Advanced Approaches to flow Analysis

5th International Advanced Course in Cytometry
University of Modena, March 2009

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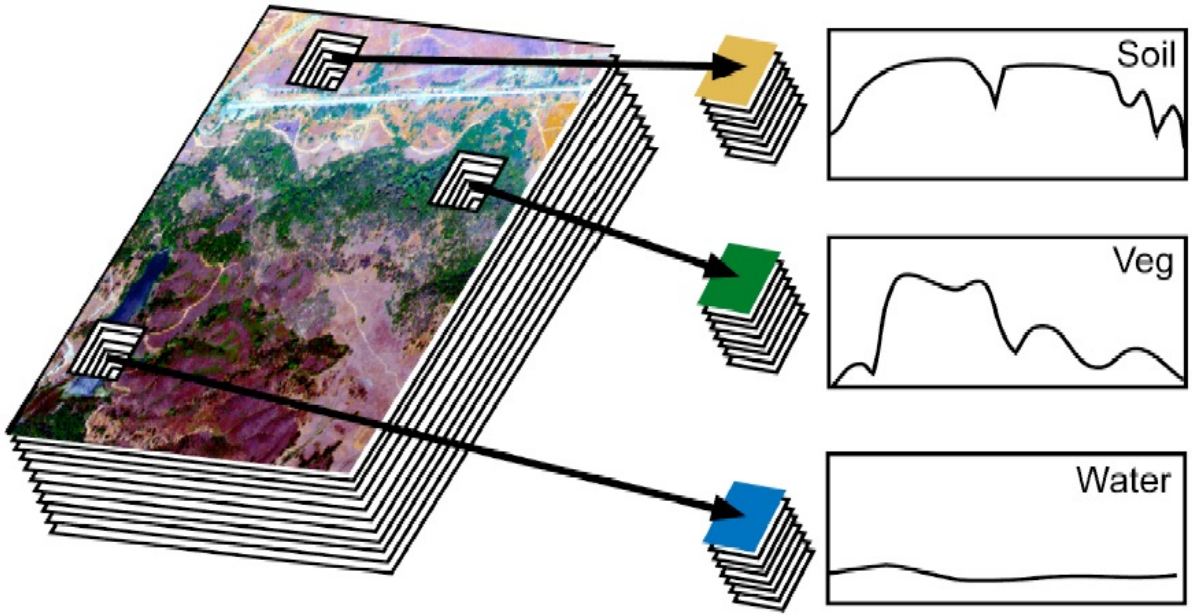
Areas of discussion

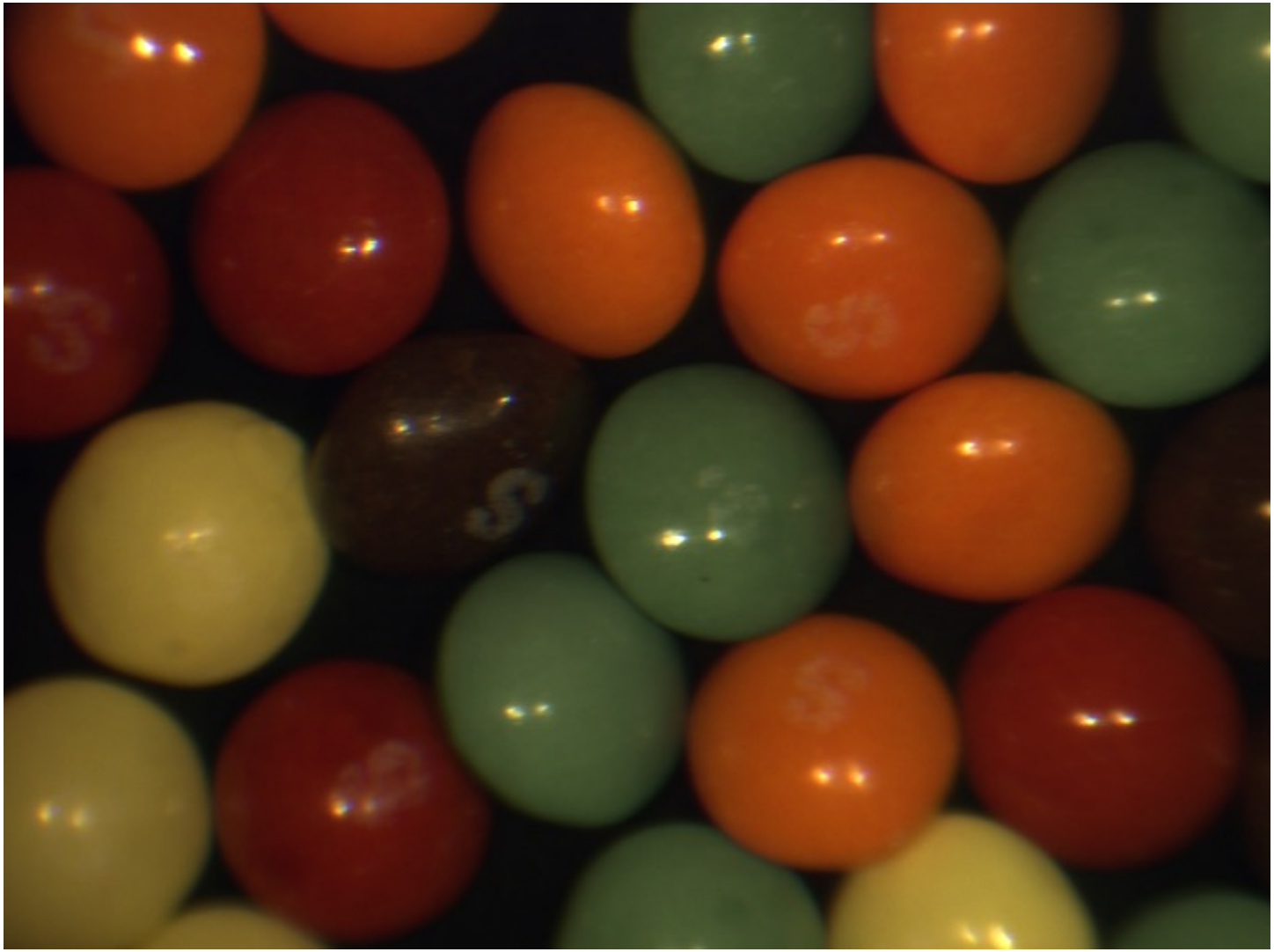
- Hyperspectral Cytometry
- Multiangle light scatter cytometry
- Advanced classification approaches

Introduction to Multispectral/hyperspectral Imaging

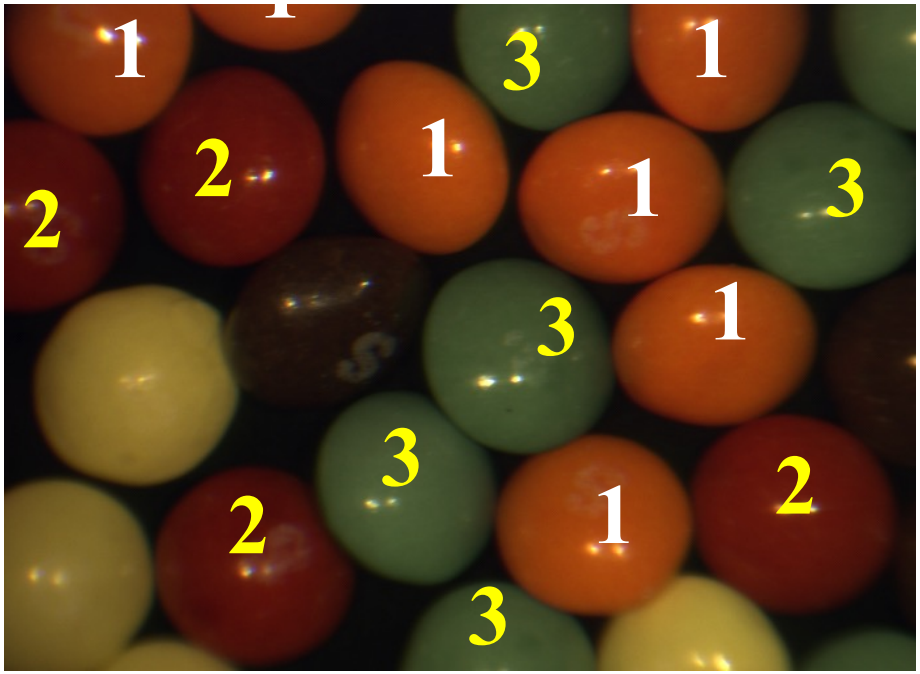
- Used by NASA in the LandSAT program.
- Many applications in biology and medicine.
- Started at Purdue University in the 1960's by Professor David Landgrebe

