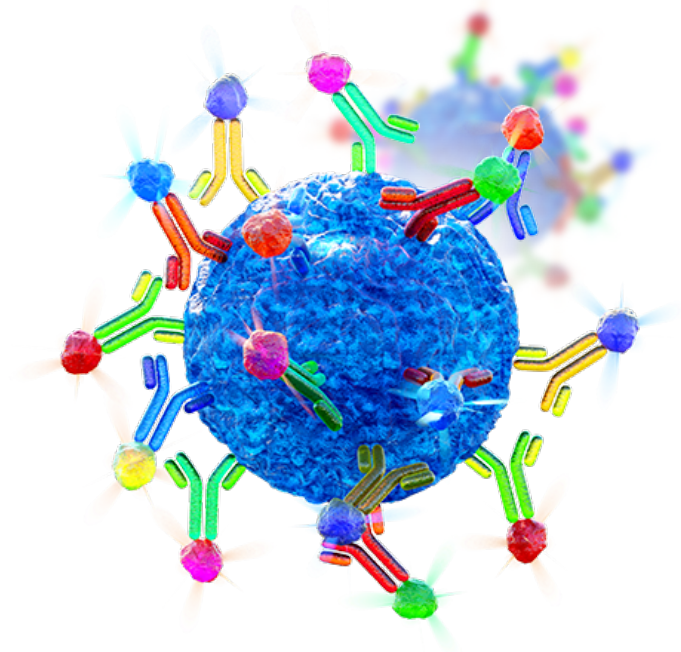


Evolutions in Flow Cytometry

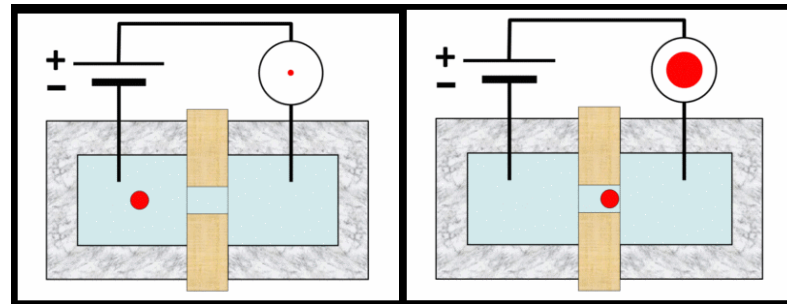
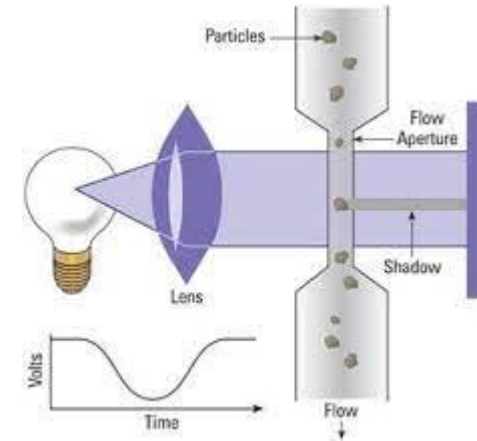


Dr Davide Brusa, PhD
Institute of Experimental and
Clinical Research (IREC)
UCLouvain

MB&C24, Hogeschool UCLL, Diepenbeek
08-09/02/24

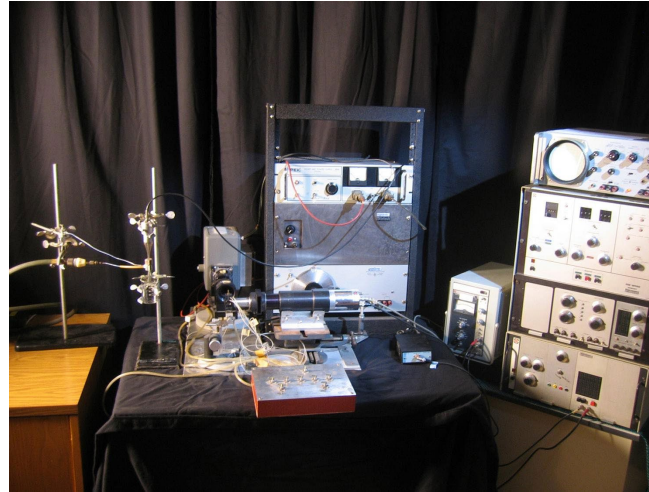
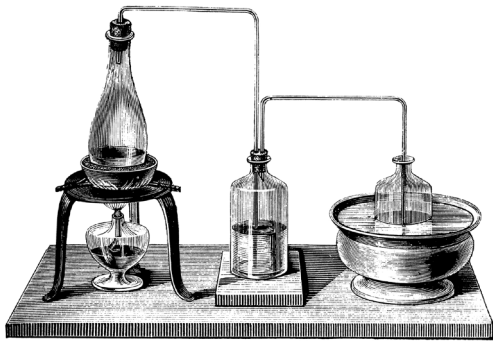
History of cell counting

- **In 1879 Lord Rayleigh** discovered that a stream of fluid emerging from an orifice is hydrodynamically unstable and breaks into a series of droplets
- **In 1934 Moldovan A.:** published on Science «Photo-electric technique for the counting of microscopical cells» → Red Blood Cells
- **In 1949 Coulter W.:** patented 'Means for Counting Particles Suspended in a Fluid' → Impedance Principle, counted RBCs in suspension



History of cell counting

- In 1965 Sweet R.: described a system that prints with ink that is electrostatically charged and deflected in accordance with the input signal potential, the ink stream is divided into regular uniform drops and the drop charge can be controlled by the input system.
- In 1965 Fulwyler adapted Sweet's principle of electrostatic inkjet droplet deflection for use with a Coulter cell sizing instrument → first Cell Sorter was born



- In 1968 Göhde W./Dittrich W.: developed a flow cytometry system that sorted by intracellular fluorescence
- In 1970 Herzenberg L.: coined the term FACS (Fluorescence-activated cell sorting)

History of cell counting



1971
Cytofluorograph
(Bio/Physics Systems)



1973
PAS8000
(Partec)



1974
FACS
(Beckton Dickinson)

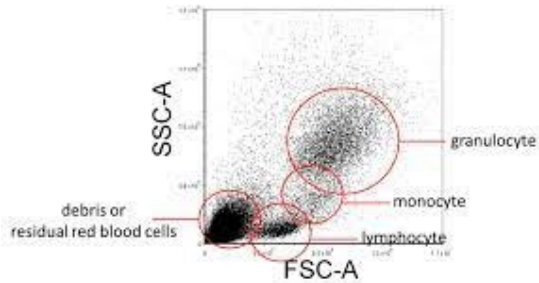


1975
ICP22
(Partec)

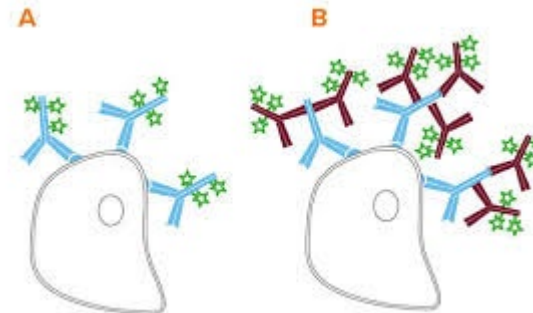
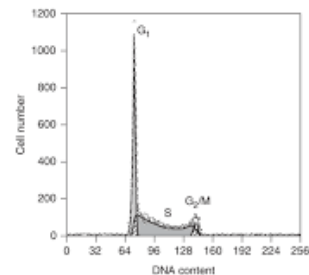


1978
Epic
(Coulter)

↓
Nucleic Acids
and Cell Size



↓
Fluorescence

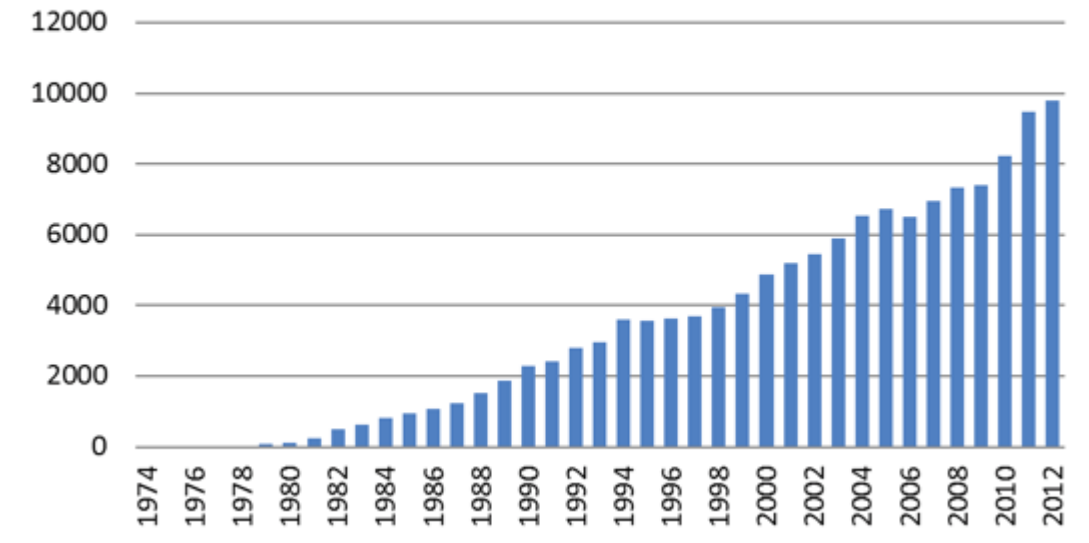


History of cell counting

- Cytometrists such as Howard Shapiro (in Boston) built their own cytometers, adding multiple lasers and detectors to their machines.
- The machines evolved to what we are using in the lab today. The now commonly used and recognized term 'Flow Cytometry' was only first used in the late 1970's;
- PubMed shows that there were 13 publications using the term 'Flow Cytometry' in 1977, which can be compared to the 9726 publications in 2012.



Flow Cytometry Publications by Year



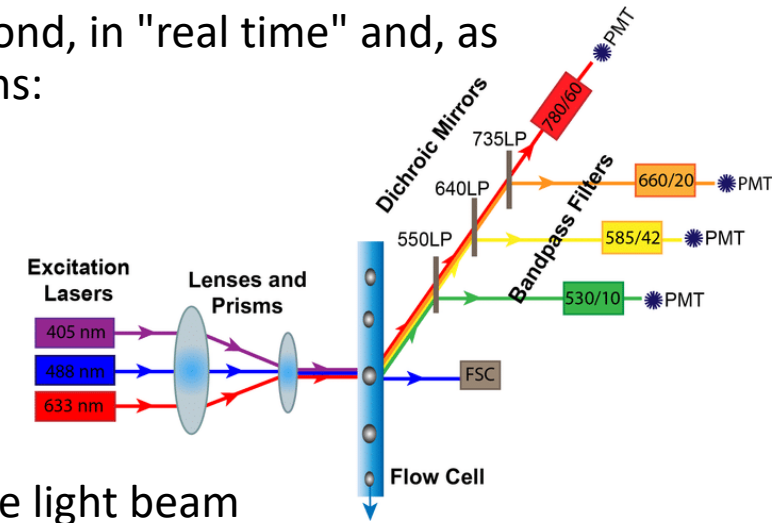
Flow Cytometers

- Modern Flow Cytometers are able to analyze many thousands of particles per second, in "real time" and, as cell sorters, can actively separate and isolate particles. They contain 3 main systems:

- Fluidics
- Optics
- Electronics

- Flow cytometers have five main components:

- flow cell → carries and aligns the cells so that they pass single file through the light beam
- measuring system → uses lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet)
- detector → analog-to-digital conversion (ADC) system converts analog measurements of forward-scattered light (FSC) and side-scattered light (SSC) as well as dye-specific fluorescence signals into digital signals
- amplification system → linear or logarithmic
- computer → analysis of the signals



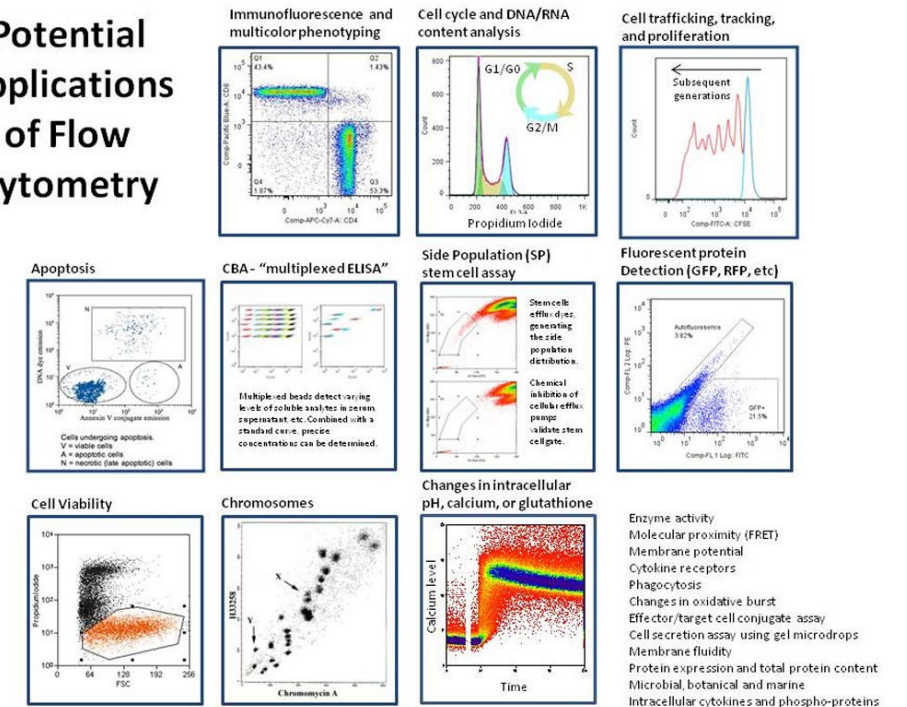
Evolution of Flow Cytometers

- **Classic/Polychromatic Flow Cytometers** use more robust photodetectors and LED lamps as emitter. The improvement is more correlate to the multidimensional analysis.

