Introduction of CytoFLEX

Beckman Coulter Korea

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BECKMAN COULTER
Life Sciences

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WE HAVE A HISTORY OF INNOVATION IN FLOW CYTOMETRY

This timeline shows the evolution of flow cytometry instrumentation. Since the 1970s, we've been continually reinvesting to bring you the next innovation, the next way to discover things you might have been missing, and the next way to take your research to new heights and smaller diameters.

EPICS TPS
2 Parameter Sorter

EPICS Profile II
Benchtop Cytometer

EPICS Altra
Sorter

FC500
Advanced Cytometer

MoFlo XDP
Cell Sorter, Early Model

Purchased ReaMetrix, the dry reagent technology foundation for DURAClone

AQUIOS CL
Load-and-go Cytometer

Navios EX
3-laser 10-color Clinical Cytometer

Launch of Kaluza for Clinical

CytoFLEX
3-laser 13-color Flow Cytometer

ClearLLab 10C first 10-color IVD system approved by US FDA to aid the detection of leukemia and lymphoma

CytoFLEX LX
6-laser 21-color Flow Cytometer

1970 · · · · **1980** · · · · **1990** · · · · **2000** · · · · **2005** · · · · **2010** · · · · **2015** · · · · **2020**

EPICS II
First Laser Analyzer

EPICS Elite
Sorter

EPICS XL
Fully Automated Clinical Cytometer

Release of IOtest3 cocktails for the diagnosis of L&L

Launch of Kaluza Analysis Software

Navios
10-color Clinical Cytometer

Release of PerFix-nc buffer, first no wash intracellular staining buffer

MoFlo Astrios
Cell Sorter

CytoFLEX S
4-laser 13-color Flow Cytometer

Acquired Cytobank
Machine Learning-Assisted Analysis software

2021

CytoFLEX SRT
Benchtop Sorter

Research (RUO) Products ■ Clinical Products

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BECKMAN COULTER *Life Sciences*

What is Flow Cytometry?

- A technology that ***simultaneously*** measures ***multiple characteristics*** of ***single cells*** at a ***rapid rate***

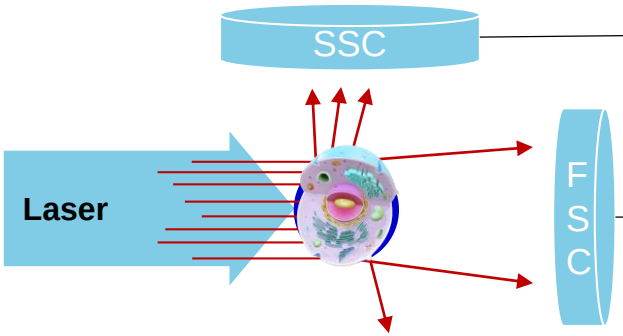
- Its relative size (Forward Scatter—FSC)
- Its relative granularity or internal complexity (Side Scatter—SSC)
- Its relative fluorescence intensity

** sample type

Cells, Bacteria, Plankton, Bead, Particles

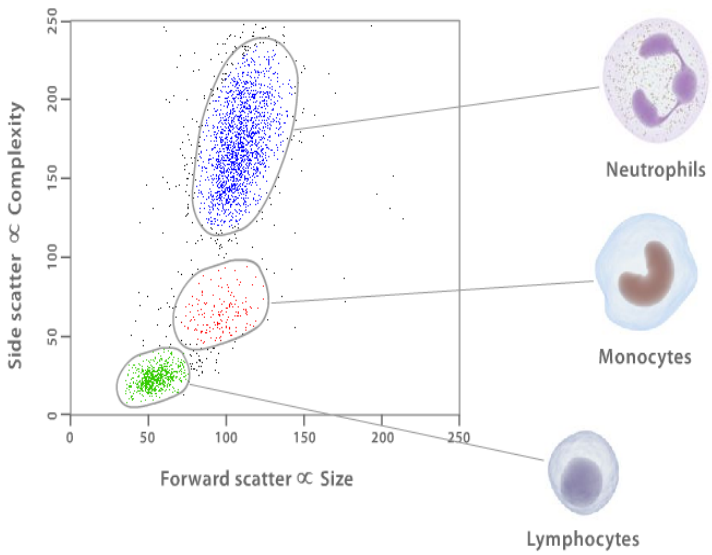
Properties of FSC and SSC

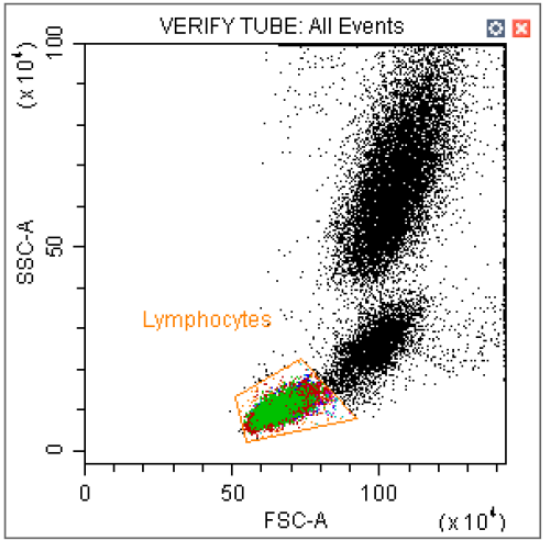
Light scattering



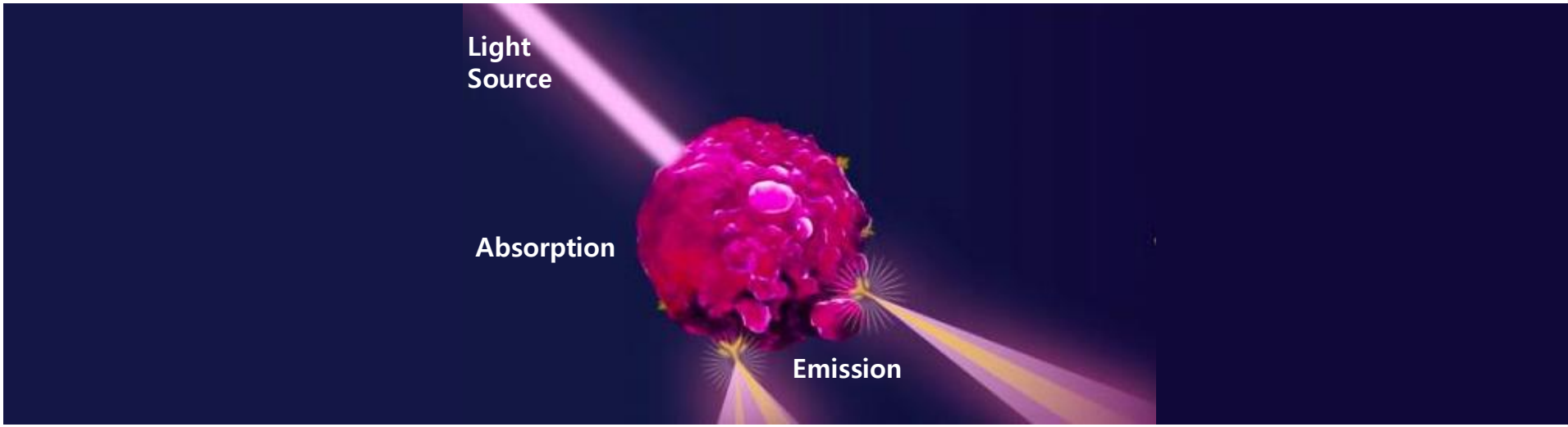
•Side Scatter (SSC) : Granularity or Internal Complexity

•Forward Scatter (FSC) : Size

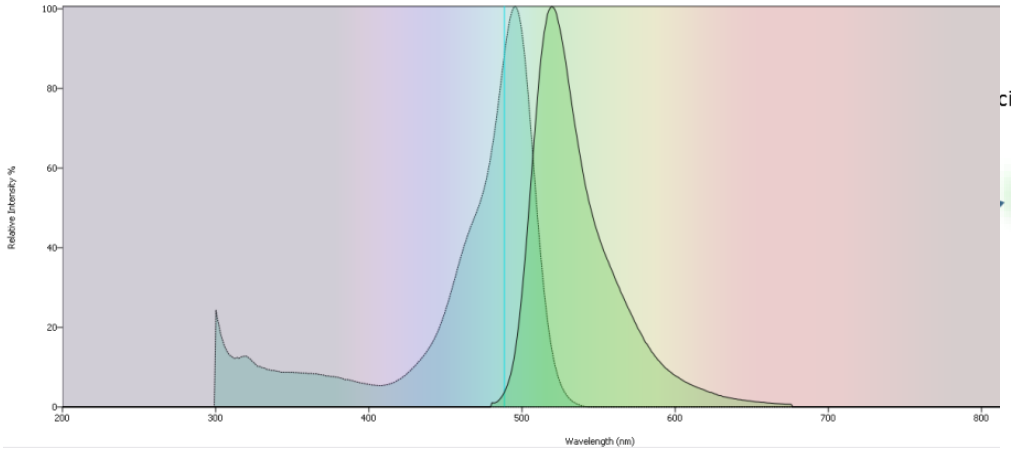




Fluorescence _Excitation and Emission



Laser
(High Energy)



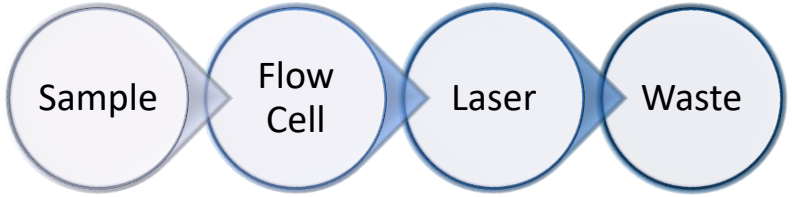
cited State

Fluorescence Light
(Lower Energy)

Subsystem

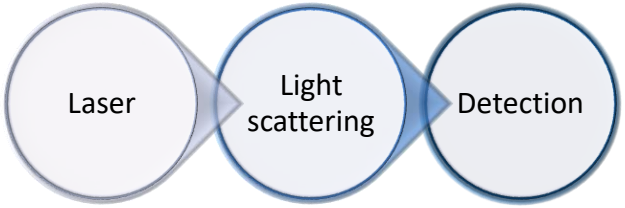
Fluidics

To introduce and focus the cells for interrogation



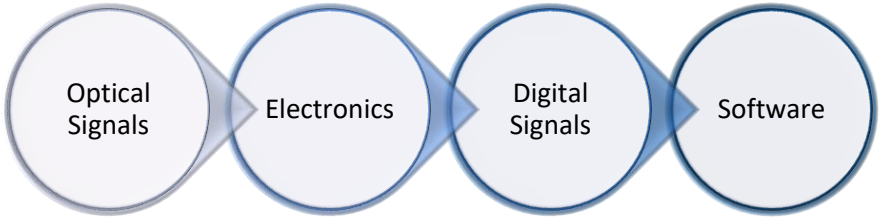
Optics

To generate and collect the light signals

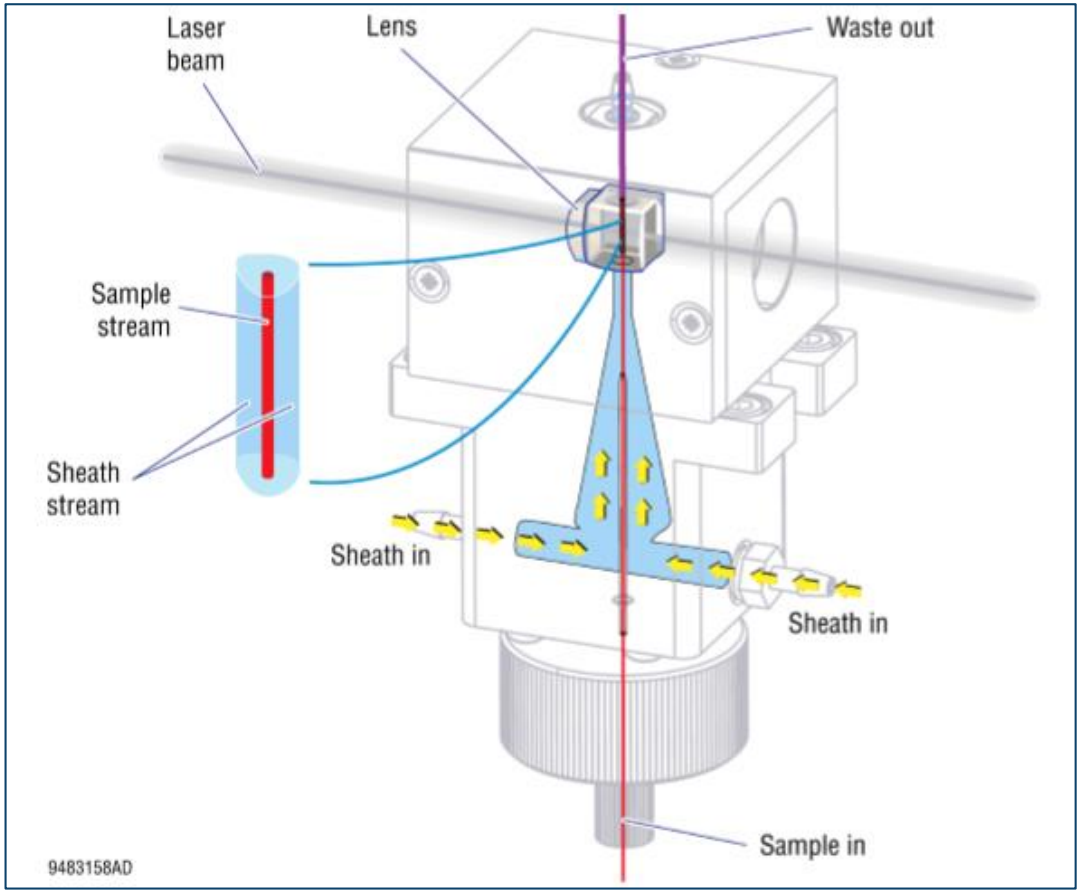


Electronics

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



Fluidics



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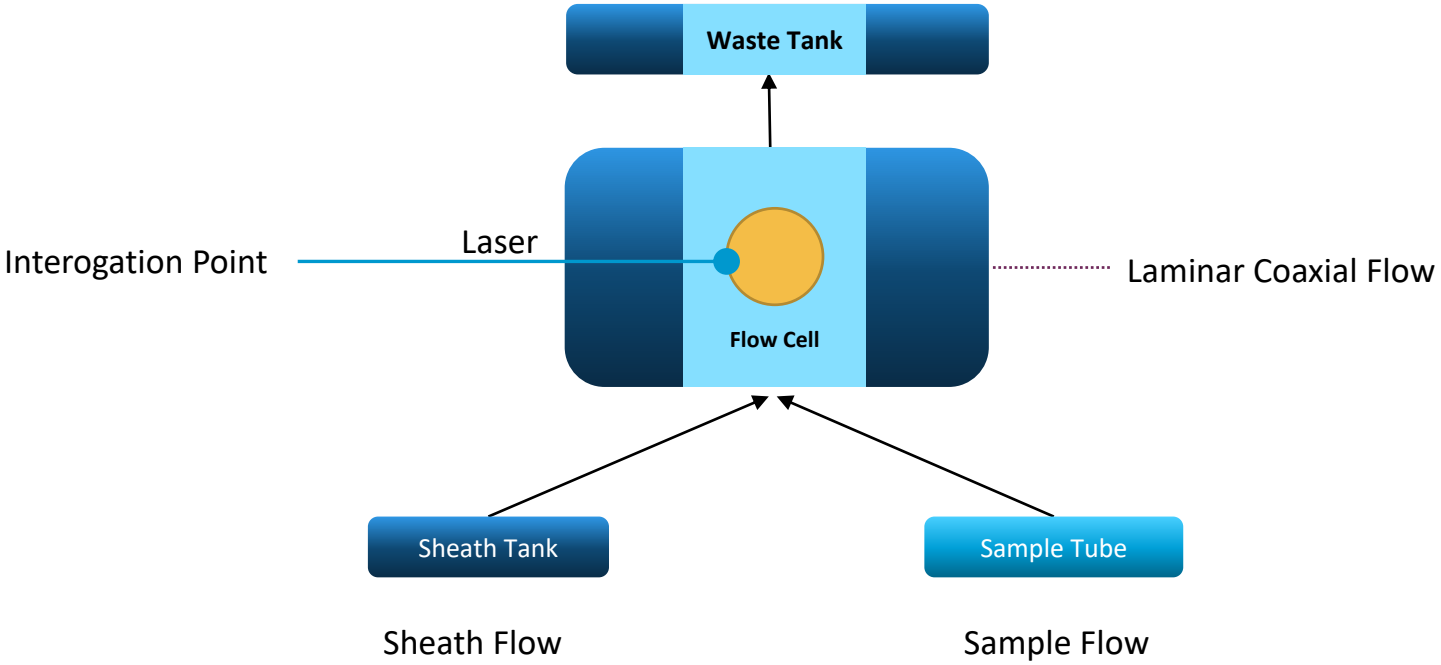