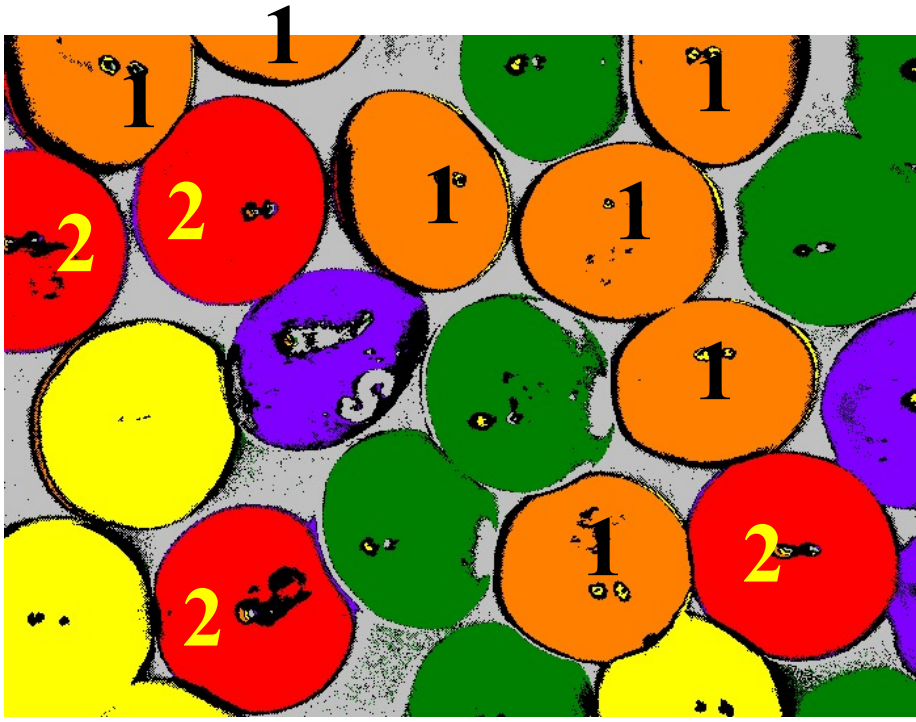


David Landgrebe

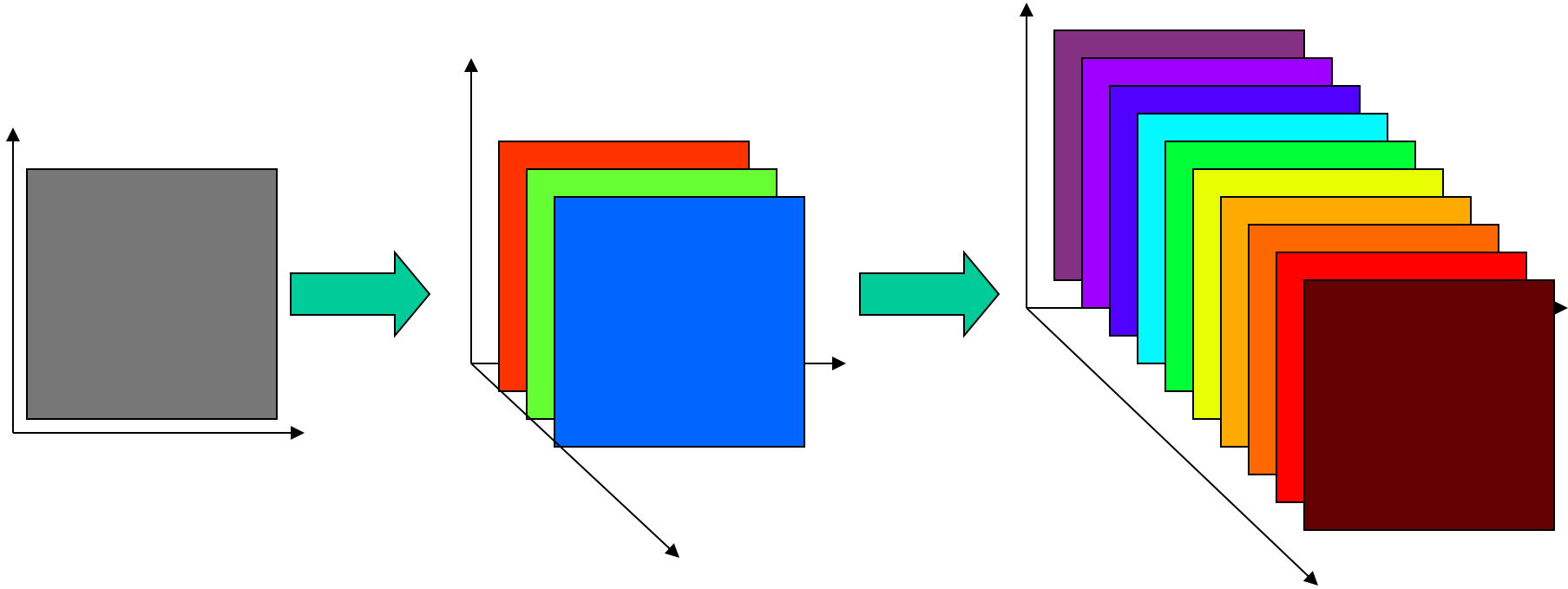


Classified Skittles





Basic imaging…

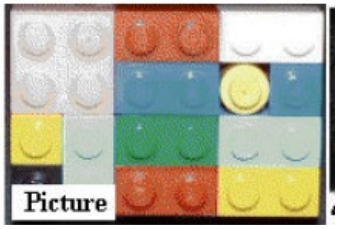


Greyscale image

Color image

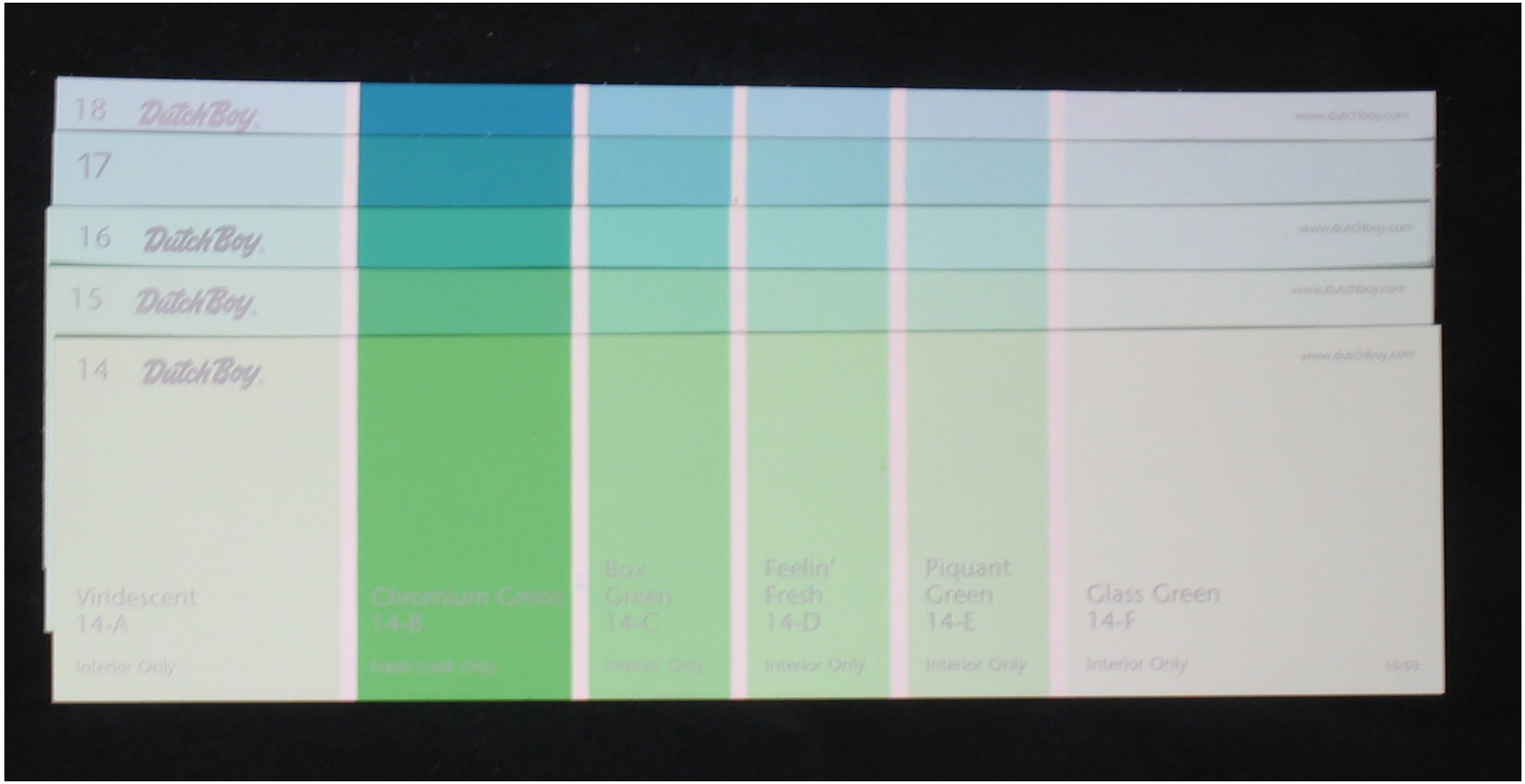
Multispectral image

Color composition is a mixture of spectral bands



Proc. SPIE Vol. 4056, p. 50-64, Wavelet Applications VII, Harold H. Szu, Martin Vetterli; William J. Campbell, James R. Buss, Ed.

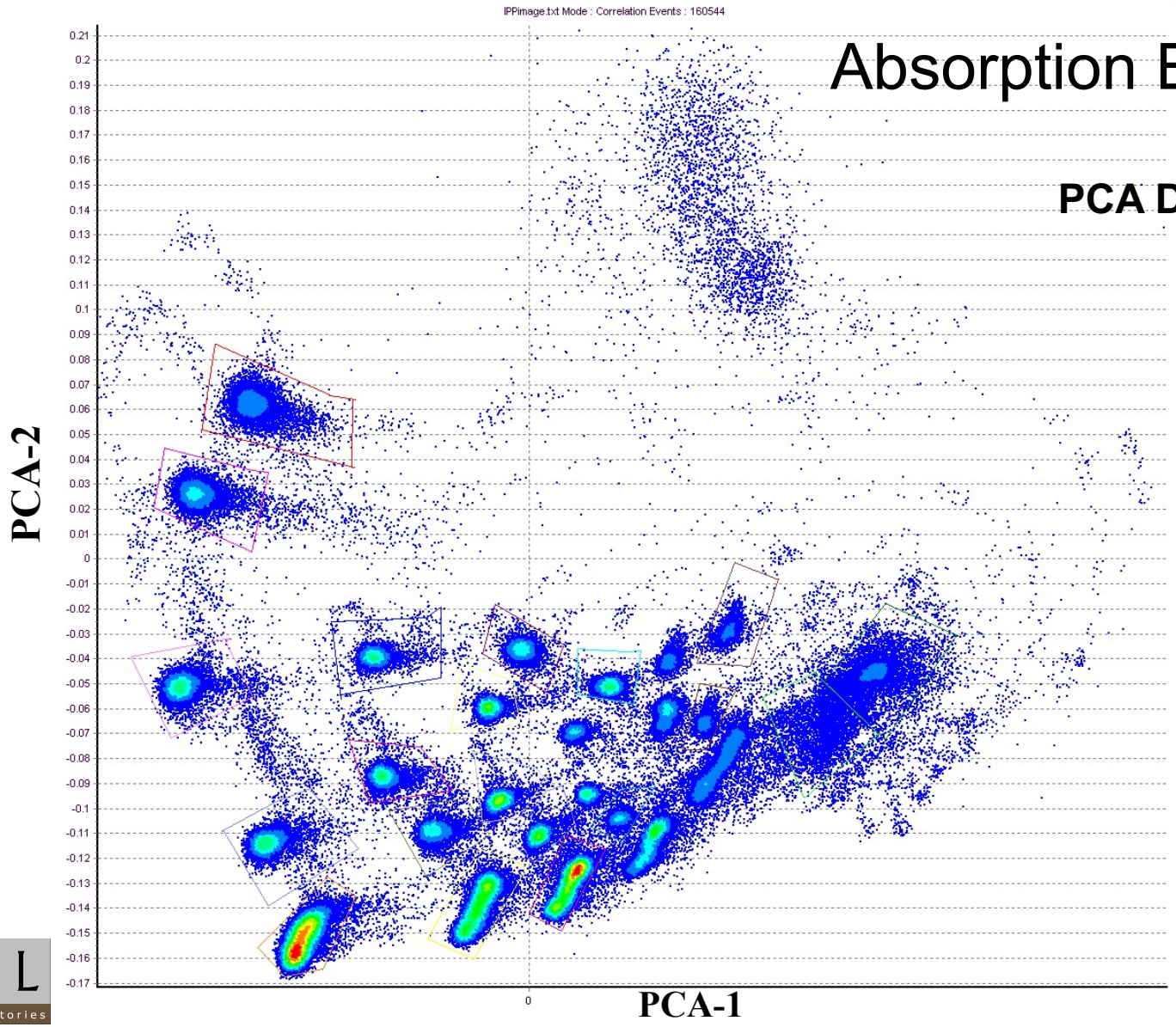
Absorption Example



- Dutch Boy paint cards
- Colors difficult to distinguish by visual inspection

Absorption Example

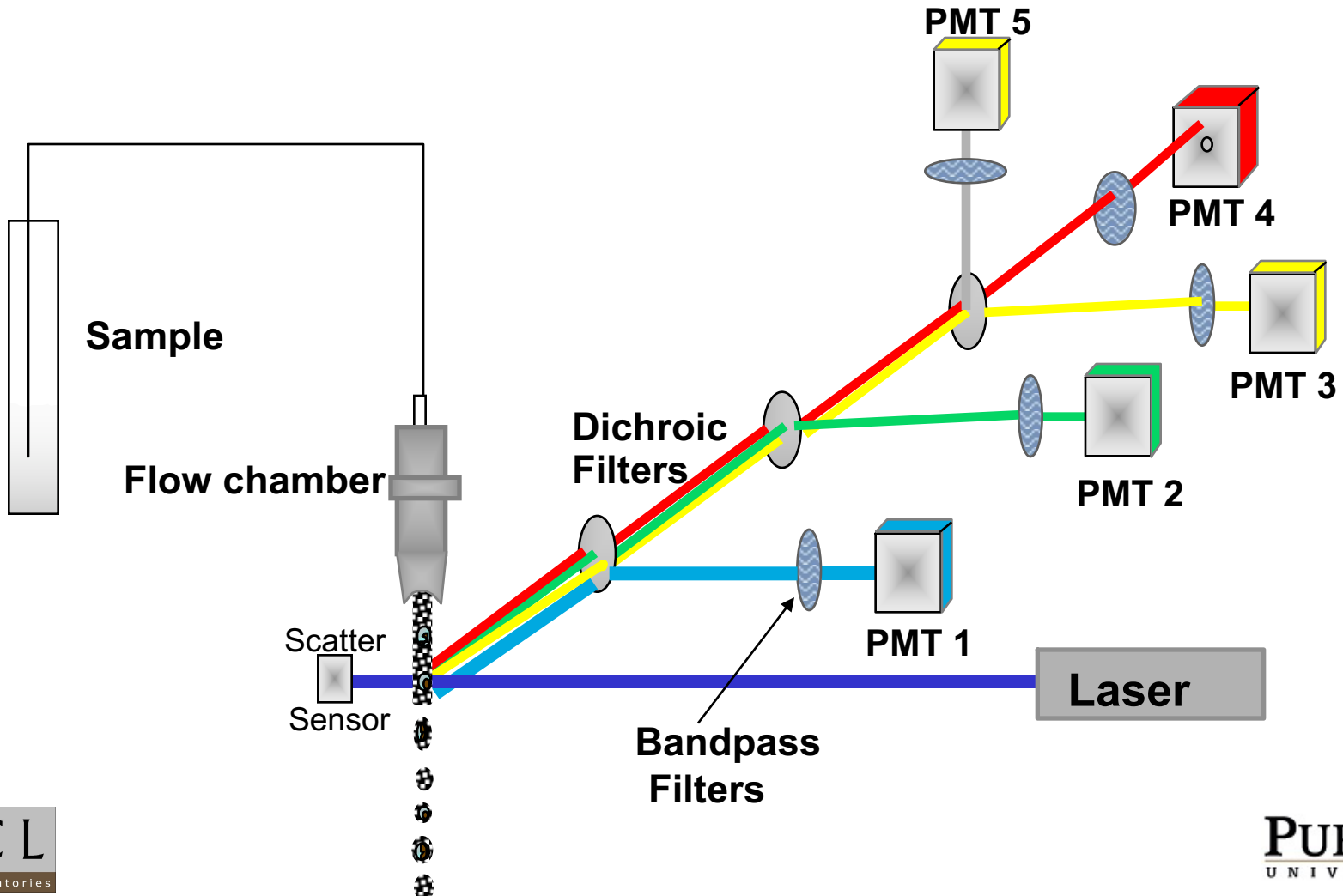
PCA Density Plot



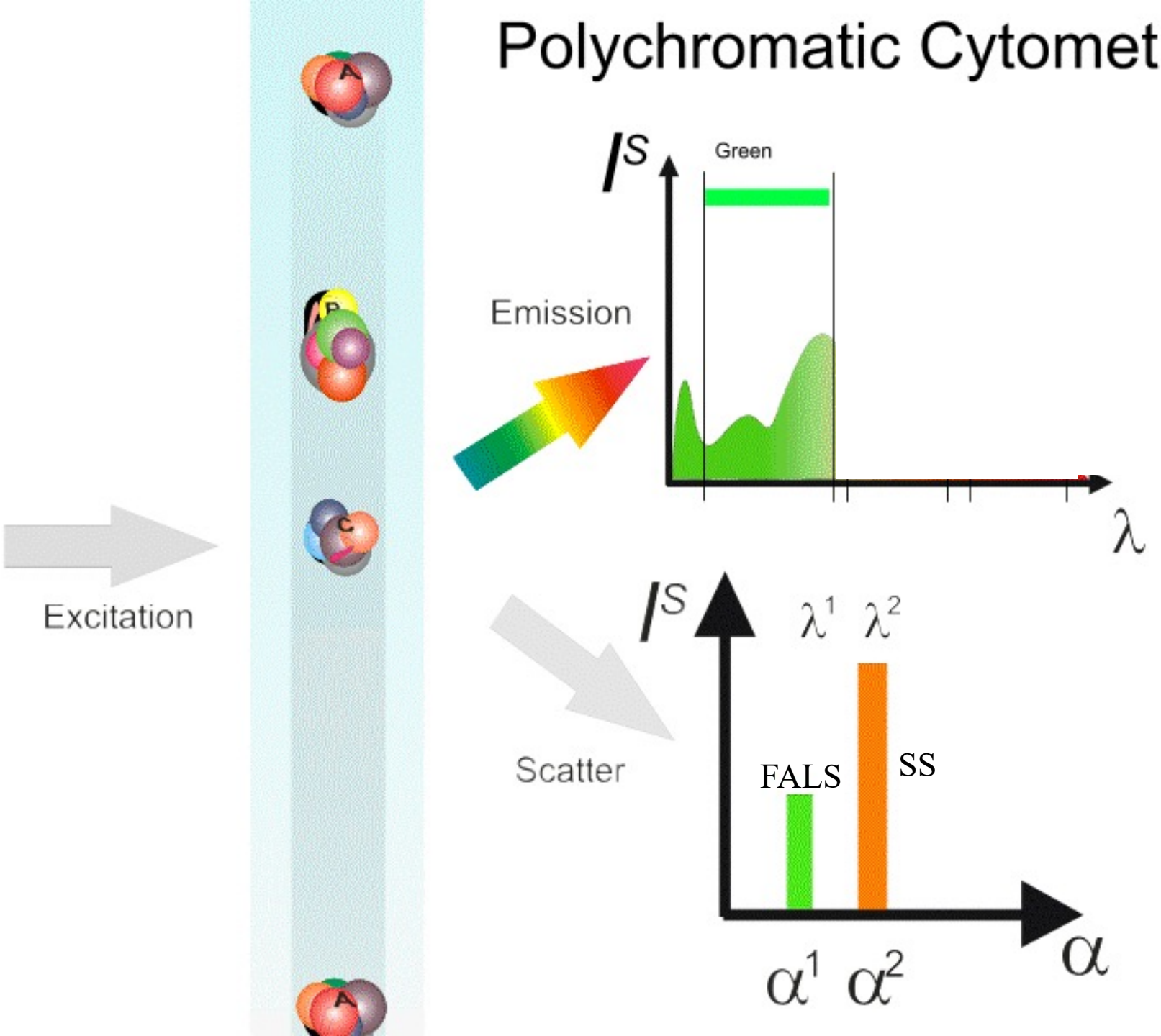
Multispectral Cytometry. Why?

