NASDC

NCI Awardee Skills Development Consortium

Flow Cytometry in a Nutshell

Rui Gardner

Head, Flow Cytometry Core Facility

Memorial Sloan Kettering Cancer Center

Flow Cytometry Core Facility - Analyzers

Polychromatic

#3 Fortessa/LSRII

Up-to 18 colors

#1 CytoFLEX LX

Up-to 21 colors

Spectral

#2 Aurora

40+ colors

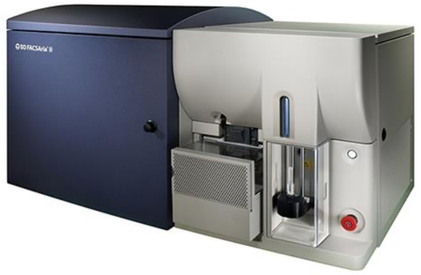
Flow Cytometry Core Facility - Sorters

Spectral

Polychromatic

#2 FACSymphony S6

Up-to 18 colors



#5 FACSAria

Up-to 18 colors

#2 SH800

Up-to 6 colors

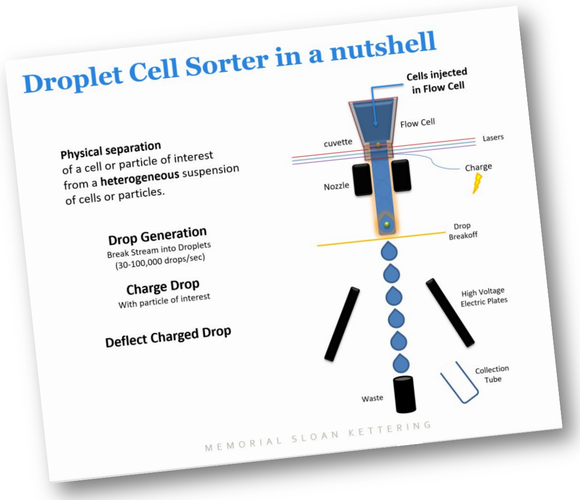
Available 10am-8.30pm

#4 Operator Assisted Sorter

#5 Self-Operated Sorters

Available 24/7

Flow Cytometry Core Facility – Edu & Training



Current Training & Courses:

- Principles of Flow Cytometry
- Multicolor Panel Design
- The art of (self) sorting
- Best Practices in Publishing Flow Cytometry Data
- Software analysis with FlowJo/FCS

Training & Courses (203):

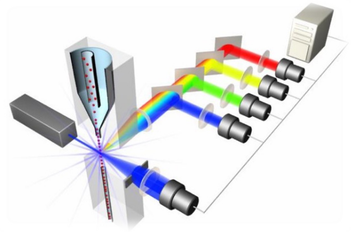
- New modules for Multicolor Flow Cytometry
- Multidimensional Data Analysis
- Cell Cycle, Proliferation, and Death

Educational Material:

- https://expertcytometry.com/

EXCYTE
EXPERT CYTOMETRY

What is Flow Cytometry?



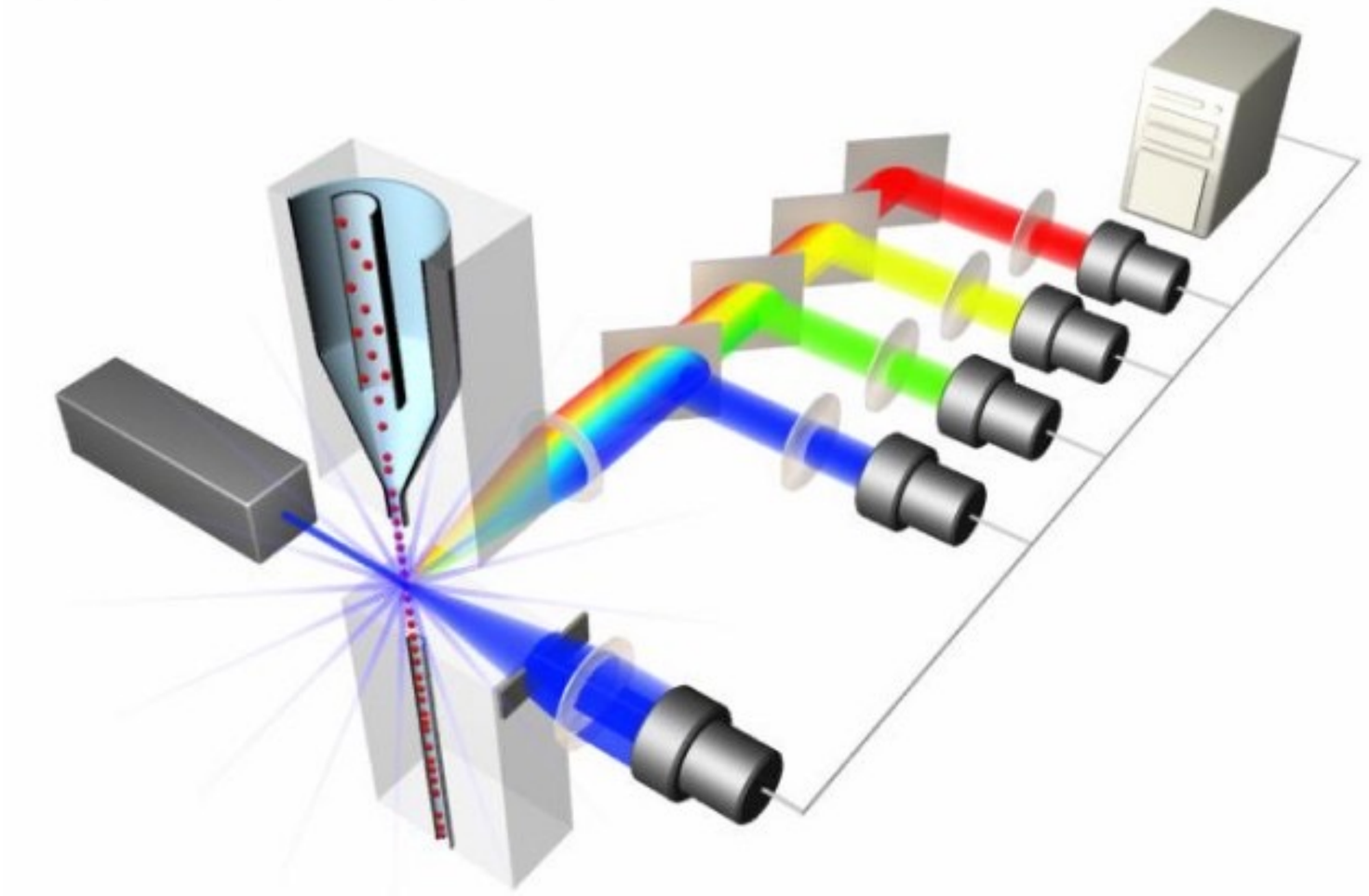
Cyto – metry: Measurement of physical and chemical properties of cells
(Cell) (measurement)

Flow Cytometry: Characterization of cells flowing in a stream of fluid

What is Flow Cytometry used for?

» Flow cytometry is typically used to count large numbers of cells or particles based on size, internal complexity, phenotype, cellular state, cell function, DNA content, gene expression, and to quantify these same cellular properties at a single-cell level.

Flow Cytometer in a nutshell



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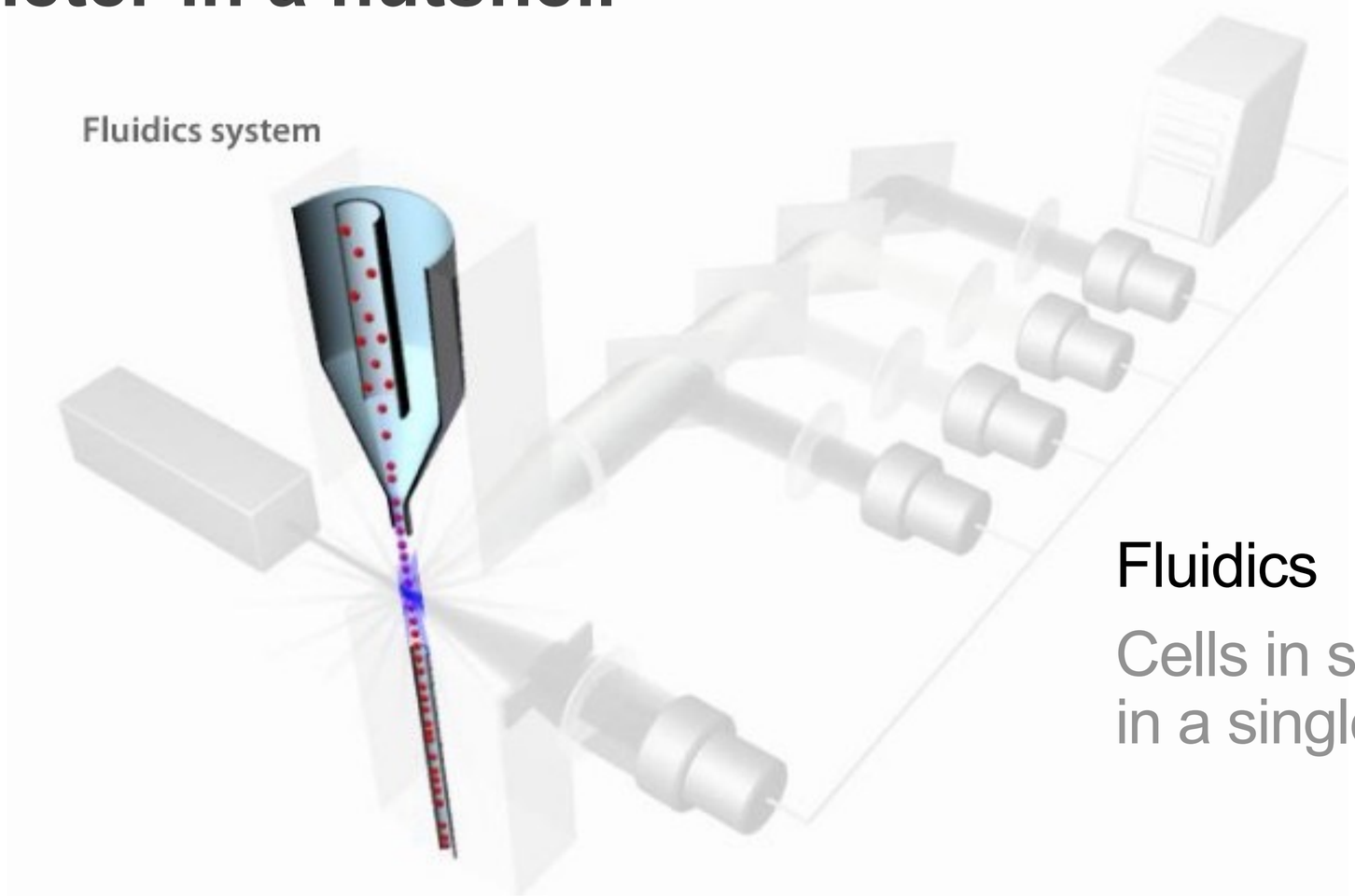
Flow Cytometer in a nutshell



Fluidics

Cells in suspension flow in a single file through…

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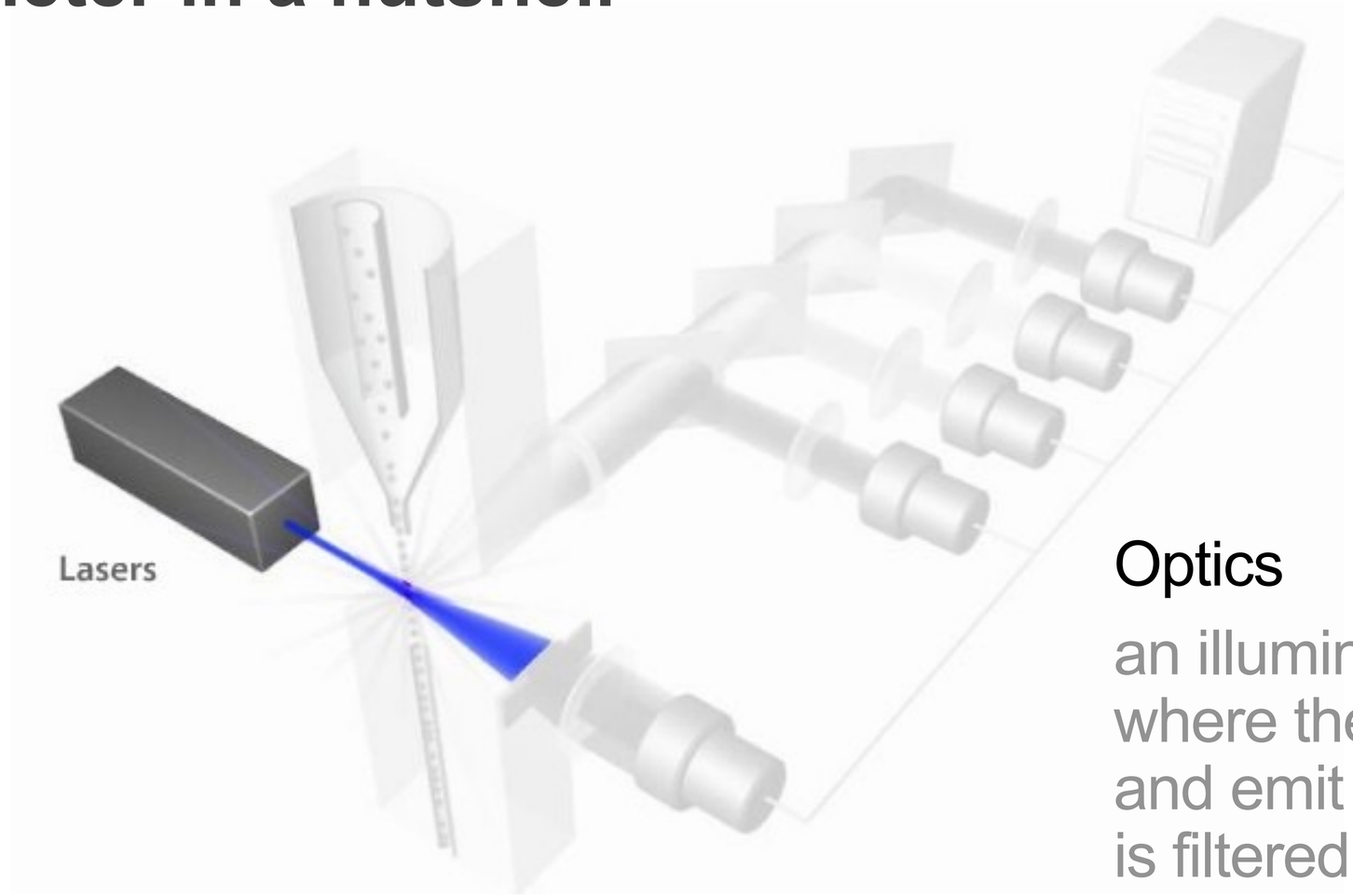
Flow Cytometer in a nutshell



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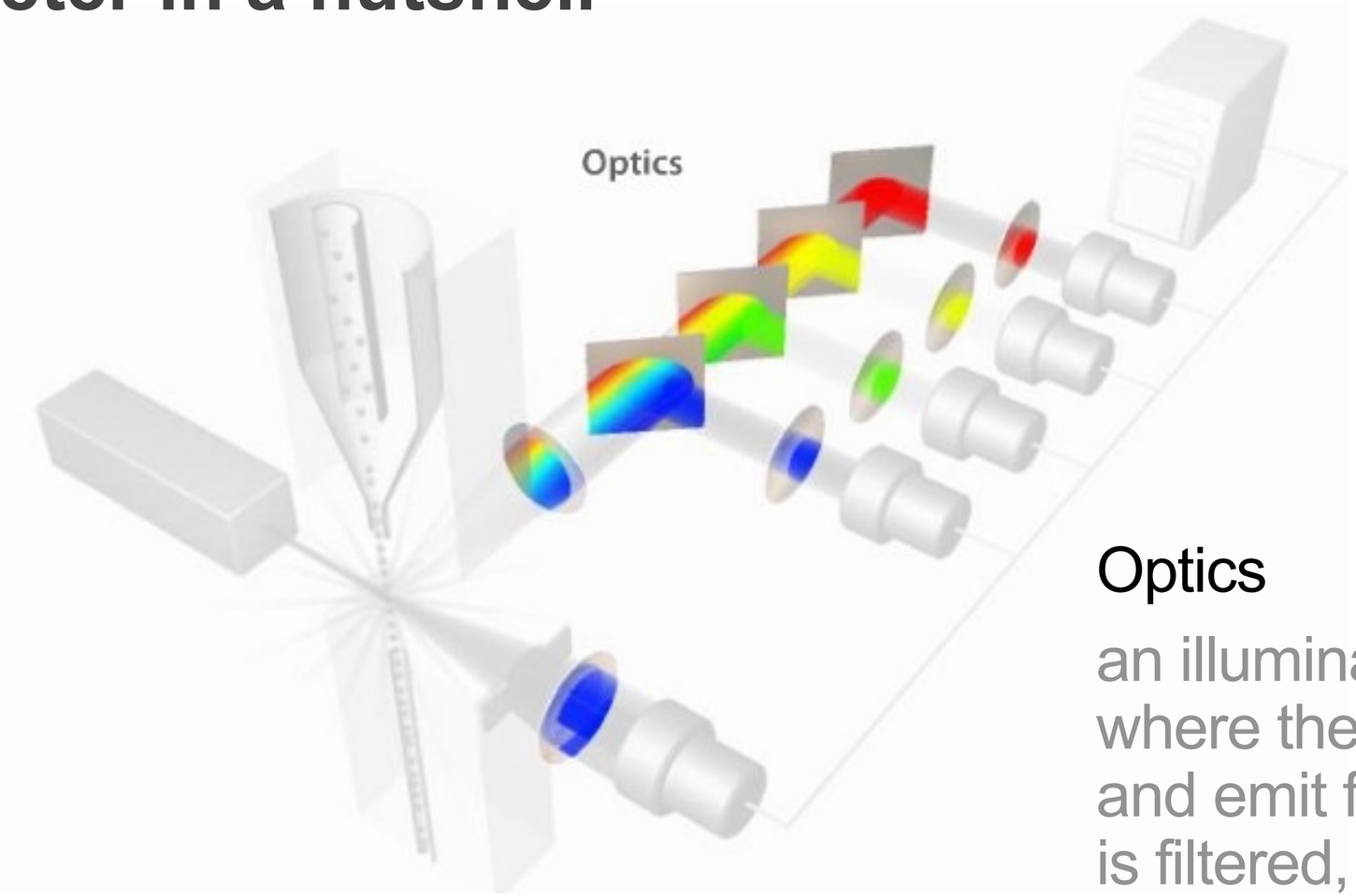
Optics

an illuminated volume where they scatter light and emit fluorescence that is filtered, collected, and…

