Avances y Perspectivas de la **Citometría de Flujo 2013**

Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional

Laboratorio Nacional de Servicios Experimentales (LaNSE)

El Centro de Investigación y Estudios Avanzados del IPN y Beckman Coulter de México tienen el honor de invitar a la comunidad científica, clínica y público en general al Simposio "Avances y Perspectivas de La Citometría de Flujo 2013" que consistirá en ponencias magistrales y talleres teórico – prácticos.

SYMPOSIUM

19 y 20 de Septiembre, 2013

CINVESTAV - Zacatenco, México, D.F.

Coordinador: Dr. José Tapia Ramírez (CINVESTAV)

Talleres Cupo Limitado. Lunch Incluido.

Pre-registro de Talleres en: vrosales@cinvestav.mx

Jueves 19 de Septiembre	8:30 – 9:30	**Registro**
	9:30 – 9:45	**Palabras de Bienvenida** *Dr. René Asomoza Palacio (Director General del CINVESTAV)*
	9:45 – 10:45	**Hemoglobinuria Paroxística Nocturna** *Dr. Alejandro Ruiz Argüelles (Laboratorios Clínicos De Puebla)*
	10:45 – 11:45	**Integridad y Daño en Espermatozoides de Humano y Roedores: Evaluación por Citometría de Flujo** *Dra. Betzabet Quintanilla Vega (CINVESTAV)*
	11:45 – 12:00	**Receso**
	12:00 – 13:00	**Inmunodeficiencias** *Dr. Leopoldo Santos Argumedo (CINVESTAV)*
	13:00 – 14:30	**Citometría de Flujo: Historia y Avances** *Dr. Diether Recktenweald (Desatoya LLC)*
Viernes 20 de Septiembre	12:00 – 13:00	**Linfocitos B Blanco de Salmonella** *Dr. Vianney Ortiz Navarrete (CINVESTAV)*
	13:00 – 14:30	**Citometría de Masas y Ciclo Celular** *Dr. Garry Nolan (Universidad de Stanford)*
	14:30 – 14:45	**Clausura** *Dr. Marco Antonio Meraz Ríos (Secretario de Planeación, CINVESTAV)*

Talleres Simultáneos — **Coordinador: M. en C. Víctor Hugo Rosales (CINVESTAV)**

Teoría
Jueves 19
8:00 – 9:15

Práctica
Jueves 19
15:00 – 18:00

Viernes 20
8:00 – 11:30

Células Troncales y Linaje
M en C. Libertad Meza (DICIPA) y M en C. Jairo Villanueva (Beckman Coulter)

Ciclo Celular y Apoptosis
M en C. Alberto Ponciano Gómez (CINVESTAV) y QFB Alfredo García Vensor (CINVESTAV)

Detección de Microvesículas Derivadas de Neutrófilos por Citometría de Flujo
M en C. Violeta Álvarez Jiménez (Ciencias Biológicas-IPN) y M en C. Israel Romo Cruz (CINVESTAV)

Leucemias: Investigación y Diagnóstico
M en C. Adriana Gutiérrez (Ciencias Biológicas-IPN)

Señalización Intracelular *Dr. Héctor Romero Moreno (CINVESTAV)*

Separación (Sort) de Linfocitos Antígeno-Específico
Dr. Luis Donis (CINVESTAV) y M en C. Juan Carlos Yam Puc (CINVESTAV)

DNA de Espermatozoides y Daño Toxicológico
M en C. María Solís (CINVESTAV)

Determinación de Citocinas en Investigación y Diagnóstico
Dra. Yevel Flores García (Instituto Nacional de Pediatría)

Detección de Células T Reguladoras
Dra. Gloria Soldevila Melgarejo (Instituto de Investigaciones Biomédicas-UNAM)

SEDE: ***Auditorio Rosenblueth*** *CINVESTAV – ZACATENCO*
Av. Instituto Politécnico Nacional 2508 Col. San Pedro Zacatenco.
Del. Gustavo A. Madero, México, D.F. C.P. 07360
Tel: +52(55) 5747 3800 Ext. 5368, 6754, 1790

Para obtener constancia de asistencia al evento favor de registrar sus datos al inicio del evento.
El evento no tiene costo.

BECKMAN COULTER

Cinvestav

Citometría de Flujo
Historia y Avances

Avances y Perspectivas de la **Citometría de Flujo**

CINVESTAV - Zacatenco, Mexico, D.F.

19 Septiembre, 2013

Dr. Diether Recktenwald, Desatoya LLC
Reno NV 89507, USA

Diether@desatoya.com

Biology Research Targets and Tools

Organism

Organ

Tissue

Single Cell

Organelle

Macromolecule

Small molecules

NMR

X-ray imaging

Ultrasound

2-photon imaging

In-vivo cytometry

Light microscopy

Electron microscopy

Flow cytometry

Cell imaging

NA sequencing

Mass spectrometry

TIRF microscopy

Electrophoresis

Contrast agents

Affinity reagents

- antibodies
- probes

Enzyme substrates

Labels

- absorbance
- fluorescence
- element tags

Sample prep

Outline

- History
- Flow Cytometry Principles
- Important applications
- New developments
- New flow cytometric technologies for single cell analysis and sorting
- Outlook
- Summary and Conclusions

The Past

- 1665 – English physicist, Robert Hooke used a microscope lens to observe “pores” in cork
- 1674 – Anton van Leeuwenhoek built a simple microscope with only one lens to examine blood cells
- 1872 – Ernst Abbe calculated the maximum resolution in microscopes
- 1932 – Frits Zernike invented the phase-contrast microscope (label-free observations)
- 1969 – Willard Boyle and George E. Smith at Bell laboratories invented the CCD
- 1971 – Intel launches 4-bit 4004 microprocessor

Cell Counters

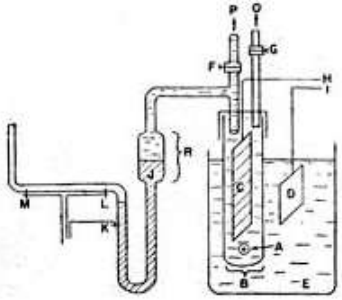
The Coulter Principle (1954-1955)

The Coulter Principle
As a particle passes through the aperture, it creates a resistance. The bigger the particle, the more the resistance, the greater the voltage. Each voltage spike is directly proportional to the size of the cell. Today every modern hematology analyzer depends in some way on the Coulter Principle.

Wallace H. Coulter
1913-1998

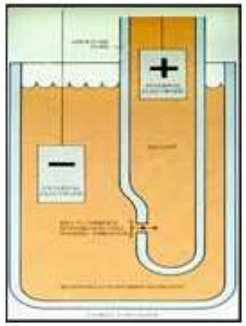
Joseph R. Coulter, Jr.
1924-1995

High Speed Automatic Blood Cell Counter and Cell Size Analyzer



High Speed Automatic Blood Cell Counter and Cell Size Analyzer Wallace H. Coulter, Coulter Electronics, Chicago, Illinois. Proc.Natl.Electronics Conf.12:1034-1042, 1956

The First Coulter Counter

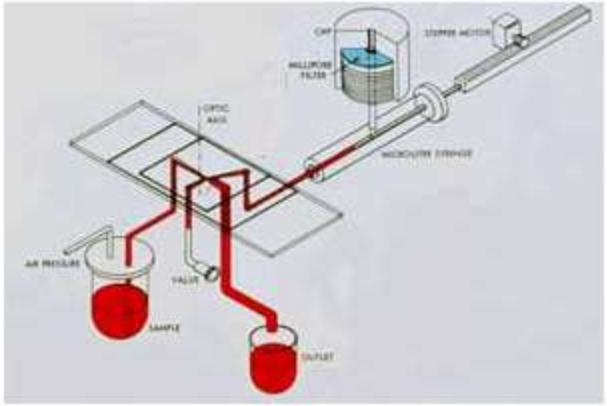


The first commercial version of the Coulter Counter

Early Flow Cytometry Pioneers

1964 Kamentsky Sorter

LA Kamentsky, MR Melamed & H. Derman, Spectrophotometer: New instrument for ultrarapid cell analysis, Science 150, 1965



Spectrophotometric Cell Sorter; Louis A. Kamentsky[1] and Myron R. Melamed[2]

1. IBM Watson Laboratory, Columbia University, New York
2. Memorial Sloan Kettering Cancer Center, New York

http://www.beckmancoulter.com/wsrportal/wsr/diagnostics/clinical-products/flow-cytometry/history-of-flow-cytometry/index.htm

Los Alamos Contributions

Los Alamos Volume Sorter -1965

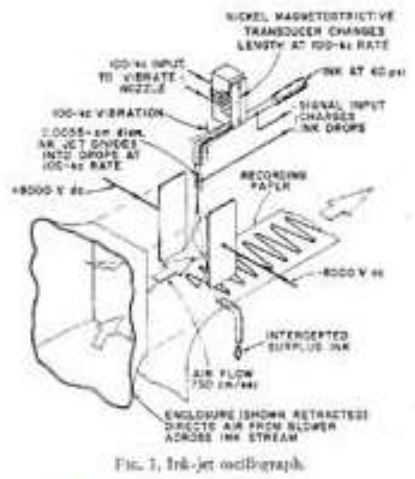


Fig. 1. Ink-jet oscillograph.

Mack Fulwyler worked in Marvin van Dilla's lab at Los Alamos. He developed the sorter in 1965. He initially used electronic cell volume at Los Alamos National Labs. This instrument separated cells based on electronic cell volume (same principle as the Coulter counter) and used electrostatic deflection to sort. The cells sorted were RBC because they observed a bimodal distribution of cell volume when counting cells. The sorting principle was based on that developed for the inkjet printer by Richard Sweet at Stanford in 1965.

The mysterious red cell problem solved

So it was determined that RBC traveling through the orifice were identified as "different" only because of the rotation of the cells (which was essentially random) After determining that the bimodal distribution was artifactual, this group were able to sort neutrophils and lymphocytes from blood.

http://www.beckmancoulter.com/wsrportal/wsr/diagnostics/clinical-products/flow-cytometry/history-of-flow-cytometry/index.htm

1st Commercial Flow Cytometer

Phywe AG of Gottingen - 1969
Produced the first commercial flow cytometer built around a Zeiss fluorescent microscope.

ICP 11 (1969) Distributed by Phywe, Göttingen The first commercial flow cytometer PDP 11 computer

Wolfgang Göhde

http://www.beckmancoulter.com/wsrportal/wsr/diagnostics/clinical-products/flow-cytometry/history-of-flow-cytometry/index.htm

Product Improvements

Lou Kamentsky - Biophysics Systems -1970

Bio/Physics Systems - 1970 commercial cytometer - the "Cytograph" He-Ne laser system at 633 nm for scatter (and extinction) - supposedly the first commercial instrument incorporating a laser. It could separate live and dead cells by uptake of Trypan blue. A fluorescence version called the "Cytofluorograph" followed using an air cooled argon laser at 488 nm excitation

Ortho Diagnostics (Johnson and Johnson) purchased Biophysics in 1976 and in 1977 the System 50 Cytofluorograph was developed - this was a droplet sorter, with a flat sided flow cell, forward and orthogonal scatter, extinction, 2 fluorescence parameters, multibeam excitation, computer analysis option. J&J exit business twice, mid 1980s and mid 1990s.

ICP 11 (1969) Distributed by Phywe, Göttingen The first commercial flow cytometer PDP 11 computer

http://www.beckmancoulter.com/wsrportal/wsr/diagnostics/clinical-products/flow-cytometry/history-of-flow-cytometry/index.htm

Stanford University Cell Sorter

Herzenberg - Stanford - 1969

Len Herzenberg - Sorter based on fluorescence (arc lamp) built after working with one of Kamentsky's RCS systems where they built an instrument they called the Fluorescence Activated Cell Sorter (FACS)

ICP 11 (1969) Distributed by Phywe, Göttingen The first commercial flow cytometer PDP 11 computer

Herzenberg -1972 - Argon laser flow sorter - placed an argon laser onto their sorter and successfully did high speed sorting - Coined the term Fluorescence Activated Cell Sorting (FACS) This instrument could detect weak fluorescence with rhodamine and fluorescein tagged antibodies. A commercial version was distributed by B-D in 1974 and could collect forward scatter and fluorescence above 530 nm.

http://www.beckmancoulter.com/wsrportal/wsr/diagnostics/clinical-products/flow-cytometry/history-of-flow-cytometry/index.htm

Particle Technology

Particle Technology Inc. - COULTER -1971

Fulwyler began consulting for Coulter in the late 1960's. Spinning out LASL FCM and Particle manufacturing technologies.

In 1971, Mack Fulwyler resigned from LASL and established PTI as a Coulter subsidiary company

1976 PTI dissolved, technology transferred to Florida

Epics II 1975, Designed by Mack Fulwyler and Jim Corell
Delivered to NCI/NIH

TPS 1974 - 1979, Designed by Bob Auer

http://www.beckmancoulter.com/wsrportal/wsr/diagnostics/clinical-products/flow-cytometry/history-of-flow-cytometry/index.htm

Blood Cell Counter

Hemalog D - 1974

Technicon - First commercial differential flow cytometer with light scatter and absorption at different wavelengths. Chromogenic enzyme substrates were used to identify neutrophils and eosinophils by peroxidase and monocytes by esterase, basophils were identified by the presence of glycosaminoglycans using Alcian Blue. The excitation for all measurements was a tungsten-halogen lamp.

