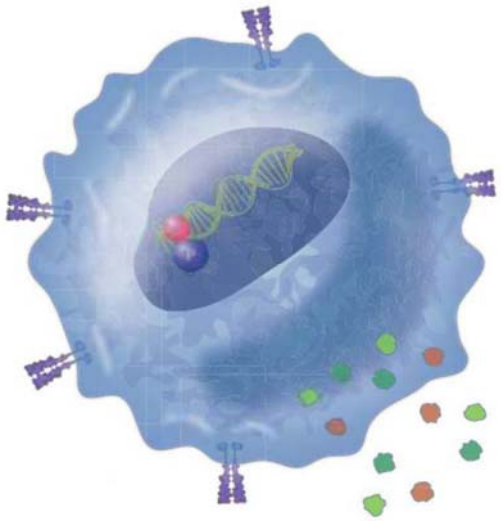
Enabling a deeper understanding of what is on, in, and made by the cell

Analyzing what is on, in, and Made by the Cell



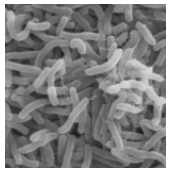
	Methods of Analysis
Cell Surface Markers	• Flow Cytometry • IF/IHC
Intracellular Proteins	• IF/IHC • WB • PCR
Cytokines	• ELISA • PCR
Cellular Processes	• Multiple

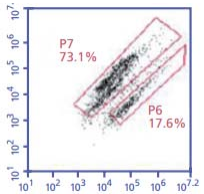
Challenges:

- **Multiple methodologies commonly used in cell biology; consume time and materials**
- **Unable to correlate expression of surface and intracellular molecules**
- **Difficult to quantitate the percentage of cells expressing markers of interest**

Flow Cytometry: a Versatile Technology for Broad Applications

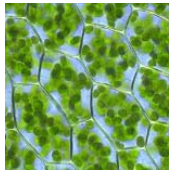
Microbiology

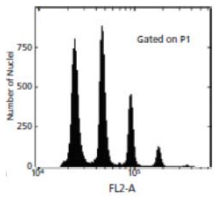




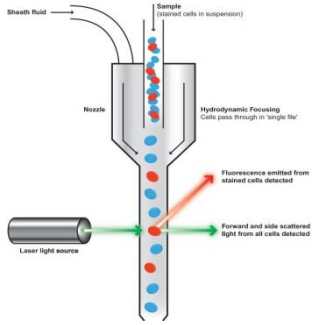
- Aquatic microbiome analysis
- Biofuel research
- Bacteria viability and concentration

Plant Biology

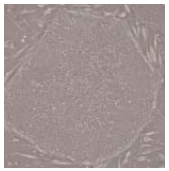


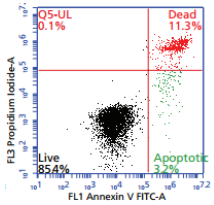


- DNA content



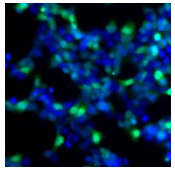
Cell Biology

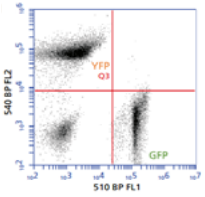




- Apoptosis
- Cell signaling
- Immunophenotyping
- Cytokine analysis

Fluorescent Protein Analysis

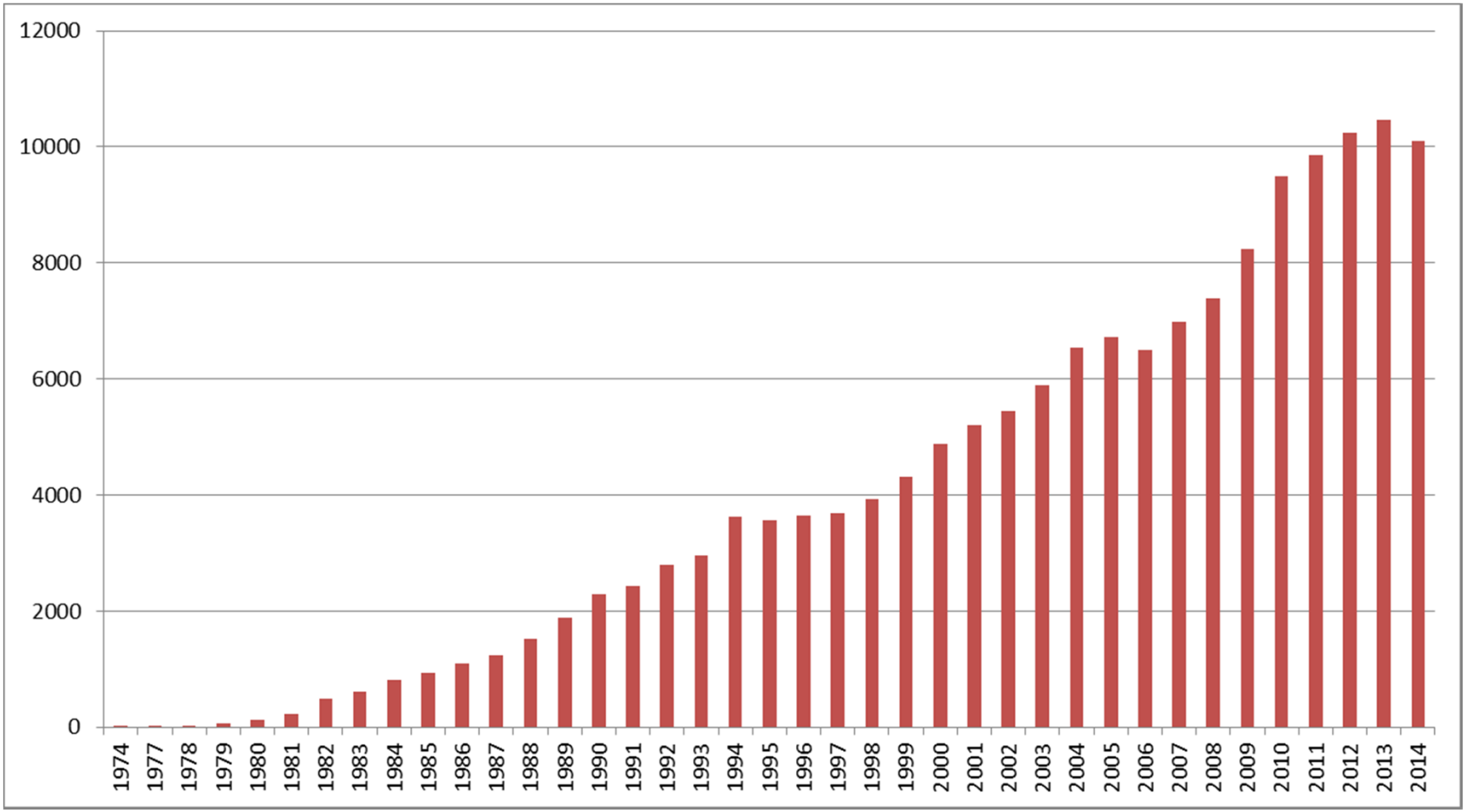




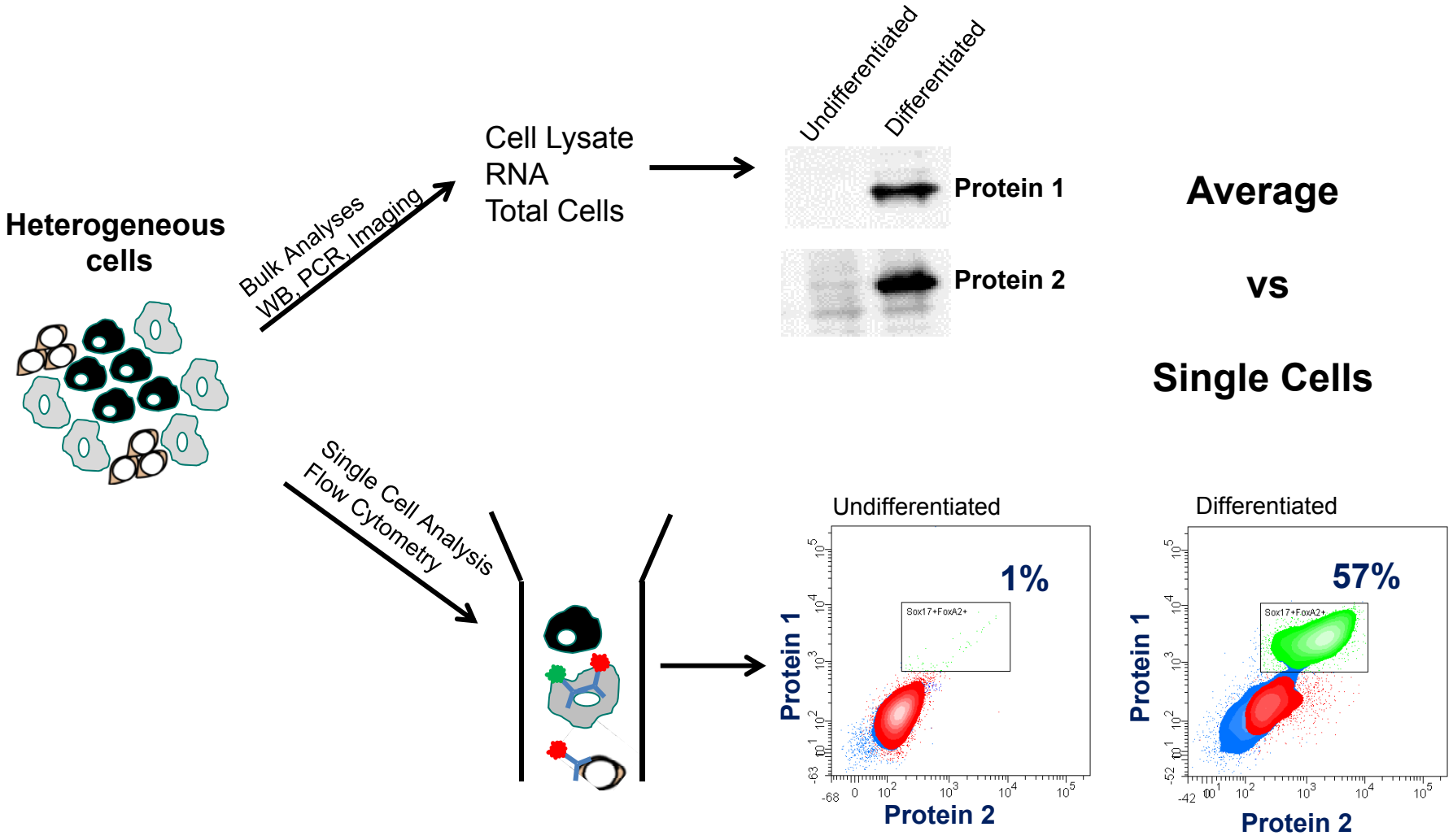
- GFP,YFP
- mCherry, RFP
- mOrange, dTomato