Flow Cytometer in a nutshell



Optics

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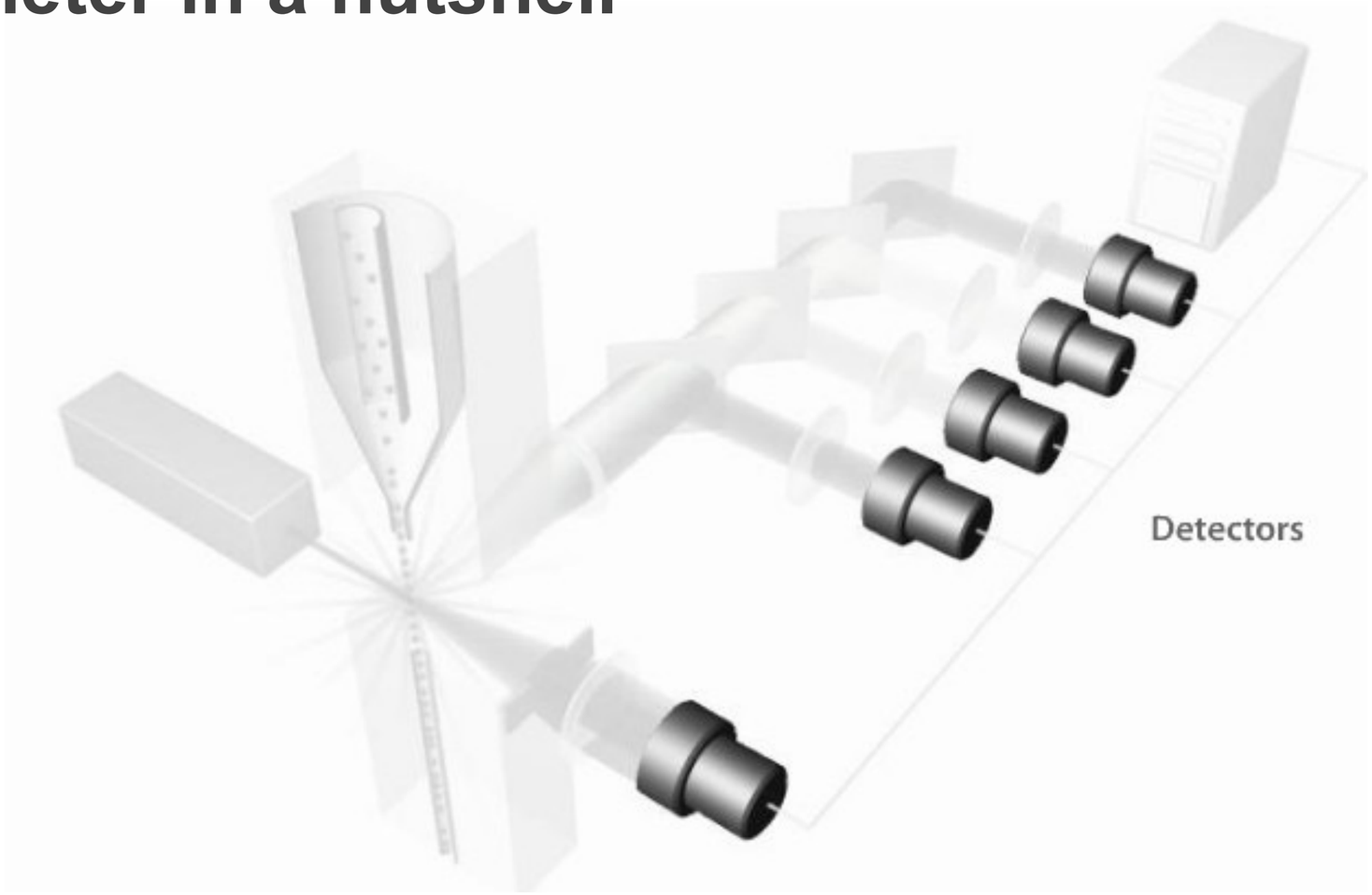
Flow Cytometer in a nutshell



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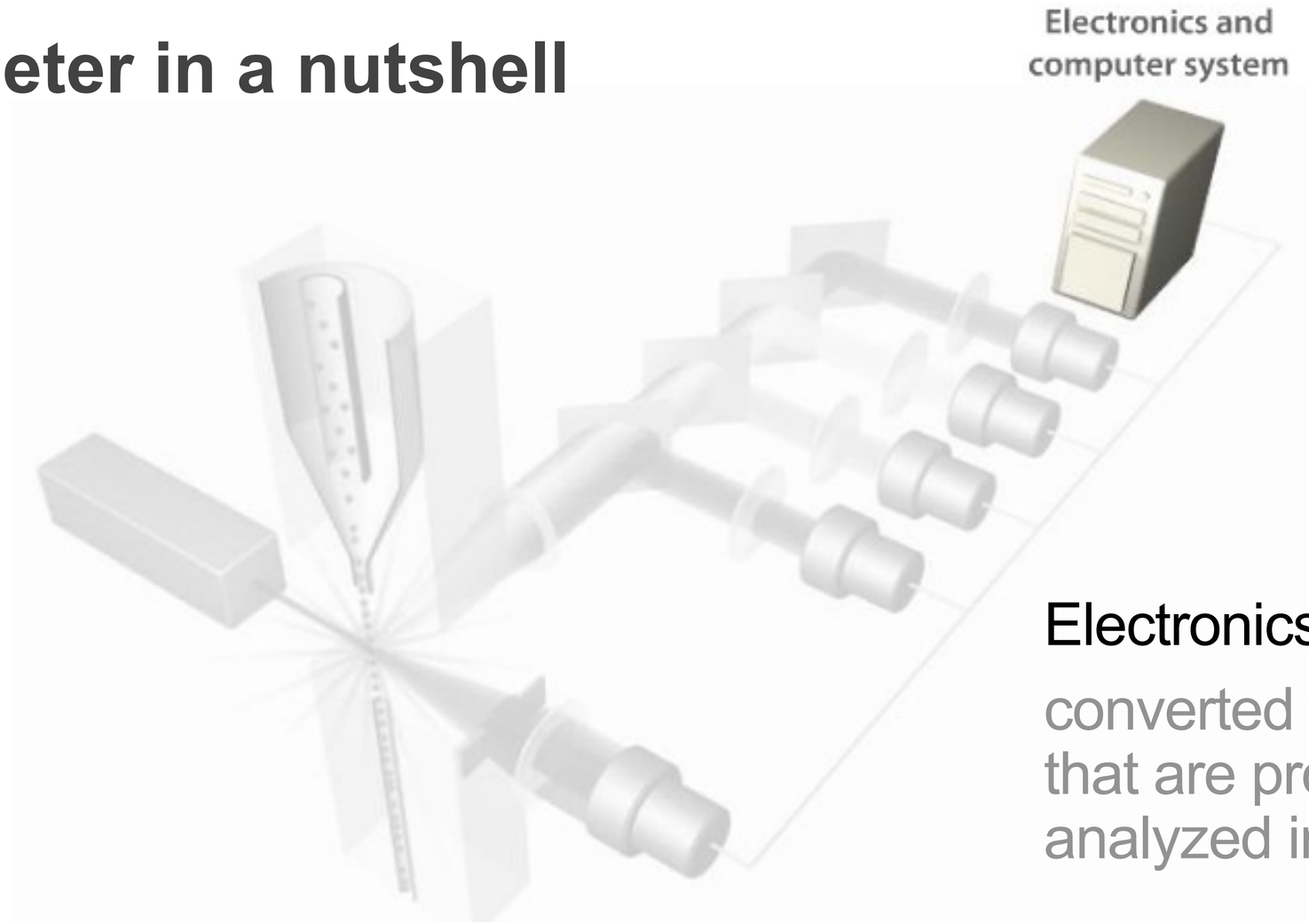
Flow Cytometer in a nutshell



Electronics

converted to digital values that are processed and analyzed in a computer

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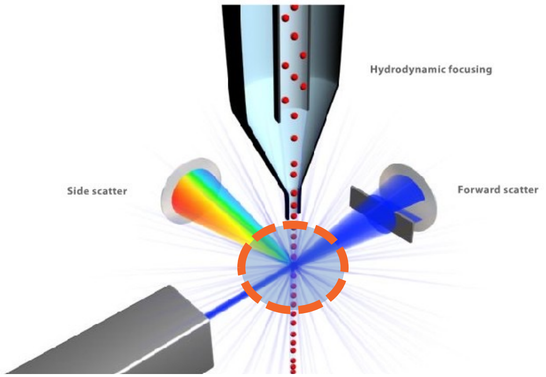
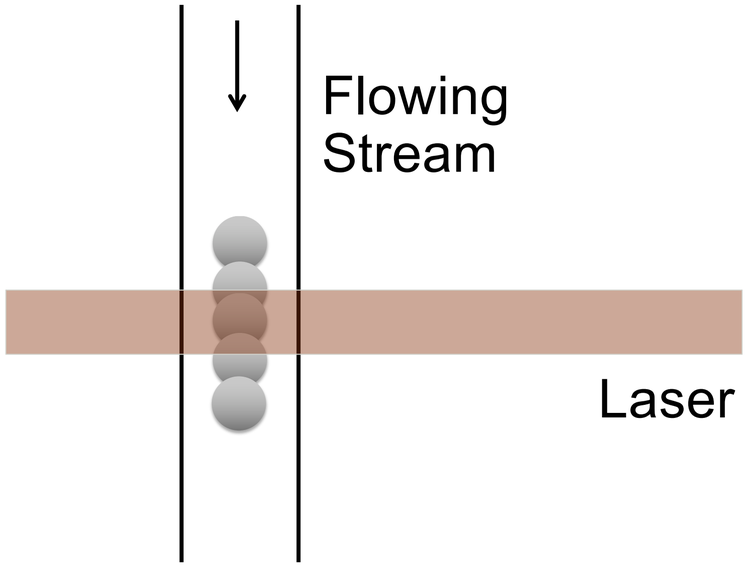
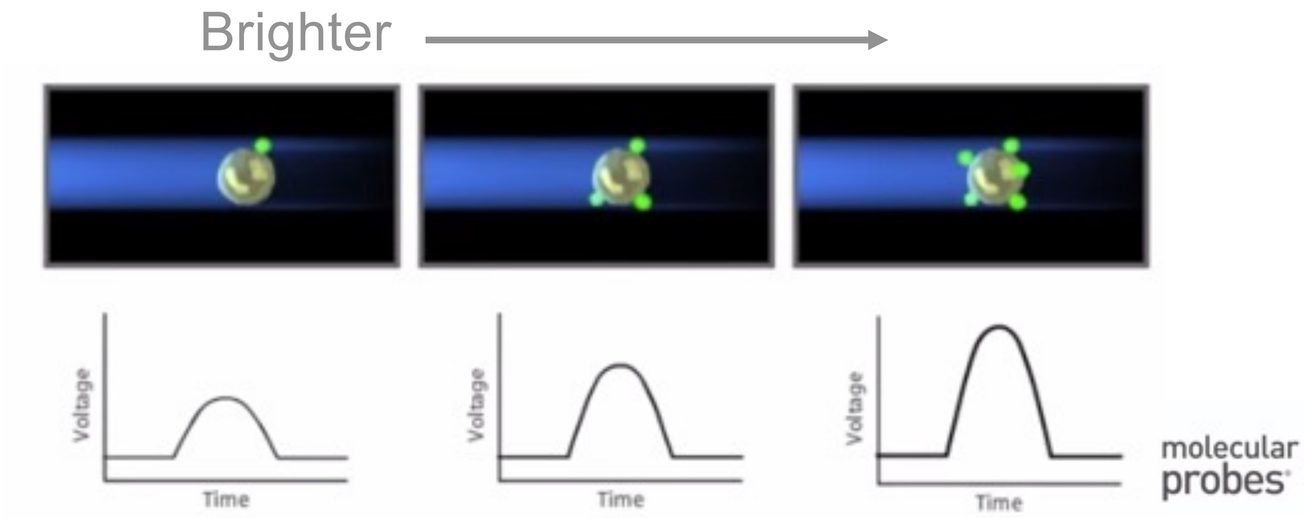
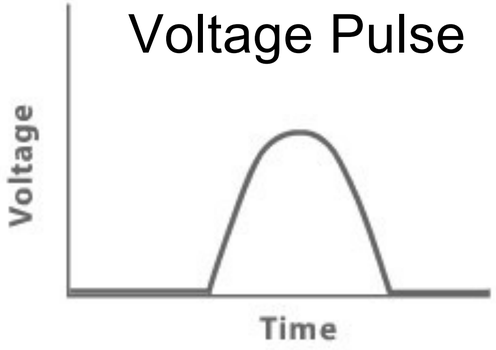
Measuring Cell Properties