Photo from Shapiro "Practical Flow Cytometry", 3rd. Ed.Wiley-Liss, 1994

http://www.beckmancoulter.com/wsrportal/wsr/diagnostics/clinical-products/flow-cytometry/history-of-flow-cytometry/index.htm

Multi-beam Flow Cytometers

Howard M. Shapiro - 1973-76

Shapiro and the Block instruments designed a series of multibeam flow cytometers that did differentials and multiple fluorescence excitation and emission

http://www.beckmancoulter.com/wsrportal/wsr/diagnostics/clinical-products/flow-cytometry/history-of-flow-cytometry/index.htm

High Speed Cell Sorter

LLNL High Speed Sorter - 1978

Marv Van Dilla and Phil Dean sorting chromosomes at LLNL around 1978, on the first fluorescence-based sorter developed there. The sorter shown was later modified to become the first dual-beam, fully computer-controlled, multi-parameter sorter. Father of the MoFlo.

http://www.beckmancoulter.com/wsrportal/wsr/diagnostics/clinical-products/flow-cytometry/history-of-flow-cytometry/index.htm

The Recent Past

1977	Epics Instrument, Coulter
2002	Microfluidic Cytometer, Caltech
2003+	Academic work on microfluidic analyzers and sorters

1986 Epics PROFILE

Clinical Flow Analyzer

1987 Q Prep

Automated Sample Prep

Persistent Supporters from Major Companies

Wallace H. Coulter
1913-1998

Joseph R. Coulter, Jr.
1924-1995

BEC

BDX

The Present

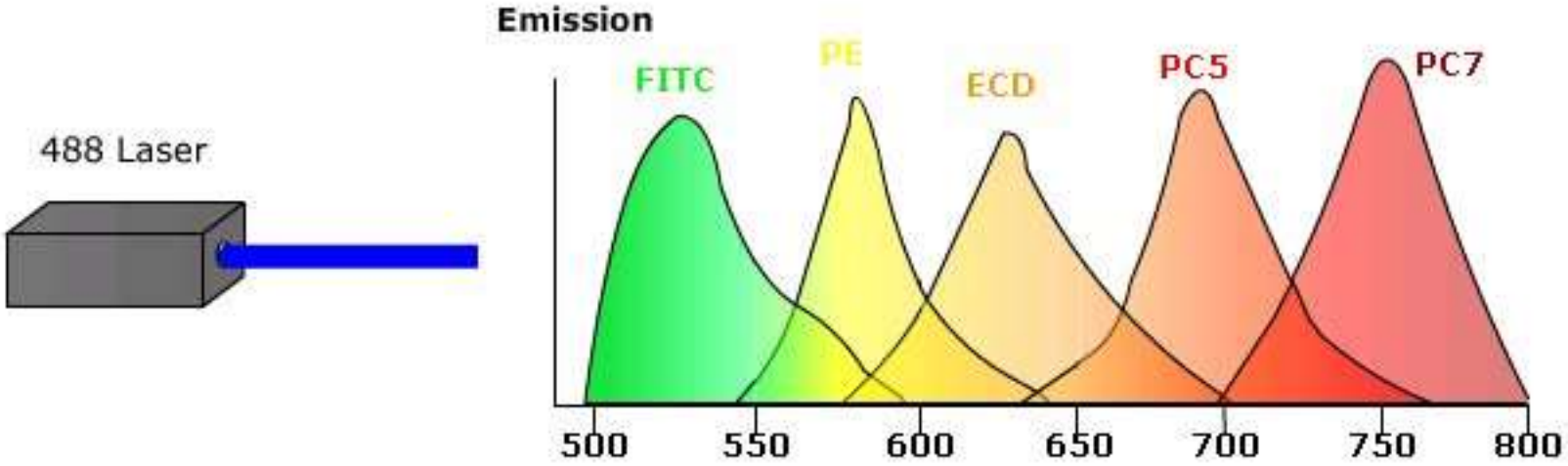
Flow Cytometry Features

Single cell analysis with

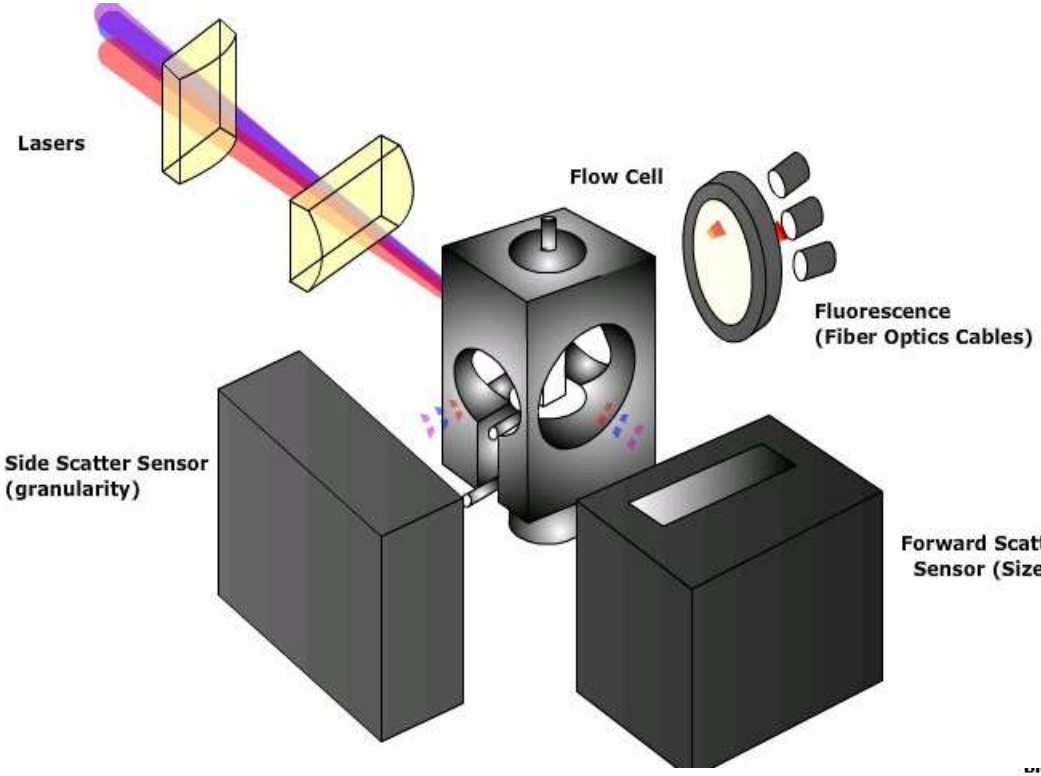
- High sensitivity (single molecule sensitivity by fluorescence)
- Wide dynamic range (10^3 to 10^7 cells mL^{-1})
- High analysis rates to ~10^5 particles sec^{-1}
- Light scatter
- Multi-color fluorescence, multi-parameter analysis
- High precision fluorescence measurement (1% CV)
- Live/dead discrimination
- Viable cells can be re-covered
- Good ease-of-use

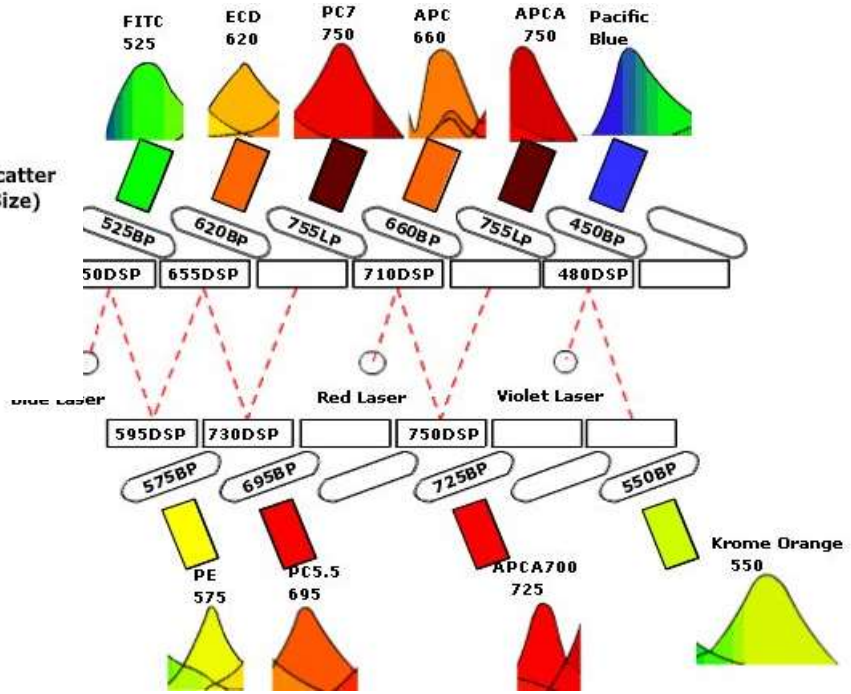
Physical Parameters used for Cytometry

- Light scatter
- Absorbance
- Fluorescence
- Phosphorescence
- Raman
- Electrical properties
- …

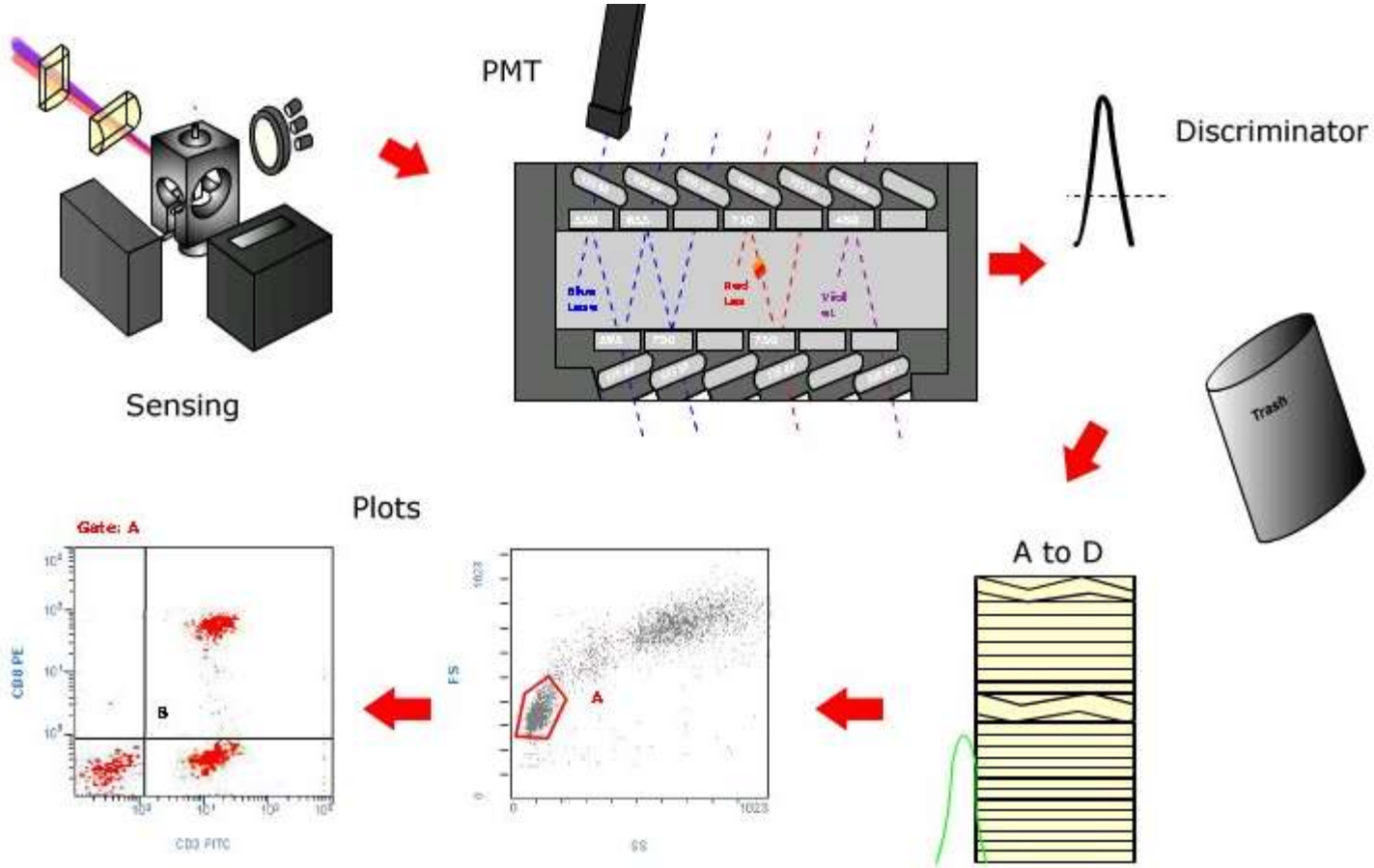


Flow Cytometer Schematics



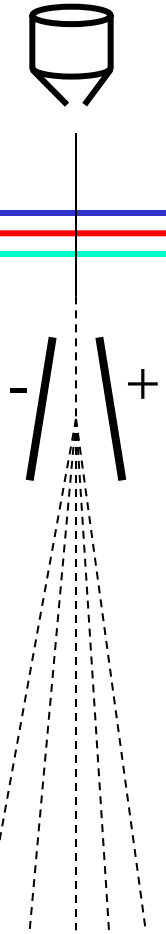


Cytometer Data Flow



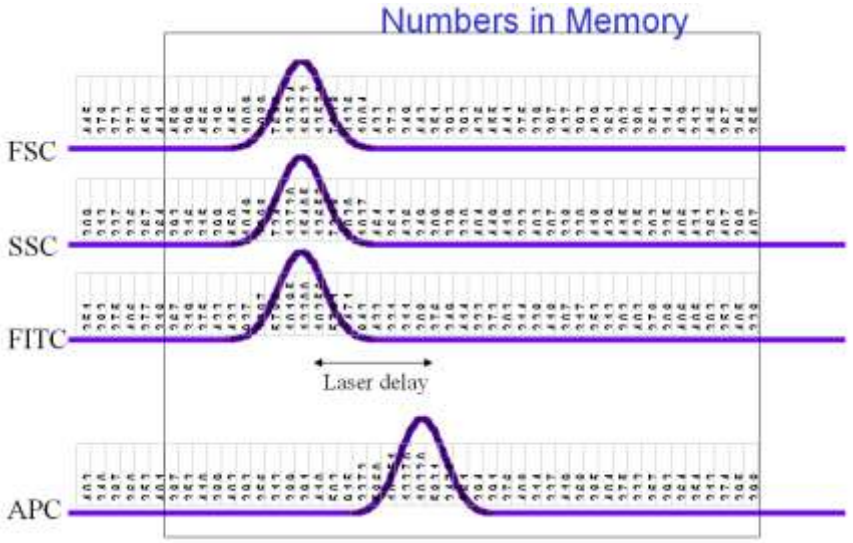
“Droplet-based” Sorting

Astrios

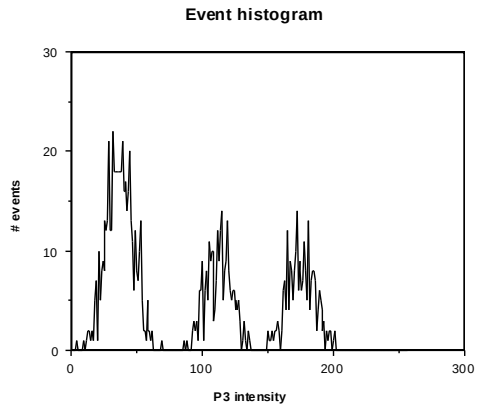


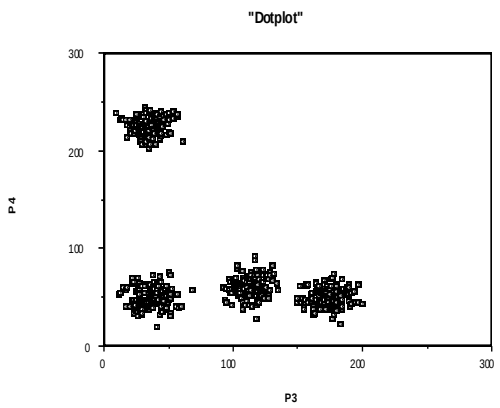
Basic Data Processing

Flow Cytometry



Cell	P1	P2	P3	P4	P5	Pop#
1	242	135	704	175	612	1
2	146	132	690	178	566	1
3	269	147	89	206	580	3
4	442	143	399	250	255	4
5	212	167	155	926	526	2
6	269	2	659	207	575	1
7	204	232	112	171	679	3
8	152	74	160	828	532	2
			…			
9997	215	119	138	936	662	2
9998	244	50	72	261	543	3
9999	214	137	174	1014	597	2
10000	312	87	110	904	560	2





- Gating
- Cluster Analysis
- Other Data Anal.

