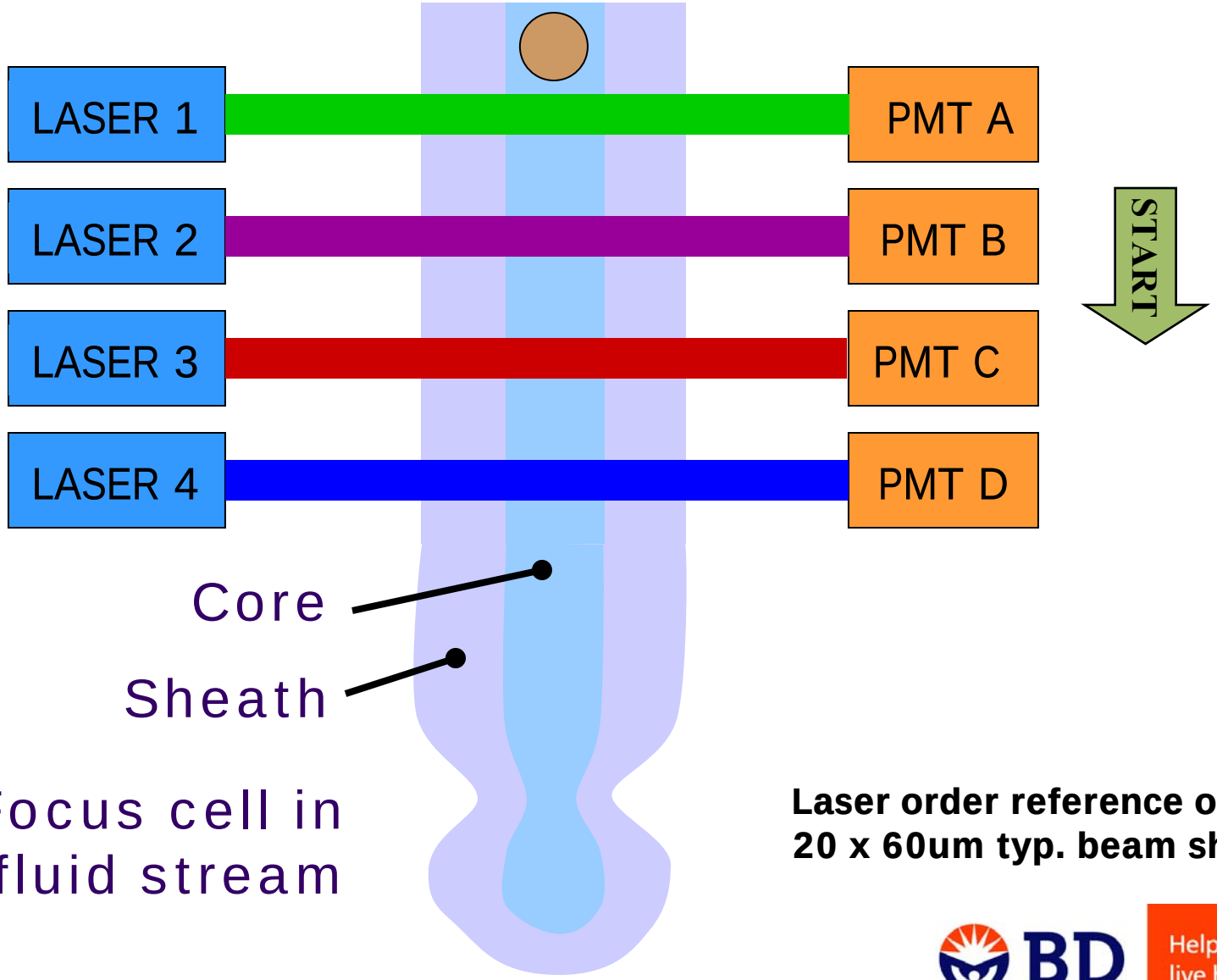


Fluorescent tags

Emissions produced by fluorescent antibody tags attached to cells