- Identification of multiple spectrally overlapping stains (multiplexing)
- Spectral barcoding
- Spectral un-mixing (multiple stains in a single particle)
- Identification of intrinsic (auto) fluorescence
- Allows “intelligent systems” approach to classification

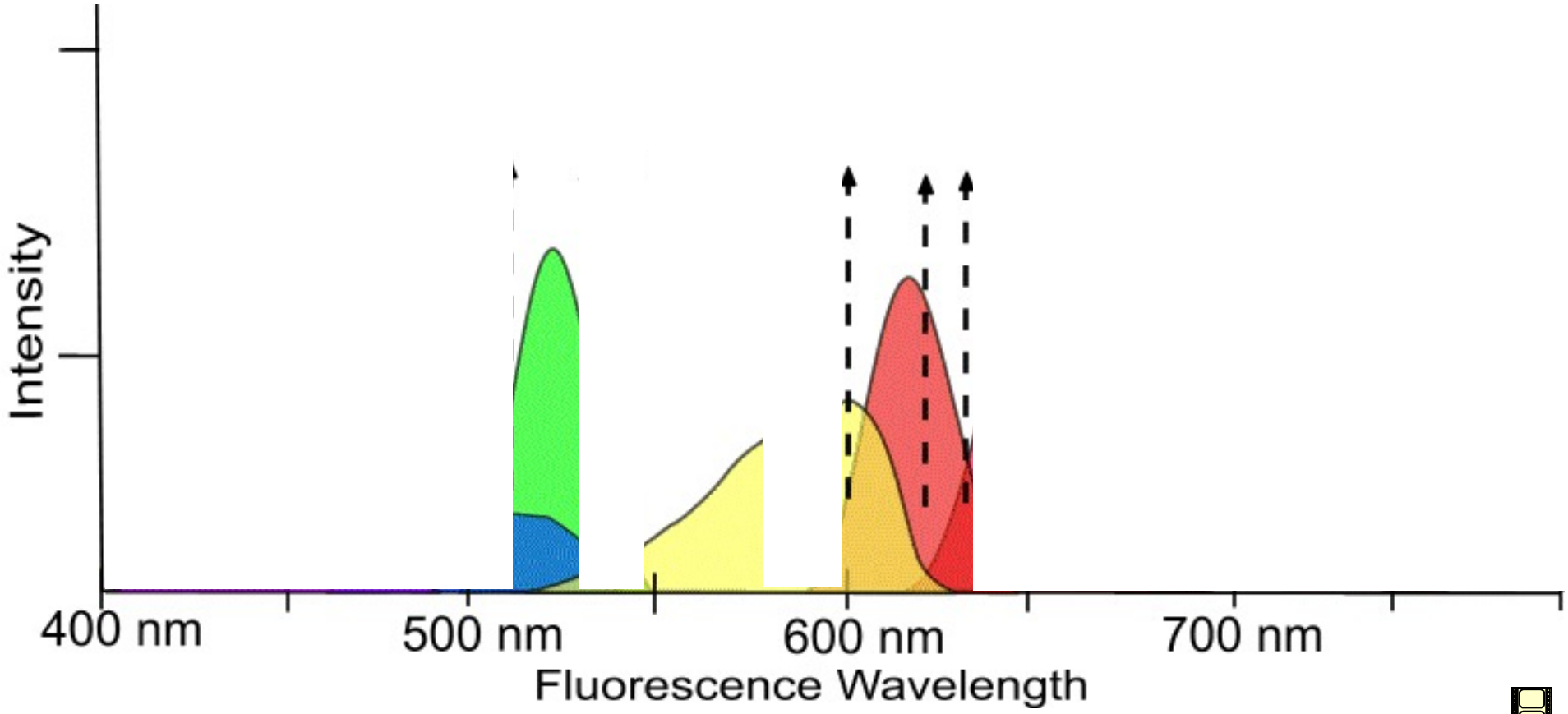
Optical Design of a basic flow cytometer



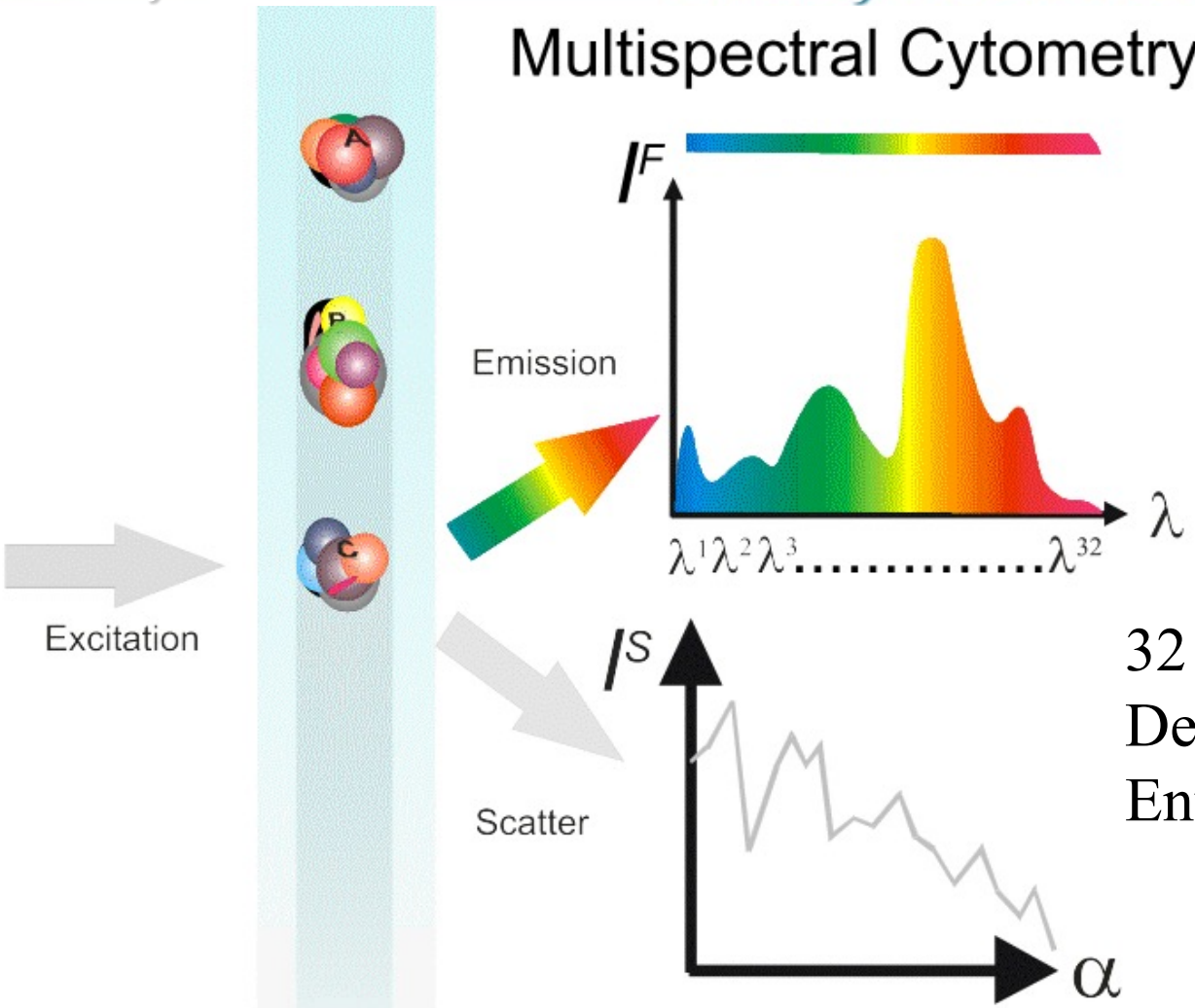
Polychromatic Cytometry



Spectral Overlap makes for very complex analysis



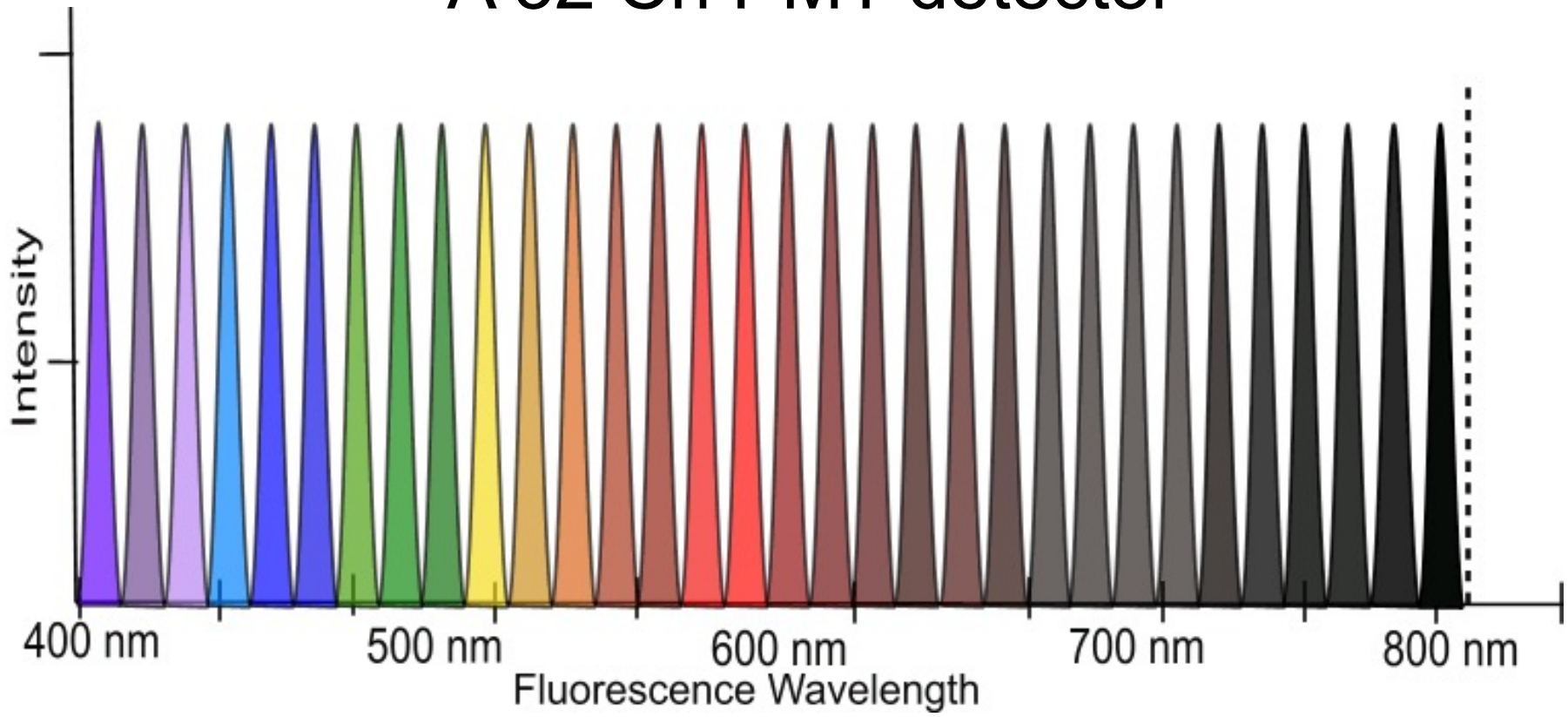
Multispectral Cytometry



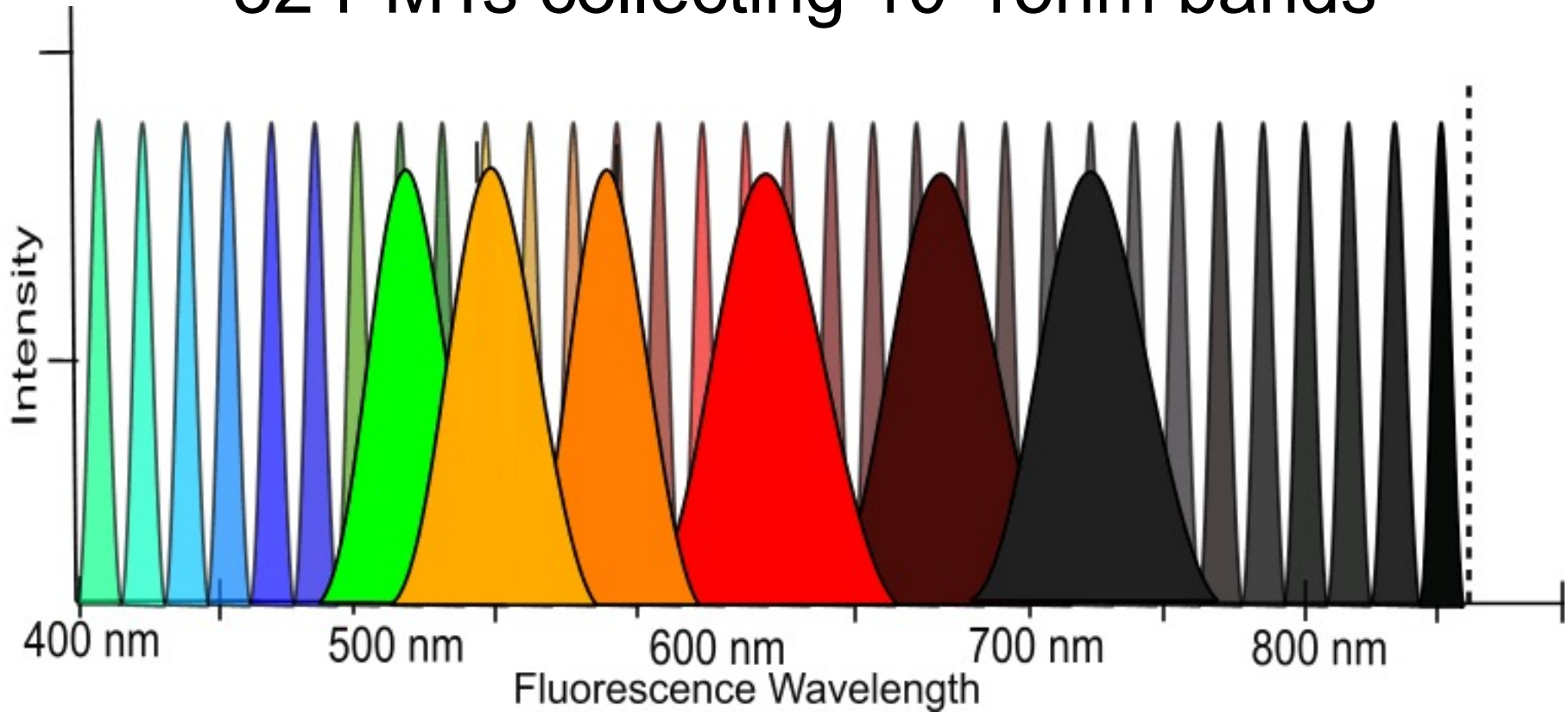
32 Channel Pulse Describes the Entire spectrum