Automated Flow Cytometry System

Potential Menu:

- lymphocyte subset panel for immune monitoring
- stem cell counting for bone marrow transplants
- leukocyte counting for blood banks
- patient transplant monitoring
- hematology test kit for WBC differentials

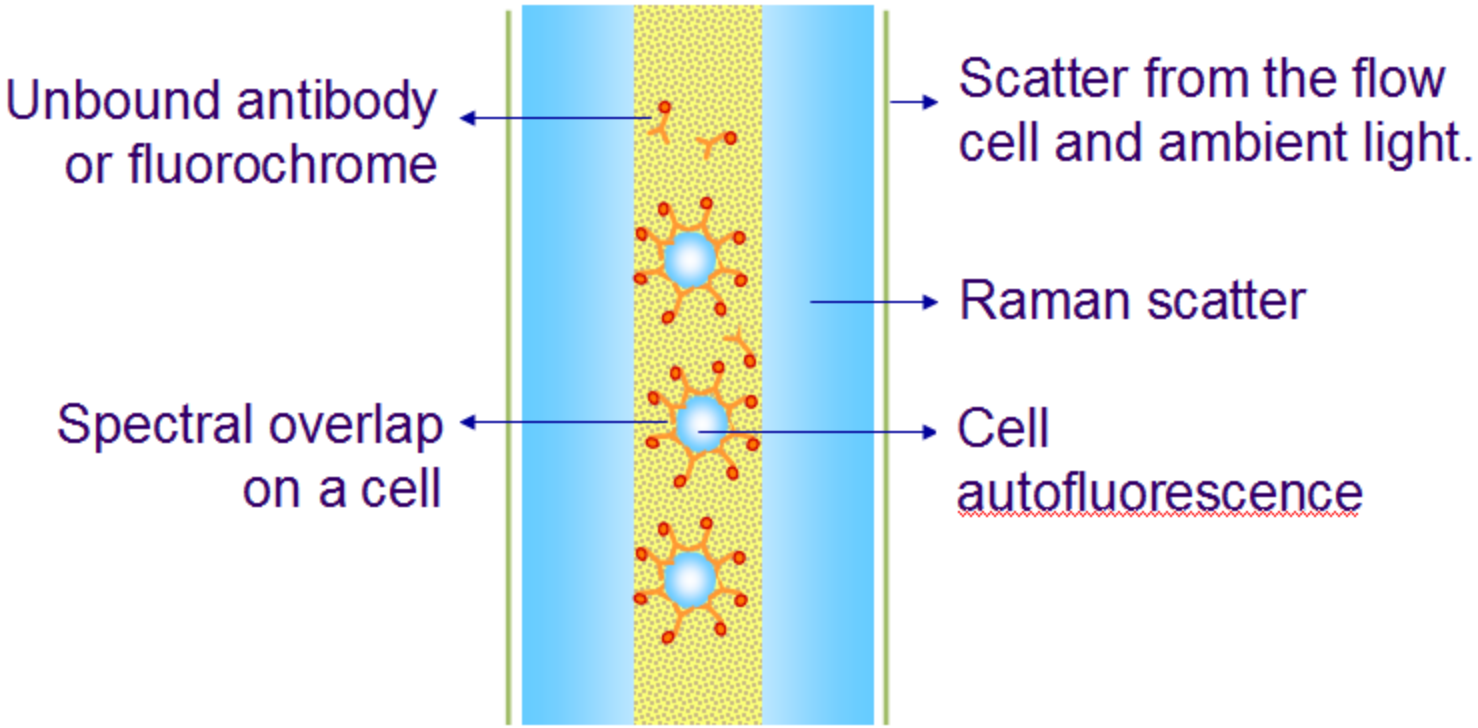
Blue Ocean Biomedical/Beckman Coulter products will provide integration of sample prep, handling, analysis and data in a SIRO (sample-in, results-out) solution for clinical flow cytometry.

Optimizing Flow Cytometry Measurements

- Know your instrument status e.g. Qr & Br for different channels
- Use high enough gain settings to maximize sensitivity
- An antibody/dye combination that marginally allows discrimination of positives/negatives in a single color assay is unlikely to contribute anything helpful in a multicolor experiment.
- Avoid spillover from bright cell populations into channels requiring high sensitivity
- Beware of tandem dye degradation
- Internal controls are essential

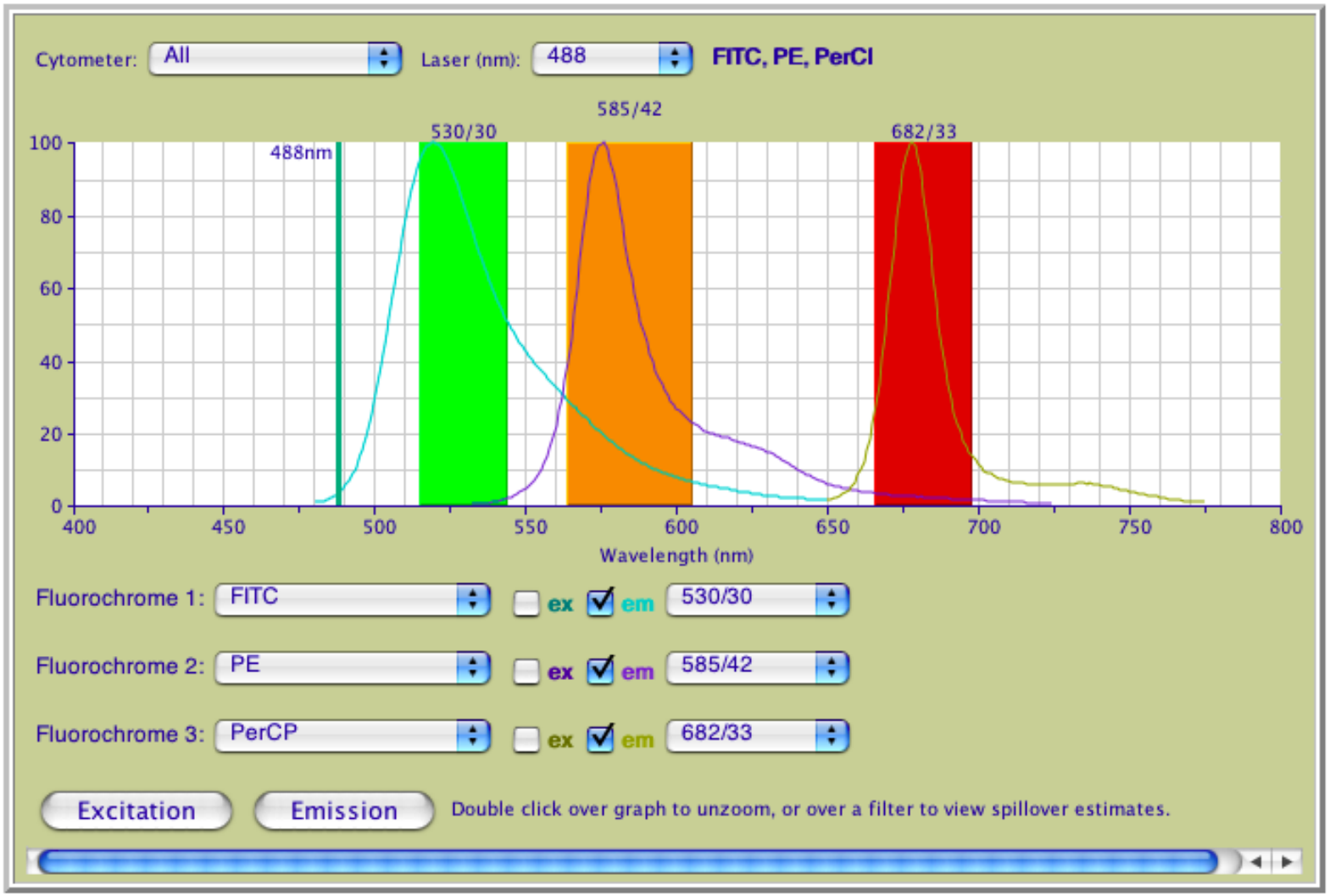
Instrument Evaluation Br

Relative B (Br) is a measure of true optical background in the fluorescence detector.

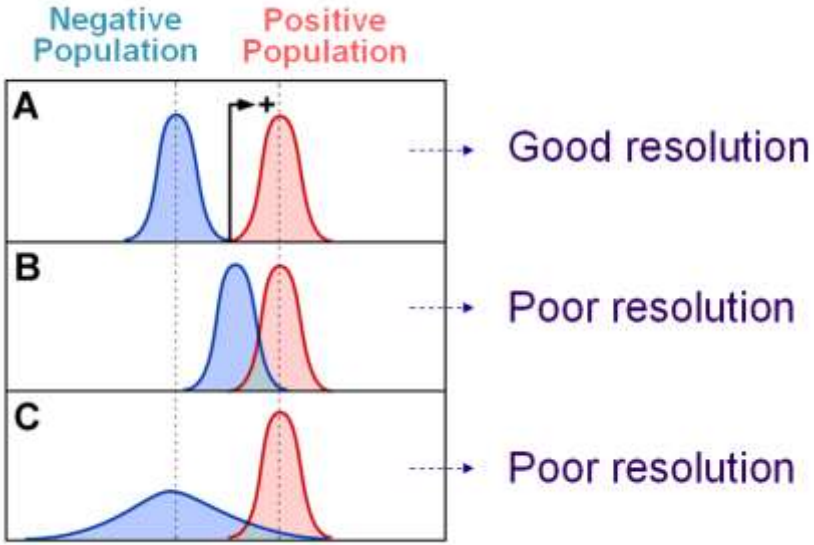


Spectral Overlap

(not relevant for element mass cytometry)

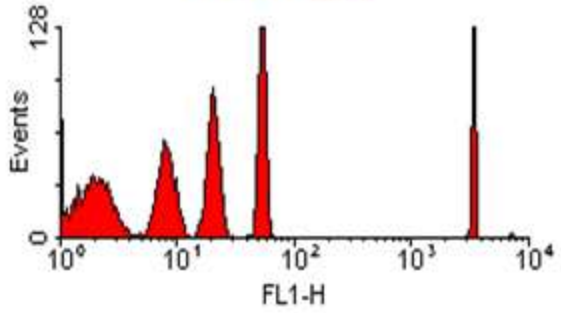


Instrument Evaluation Qr

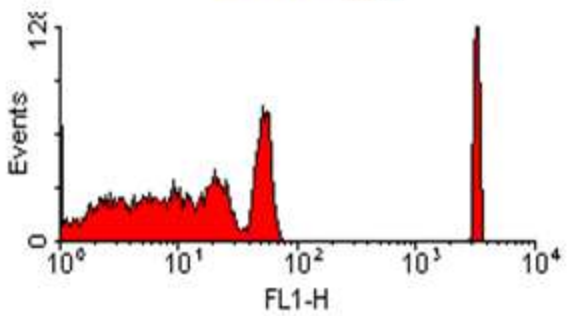


$$Qr = \frac{\#\ \text{photoelectrons}}{\#\ \text{fluorescence molecules}}$$

High Qr



Low Qr



Optimizing cytometry measurements

- Background light

- The total measurement SD is the sum of the error contributions from all sources:

$$SD = \sqrt{SD^2_{optical} + SD^2_{intrinsic} + SD^2_{illumination} + SD^2_{noise}}$$

- When two dyes (PE and FITC) are measured by a single detector the SD is additive:

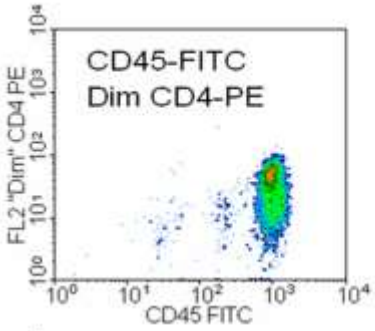
$$SD_{optical} = \sqrt{SD^2_{PE} + SD^2_{FITC} + SD^2_{background}}$$

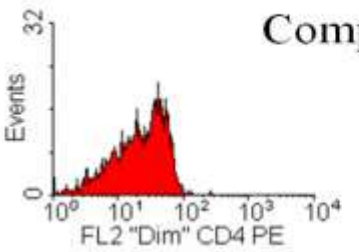
Reagent performance

Stain index

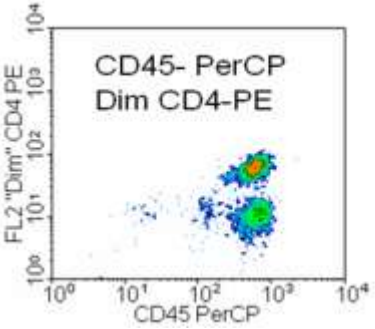
$$\frac{Medium_{pos} - Medium_{neg}}{2 * SD_{neg}}$$

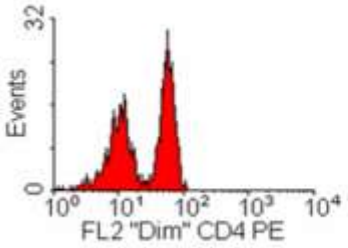
- Dye properties (brightness and spectral overlap)





Compensated data

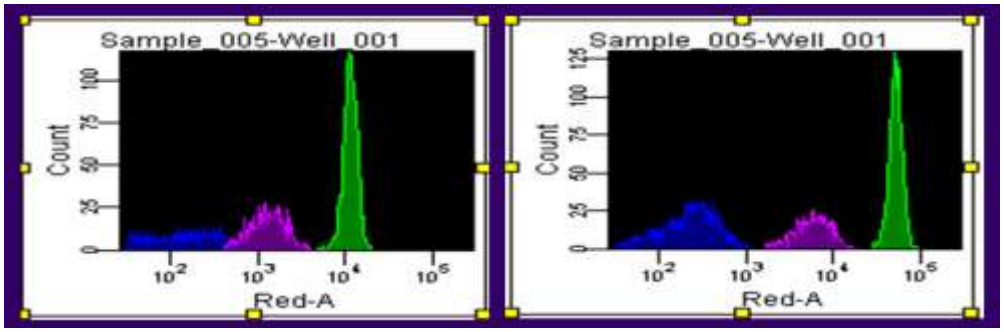




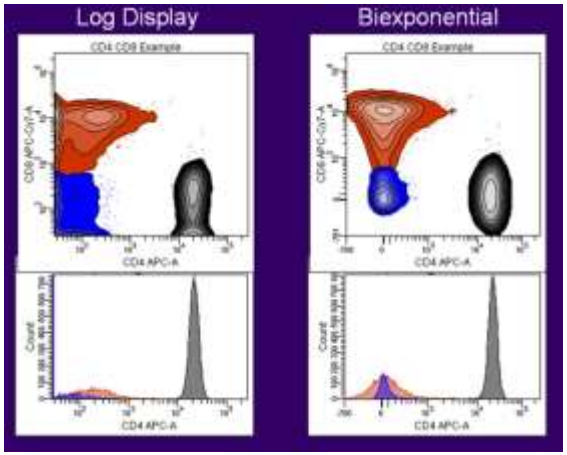
Better separation with less spectral overlap.