Flow Cytometry and Cell Biology

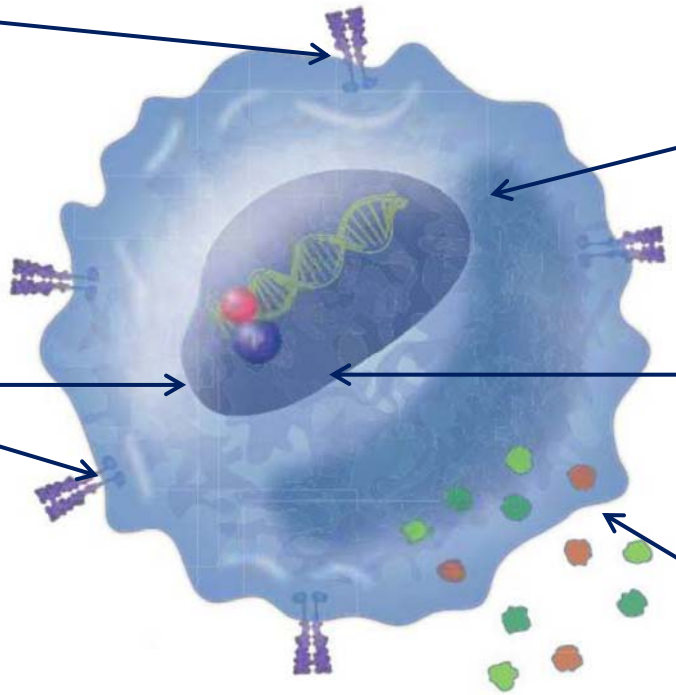
Publications Using the Keyword "Flow Cytometry" from PubMed



The Relevance of Flow Cytometry in Cell Biology



Analyzing a Cell by Flow Cytometry: More Than Surface Marker Analysis



Cell Surface

Lineage marker

Activation marker

Cytokine and chemokine receptors

Intracytoplasmic or Intranuclear

Cell signaling proteins

BD Phosflow™ technology

Cell Processes

Apoptosis

Cell viability

Proliferation

Antibodies and Functional Dyes

Intranuclear

Transcription factors

Intracytoplasmic

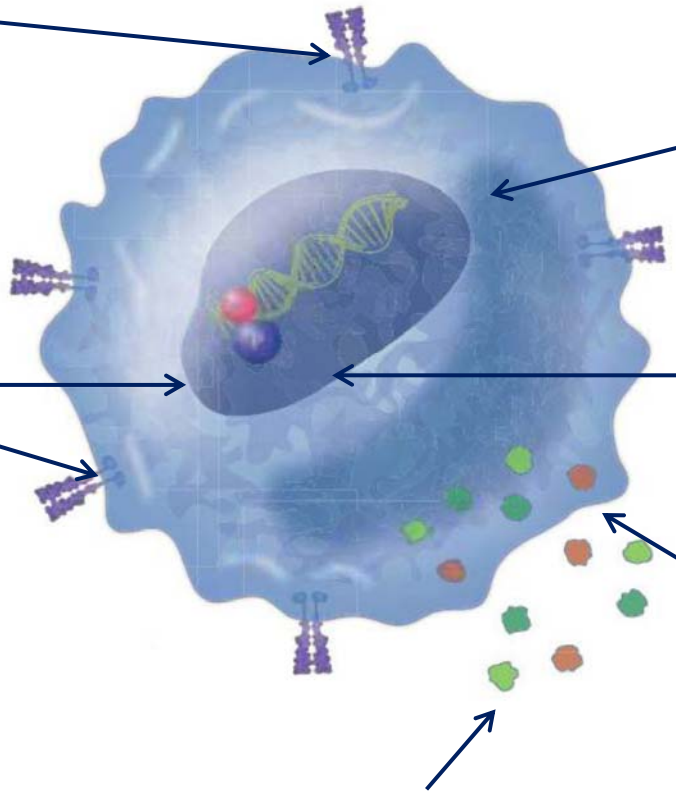
Cytokines, chemokines, various proteins

Antibodies and BD™ Cytometric Bead Array (CBA)

Quantification in supernatant, plasma, serum, or cell lysates:

cytokines, chemokines, immunoglobulins, or signaling proteins

Analyzing a Cell by Flow Cytometry: More Than Surface Marker Analysis



Cell Surface
Lineage marker
Activation marker
Cytokine and chemokine receptors

Intracytoplasmic or Intranuclear
Cell signaling proteins
BD Phosflow technology

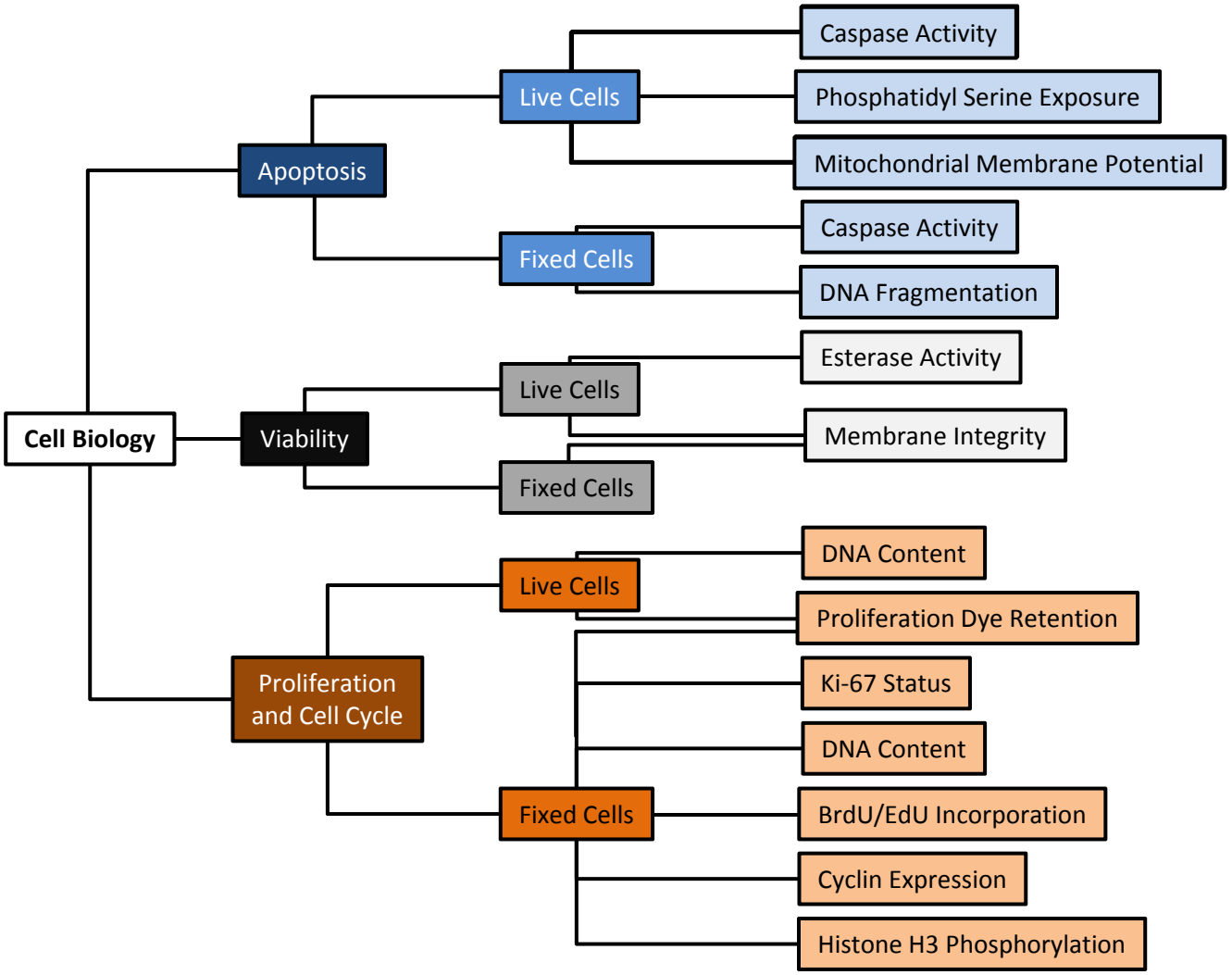
Cell Processes
Apoptosis
Cell viability
Proliferation
Antibodies and Functional Dyes

Intranuclear
Transcription factors

Intracytoplasmic
Cytokines, chemokines, various proteins
Antibodies and BD Cytometric Bead Array (CBA)

Quantification in supernatant, plasma, serum, or cell lysates:
cytokines, chemokines, immunoglobulins, or signaling proteins