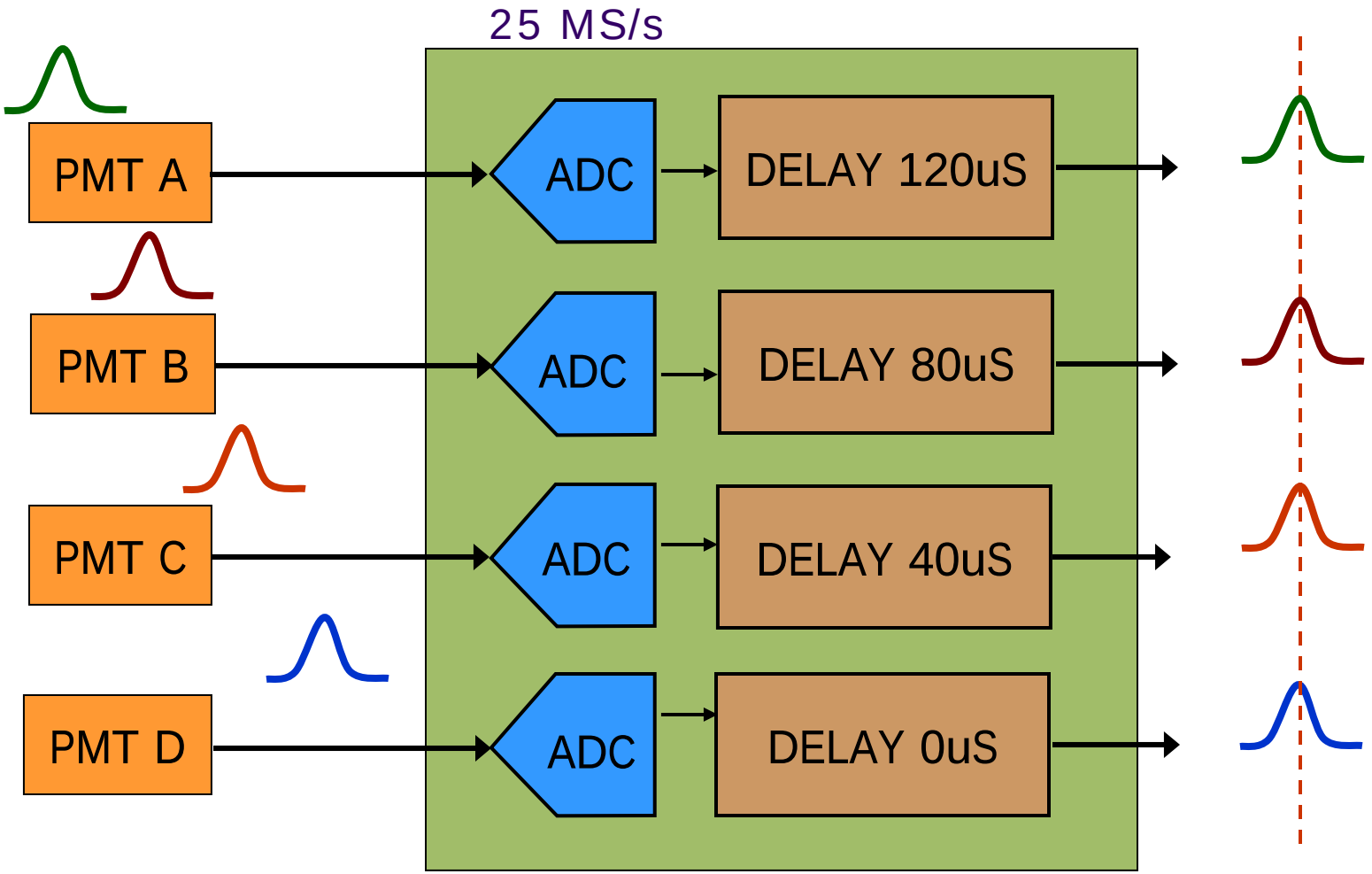


Cell through multiple lasers

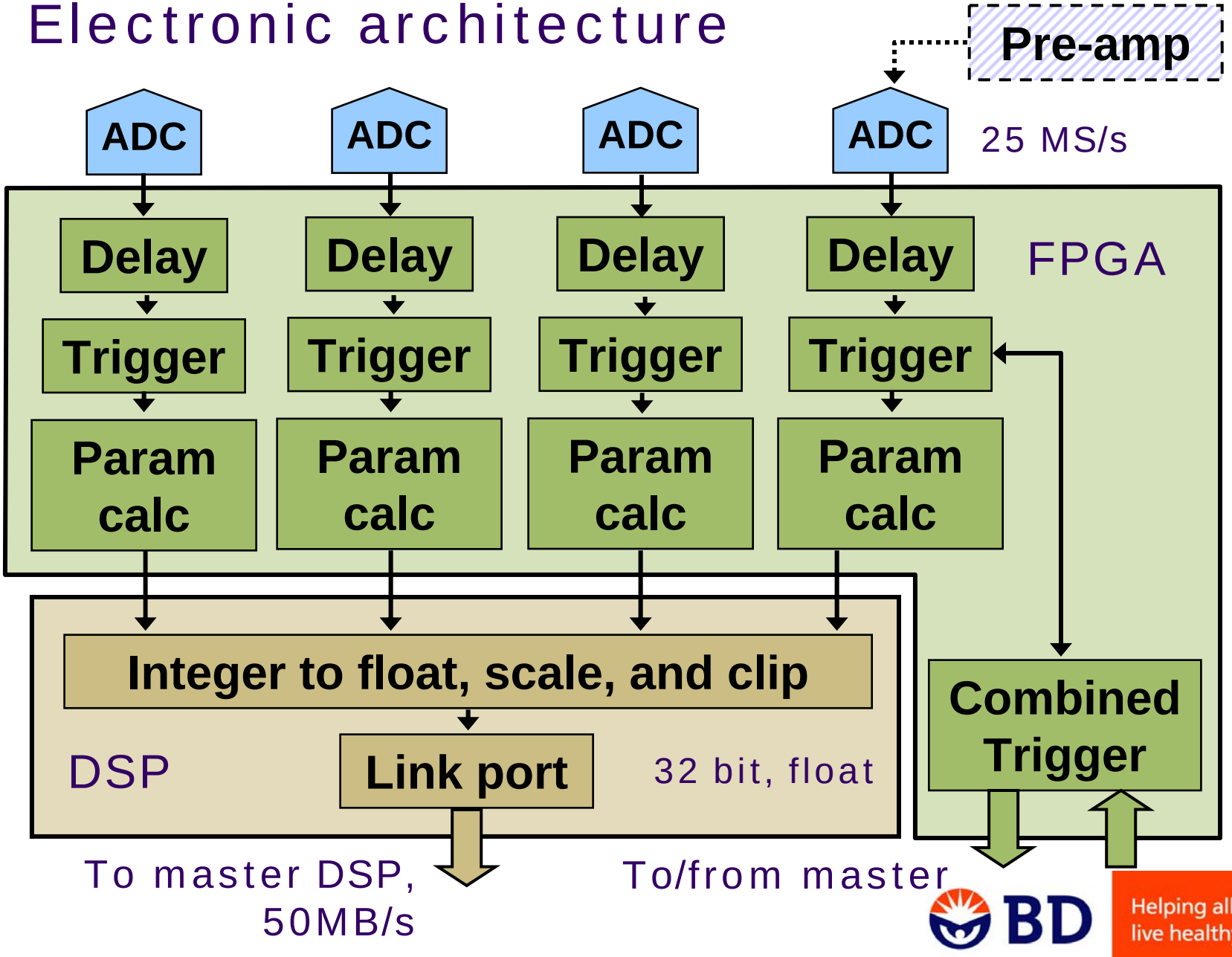