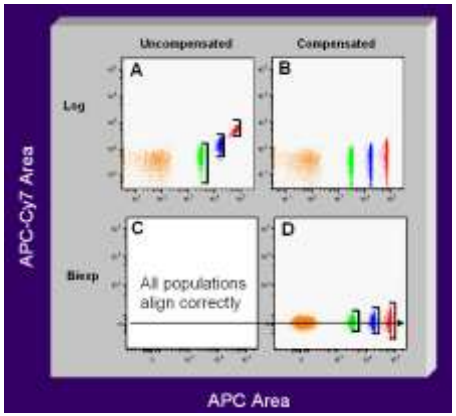
Optimizing cytometry measurements

- Gain (PMT, CMOS, CCD) settings

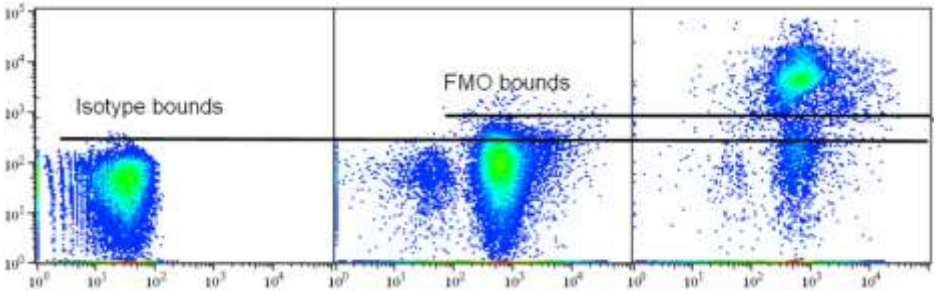


- Data Display





- Controls

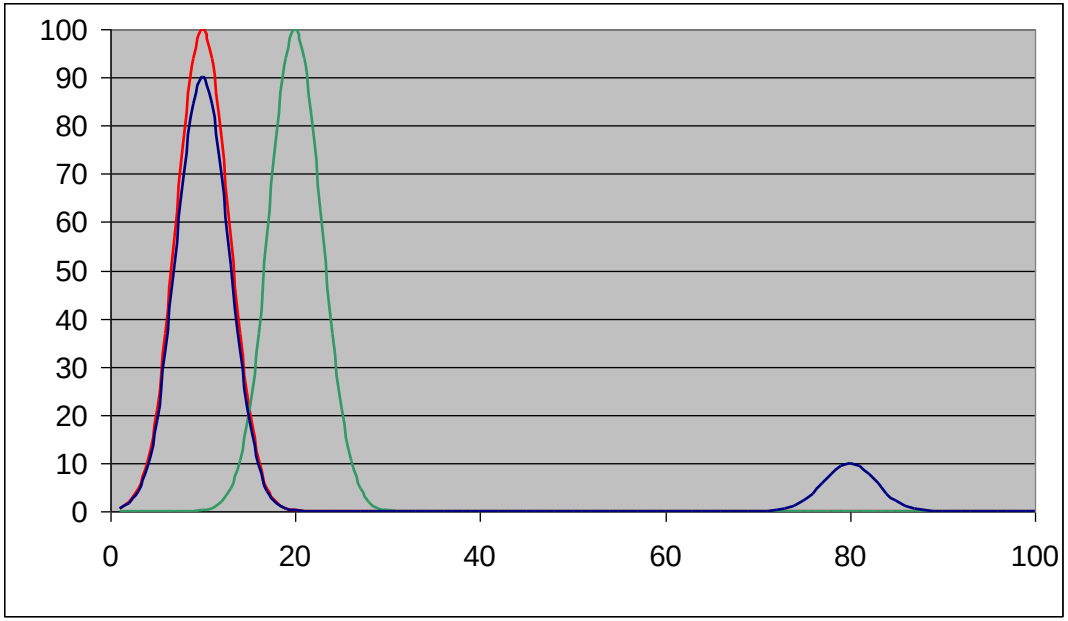


Multi-parameter Fluorescence Cytometry Points To Consider

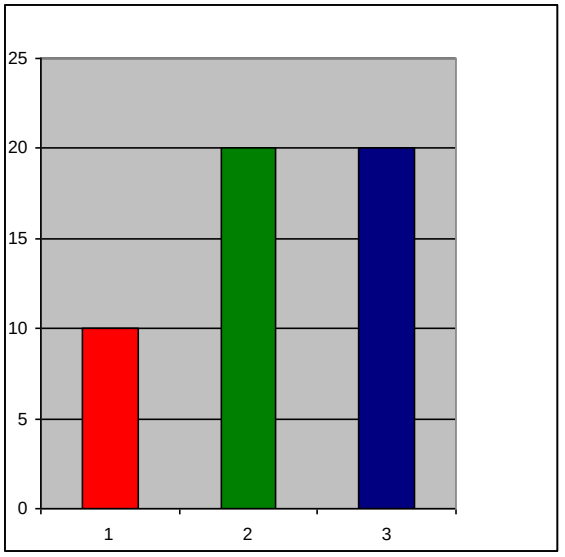
- Know your instrument status e.g. Qr & Br for different channels
- Use high enough gain settings to maximize sensitivity
- An antibody/dye combination that marginally allows discrimination of positives/negatives in a single color assay is unlikely to contribute anything helpful in a multicolor experiment.
- Avoid spillover from bright cell populations into channels requiring high sensitivity
- Beware of tandem dye degradation
- Internal controls are essential

Single Cell Cytometry vs. Bulk Analysis

Intensity Histogram for Single Particles



Intensity per Sample



Cell by cell intensity analysis detects population heterogeneity.

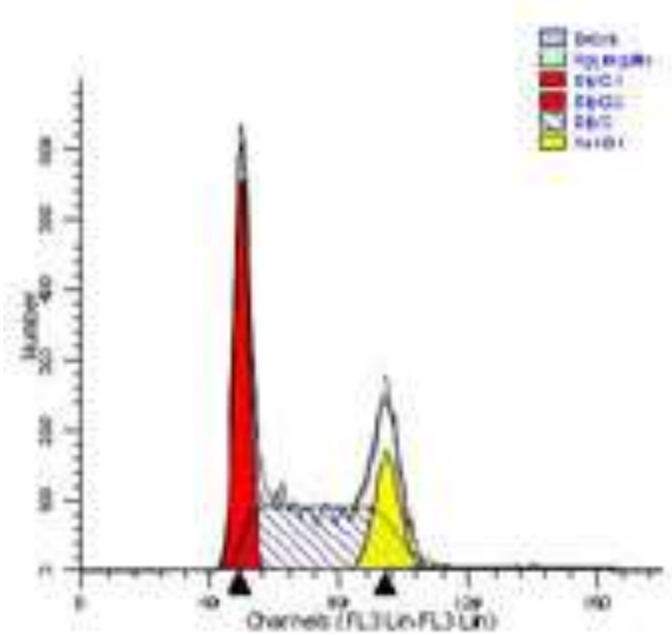
Key Applications

- Cell Cycle Analysis
- Immunology, Cell Biology, Stem Cell Research
- Microvesicles
- Clinical Diagnostics
  - Immune status
  - Tumor Cell Cycle
- Cell Sorting
  - Single cell genomics
  - Cell population proteomics
  - Cloning for research and industrial biotechnology
- Marker quantitation
- Molecule counting

Cell Cycle Analysis

High Precision Measurement of

- Cell cycle phases G0/G1, S, G2M
- Aneuploidy
- Proliferation rate also with BrDU
- …

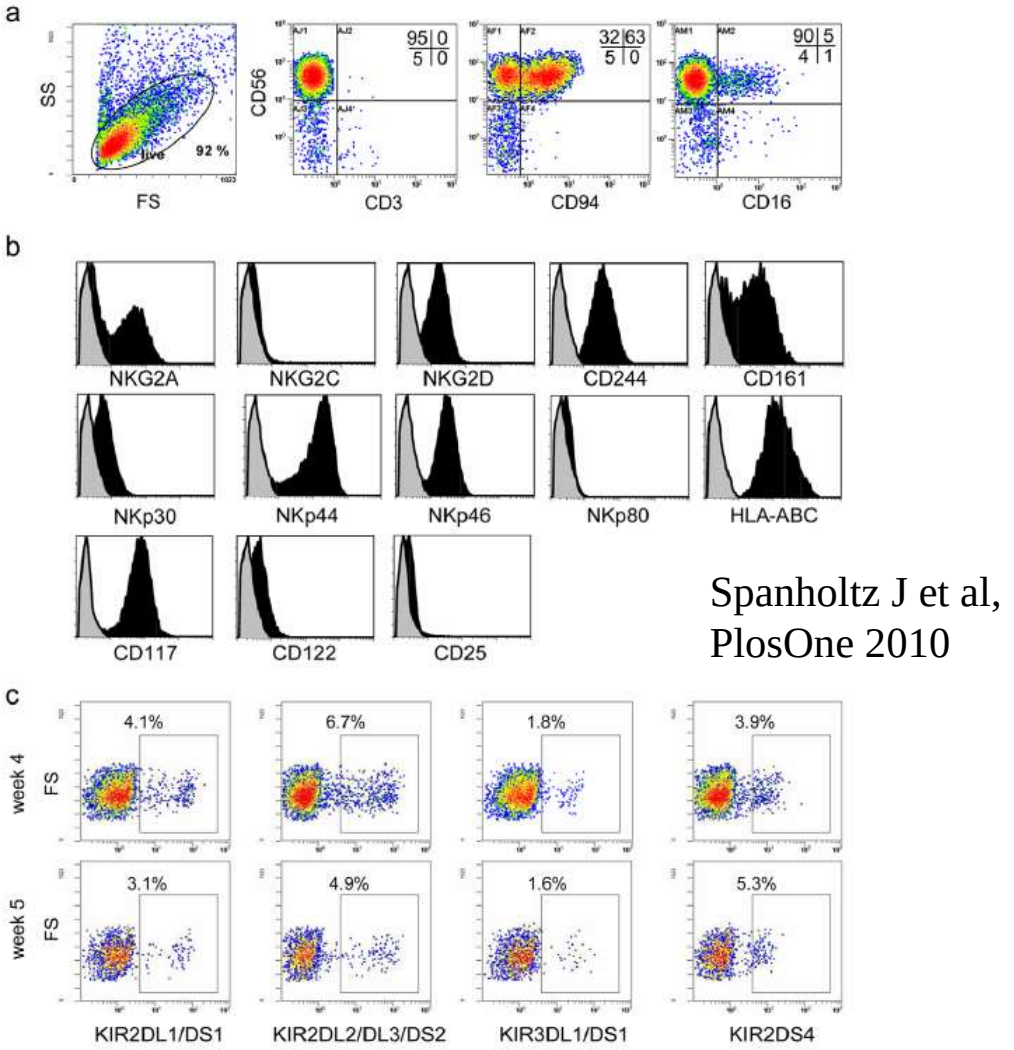


Immunofluorescence

Measurement of

- Biomarkers
  - Cell surface
  - Intracellular
- Phosphoproteins

Many simultaneous measurements with multi-laser systems or mass labels (CyTOF)



Spanholtz J et al, PlosOne 2010

Figure 4. Phenotypical profile of *ex vivo*-generated NK cells using Method II with GBGM. (a) Flow cytometric analysis of a representative NK cell product generated from $CD34^+$ UCB progenitor cells. Cells at 5 weeks of culture were analyzed for expression of CD56, CD3, CD94 and CD16. (b) Expression of a repertoire of receptors important for regulating NK cell activity, including C-type lectin receptors, natural cytotoxicity receptors and cytokine receptors. Histograms show expression of the antigen of interest (black histogram) compared to the specific isotype control (grey histogram). (c) Acquisition of KIR^+ NK cell subsets during *ex vivo* NK cell generation from expanded $CD34^+$ UCB cells. KIR expression was determined at week 4 and 5 during the differentiation step by FCM.
doi:10.1371/journal.pone.0009221.g004

Intracellular Enzyme Activity

Measurement of enzyme activities e.g. esterases or peptidases with fluorogenic substrates.
(Continuous measurements of kinetics of changes in cell sub-populations are possible.)

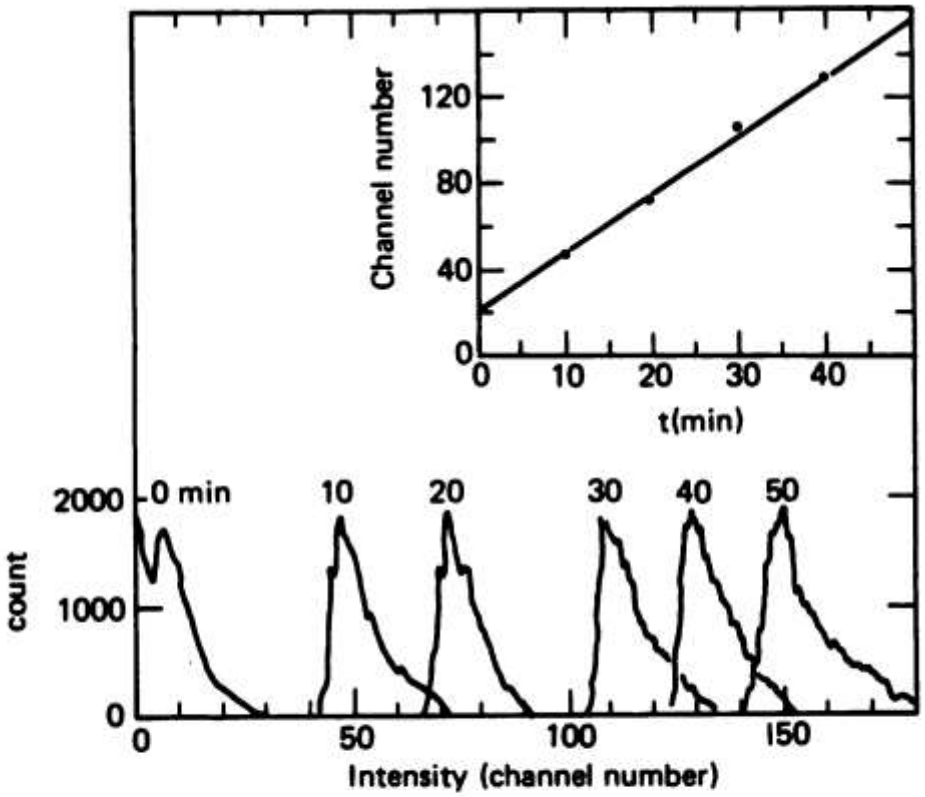
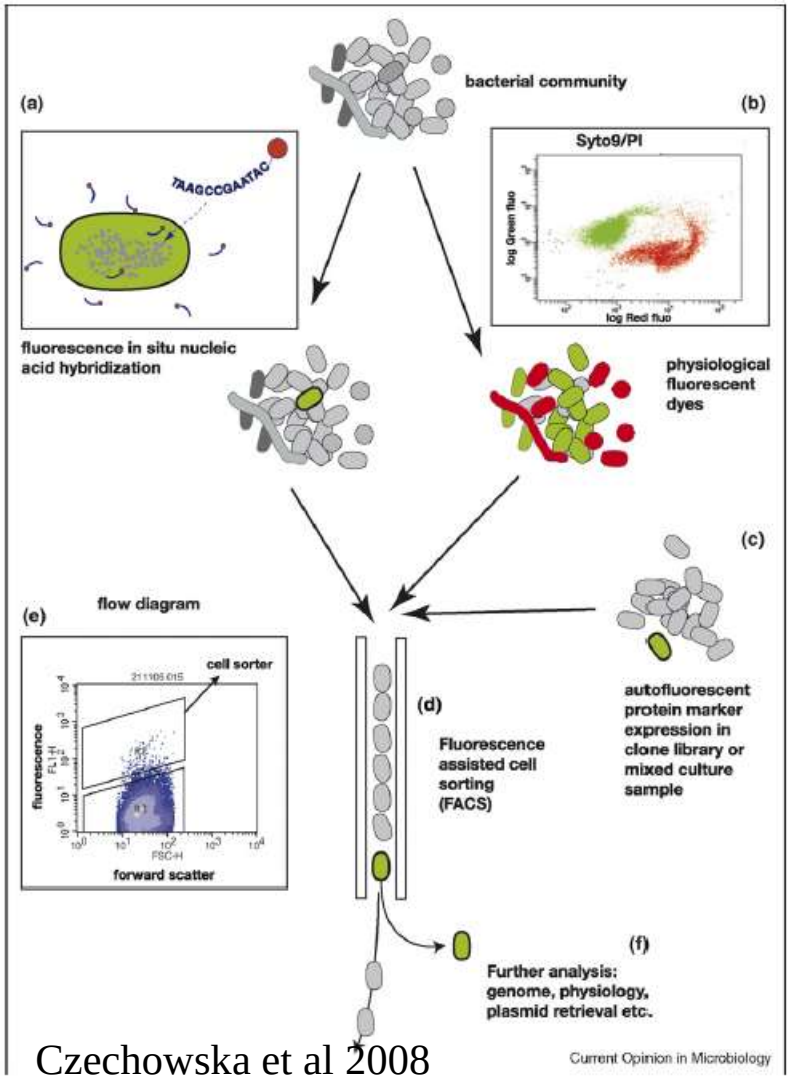