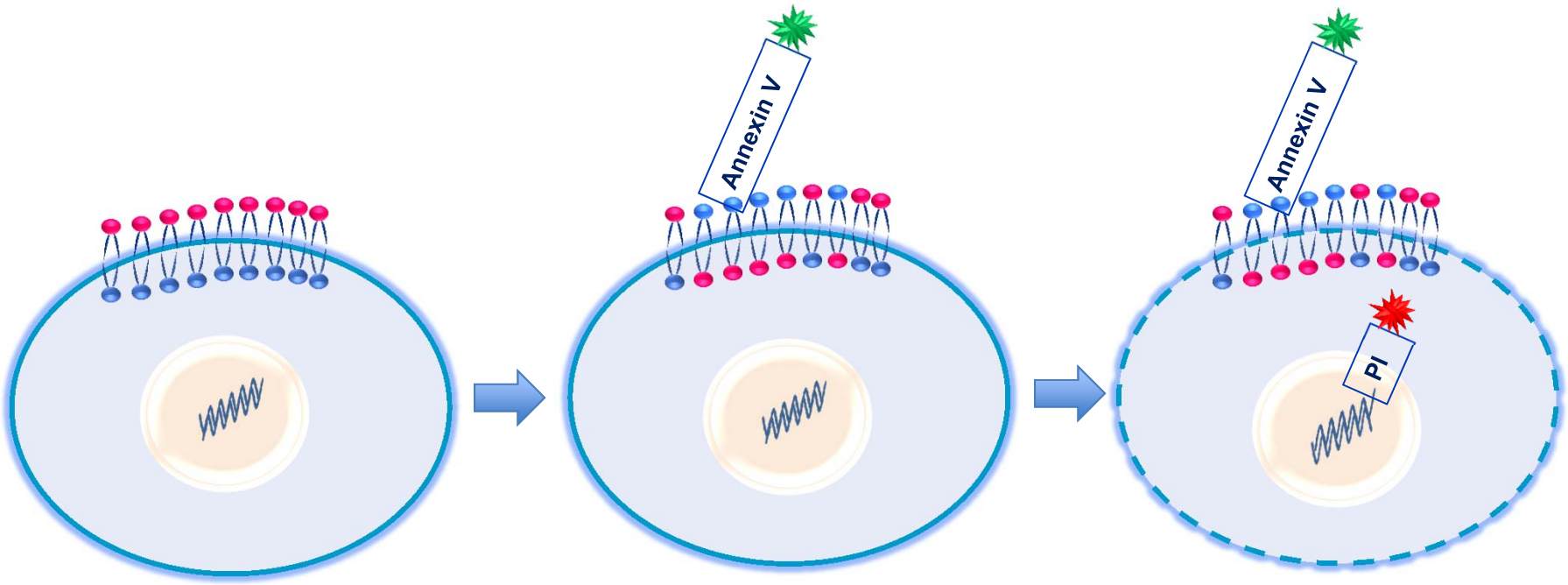
Cell Function Assay Landscape



The BD Accuri™ C6 Personal Flow Cytometer

- Easy to use
- Two lasers, six parameters
- Fixed voltages
- Cell counting
- Continuous sampling
- Kits and templates

Apoptosis/Viability Assay

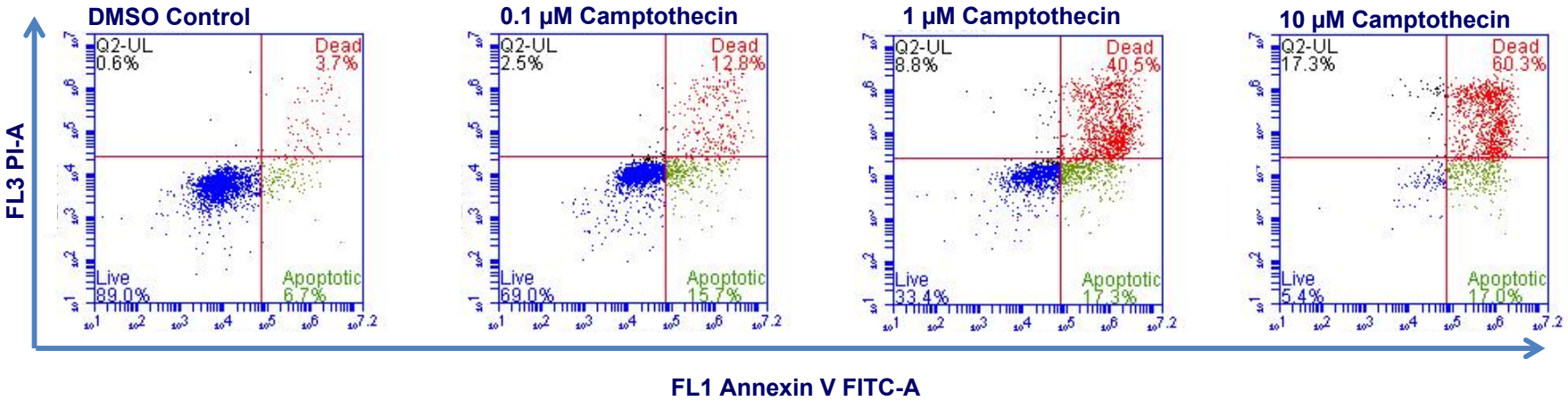


Live
Annexin V^-/PI^-

Apoptotic
Annexin V^+/PI^-

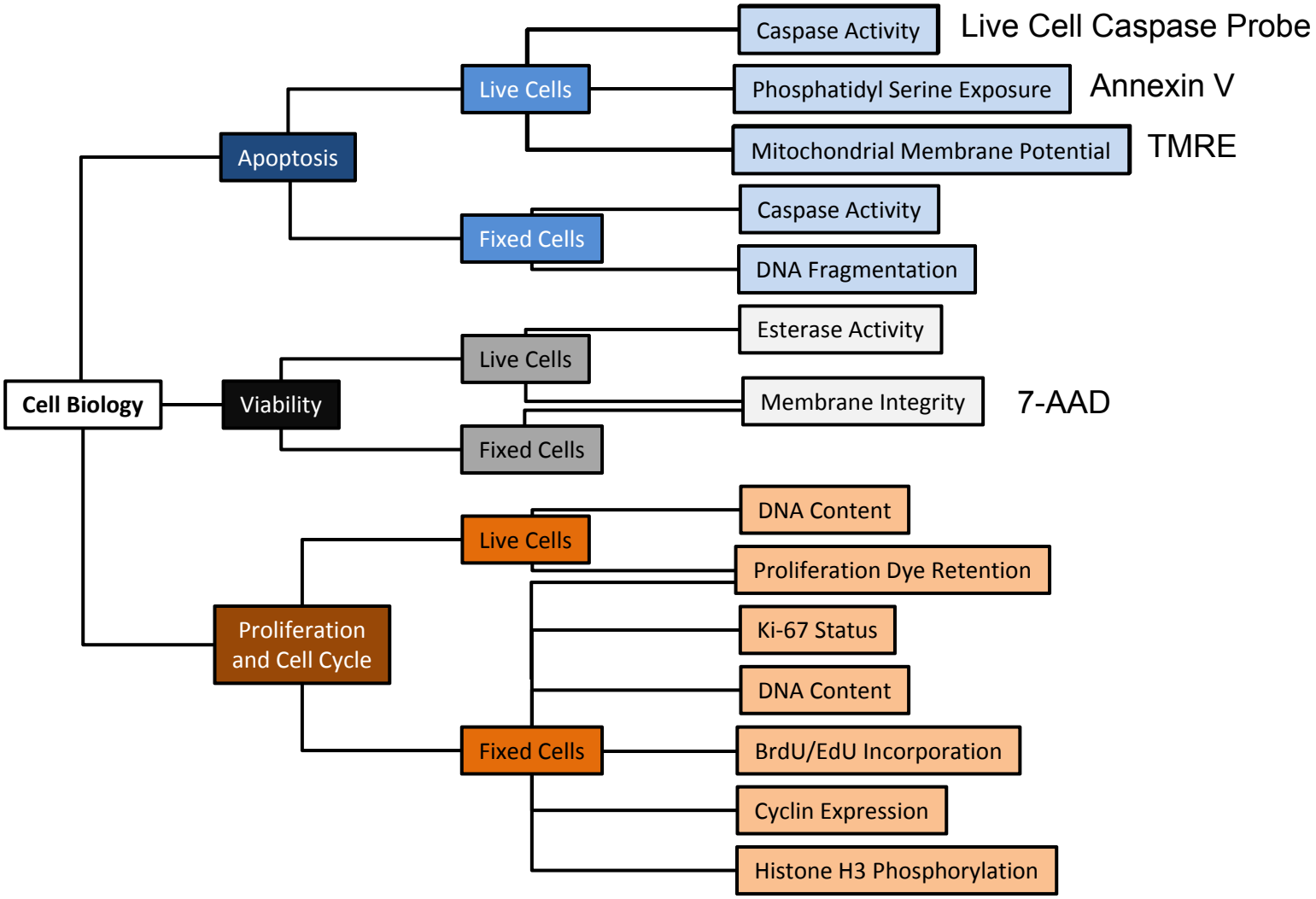
Dead
Annexin V^+/PI^+

Dose Response Assay



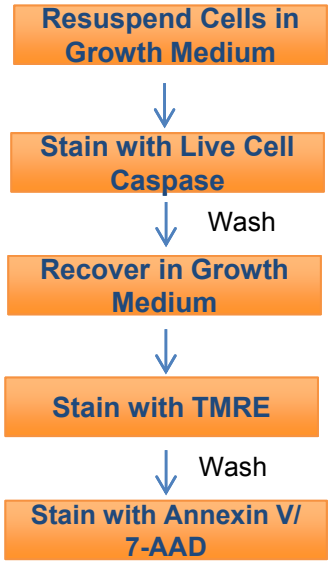
- **MDA-MB-231 cells were treated for 48 hours with varying doses of camptothecin (0.1–100 µM).**
- **Cells were stained with the BD™ Annexin V Apoptosis Detection Kit and analyzed using the kit template.**