Laser order reference only
20 x 60um typ. beam shape

Acquisition sample delay



Electronic architecture



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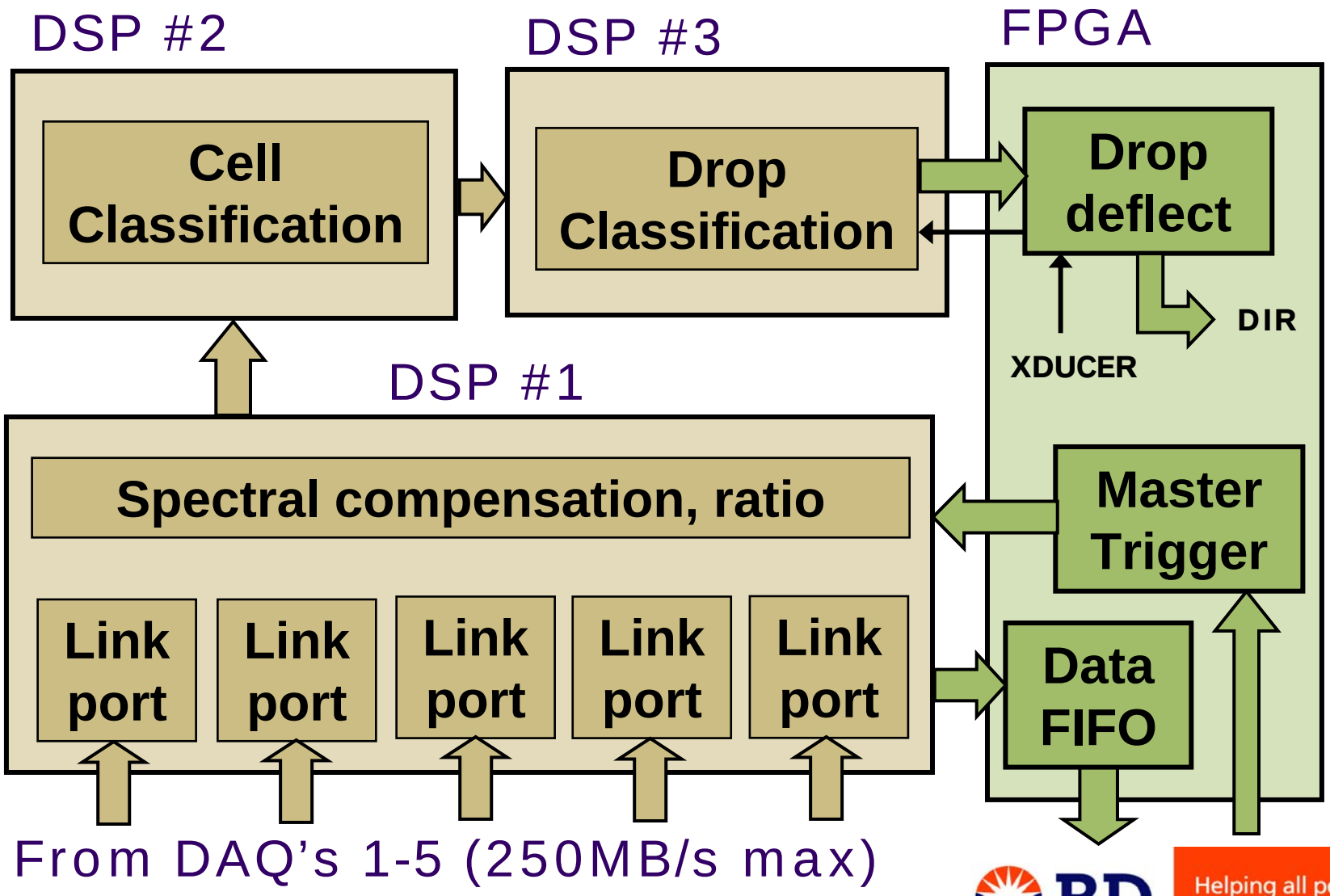
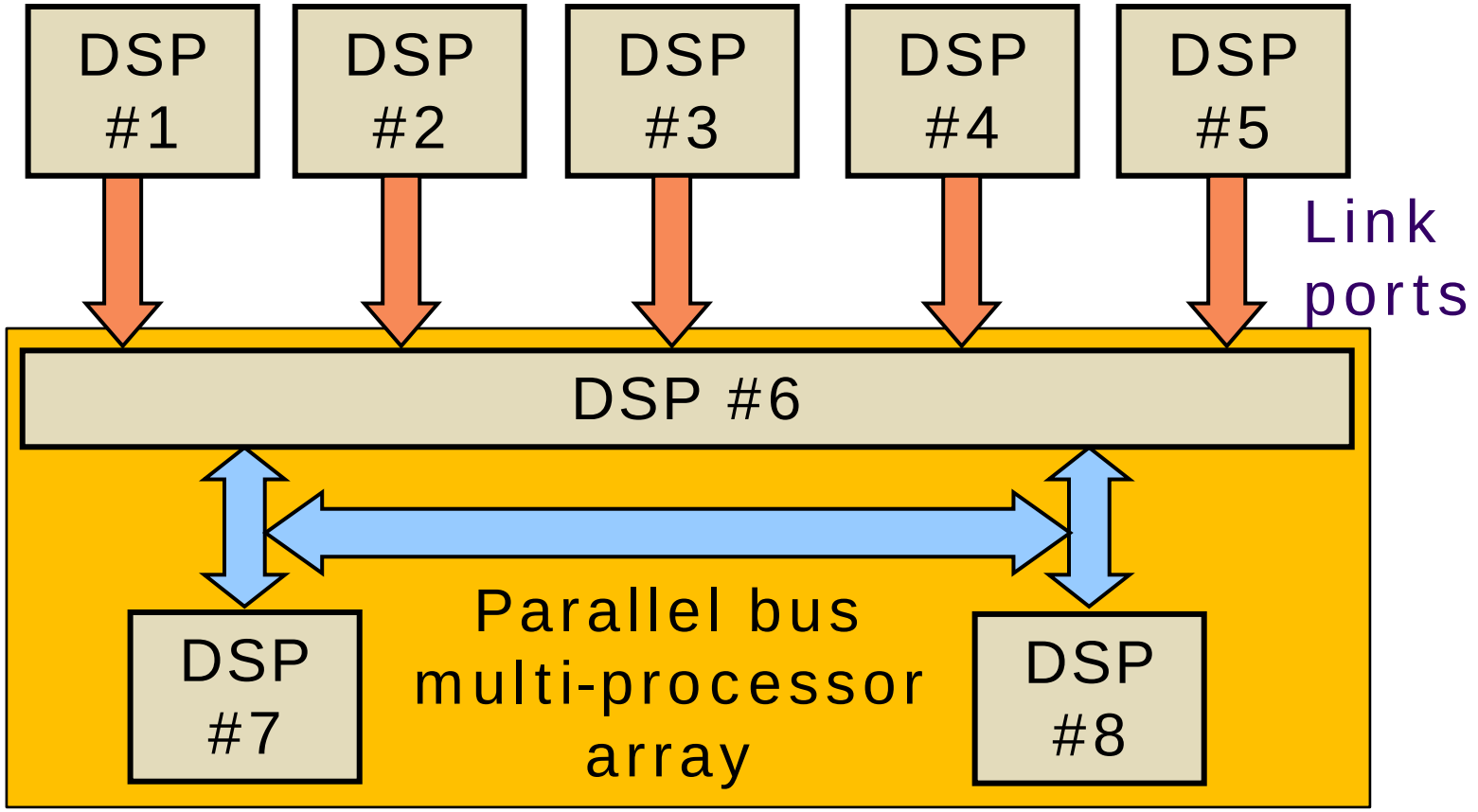
Master DAQ/DSP's