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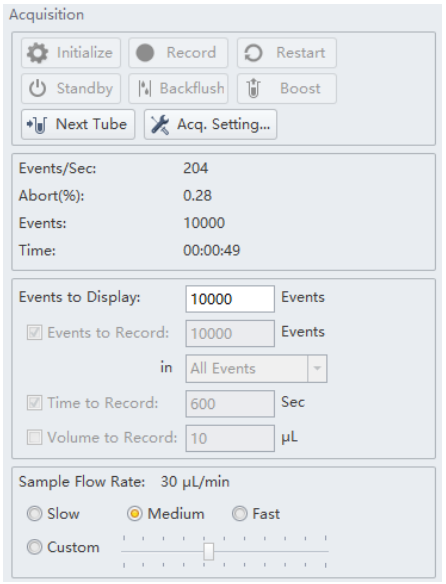
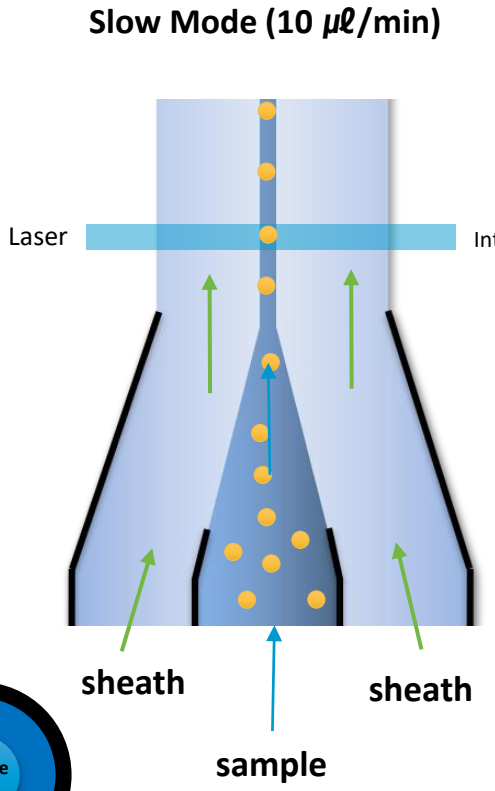
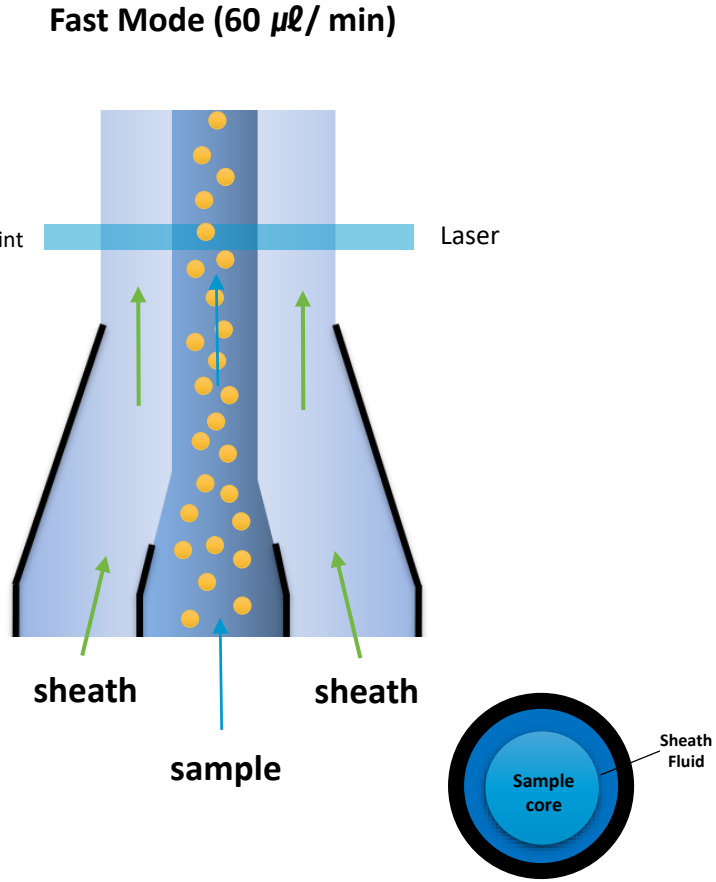


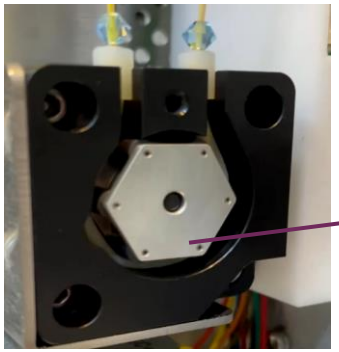


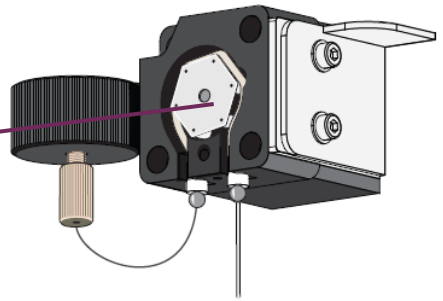
Low sample pressure
Low sample rate



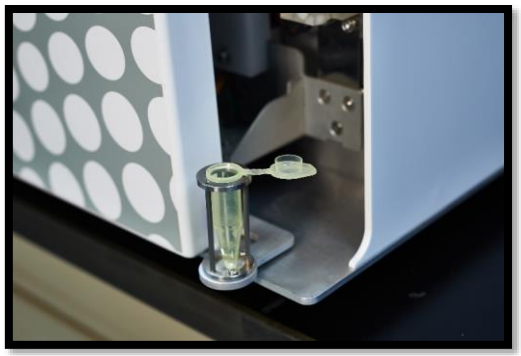
High sample pressure
High sample rate

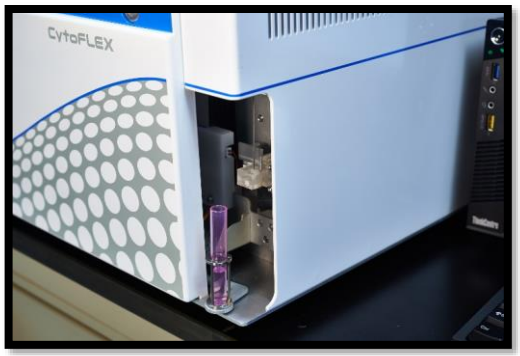
Fluidics - Sample Loader

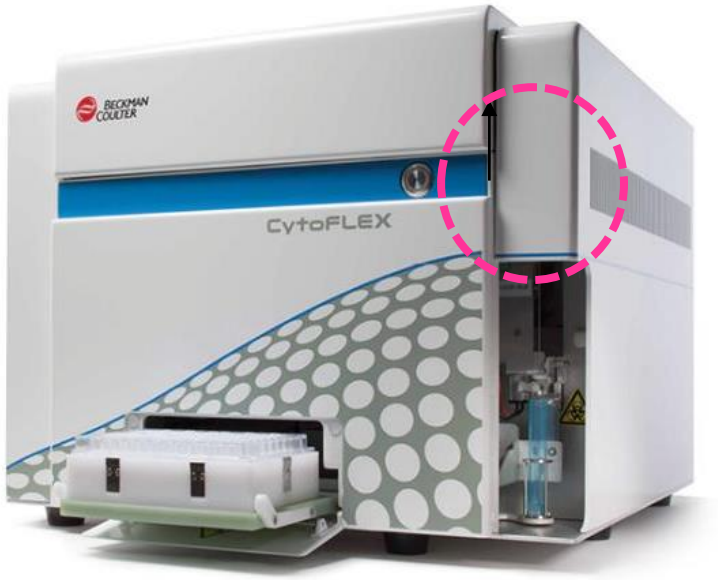




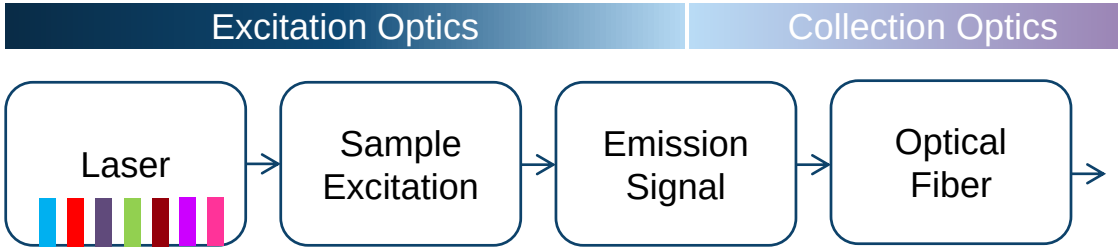
- Accepts 12 x 75 mm and micro-vol. tubes
- Minimum Sample Volume: 10 µL
- 96 well Plate
  - Flat bottom type
  - V-bottom type
  - U-bottom type
  - Deep well type

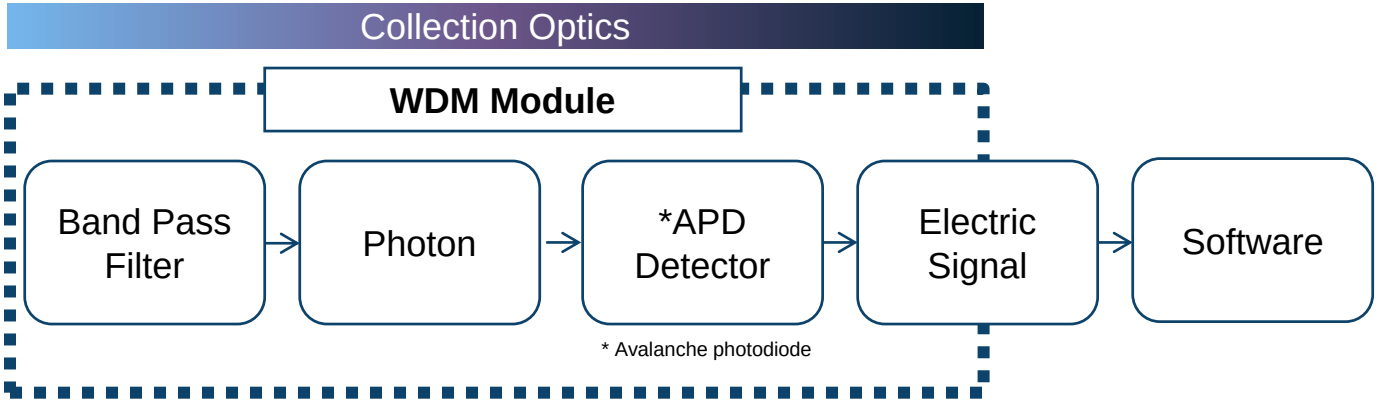






Optics





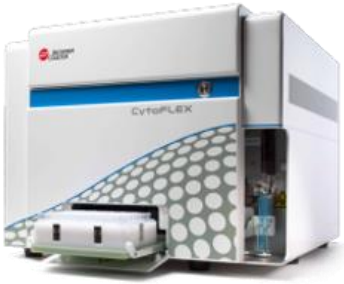
Optics _Excitation

CytoFLEX

3 Lasers

- Blue 488nm
- Red 638nm
- Violet 405nm

CytoFLEX S



4 Lasers

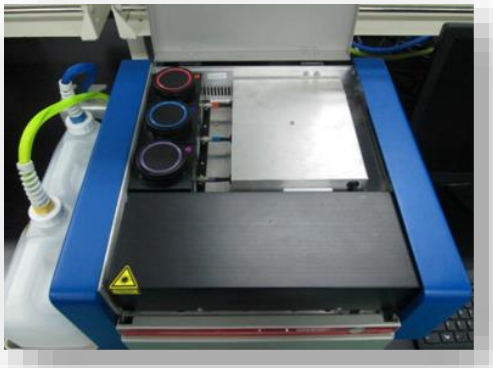
- Blue 488nm
- Red 638nm
- Violet 405nm
- Yellow Green 561nm
- Near UV 375nm
- Infrared 808nm

CytoFLEX LX

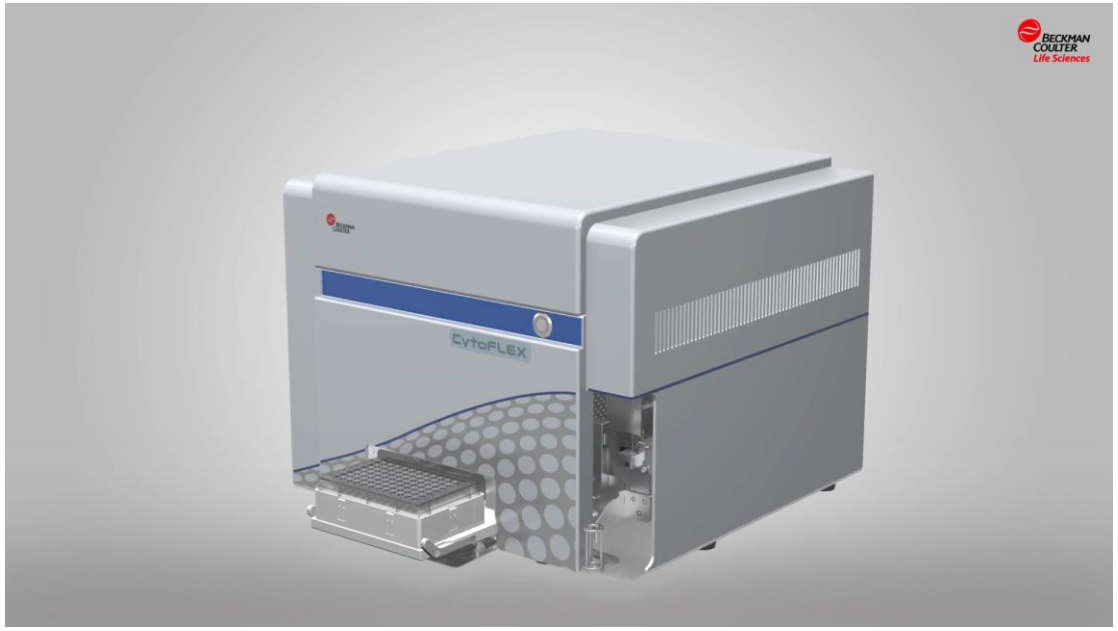
6 Lasers

- Blue 488nm
- Red 638 nm
- Violet 405nm
- Yellow Green 561nm
- Infrared 808nm
- Near UV 375nm
- UV 355nm