- Apoptosis
- Cell adherence
- Cell pigments
- Cell surface antigens
- Cell viability
- Circulating tumor cells
- Characterizing multidrug resistance (MDR)
- Chromosome analysis
- Flow-FISH, DNA copy number variation
- Protein expression and modifications
- Membrane fluidity
- Total DNA and RNA content
- Monitoring intracellular parameters: pH, calcium and magnesium levels, membrane potential, glutathione levels, and oxidative burst
- Light scattering: Forward scatter (FSC) and side scatter (SSC) measures are used in flow cytometry to assess cell volume and morphological complexity, respectively. These metrics describe the size, granularity, and shape of cells.
- Transgenic products

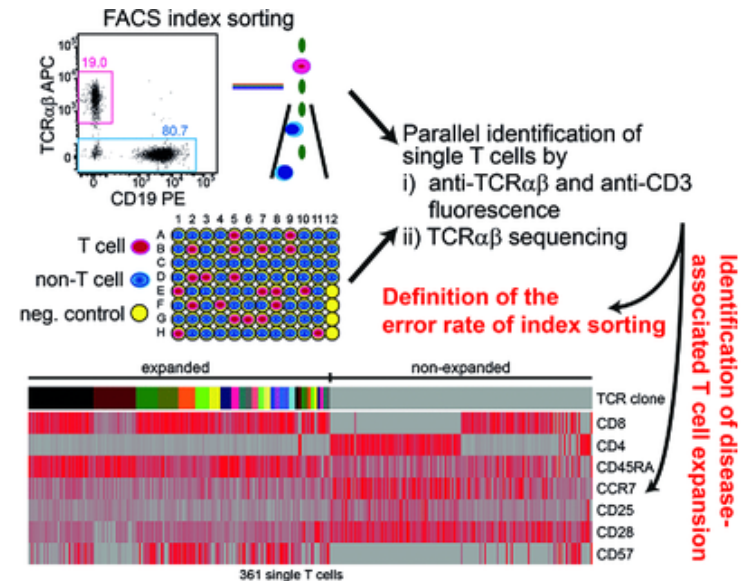
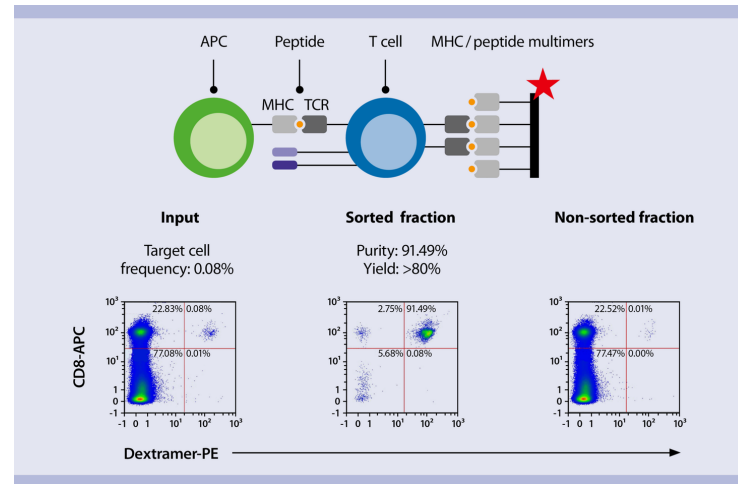
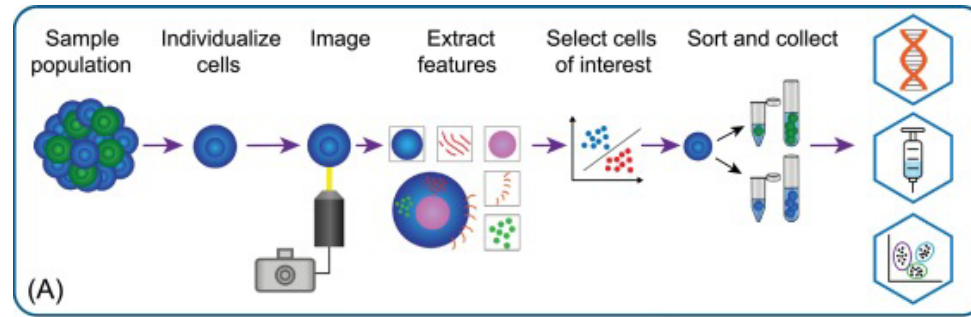
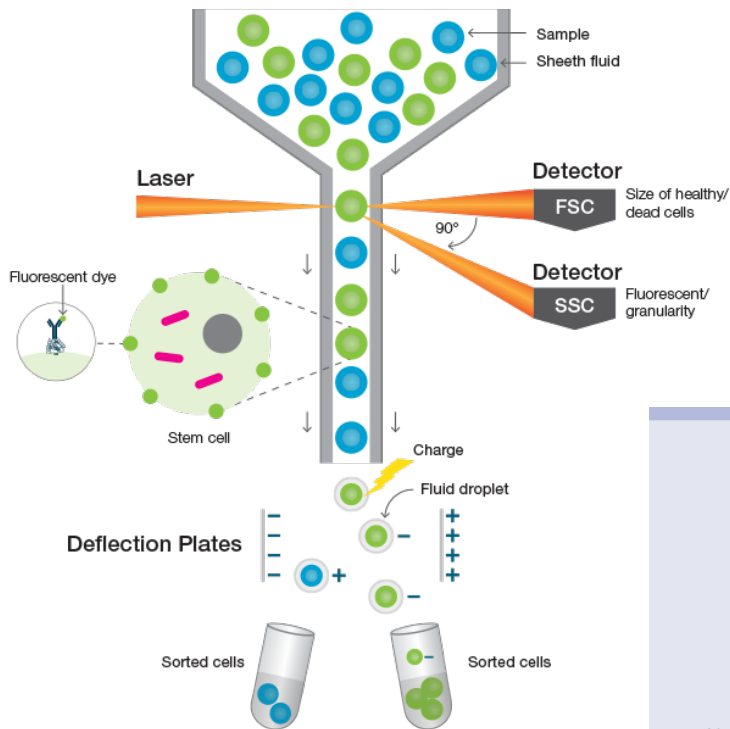
- Cytology
- Hematology
- Immunology
- Microbiology
- Virology
- Pathology
- Transplantation
- Plant Biology
- Marine Biology
- Neuroscience
- Chemotherapy
- Genetics

Potential Applications of Flow Cytometry



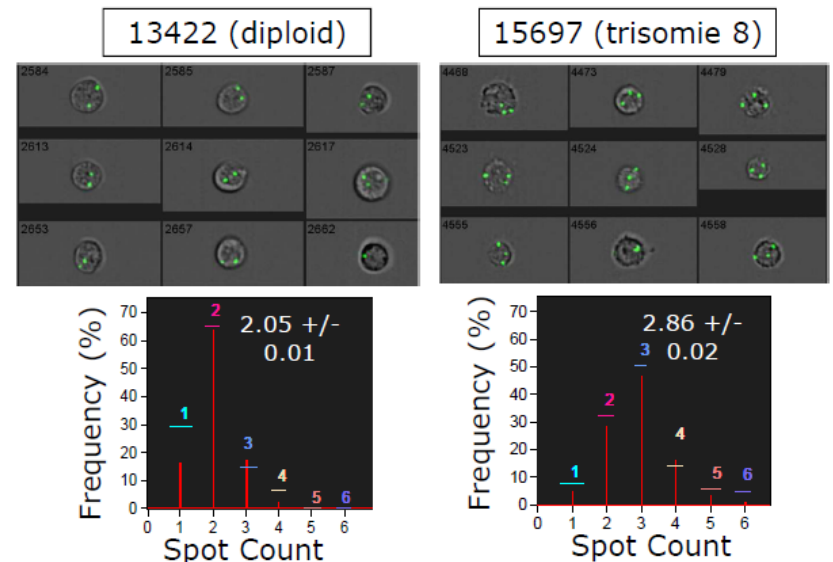
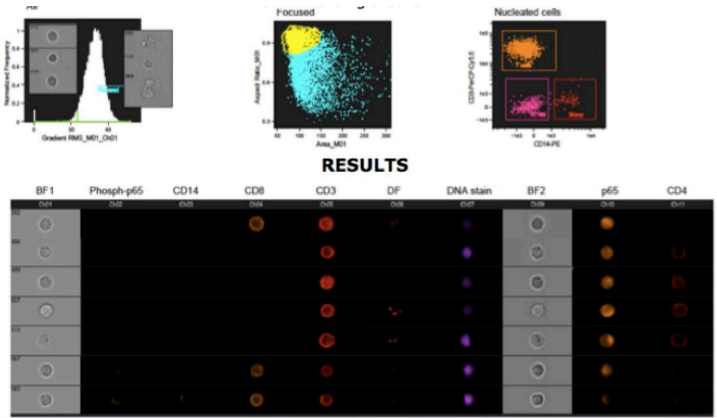
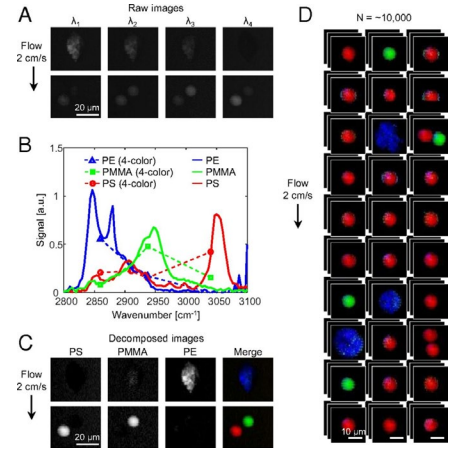
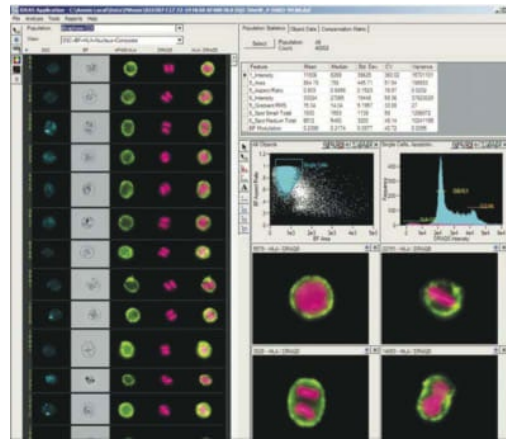
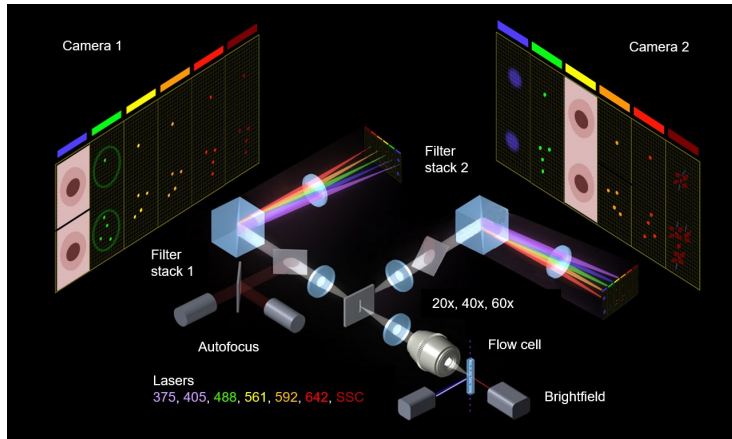
Evolution of Flow Cytometers

- Cell sorters** are flow cytometers capable of sorting cells according to their characteristics. The sorting is achieved by using technology similar to what is used in inkjet printers. The fluid stream is broken up into droplets by a mechanical vibration. The droplets are then electrically charged according to the characteristics of the cell contained within the droplet. Depending on their charge, the droplets are finally deflected by an electric field into different containers.