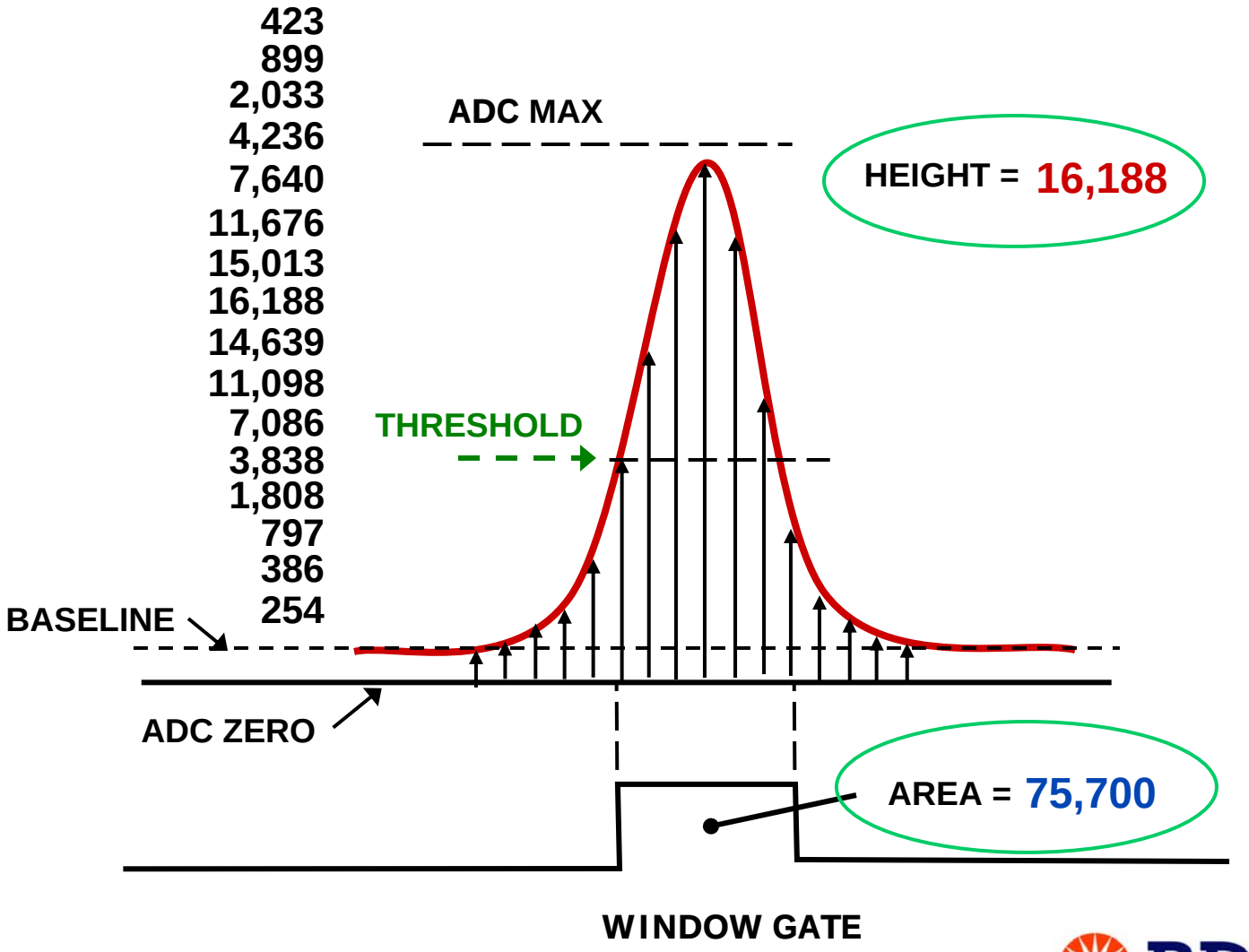


DSP Architecture

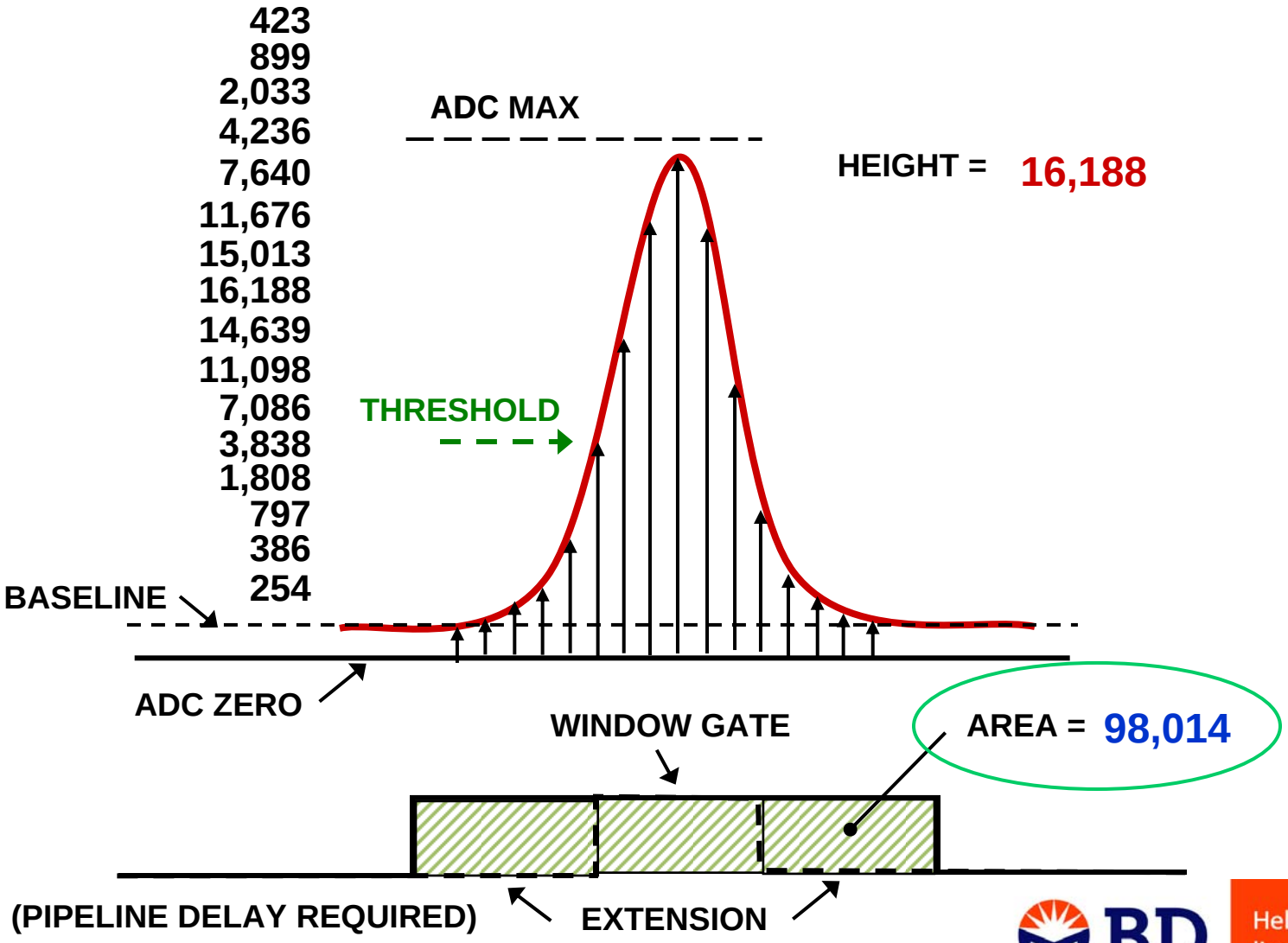
32 bit, floating point



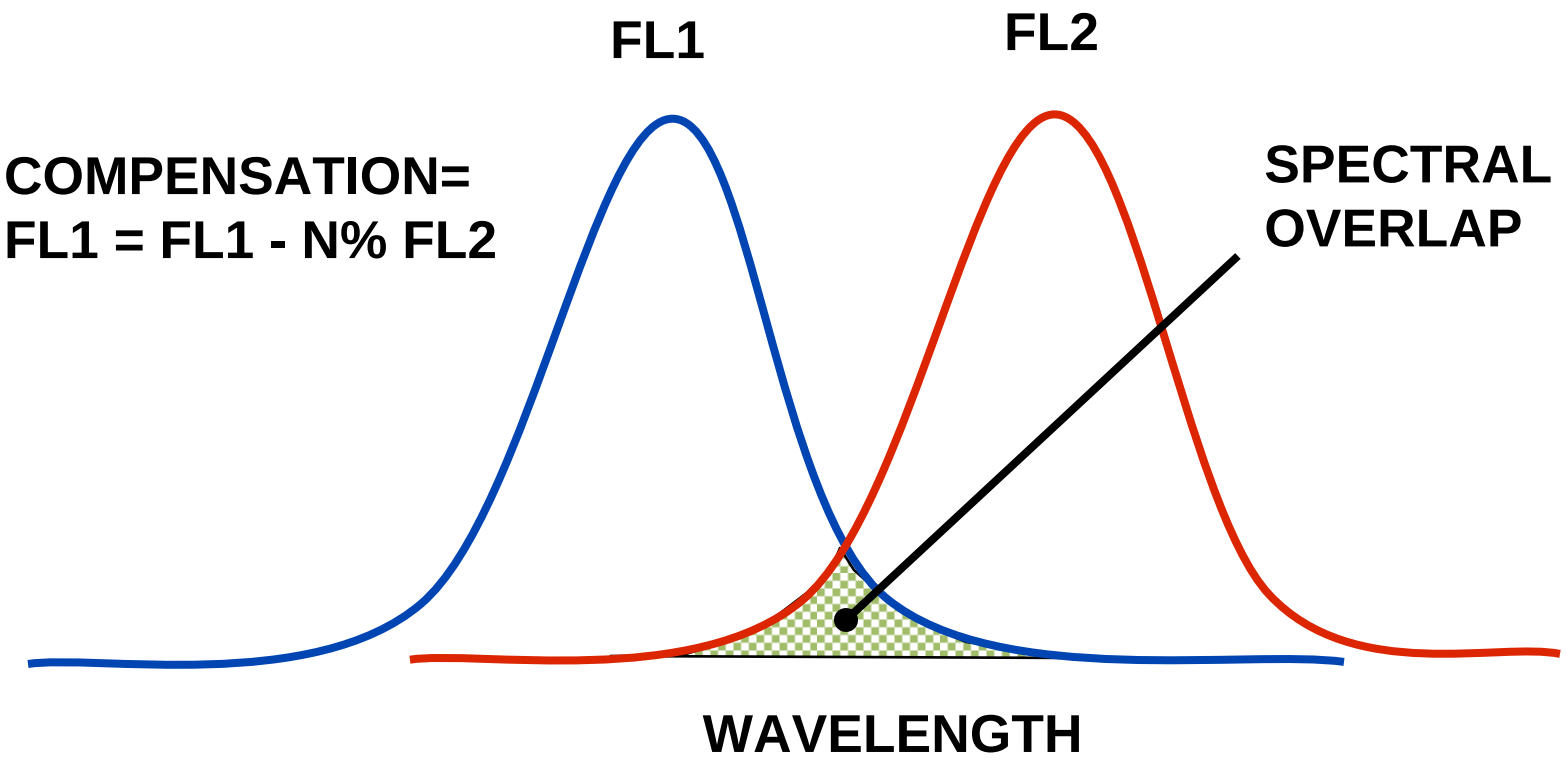
Digital measurements