Collection Optics

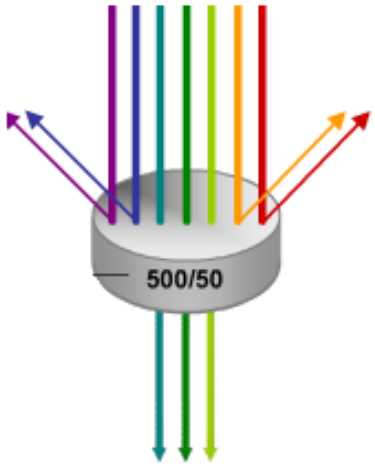


WDM Module

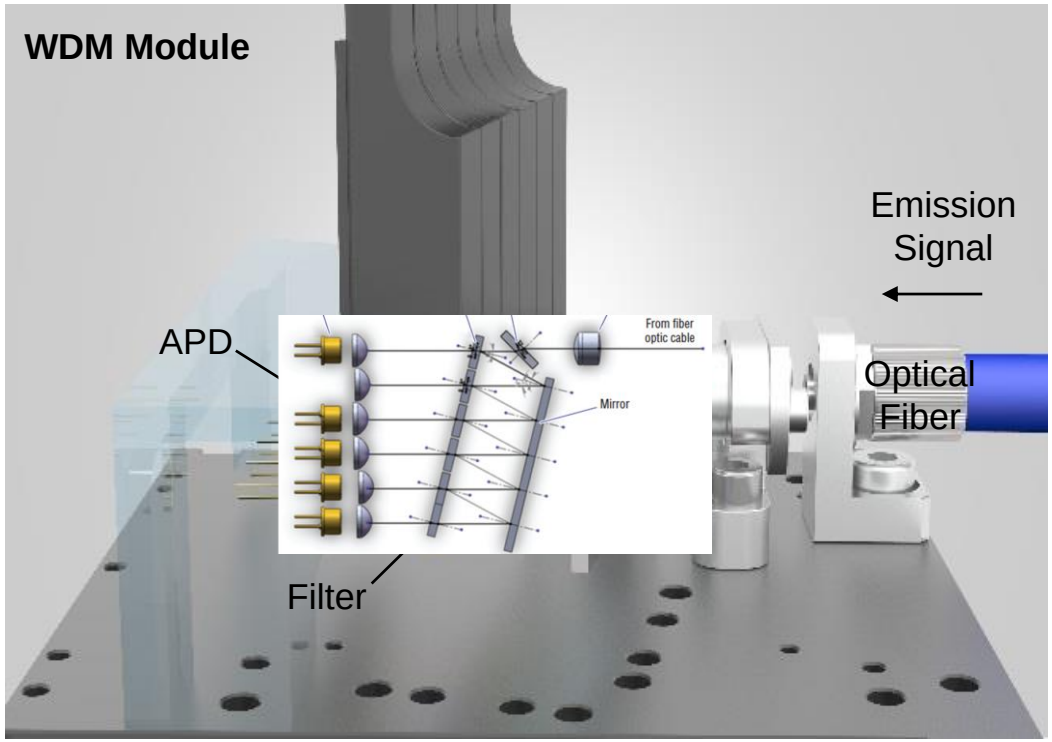


Collection Optics – Bandpass Filter

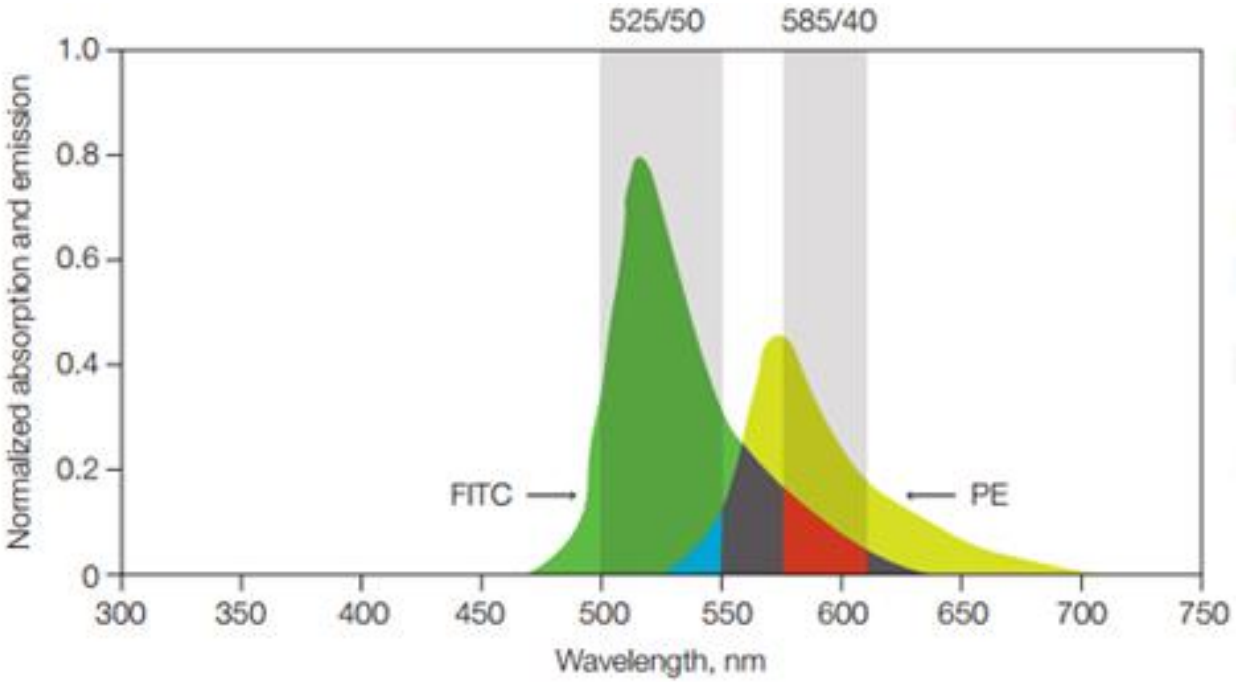
500/50 band pass filter(BP)



475-525nm

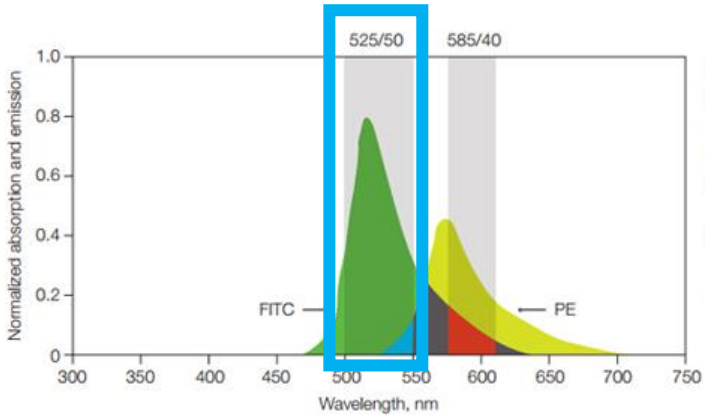


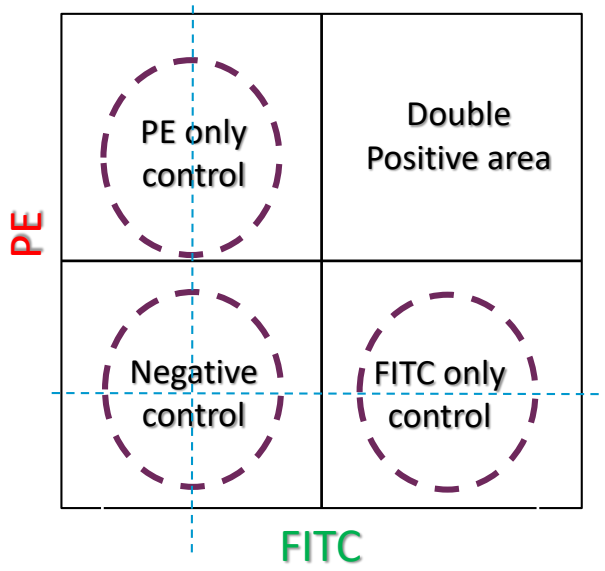
Optics –Spill Over



- Run single color controls for each fluorescent marker in the combination used to stain the sample.
- Compensation can be calculated based on spectral overlap (spillover) of each single color control

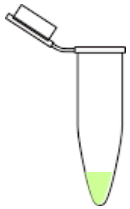
Optics -compensation



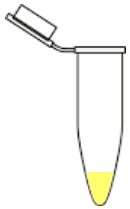


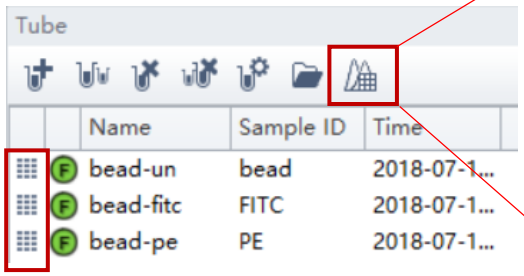
Negative control

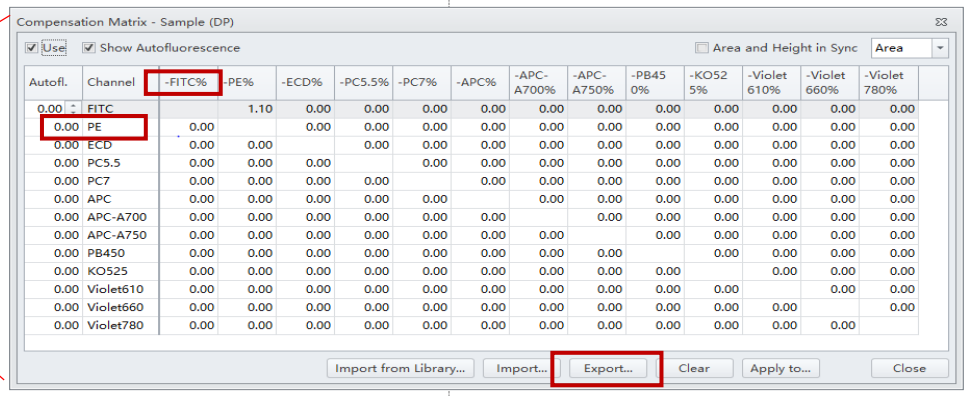
Positive control - FITC



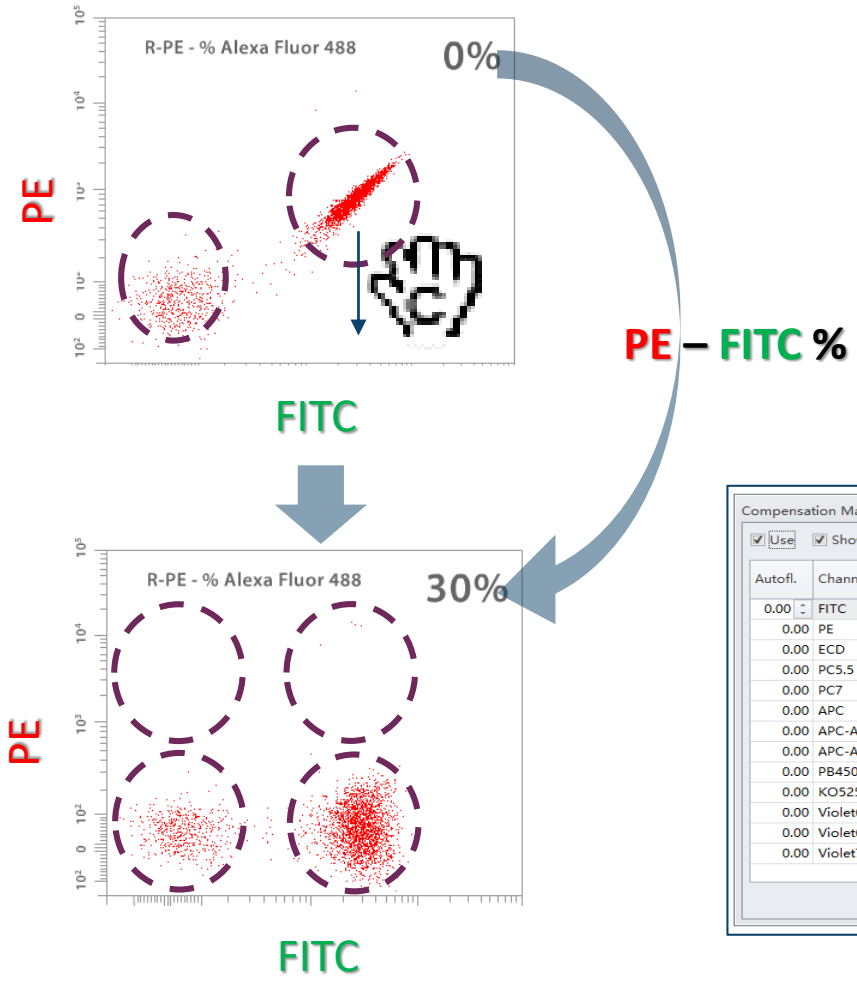
Positive control - PE

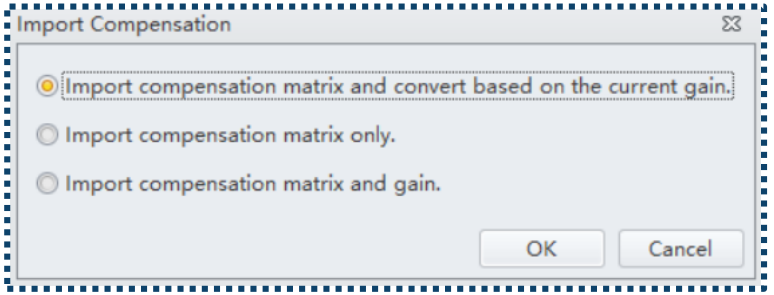






Optics -compensation





Compensation Matrix - Sample (DP)

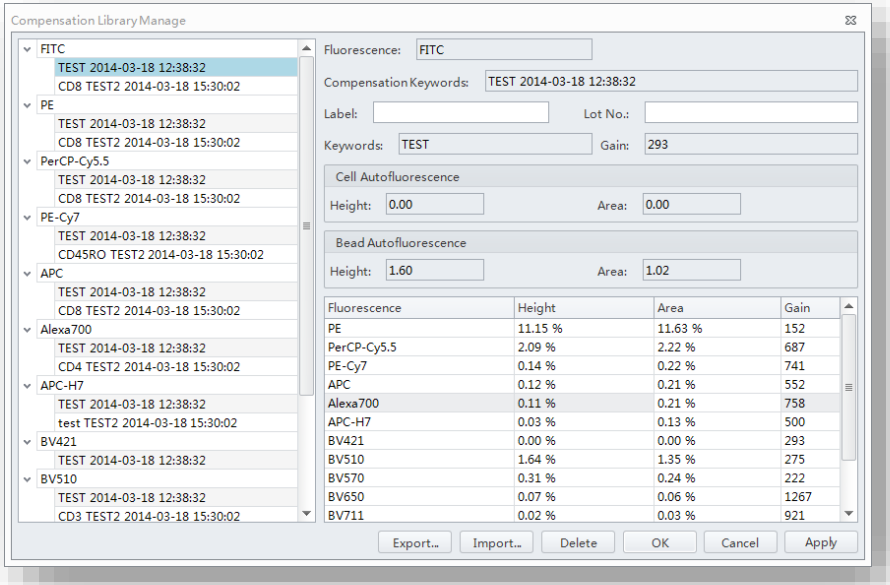
Use | Show Autofluorescence | Area and Height in Sync | Area

