CCR Flow Cytometry Core Training

NCI CCR FACS Core Managers

Section 1
Overview of Flow Cytometry

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CCR/LGI Flow Core in BD37

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Flow Cytometry Services at the National Cancer Institute

Flow "Core "*vs*. "Facility"

A Flow Core is funded by CCR and is generally *open to all members* of all NCI-affiliated Labs or Branches.

A Facility is funded by and operated from an NCI Branch and is generally *restricted* to members of that Branch.

CCR Flow Cytometry Cores:

CCR LGI Flow Cytometry Core in BG37.
CCR Vaccine Branch Core in BG41.
NCI-Frederick CCR Flow Cytometry Core Cancer Inflammation Program.
(*in Frederick, MD in BG560*)

NCI Flow Cytometry Facilities:

NCI EIB Flow Cytometry Facility in BG10.
NCI ETIB Flow Cytometry Facility in BG10.
NCI Surgery Branch Flow Cytometry Facility in BG10.

NOTE: Special technologies available only at any one Flow service may be available to all NCI users – please inquire!

Outline

- Theory of flow cytometry
- Technical considerations
- Applications

What is Flow Cytometry

- ***Cytometry*** - the measurement of physical/chemical characteristics of cells or *other* particles (typically microscopy-based).

- ***Flow Cytometry*** - the process whereby cytometry measurements are made on cells/particles as they pass through suspended in a fluid stream.

- ***Flow Cell Sorting*** - the application of flow cytometry to separate and collect cells in real time during flow acquisition.

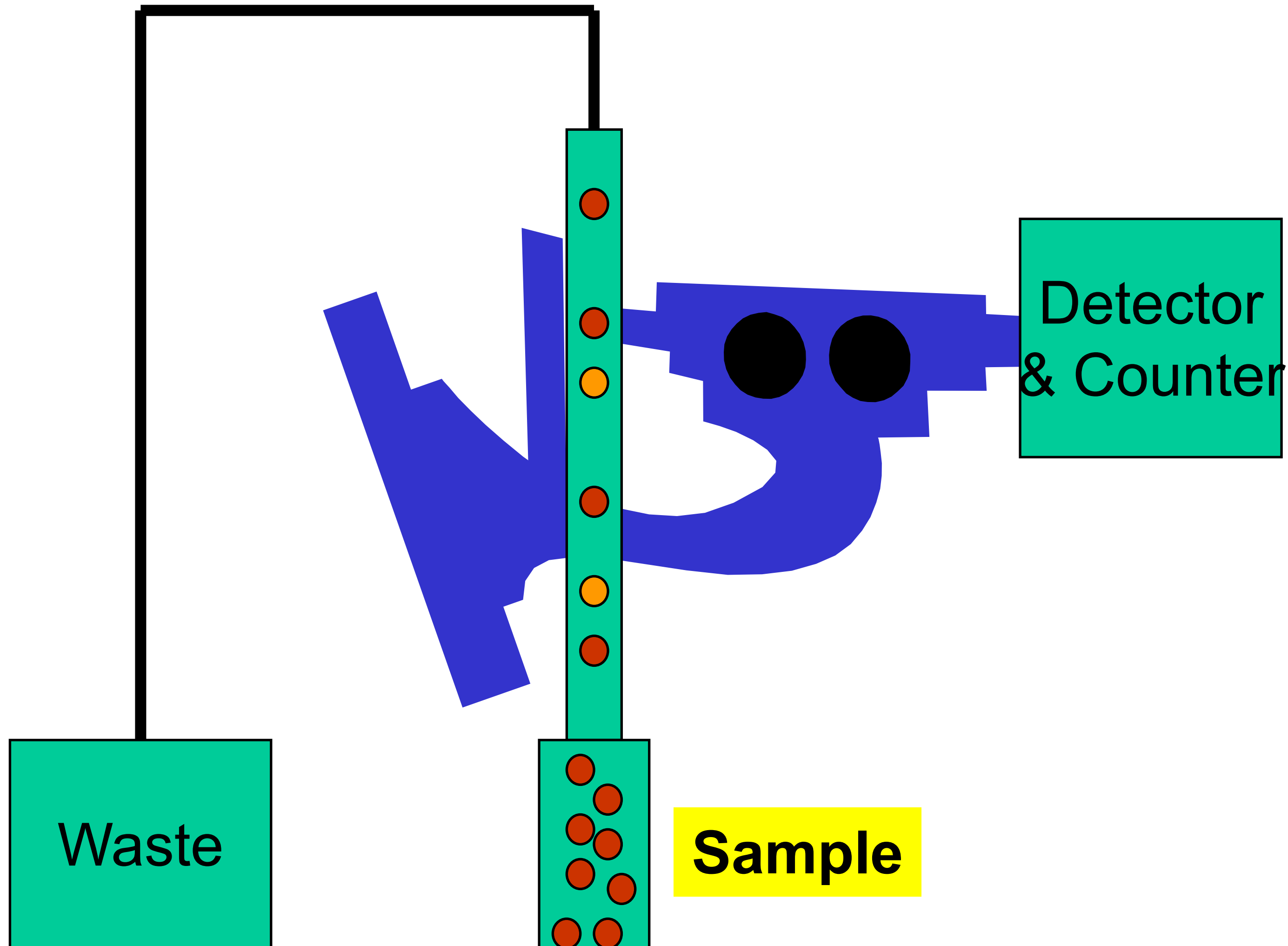
- *FACS* - Fluorescence Activated Cell Sorting. FACS™ is a trademark of Becton Dickinson Immunocytometry Systems (BDIS, now BD Bioscience).

All FACS instruments are BD cytometers, but not all cytometers are FACS.

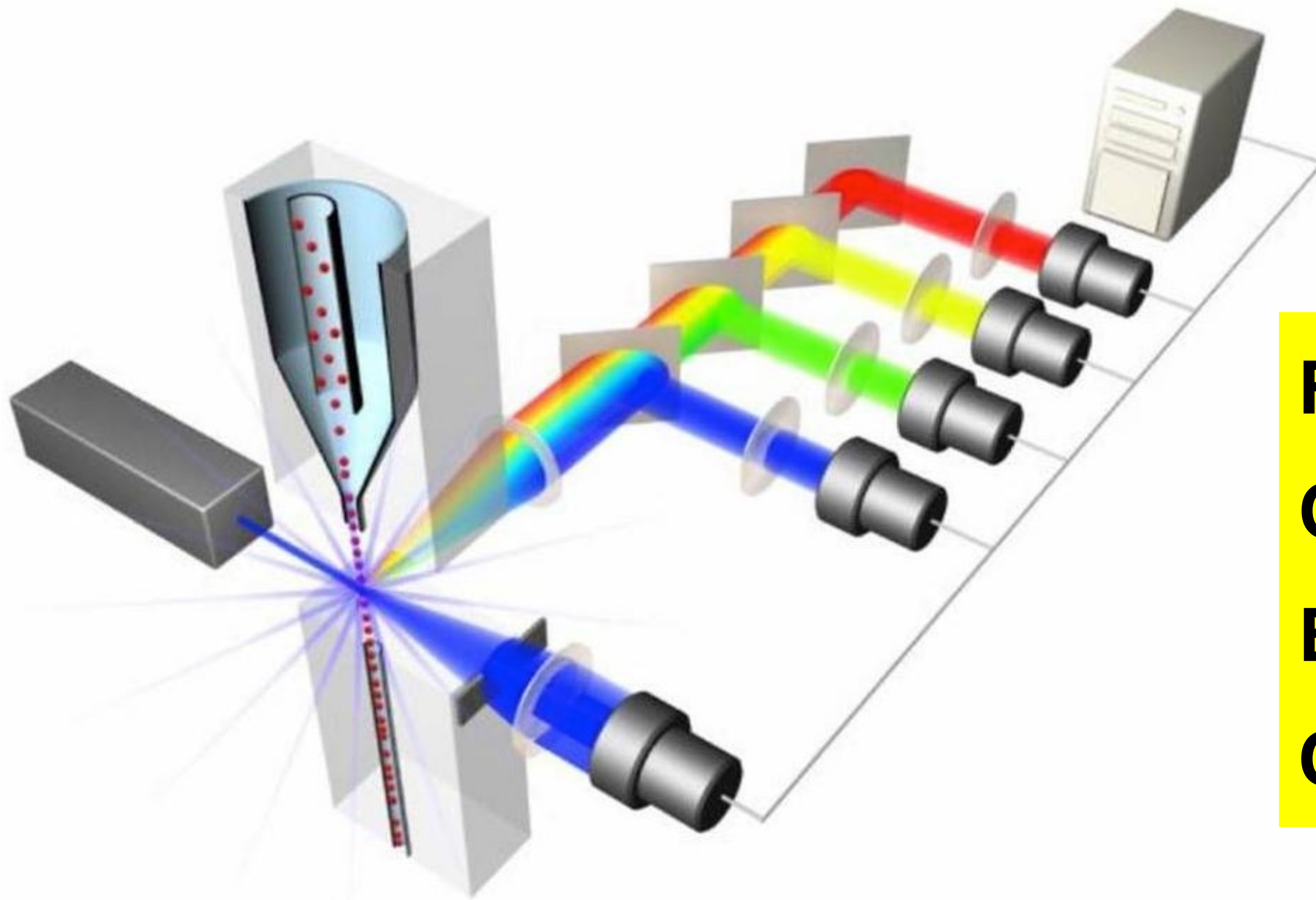
Flow Cytometry Dictionary at FluoroFinder