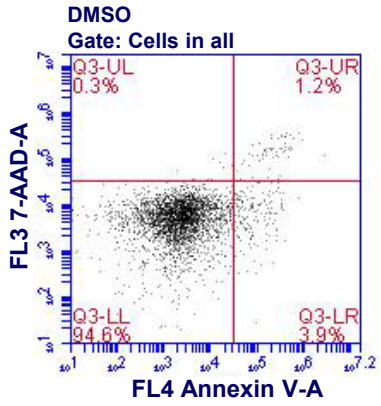
Cell Function Assay Landscape

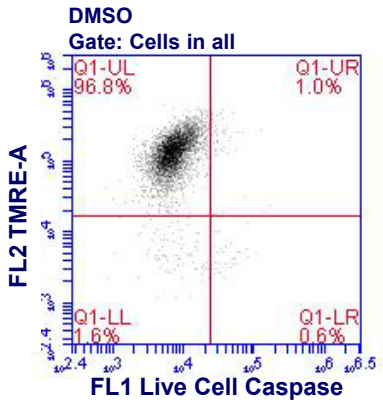


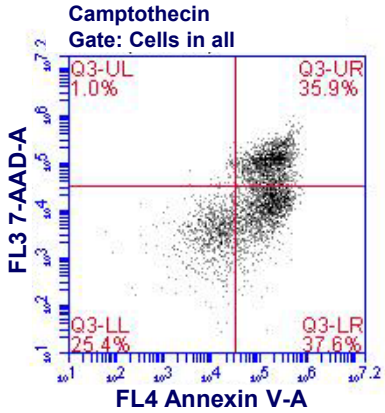
Multiparameter Apoptosis/Viability Analysis on Live Cells

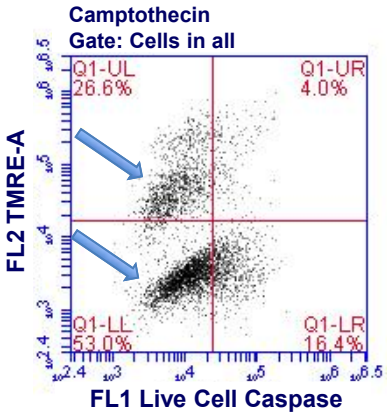
Function	Target	Probe
Apoptosis	Caspase Activity	Blue Live Cell Caspase (FL1)
Apoptosis	Mitochondrial Membrane Potential	TMRE (FL2)
Viability	Membrane Integrity	7-AAD (FL3)
Apoptosis	Phosphatidyl Serine Exposure	Annexin V (FL4)











MDA-MB-231 cells were treated for 48 hours with 10 µM of camptothecin.

Designing Multiparameter Cell Function Panels

Cell Cycle/Proliferation

Function	Target	Probe
Proliferation	BrdU Incorporation	α-BrdU antibody (FL1)
Cell Cycle	Cyclin-B	α-Cyclin-B antibody (FL2)
Cell Cycle	DNA Content	7-AAD (FL3)
Cell Cycle	Phosphorylated Histone 3	α-pH3 antibody (FL4)

DNA Damage/Proliferation

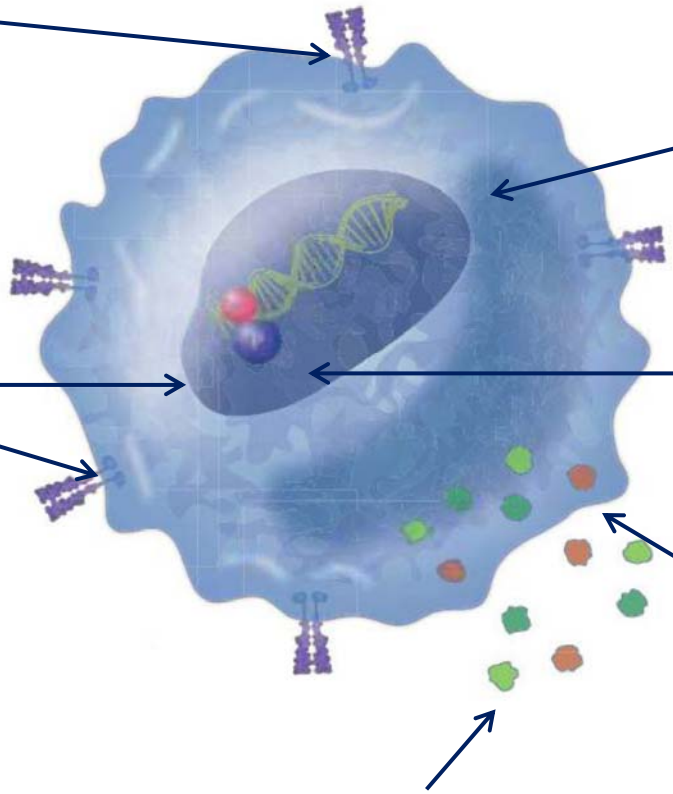
Function	Target	Probe
Apoptosis	Cleaved PARP	α-PARP cleaved form antibody (FL2)
Proliferation	BrdU Incorporation	α-BrdU antibody (FL3)
DNA Damage	Phosphorylated H2AX Histone	α-pH2AX (FL4)

Cell Cycle/Viability/Apoptosis

Function	Target	Probe
Proliferation	BrdU Incorporation	α-BrdU antibody (FL1)
Apoptosis	Caspase	α-Caspase 3 antibody (FL2)
Cell Cycle	DNA Content	7-AAD (FL3)
Viability	Membrane Integrity	FVS660 (FL4)

- **Multiparameter panels can be designed to analyze different cell functions simultaneously.**
- **Cell function reagents are offered in a variety of colors for increased panel design flexibility.**
- **BD Horizon™ fixable viability stains allow for simultaneous analysis of viability and intracellular molecule expression.**

Analyzing a Cell by Flow Cytometry: More Than Surface Marker Analysis



Cell Surface
Lineage marker
Activation marker
Cytokine and chemokine receptors

Intracytoplasmic or intranuclear
Cell signaling proteins
BD Phosflow technology

Cell Processes
Apoptosis
Cell viability
Proliferation
Antibodies and Functional Dyes

Intranuclear
Transcription factors

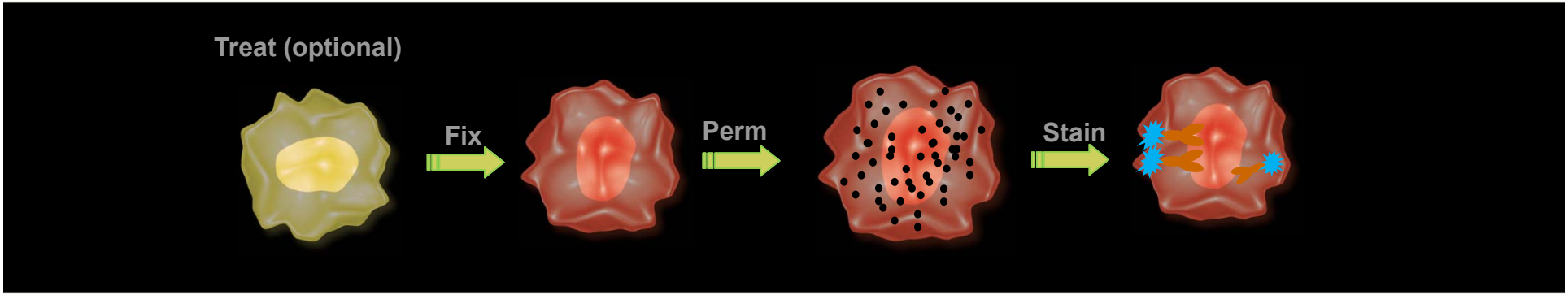
Intracytoplasmic
Cytokines, chemokines, various proteins
Antibodies and BD Cytometric Bead Array (CBA)

Quantification in supernatant, plasma, serum, or cell lysates:
cytokines, chemokines, immunoglobulins, or signaling proteins

Advantages of Phosphorylation Analysis by Flow Cytometry

- Single-cell analysis
- Rapid assay
- Reduced number of cells per test
- Quantitative
- Multiparametric
- Increased throughput

Standard Protocol for Analyzing Protein Phosphorylation by Flow Cytometry

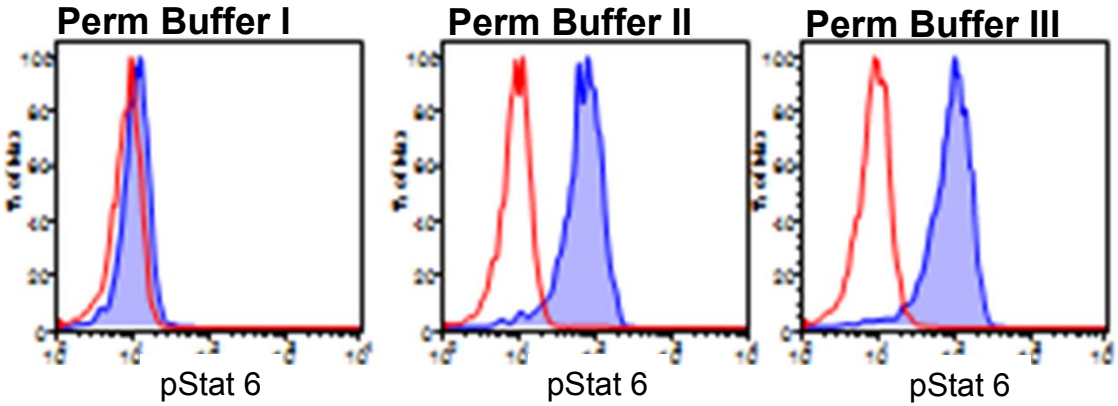


Protocol Considerations

- Stimulating conditions
- Kinetics
- Assay controls
- Cell culture conditions (suspension vs adherent)
- Fix and permeabilization buffer selection