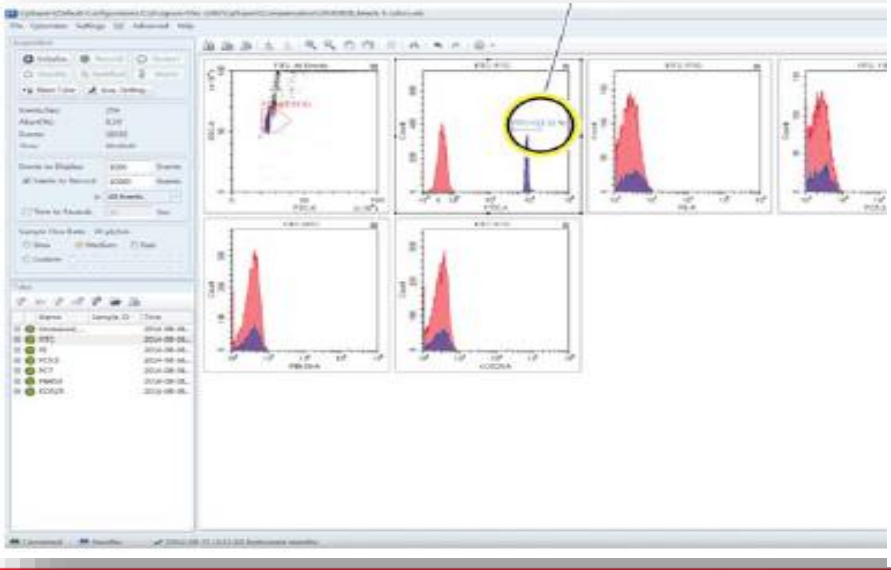
Autofl.	Channel	-FITC%	-PE%	-ECD%	-PC5.5%	-PC7%	-APC%	-APC-A700%	-APC-A750%	-PB450%	-KO525%	-Violet610%	-Violet660%	-Violet780%
0.00	FITC		1.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PE	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	ECD	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PC5.5	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PC7	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC-A700	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC-A750	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
0.00	PB450	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00
0.00	KO525	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00
0.00	Violet610	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00
0.00	Violet660	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00
0.00	Violet780	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

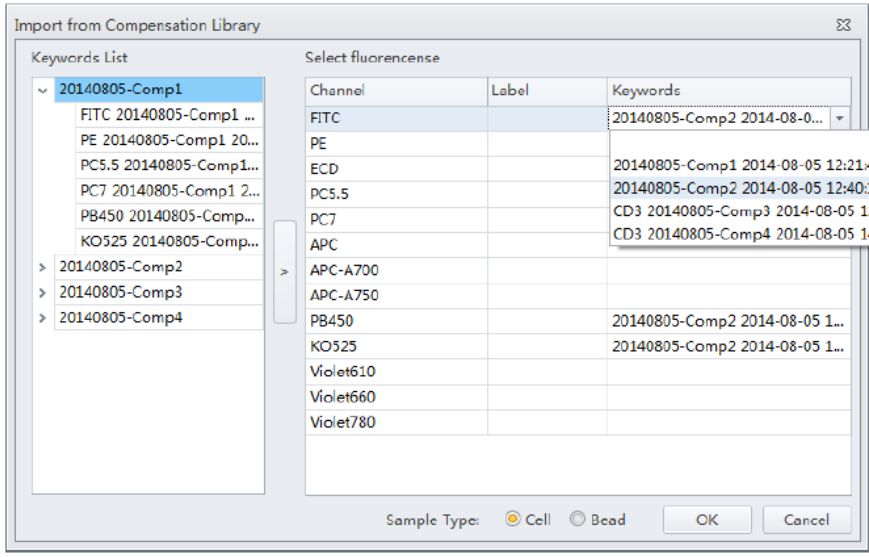
Import from Library... | Import... | Export... | Clear | Apply to... | Close

Auto Compensation

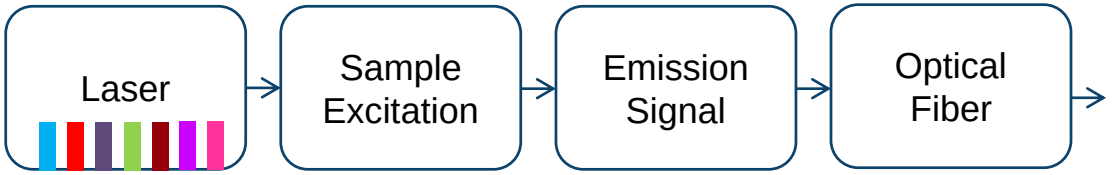
- Automatic and manual
- Compensation Library
  - Store lot-specific dye spillover information
  - Auto adjustment to compensation
  - Absolute Linearity in gain adjustment

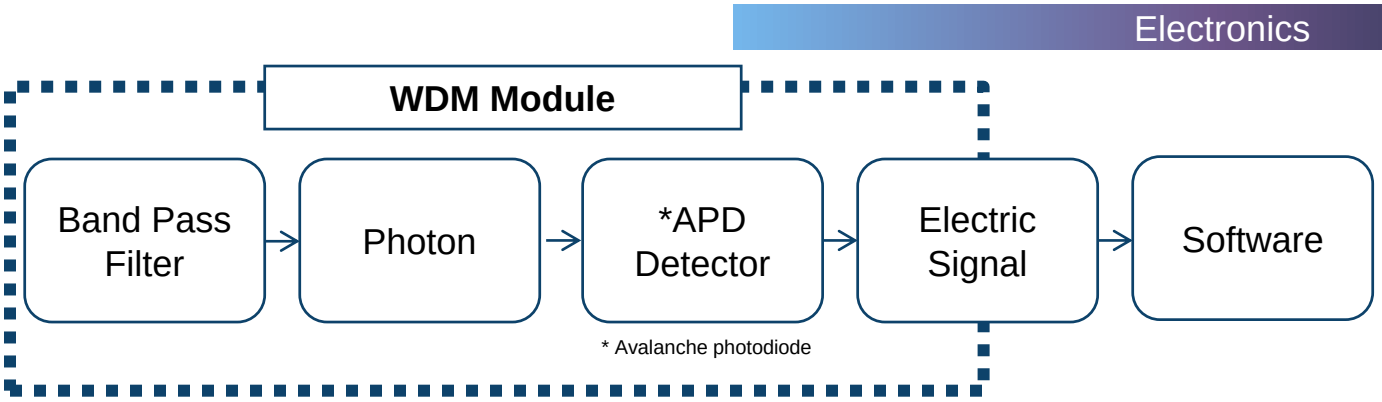




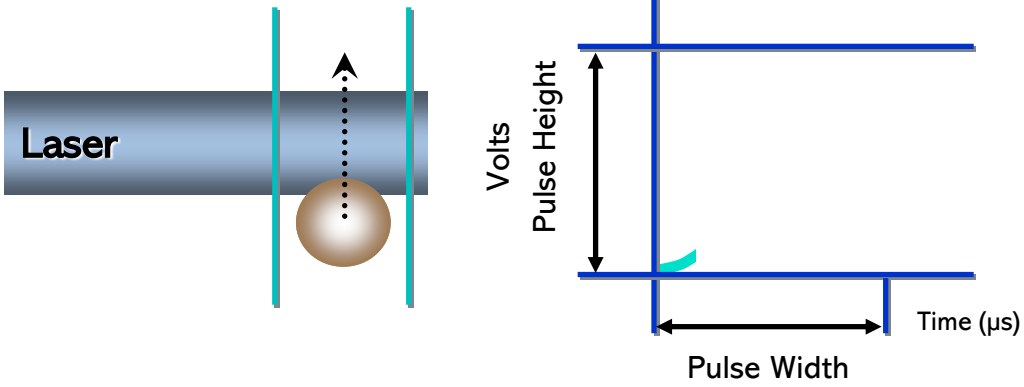


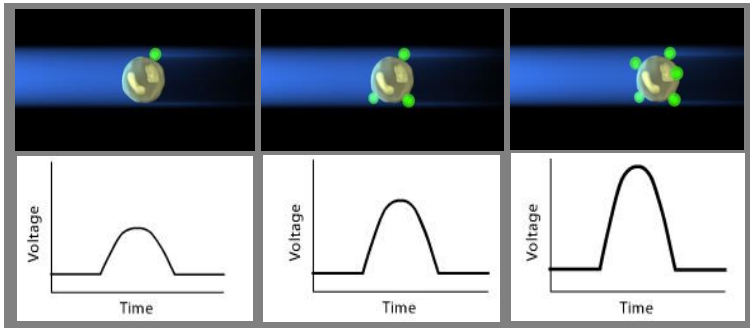
Electronics





Creation of a Voltage Pulse

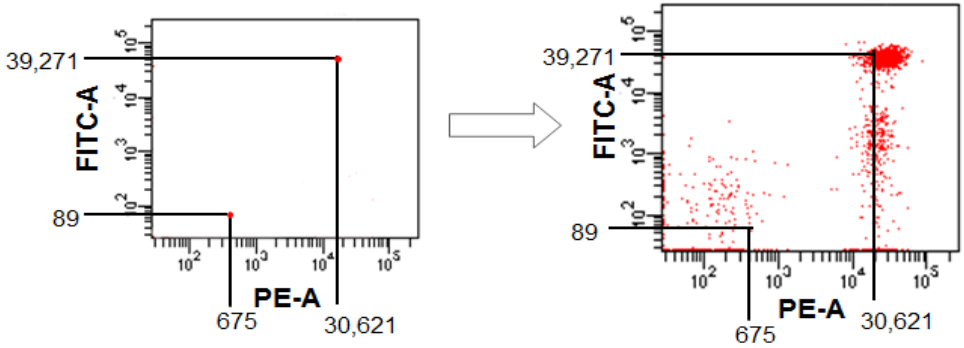




List-Mode Data

	Time	FSC	SSC	FITC	PE
Event 1	0	60	120	89	675
Event 2	10	160	65	39,271	30,621
Event 3	30	650	160	22,688	6,189

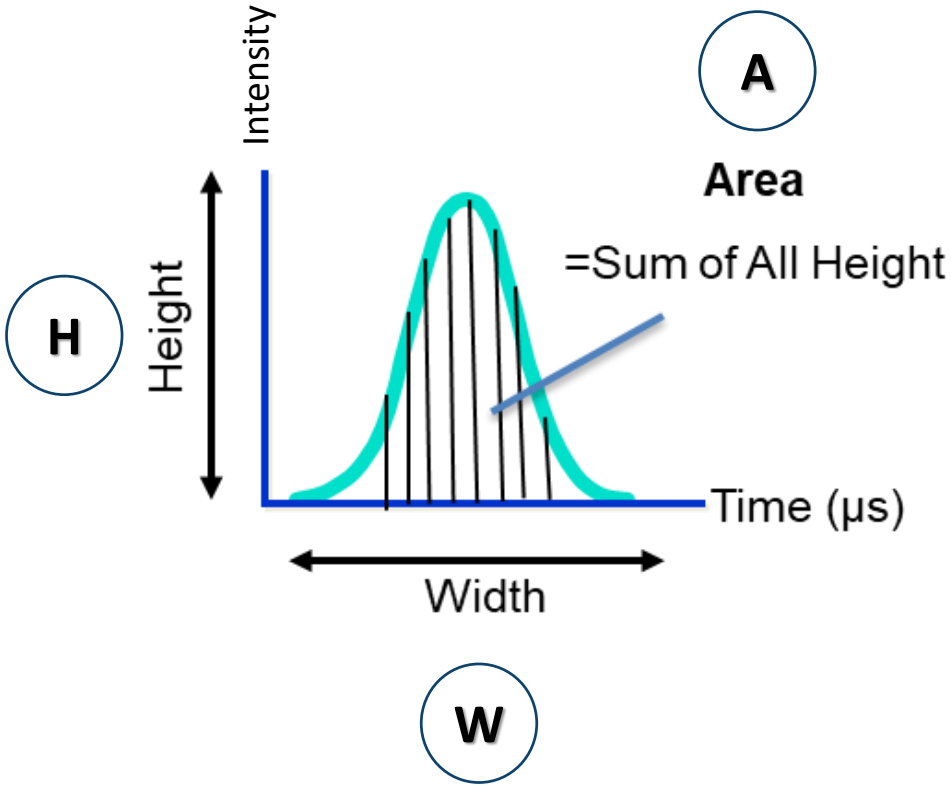
May be exported as FCS file

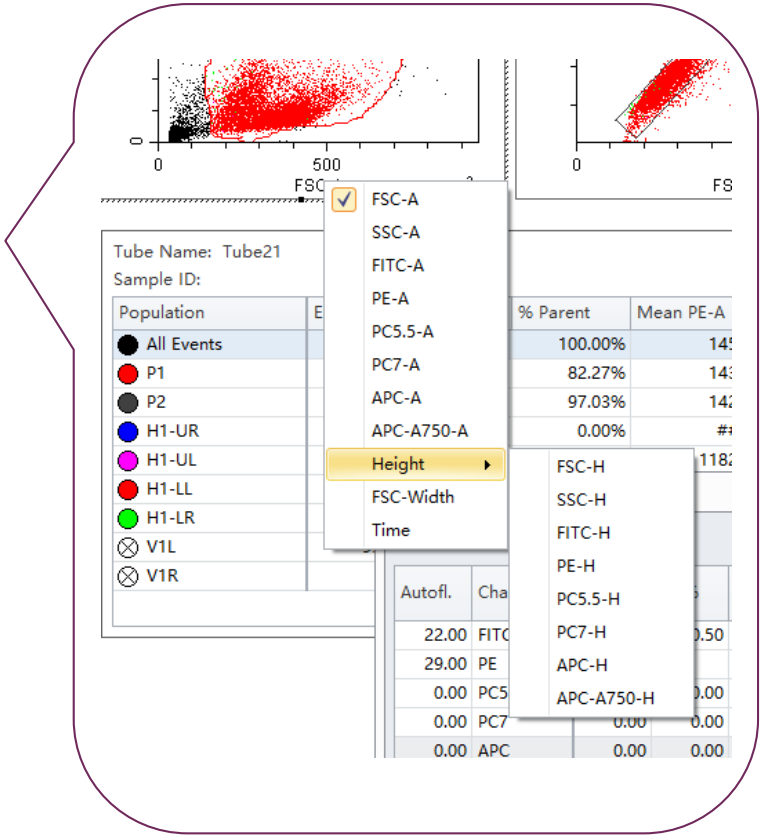


- Height = maximum digitized value
- Area = sum of all height
- Width = Area/Height