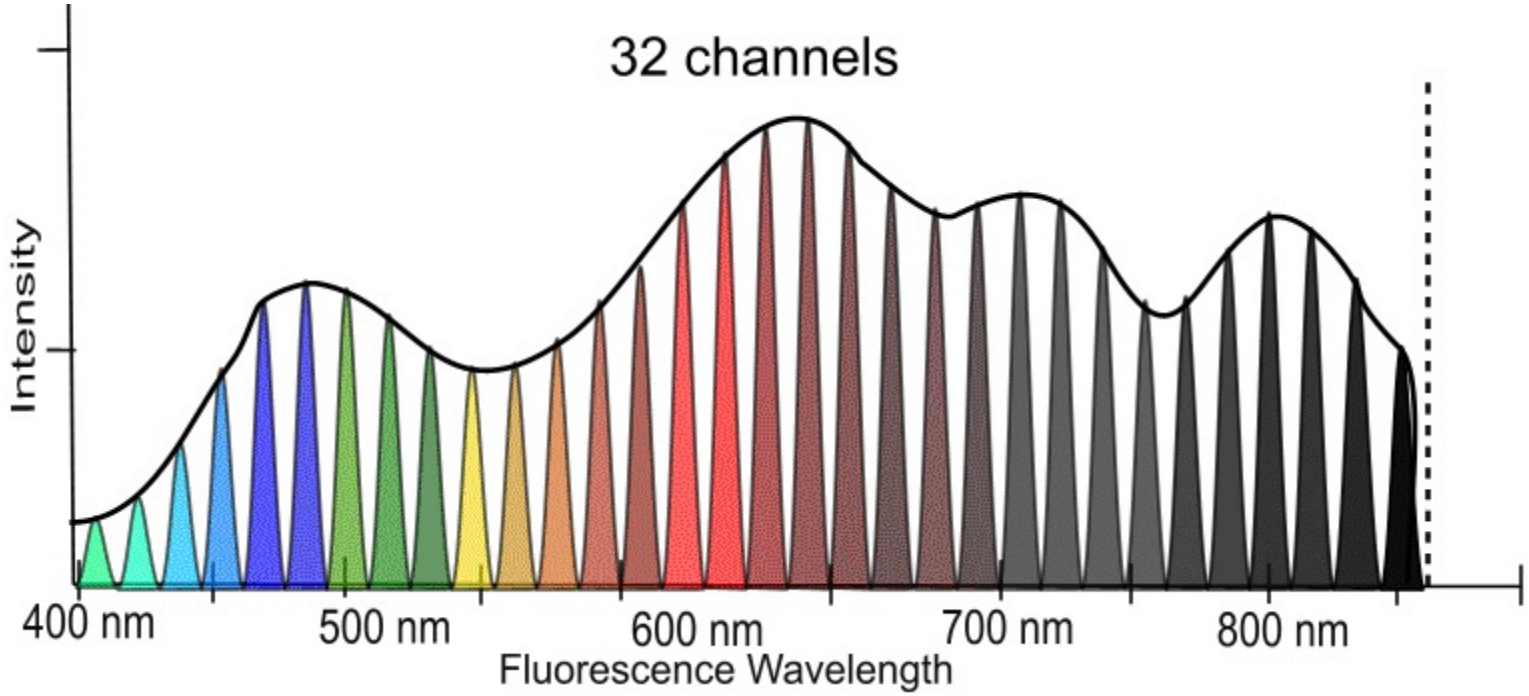
Spectrum becomes a parameter

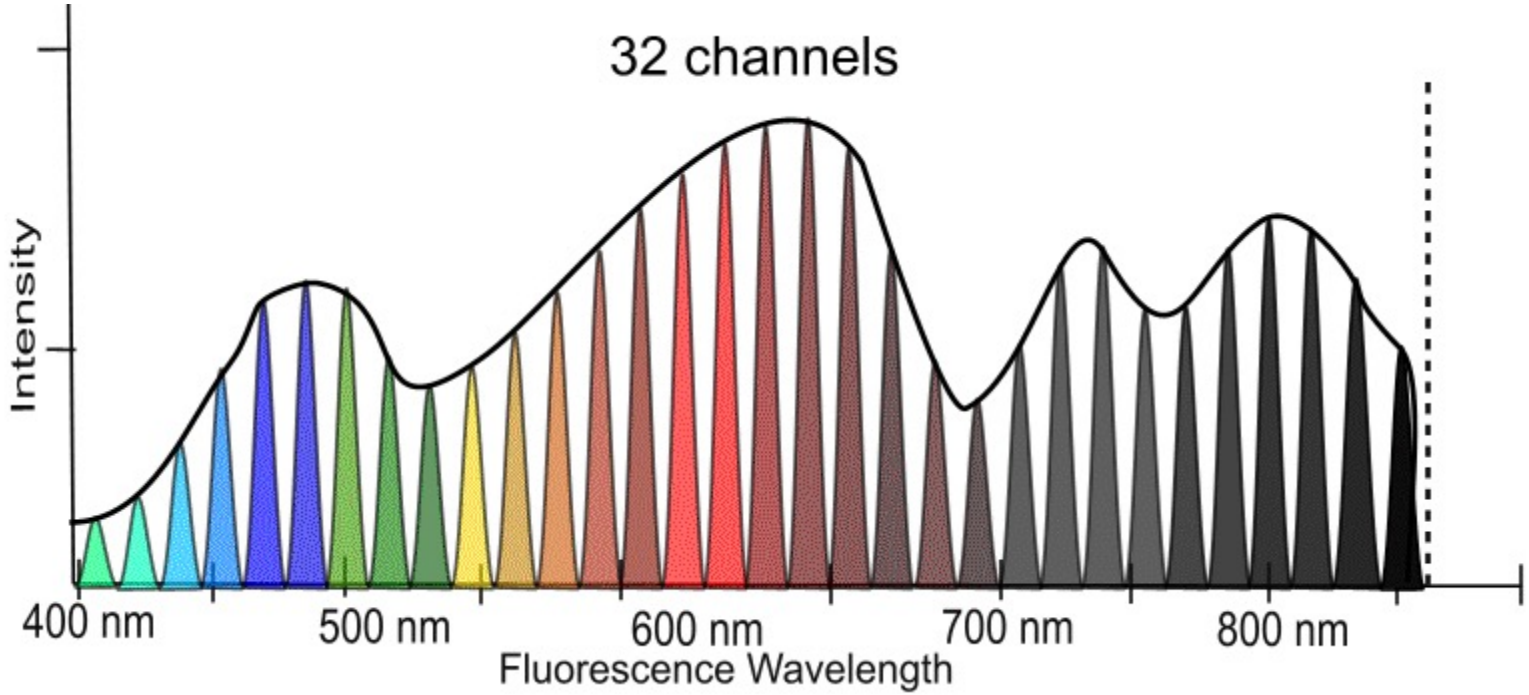
A 32 Ch PMT detector



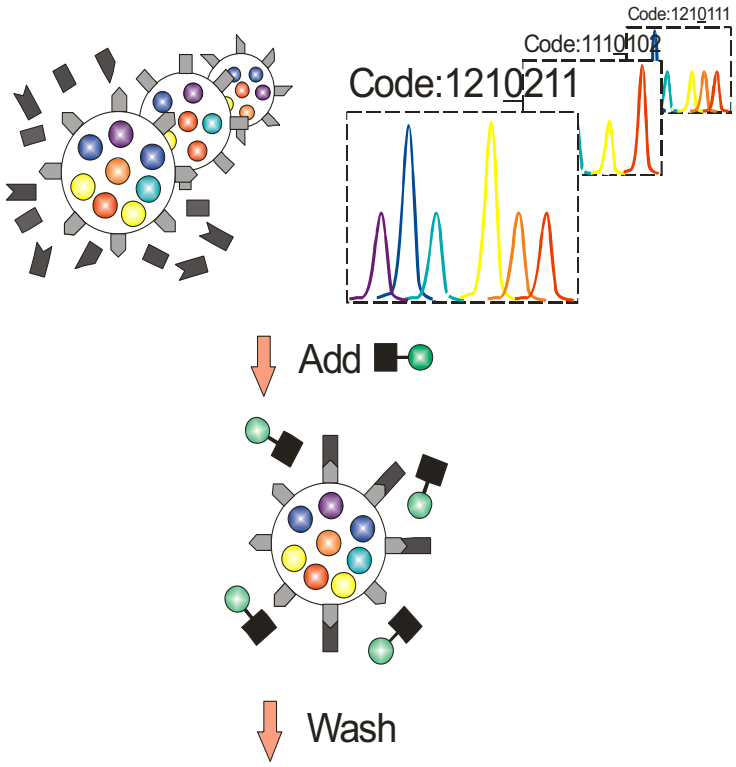
32 PMTs collecting 10-15nm bands




32 channels
Intensity
400 nm
500 nm
600 nm
700 nm
800 nm
Fluorescence Wavelength

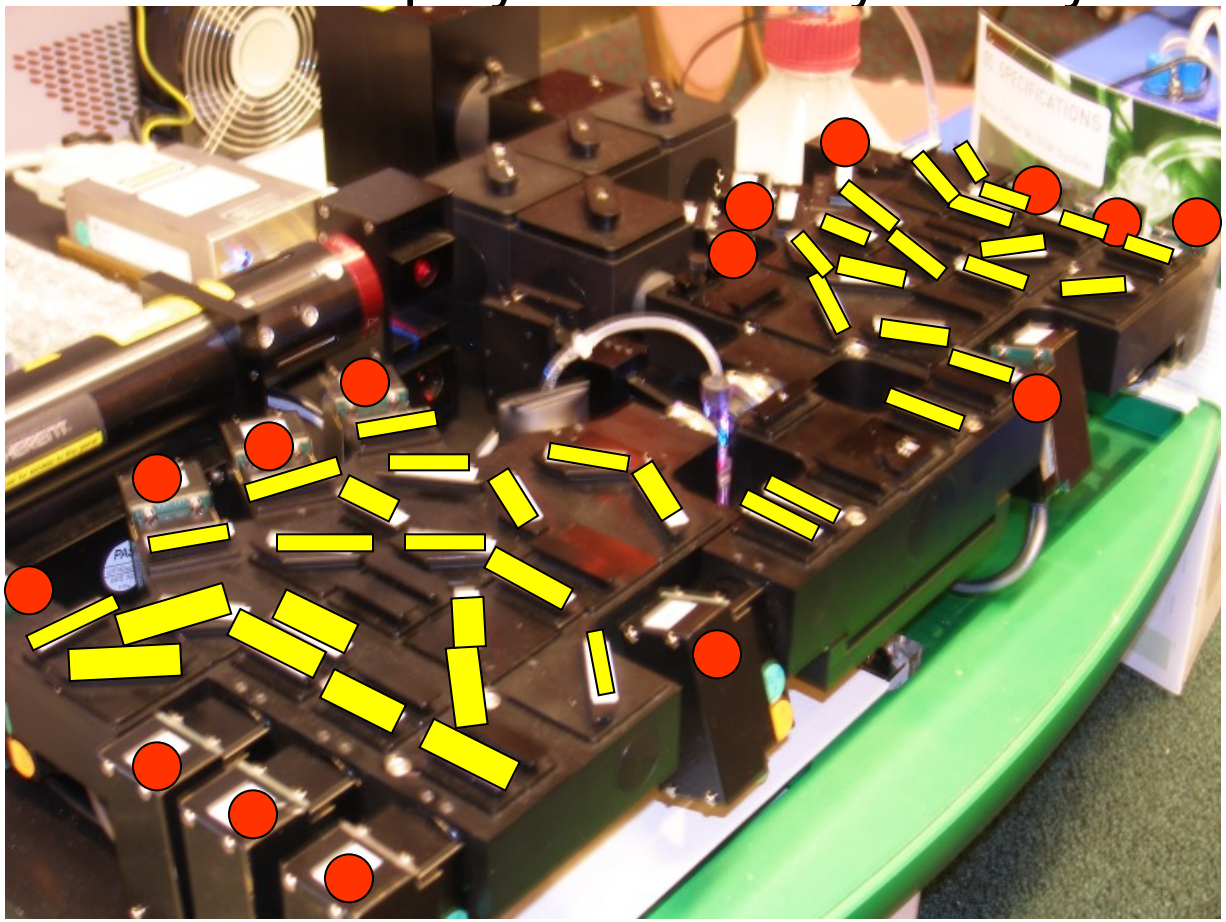

32 channels
Intensity
400 nm
500 nm
600 nm
700 nm
800 nm
Fluorescence Wavelength

Nanocrystals/Micro-Dots multiplexed systems



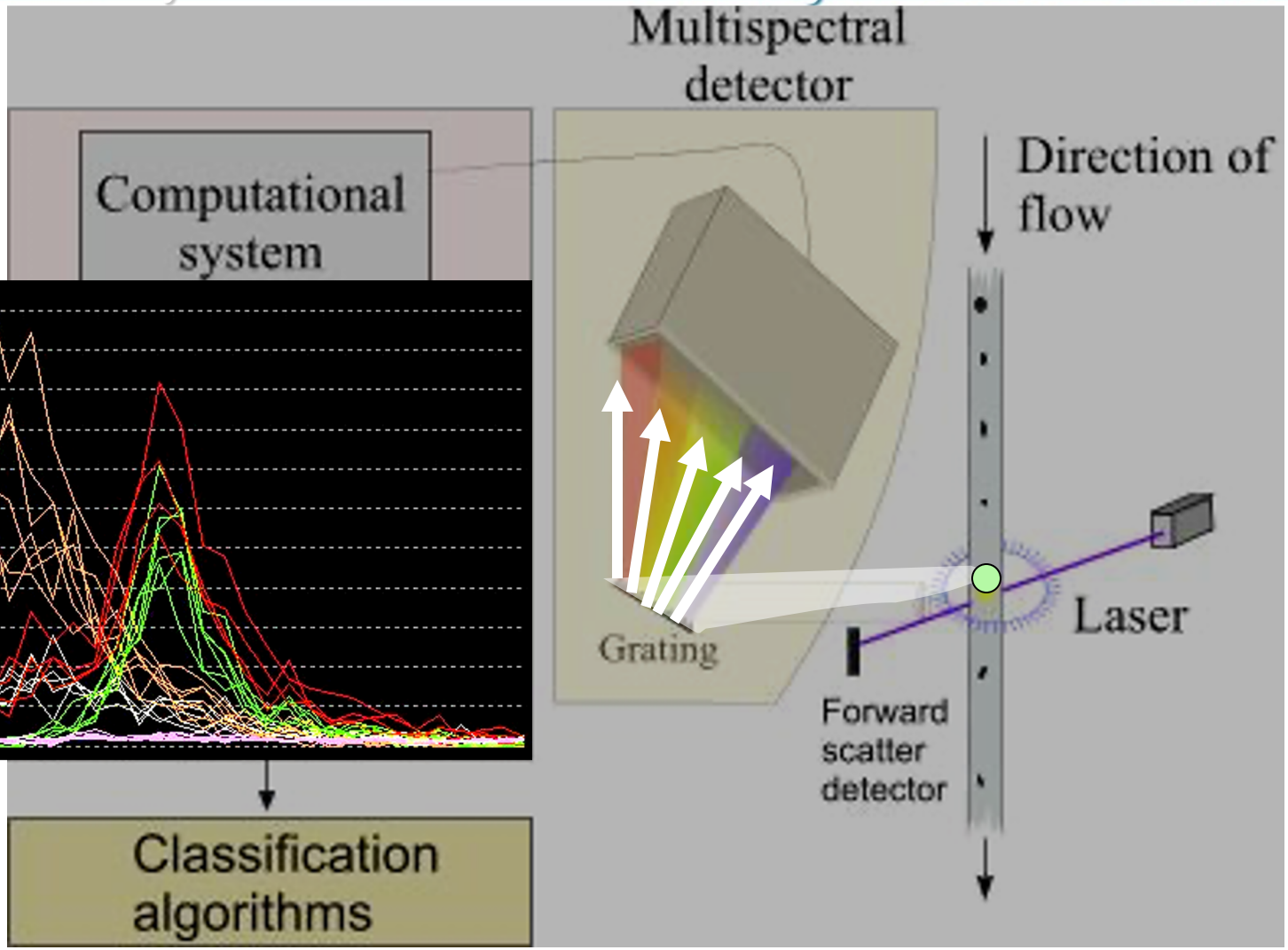
- New probes