Extended Measurement Window

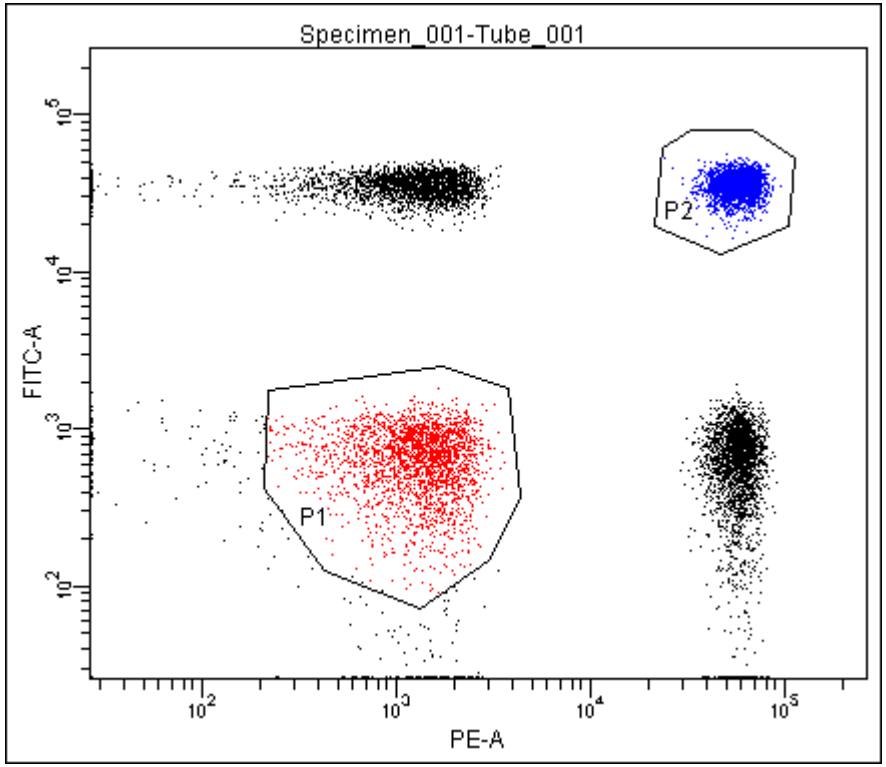


Spectral overlap compensation

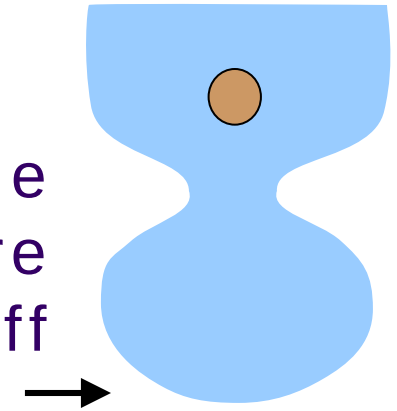


Sorting Software Setup

- Define sort mode: yield or purity
- Define regions: P1, P2.
- Regions can be combined into gates
- Define droplet/cell destination
  - Collection tube, left, right, etc.
  - Example; P1 deflect left, P2 right