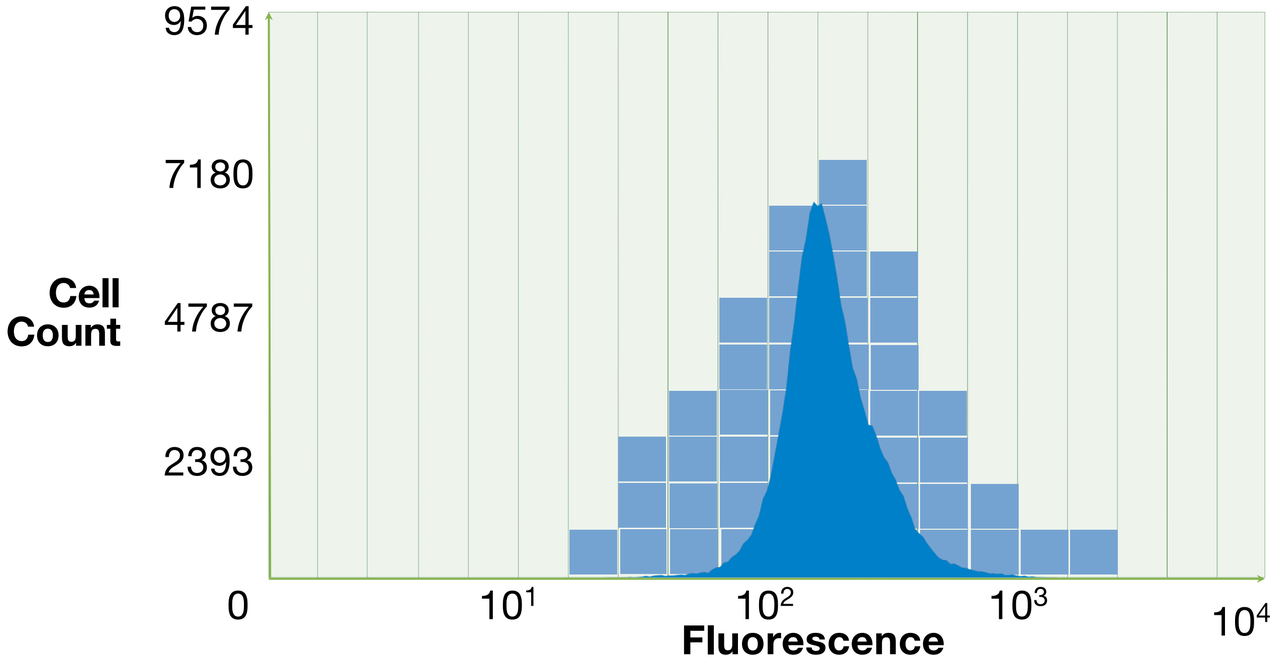




Measuring Cell Properties

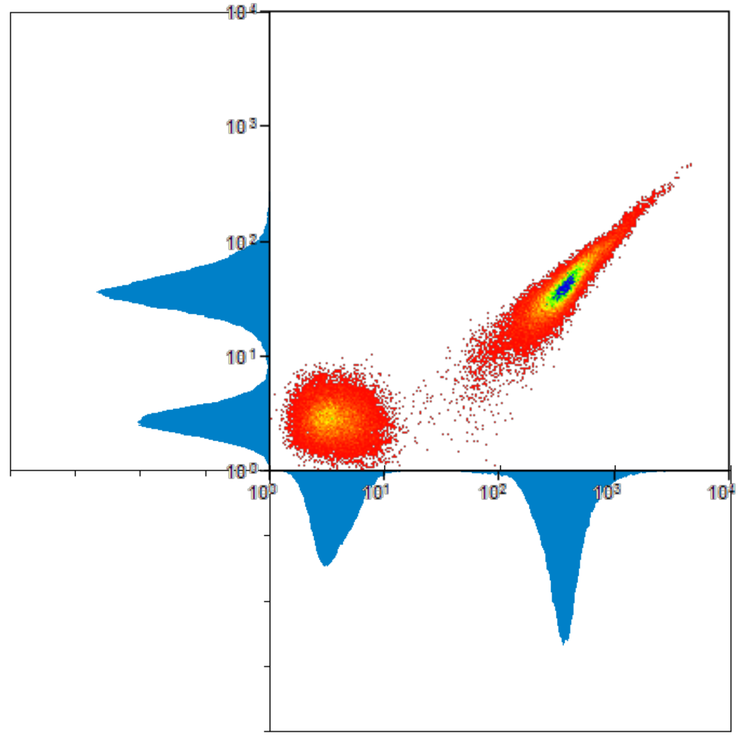
1-D Histogram

Fluorescence/cell

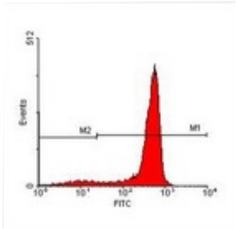


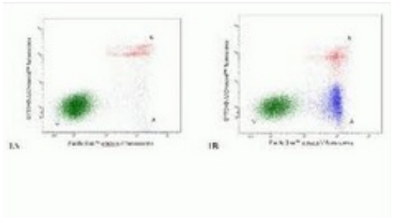
2-D Histogram

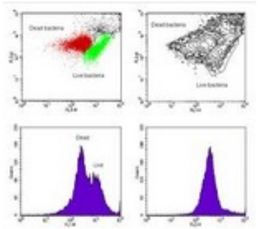
Fluorescence1 vs Fluorescence2

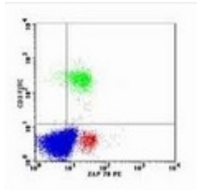


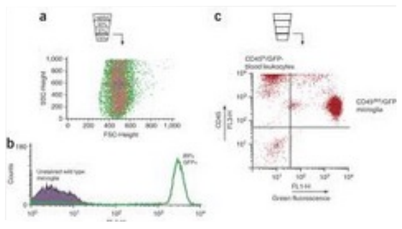
Measuring Cell Properties

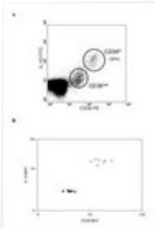


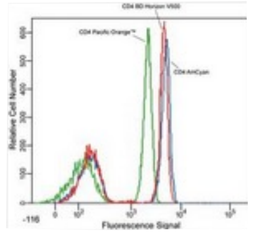


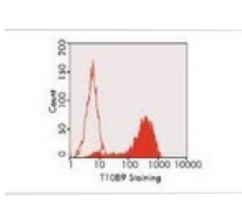


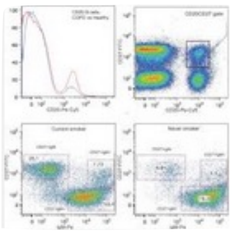


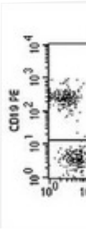


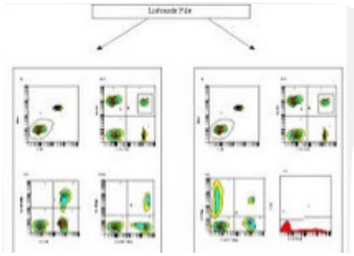


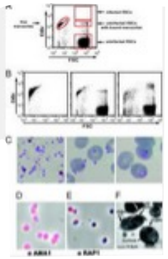


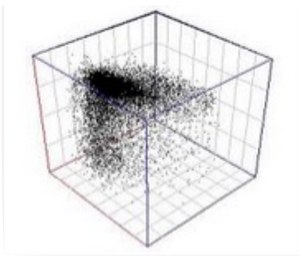


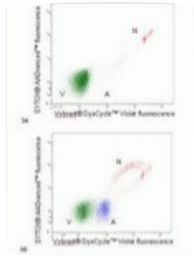


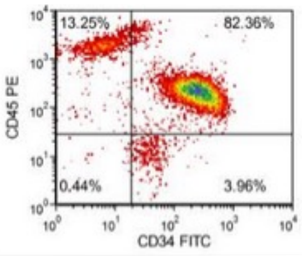


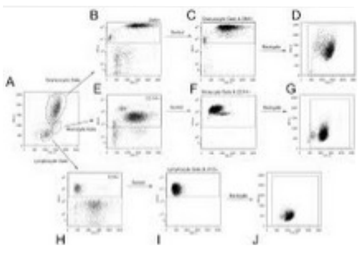


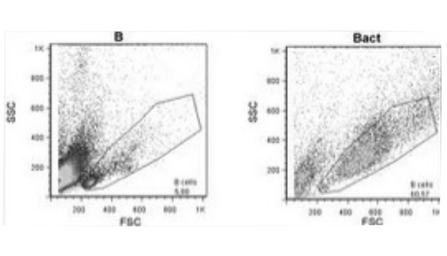


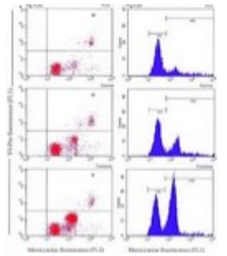


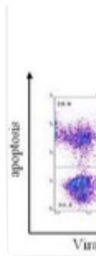


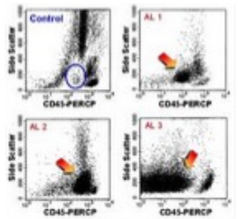


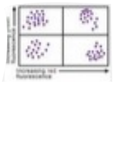


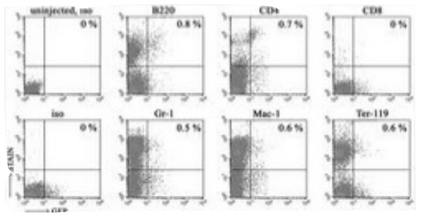


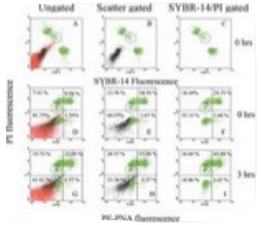


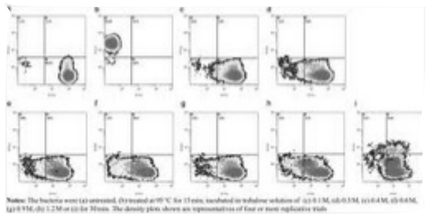


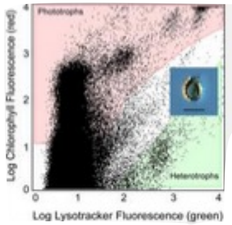


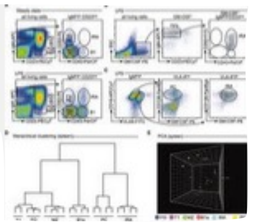


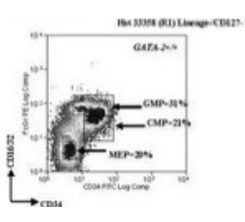


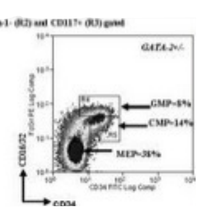


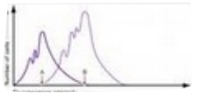


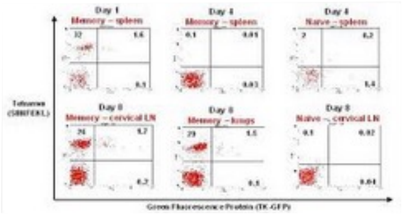


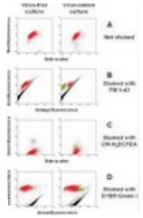


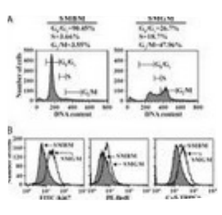


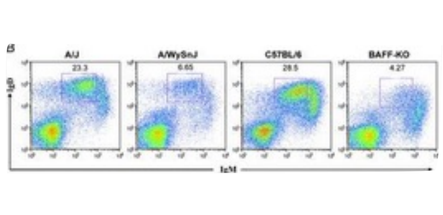


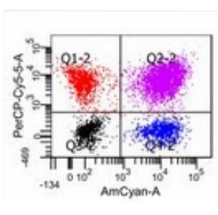


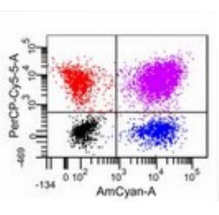


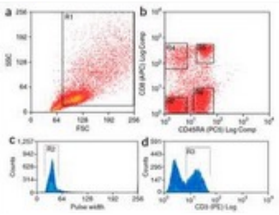


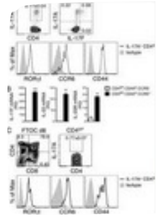


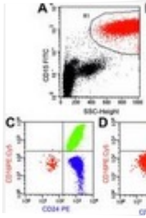






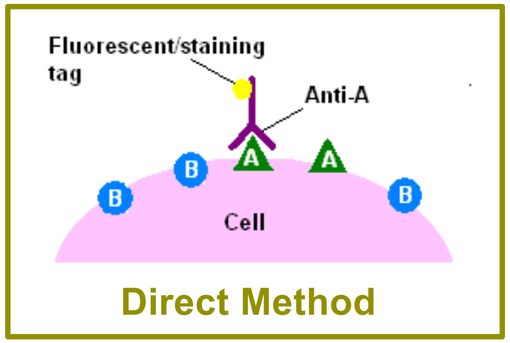


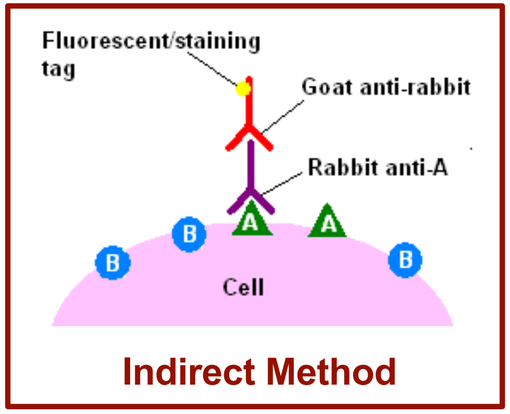


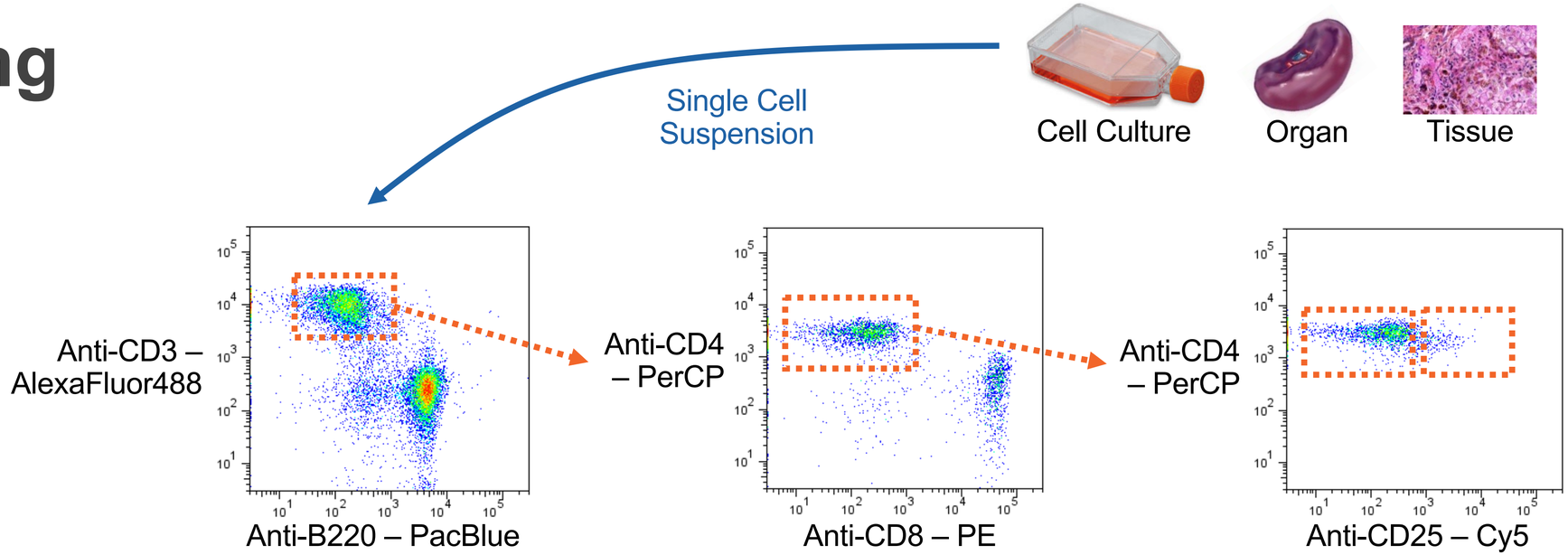


Immunophenotyping

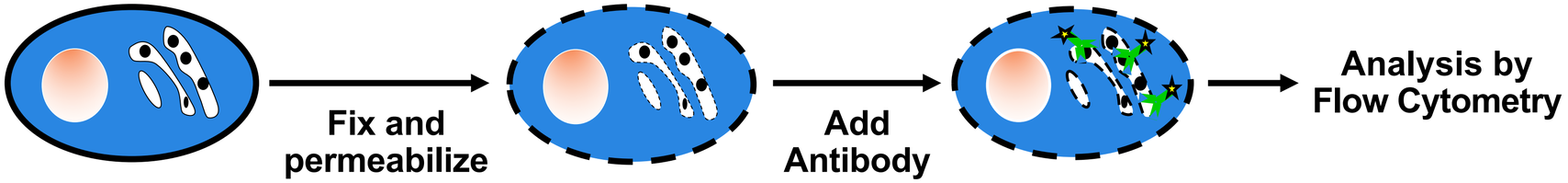
Antibody Fluorescence Labeling



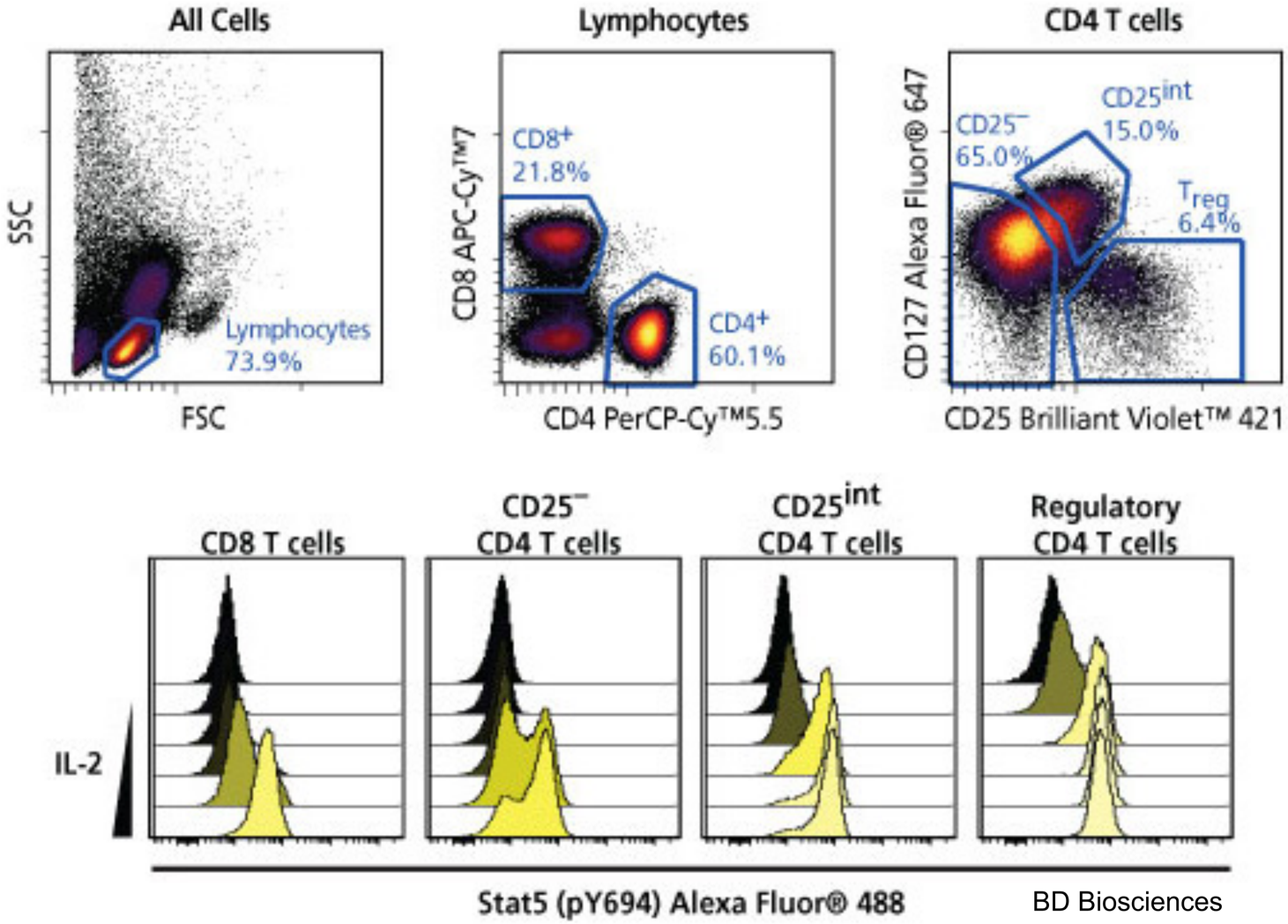




Intracellular Staining



Flow Cytometry Analysis



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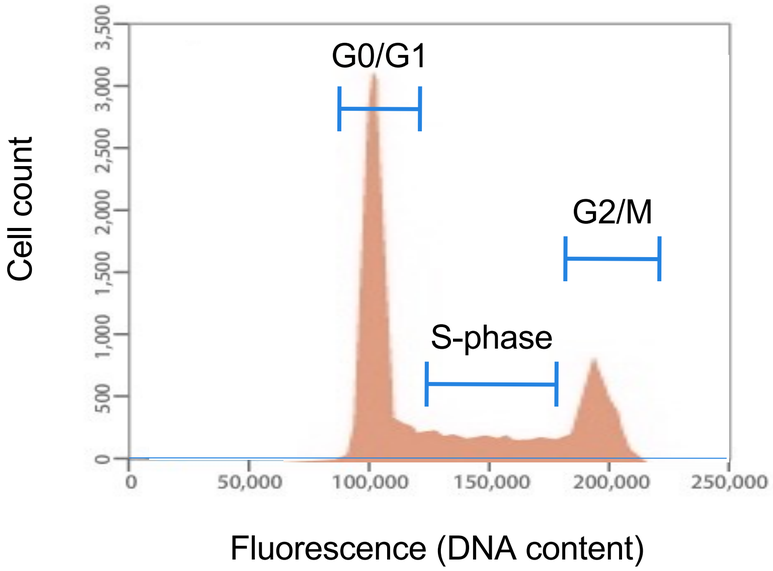
Applications in Flow Cytometry