- Potentially 1000's of combinations
- Sensitive, long lived, less bleaching
- Difficult to make
- Will require some advanced classification

Advanced polychromatic cytometry



14 PMTs

41 filters



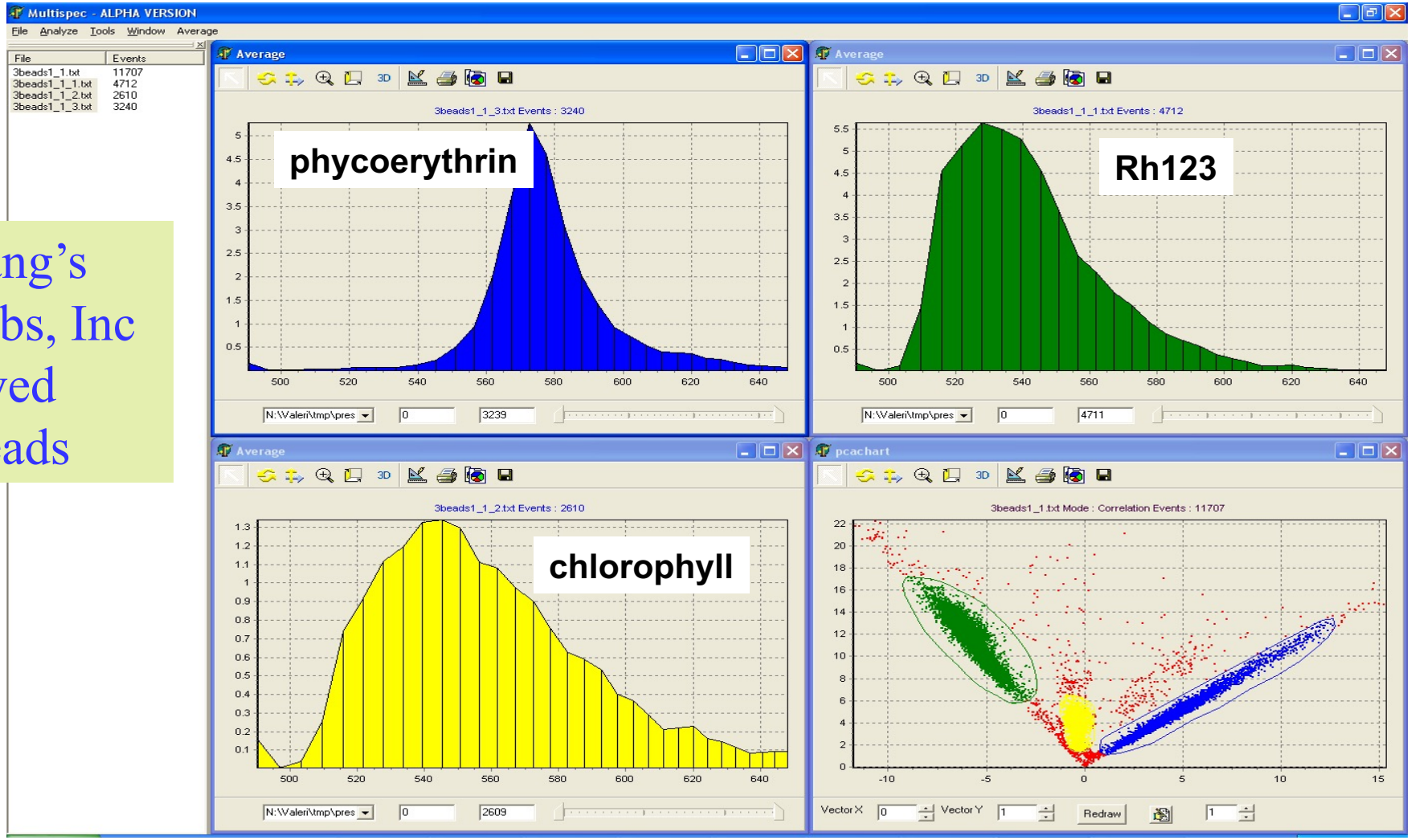
US & foreign patents pending

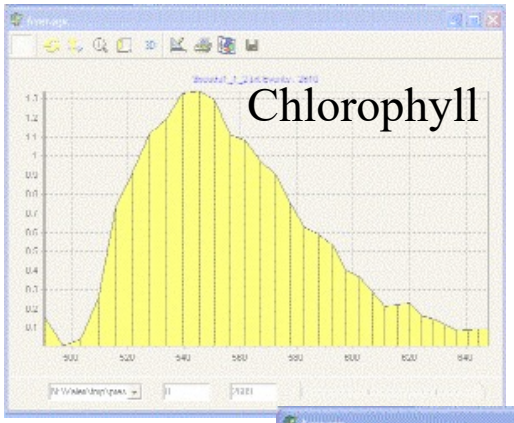
46 parameter cytometer

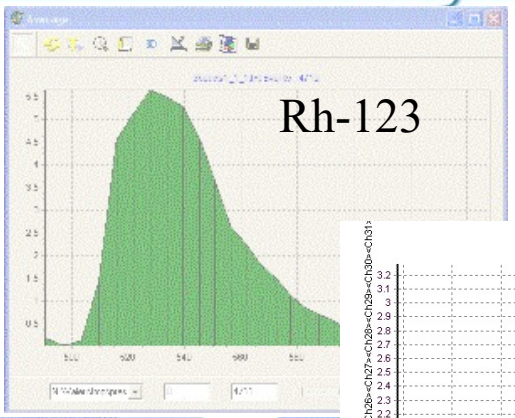
- 32 channel PM array
- 6 channels of regular PMTs
- 1 PMT for side scatter
- 5 detectors for forward scatter
- 1 channel for boxcar (pulse width)
- Time (microsecond resolution)