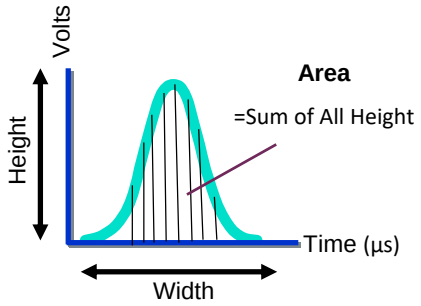
Creation of a Voltage Pulse

Electronics - Area, Height, Width

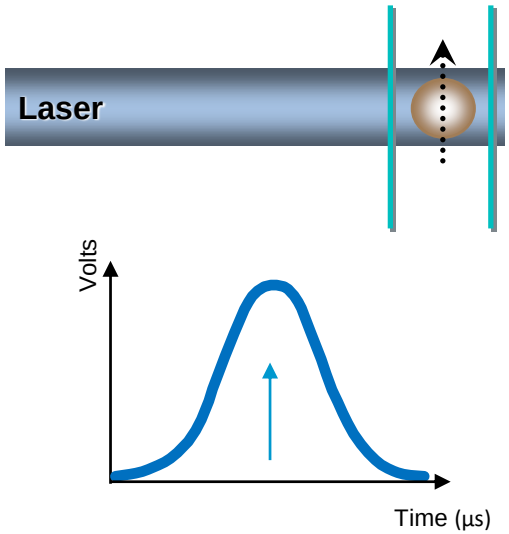


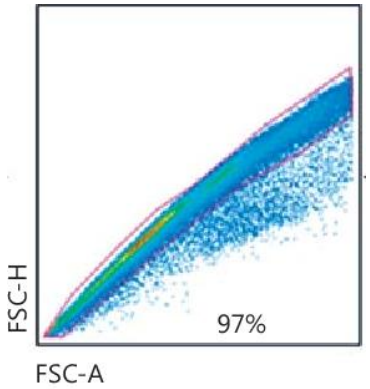


Creation of a Voltage Pulse

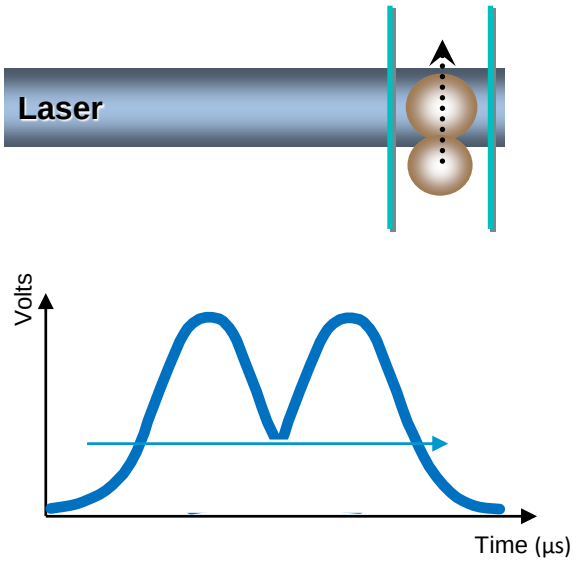


Singlet

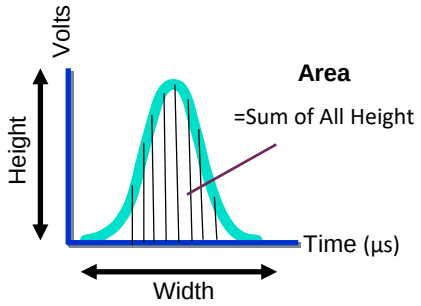


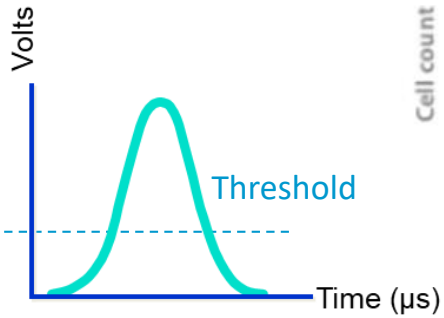


Doublet

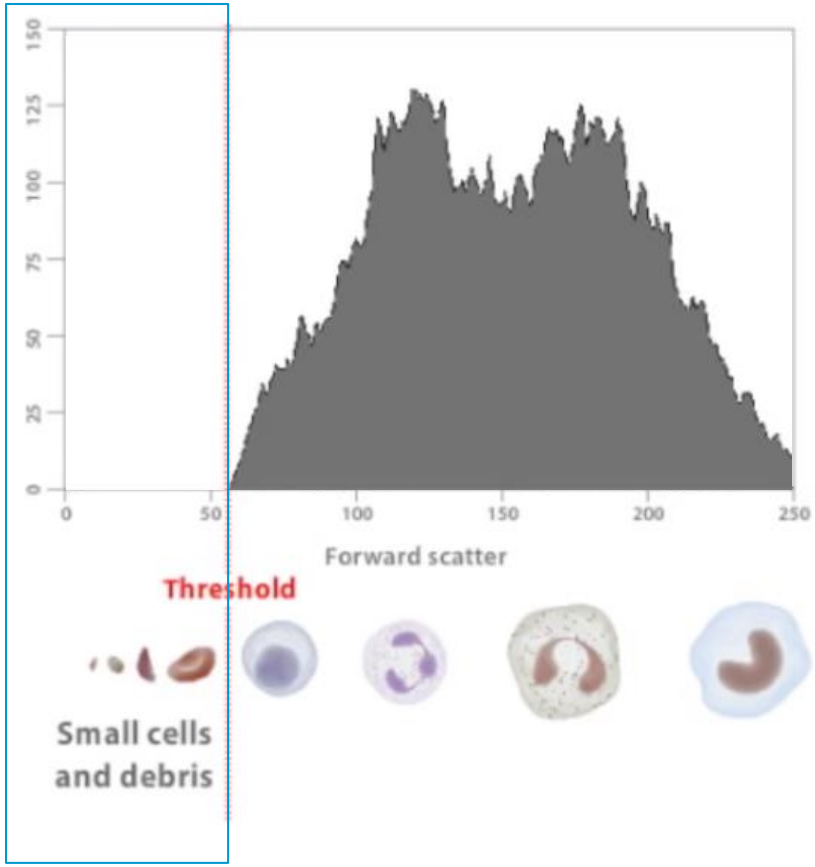


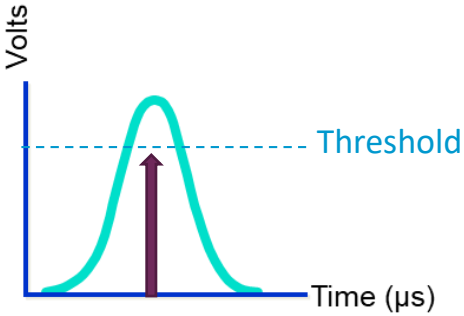
Creation of a Voltage Pulse





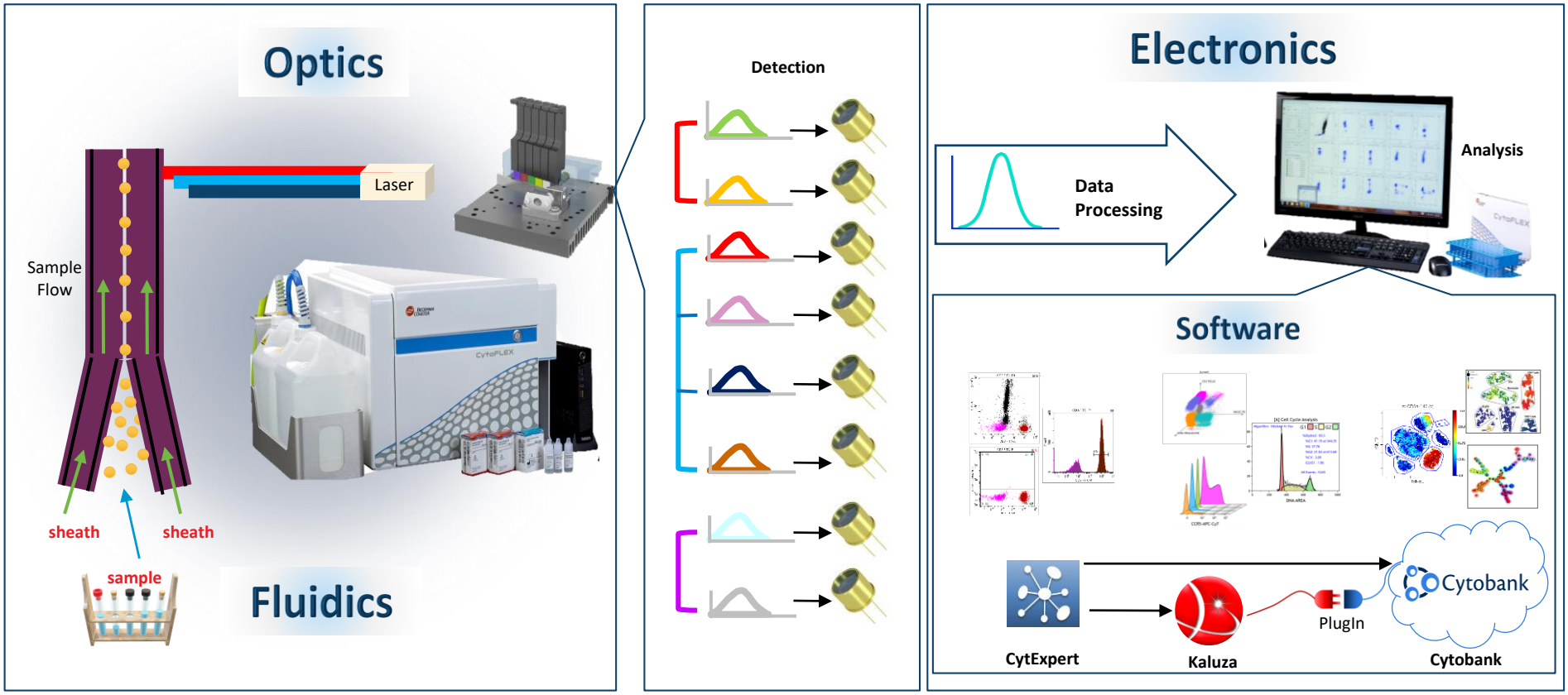
Above threshold
= an event





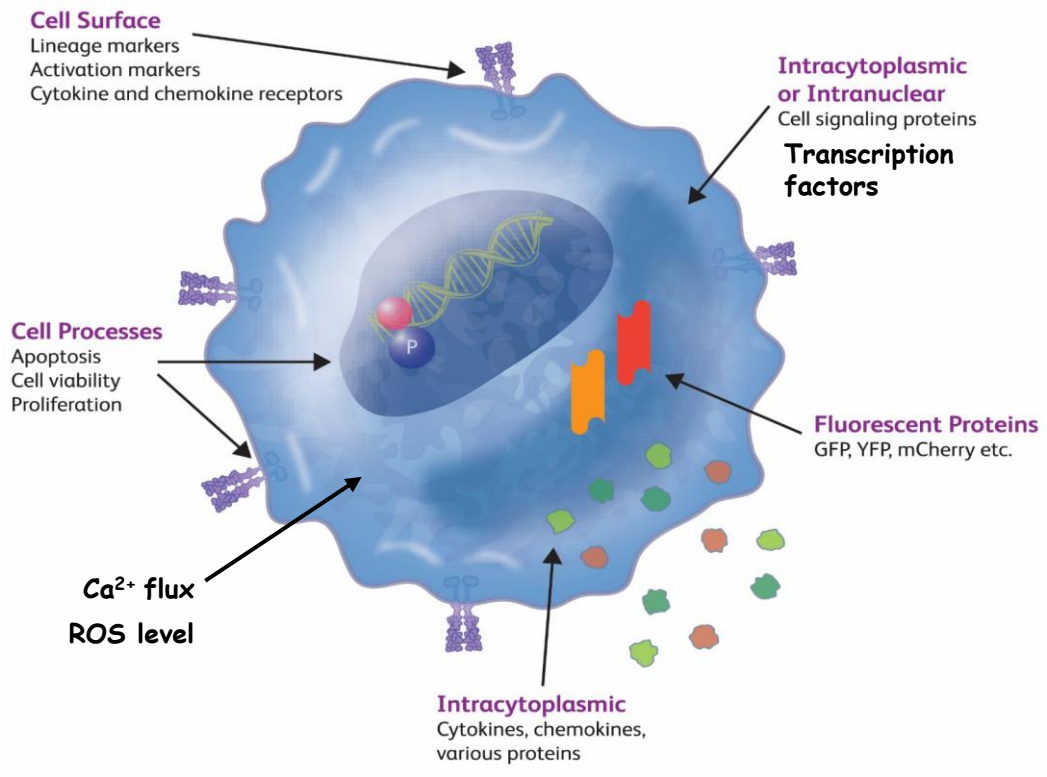
Below threshold
= not an event

Summary



Applications

Application

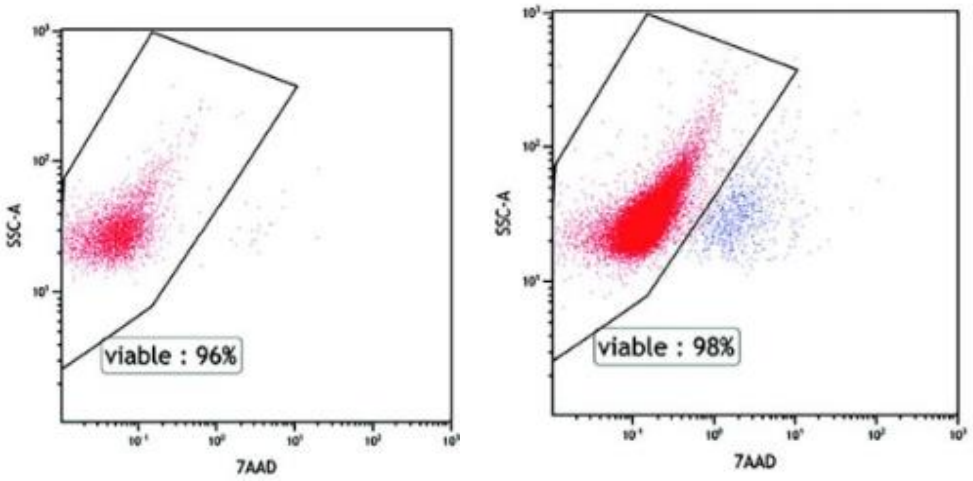


Applications

- Absolute Cell Counting
- Aneuploids and Allopolyploids
- Apoptosis
- Biomonitoring
- Bioreactor Process Optimization
- Cancer Research
- Cell Counting
- Cell Cultures
- Cell Cycle Analysis
- Cell Proliferation
- Cell Type Identification in Natural Populations
- Cytokines
- Detection of Hybrids
- Detection of Microorganisms
- Detection of Ploidy Level
- DNA Analysis
- Fermentation Process Control
- Fluorescent Protein Detection
- FRET Studies
- Gender Determination
- Immunology
- Leukocyte Depletion
- Live/Dead Analysis
- Marine Biology & Algae
- Microbiology
- Nucleic Acid Composition
- Particle Counting
- Particle Sizing
- Phagocytosis
- Phosphorylation Studies
- Signal Transduction Studies
- Plant Genome Size
- Platelet Counting
- Polysomaty/Plant Chimera
- Quality Assurance in Food & Beverage Industry
- Quality Control in Dairy Industry
- Research Agrosciences
- Research Microbiology
- Sperm Cell Counting
- Sperm Cell Function
- Sperm Cell Viability
- Stem Cells
- Viability

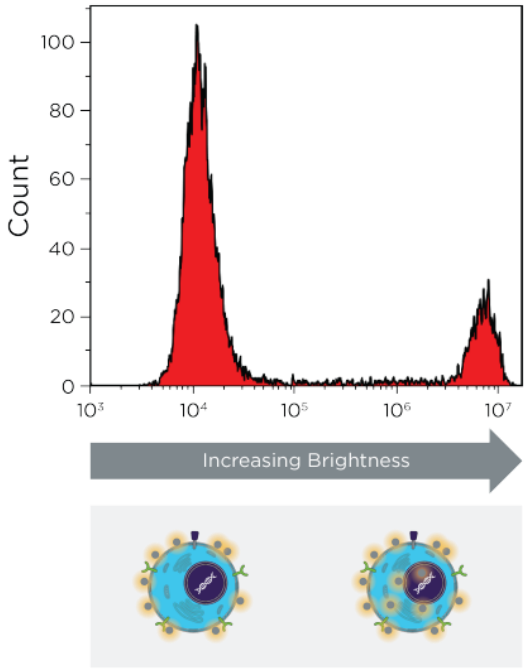
Cell viability

- Pi (Blue)
- 7-AAD (Blue)
- DRAQ7 (Red)
- DAPI (Violet)