https://fluorofinder.com/newsletter-flow-dictionary/?utm_source=FluoroFinder+Newsletter&utm_campaign=2b56067893-EMAIL_CAMPAIGN_2017_03_31&utm_medium=email&utm_term=0_52a52ca7fe-2b56067893-413101109

The “automated microscope“



Four Major Technical Considerations



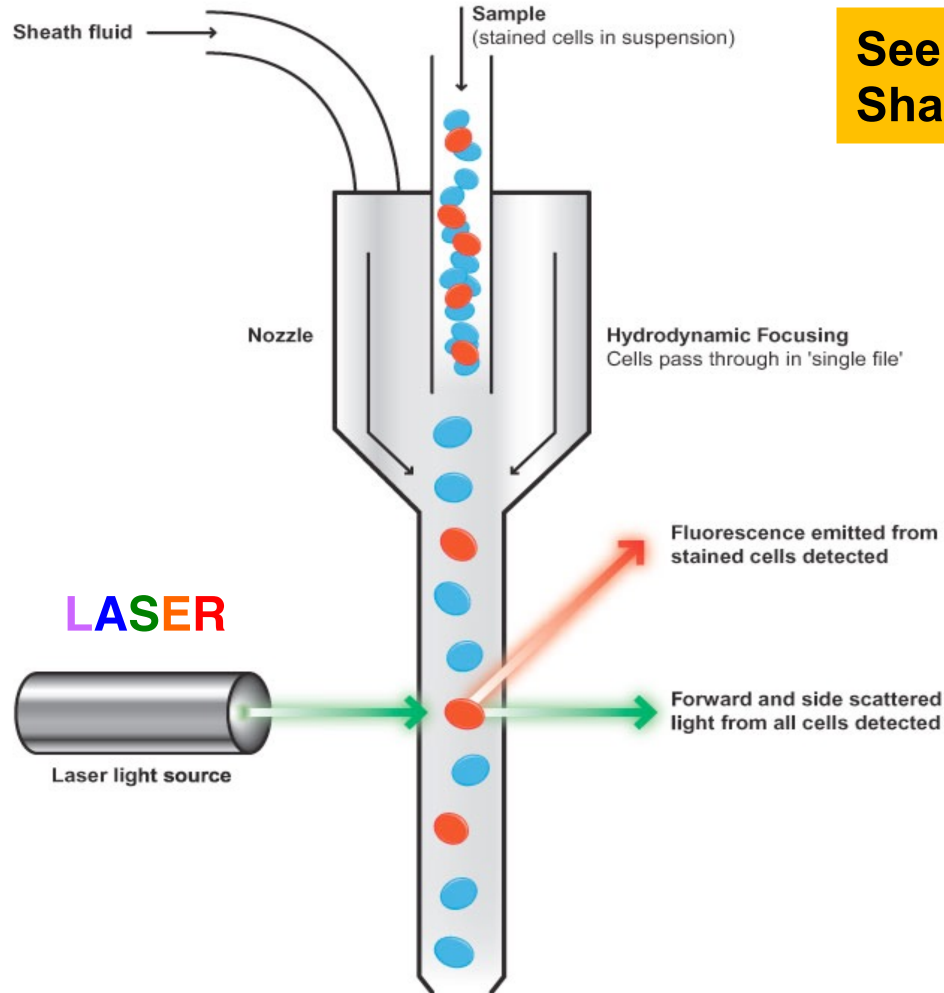
Fluidics

Optics

Electronics

Computing

Fluidics: Hydrodynamic Focusing

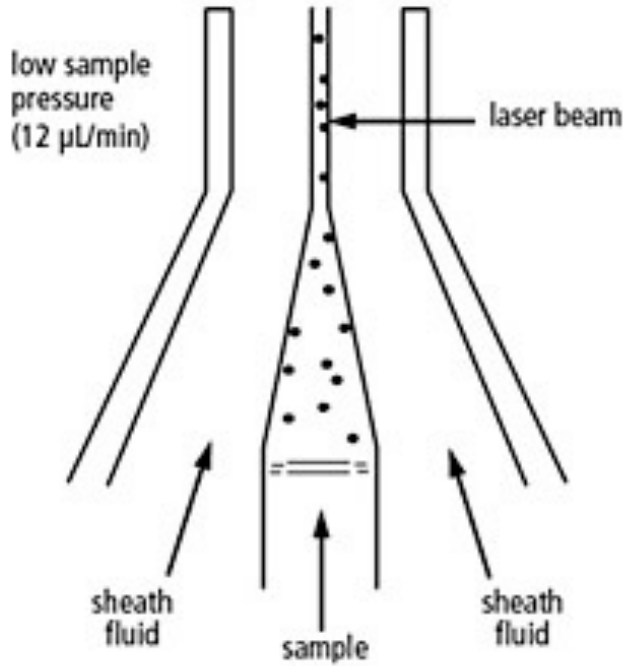


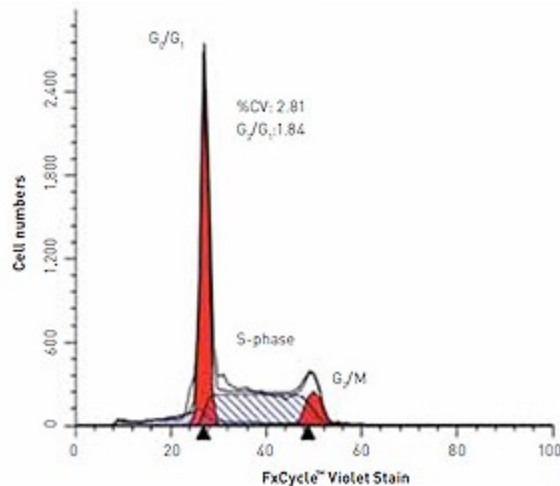
See further Sue Sharrow's presentation!

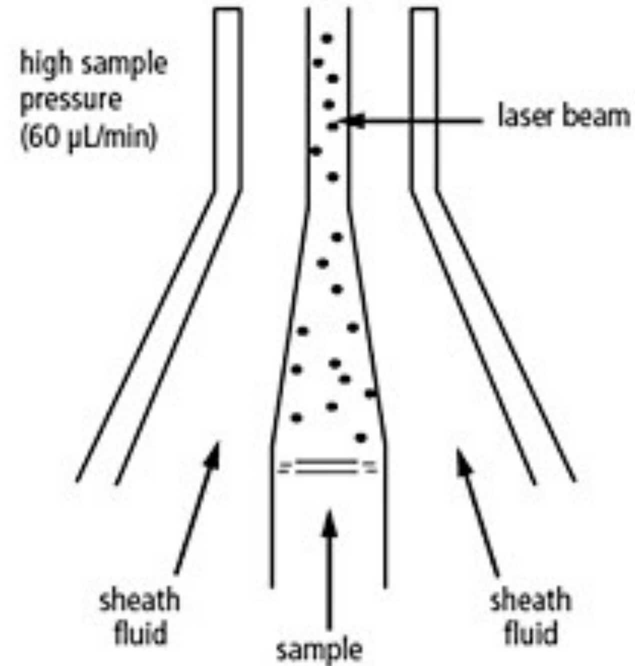
NOTE: Good single cell suspension – good flow data