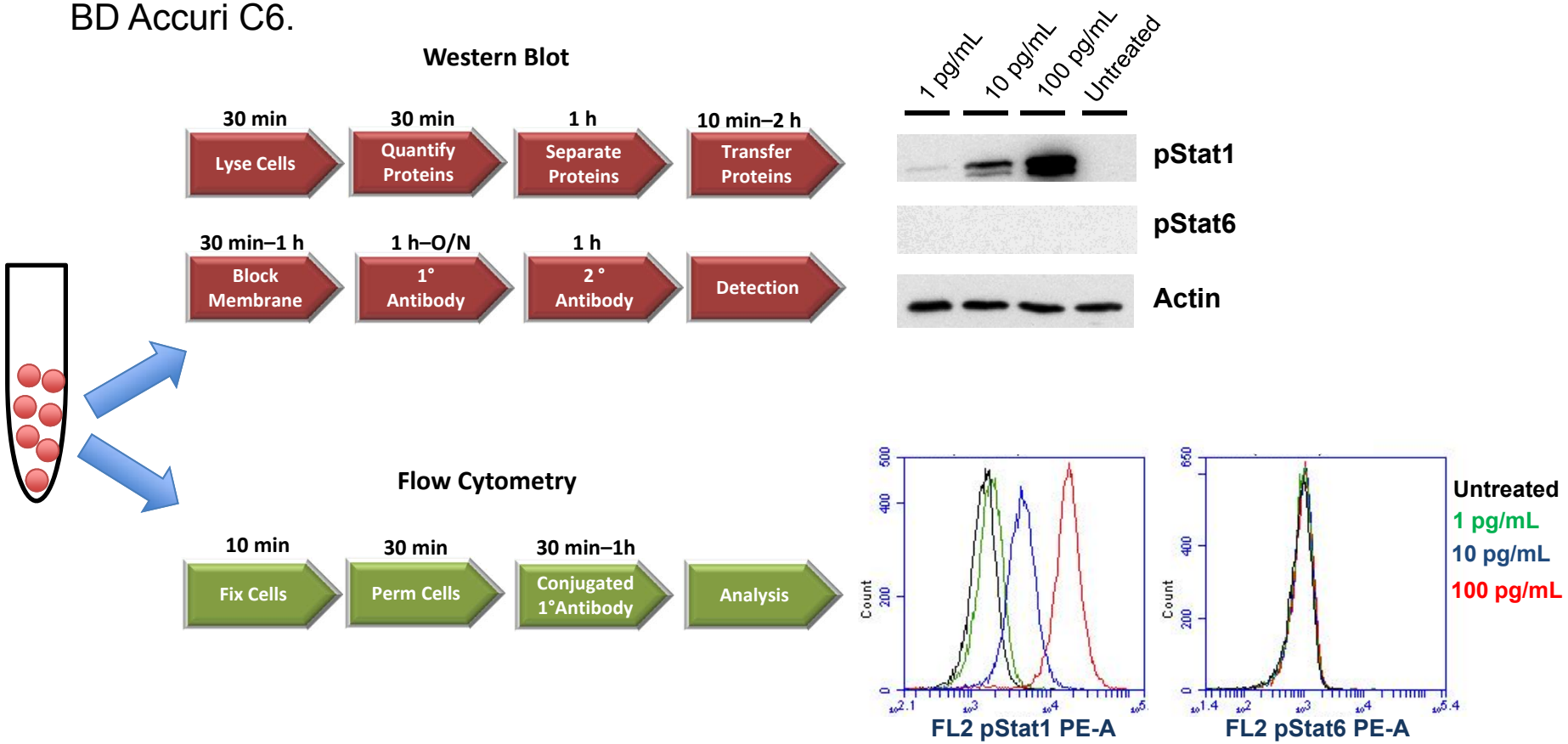
Protocol Considerations

- Stimulating conditions
- Kinetics
- Assay controls
- Cell culture conditions (suspension vs adherent)
- Fix and permeabilization buffer selection



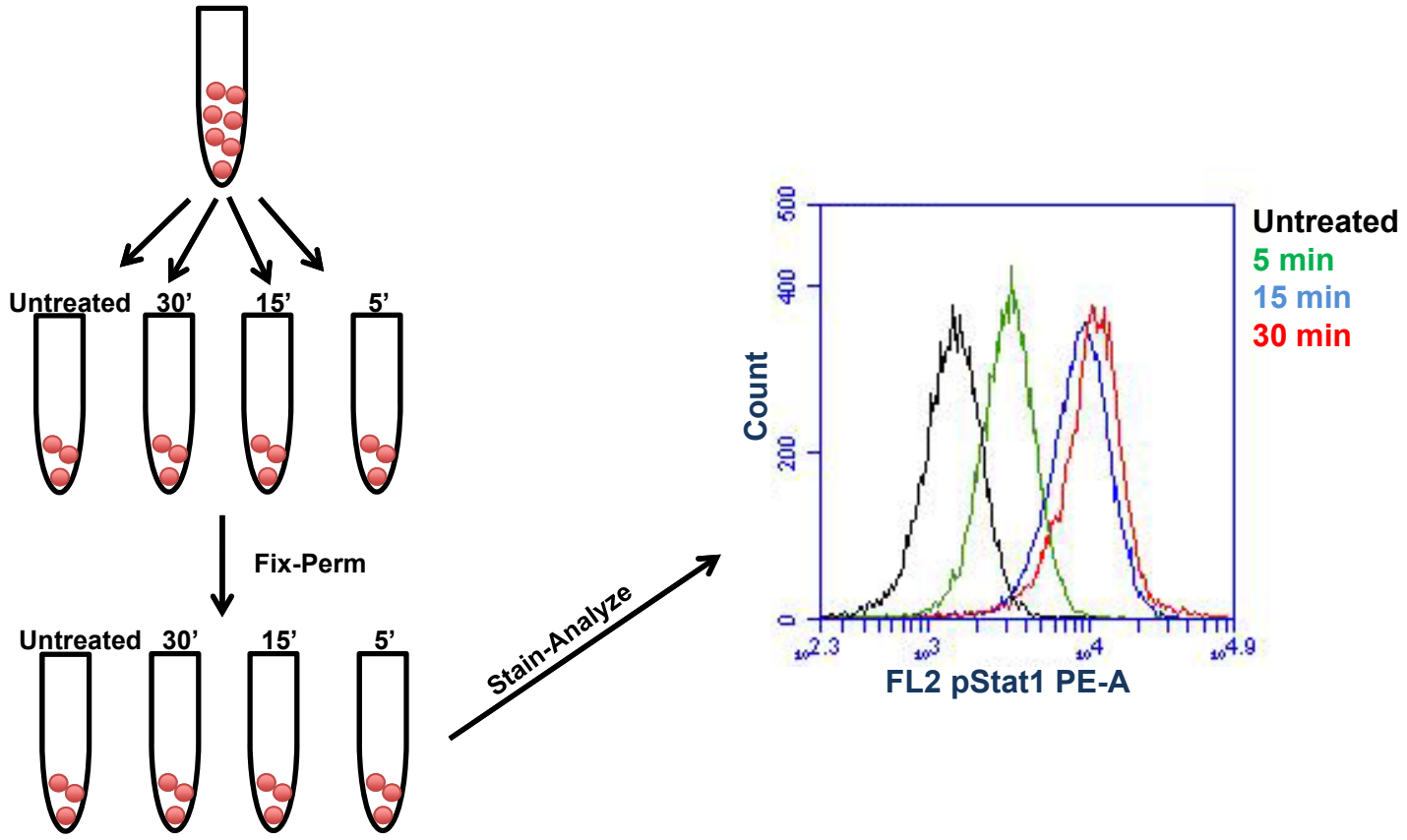
Analysis of Stat Phosphorylation on the BD Accuri C6: Dose Response

- Human lymphoma U-937 cells were stimulated with increasing doses of IFN-γ for 15 minutes.
- Stat1 and Stat6 phosphorylation was assessed by WB or flow cytometry on the BD Accuri C6.



Analysis of Stat Phosphorylation on the BD Accuri C6: Time Course

- Human lymphoma U-937 cells were stimulated with 10 pg/mL of IFN-γ for 30, 15, and 5 minutes.
- Each tube was individually stained with pStat-1 PE antibody and analyzed on the BD Accuri C6.



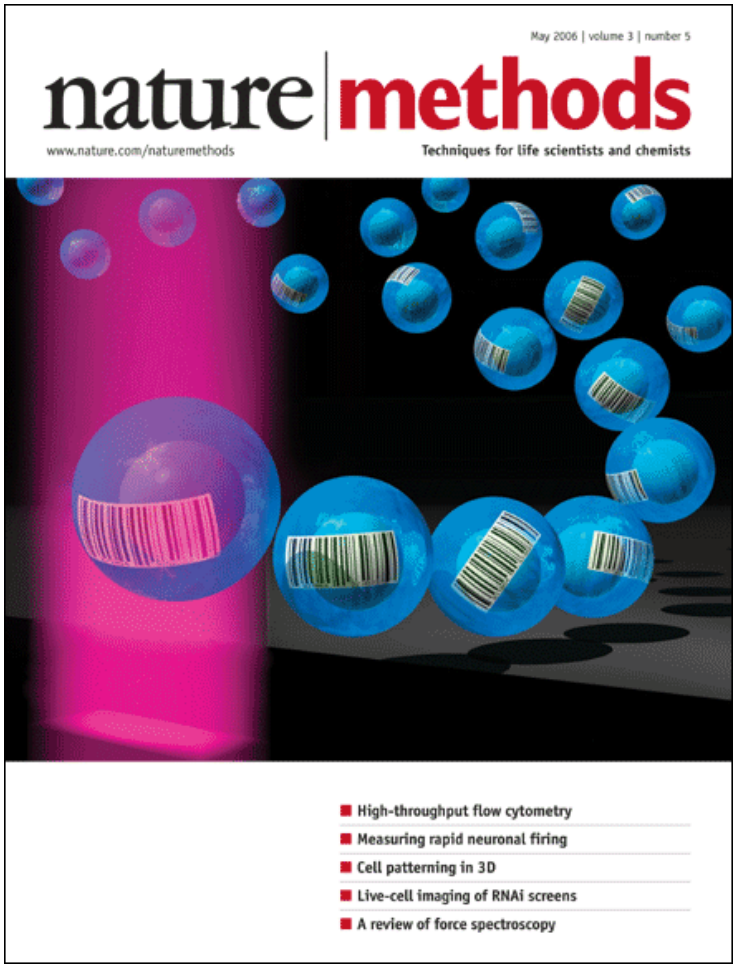
Cell Barcoding

How can we track populations of cells?