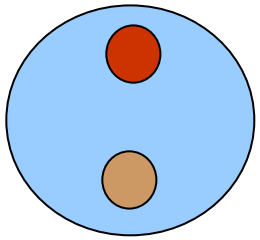
Droplet break-off conflict resolution



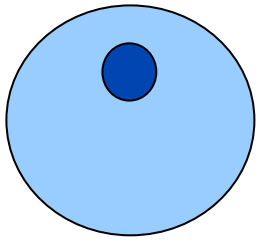
Apply charge (+/-) before break-off

Transducer vibrates stream

No cell in this drop, goes to waste

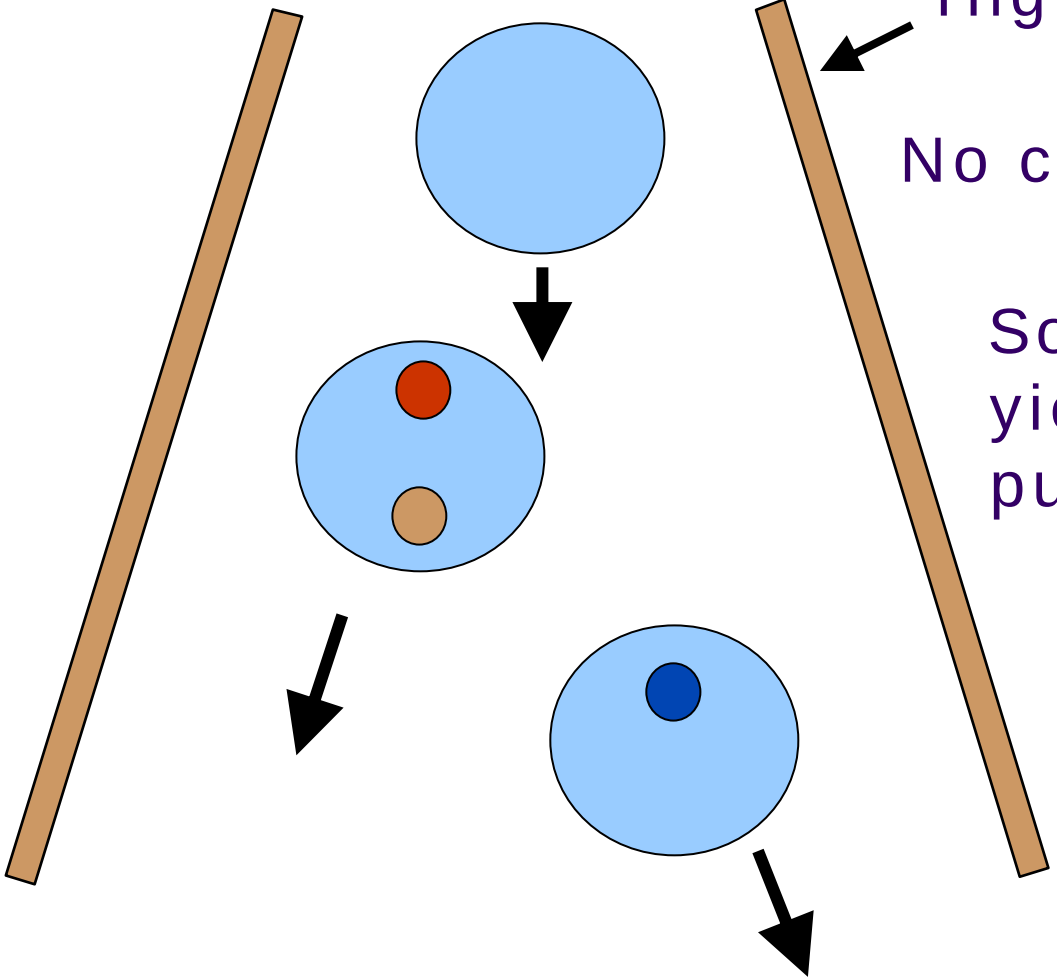


Sort if yield mode, not if purity



Sort if yield or purity mode

Droplet deflection



High voltage plates

No cell in drop

Sort RED cell if yield mode, not purity

Sort BLU cell if yield or purity mode

Question & Answers