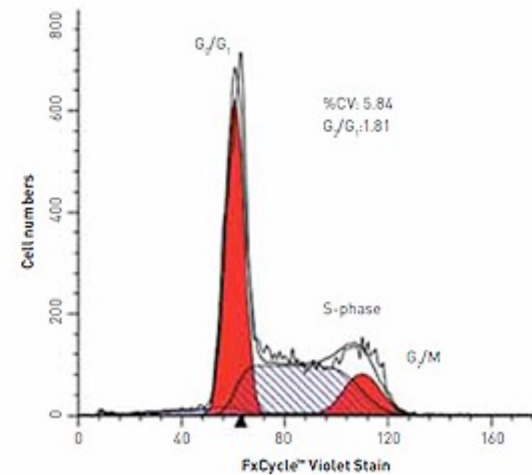
Sample Pressure/Flow Rate Affect Data Quality

Low flow – Better resolution (e.g. cell cycle)



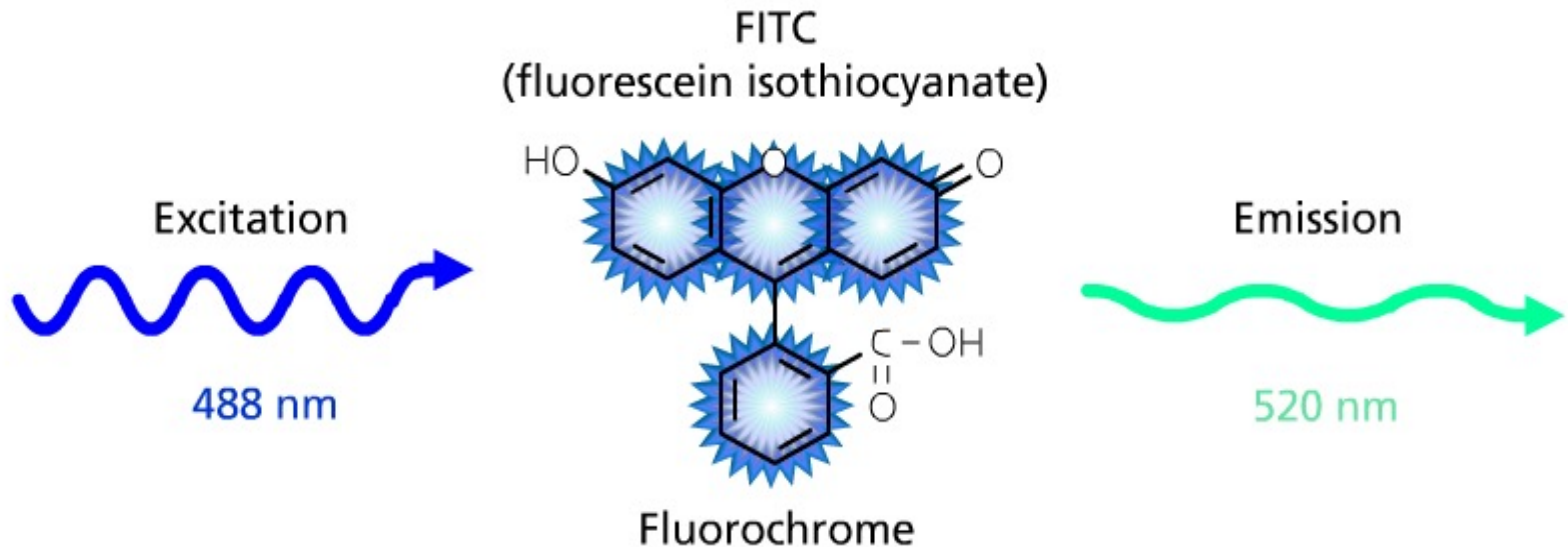






High flow – Lesser resolution (rare event analysis)

What is Fluorescent Light?



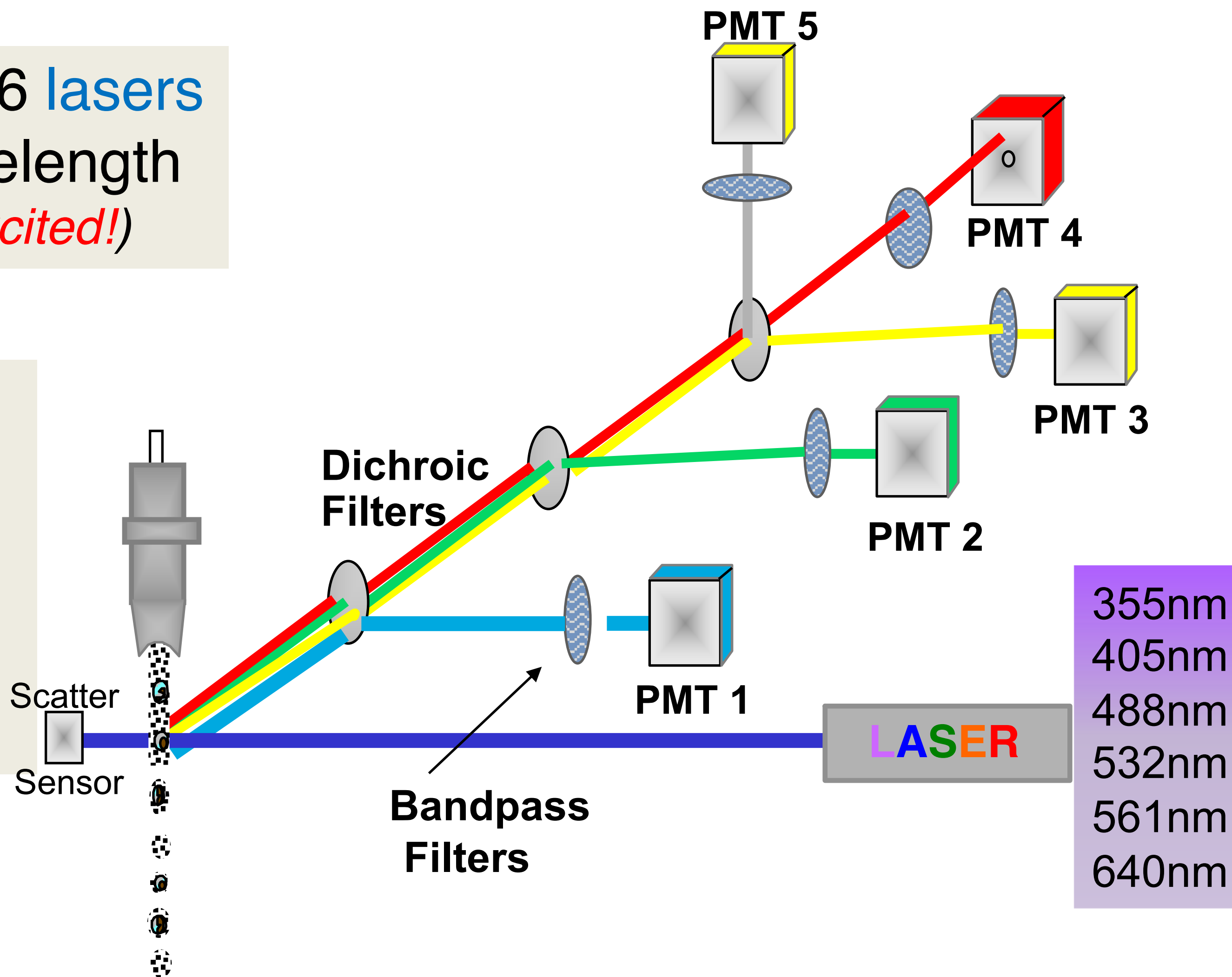
- Fluorochromes absorb energy from a *shorter* wavelength light and emits a *longer* wavelength light.
- The difference in the wavelength of absorption and the emission wavelengths of a fluorochrome is called the Stokes shift.
- There is a range of the absorbed and a range of the emitted light (about 100-300nm) – both are characteristic of the fluorochrome.

See further Bill Telford's presentation!