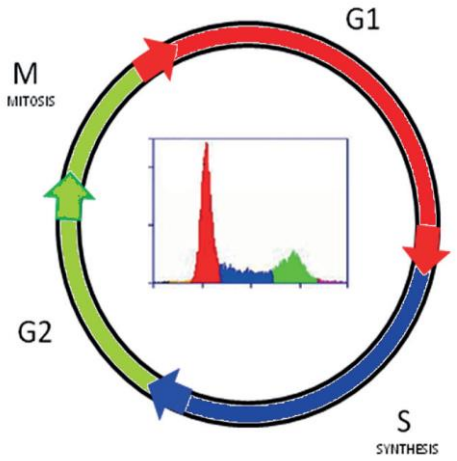


Viakrome Fixable viability Dye

Part Number	Reagent	Excitation/Emission (nm)	Excitation Laser	Bandpass
C36614	ViaKrome 405 Fixable Viability Dye	401/420	Violet 405 nm	450/45
C36620	ViaKrome 561 Fixable Viability Dye	555/565	Blue 488 nm	585/42
C36620	ViaKrome 561 Fixable Viability Dye	555/565	Yellow Green 561 nm	585/42
C36624	ViaKrome 638 Fixable Viability Dye	638/655	Red 638 nm	660/10
C36628	ViaKrome 808 Fixable Viability Dye	854/878	Infrared 808 nm	885/40



Cell cycle



G0 : 2n
(Gap0) resting state

G1 : 2n

(Gap1) RNA & protein synthesis to prepare for S phase

S : 2n~4n

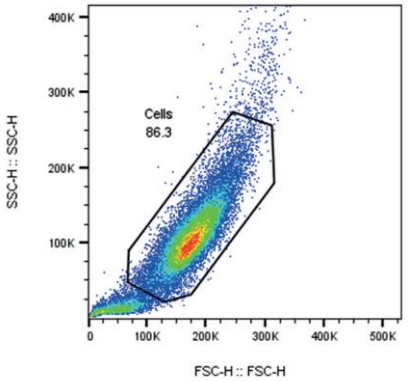
(Synthesis) DNA Synthesis

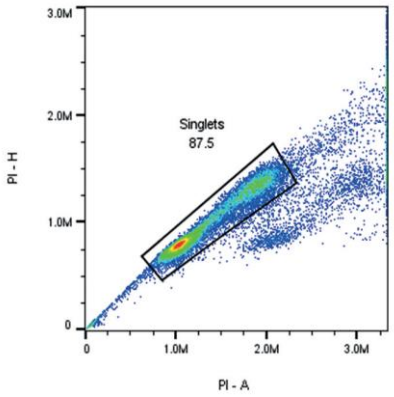
G2 : 4n

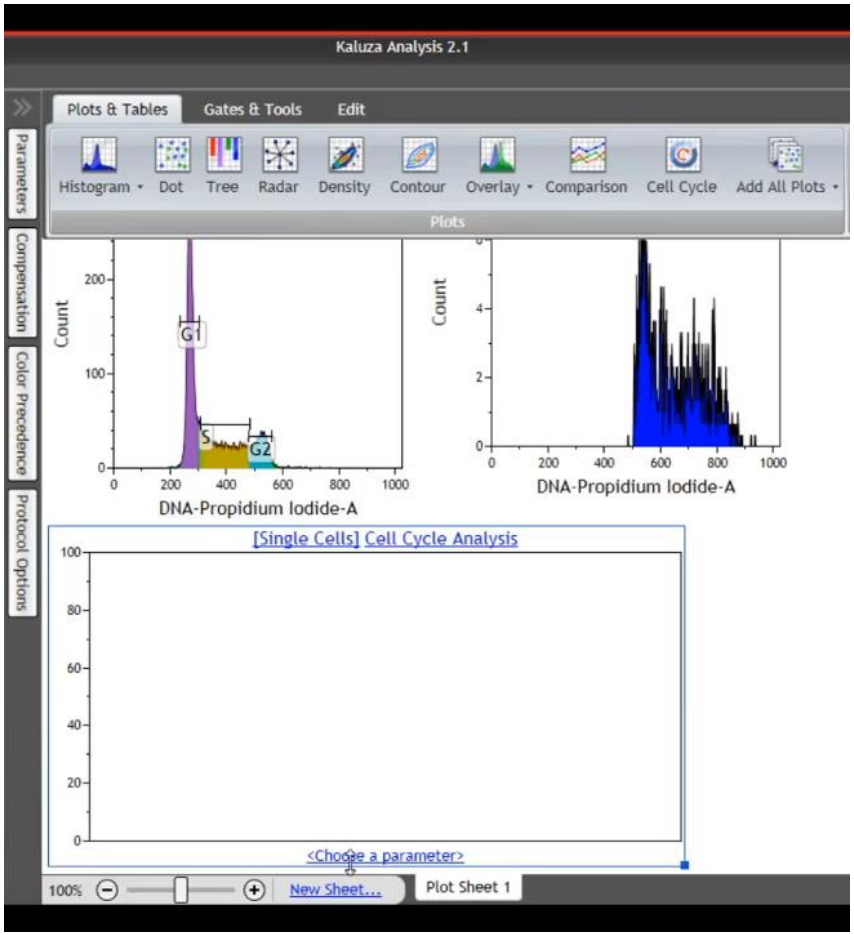
(Gap2) RNA & protein synthesis before cell division

M : 4n

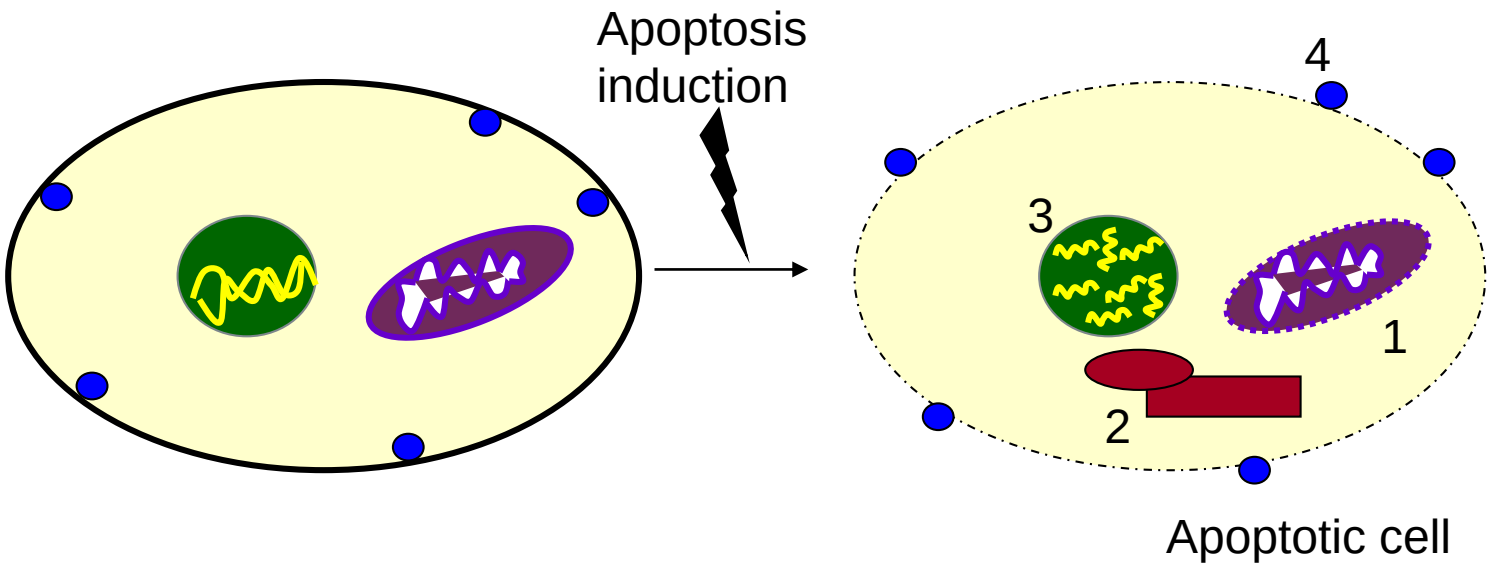
(Mitosis) preparation for daughter cell production







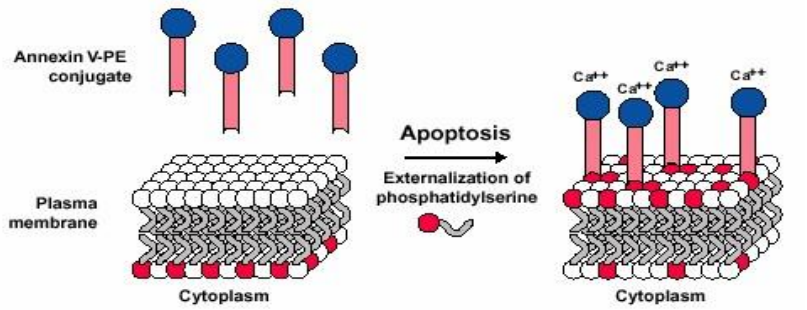
Apoptosis



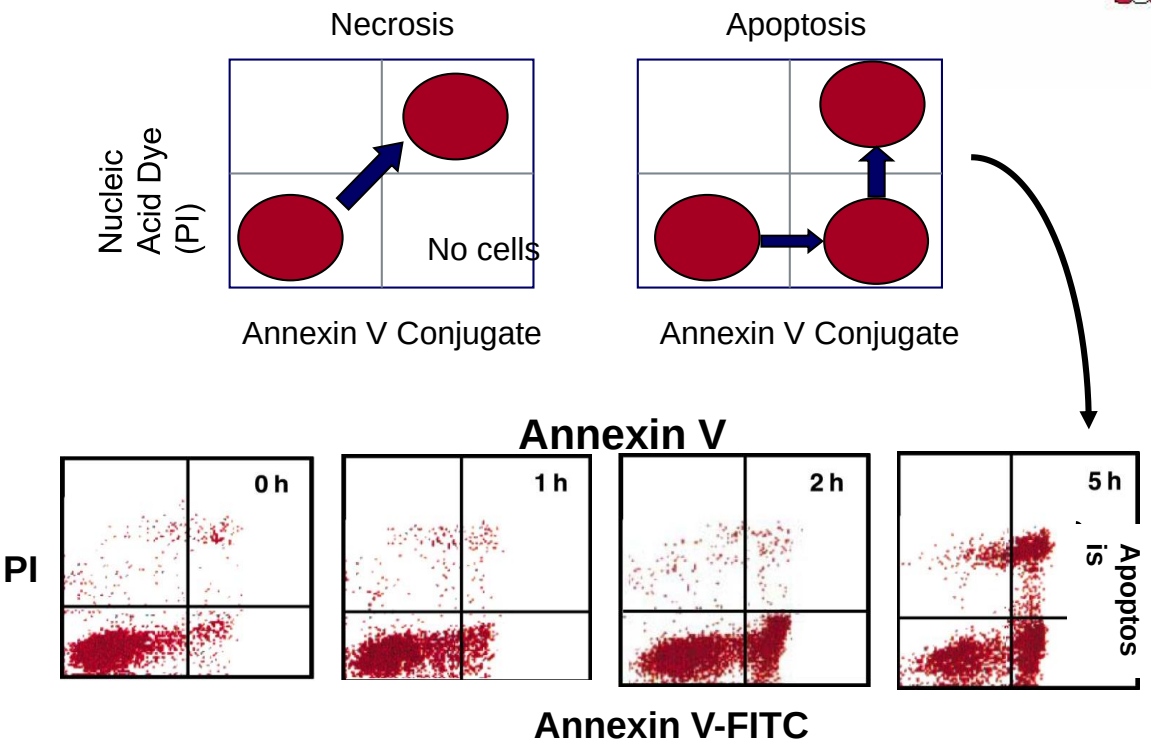
1. Mitochondrial potential decreases
2. Caspases activated
3. DNA fragmentation
4. Phosphotidyl serine (PS) exposed

Apoptosis

- Annexin V assay



Schematic representation of the Annexin V assay.



Ca Flux

- Calcium-mediated signal transduction
- No pressure on tubes:
  - ✓ Add reagents during acquisition of sample

The unique peristaltic sample delivery system of the CytoFLEX analyzer enables optimized measurements of transient changes in intracellular calcium in cells following agonist activation

APPLICATION NOTE

Authors:
Ira Schieren[1]
Peter Racanelli[2]
Damian Williams[3]

Affiliation:
1 Howard Hughes Medical Institute, Columbia University Medical Center
2 Beckman Coulter Inc., Miami, United States
3 Department of Pathology and Cell Biology, Columbia University Medical Center

IN THIS PAPER YOU WILL LEARN

How to set up a flow cytometeric assay to measure changes in cellular ion calcium

How to adapt the CytoFLEX flow cytometer to make real-time measurements for cellular assays

About a rapid screening method of calcium flux by flow cytometry

Principal of the Technique

Generation of fluorescent antibody or genetic labels to identify hormone and neurotransmitter receptor activity can be difficult and time consuming. A useful alternative is recording physiological changes in response to agonist binding its cognate receptor, many of which are G-protein coupled. When an agonist binds a G-protein coupled receptor, it triggers a quick cascade of events that often results in a transient release of calcium from intracellular stores. Alterations in transient intracellular calcium ($[Ca^{2+}]_i$) levels have been used previously in flow cytometry to identify functional receptor expression in cellular subpopulations [2], here we show that peristaltic sample delivery of the new CytoFLEX analyzer is particularly well suited to agonist-based calcium studies. Using the ester based, green fluorescent calcium indicator, Fluo-4 AM (Life Technologies), $[Ca^{2+}]_i$ changes were measured in HEK-293 cells in response to ATP stimulation. Simple plumbing modifications to the CytoFLEX allowed easier access to the sample tube for agonist application; further modifications were made to implement a "stop time" technique. By using response to agonist as our physiological criteria, we have fundamentally enabled receptor identification and conclusively demonstrated its functionality.

Materials and Methods

Calcium indicator and cell loading

5 x 10⁶ HEK-293 cells were dissociated and re-suspended in 1.6 mL Dulbecco's phosphate-buffered saline (DPBS) containing 3 µM Fluo-4 AM [1]. The cells were incubated at room temperature in the dark for 20 minutes. Following incubation, cells were spun down and resuspended in 2 mL DPBS. 10 mM ATP was freshly prepared in DPBS and added directly to the cells during the experiment to reach a final concentration of 100 µM.