- Fluorescent markers (example, GFP, mCherry)
- Cellular dyes (example, CFSE)
- Genetic barcoding

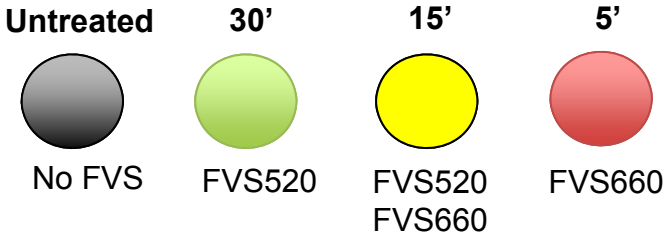
Fluorescent cell barcoding for flow cytometry

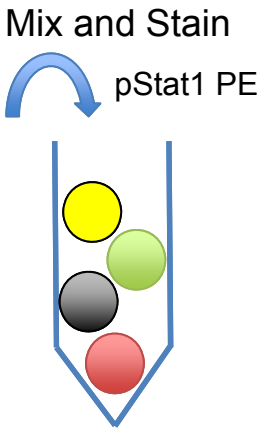
- Increases throughput
- Enables larger screens/profiles
- Improves robustness of assays
- Decreases acquisition times

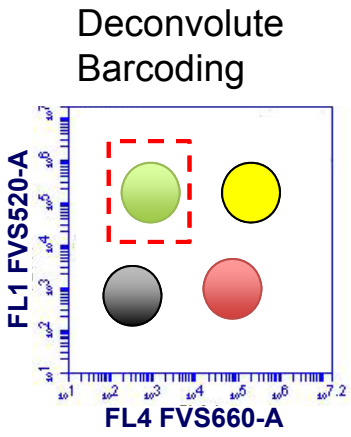


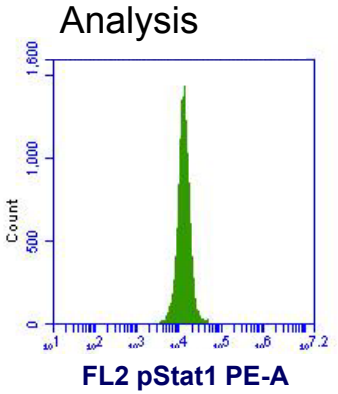
Barcoding: Kinetic Assay in One Tube

- Fixed and permeabilized cells were individually color coded using BD Horizon fixable viability stains.
- Cells were then mixed and stained with a single aliquot of anti-pStat1 PE antibody.



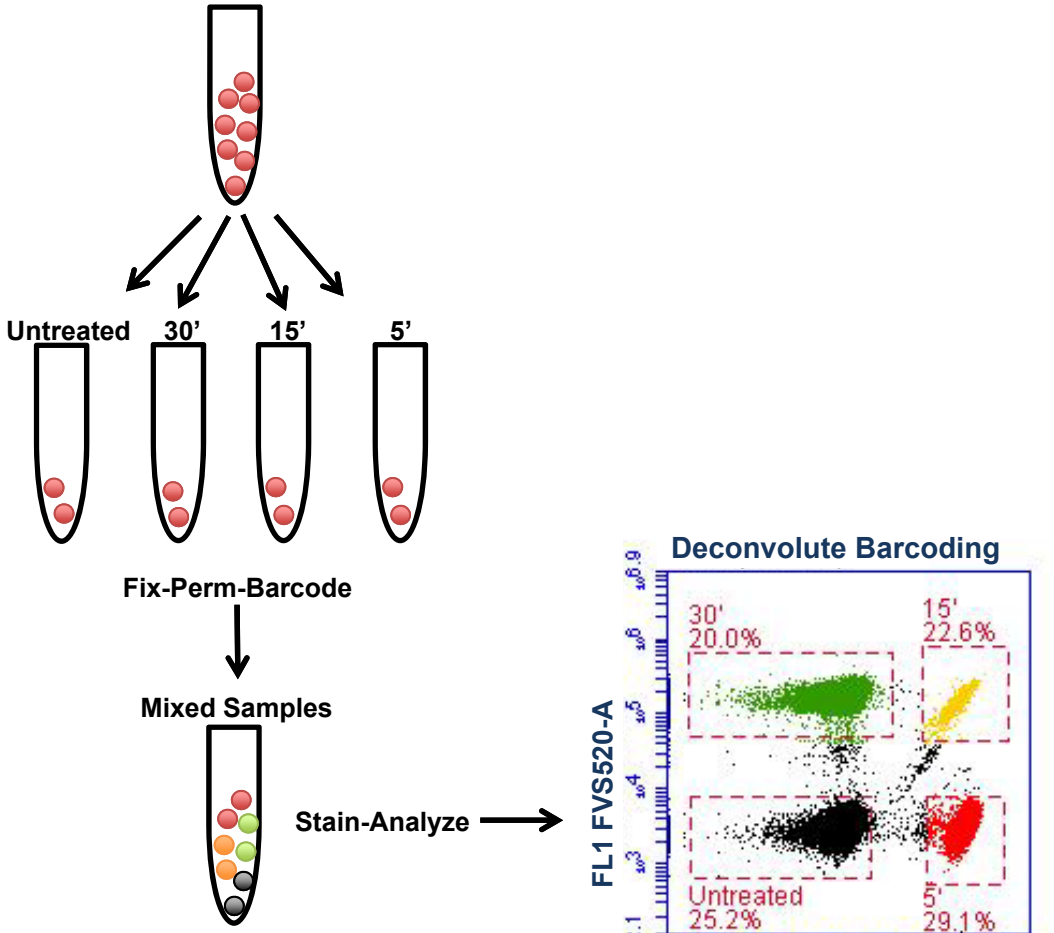




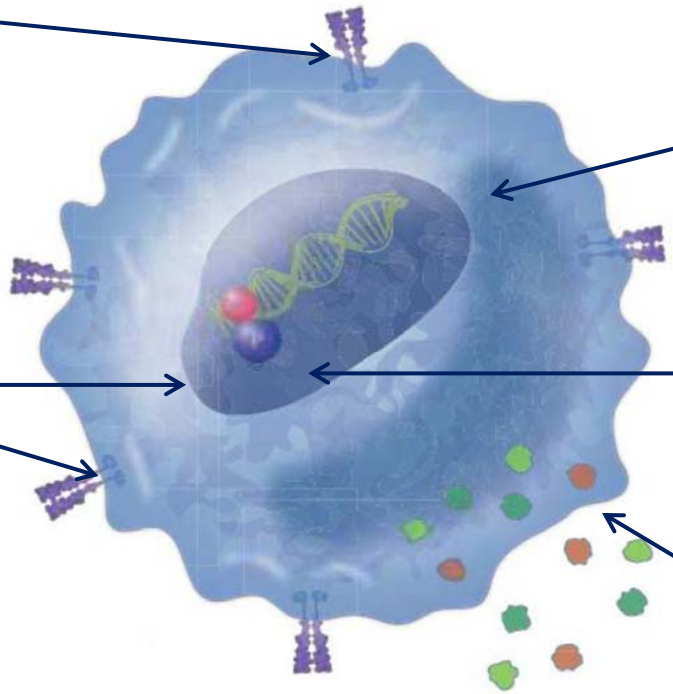


Barcoding: Kinetic Assay in One Tube

- Only one tube containing all four samples was analyzed on the BD Accuri C6.



Analyzing a Cell by Flow Cytometry: More Than Surface Marker Analysis



Cell Surface

Lineage marker

Activation marker

Cytokine and chemokine receptors

Intracytoplasmic or Intranuclear

Cell signaling proteins

BD Phosflow technology

Cell Processes

Apoptosis

Cell viability

Proliferation

Antibodies and Functional Dyes

Intranuclear

Transcription factors

Intracytoplasmic

Cytokines, chemokines, various proteins

Antibodies and BD Cytometric Bead Array (CBA)

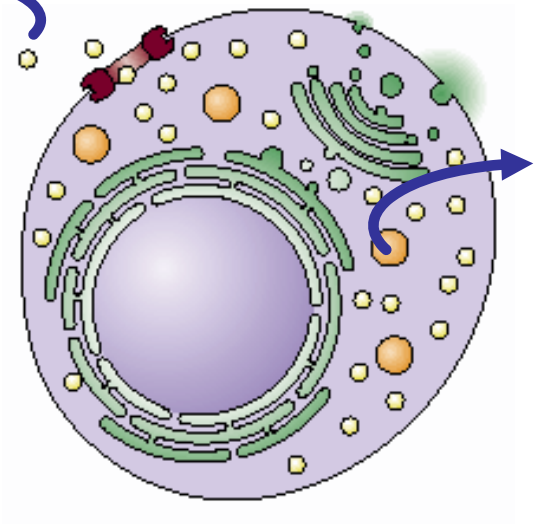
Quantification in supernatant, plasma, serum, or cell lysates:

cytokines, chemokines, immunoglobulins, or signaling proteins

Multiple Methods to Analyze Cytokine Expression

Soluble Proteins

- ELISA
- ELISPOT
- BD CBA Array



Intracellular Proteins

- Flow Cytometry
- Western Blot
- Immunohistochemistry

Two distinct flow cytometry assays to analyze cytokine expression

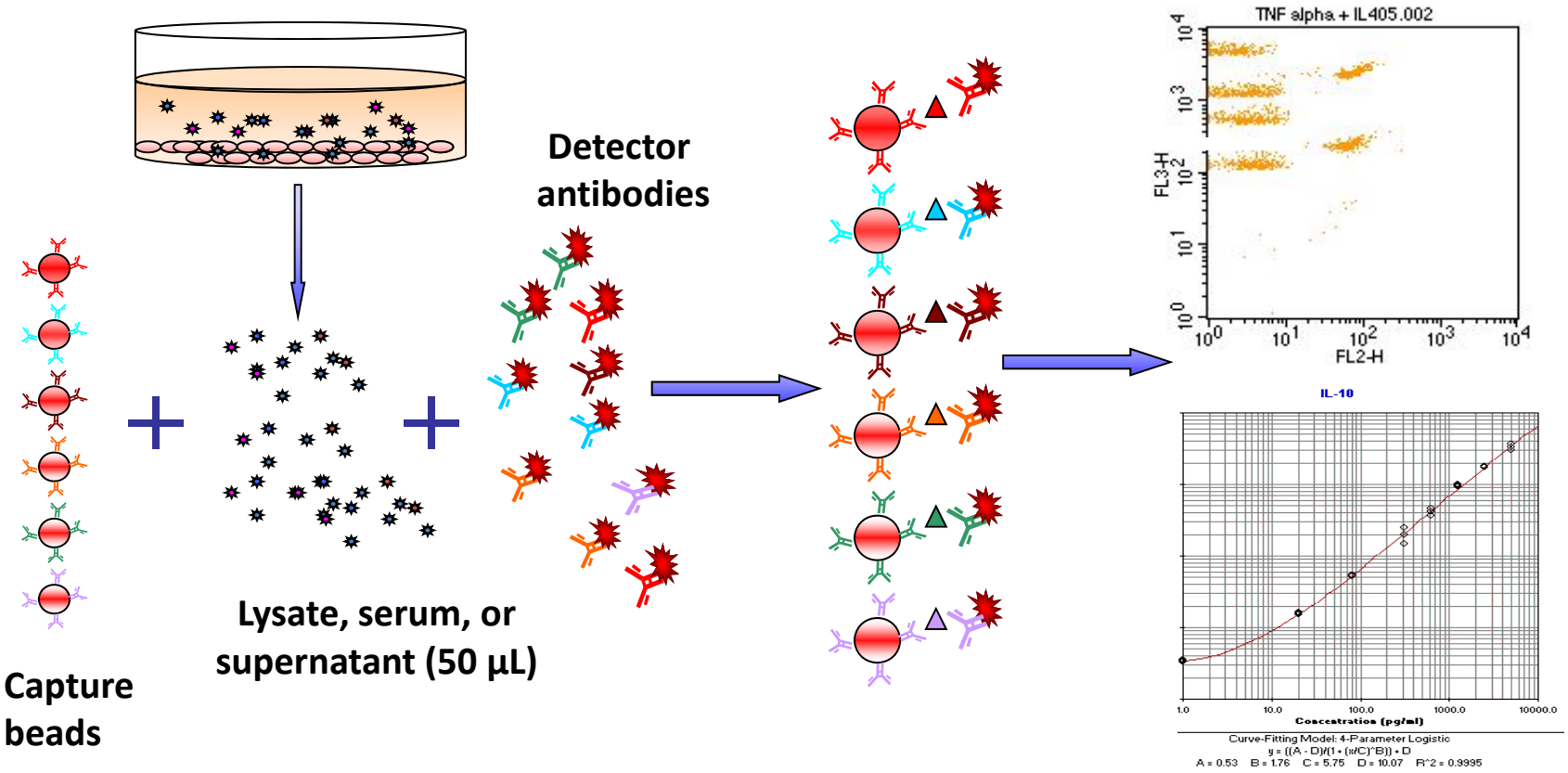
- Bead-based immunoassay (BD CBA)
- Intracellular flow cytometry

Advantages of Cytokine Analysis Using Flow Cytometry

- BD CBA:
  - Quantitative and sensitive
  - Analysis of multiple cytokines simultaneously
  - Reduced sample volume requirement
  - Requires less sample dilution
- Intracellular flow cytometry:
  - Cytokine analysis at the single cell level
  - Compatible with simultaneous surface marker analysis

BD CBA and intracellular flow cytometry can be used as complementary techniques for a more comprehensive cytokine analysis.

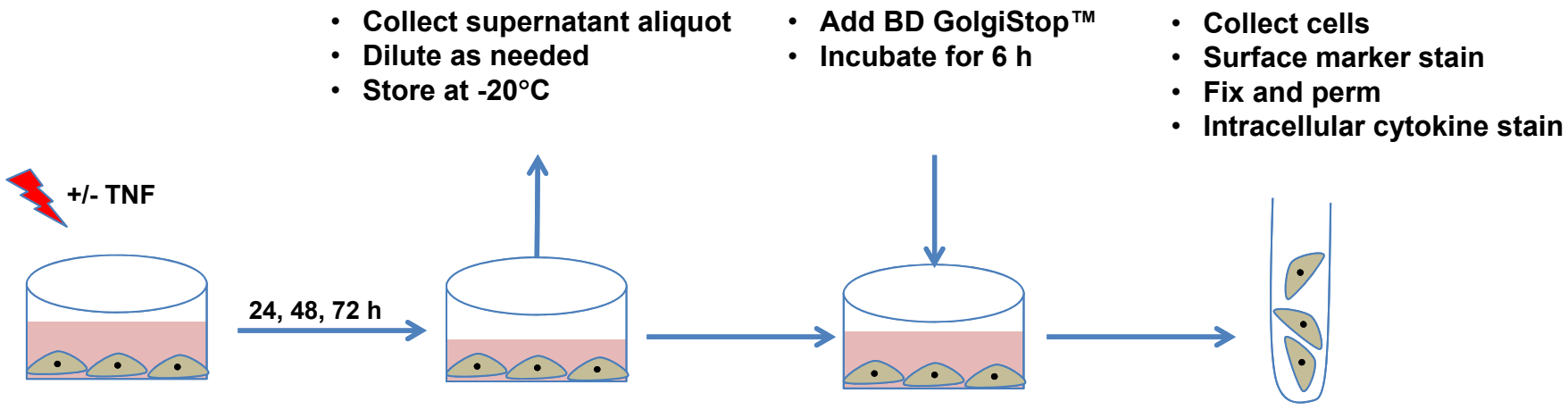
BD CBA Assay



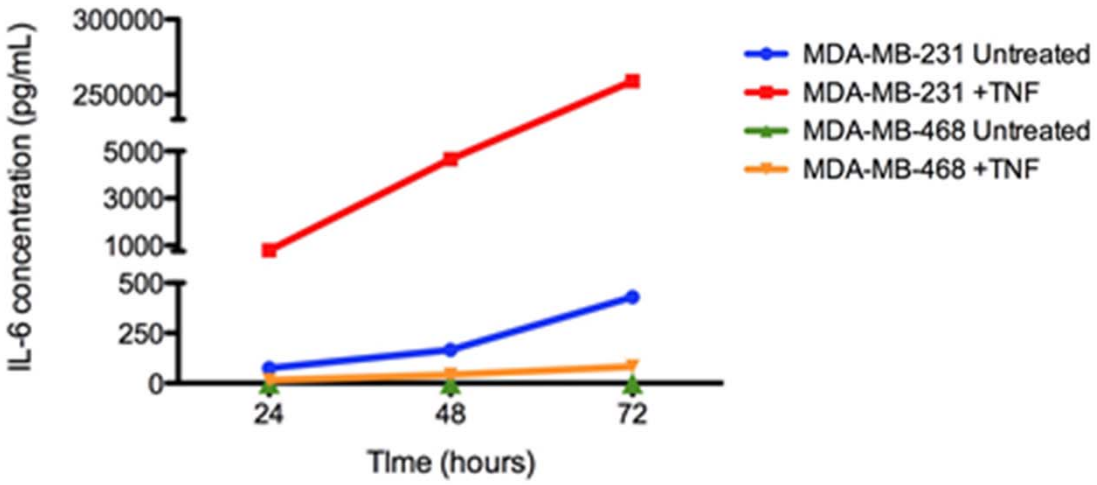
- More than 20 analytes can be detected.
- The BD CBA array is like running multiple ELISA assays in one single tube.

Cytokine Analysis in Cancer Cells

- Pro-inflammatory stimuli induce expression of cytokines involved in cancer progression.
- Breast cancer cell lines MDA-MB-231 and MDA-MB-468 were stimulated with TNF.
- Cytokine expression was evaluated on the same sample using BD CBA and intracellular flow cytometry.



Quantification of IL-6 in Cancer Cell Cultures using a BD CBA Array

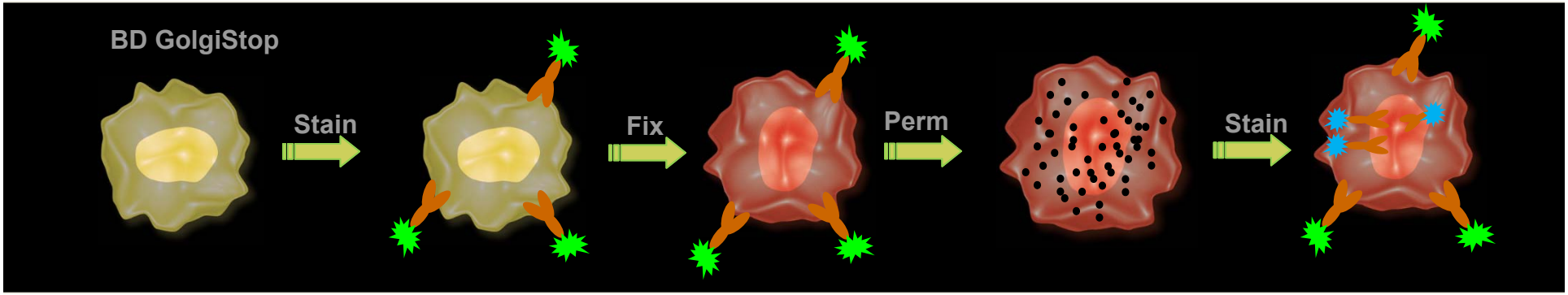


IL-6 Concentration (pg/mL)

Sample	24 h	48 h	72 h
MDA-MB-231	75.7	165	429.7
MDA-MB-231 +TNF	791.2	4,564.5	258,805
MDA-MB-468	0.26	0.54	1.3
MDA-MB-468 +TNF	15.5	42.4	82.7