Optics: Lasers and Photo Multiplier Tubes

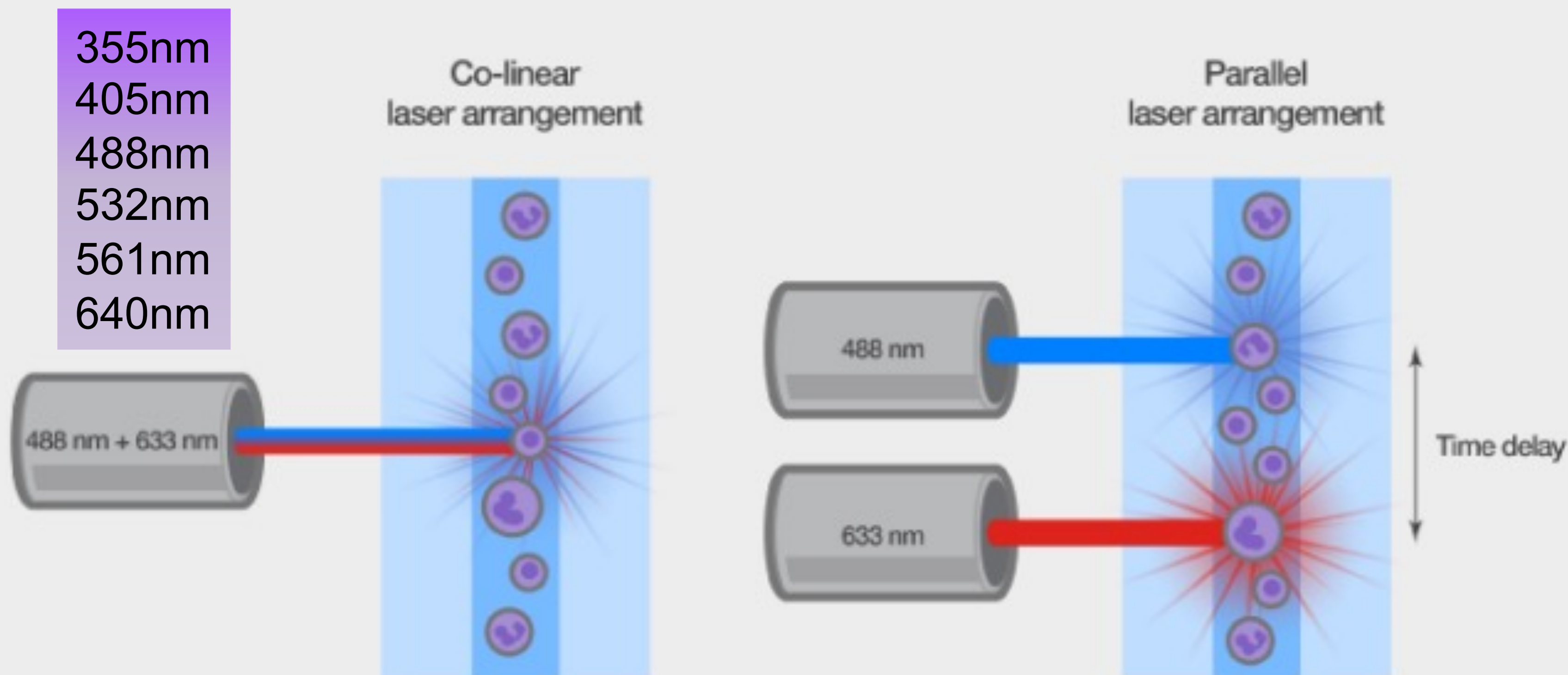
Light source: 1-6 lasers
very narrow wavelength
(*not everything is excited!*)

Light detection:
photodiode and photomultiplier tubes (PMT)
(*not a camera!*)
(*user adjustable*)



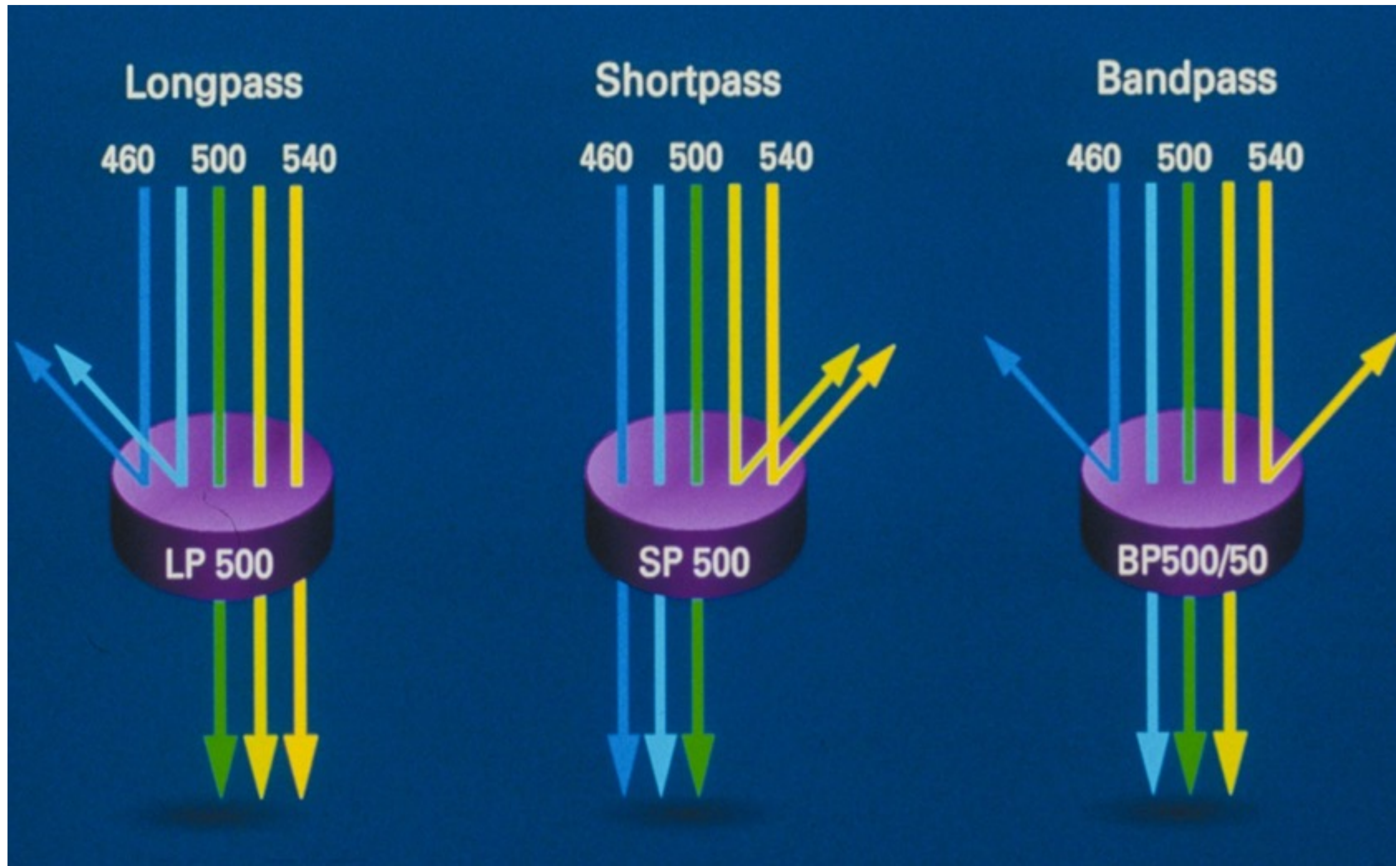
See further Arnold Mixon's presentation!