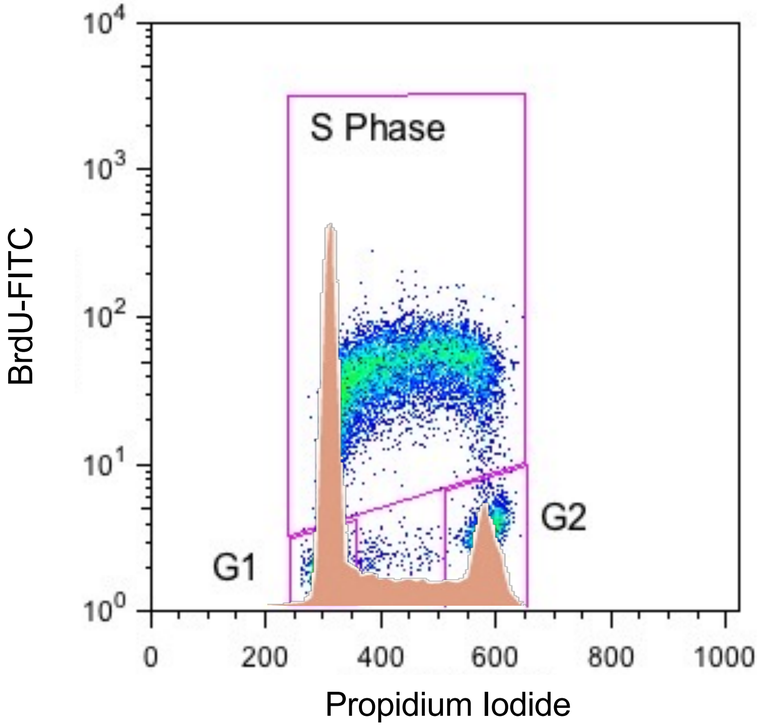
Cell State: Cell Cycle

Cell Cycle based on DNA Content

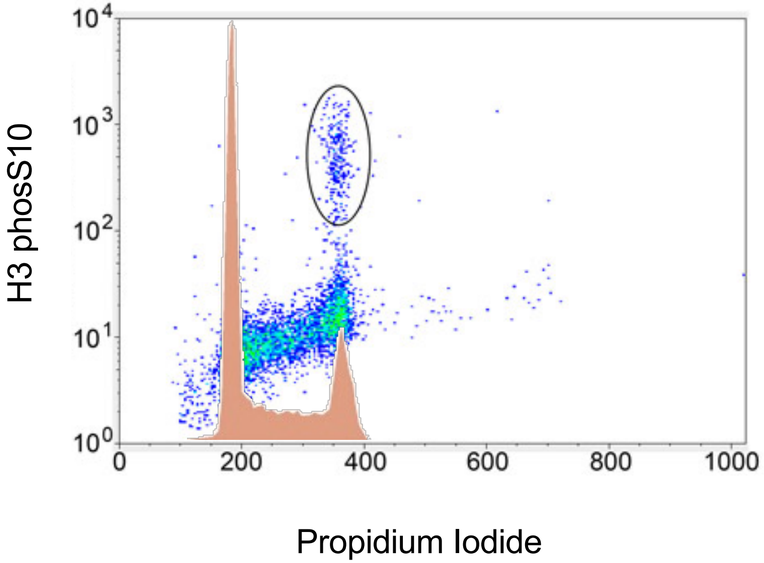


(http://www.abcam.com)

Discriminating S-phase



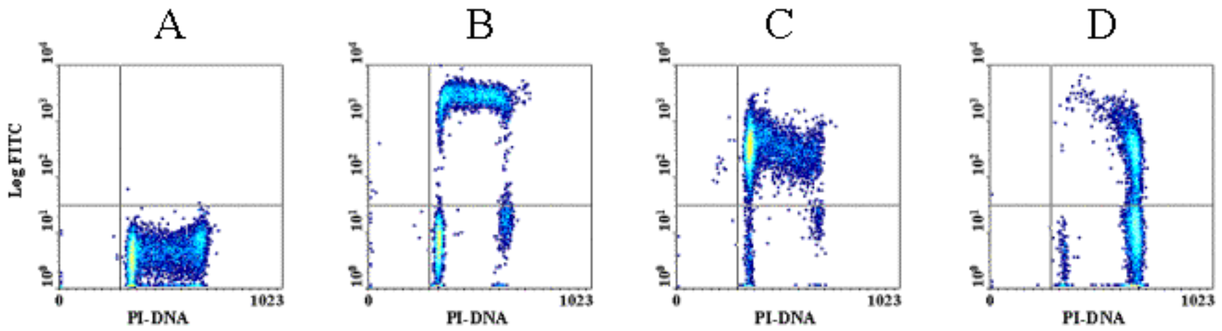
Discriminating Mitosis



Derek Davies (http://bitesizebio.com)

Cell State: Proliferation

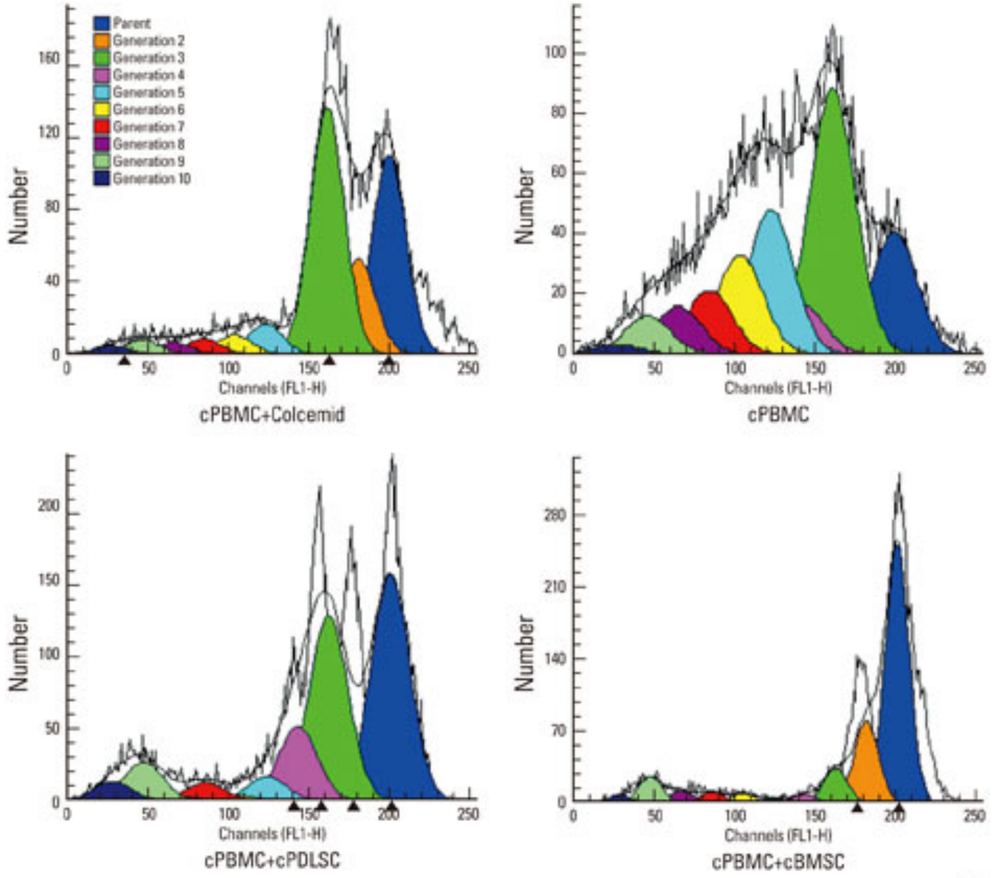
Cell Proliferation



A. Antibody control for SW620 cells. (No BrdUrd)
B. Exponentially growing SW620 cells.
C. SW620 cells after 24 hr incubation with 2mM hydroxyurea.
D. SW620 cells 24 hr after 7.5 Gy of ionizing radiation

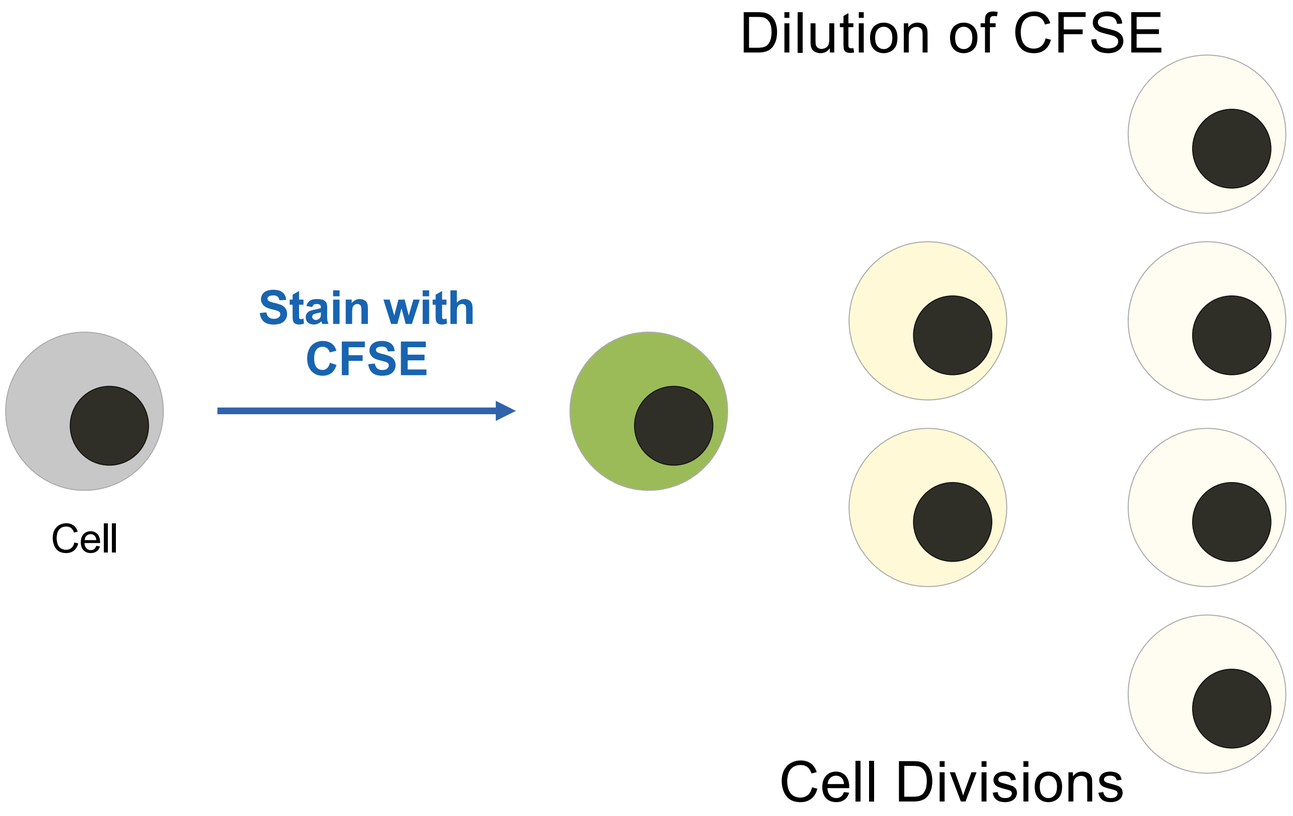
University of Michigan: Medical School lessons