Fig. 8. Hydrolysis of CBZ-ala-arg-arg-MNA in 3T3 cell suspensions analyzed by flow cytometry

Inset is a plot of peak values obtained from individual histogram channel numbers at times indicated

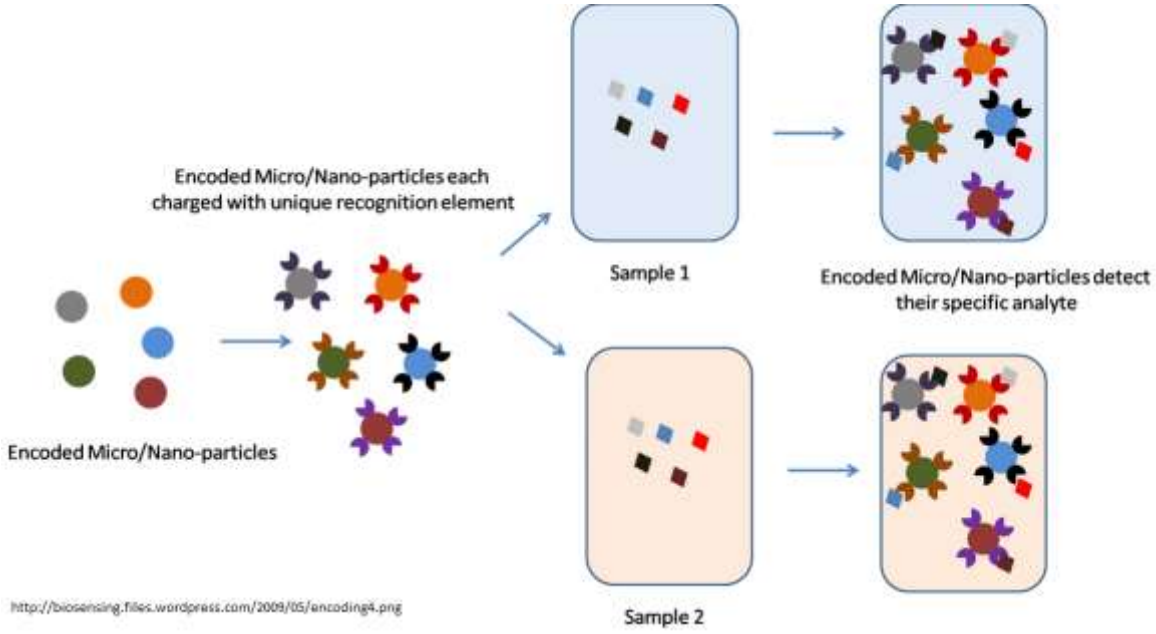
Microbiology

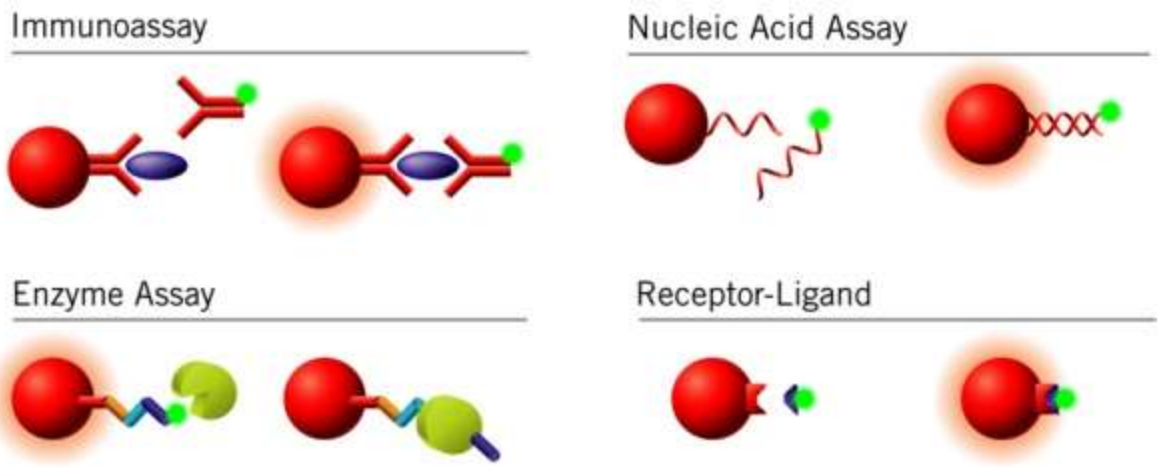
- Cell counting
- Identification
  - Antibodies
  - FISH probes
- Antibiotics resistance
- Strain improvement
- …



Czechowska et al 2008

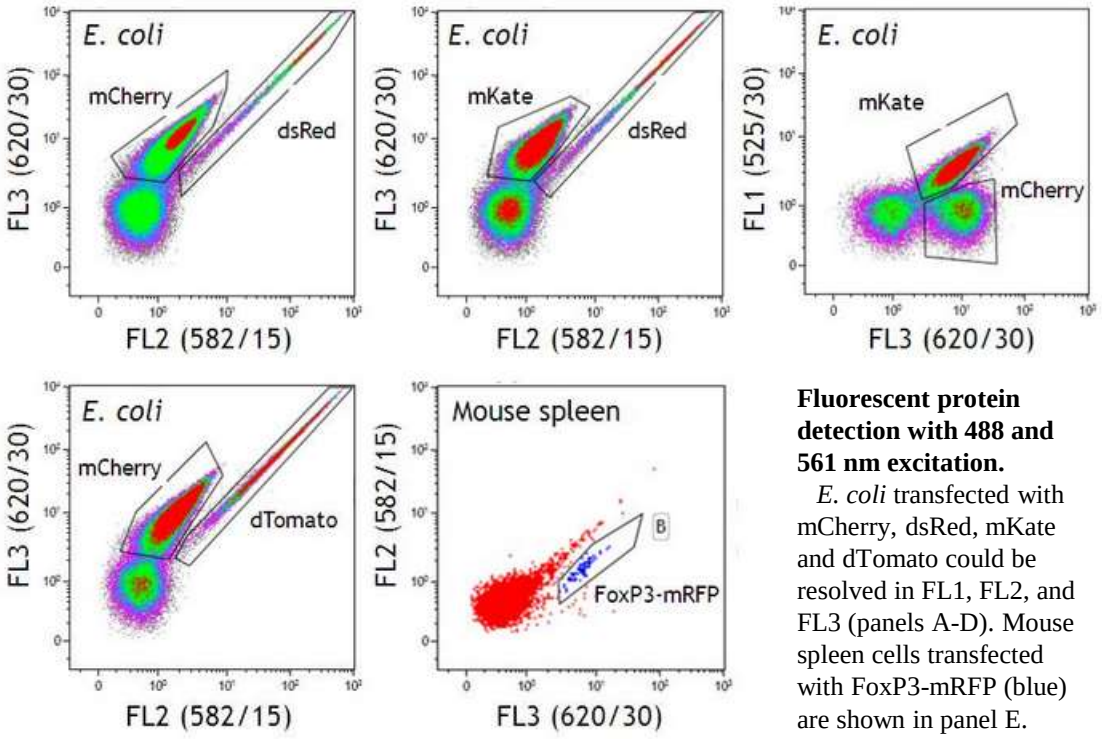
Bead Based Assays





Measurement of Fluorescent Protein Expression

Lasers	488 nm / 561 nm					638 nm			405 nm	
Channels	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8	FL9	FL10
Fluorochromes	Fluorescein	PE	ECD	PC5.5	PC7	APC	APC A700	APC A750	Pacific Blue	Krome Orange
Fluorescent Proteins & Viability	GFP	dTomato mKate dsRed	mCherry mKate							

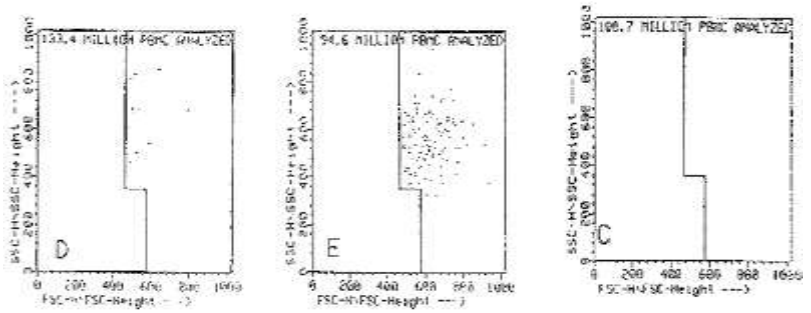


Fluorescent protein detection with 488 and 561 nm excitation.

E. coli transfected with mCherry, dsRed, mKate and dTomato could be resolved in FL1, FL2, and FL3 (panels A-D). Mouse spleen cells transfected with FoxP3-mRFP (blue) are shown in panel E.

Rare Cell Analysis

- Ag-specific T-cells
- Ag-specific B-cells
- Circulating epithelial cells
- Circulating endothelial cells
- Fetal cells in maternal blood
- …



10^{-6} **10^{-5}** **control**

Gross HJ et al, Cytometry 14 (1993) 519-526

Gross HJ et al, PNAS 92 (1995) 537-541

Limit of Detection

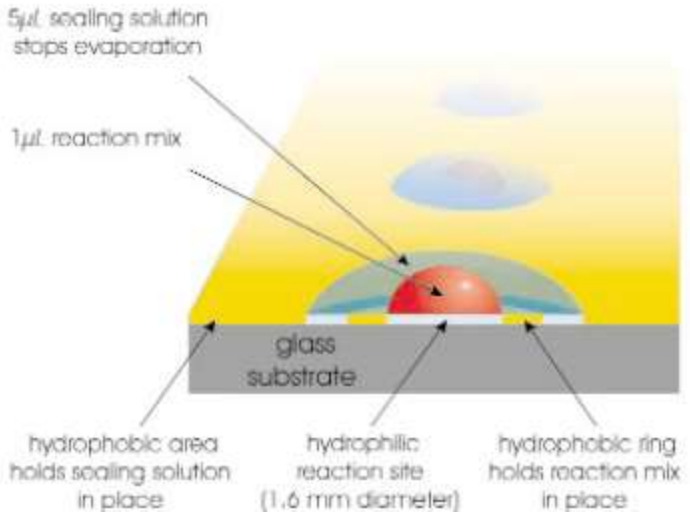
Routine >0.2%

Optimized instrument >0.01%

Optimized system $>10^{-7}$

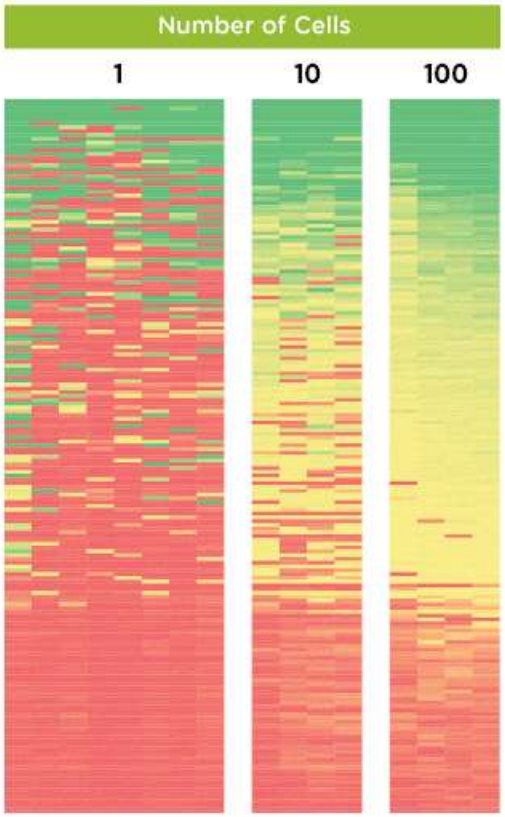
Single Cell Sorting for PCR

Flow sorting of single cells onto a slide (Ampligrid) followed by automated miniaturized single cell PCR (Advalytix).



Single Cell Genomics

Single cell analysis reveals heterogeneity, which is masked by averaging, when analyzing groups of cells.