Temporal and Spatial Separation of Multiple Lasers



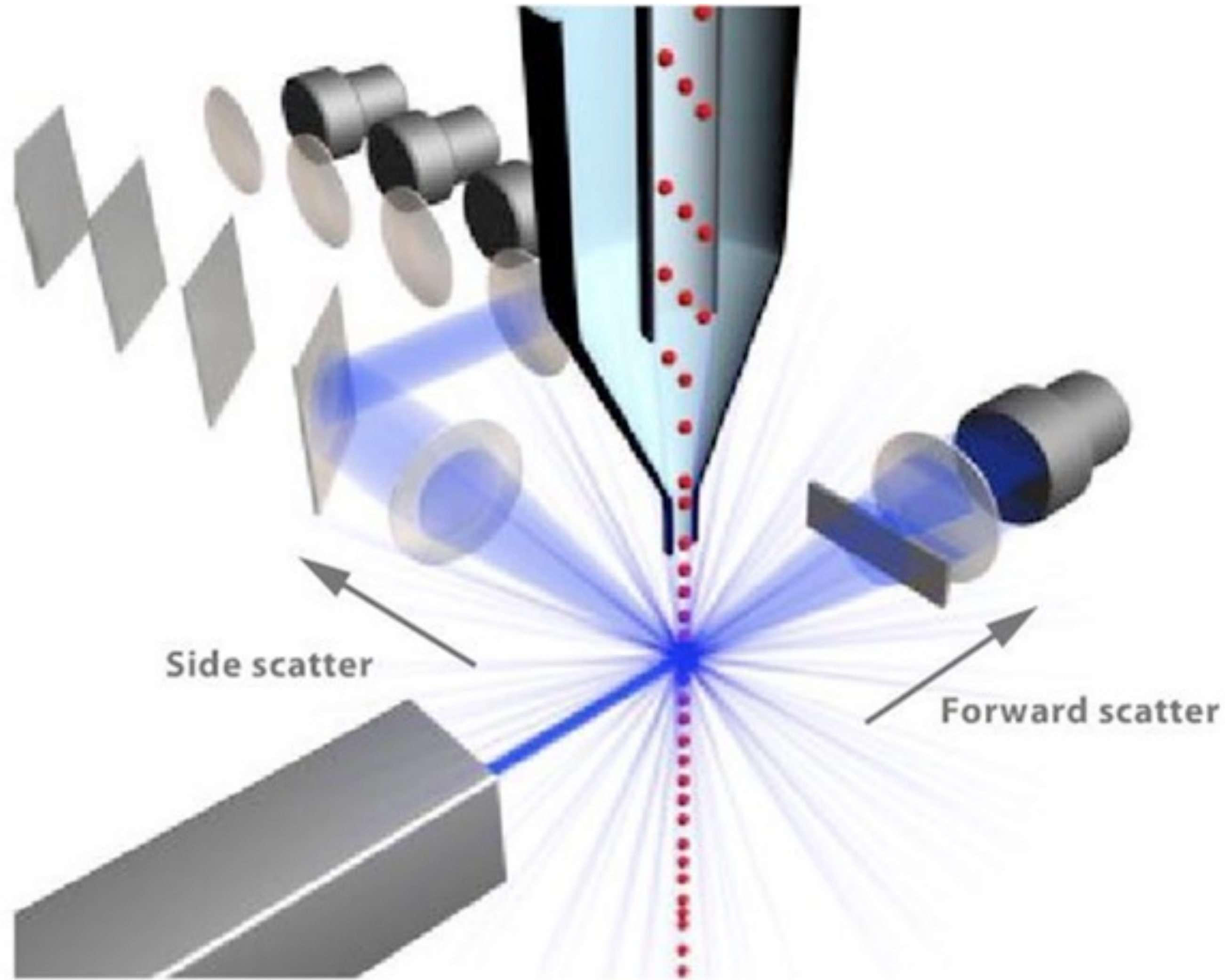
Laser time delay is set during daily QC by the Core staff and normally should not be changed by users!

Types of Optical Filters



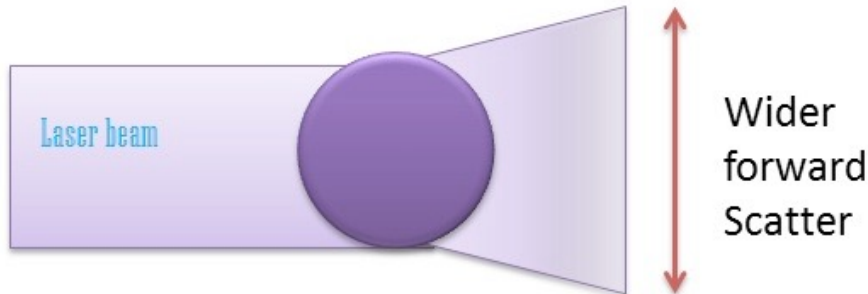
The "color" of the detector ("channel") Is determined by the combination of optical filters in front of the PMT.

Light scatter – independent of fluorescence

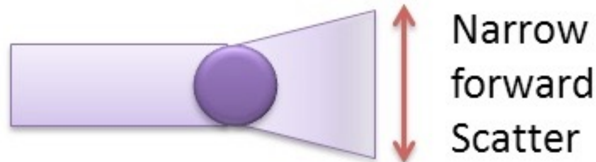


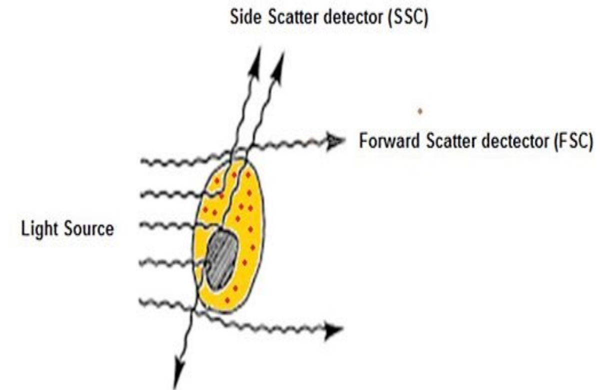
See further Kim Klarmann's presentation !

Forward Light Scatter (FSC)



With larger particle forward scatter increases





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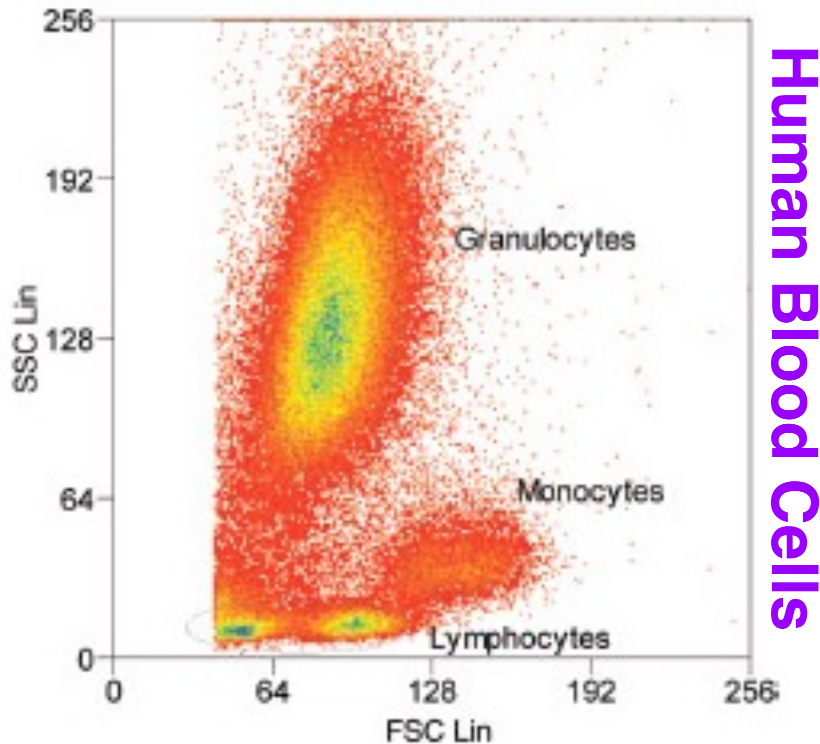
NOTE: FSC is proportional to particle diameter, but also depends on optical reflective properties – allows only relative comparison!

FSC and SSC help distinguish cells from debris



- FSC and SCC apply to all particles regardless of fluorescent signals.
- FSC and SSC are typically acquired on *linear* scale (*not for bacteria*).
- FSC/SSC plots help distinguish intact cells from debris.
- FSC/SSC may help identify and discriminate certain cell types.
- ***Always start with FSC/SSC plot for both acquisition and analysis!***

See further Kim Klarmann's and Cherry Li's presentations!