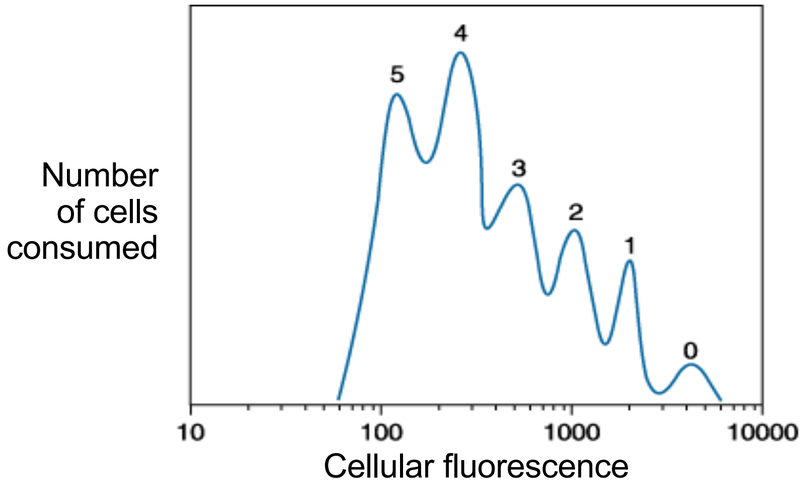
Quantifying Cell Proliferation

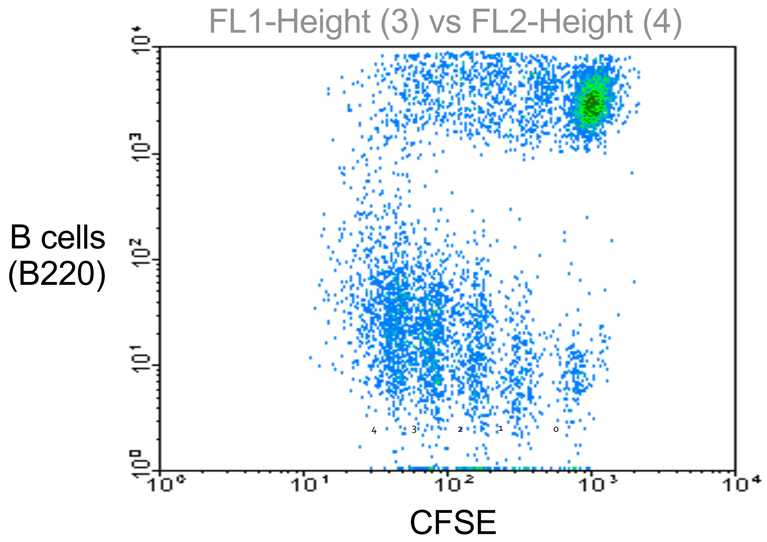


http://dx.doi.org/10.5051/jpis.2010.40.6.265

Cell State: Proliferation

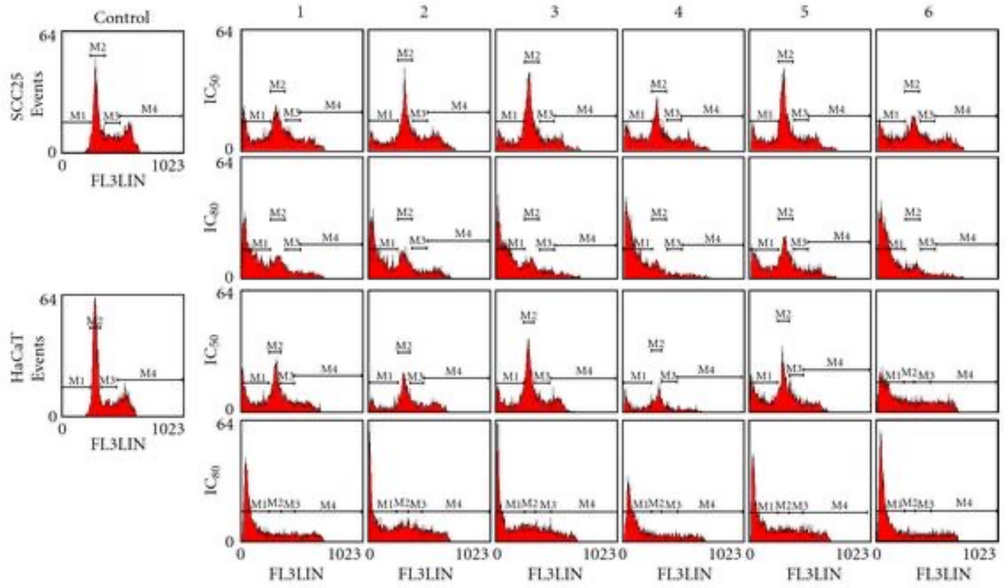






Cell State: Death

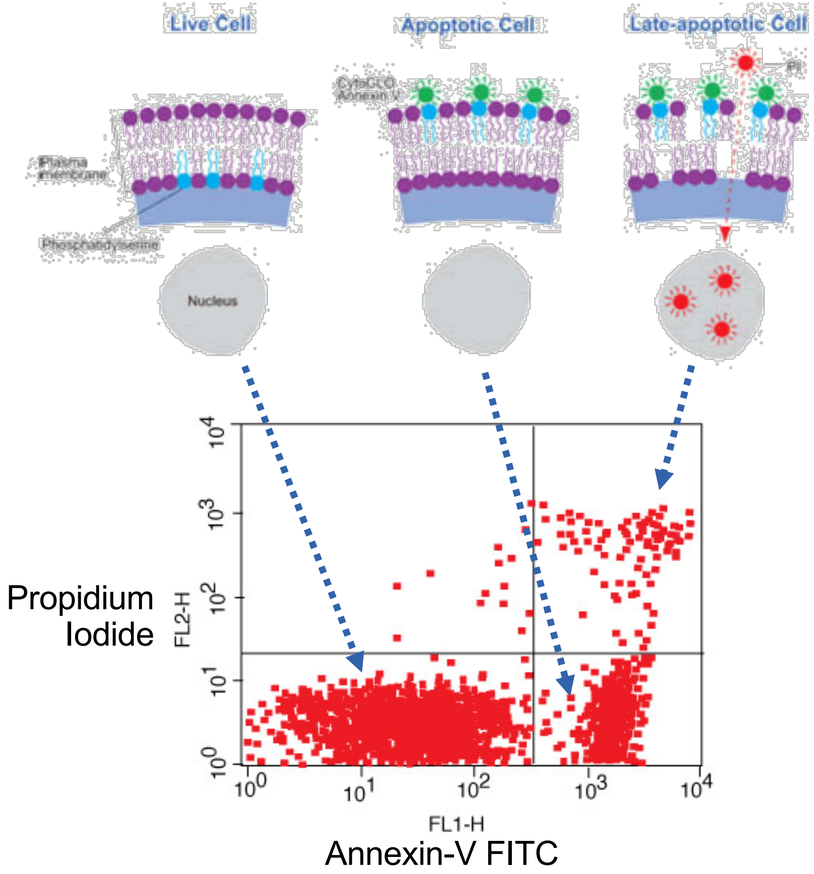
Apoptosis based on DNA Content



Cytotoxic Effect of the Genus Sinularia Extracts on Human SCC25 and HaCaT Cells: http://dx.doi.org/10.1155/2009/634868

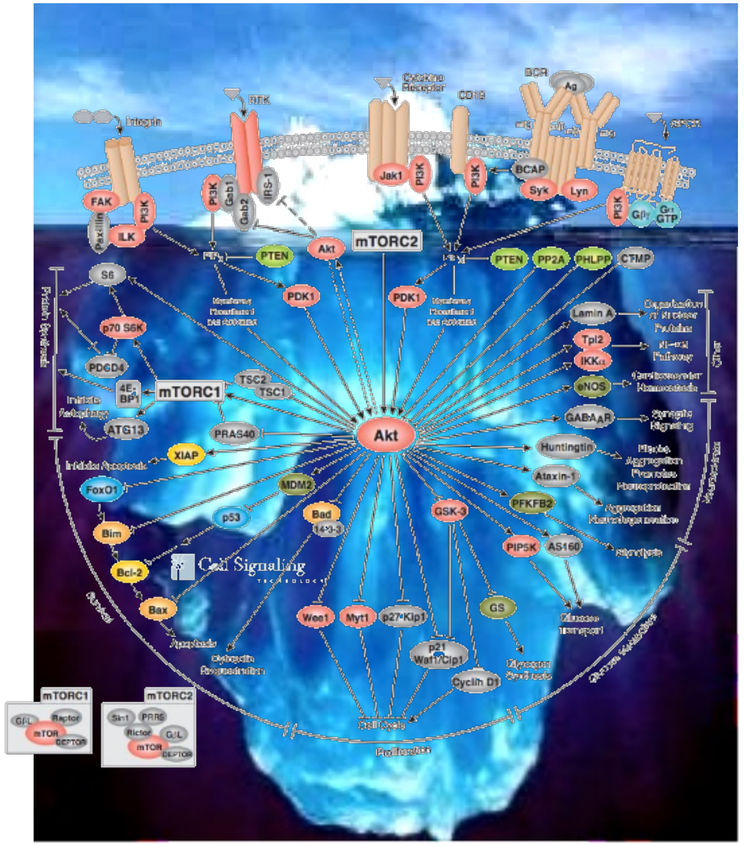
Annexin-based assay to distinguish apoptosis from necrosis

Annexin V-fluorochrome + **Propidium Iodide**

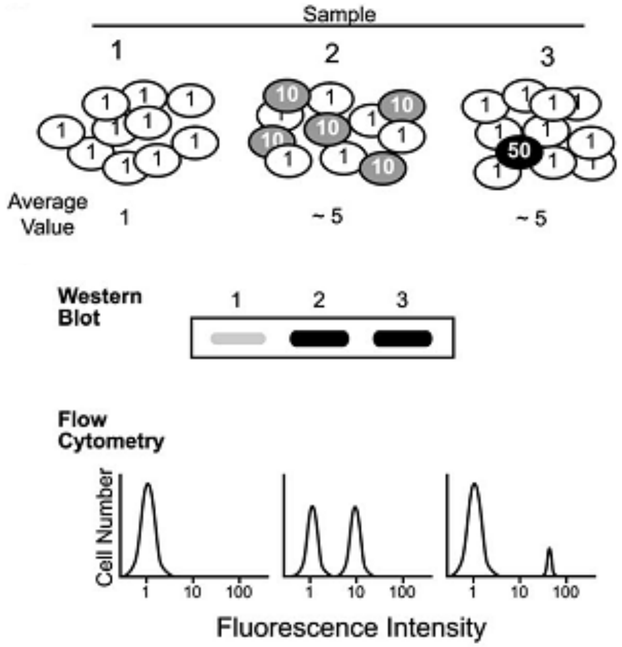


Cell Function: Signaling

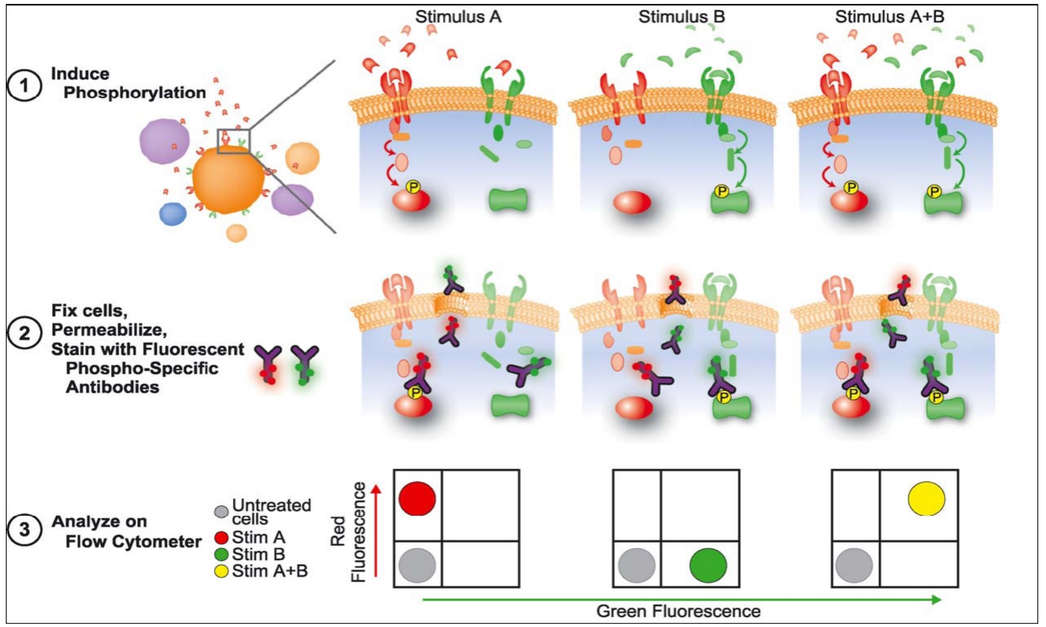
Cell Signaling by intracellular staining



Cell Signaling Technology



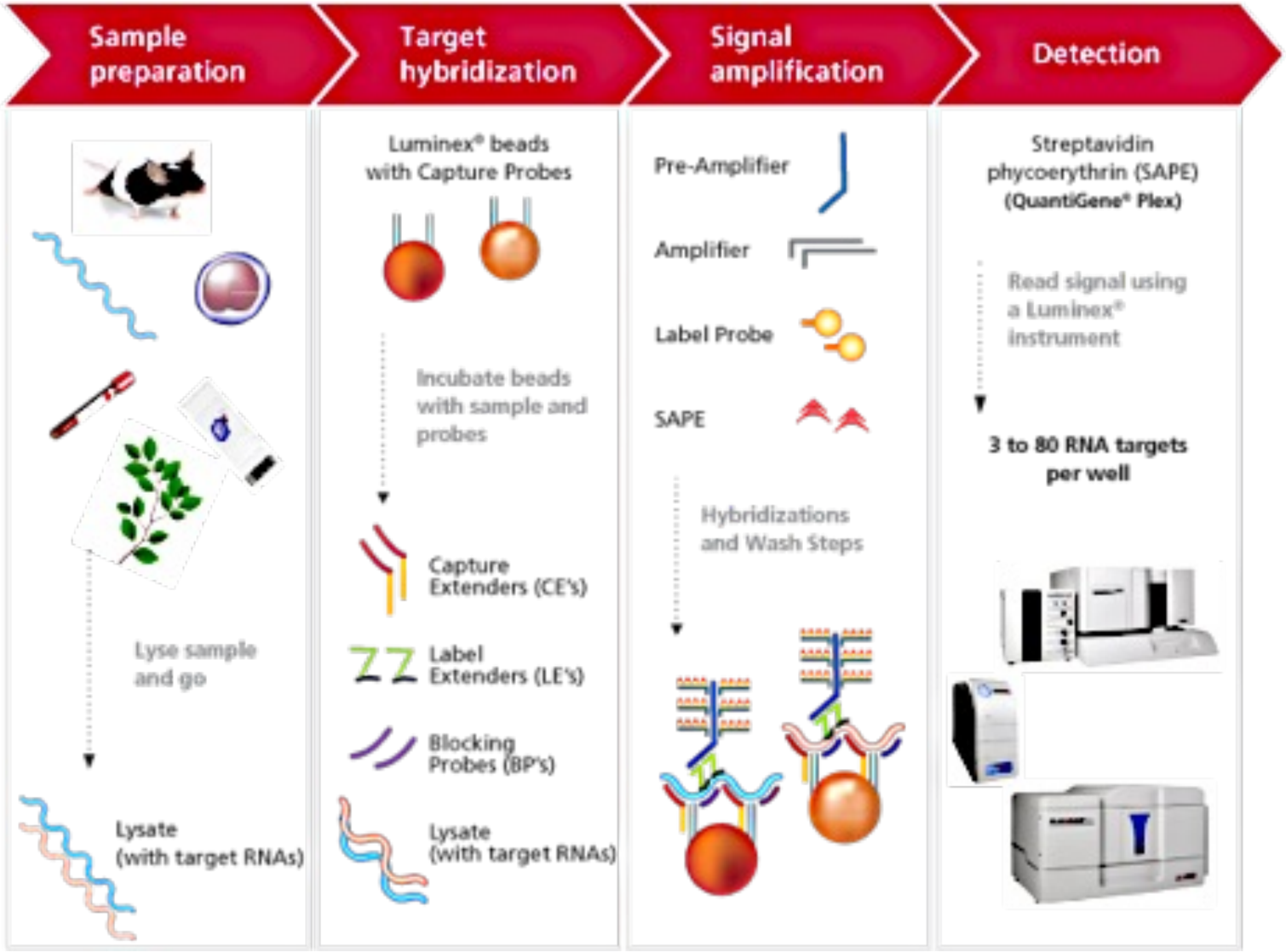
Powerful Multivariate Multiparametric Technique



Krutzik et al. Clin Immunology (2004)

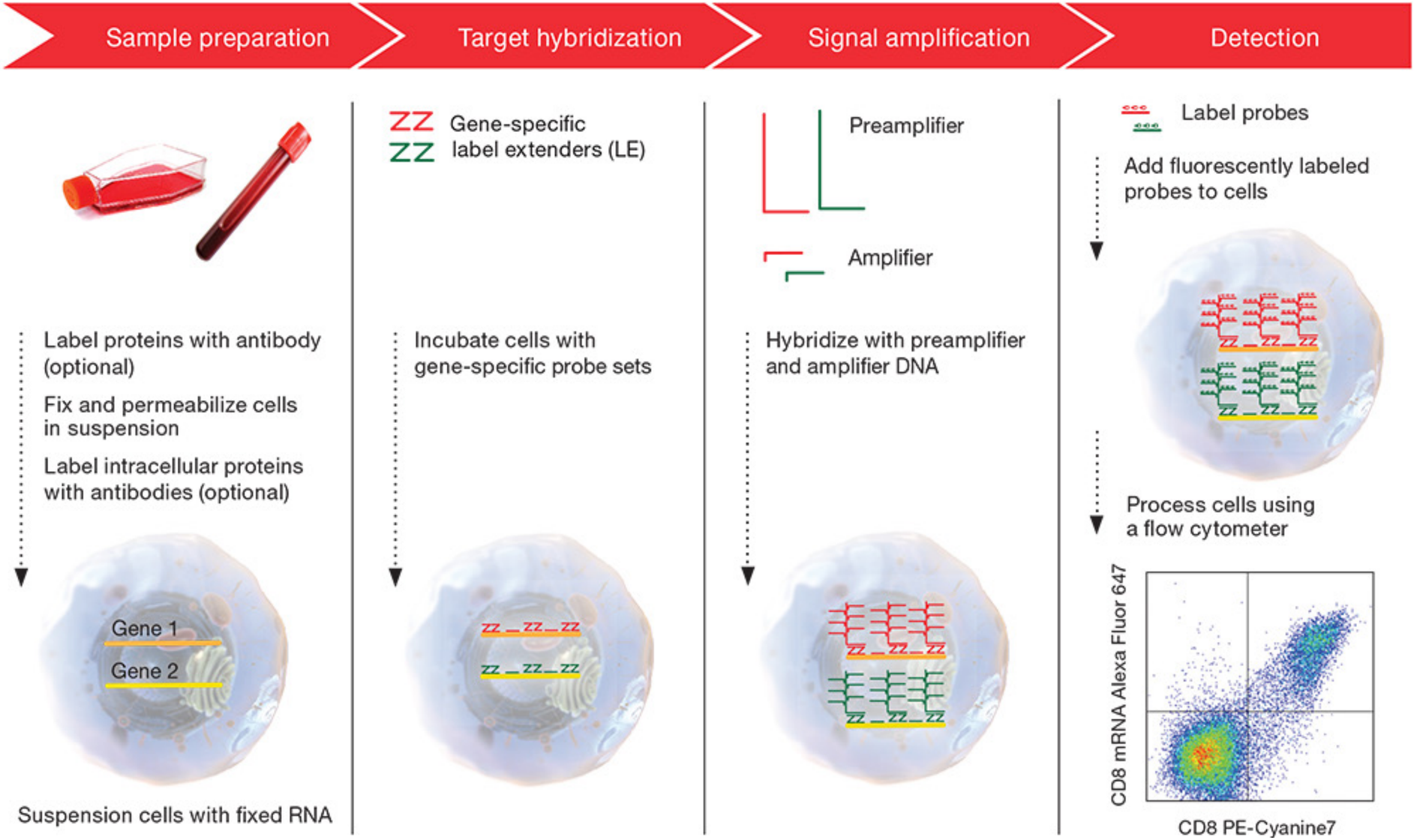
Measuring Soluble Analytes

Multiplexed ELISAs: Luminex



RNA Detection by Flow Cytometry

PrimeFlow RNA Assay



https://www.thermofisher.com/us/en/home/references/newsletters-and-journals/bioprobes-journal-of-cell-biology-applications/bioprobes-75/bioprobes-75-primeflow-rna-assay.html

Drinking the Data

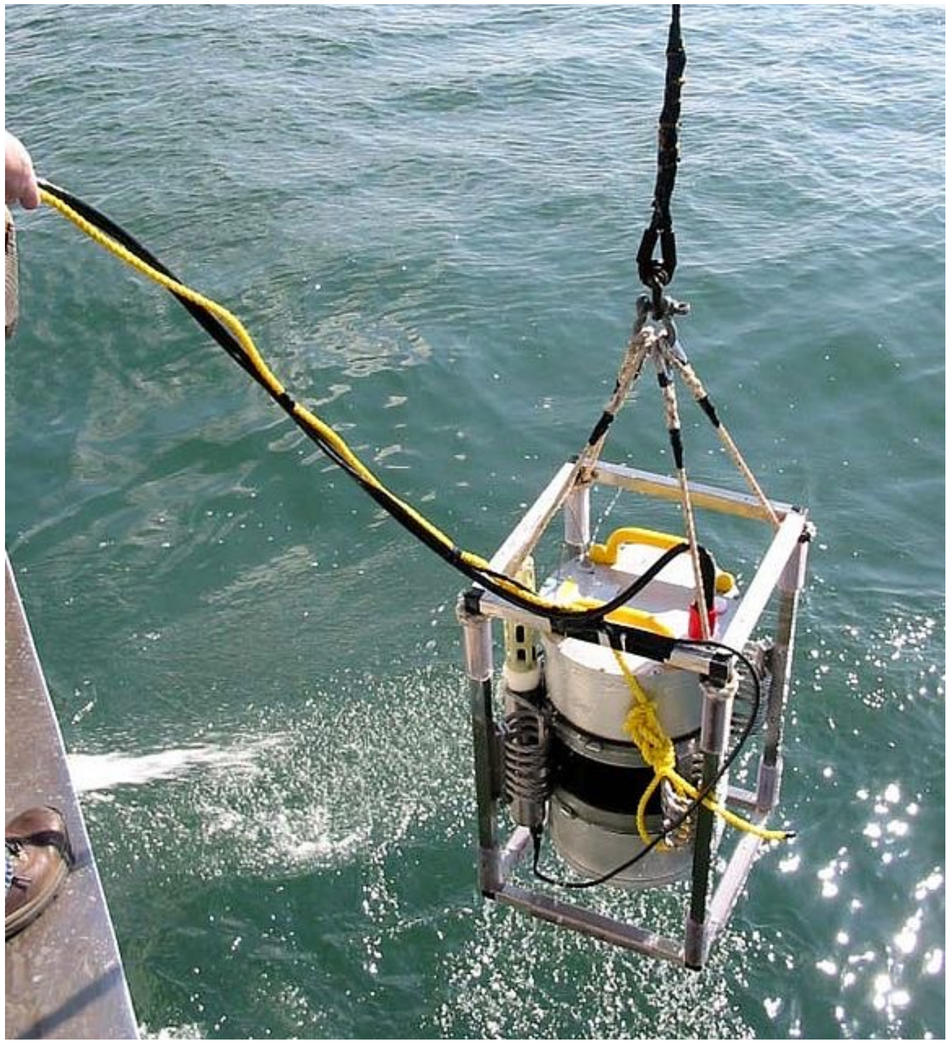
The technology has not only been utilized in clinical and research environments, but also throughout the food and beverage industry.