Evolution of Flow Cytometers

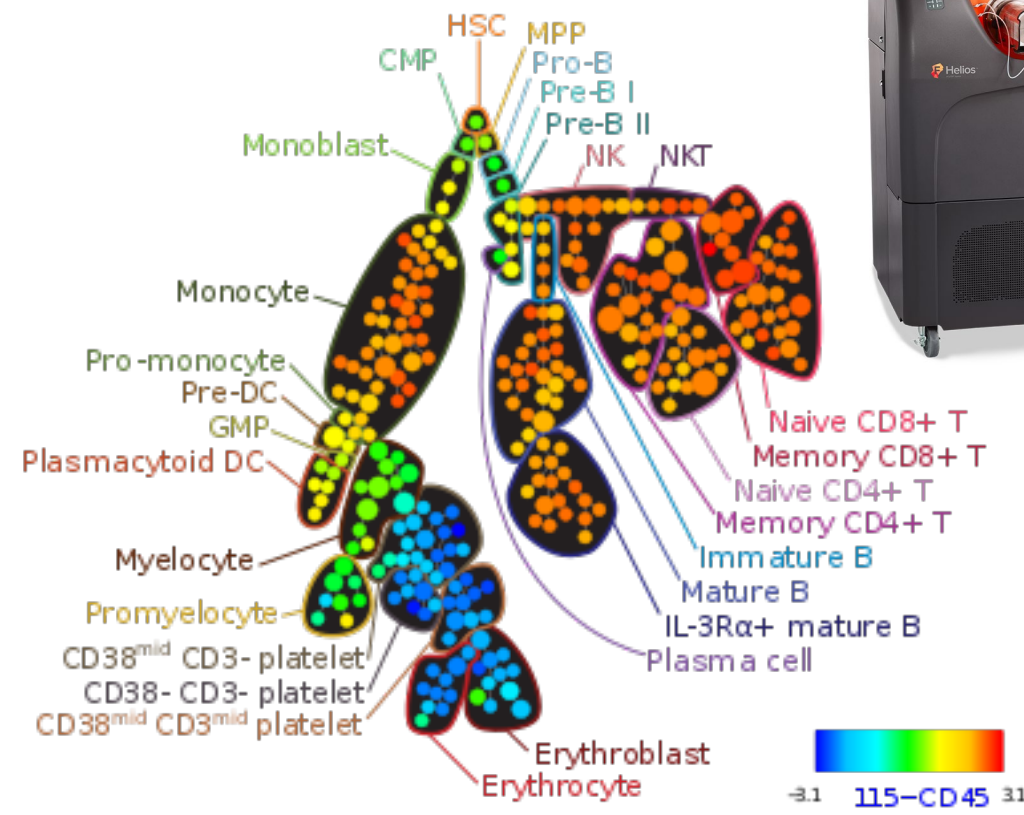
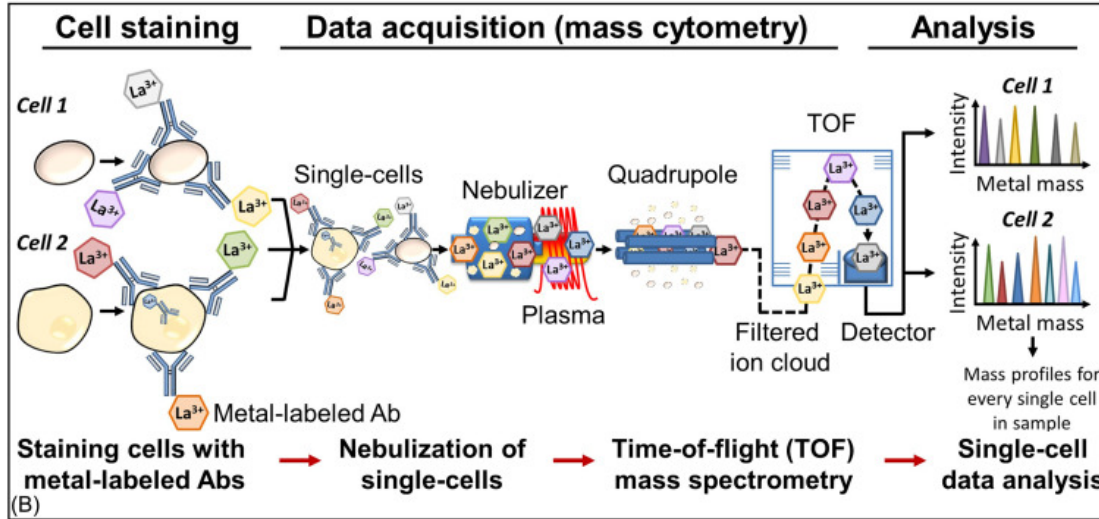
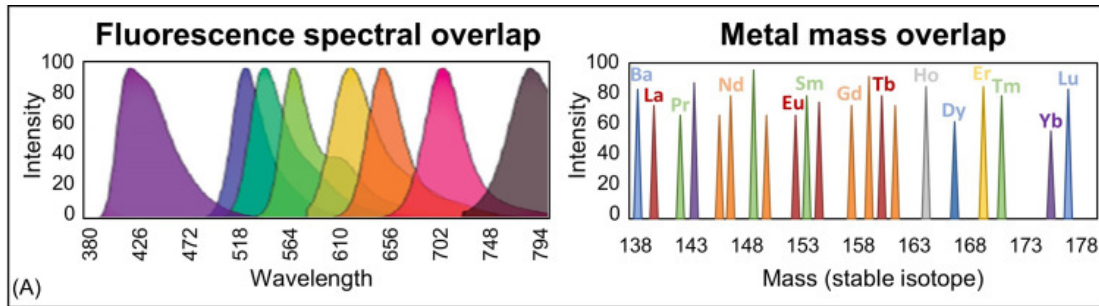
- **Image Cytometers** acquire images of cells in real time as they pass by the interrogation point using optical microscopy. Since the introduction of the digital camera, in the mid-1990s, the automation level of image cytometers has steadily increased.



Field of Study	Example from ImageStream
Cell-cell interaction	Immune synapse formation, T-cell APC conjugation
Cell signaling	NF-κB Translocation, HIV induced NFAT, FoxP3 localization
Shape change & Chemotaxis	MCP-1 activation of monocytes, Differentiation of FDCP cells
Internalization & Phagocytosis	CpGB, Internalization, phagocytosis of Bacteria by monocytes
Intracellular co-localization	Ligand colocalization to lysosomes
Field of Study	Example from ImageStream
Cell death & Autophagy	Apoptosis, nuclear fragmentation, caspase3 activation
Cell cycle & Mitosis	Morphological classification of mitosis
Hematology & Stem cell biology	WBC differential, Erythroid differentiation, FISH analysis
Microbiology	Bacterial phagocytosis in PBMC
Parasitology	Babesia infection in RBCs, Trypanosomiasis

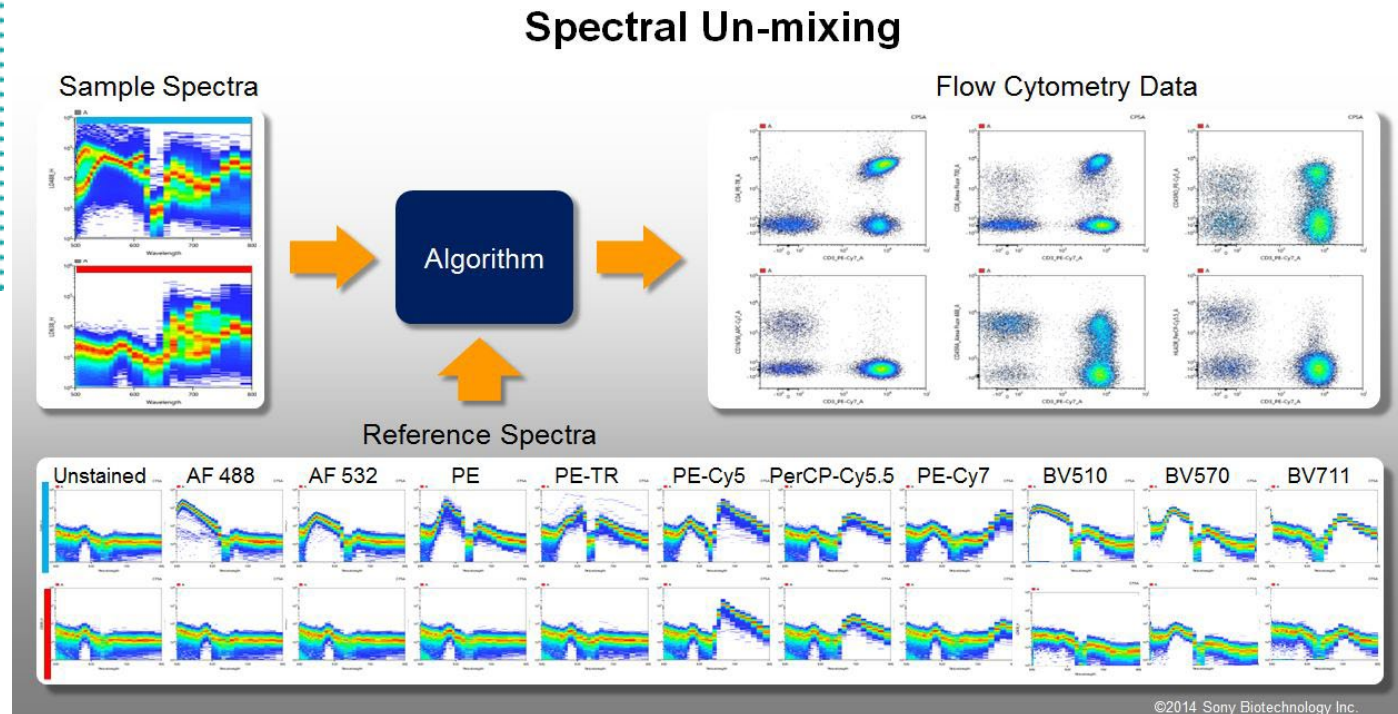
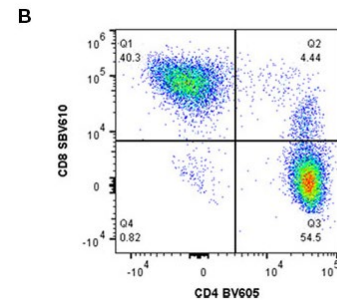
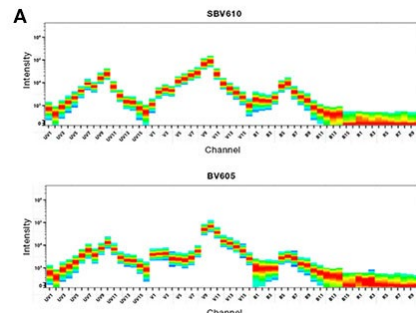
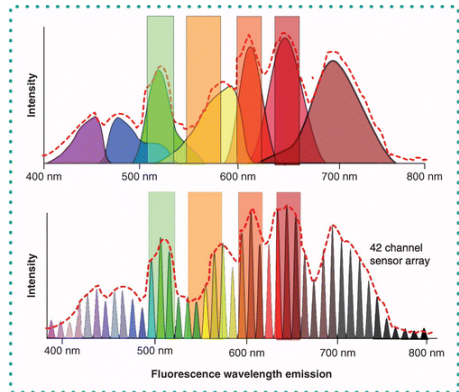
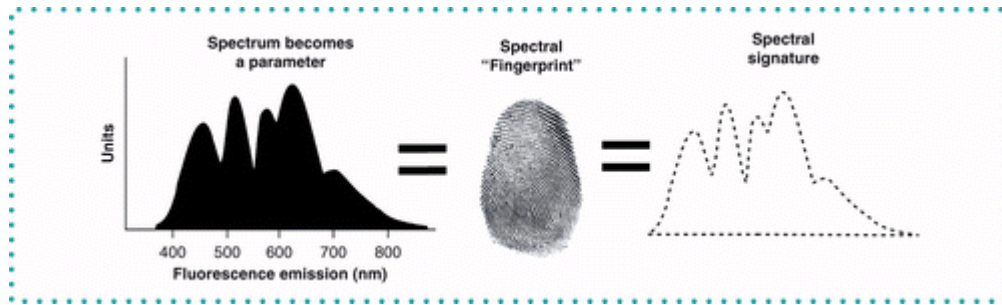
Evolution of Flow Cytometers

- **Mass Spectrometry Flow Cytometers (TOF)** allow separation of cells with the resolution of mass spectrometry. They use antibodies labeled with non-fluorescent heavy metals. In a ionization chamber the cells are nebulized and the metals identified by their TOF characteristics. It's possible to detect up to 100 markers