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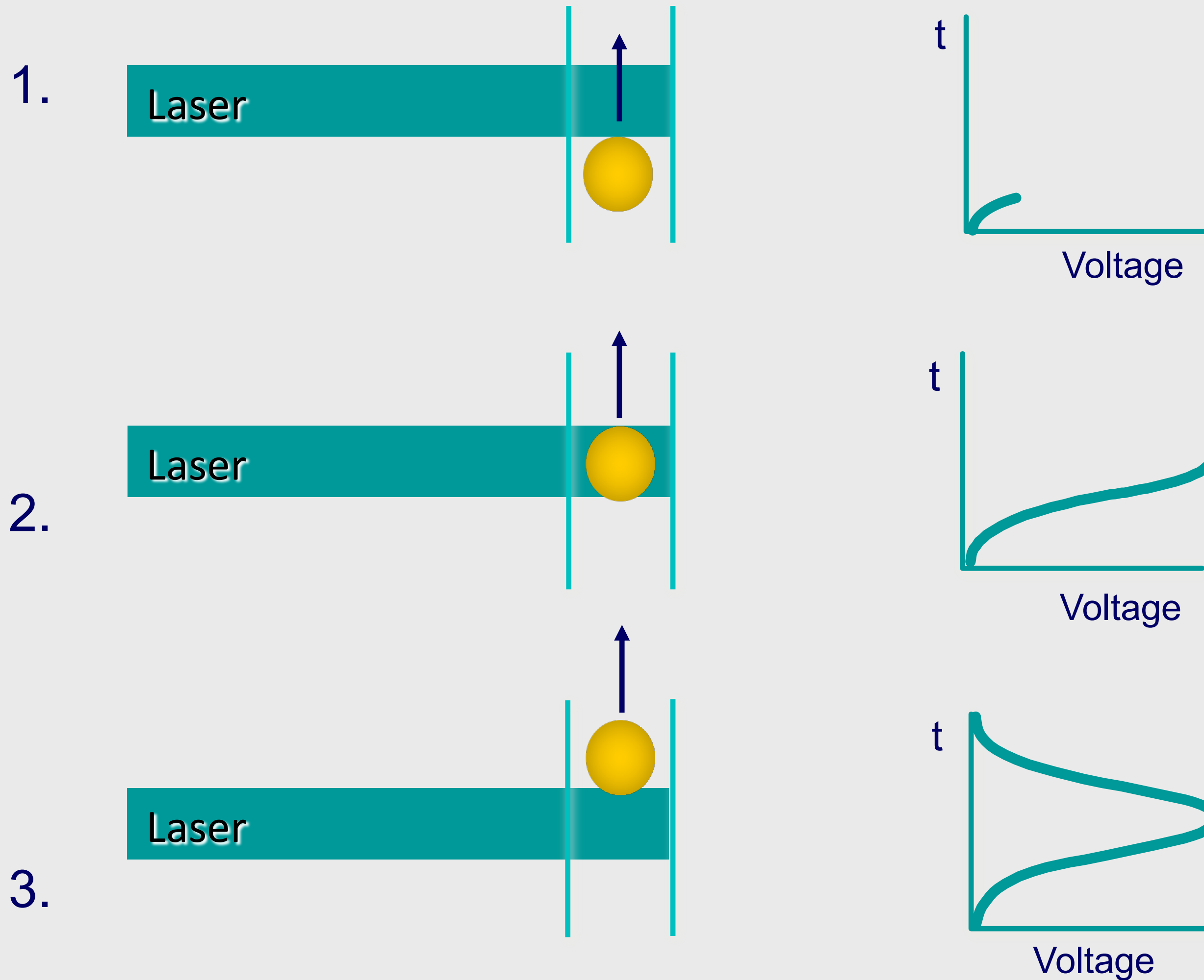
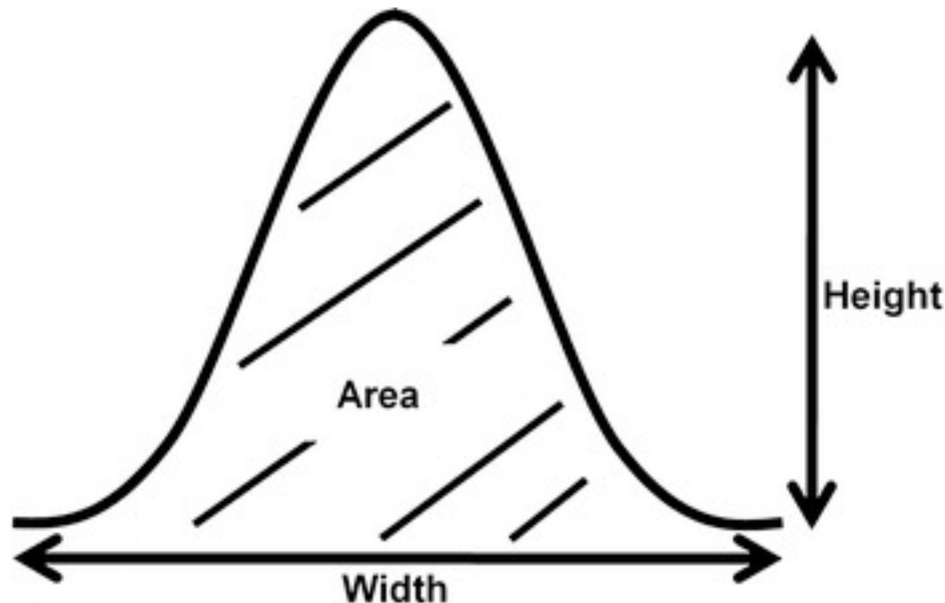
Electronics: Voltage Pulse



Three qualifiers for every parameter

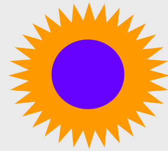


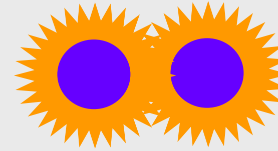
H = Height
W = Width
A = Area

Area is the default qualifier but the other aspects are also useful. Signal threshold is used to exclude electronic noise or debris.

See further Sue Sharrow'spresentation !

Pulse width is used to identify single events





After FSC-A vs. SSC-A plot always create a W vs. H plot to exclude cell aggregates!

Computing: One Parameter Histogram

Negative

Positive

Count

Dimmer

Brighter

6

4

1

1 2 3 4 6 7 8

150 160170 …190 192 **Channel Number**

Fluorescence detected on the FITC-A channel

NOTE: Every particle has some autofluorescence!

Hector Nolla

See further Cherry Li's presentation !

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Computing: Two Parameter Scatter Plot

Single PE+ Population

Double Positive Population

PE -FL2-A

Negative Population

FITC-FL-A

Single FITC+ Population

NOTE: Two dimensions increase the resolution of populations

Hector Nolla

See further Cherry Li's presentation !

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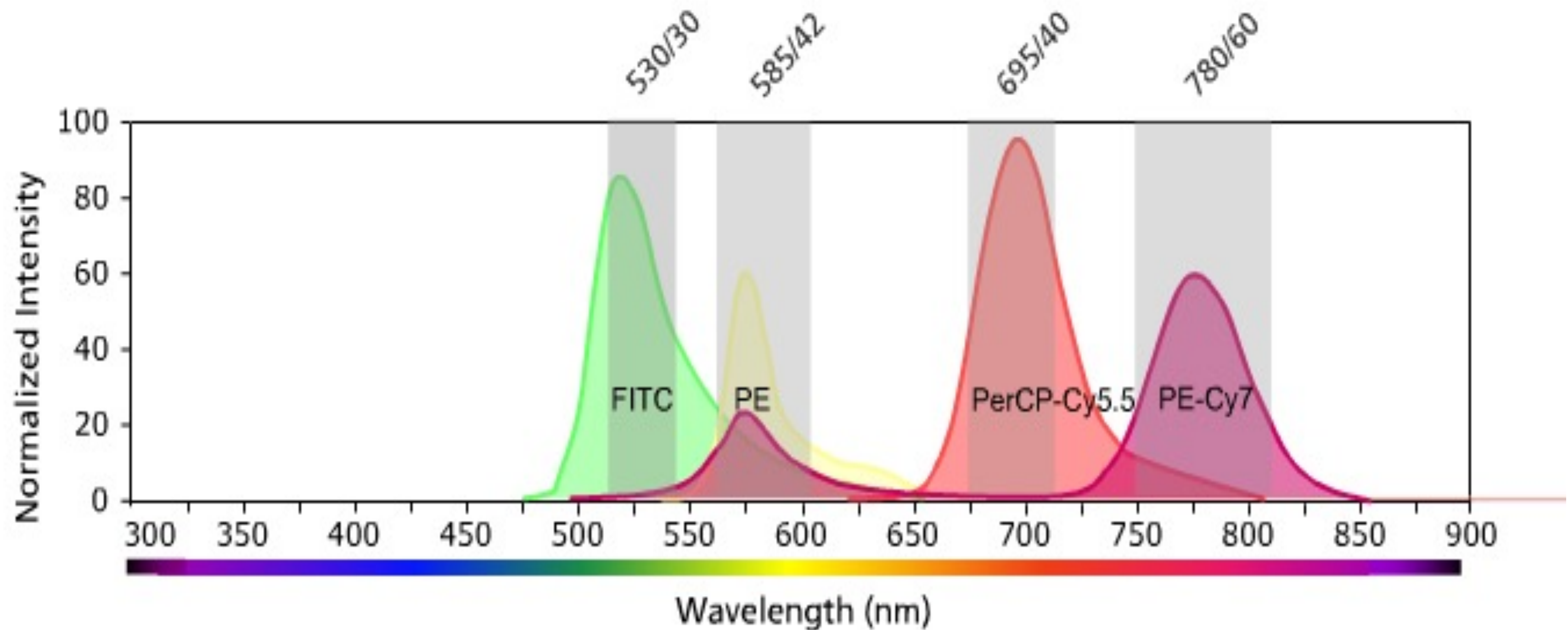
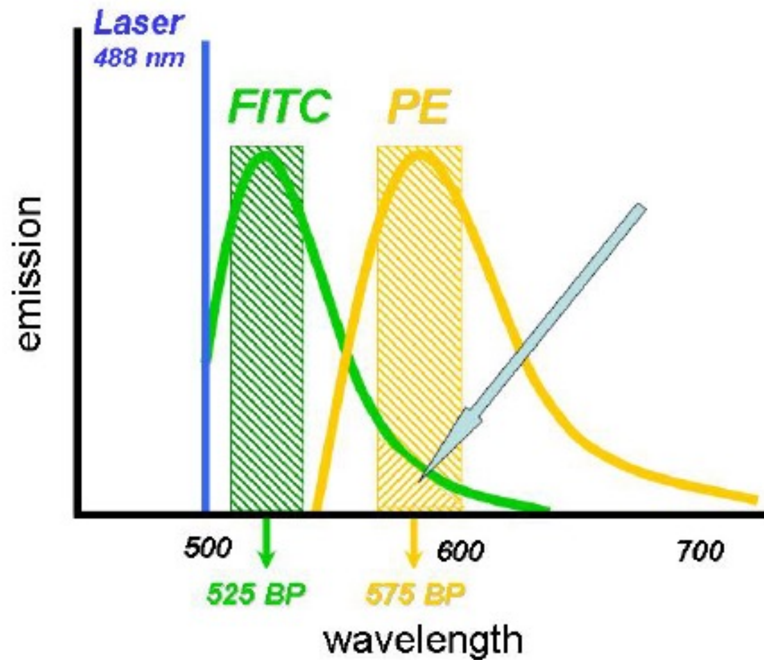
The Emission Spectra of Different Fluorochromes can Significantly Overlap