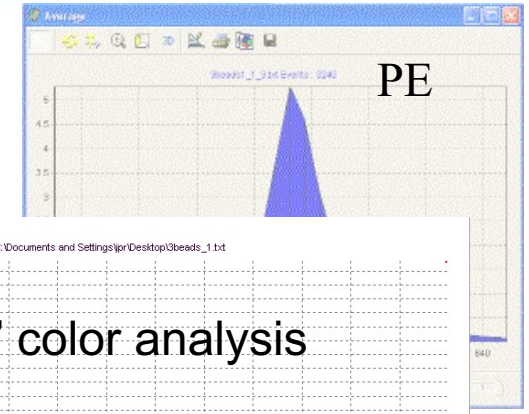
46 measurements per cell

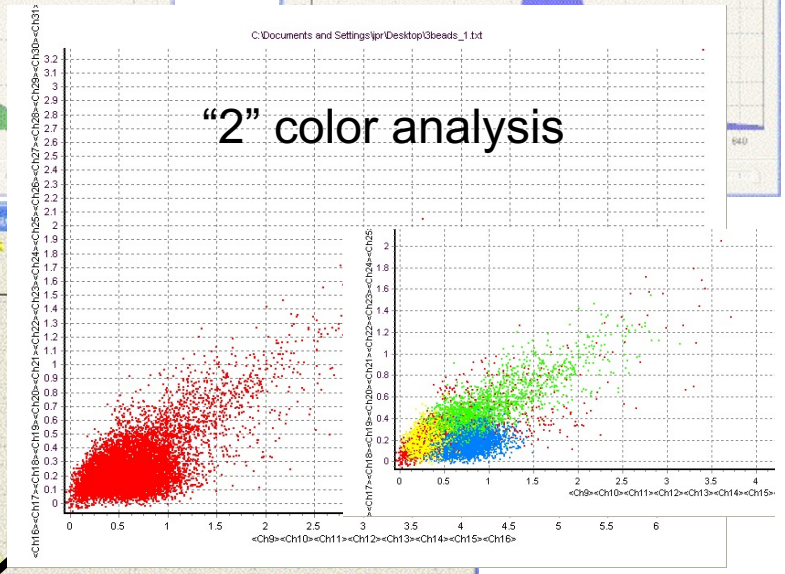
Bang's Labs, Inc Dyed Beads



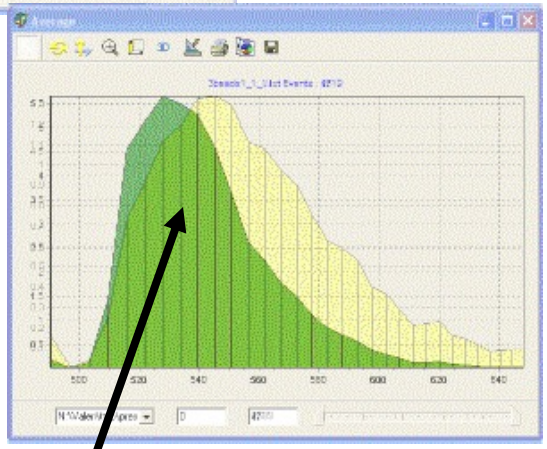


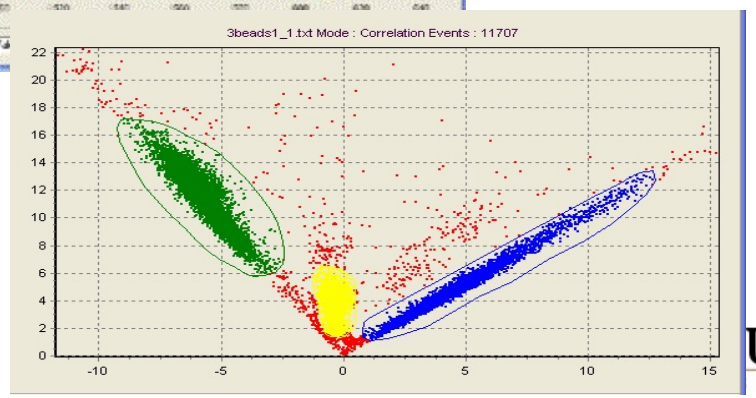






Bang's Labs Dyed Beads*

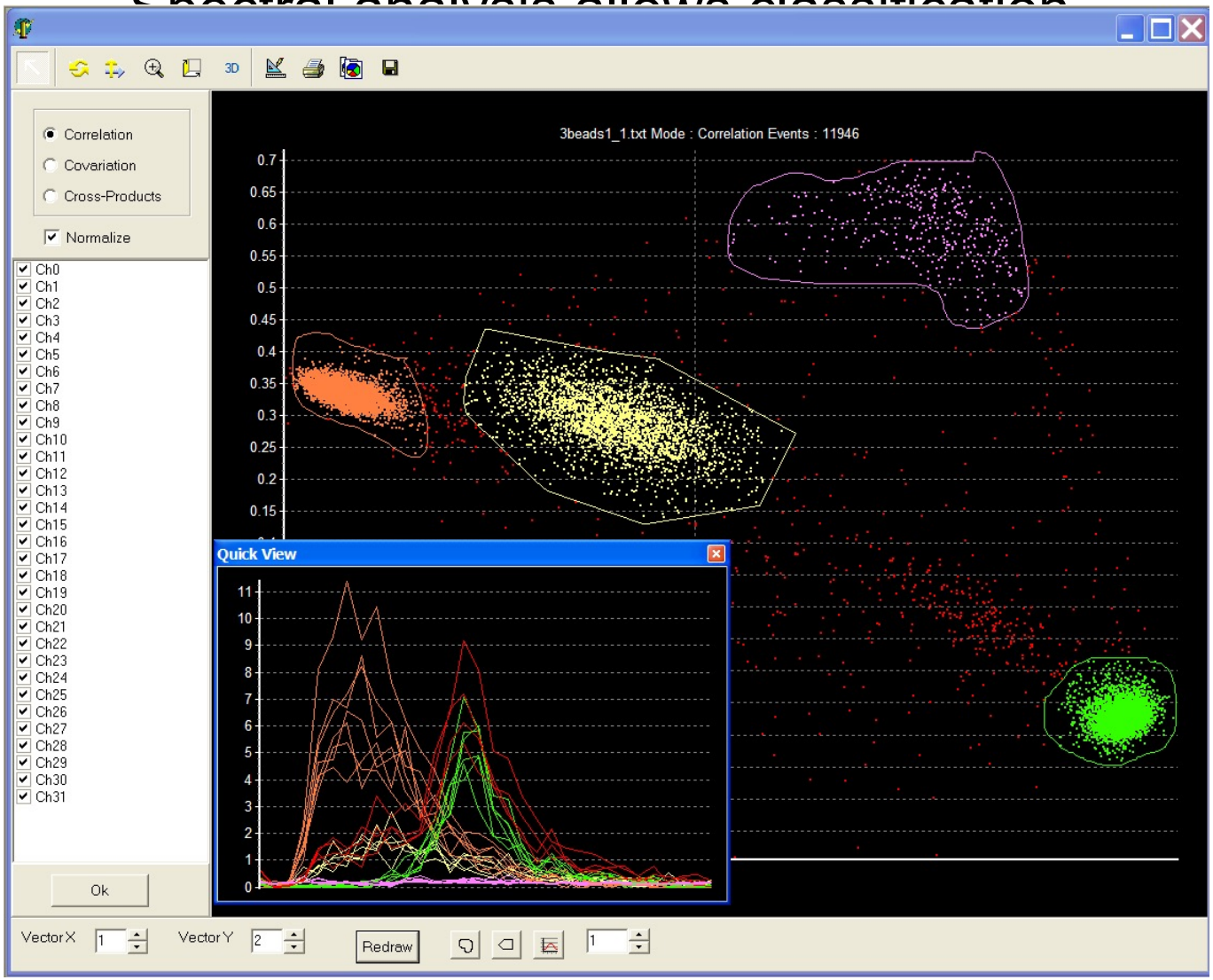




These CAN now be resolved

Except using advanced Classification tools like PCA

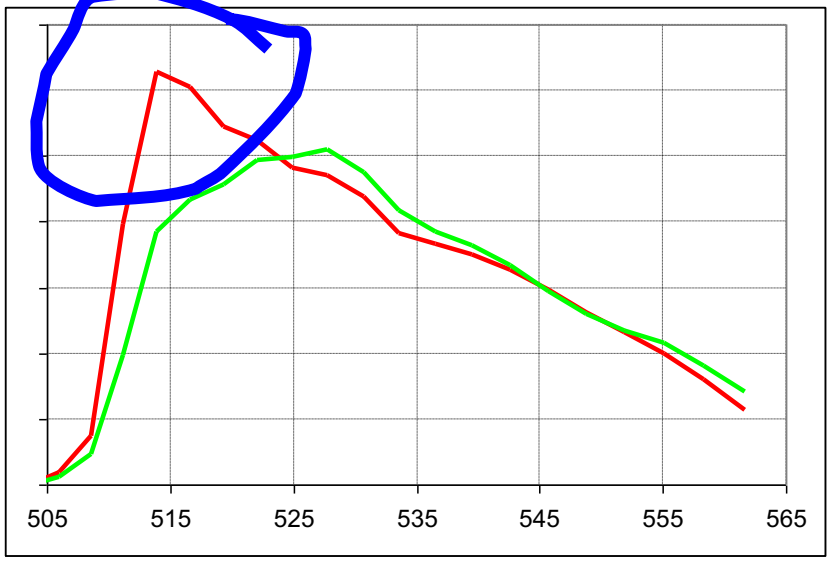
Spectral analysis allows classification



Dye 1: bis-(1,3-dibutylbarbituric acid)trimethine oxonol, $DiBAC_4(3)$
Dye 2: 3,3'-dihexyloxacarbocyanine iodide, $DiOC_6(3)$

Spectral "unmixing"

FITC filter block

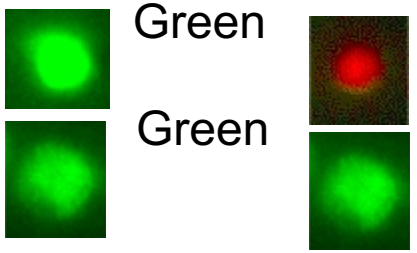


Before

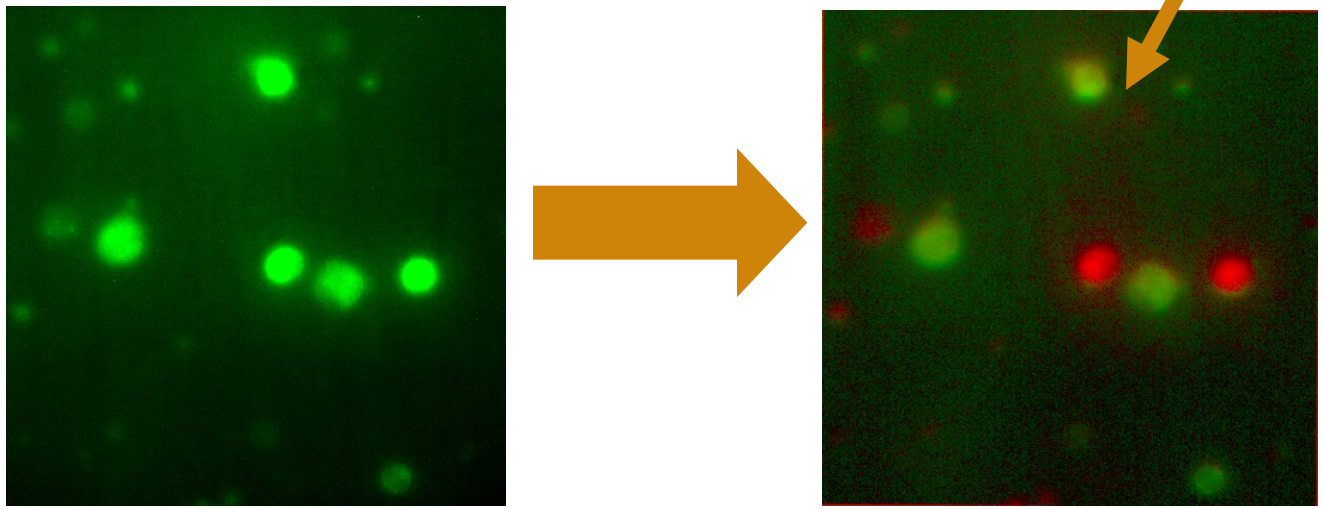
After

Green

Green



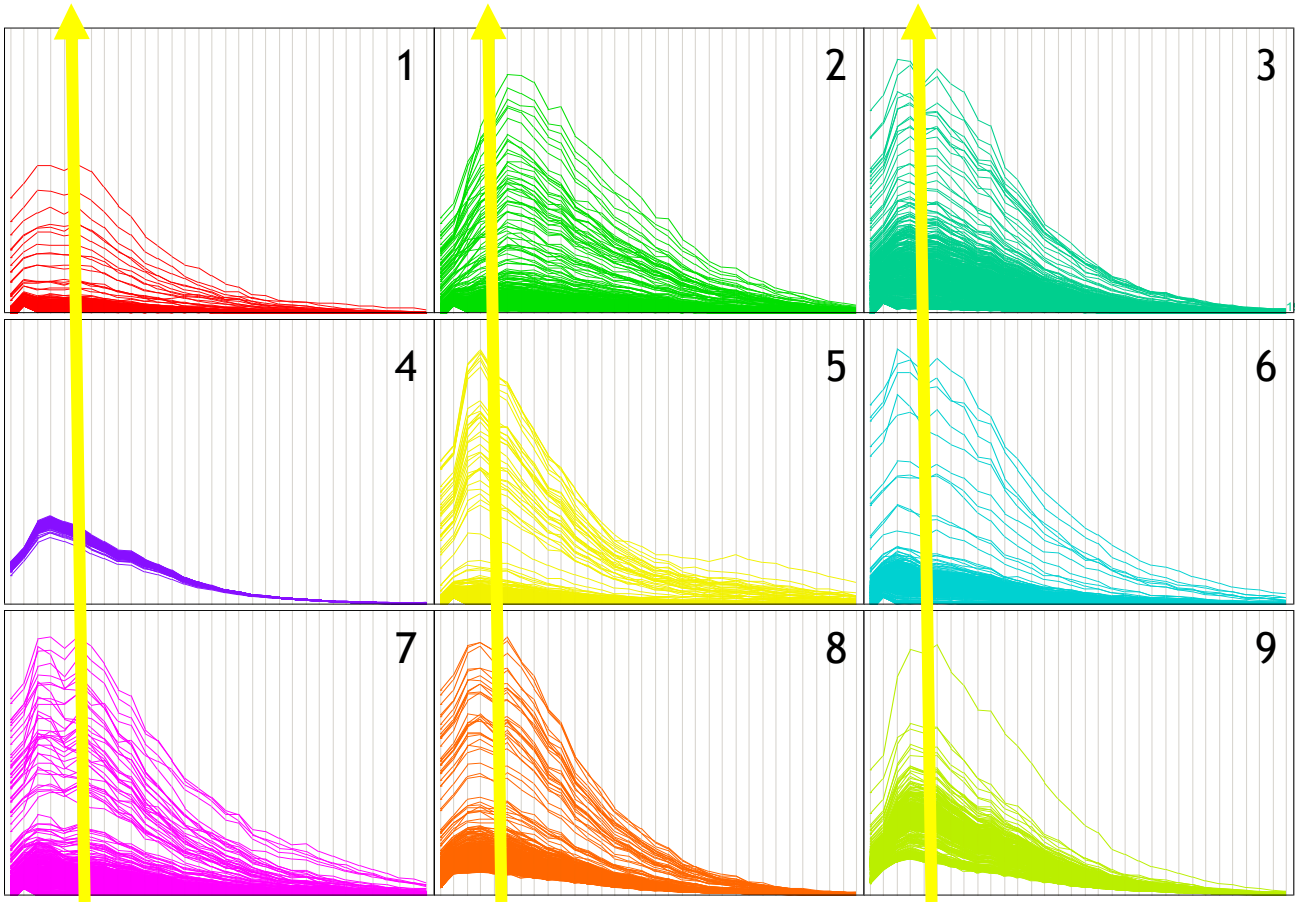
Unmixed
$DiOC_6(3)$
And bis-oxonol



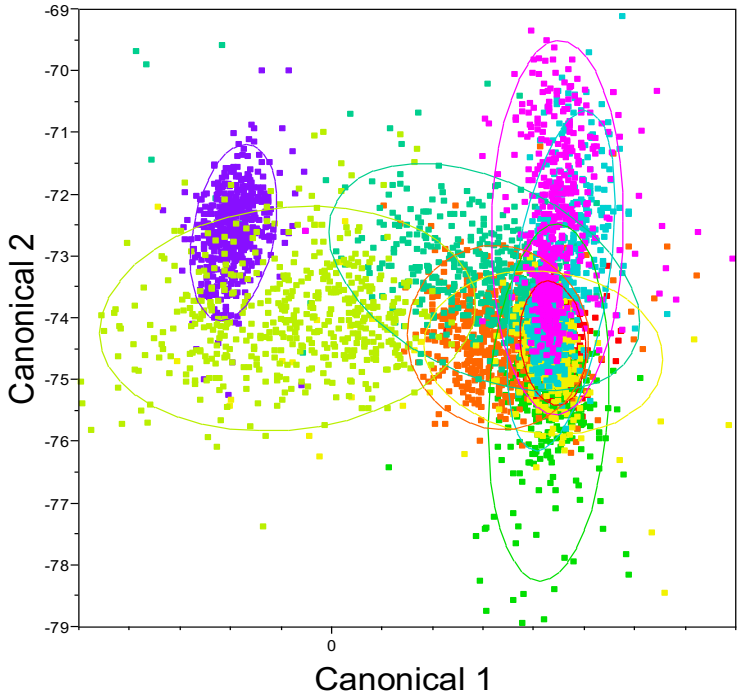
Spectral plots

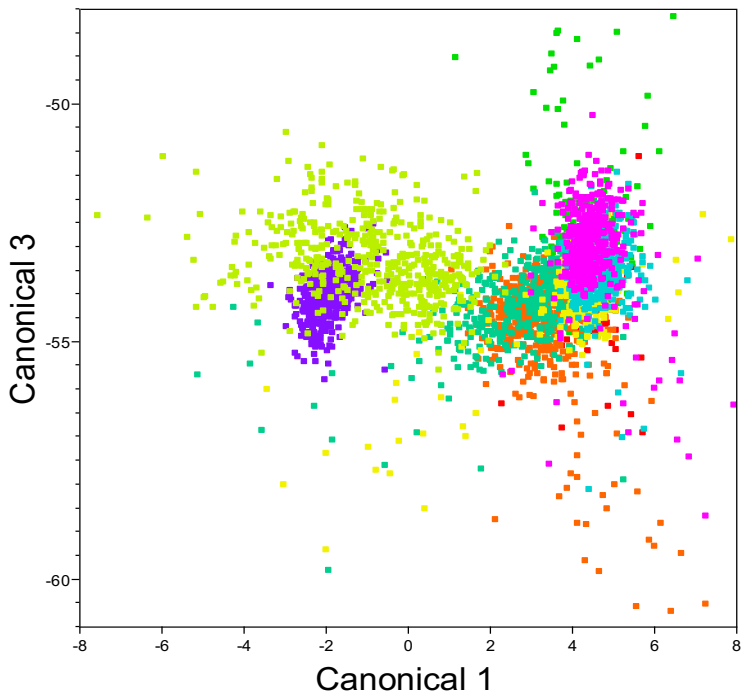
515-535 nm

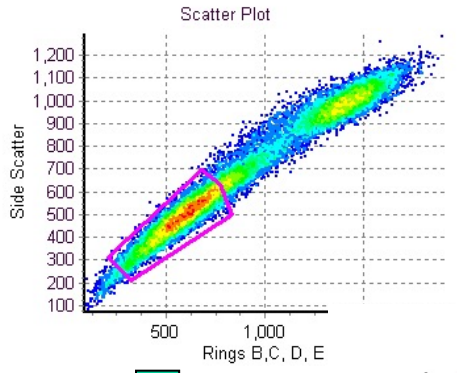
1. 5-(and-6)-carboxy-2´,7´-dichlorofluorescein diacetate (CDCFA)
2. 5(6)-carboxy-4',5'-dimethylfluorescein (CDMFA)
3. 5-sulfofluorescein diacetate (SFDA)
4. Cell Tracker Green – 5-chloromethylfluorescein diacetate (CTG)
5. 5-(and-6)-carboxy-2´,7´-dichlorofluorescein diacetate, succinimidyl ester (DCF)
6. bis-(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4(3)$)
7. 3,3'-dipentyloxacarbocyanine iodide ($DiOC_5(3)$)
8. 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6(3)$)
9. Rhodamine 110

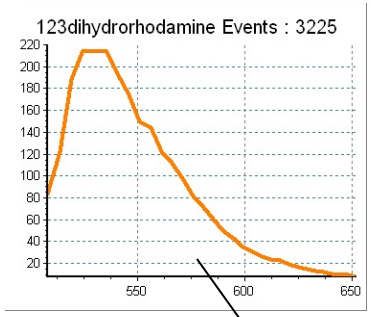


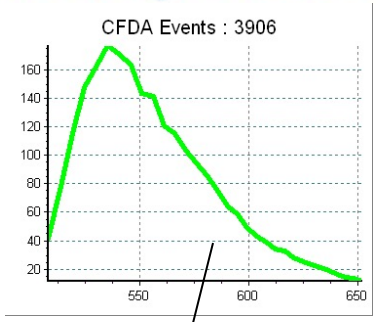
Linear classifiers fail!