Evolution of Flow Cytometers

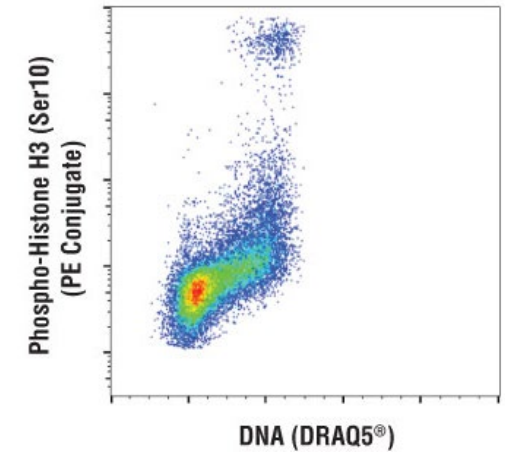
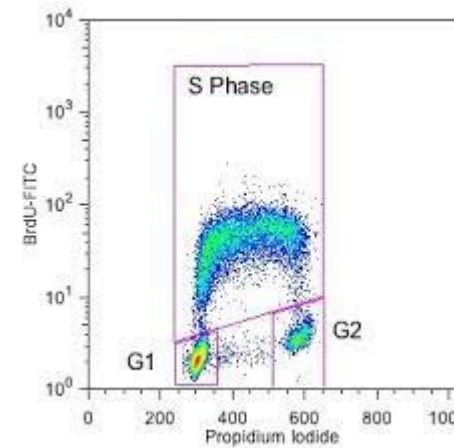
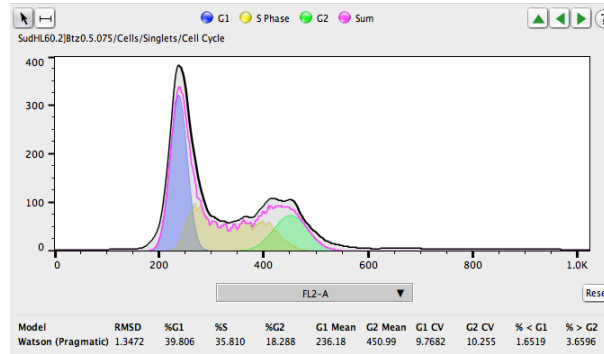
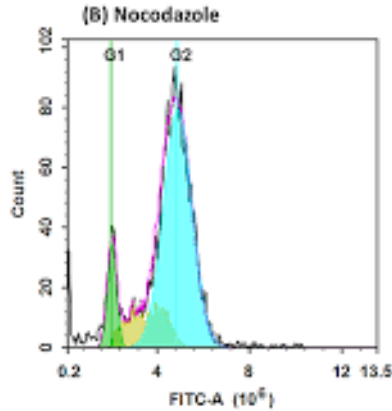
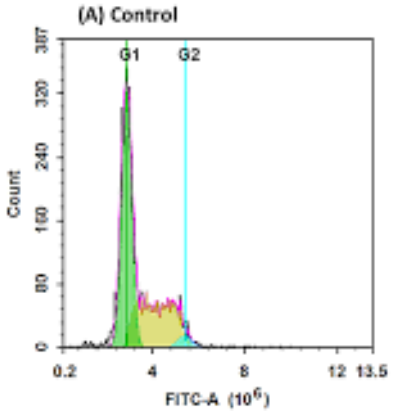
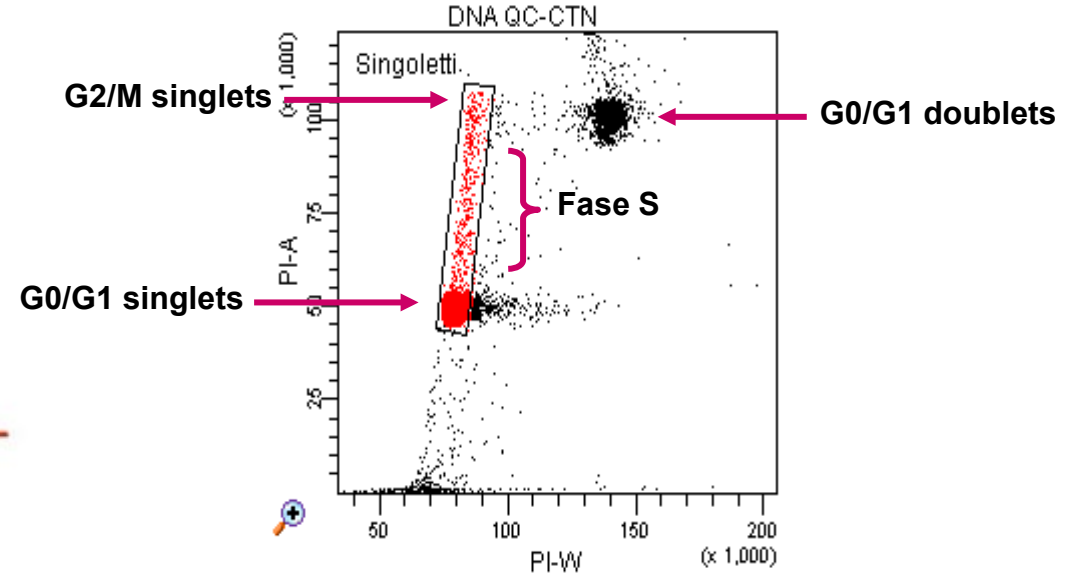
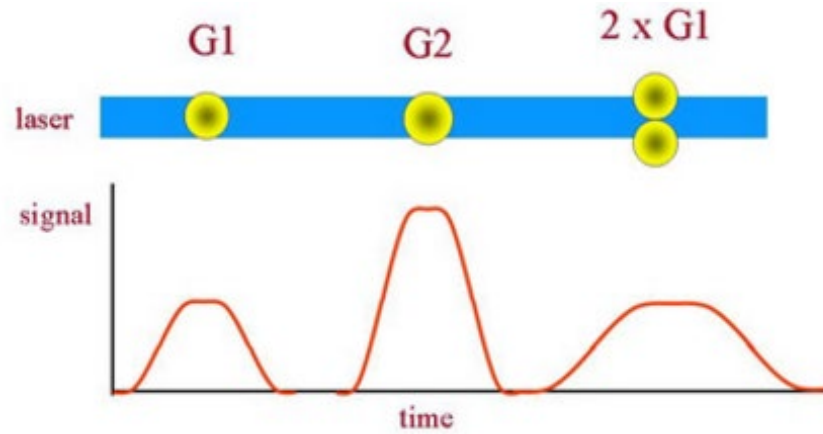
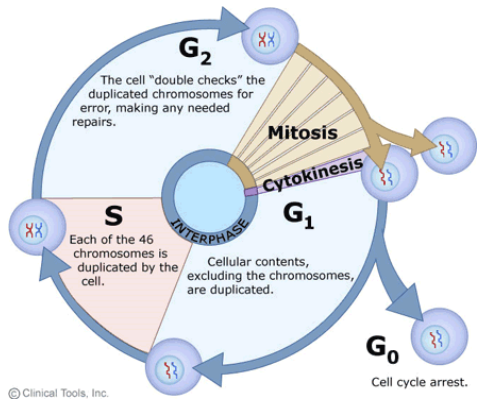
- **Spectral Flow Cytometers** provides more information for each fluorophore which allows for increased resolution and sensitivity. This allows the use of more existing fluorophores that would otherwise be incompatible on a conventional flow cytometer and the expansion of immunophenotyping panels beyond 40 fluorescent parameters



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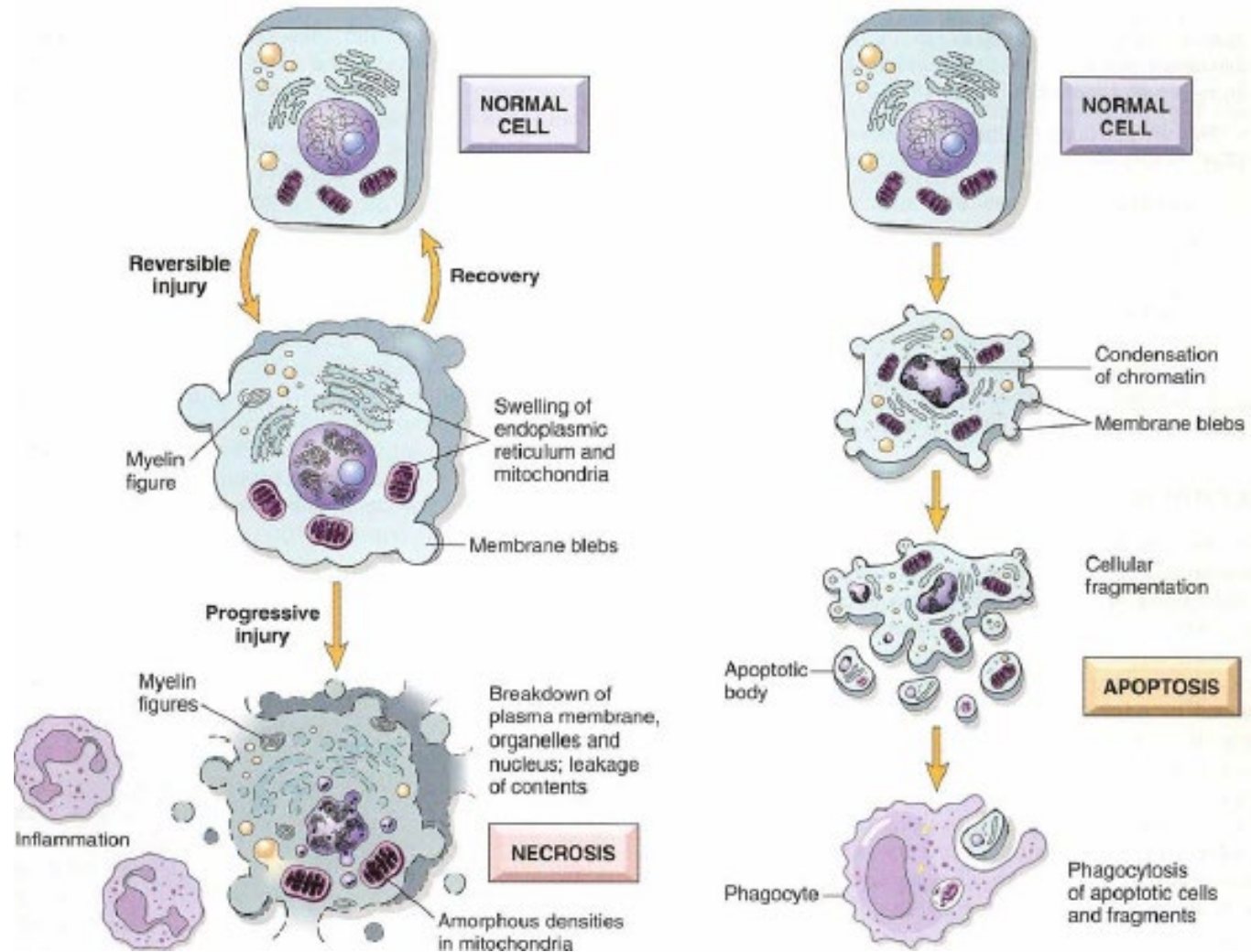
Applications

Cell Cycle, DNA analysis

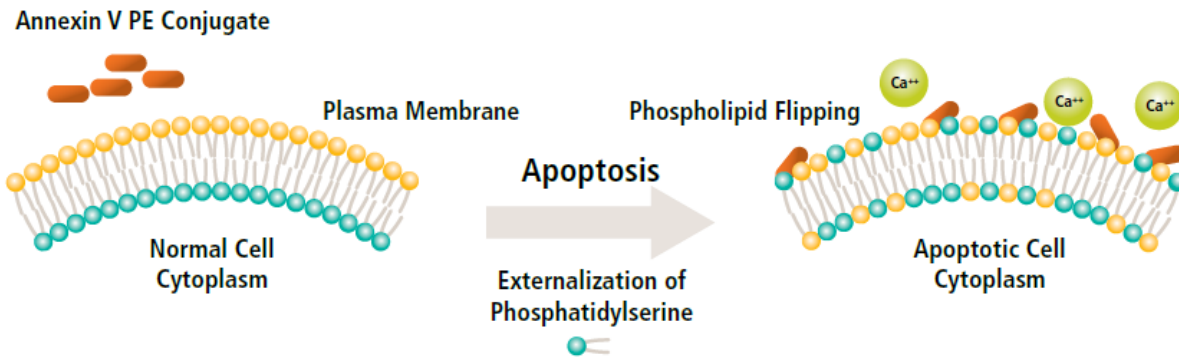
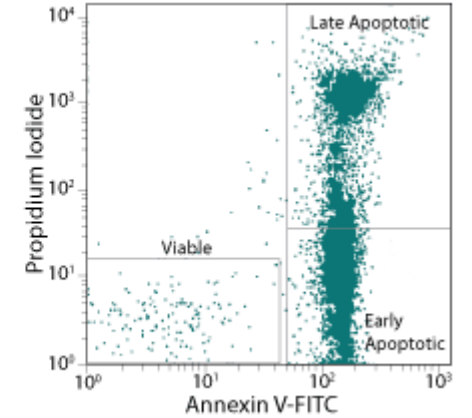
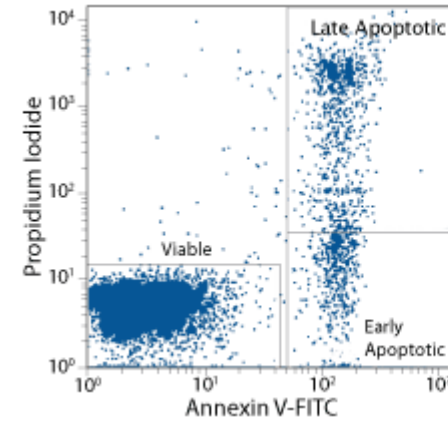
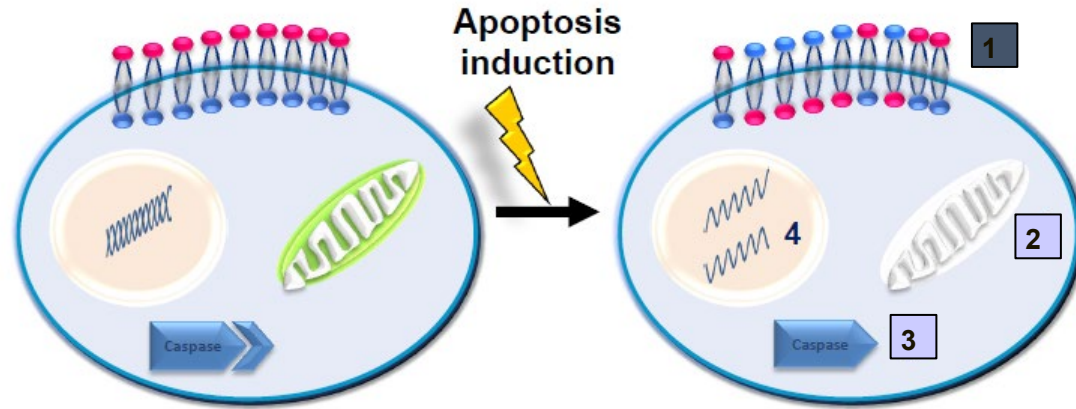


Cell Death

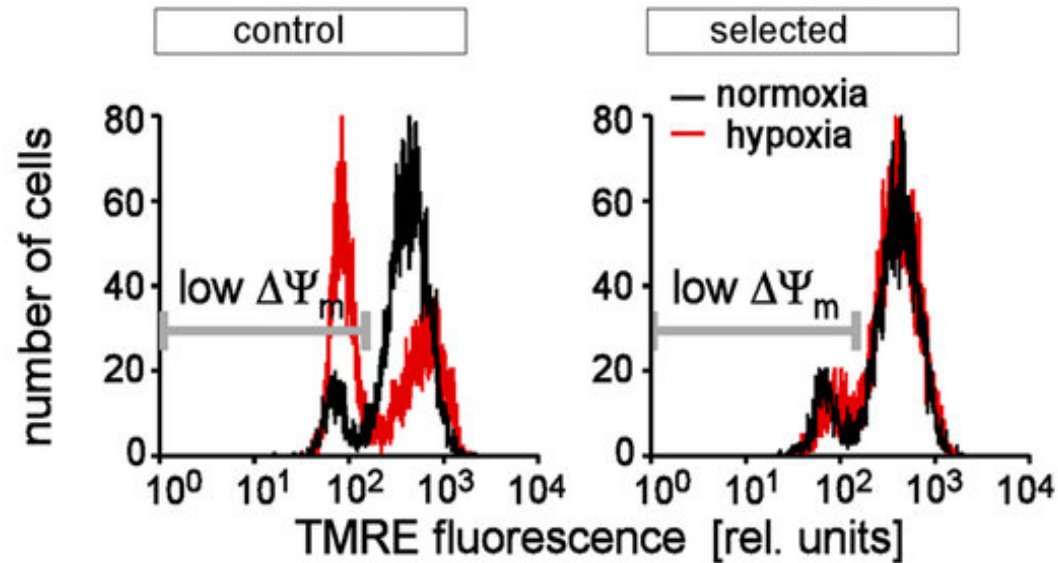
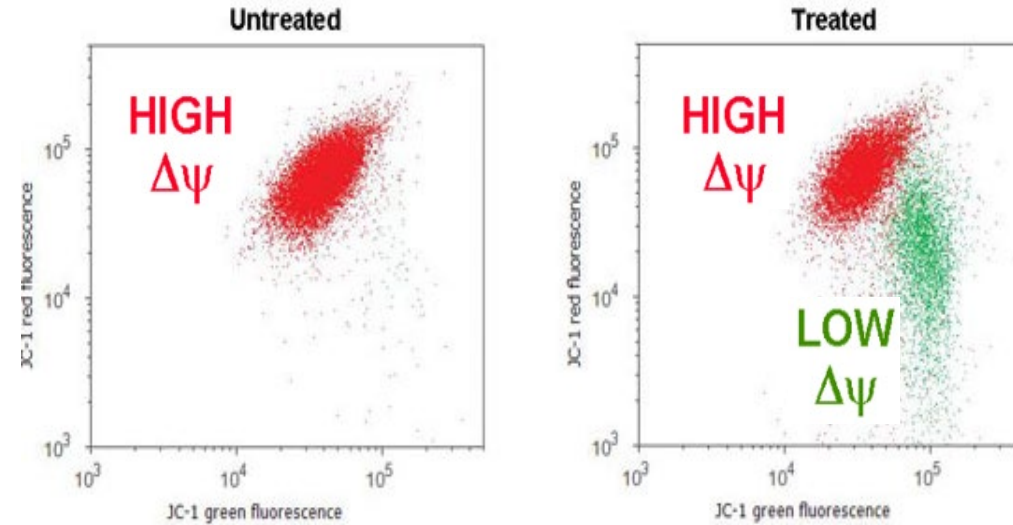
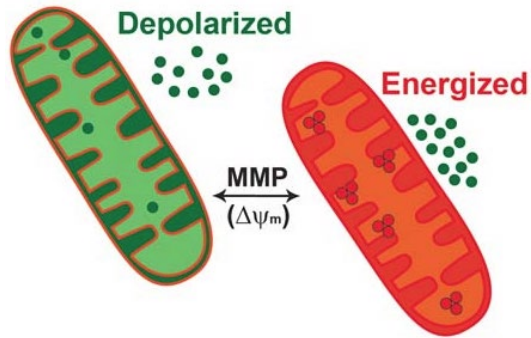
1. Plasma membrane alterations
2. Mitochondrial changes
3. Activation of caspases
4. DNA fragmentation