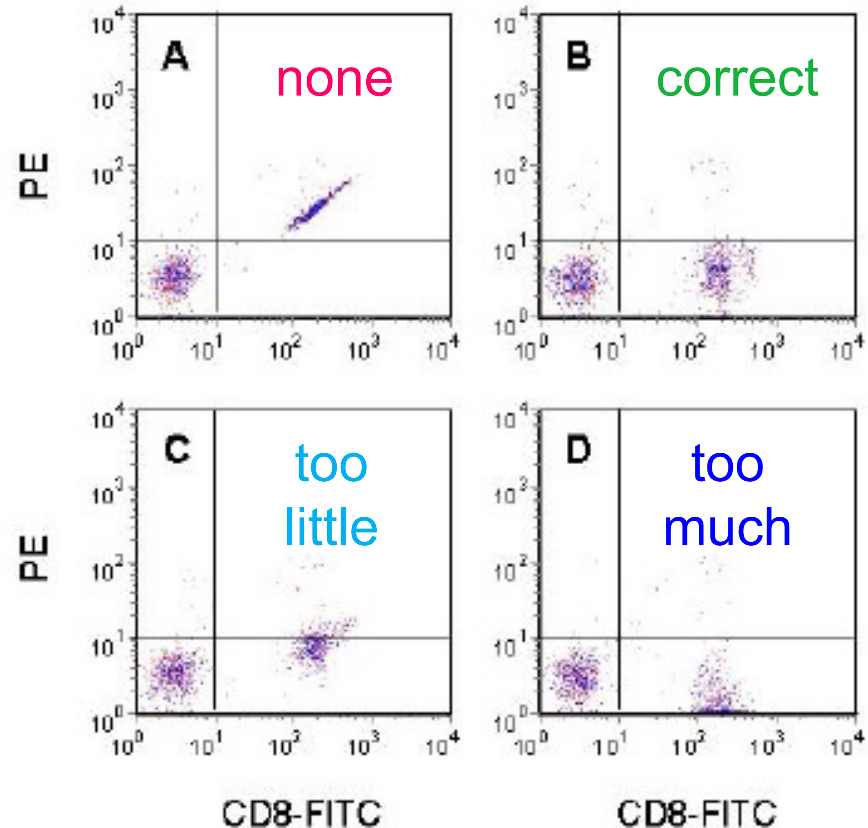
See further Sue Sharrow's, Bill Telford's, Kathy McKinnon's presentations!

Spectral Overlap Needs to be Corrected



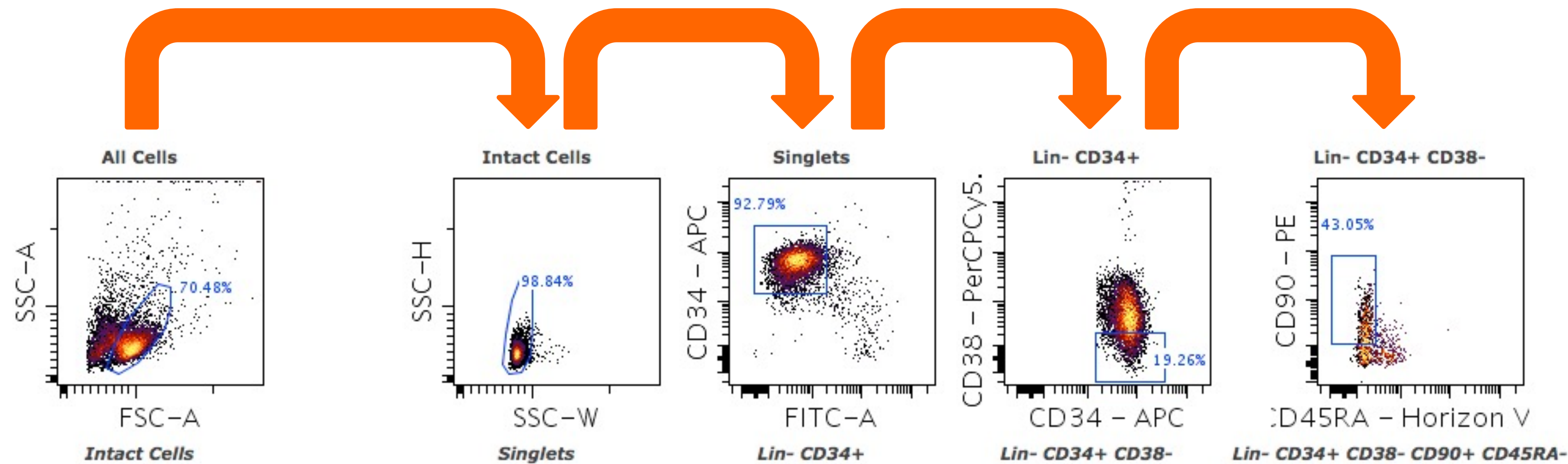
Spectral overlap between two fluorochromes (FITC and PE)

Single FITC-stained sample



Electronic compensation corrects spectral "spill over"

Analysis: Hierarchical Gating Allows Systematic Analysis of Cellular Subpopulations



NOTE: The flow cytometer "sees" only particles. It is the operator, who will establish what cells, singlets, positives and negatives are by setting the gates!