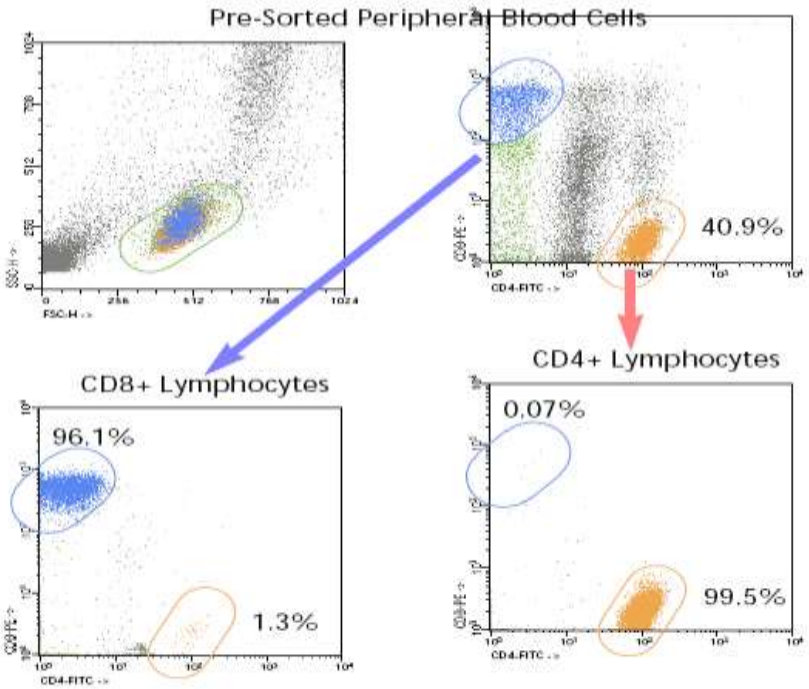


Source:
http://www.nanostring.com

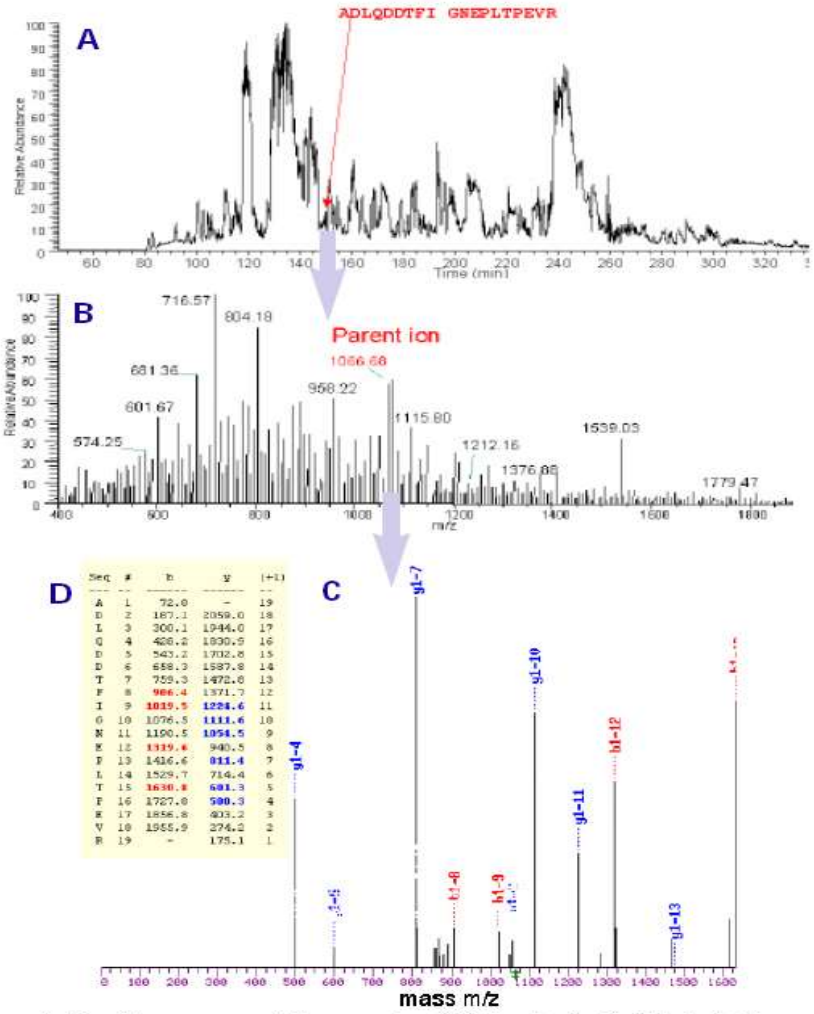
Sorting for Cell Surface Proteomics

Cell surface proteome by FACS sorting, followed by LC MS

(in collaboration with Thermo Finnigan, San Jose, CA)



The dot plots show the sorting strategy used for stained peripheral blood cells and population purity after sorting for CD4- and CD8-positive cells. CD4 cells were gated on scatter and FITC fluorescence; CD8 bright cells were gated on scatter and RPE fluorescence. Sorted populations showed >95% purity.

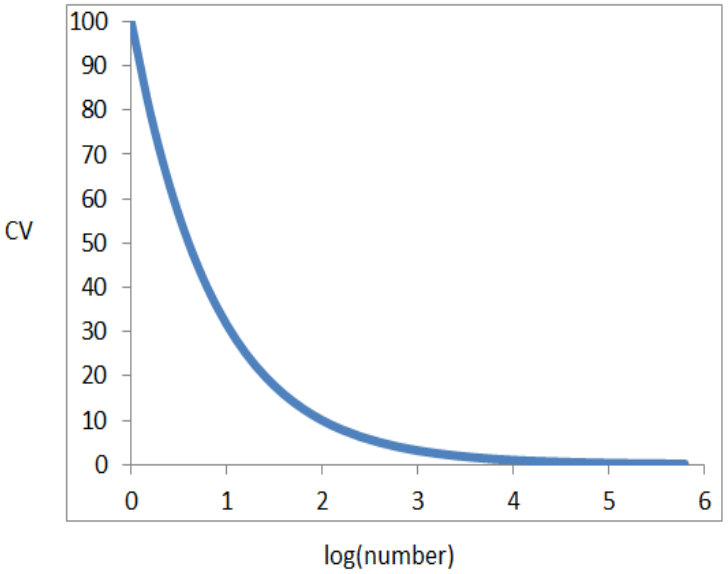


Peptide mixtures were separated by reverse phase HPLC **(A)** as described in Methods. Eluted peptides were subjected to electrospray injection into the mass spectrometer and analyzed for their mass/charge ratio (m/z value) **(B)**. Selected ions were collected in the ion trap. These parent ions were cracked by collision ion dissociation to produce a range of fragment sizes **(C)** that were compared to predicted peptide sequences in the human database using TurboSequest **(D)**.

Cell Counting

Counting Statistics

	Sample 1	Sample 2	Sample 3	Sample 4
	6	2	6	8
	3	7	1	6
	1	3	5	3
	1	4	5	6
	1	4	6	3
Mean	2.4	4	4.6	5.2
St.Dev	2.2	1.9	2.1	2.2
		Overall	Mean	4.1
			St.Dev	2.2

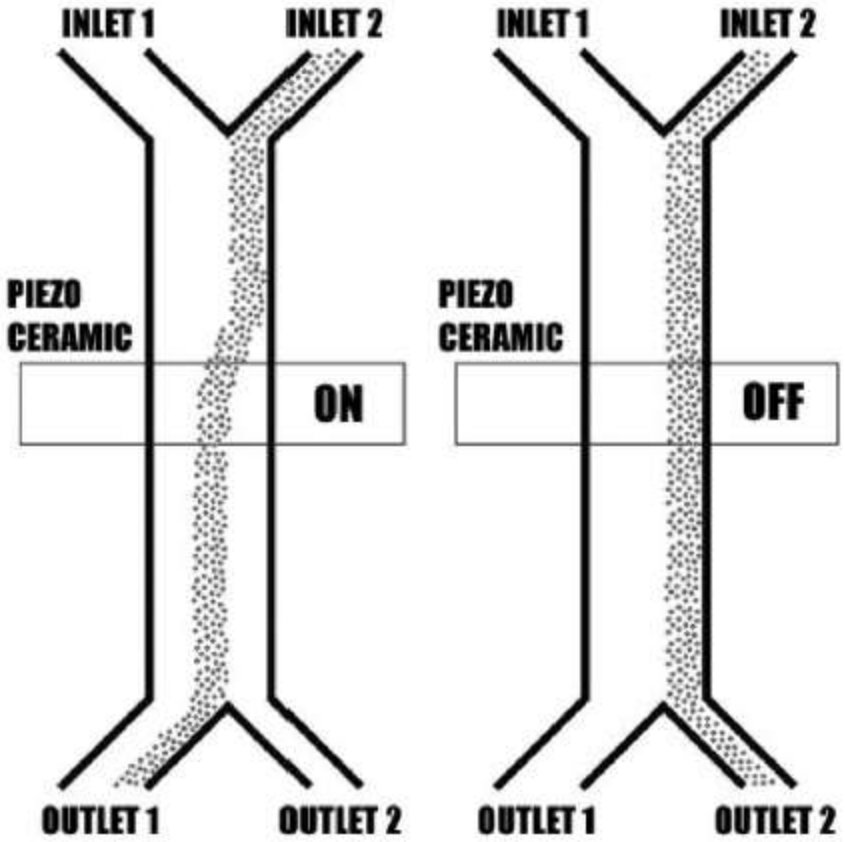


Ignoring Counting Statistics Can Lead to Erroneous Conclusions (abs. counts or percentages)

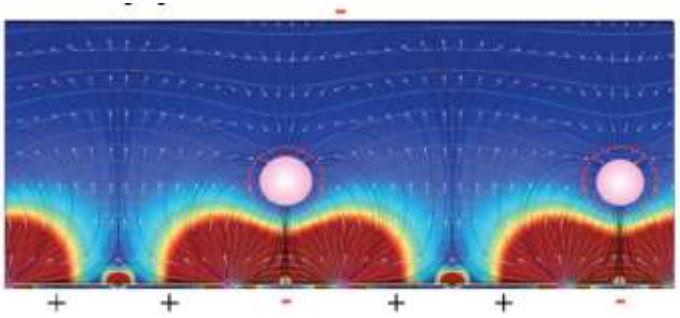
Recent Novel Products

- Fluidics
  - New particle focusing technologies
- Sorting
  - New single cell sorter
- Systems
  - More parameters

Acoustic Particle Focusing



Laurell T et al 2006, Chem. Soc. Reviews

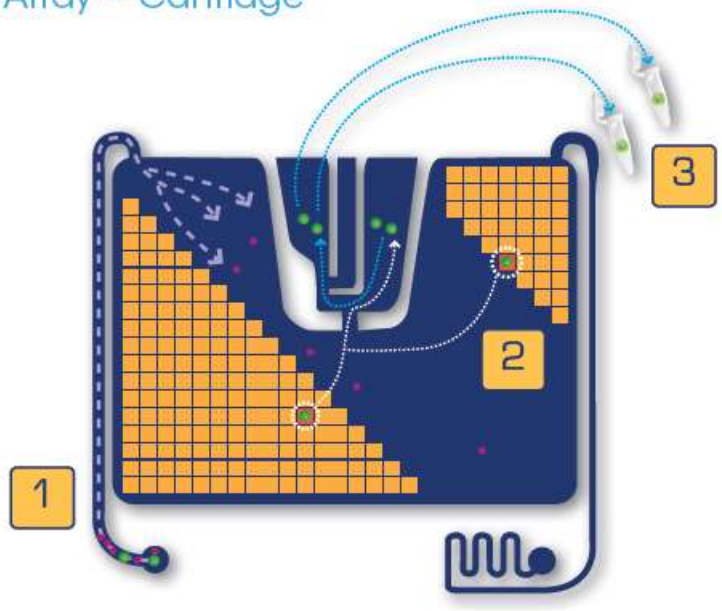


Single Cell Sorter with Microscopic Detection

Cell movement with dielectric forces.
DEPArray
Silicon Biosystems, Bologna, IT

Cells are transferred to a special slide with 40,000 “cages”. Cells of interest are identified by fluorescence microscopy and sorted by the instrument.

The DEPArray™ Cartridge



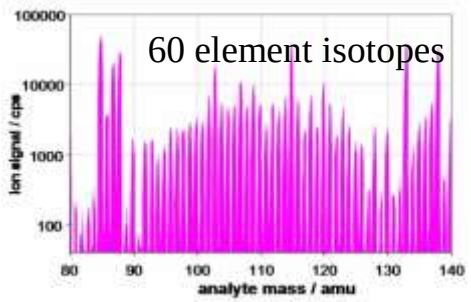
1
Inject, trap and image cells

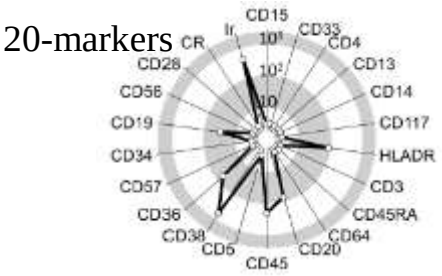
2
Move cells of interest into parking chamber

3
Move individual or multiple cells into recovery chamber and flush

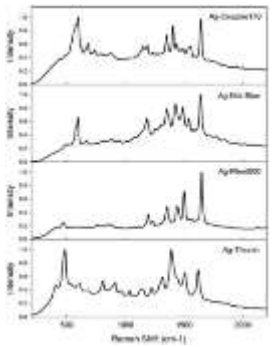
New Developments for Multi-parameter Cytometry

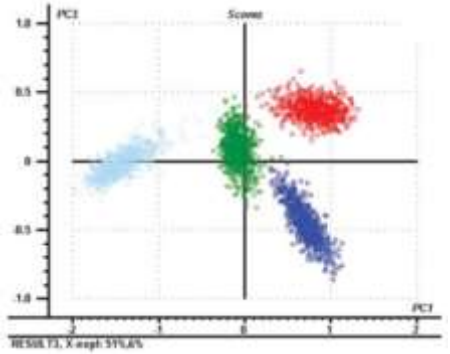
- Element-Label Flow Cytometry (CyTOF, addresses fluorescence spectral overlap issue by using elements as labels, Anal. Chem., 2009, 81 (16), pp 6813–6822)
- SERS-Label Flow Cytometry (uses spectral fine-structure to distinguish labels, Cytometry, 2008, 73A(2), pp 119-128)
- Sequential Stain De-stain Cytometry (Cytometry, 2009, 75A(4), pp 362-370)



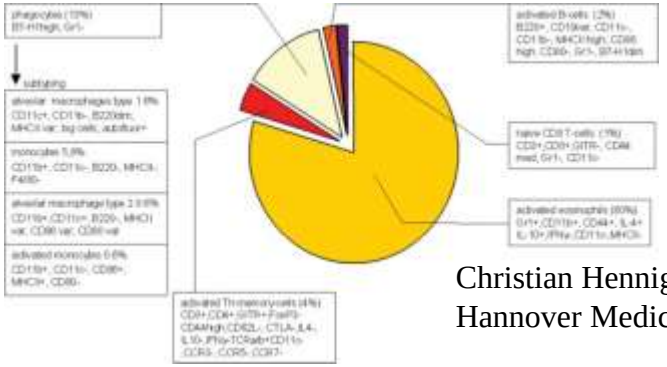


Scott Tanner, DVS Sciences Inc





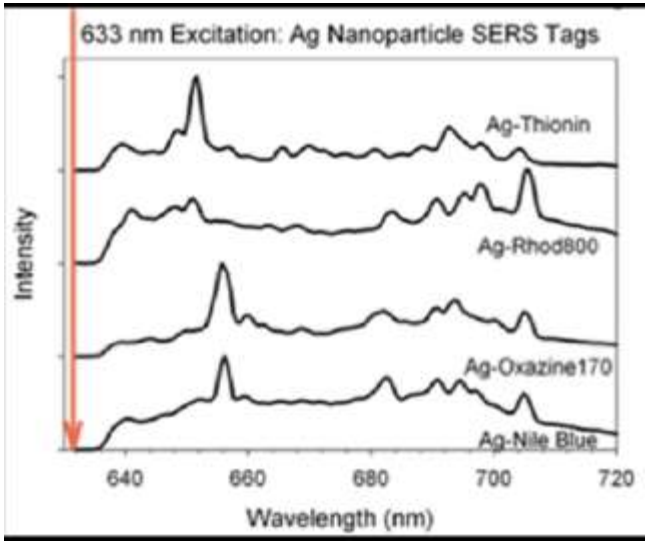
John Nolan, La Jolla Bioengineering Institute

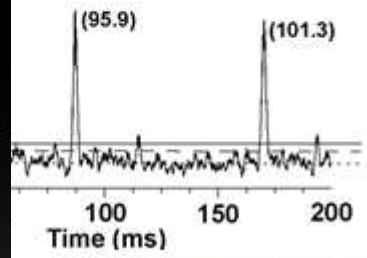


31-marker analysis

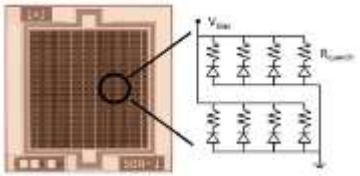
Christian Hennig, ChipCytometry
Hannover Medical School