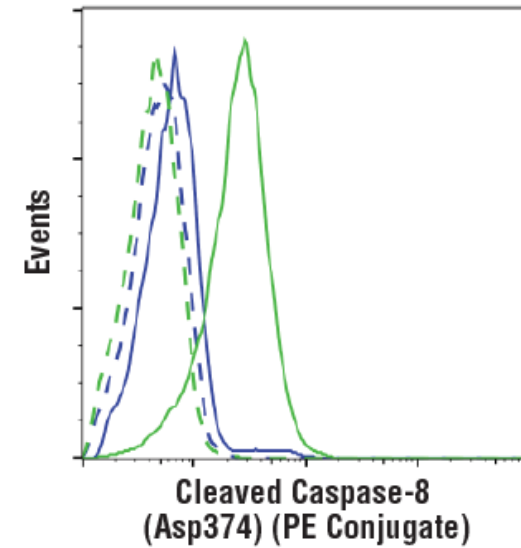
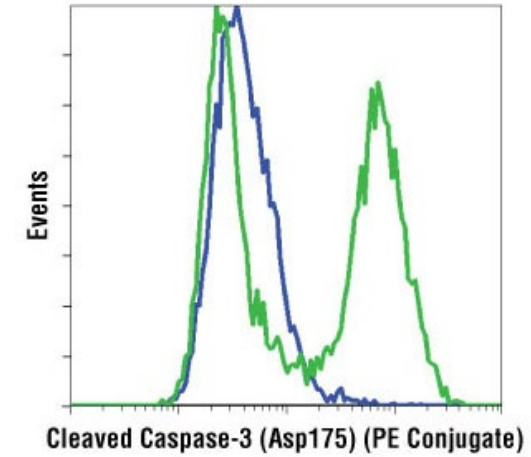
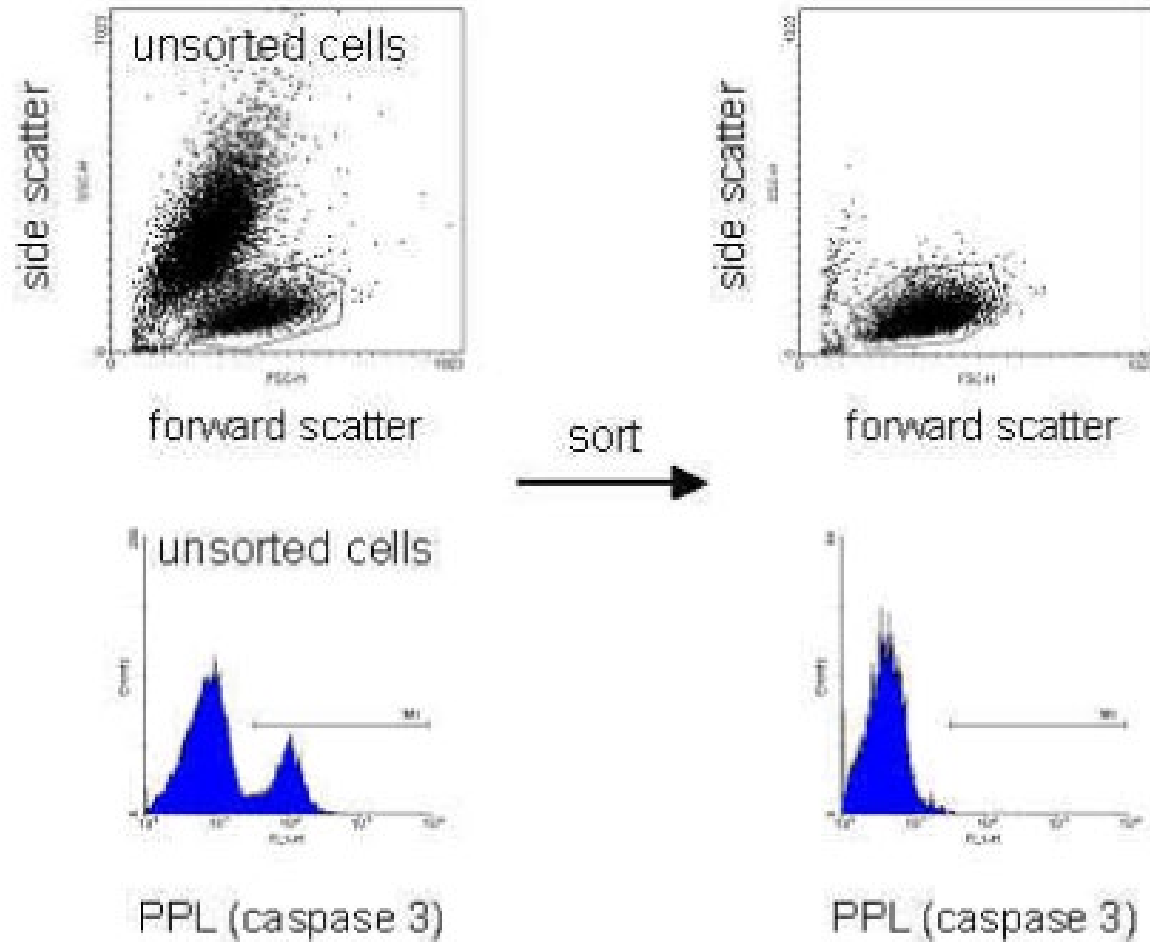
Cell Death: plasmamembrane alterations



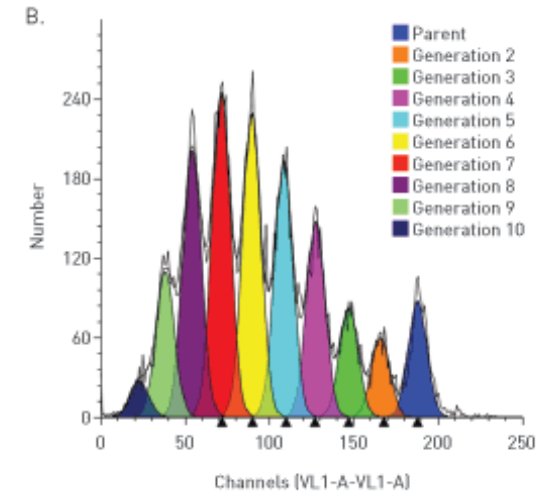
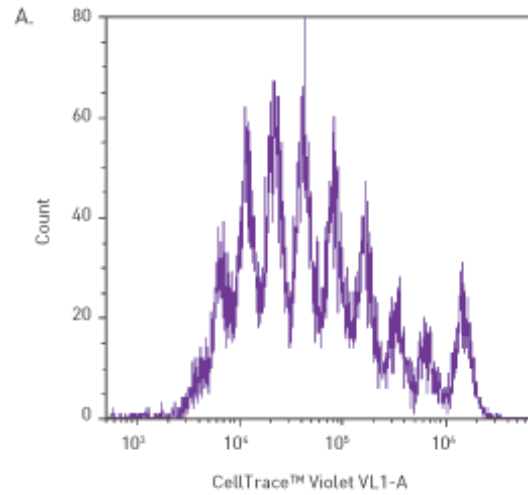
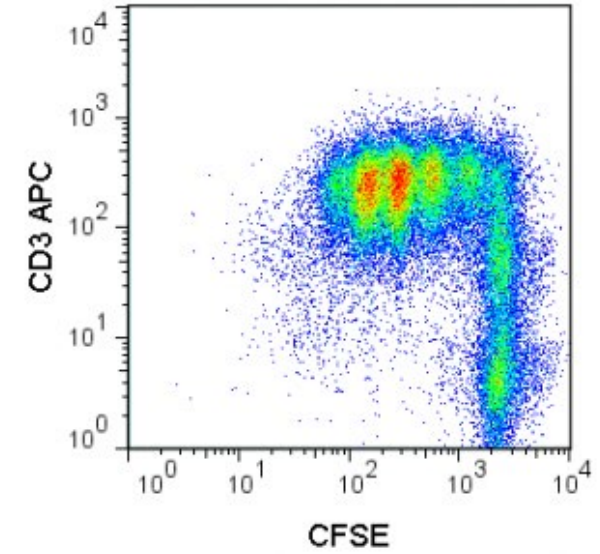
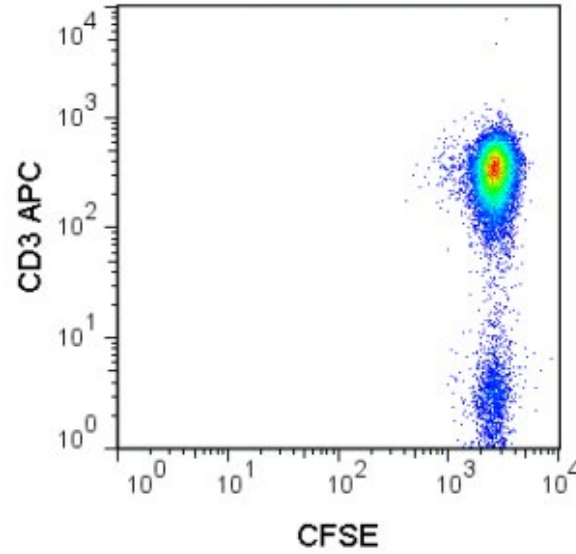
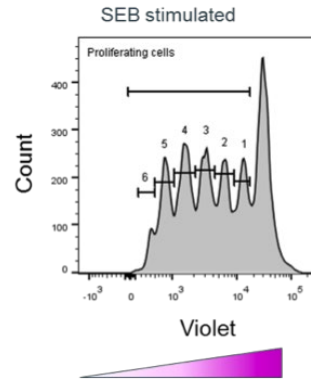
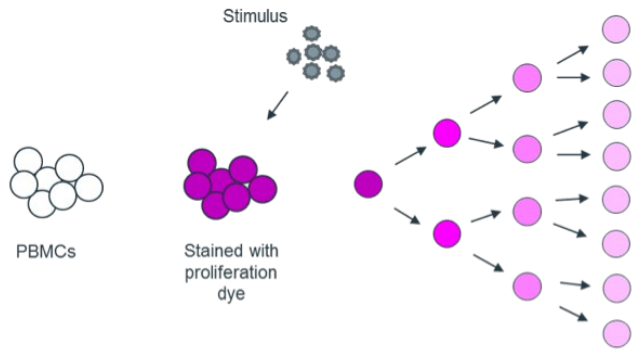
Cell Death: mitochondrial potential changes