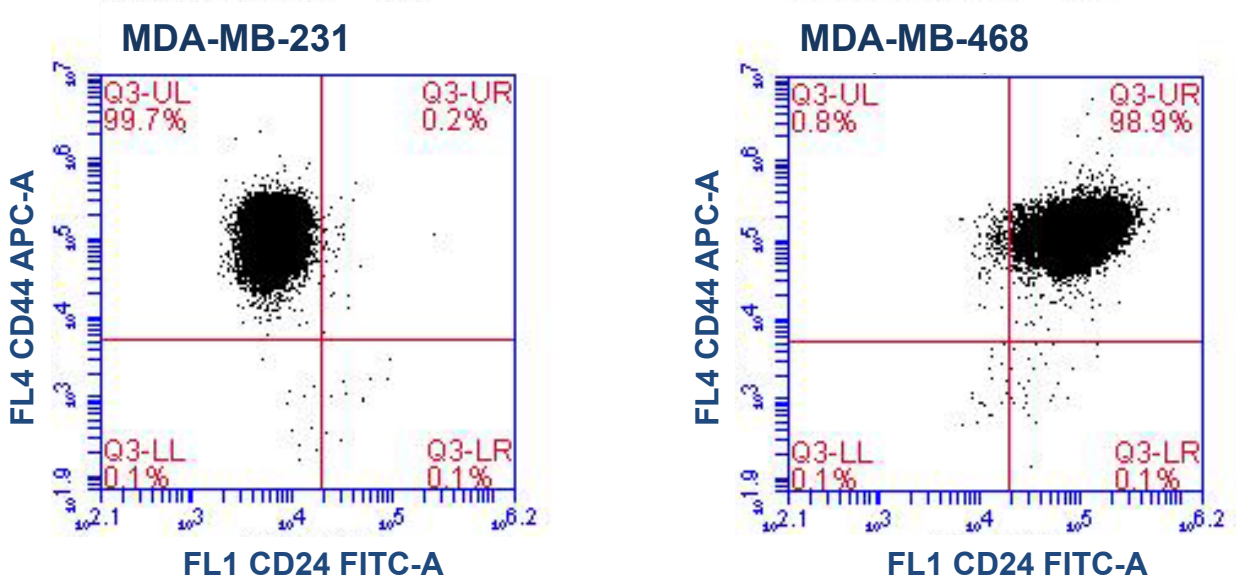
- The more aggressive cell line MD-MB-231 expressed a higher basal level of IL-6.
- Upon TNF stimulation, MDA-MB-231 responded by robustly increasing IL-6 secretion.

Combining Surface and Intracellular Stain for Single Cell Cytokine Analysis



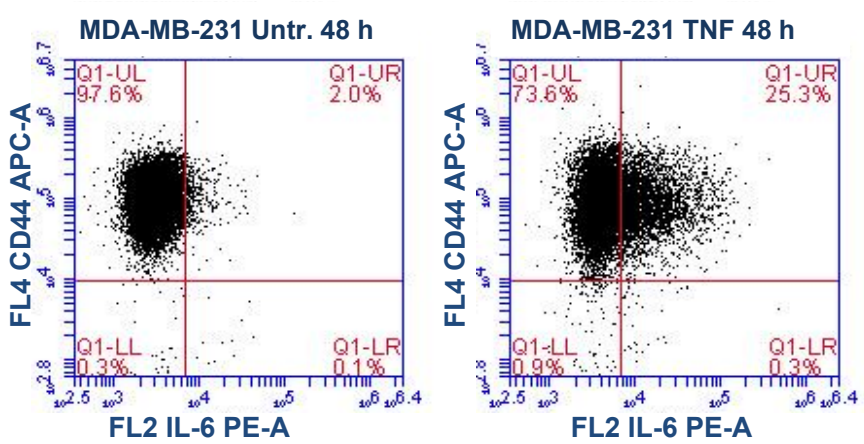
- Treat with BD GolgiStop™ inhibitor for six hours to block cytokine secretion.
- Detach with BD Accutase™.
- Stain for surface markers CD24 and CD44.
- Fix and perm.
- Stain with antibodies against IL-6.

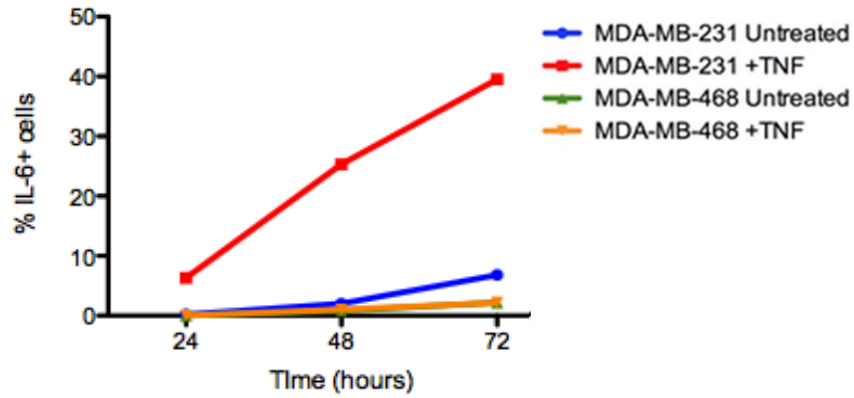
Surface Marker Analysis

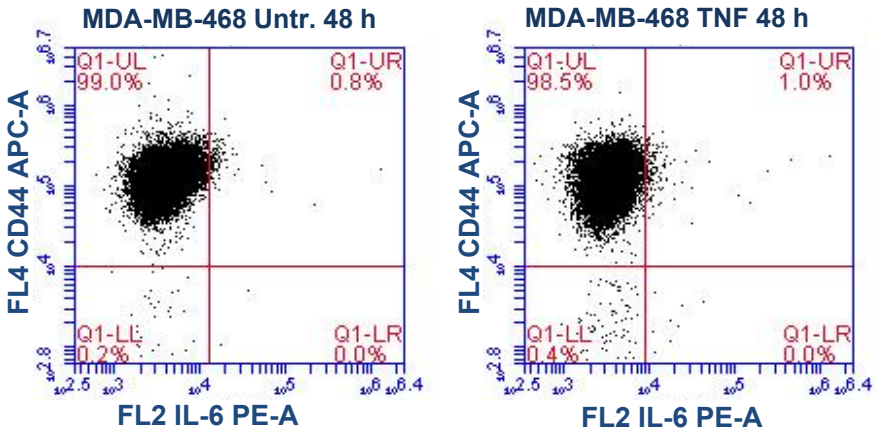


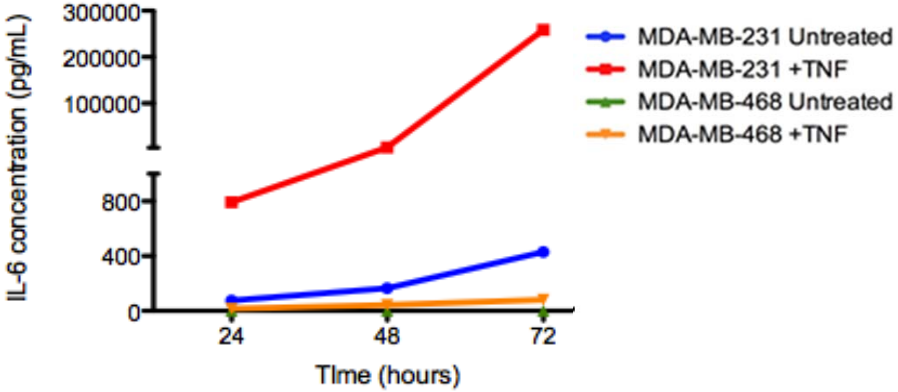
MDA-MB-231 cells displayed a $CD44^{+}CD24^{-}$ cancer stem cell signature correlating with a more aggressive cancer phenotype.

Surface and Intracellular Analysis: CD44 and IL-6









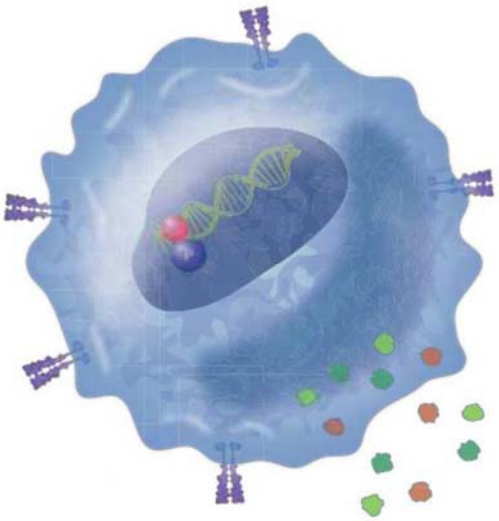
Intracellular flow cytometry results correlate with BD CBA data.

Cytokine Analysis Summary

- The BD CBA assay allowed us to test multiple cytokines simultaneously and to identify IL-6 as a cytokine regulated by TNF treatment.

- The combination of surface and intracellular flow cytometry allowed us to:
  - Confirm the different phenotype of the two cell lines tested
  - Determine that only a discrete subset of MDA-MB-231 cells express IL-6 upon TNF stimulation

Analyzing what is on, in, and Made by the Cell



	Method of Analysis
Cell Surface Markers	• Flow Cytometry
Intracellular Proteins	• Flow Cytometry
Cytokines	• Flow Cytometry
Cellular Processes	• Flow Cytometry

- **One rapid methodology for broad cell biology applications**
- **Ability to correlate expression of surface and intracellular molecules**
- **Ability to quantitate the percentage of cells expressing markers of interest and the amount of secreted cytokines**

Research Solutions for Cell Biology

- **Free Downloadable Templates**
- **Broad Reagent Portfolio**
- **Product Information Sheets**
- **Technical Documents**
- **Webinars**
- **BD Accuri News**

www.bdbiosciences.com/resources/accuri

Acknowledgments

BD Biosciences:

San Diego

- Mirko Corselli
- Nil Emre
- Guo-Jian Gao
- Rosanto Paramban
- Jacob Rabenstein
- Stephanie Widmann
- Lissette Wilensky

Ann Arbor

- Stacey Roys
- David Draper

San Jose

- Ranga Partha
- Andy Wang

Questions?

If you have further questions:
Contact Technical Support (US) at:
877-232-8995, Prompt 3, 2
or email: ResearchApplications@bd.com