During fermentation, the microorganisms responsible for the production of wine can be analyzed via Flow Cytometry. This allows for a monitoring of the quantity and viability of desired species which also determining contamination levels of unwanted organisms which can spoil the process.

Marine Cytometry

Many researchers use Flow Cytometry to analyze aquatic samples. Instruments have been specially developed to spend extended periods of time under water for various purposes.

This monitoring has been used to track impacts of environmental changes on phytoplankton and blooms in different settings.



CytoSub submersible Flow Cytometer

Cosmic Flow Cytometry

Flow is a technology that can be used not only here on Earth, but also in ventures beyond. “Micro” flow cytometer instruments are in development and have shown positive preliminary results.

This instrumentation will allow for real time monitoring of physiological changes that occur while in space along with routine blood screens.

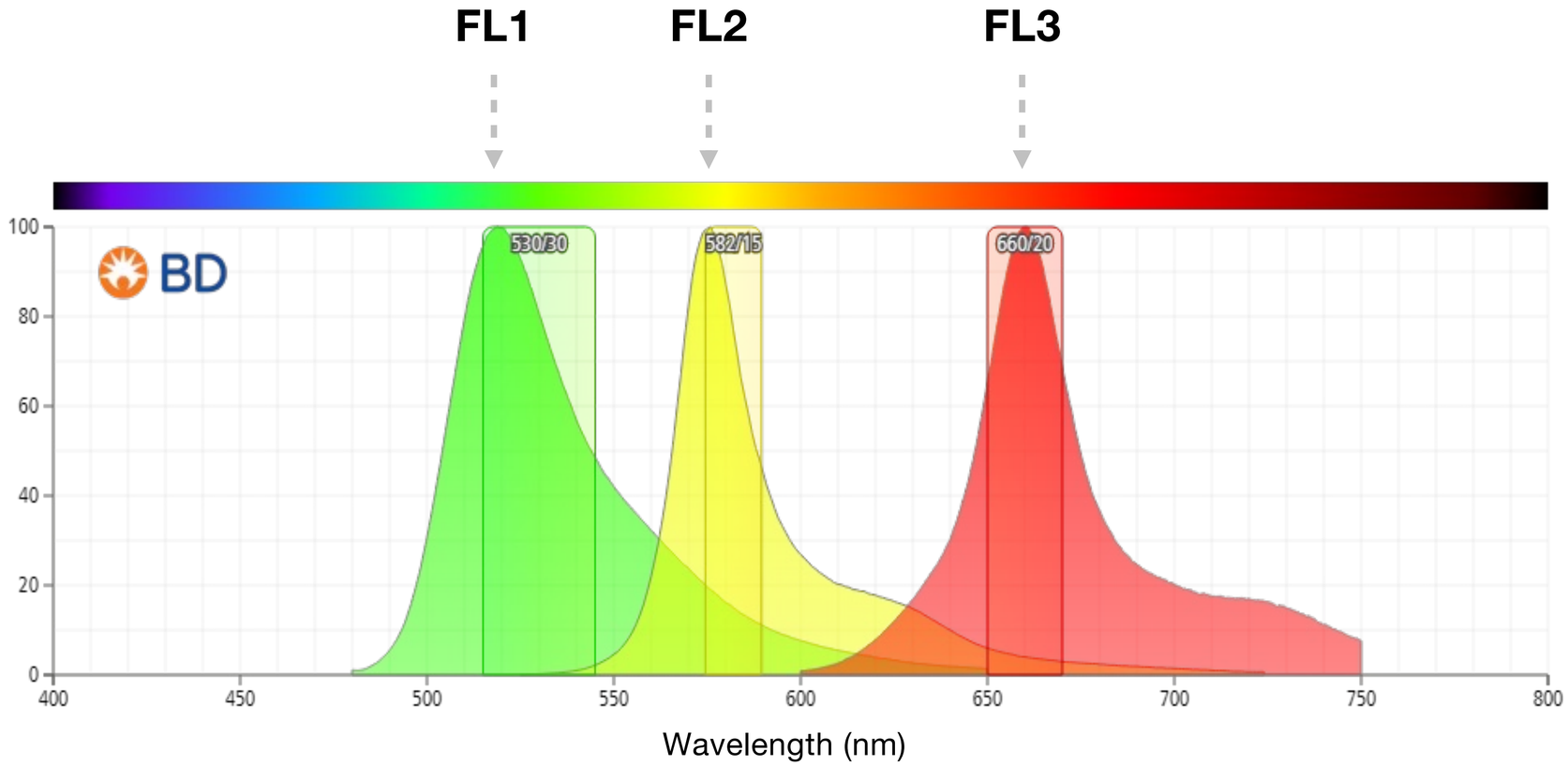
Pictured above: Canadian Space Agency Astronaut Chris Hadfield with the Microflow 1 miniature Flow Cytometer aboard the ISS.

NASDC

NCI Awardee Skills Development Consortium

Spectral Flow Cytometry

Polychromatic Flow Cytometry



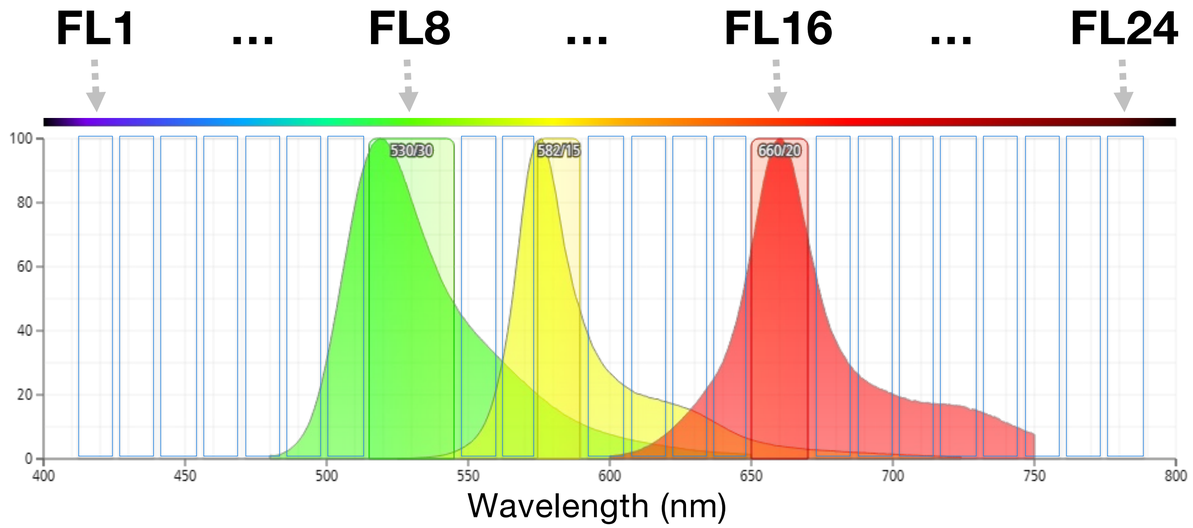
FL1 ------> FITC

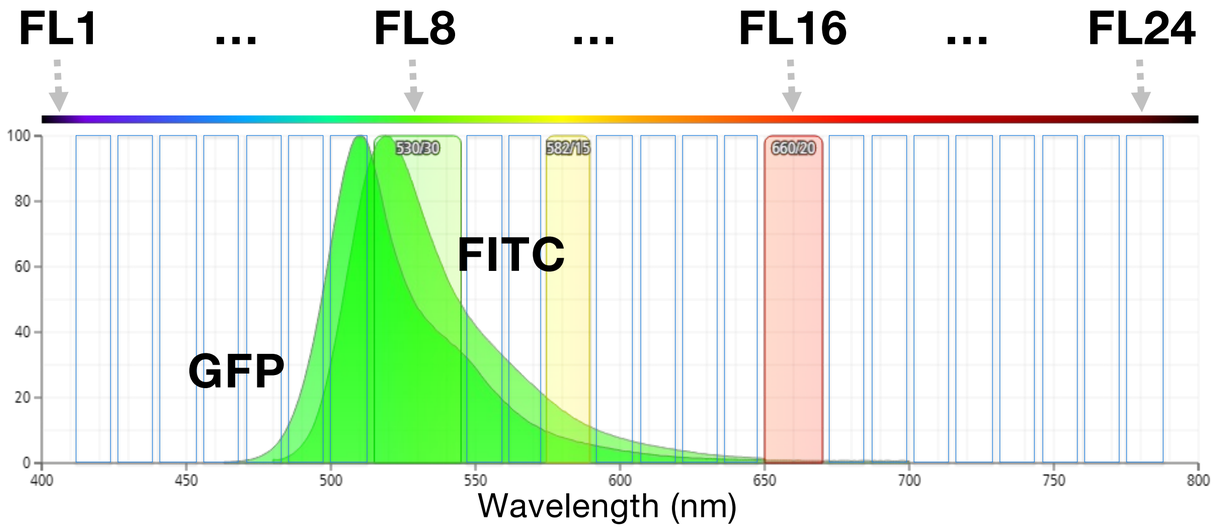
FL2 ------> PE

FL3 ------> APC

One detector per fluorochrome

Spectral Flow Cytometry





FL1 … FL24 ⇢ **FITC**, **PE**, **APC**

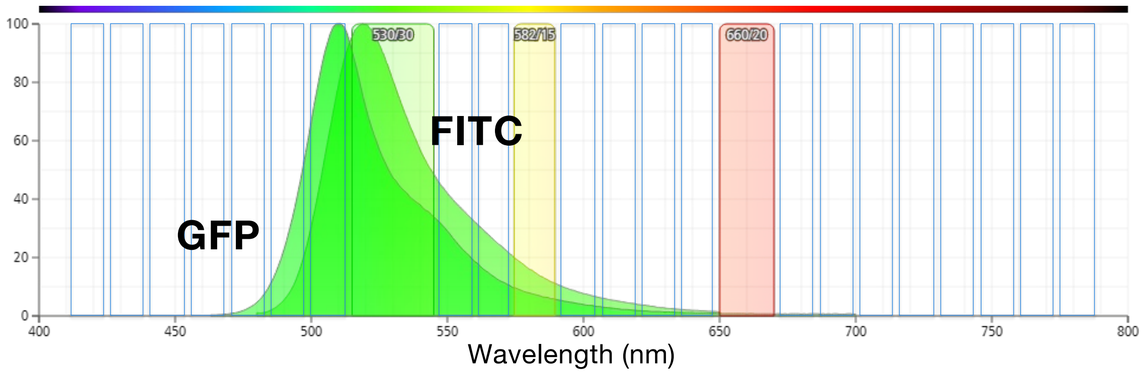
Several detectors per fluorochrome

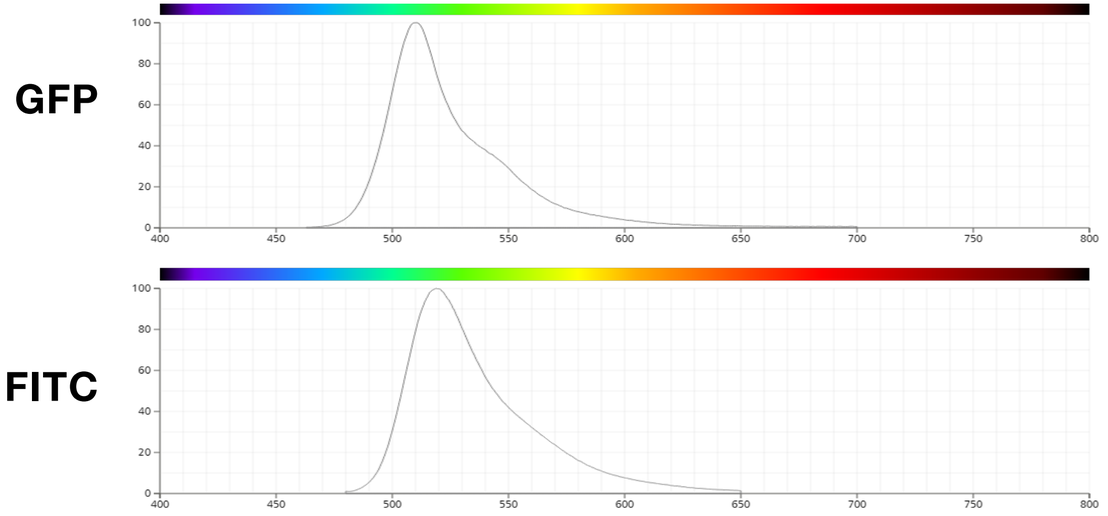
FL1 … FL7 … FL24 ⇢ **GFP**

FL1 … FL8 … FL24 ⇢ **FITC**

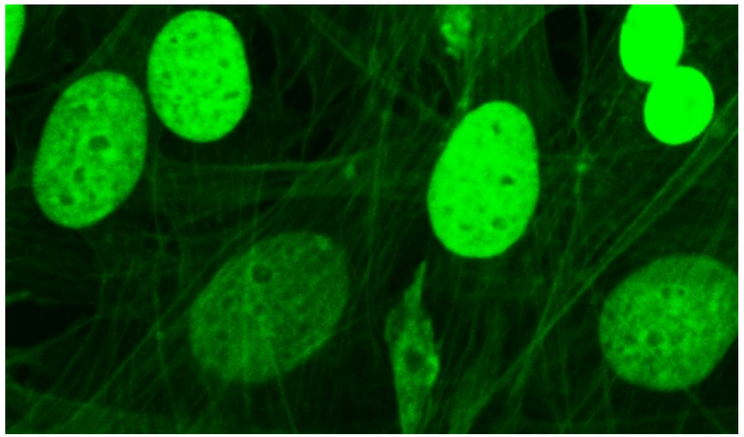
Spectral Flow Cytometry performs as good or better than Polychromatic Flow Cytometry

Seeing is Believing





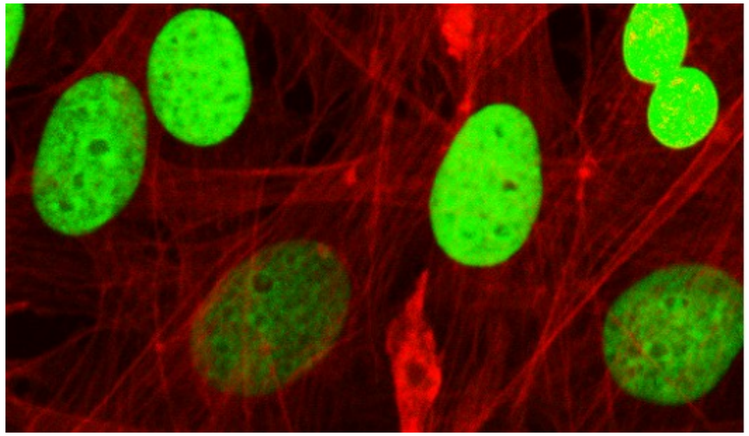
Through the Eyepieces



Unmixing

GFP-Histone2B

FITC-phalloidin (actin filaments)



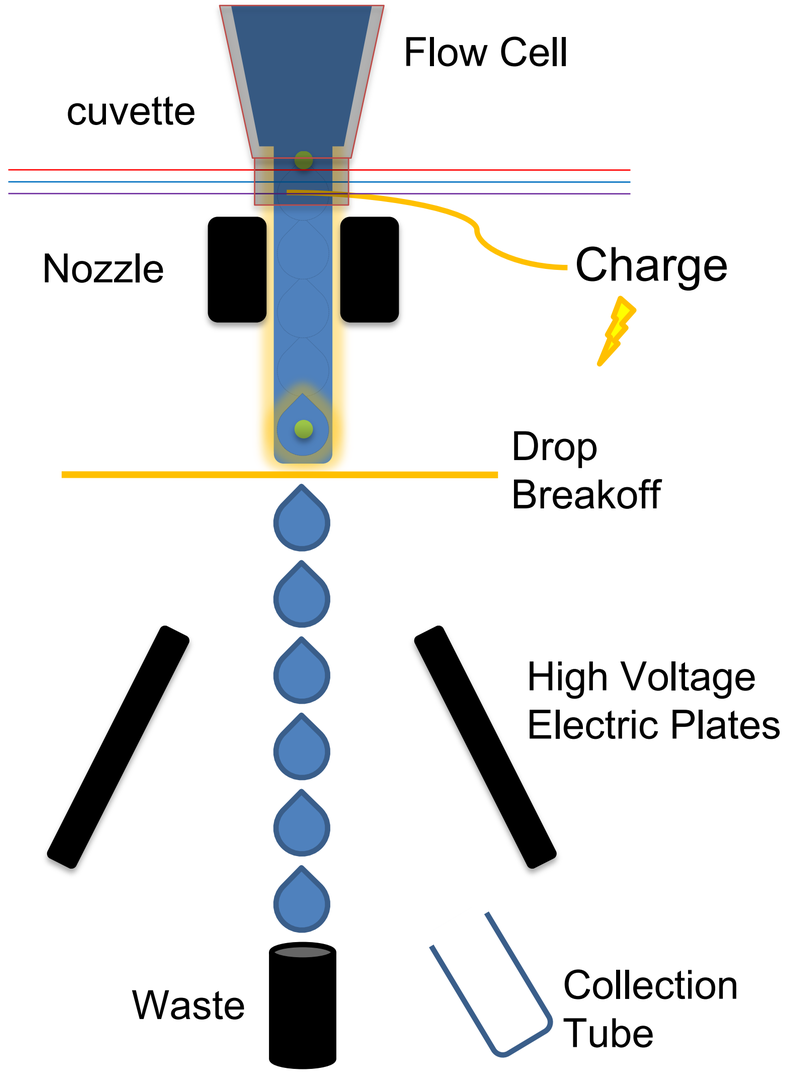
Images taken from Zeiss: “Linear Unmixing for Dummies”

NASDC

NCI Awardee Skills Development Consortium

Cell Sorting

Cell Sorting



Physical separation

of a cell or particle of interest from a heterogeneous suspension of cells or particles.