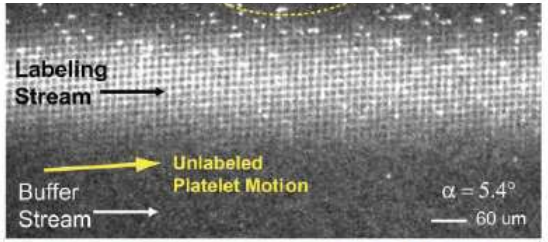
Thoughts About the Future

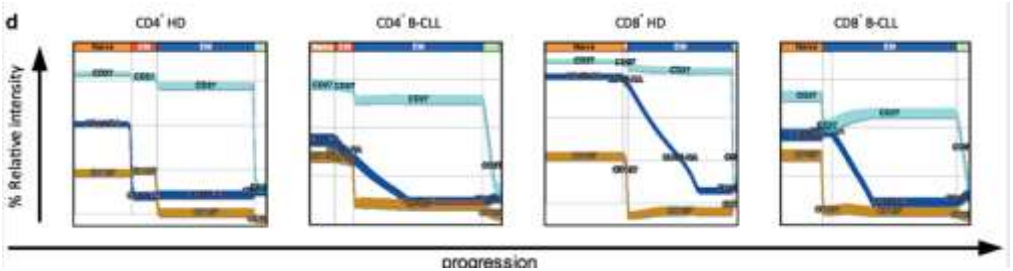


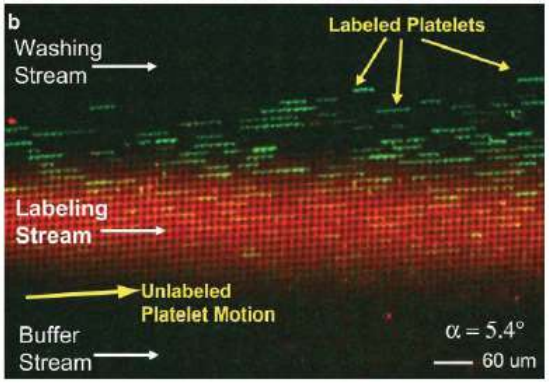


Rob Habbersett & Jim Jett, LANL









Technology Developments For Changes in Cytometry

- Labels
  - High brightness fluorescent labels ,e.g. polymers, nanoparticles
  - Raman labels
- Light sources
  - Solid state lasers
  - LEDs
- Detectors
  - Photomultiplier arrays
  - CMOS detectors
- Fluidics
  - Microfluidic channels for manipulating particles
- Computing
  - Fast multi-parallel processing

The Future Of:

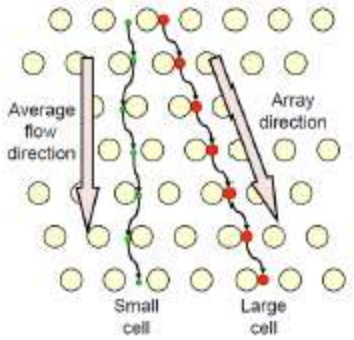
- Sample Handling and Preparation
- Instrumentation including Calibration
- Cell Sorting
- Reagents
- Software and Algorithms
- Systems

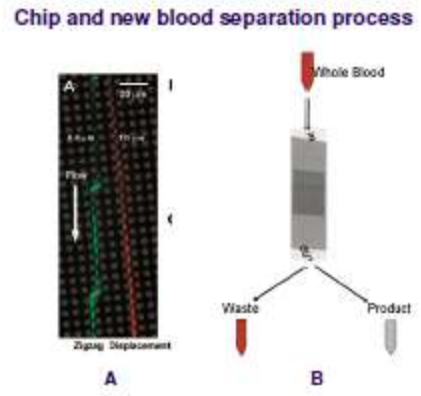
Particle Control for Sample Handling

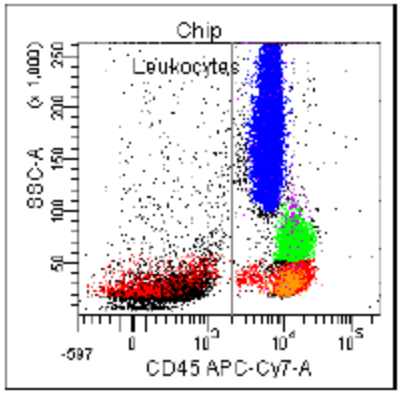
- Acoustic Forces e.g. UNM, Lund U, …
- Mechanical Forces e.g. Aviva filters
- Photon Pressure
- Dielectric Forces
- Hydrodynamic forces e.g. Princeton, UCLA
- …

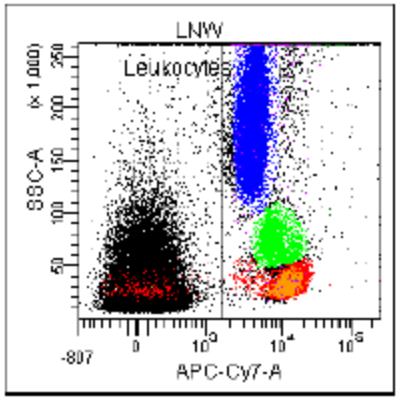
Innovative Sample Preparation

Microfluidic system
for leukocyte isolation
(deterministic lateral displacement)









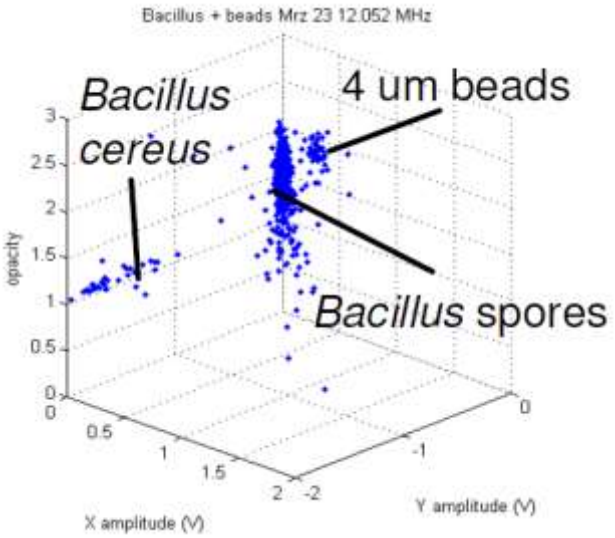
Cyto 2012 poster, Liping Yu et al, GPB and others

Instrumentation including Calibration

- Spectral Analysis
- Raman Labels
- Label-free Analysis
- High speed imaging in flow
- Single molecule sensitivity
- Automated Setup
- …

Label-free Cell Analysis

LEISTER : Axetries Impedance flow cytometry



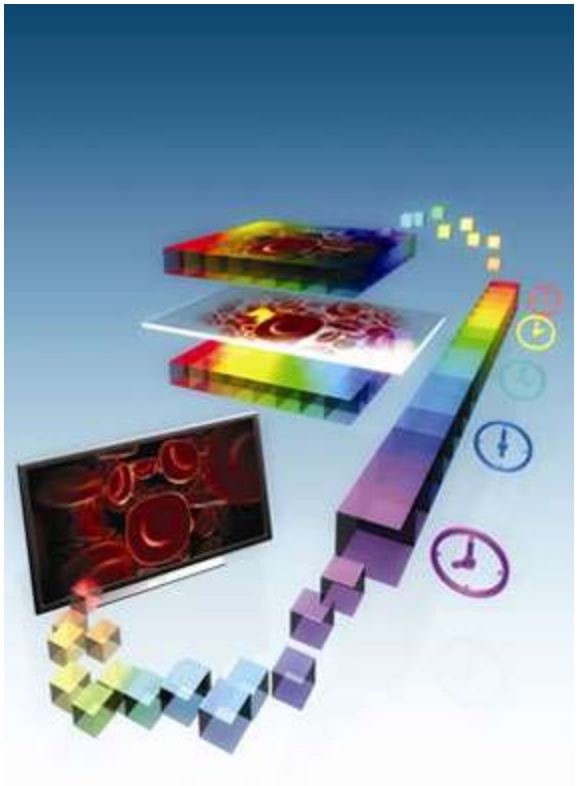
Marco DiBeradino, Leister Axetris

Electrical parameters of living cells (no label required).

Other parameters: fluorescence polarization, fluorescence lifetime, compressibility, …

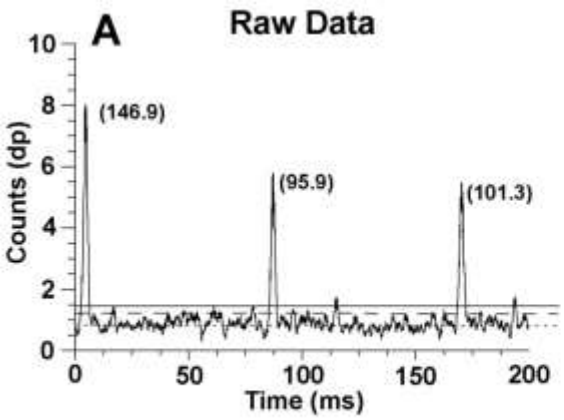
High speed imaging in flow

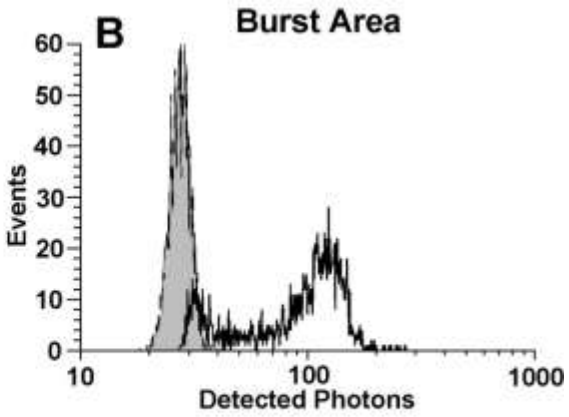
- ImageStream (EM Merck)
- Bahram Jalali group, UCLA
- …

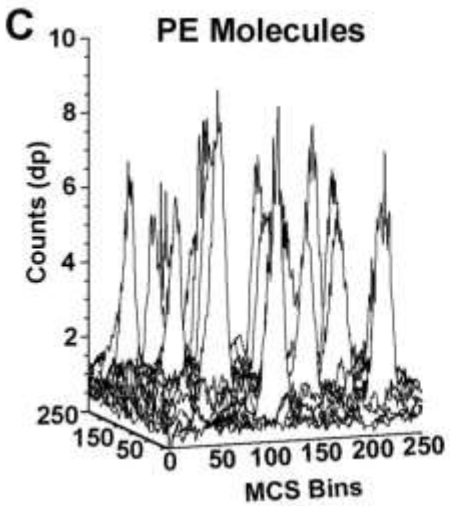


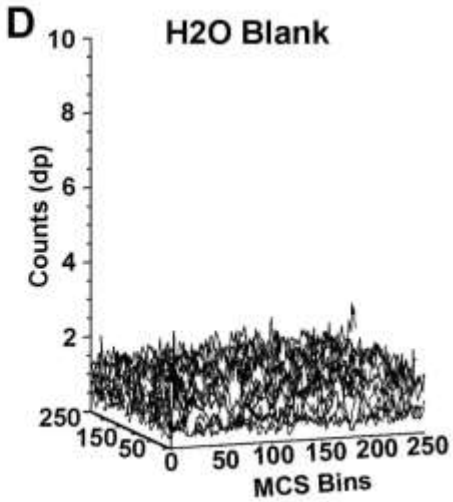
http://www1.ee.ucla.edu/Research-highlights-jalali-4.htm

Single molecule sensitivity with a special flow cytometer









A: 200 ms corrected data showing 3 molecules of B-PE

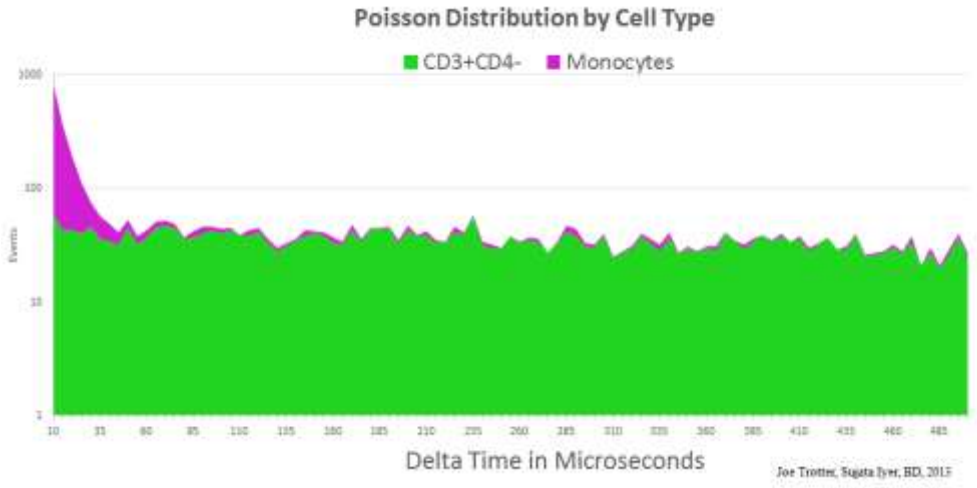
B: 2645 photon burst areas (background-grey)

C,D: each 256 bin (row) = 25.6 ms data. **C** is B-PE showing single molecules. **D** is H_20 control

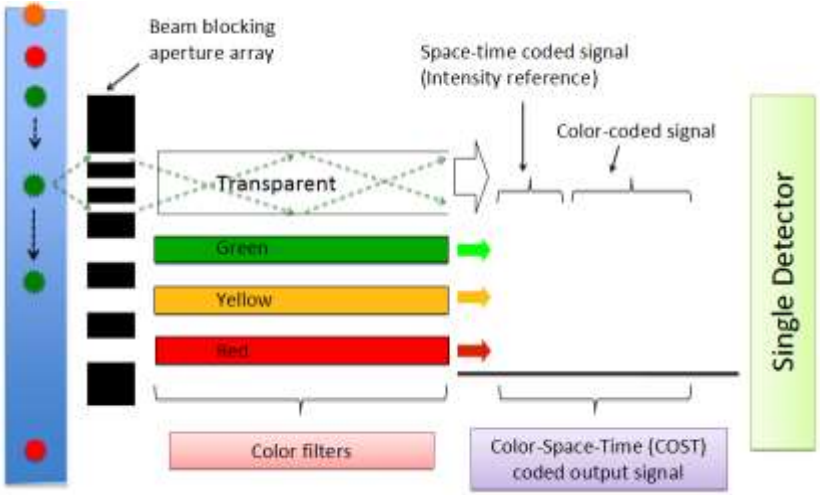
(Rob Habbersett & Jim Jett, LANL)