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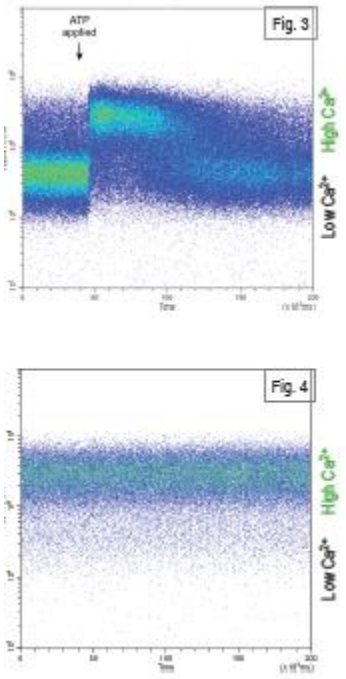
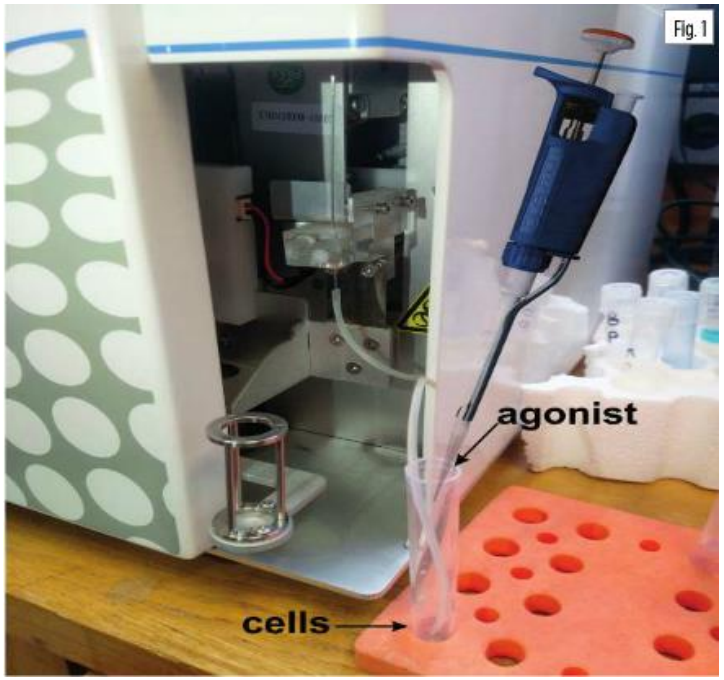
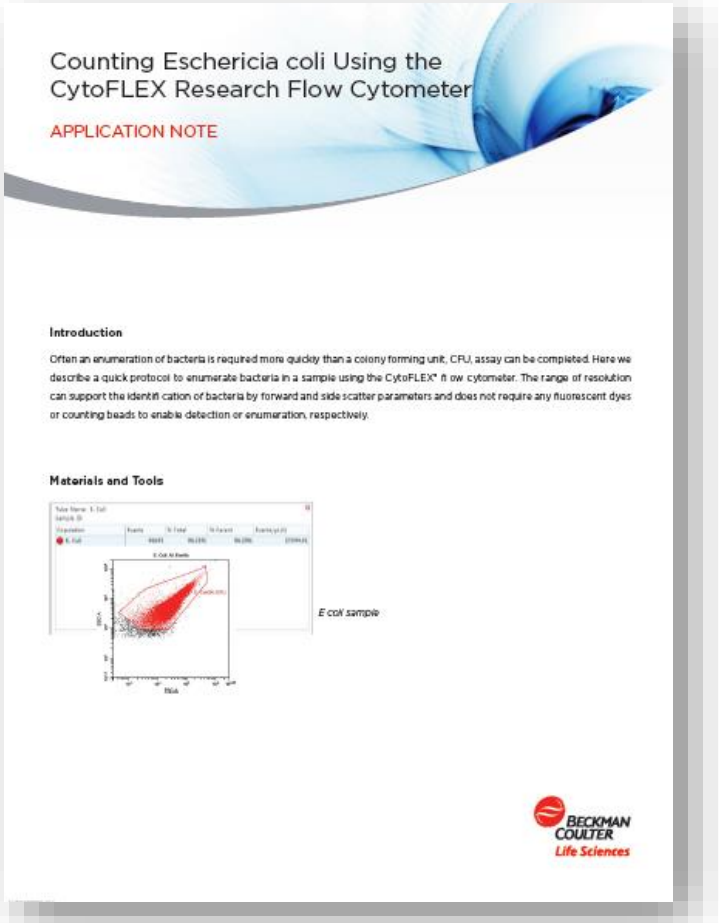




Bacteria counting

- Beadless volumetric counting for single platform enumeration



Counting Eschericia coli Using the CytoFLEX Research Flow Cytometer

APPLICATION NOTE

Introduction

Often an enumeration of bacteria is required more quickly than a colony forming unit, CFU, assay can be completed. Here we describe a quick protocol to enumerate bacteria in a sample using the CytoFLEX* flow cytometer. The range of resolution can support the identification of bacteria by forward and side scatter parameters and does not require any fluorescent dyes or counting beads to enable detection or enumeration, respectively.

Materials and Tools

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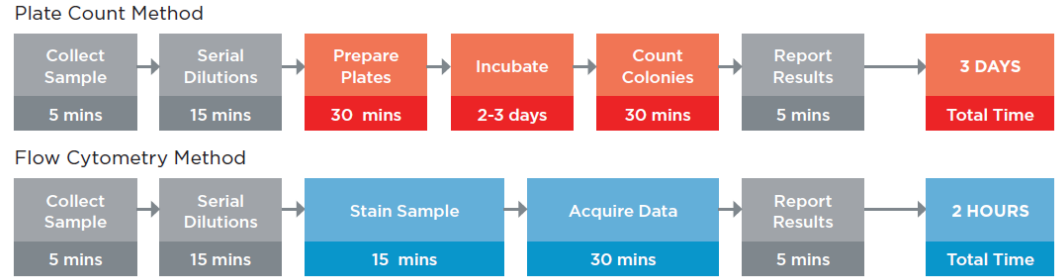


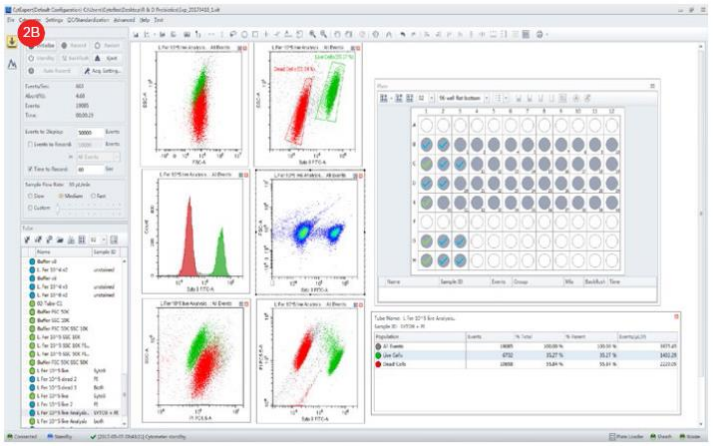
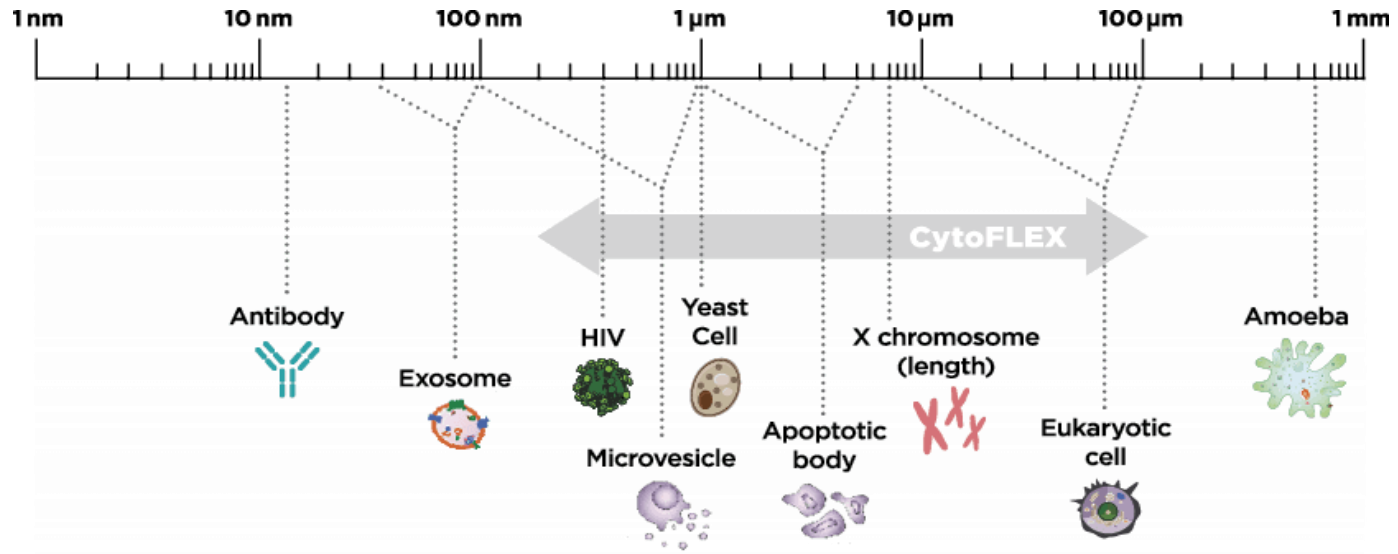
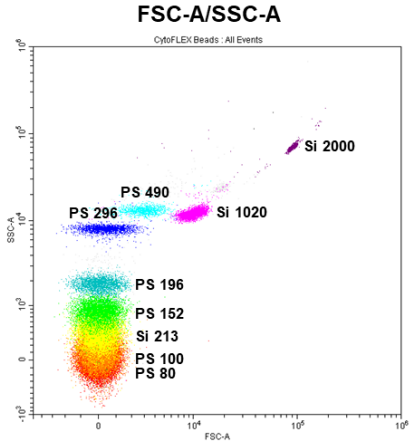
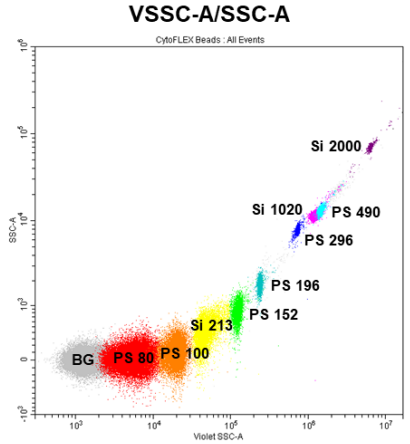


Figure 2. Comparison of the data collection read outs for the Plate Count and Flow Cytometry methods. Panel A is a typical cultured plate from which bacterial colonies are counted. This method requires a technician to accurately discriminate between overlapping or differently sized colonies to obtain counts, typically done in duplicate. Panel B shows the software screen during acquisition using flow cytometry. The instrumentation counts individual bacteria and live versus dead organisms can be differentiated based upon staining characteristics.

Extracellular Vesicle Measurement







Extracellular Vesicle Measurement

Set-Up of the CytoFLEX for Extracellular Vesicle Measurement

APPLICATION NOTE

Authors:
Andreas Spittler, MD, Associate Professor for Pathophysiology,

Affiliation:
Medical University of Vienna, Core Facility Flow Cytometry & Department of Surgery, Research Laboratories

IN THIS PAPER YOU WILL LEARN

- How to setup the CytoFLEX for small particle analysis
- How to eliminate background particles found in buffers and reagents to enhance your small particle detection
- How to use control beads to calibrate the CytoFLEX for microparticle detection

Background

The measurement and the characterization of Extracellular Vesicles (EV) have been of growing interest over the last 20 years. Flow cytometry instruments were not the most appropriate way to analyse these particles as the optical resolution of instruments was insufficient to detect particles below 250 nm. However, the Beckman Coulter CytoFLEX now offers the ability to measure EV down to at least 150 nm and allows the detection of their cellular origin using up to 13 fluorescence parameters. Regardless of the technical improvements the set-up of the instrument is still a critical point and several requirements need to be met which are illustrated here.

Introduction

Extracellular vesicles are a heterogeneous cell-derived particle population in a size range between 50 nm to 1,000 nm. There is a growing interest not only from academic research groups to determine EV in several fluids such as cell culture supernatant, in plasma samples or in whole blood but also in clinical research since it has been shown that the measurement of microparticles (MPs)[1] might be of clinical relevance. The methods to identify EV are many and involve high speed centrifugation, Western blotting, proteomics, electron microscopy, imaging methods and flow cytometry. Methods for the detection of EV by flow cytometry have been developed in the last years and special attention has been paid to standardization protocols. Compared with other methods, flow cytometry has the big advantage that EV can be detected as rare events, in high numbers and by antigens on the surface, which characterize their cellular origin.

However, until now flow cytometry technology has had some unfortunate limitations. It was not possible to detect microparticles below 250-300 nm in size in a meaningful manner. This size range does not appear to be very far from the smallest particles of 50 nm in size, however we have to consider that MPs in a size greater than 300 nm are only the "tip of the iceberg" of visible particles and at least as many particles are smaller than 300 nm in size. The importance in clinical research and the technical requirements to detect smaller microparticles was clearly demonstrated in 2013 by Sarlon-Bartoli et al[2]. Using a Beckman Coulter Gallios

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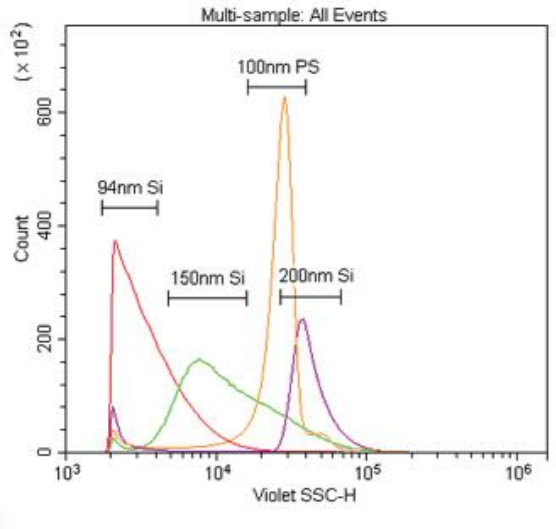
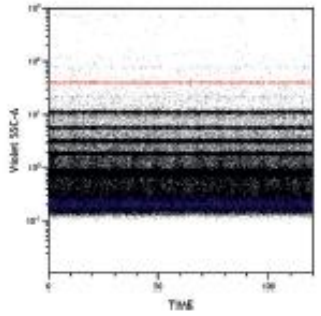
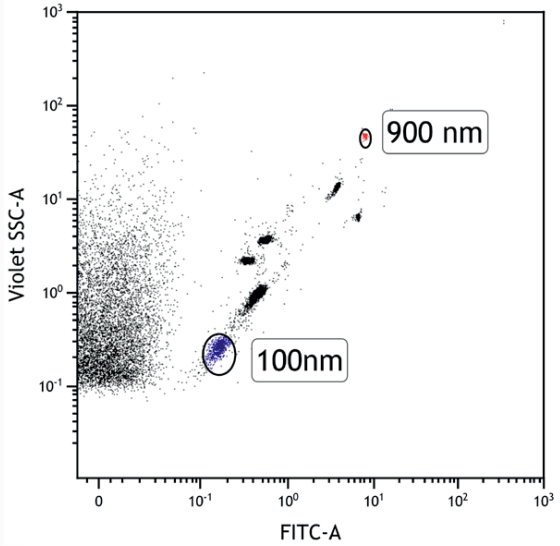
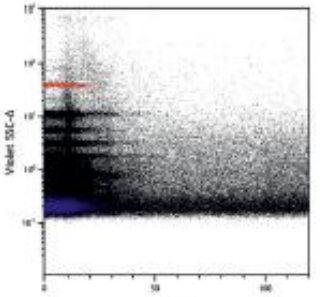




amix





Washing check

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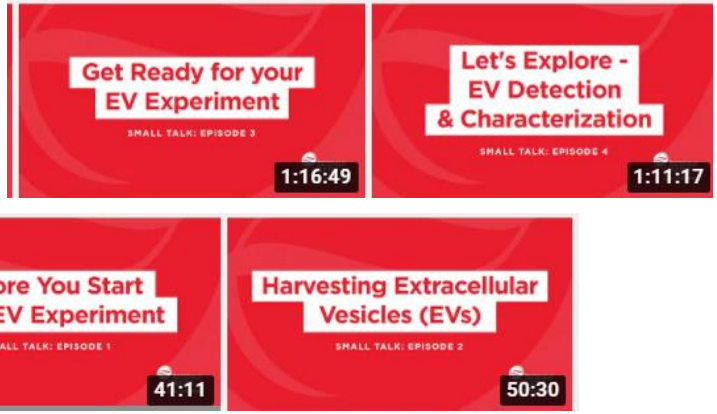
Extracellular Vesicle Measurement