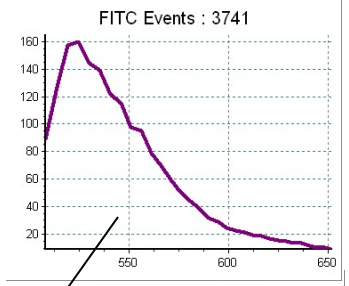


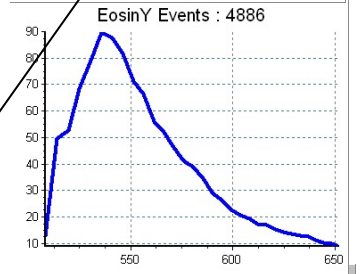


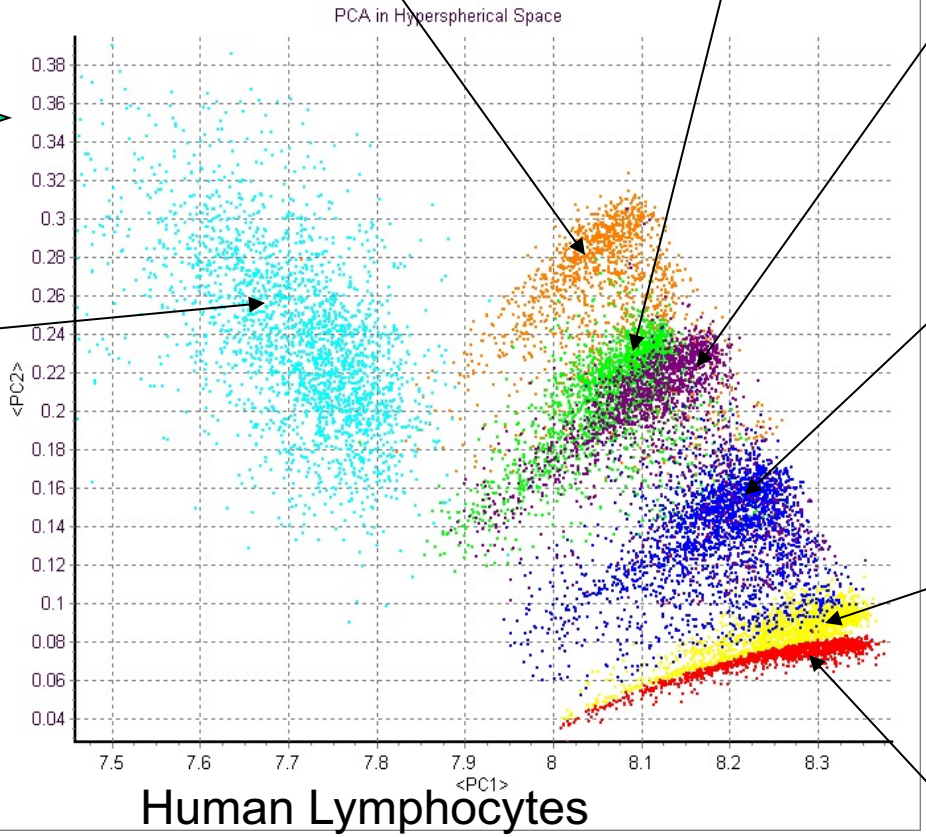


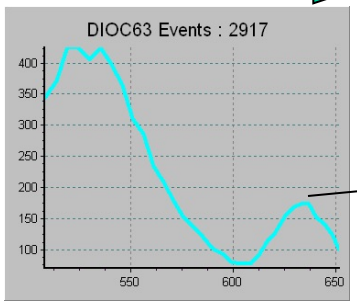


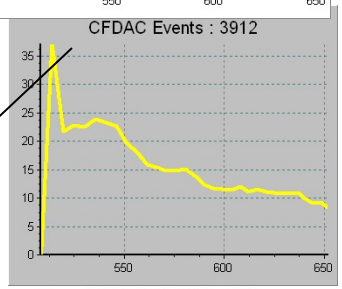


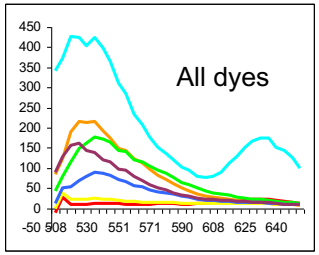


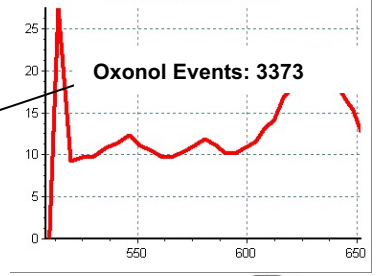












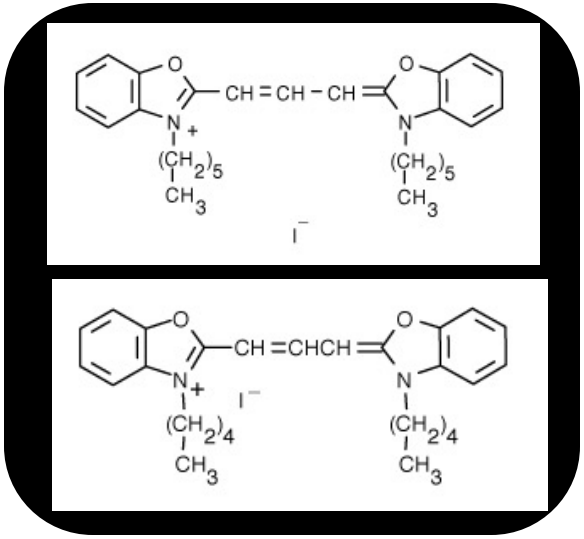
Human Lymphocytes

PUCL cytometry laboratories

PURDUE UNIVERSITY

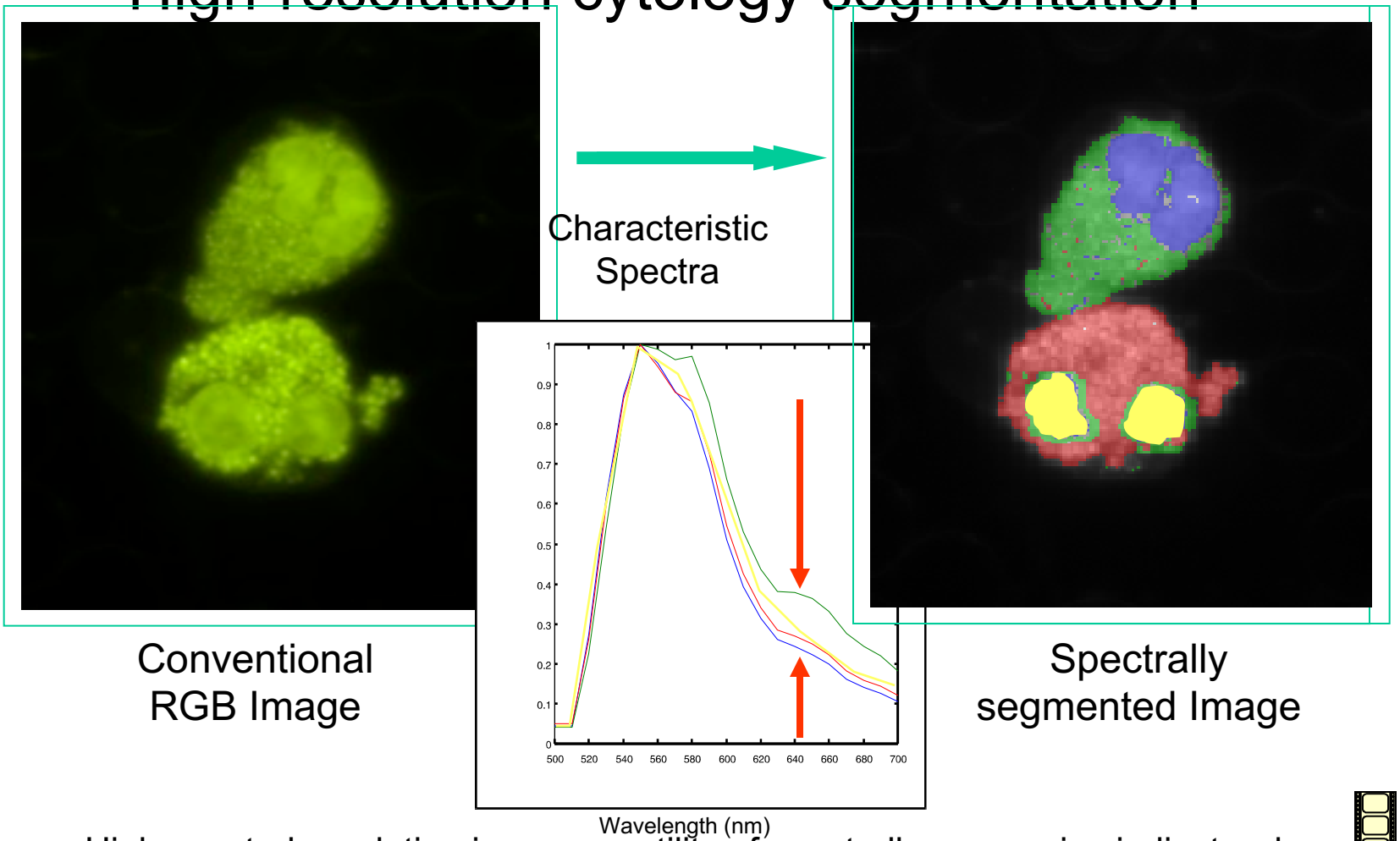
Confusion matrix

1. 5-(and-6)-carboxy-2´,7´-dichlorofluorescein diacetate (CDCFA)
2. 5(6)-carboxy-4',5'-dimethylfluorescein (CDMFA)
3. 5-sulfofluorescein diacetate (SFDA)
4. 5-(and-6)-carboxy-2´,7´-dichlorofluorescein diacetate, succinimidyl ester (DCF)
5. Cell Tracker Green – 5-chloromethylfluorescein diacetate (CTG)
6. bis-(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4(3)$)
7. 3,3'-dipentyloxacarbocyanine iodide ($DiOC_5(3)$)
8. 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6(3)$)
9. Rhodamine 110



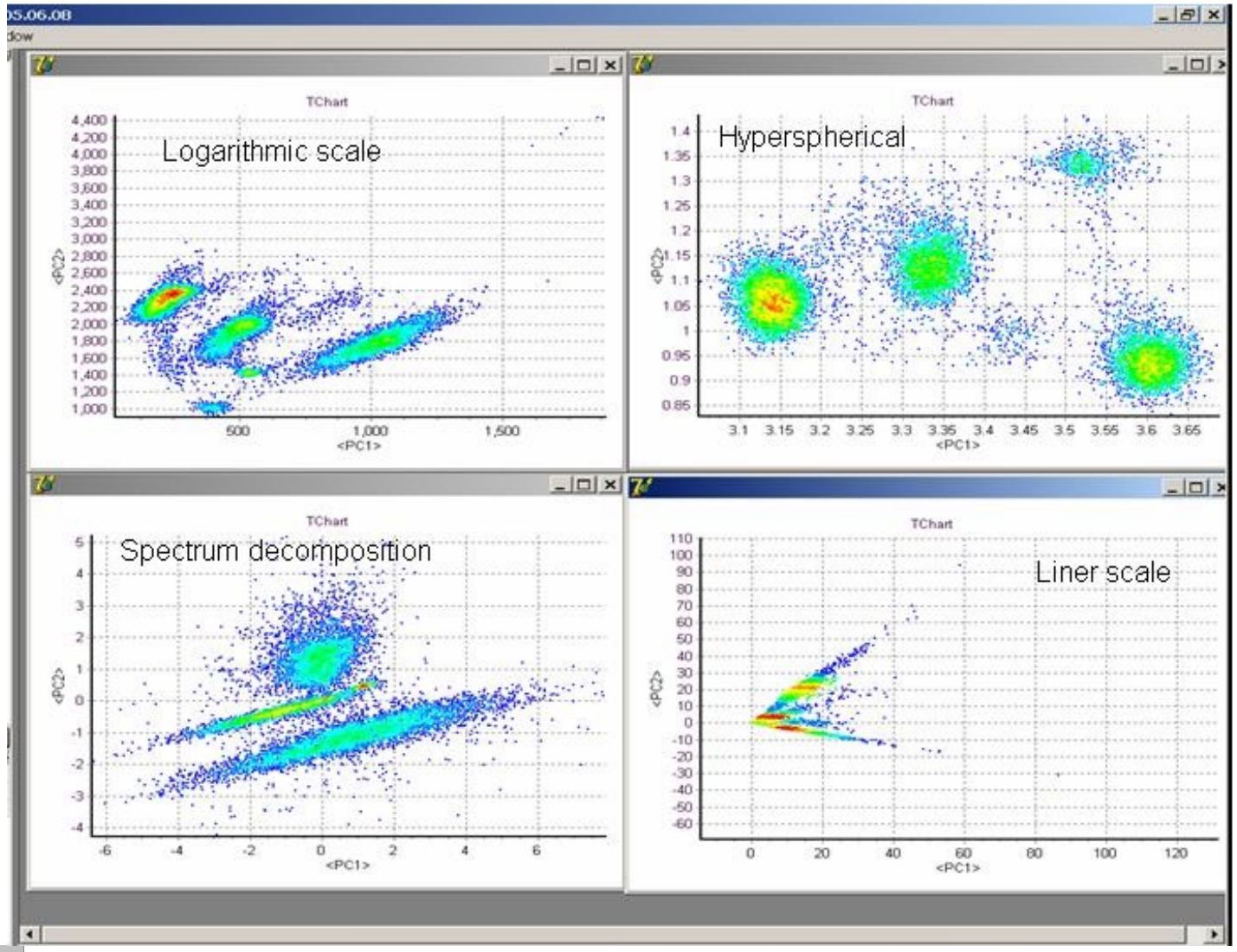
	CDCFA	CDMFA	SFDA	CTG	DCF	DiBAC43	$DiOC_5(3)$	$DiOC_6(3)$	RH110
CDCFA	**87.92%**	0.00%	0.76%	2.72%	0.00%	6.04%	1.92%	0.64%	0.00%
CDMFA	0.24%	**97.76%**	0.52%	0.04%	0.00%	0.00%	0.16%	0.88%	0.40%
SFDA	0.04%	0.00%	**94.36%**	4.88%	0.00%	0.00%	0.00%	0.72%	0.00%
CTG	5.44%	0.00%	5.04%	**86.44%**	0.00%	0.20%	0.80%	2.04%	0.04%
DCF	0.00%	0.00%	0.00%	0.00%	**100.00%**	0.00%	0.00%	0.00%	0.00%
DiBAC43	3.72%	0.20%	0.04%	0.40%	0.00%	**92.76%**	0.96%	1.92%	0.00%
$DiOC_5(3)$	4.12%	0.28%	0.56%	1.92%	0.00%	1.32%	**77.60%**	14.20%	0.00%
$DiOC_6(3)$	1.92%	0.12%	0.76%	1.72%	0.00%	1.24%	17.72%	**76.52%**	0.00%
RH110	0.00%	0.00%	0.08%	0.36%	0.00%	0.00%	0.00%	0.20%	**99.36%**