Cell Sorting

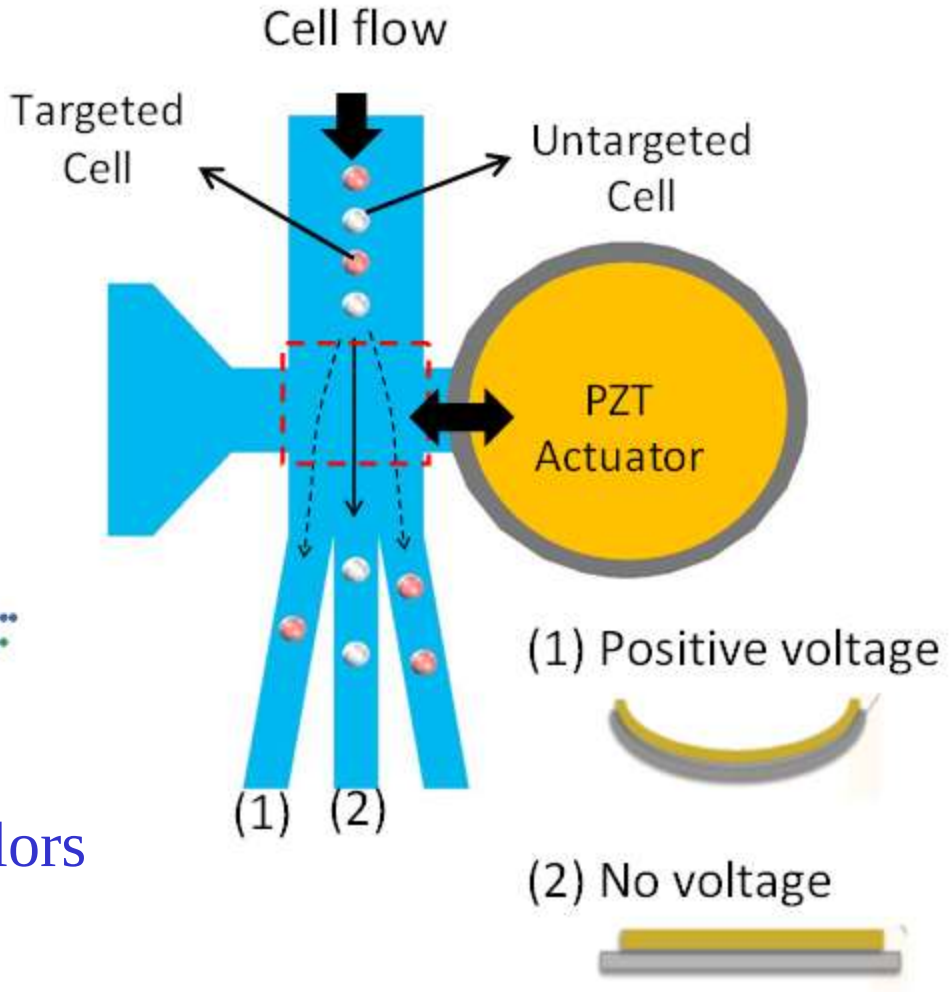
- Optimized position control in droplets
- Specialized microfluidics sorters
- New sorting technologies e.g. OWL
- …



Microfluidic Analyzer/Sorter



nanocellect Biomedical, Inc.



- microfluidics fabrication
- single detector for multiple colors
- in-channel cell deflection

Reagents

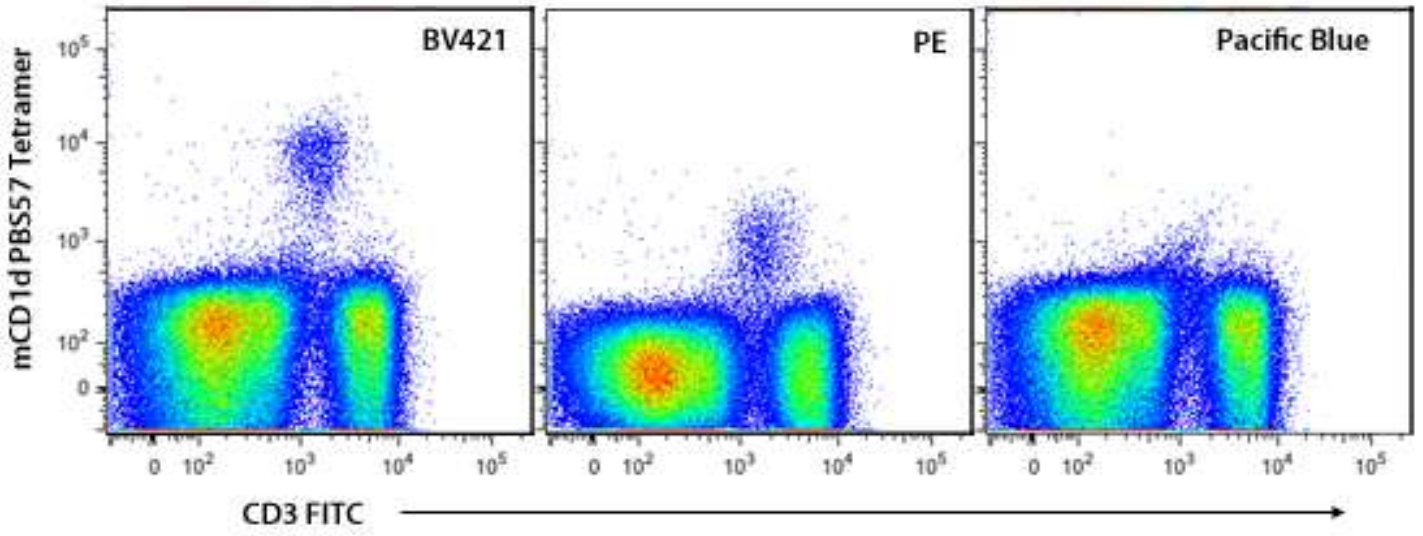
- Advances in affinity reagents
- New amplification methods for single molecule sensitivity
- More and brighter polymer and nano-particle dyes
- Concentration measurments by molecule counting

Novel Affinity Reagents

- Antibodies
  - Antibodies from different species (e.g. Llama 15 kDalton fragments with 10^{-9}M Kd and high stability, potential for intracllular use)
  - Recombinant antibody fragments
  - …
- Synthetic affinity reagents
  - Aptamers
  - Protein scaffolds
  - Molecular Imprinted Polymers

Recent review: Fodey T et al; Trends in Anal. Chem. 30(2011) 254ff

Use of Brighter Labels



http://www.biolegend.com/brilliantviolet

Software and Algorithms/ BioInformatics

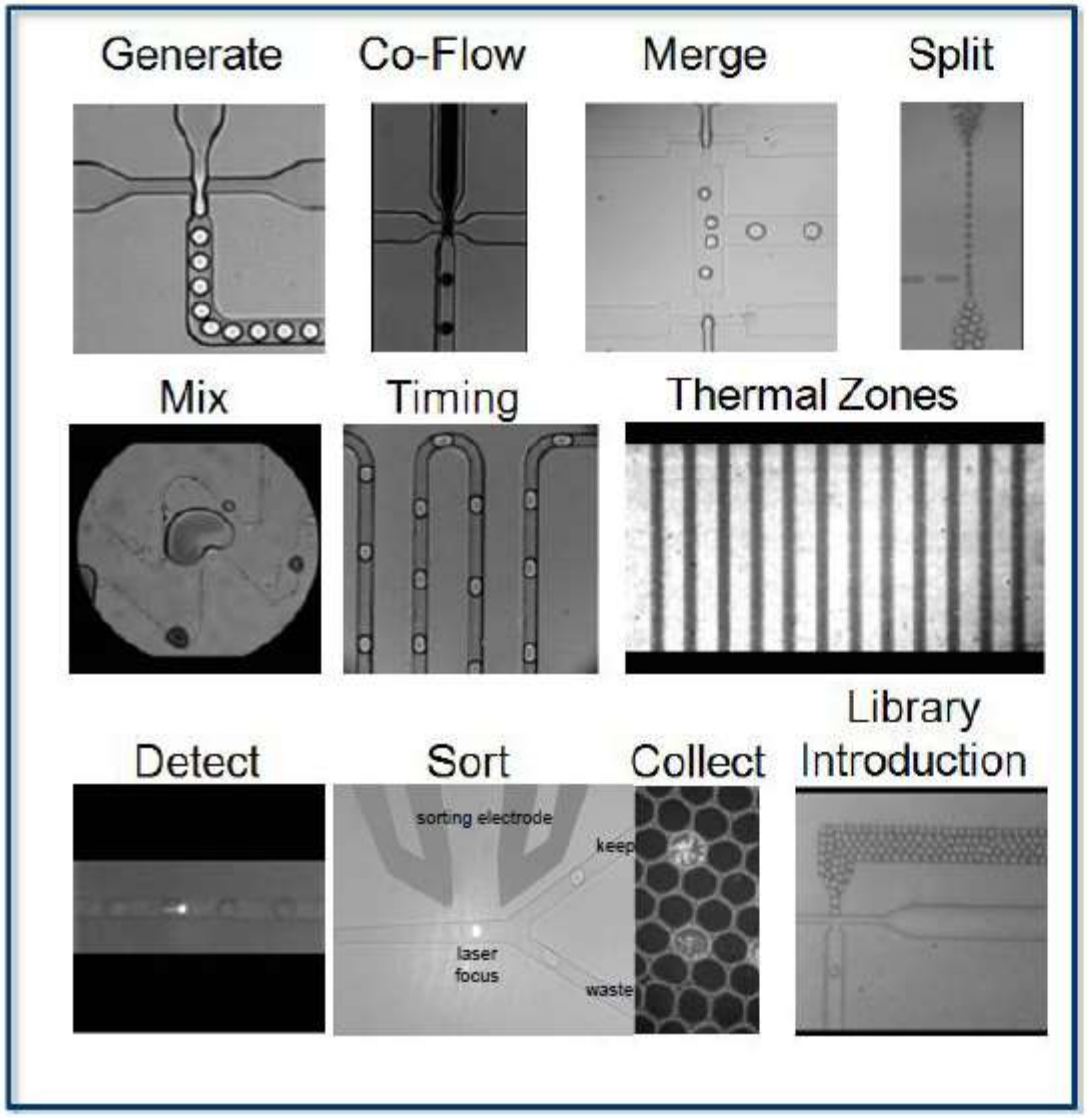
Integration, enhancements, and additions to:

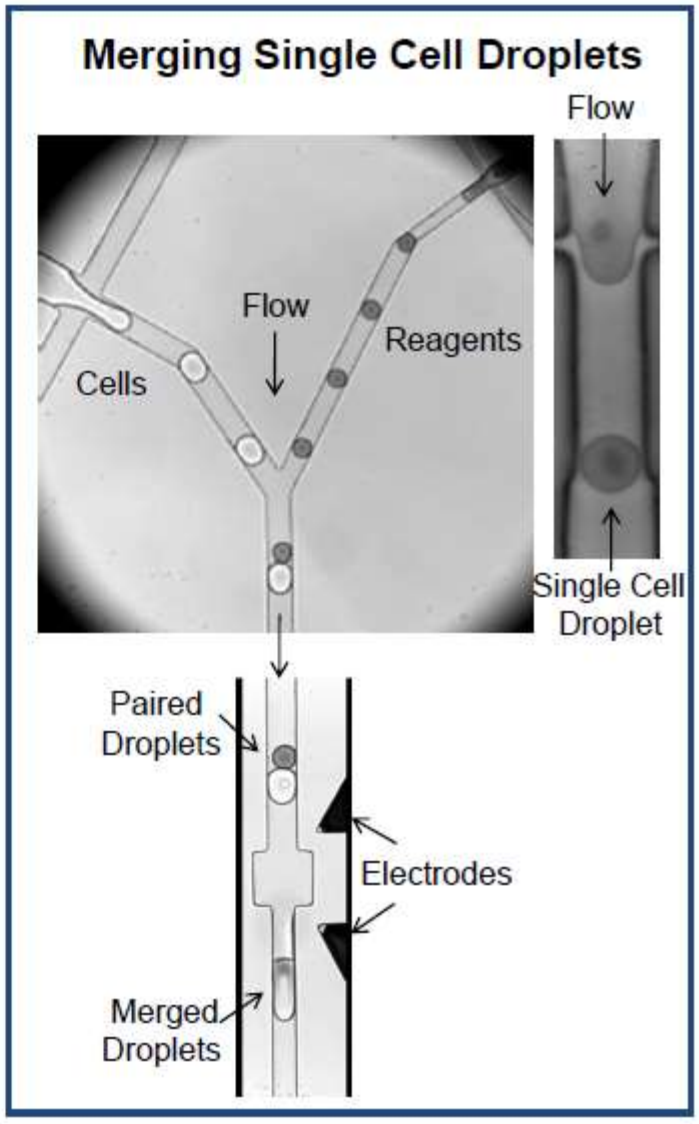
- FLOCK
- Gemstone
- Spades
- Cytobank
- …

Systems

- Fully integrated user-programmable research systems
- Fully automated, pre-programmed, validated clinical systems
- …

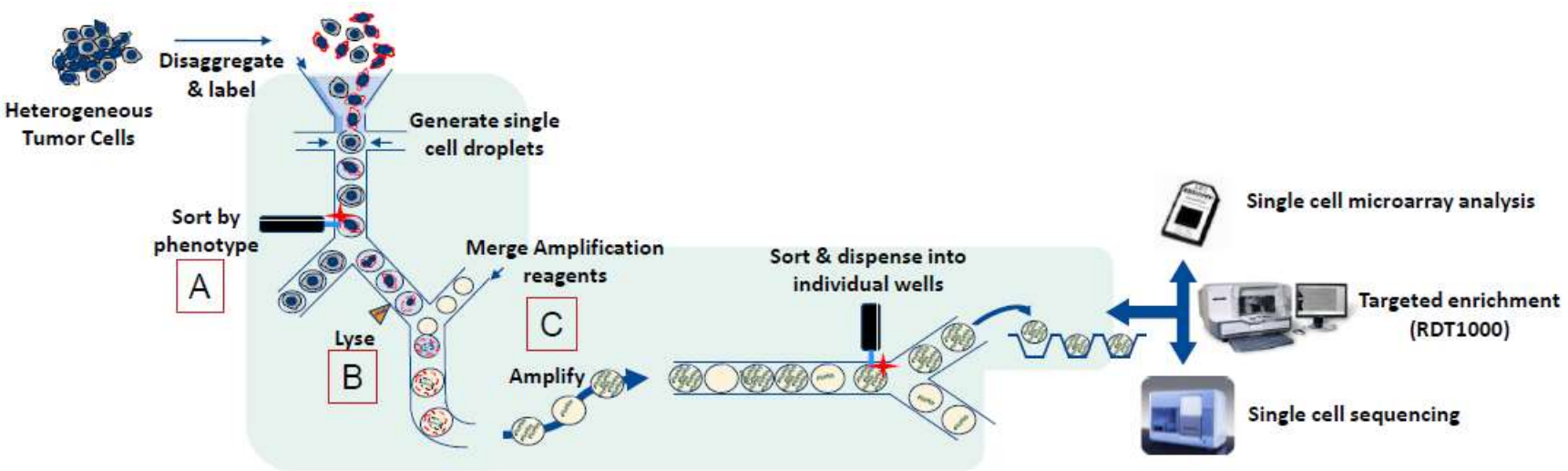
Advanced Single Cell Analysis in Droplets





Source: RainDance Technologies

Fully Integrated Single Cell Analysis



Source: Raindance Technologies

Conclusion

After more than 30 years, cytometry is at the beginning of new era to enable revolutionary discoveries in biology, higher quality in monitoring of biotechnological processes, and better patient care through clinical diagnostics and cellular therapy.

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- Beckman Coulter
- BD

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Conclusion

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END