YouTube



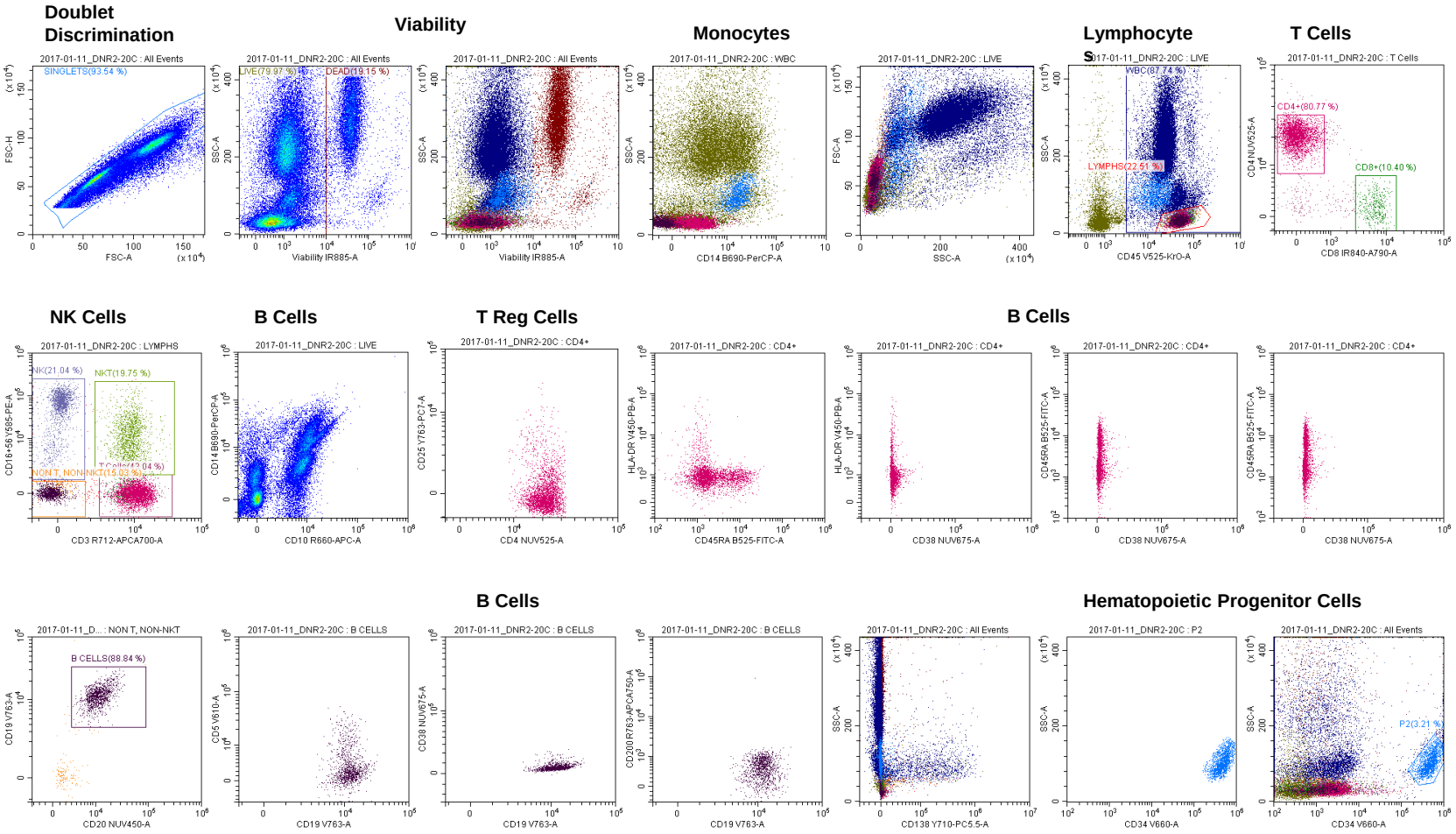
Learning Objectives

- **Defining the Biopharma Landscape**
  - The Rapid & Dynamic Evolution of Biopharma
  - Scale Up/Out in the Production of Biologics
  - Associated Challenges with Scaling Up/Out
- **Exosomes as a Model for Biologics Scale Up/Out**
  - Exosomes - General Considerations
  - Isolation & Purification
  - Associated Challenges in Scale Up/Out Workflows
- **Centrifugation in Exosome Scale Up/Out**
  - Isolation & Characterization
  - Increasing Purity (Capacity)
  - Improving Yields (Throughput)

CytoFLEX Applications

- Application Notes including
  - Cell Viability and Vitality (4)
  - Immunophenotyping (9)
  - Functional Assays (6)
  - Microbiology (6)
  - Nanoparticles (5)
  - Etc (4)

Phenotyping – 18c



DURA Innovations

Dry Unitized Reagent Assays

Defects

휴먼에러 최소화!

잘못 만든 Cocktail 시약 우려로 부터 해방!

Overproduction

Antibody 낭비도, 실험자의 시행착오도
일어나지 않습니다.

Waiting

반복되는 centrifugation, washing,
incubation 으로부터 해방!

Non-utilized talent

고도의 기술을 누구나 어디서나 동일하게!

쉽고, 간단하게 해결!

Transportation

상온 보관 시약으로서,

운송 중 온도변화 우려로 부터 해방!

서로 다른 single color reagent 운송기한으로 부터 해방!

Inventory

유효기간도 각기다른

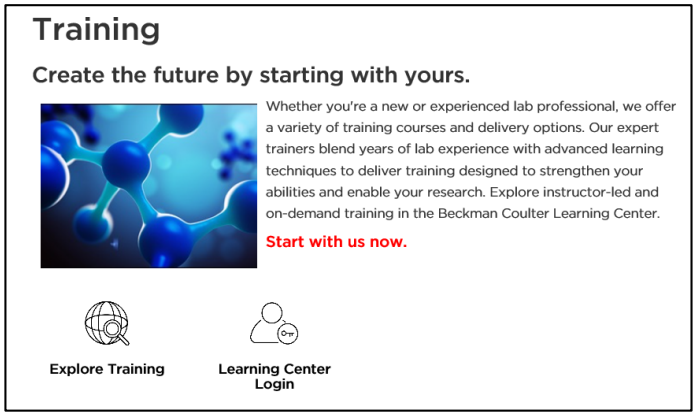
복잡한 single-color reagent 관리로부터 해방!

Motion

시약을 옮기고 – Pipetting 후 – 반응시켜서 – 측정! 끝!

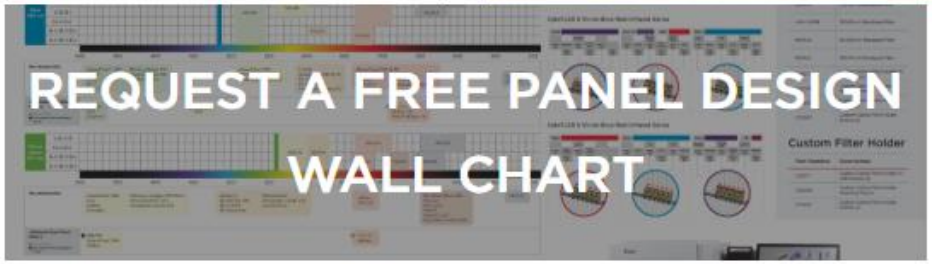
Extra-processing

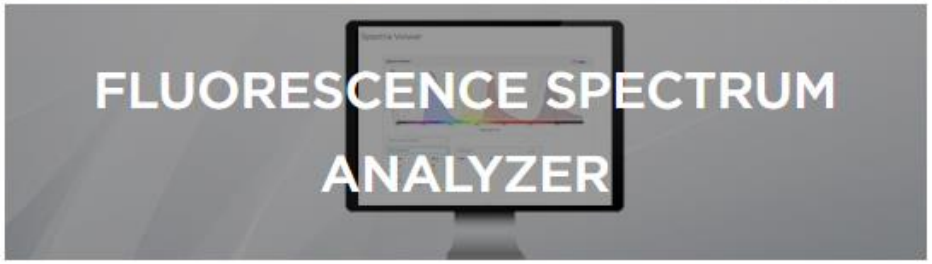
불필요한 과잉 Antibody 사용, 튜브 사용과 반복 시험
우려로 부터 해방!



https://www.beckman.kr/training

https://www.beckman.kr/resources/techniques-and-methods/immunophenotyping





Questions? 😊

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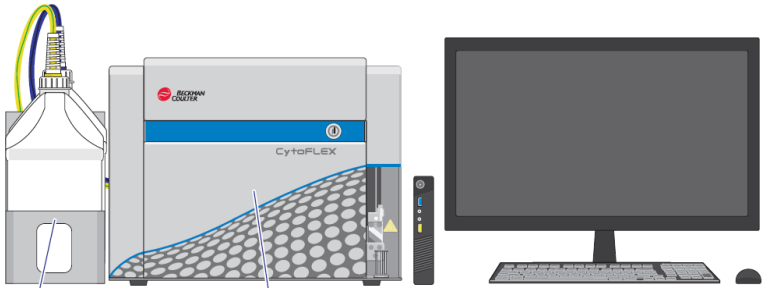
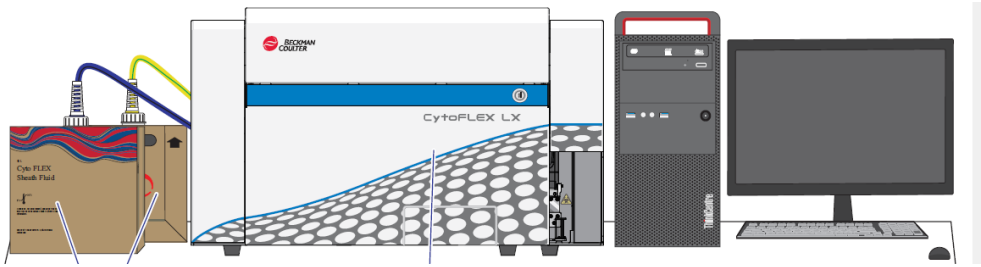
CytoFLEX Start Up!

Start up





장비 구성
장비 켜기 전 tank check 하기

✓ **Sheath & Waste bottle을 장비와 동일선상에 설치**

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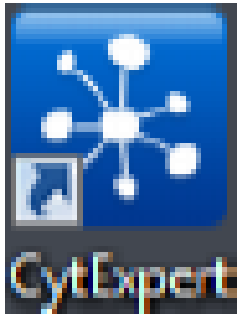
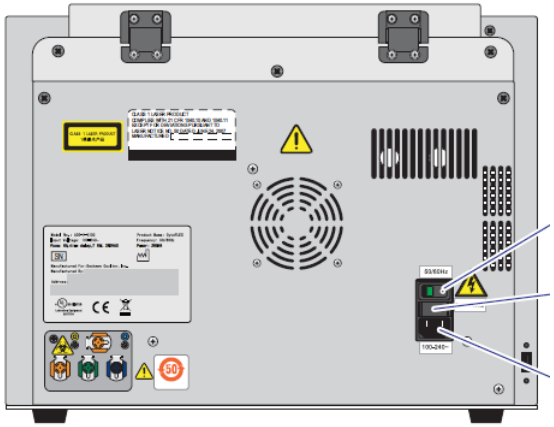
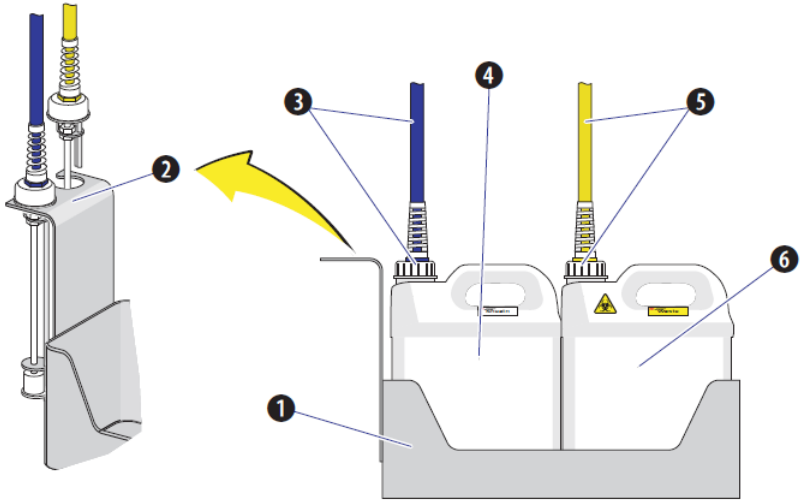
Start up

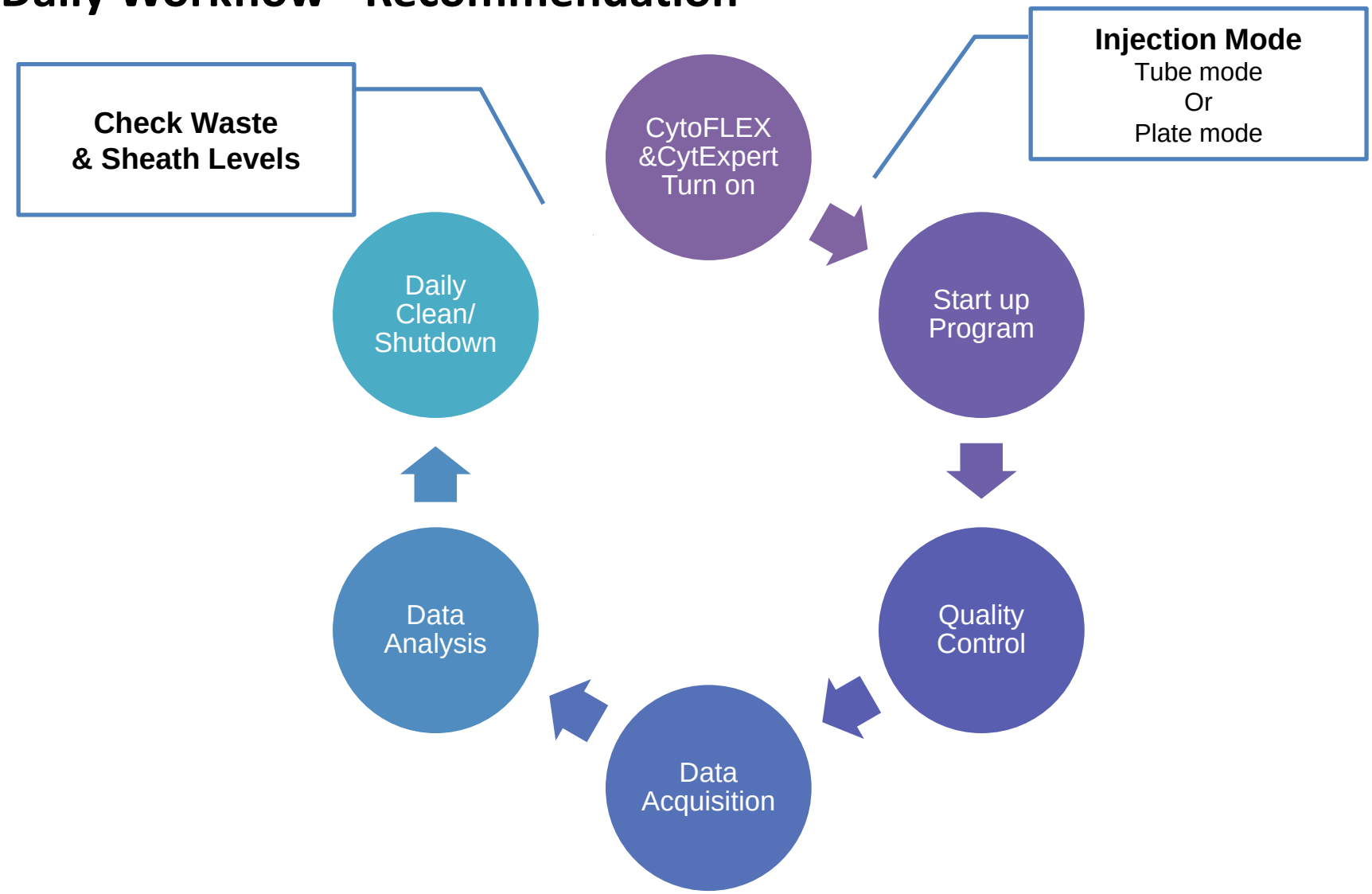




- ✓ **Don’t fasten the cap!**



Daily Workflow - Recommendation



CytExpert Go!