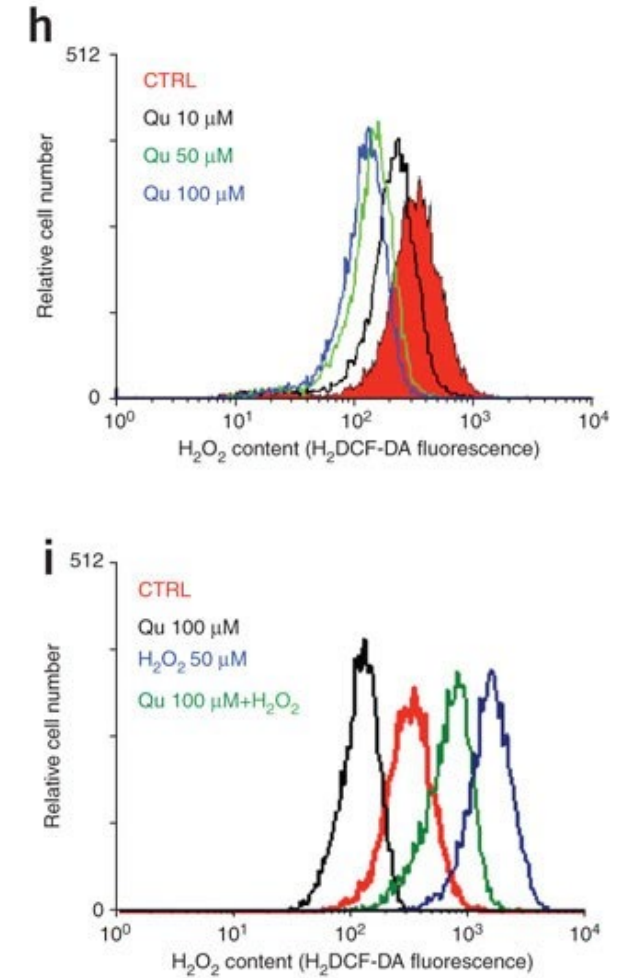
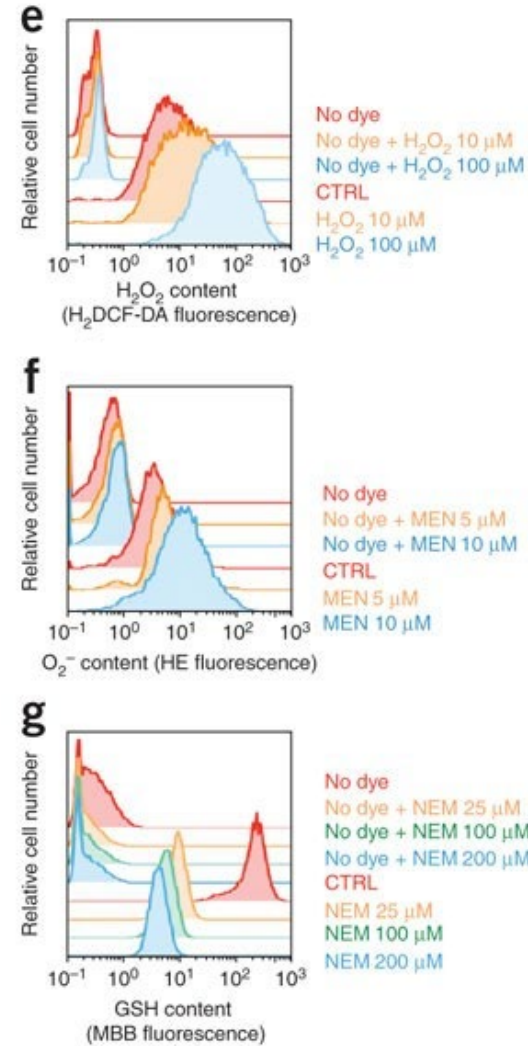
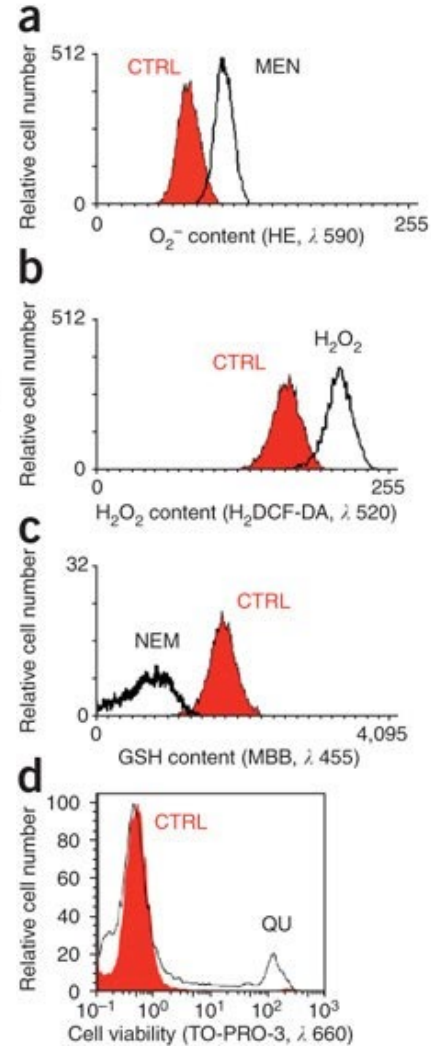
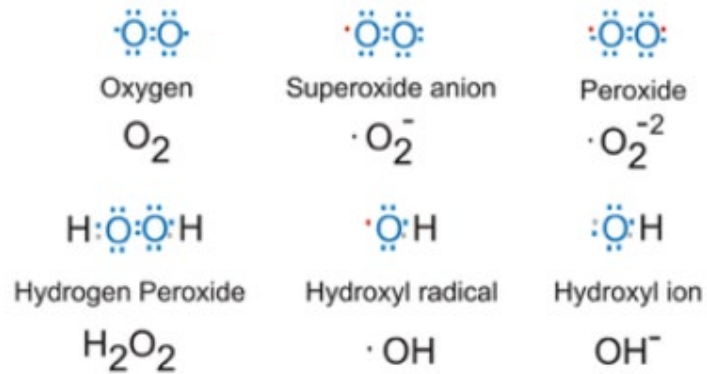
Cell Death: Caspase activation



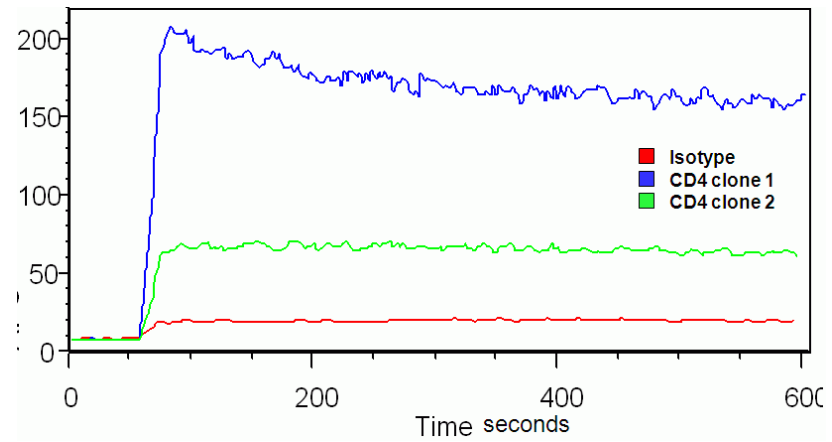
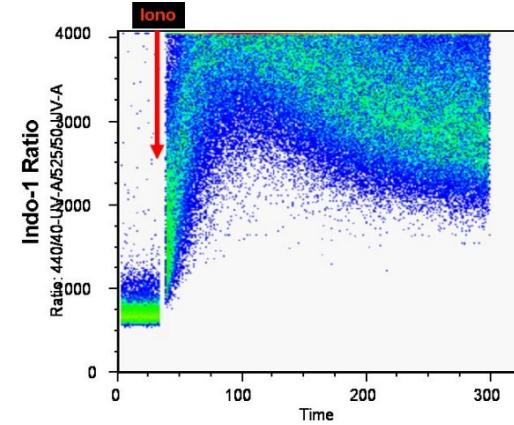
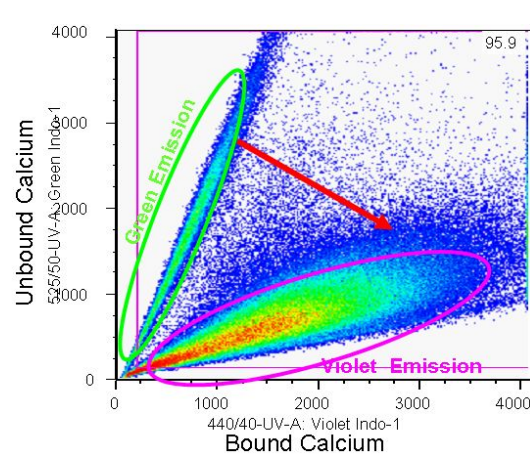
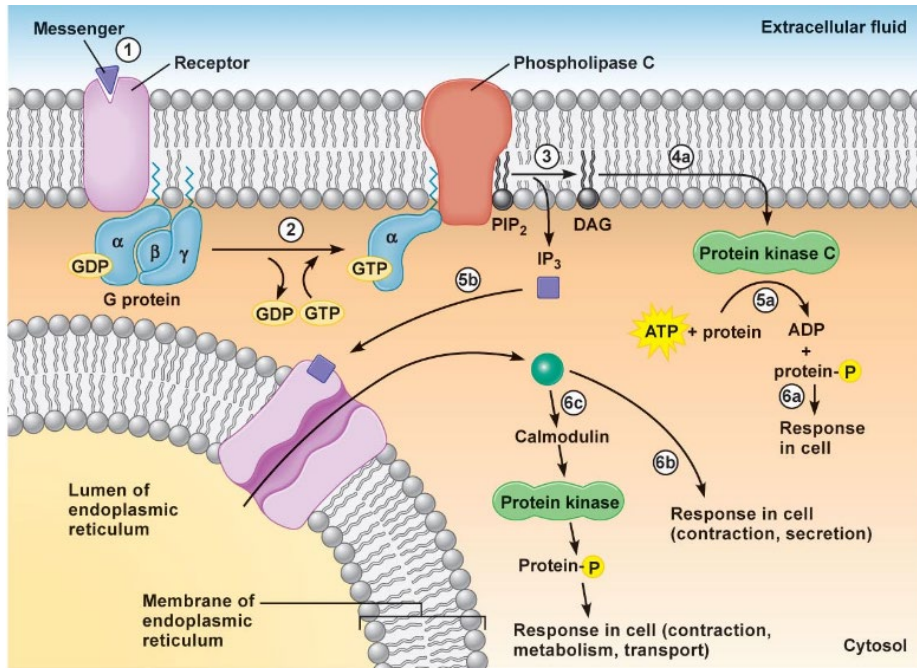
Lymphocytes proliferation



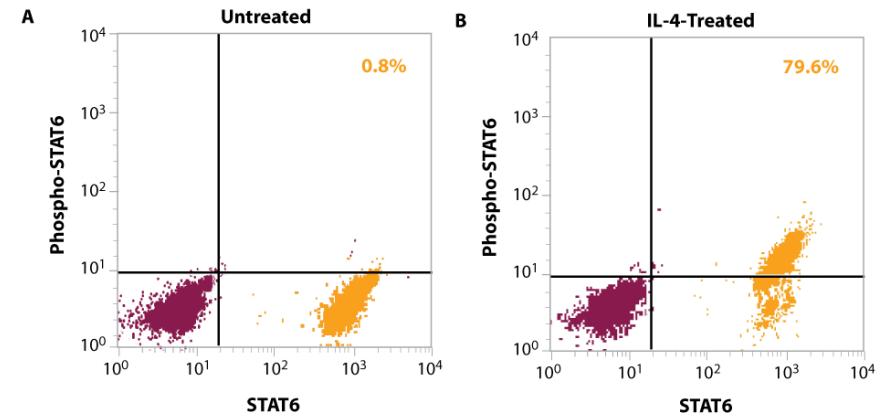
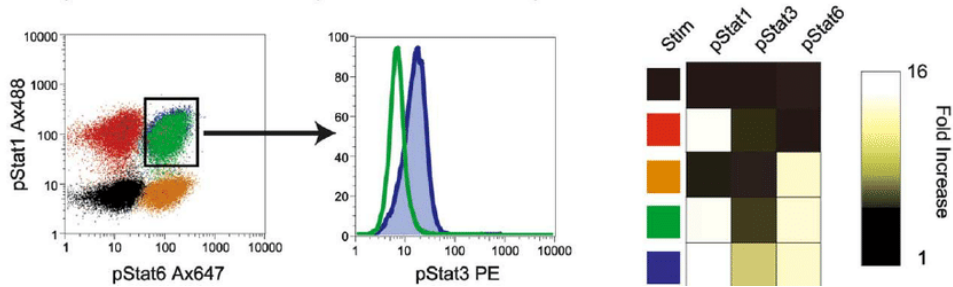
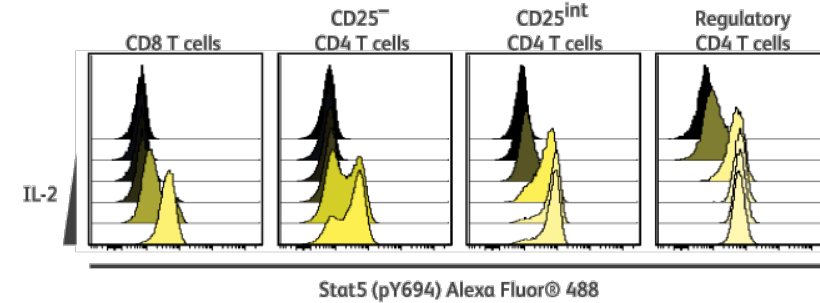
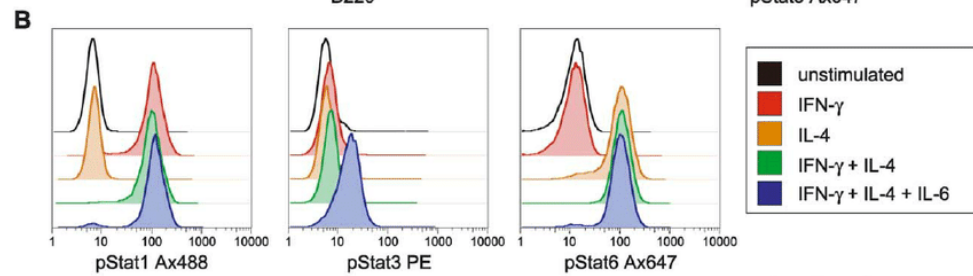
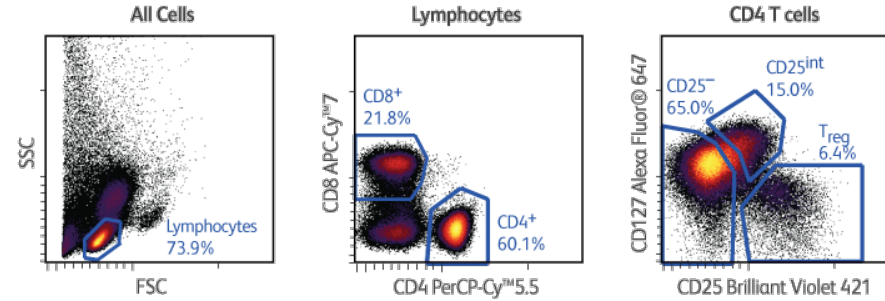
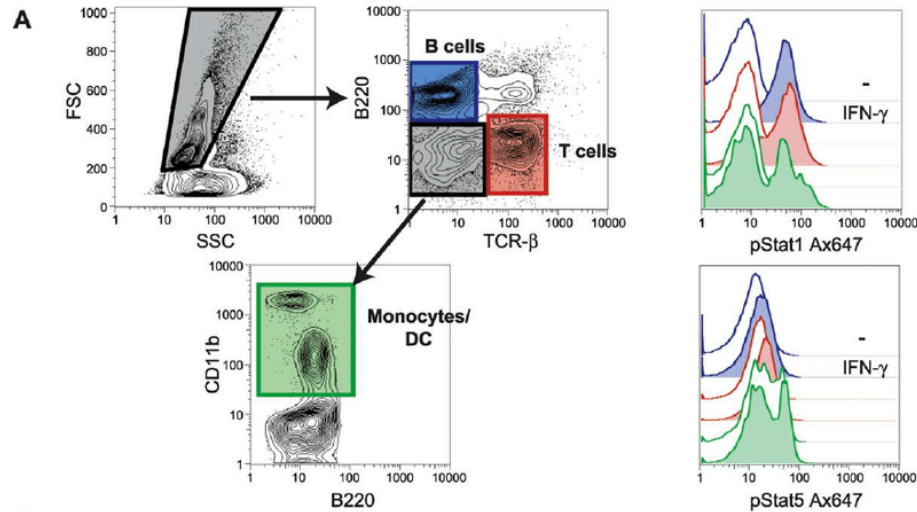
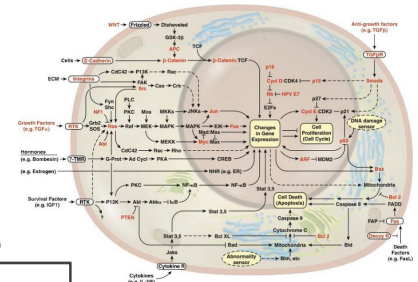
Reactive Oxygen Species (ROS)