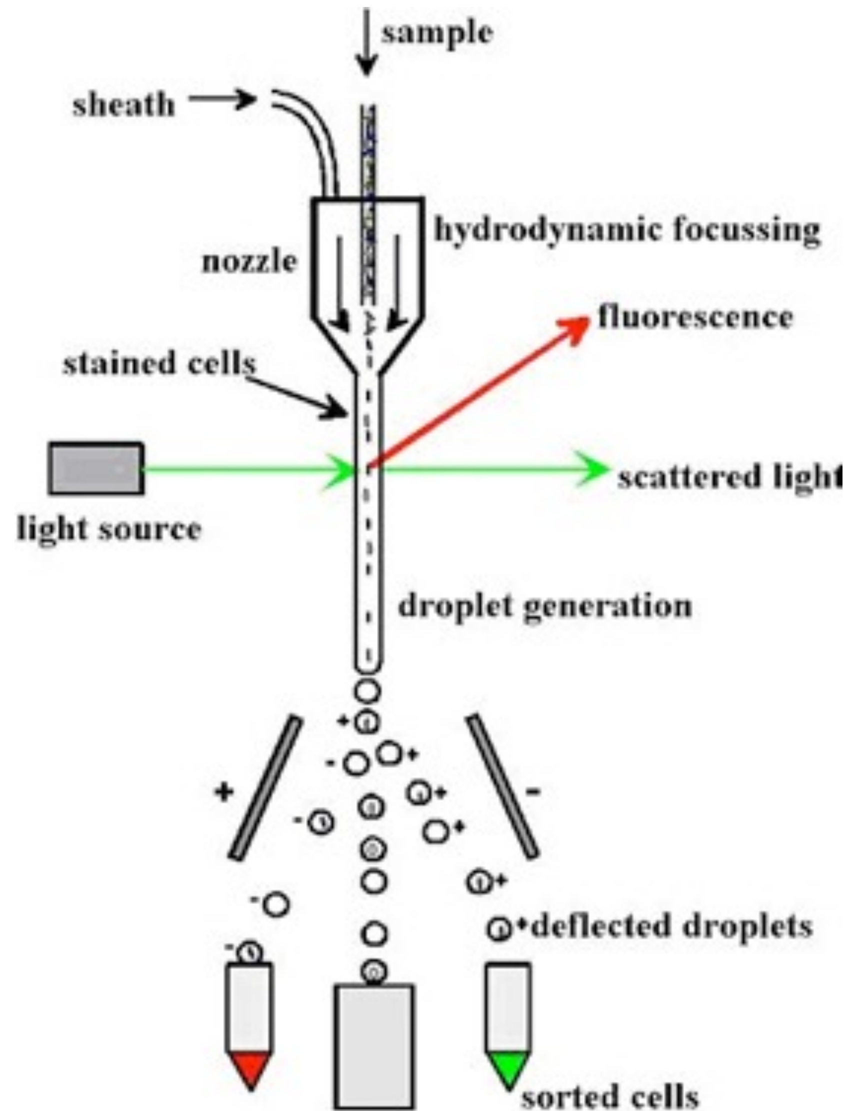
See further Cherry Li's presentation!

Applications of Flow Cytometry

- ***Immunophenotyping*** - characterization of cells with fluorescent labeled-antibodies reactive with surface or intracellular antigens, fluorescent proteins.
- ***Cell cycle analysis*** - Determination of G0-G1-S-G2-M phases of the cycle.
- ***Cell proliferation and tracking*** - Measuring the proliferation history of cells based on fluorescent dye dilution. Tracking fluorescent-labeled cells.
- ***Apoptosis*** - Determination of early and late stages of programmed cell death.
- ***Metabolic reactions*** - Measurement of oxidative burst, accumulation of free radicals, pH changes or mitochondrial function.
- ***Signal transduction*** - Measurement of intracellular ion concentrations (Ca-flux) and phosphorylated protein levels.
- ***Small particle analysis*** – Extracellular vesicles (EV), microparticles (MP).
- ***RNA detection*** – Flow cytometry detection of *in situ* RNA hybridization.
- ***Flow Cytometry Cell Sorting*** - the application of flow cytometry to separate and *collect* cells exhibiting a set of optical/fluorescent characteristics.

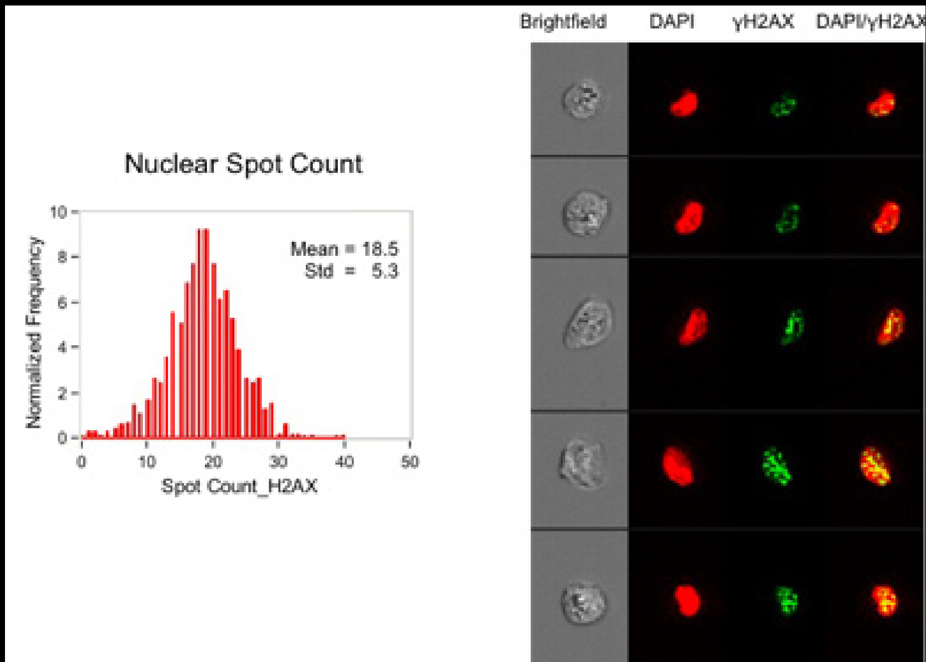
See further Kim Klarmann's presentation !

Flow Cytometry Cell Sorting

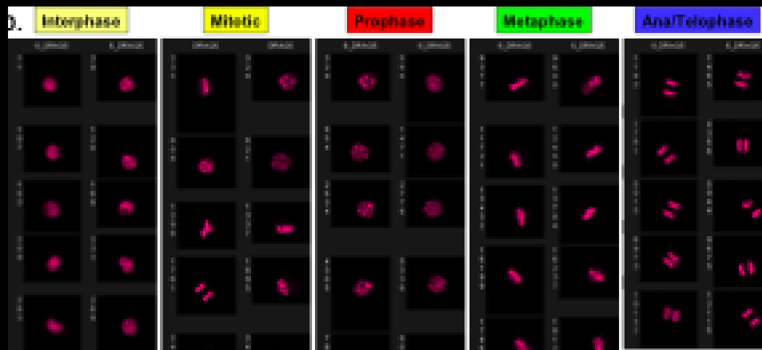


See further Karen Wolcott's presentation !

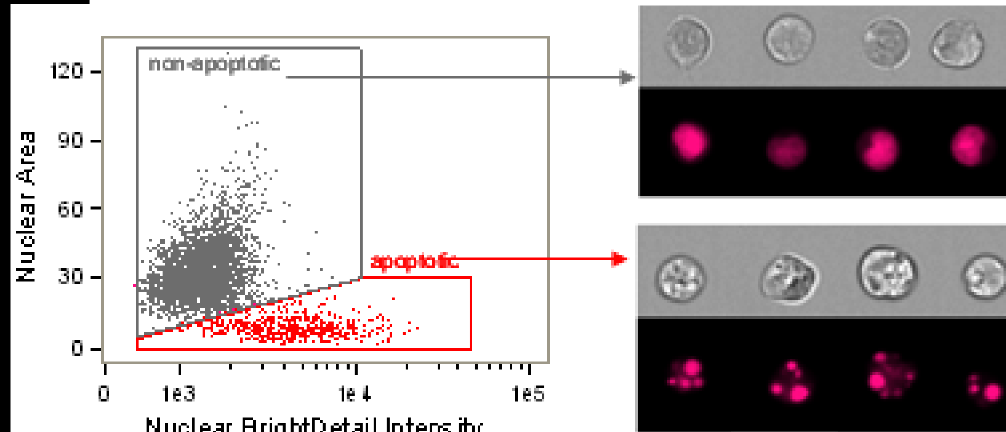
The true automated microscope - ImageStream®



Visualization and quantitation of γ-H2AX foci



Visualization of cell cycle stages



Demonstration of apoptotic nuclear fragmentation

amnis®

Flow Cytometry Technologies at NCI Flow Services

Flow Service	Location	Analysis standard	Analysis high end	Sorting standard	Sorting high end	Special technology
CCR LGI	BG 37	3	2	2	2	Spectral flow (Sony) Imaging flow (Amnis) FACSymphony Chromosome sorting
CCR VB	BG41	2	2	1	1	28-color BD FACSymphony
CCR CIP Frederick	BG 560	2	3	1	2	
NCI EIB	BG 10	3	2	1	1	
NCI ETIB	BG 10	5	1	2	1	Laser scanning (iCys) Modular laser flow Radioactive flow
NCI SB	BG 10	3	1	1	1	Spectral flow (Sony)

Standard: up to 3-4 lasers and <13-colors
High end: up to 4-5 lasers and >14-colors

For detailed instrument capabilities and configurations see handout!
CCR LGI and NCI ETIB instrument configurations are also available from the NIH CCR FluoroFinder 2.0 website:
https://app.ccr-fc37.fluorofinder.com/www/panels/new