Drop Generation

Charge Drop

Break Stream into Droplets

With particle of interest

(30-100,000 drops/sec)

Up to Millions of cells/h

Deflect Charged Drop

can be sorted with:

High Purity

High Yield

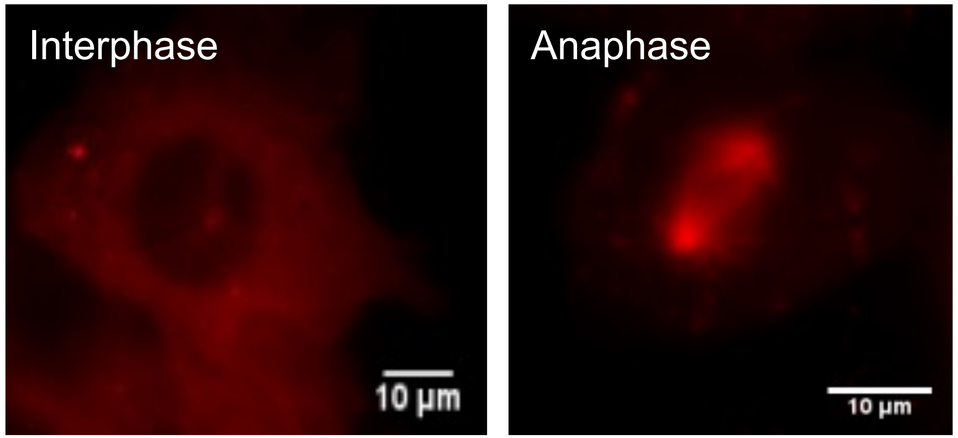
High Speed

High Viability

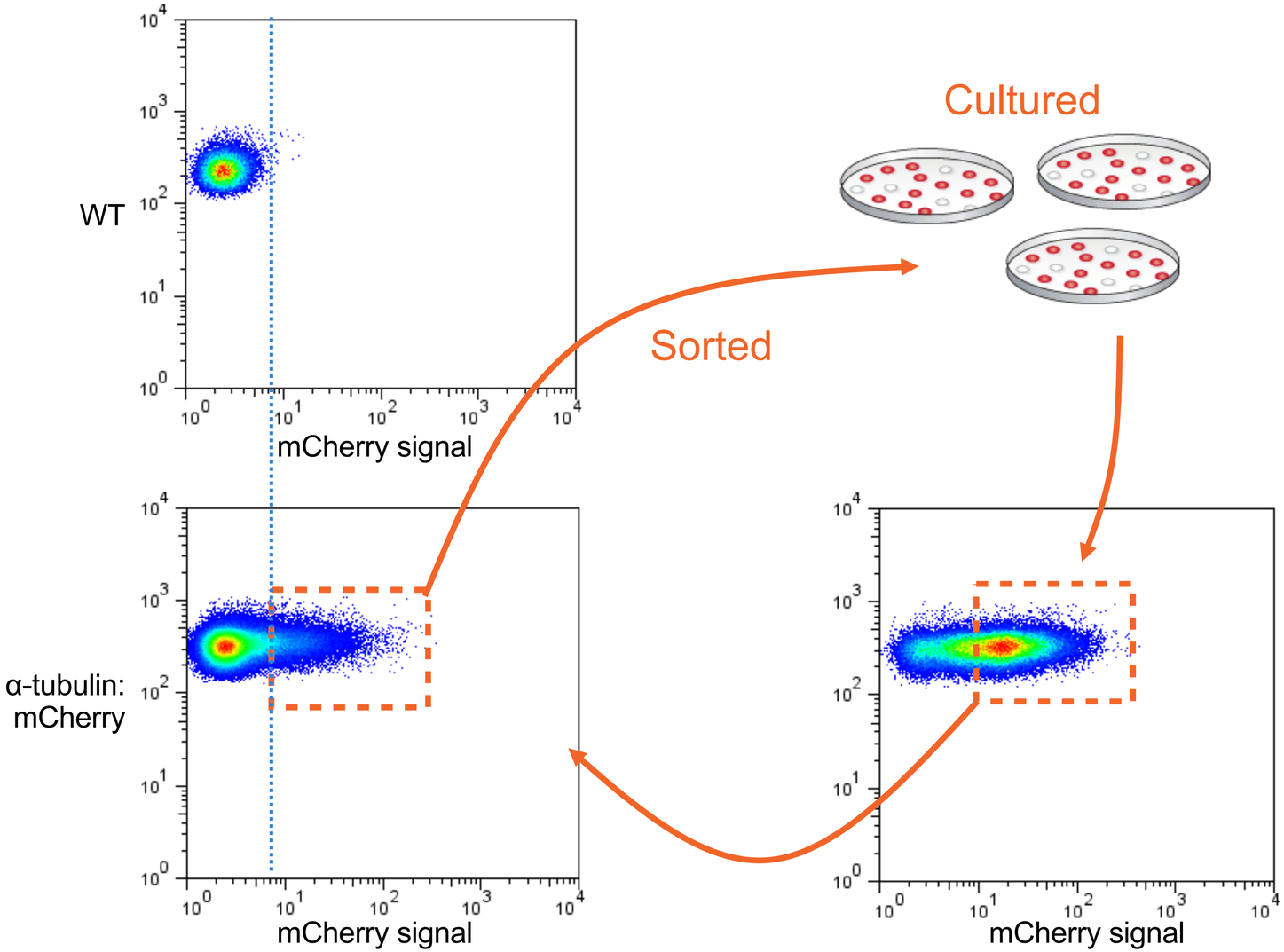
Memorial Sloan Kettering Cancer Center

Cell Sorting

Establishing Fluorescent Cell Lines

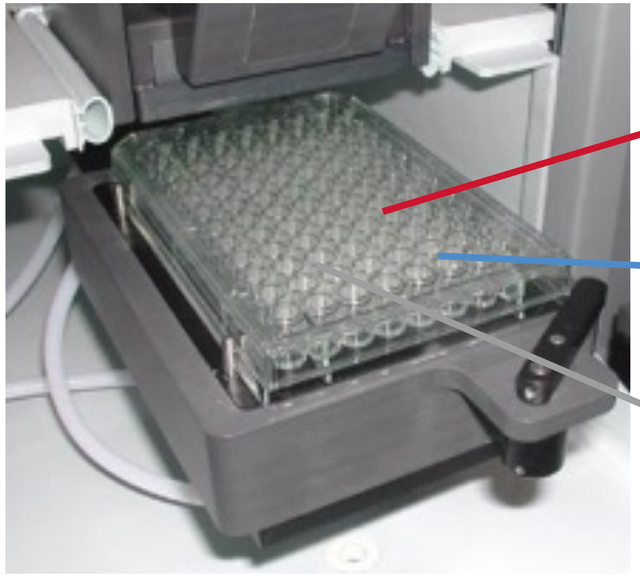


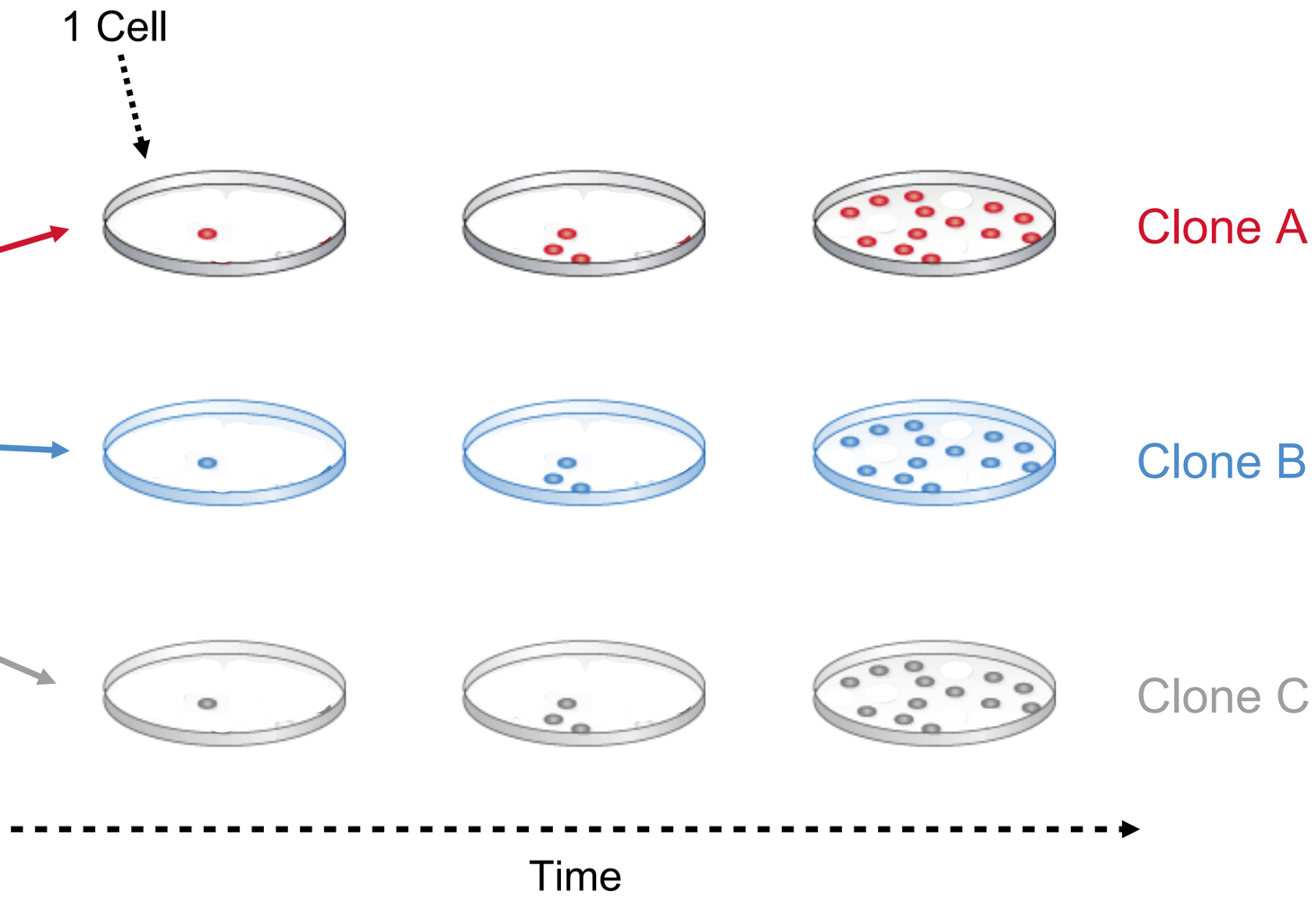
Human hepatoma cell line
Expressing α-tubulin fused with mCherry



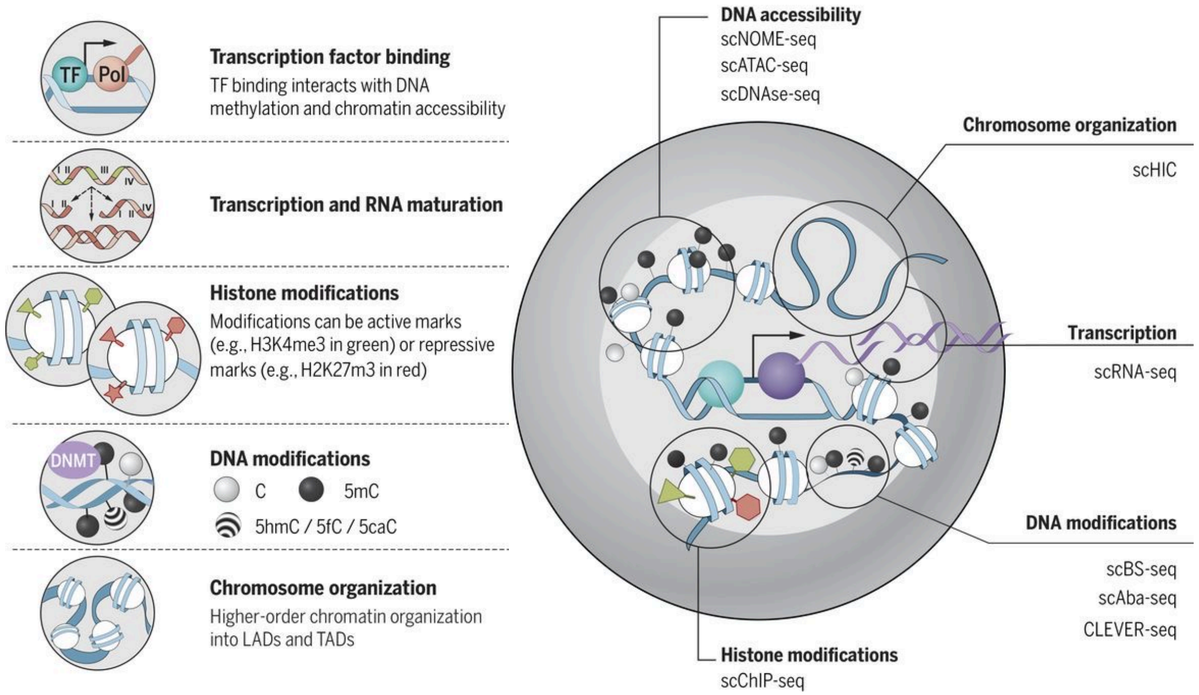
Cell Sorting

Establishing Cell Clones with Single-Cell Sorting





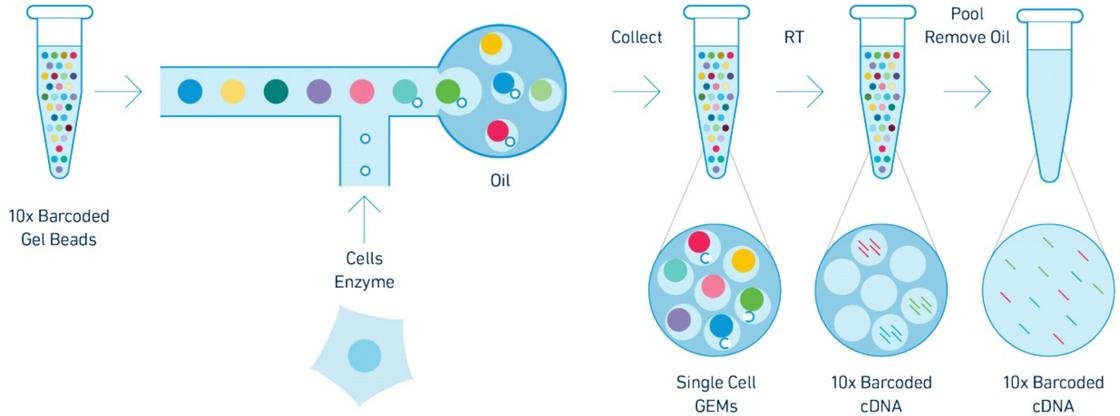
Cell Sorting for Single Cell Genomics



Gavin Kelsey et al. Science 2017;358:69-75

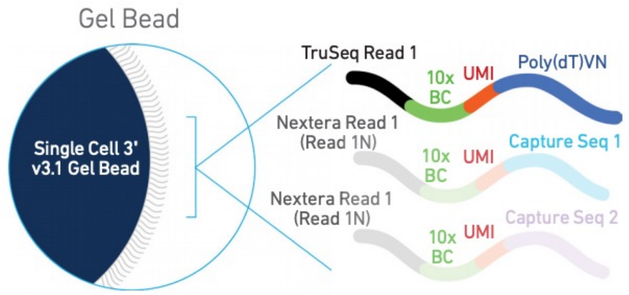
http://www.fluidigm.com/single-cell-genomics.html