Calcium Flux Kinetics

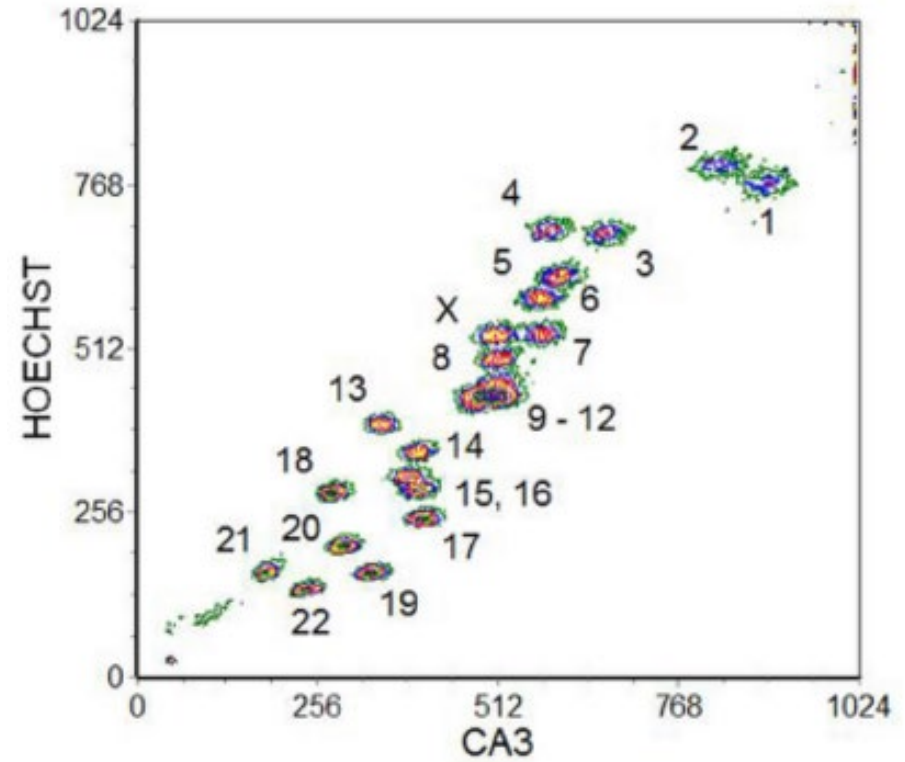
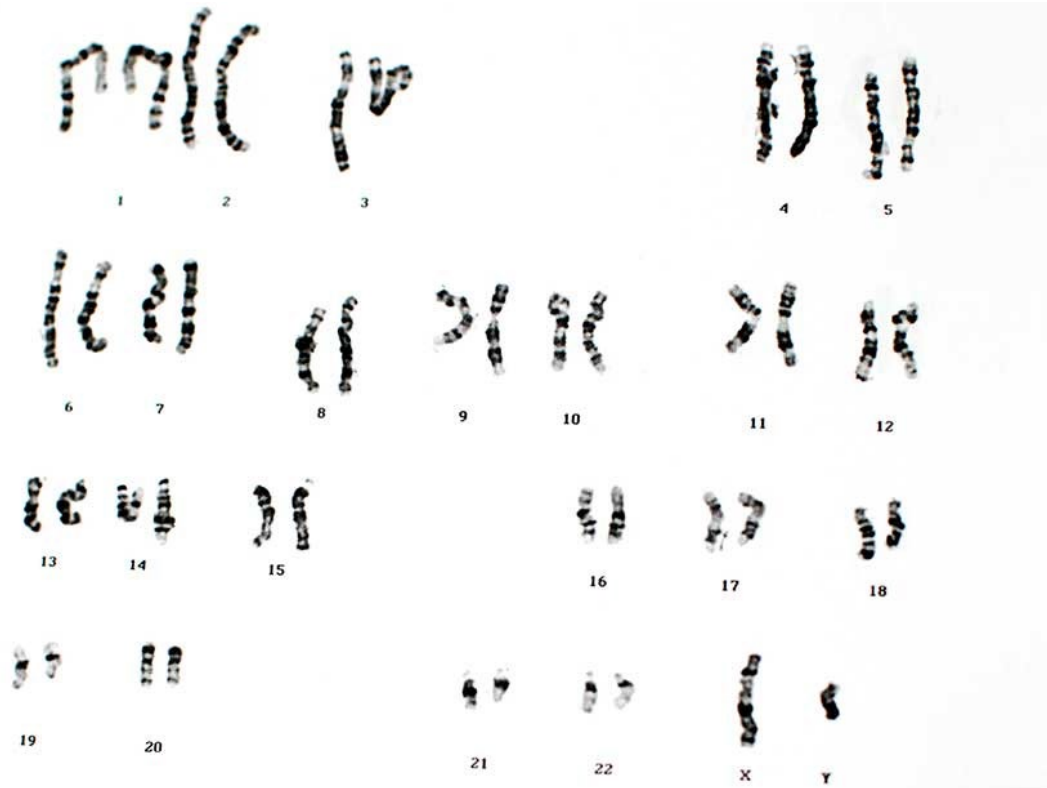


Signal transduction

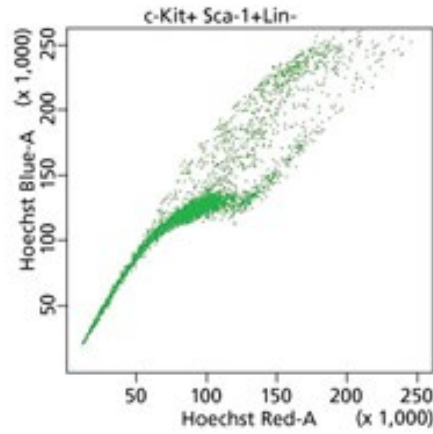
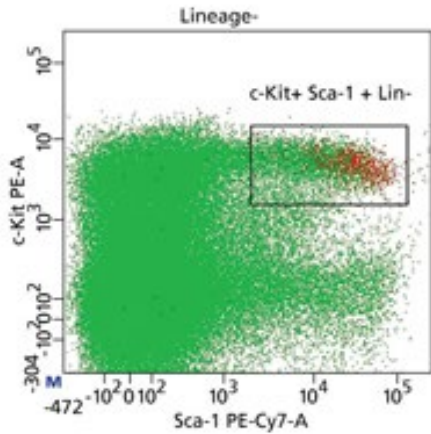
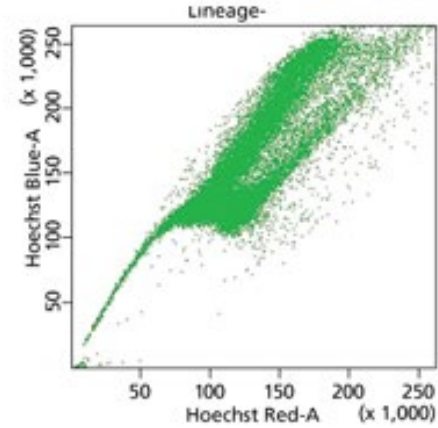
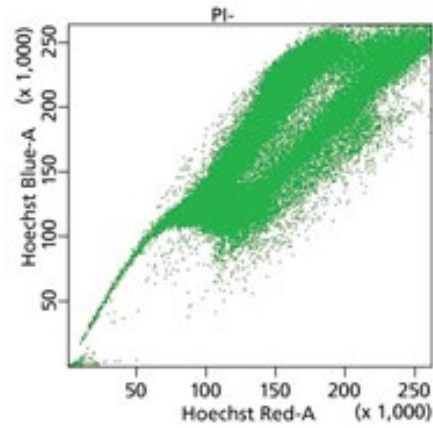
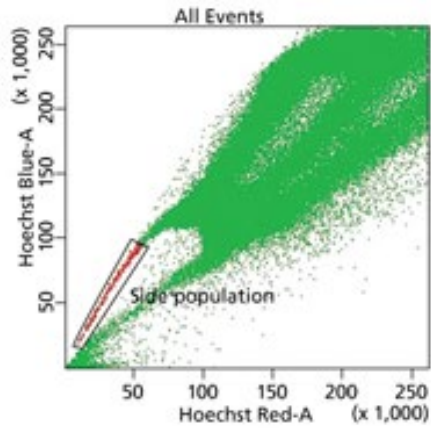


Irish et al. Clin Immunol 2004

Karyotype



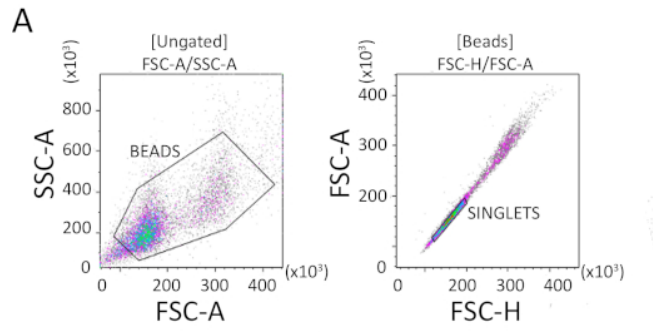
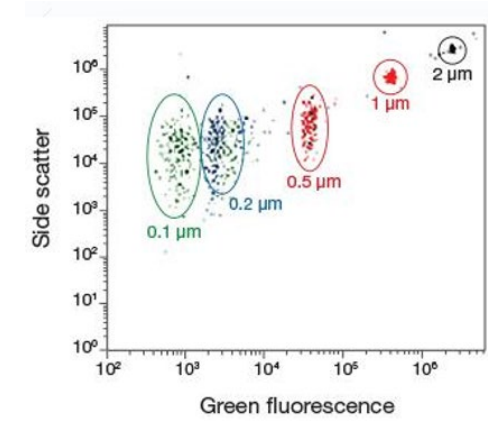
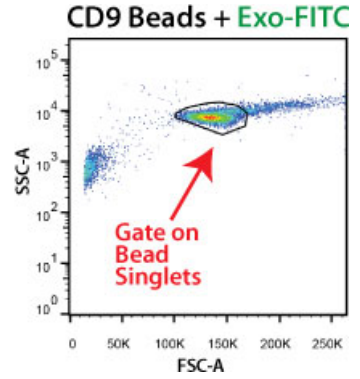
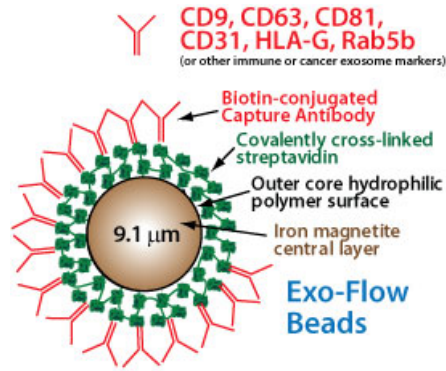
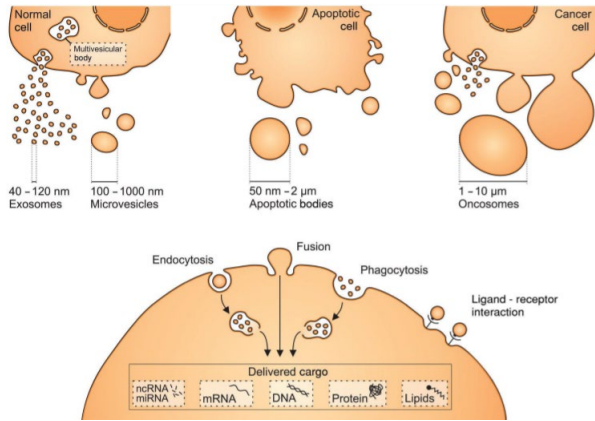
Stem cells



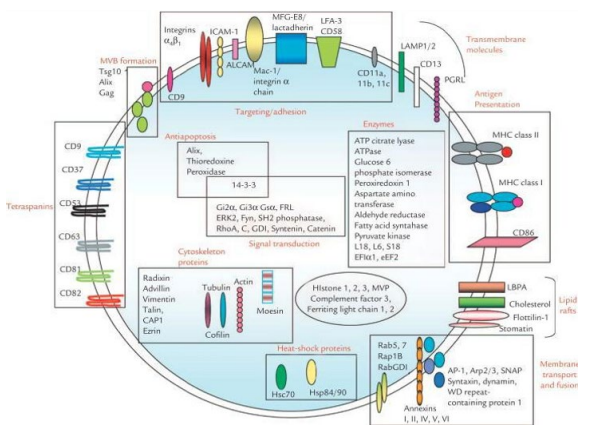
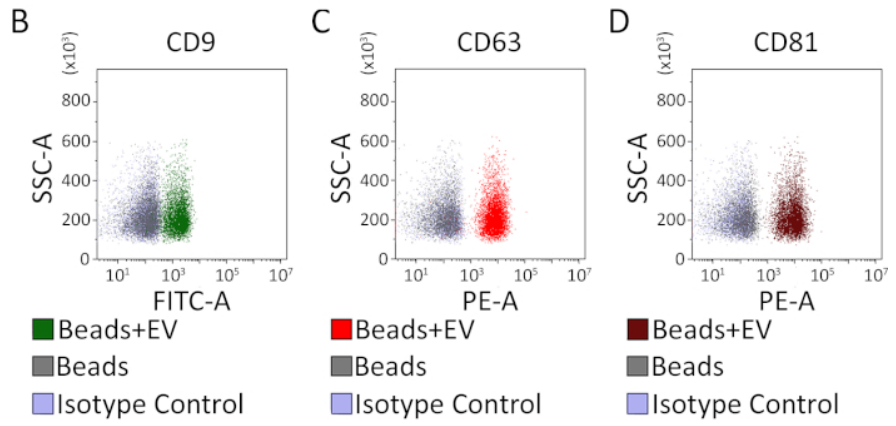
Tube: mouse BM CD34KSL Hoechst 5 *Duglml

Population	#Events	%Parent	%Total
All Events	1,000,000	###	100.0
Cells	859,338	85.9	85.9
PI-	808,841	94.1	80.9
Doublet	800,858	99.0	80.1
CD45+	799,149	99.8	79.9
Lineage-	790,724	98.9	79.1
c-Kit+ Sca-1 + Lin-	144,071	18.2	14.4
Side population	4,610	3.2	0.5
c-Kit+ Sca-1 + Lin- AND Side population	698	0.5	0.1
c-Kit+ Sca-1 + Lin- AND Side population	665	0.5	0.1

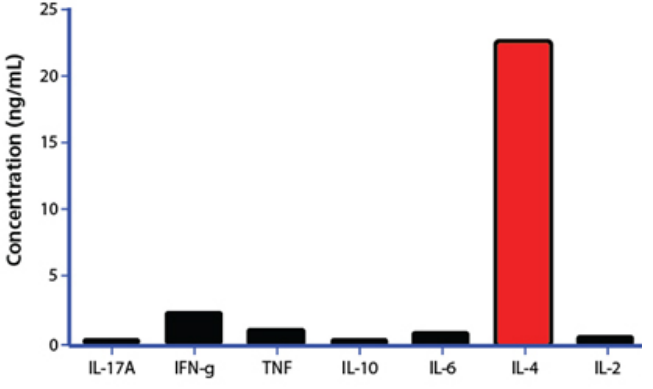
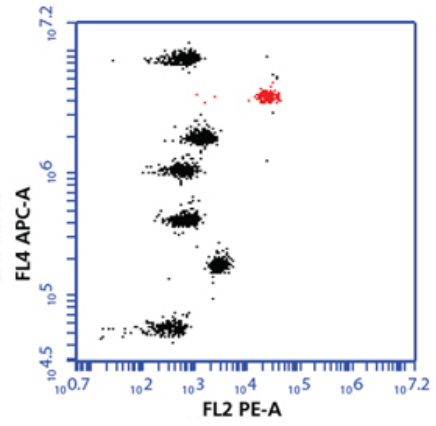
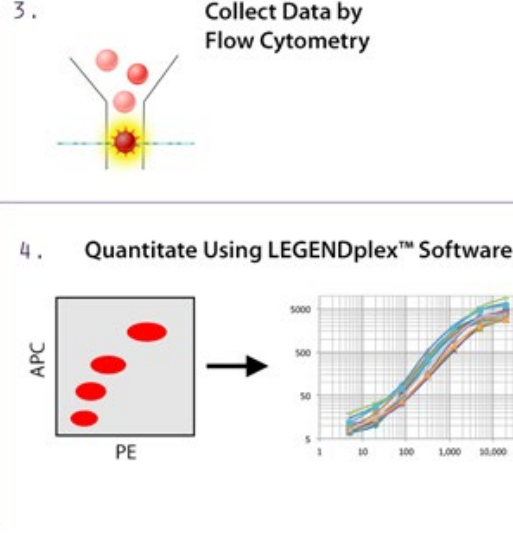
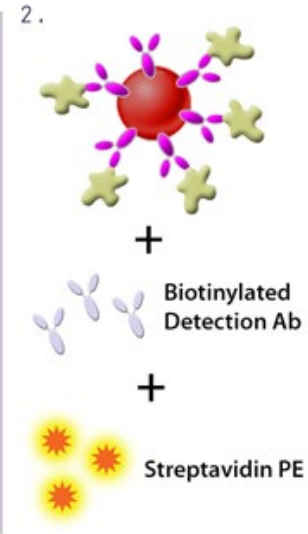
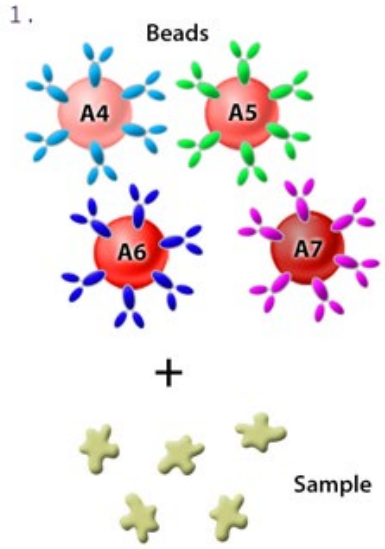
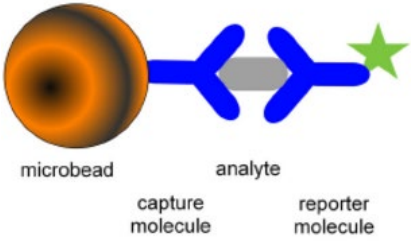
Extracellular Microvesicles and Exosomes



Balbi C et al. JoVE 2019



Cytometry Beads Array (CBA)

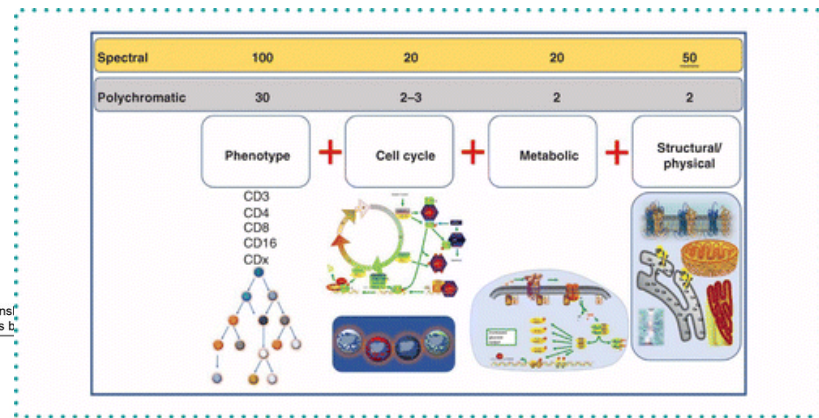


Future perspective: where FC is going?

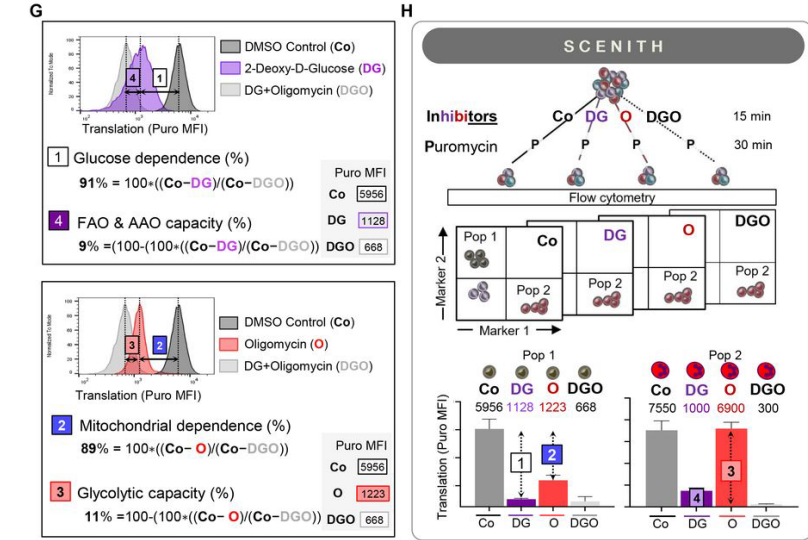
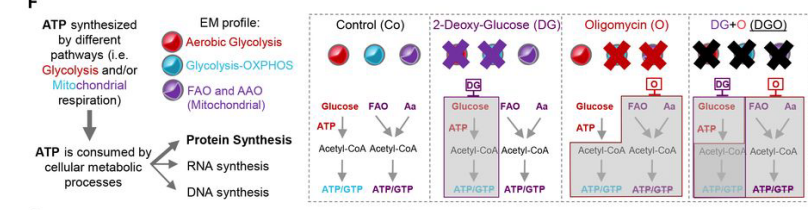
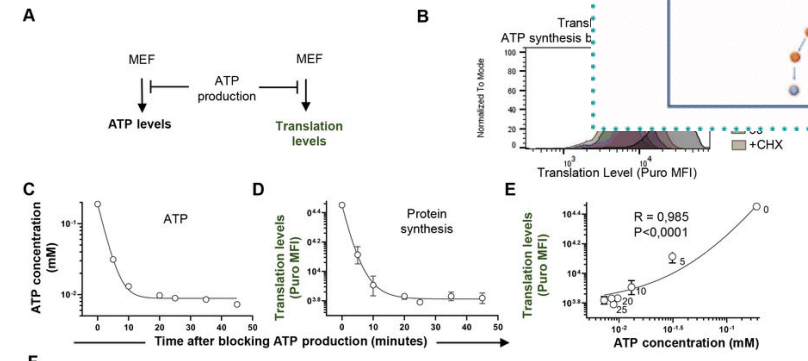
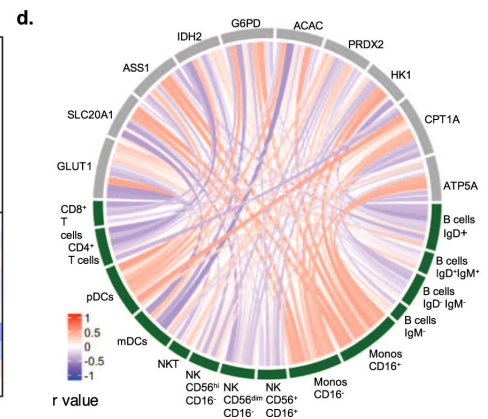
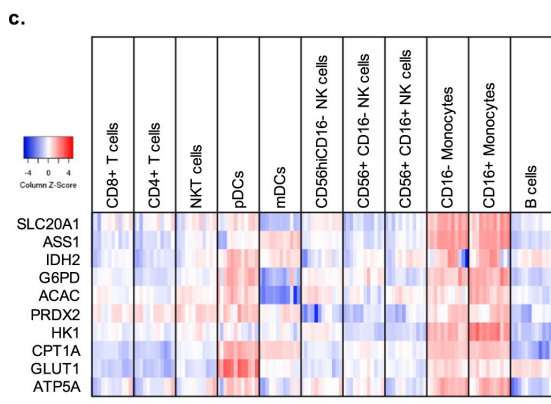
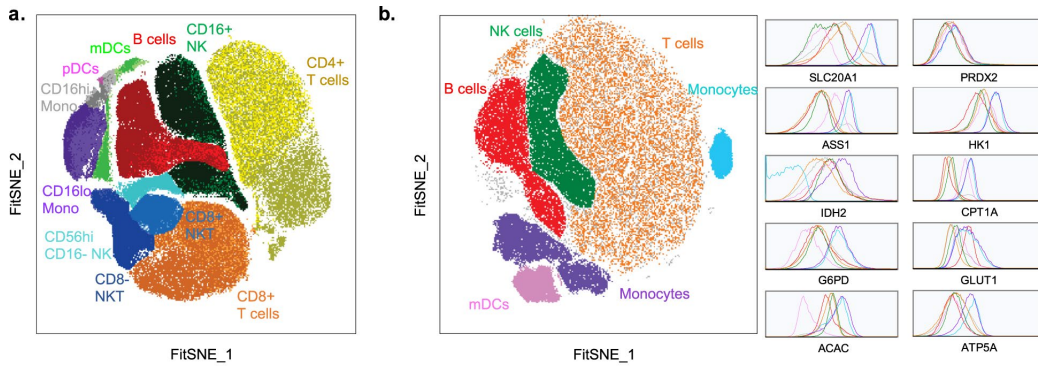
- Flow Cytometry has a bright future because it is the only viable technology for comprehensive and multiplexed analysis of single cells.
- With the latest spectral cell sorting technology, a single cell identifiable only by a complex algorithm, can be separated for cloning or sequencing.
- The advantage of being able to use any dye under almost any circumstance is a game-changing feature of spectral flow cytometry.
- There is no longer an issue with using dyes with almost overlapping spectra.
- Current spectral instruments have many advantages over polychromatic instruments but still suffer from some of the same problems, including background noise, lack of true calibration capacity and operation that remains in the analog domain.
- The next generation will function entirely in the digital domain, have features currently available only on custom-built instruments and have the capacity for potentially 150–200 parameters, which will engage metabolic, structural and functional probes, extending the field for several decades.

Future perspective: where FC is going?

Met-Flow, a strategy for single-cell metabolic analysis



SCENITH



Arguello RJ et al. Cell Metab 2020

Ahl PJ et al. Nature 2020

Thank you!