10x Genomics



Applications:

- Fixed RNA Profiling
- Gene Expression
- Nuclei Isolation
- ATAC
- Immune Profiling



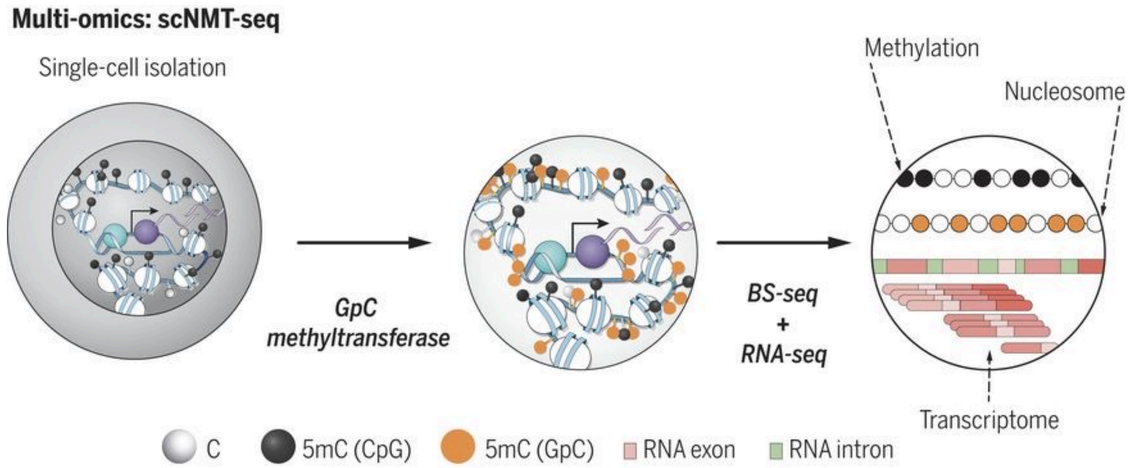
https://www.10xgenomics.com/

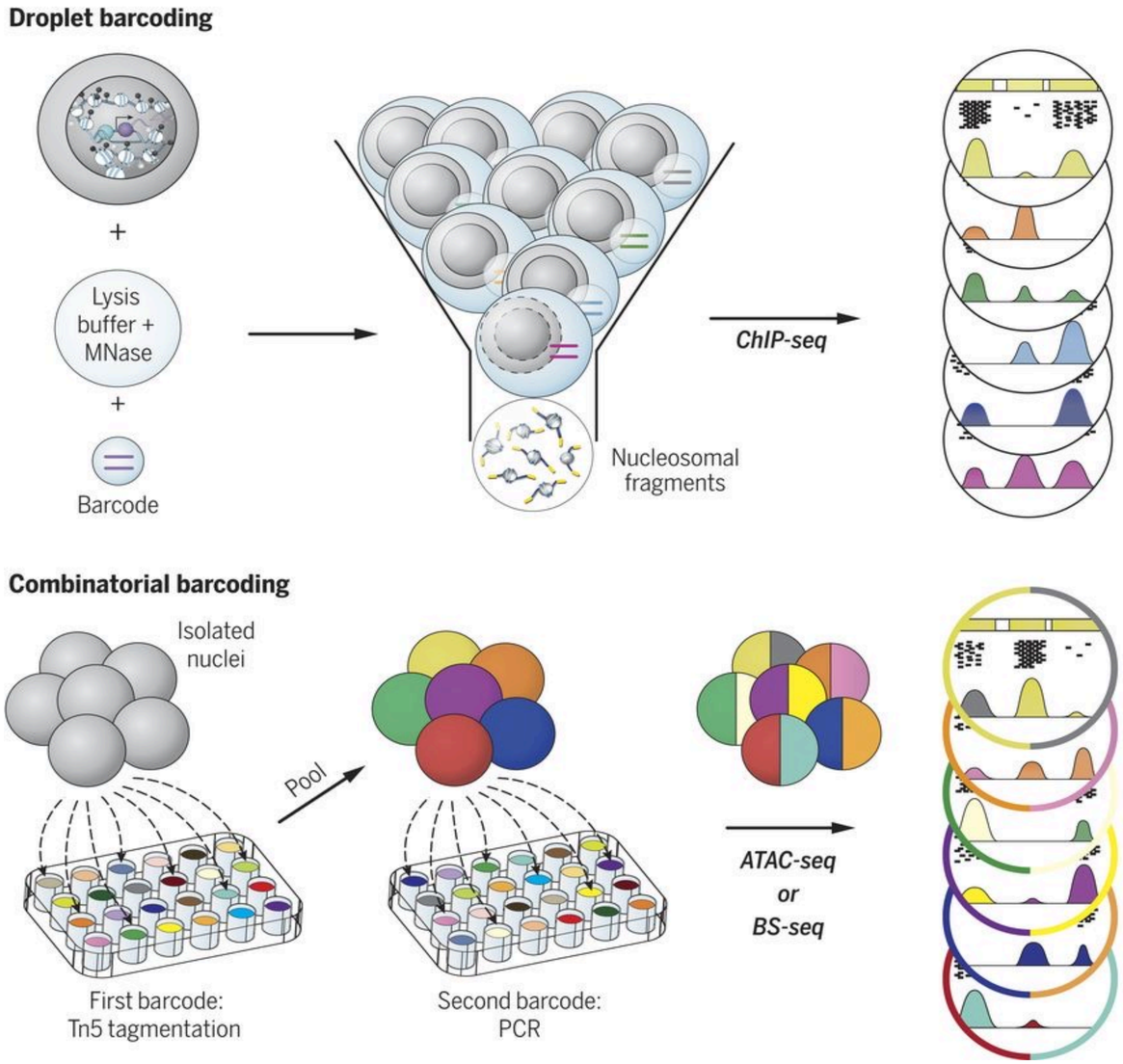
Other technologies:

BD Rhapsody

Nanostring nCounter

Single Cell Sorting for Molecular Profiling





Gavin Kelsey et al. Science 2017;358:69-75

Index Sorting single cells

Isolation of Mouse Hematopoietic Stem Cell Side Populations Using SPKLS and Post-Sort Confirmation Using Single-Cell Gene Expression

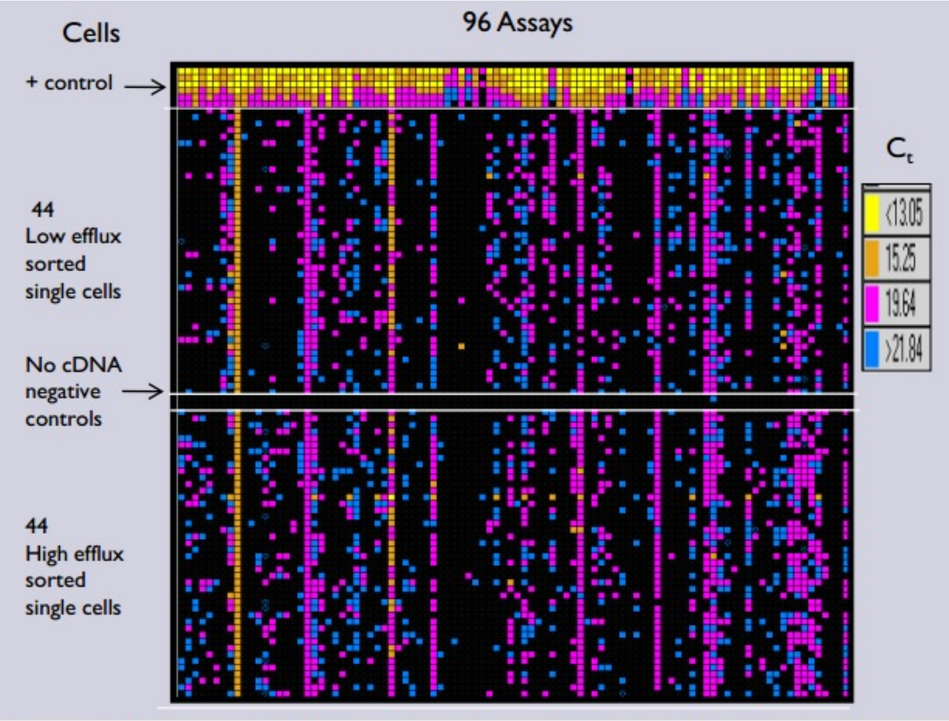
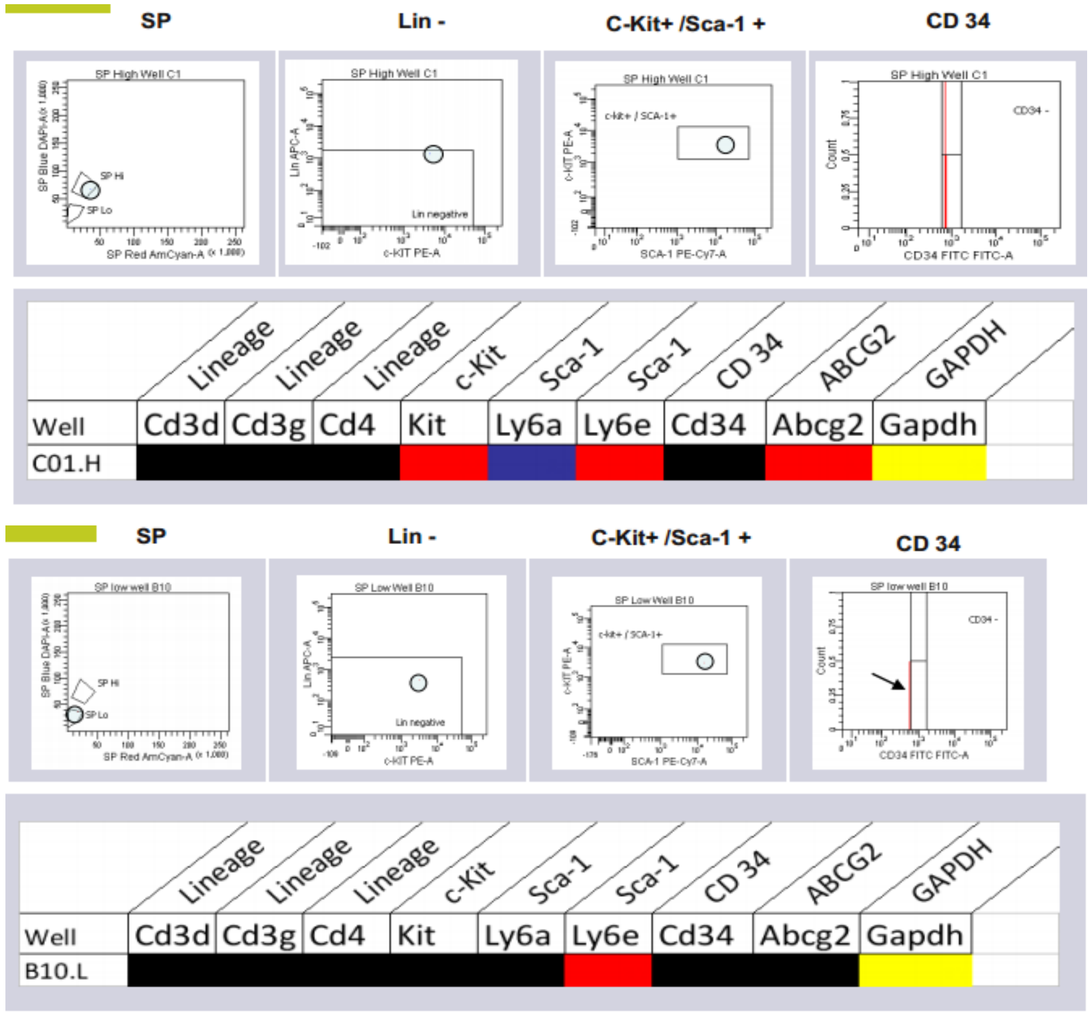


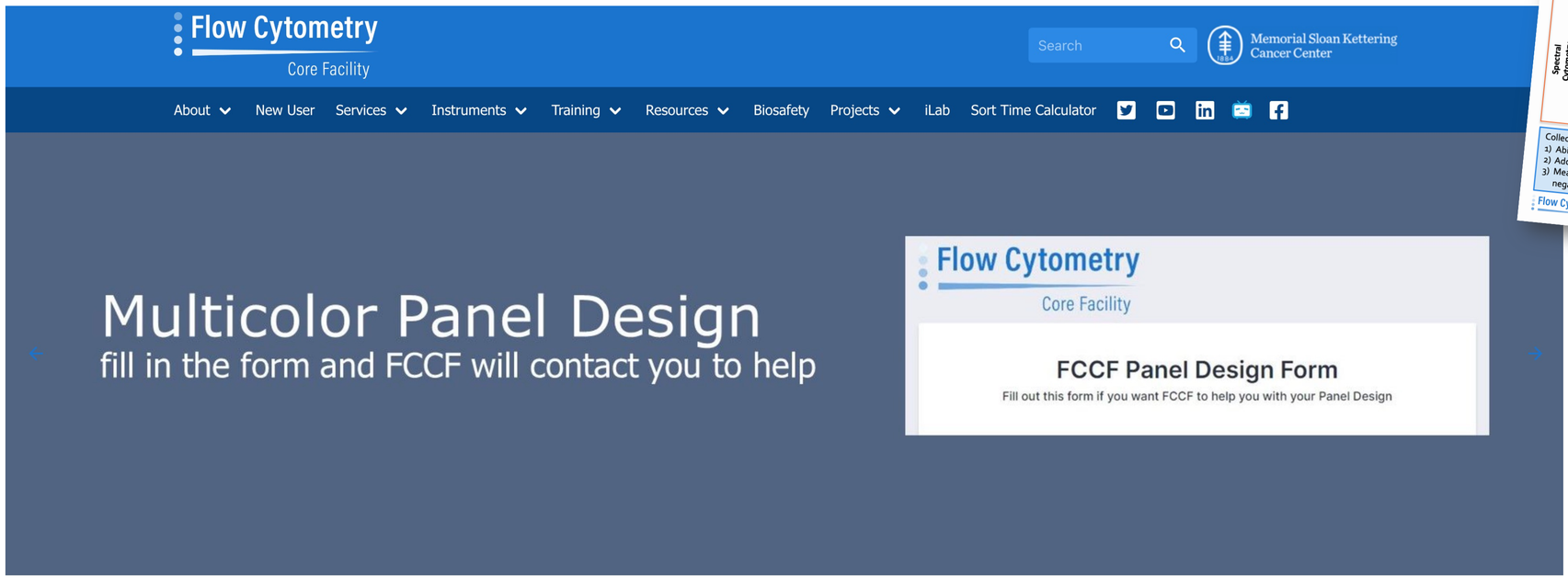
Figure 9: C_t heat map from 96 PCR assays, are shown on a 96.96 chip. Each point represents gene expression within a single cell. Some genes are more highly expressed in SP High than SP Low cell population.

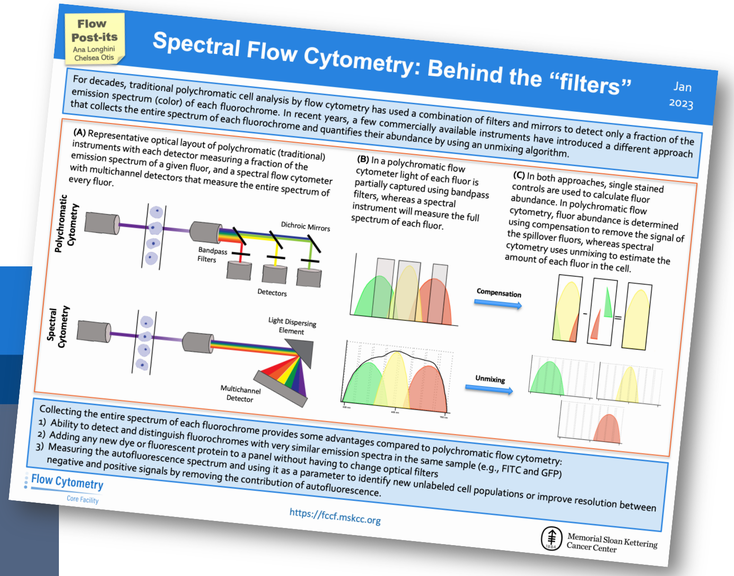


http://www.fluidigm.com/home/fluidigm/Posters/BD_FLDM_poster_Final_Red.pdf

Check out the latest news/courses

https://fccf.mskcc.org





@flowMSKCC

flowMSKCC

flowMSKCC

https://fccf.mskcc.org

Mission

The aim of the Flow Cytometry Core Facility at MSKCC is to provide advanced instrumentation as well as high-level technical and scientific expertise in multi-dimensional Flow Cytometry and Cell Sorting, to facilitate science, improve the quality, and advance the scope of MSK research.

Acknowledging the facility

All peer-reviewed publications by MSK faculty or employees must acknowledge the MSK Cancer Center Support Grant/Core Grant (P30 CA008748) when publishing work performed/assisted by FCCF.

NASDC
NCI Awardee Skills Development Consortium

Flow Cytometry in a Nutshell

Questions?