High-resolution cytology segmentation



High spectral resolution increases utility of spectrally responsive indicator dyes

Slide from Dr. Richard Levenson, CRi, Inc.,35B Cabot Rd.,Woburn, MA 01801, www.cri-inc.com


TChart
Logarithmic scale
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TChart
Hyperspherical
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TChart
Spectrum decomposition
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TChart
Liner scale
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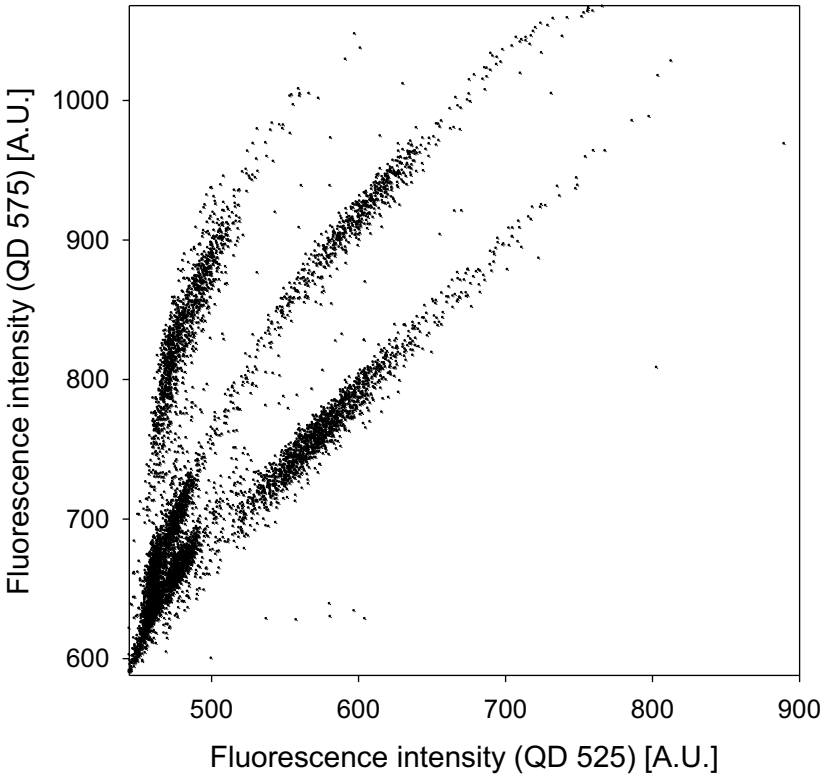
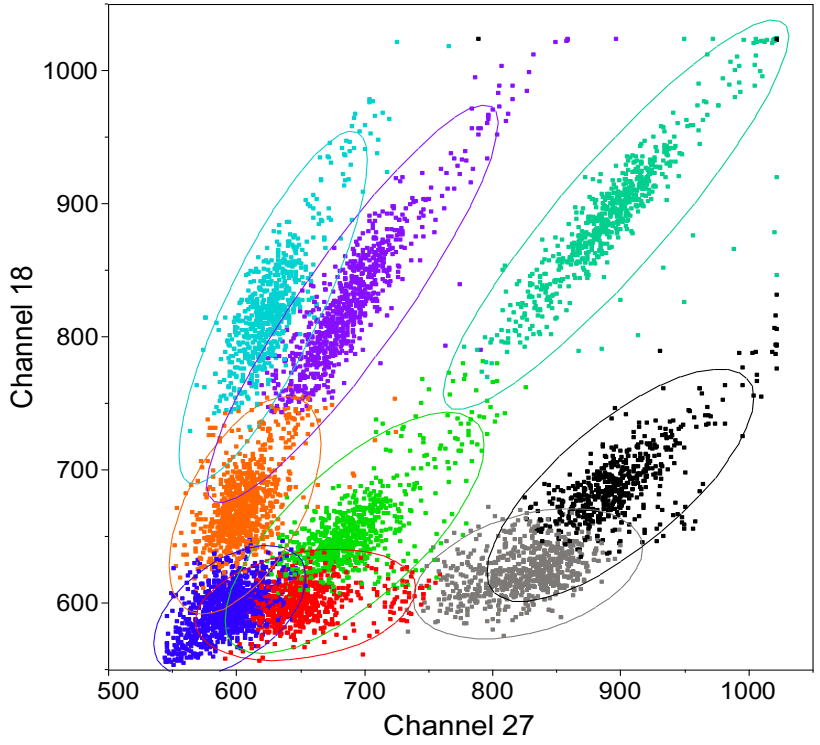
Analysis of complex samples (mixed nanocrystals)



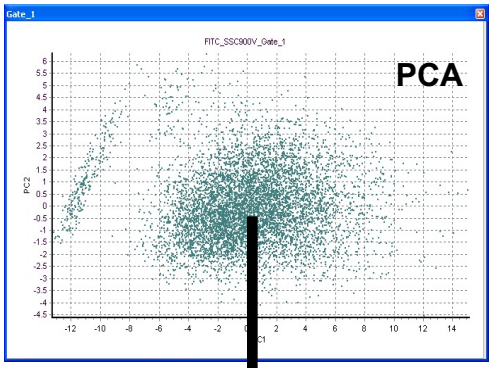
2 color system

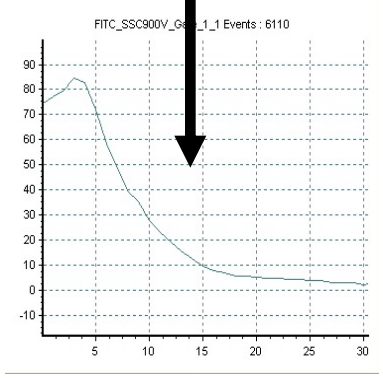


32 channel system

Spectral Distribution of labeled lymphocytes

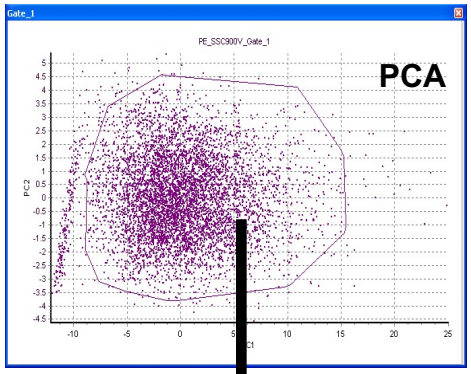
CD45FITC

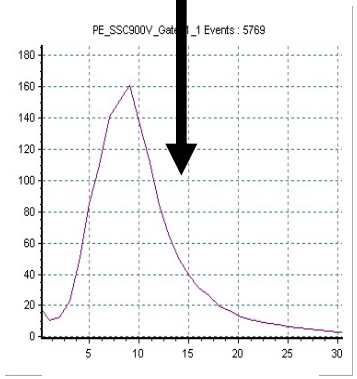




Average Spectra
(500-750 nm)

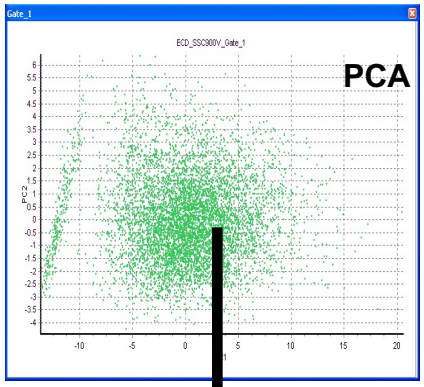
CD4PE

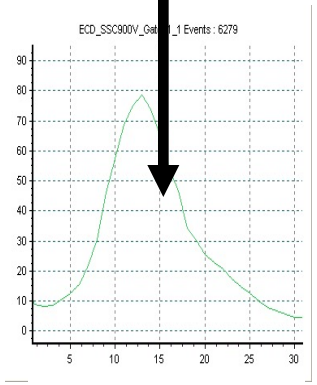




Average Spectra

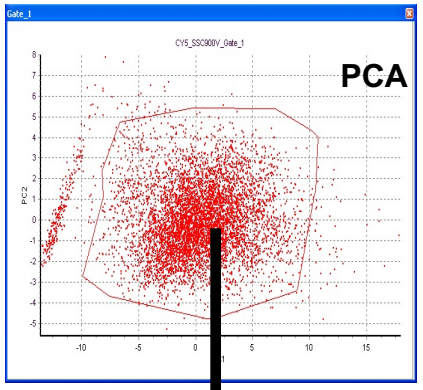
CD8ECD

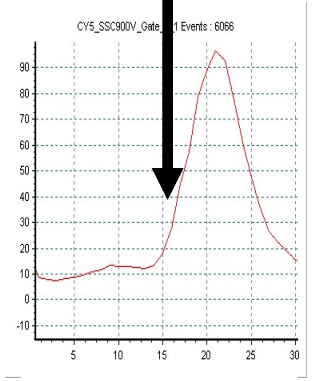




Average Spectra

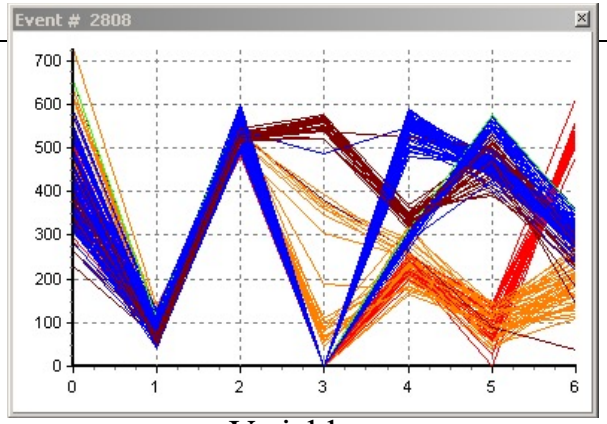
CD3CY5





Average Spectra

Note: the full (32 point) spectrum of every cell in the analysis is performed



Variables

(CD19-Cy3 not displayed)

PUCL cytometry laboratories

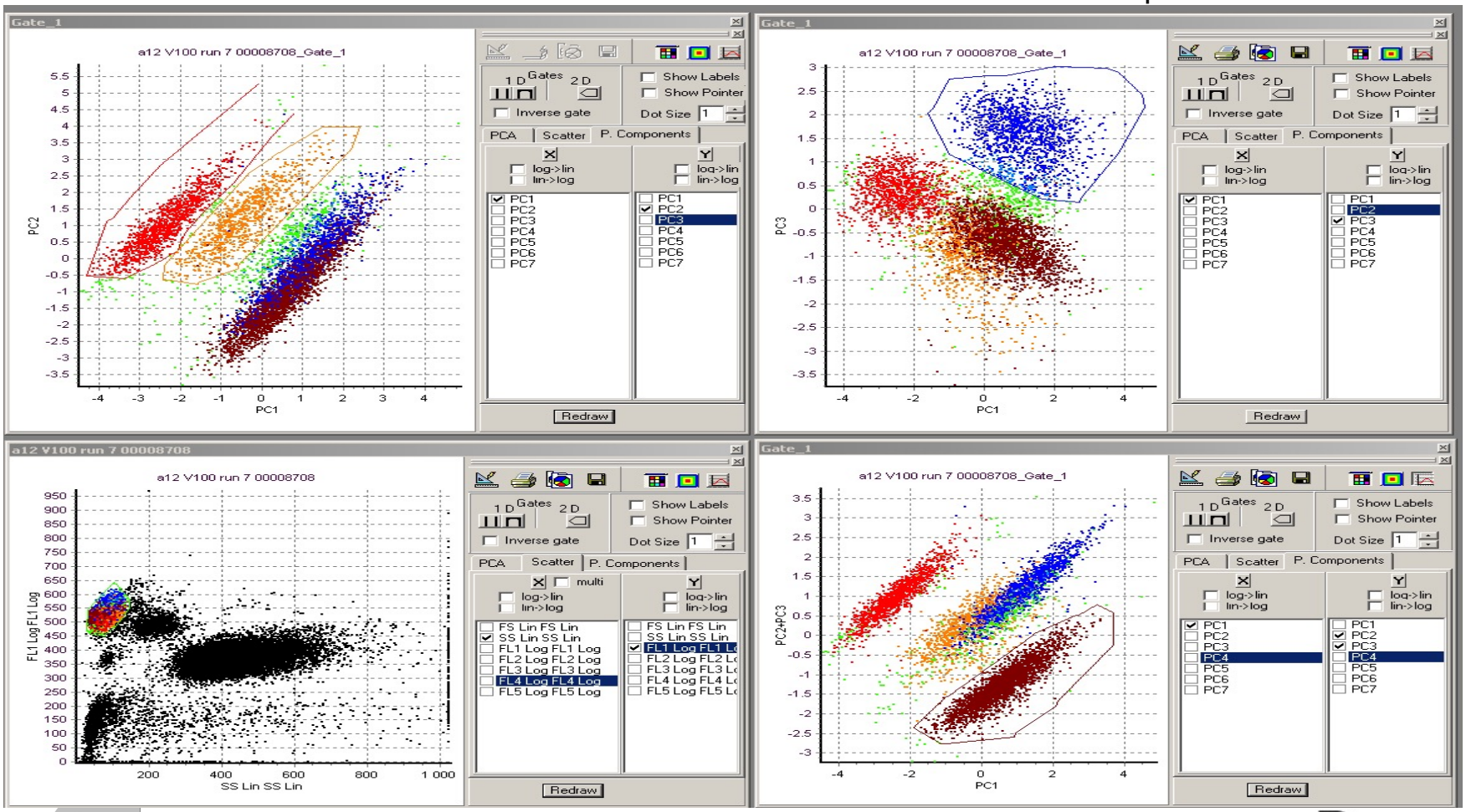
PURDUE UNIVERSITY

PCA with 5 colors and at least 2 scatter analysis on a 32 color system

Each panel represents PCA analysis of different components

Note the mix of blue & brown cells here

Now the blue cells are separated

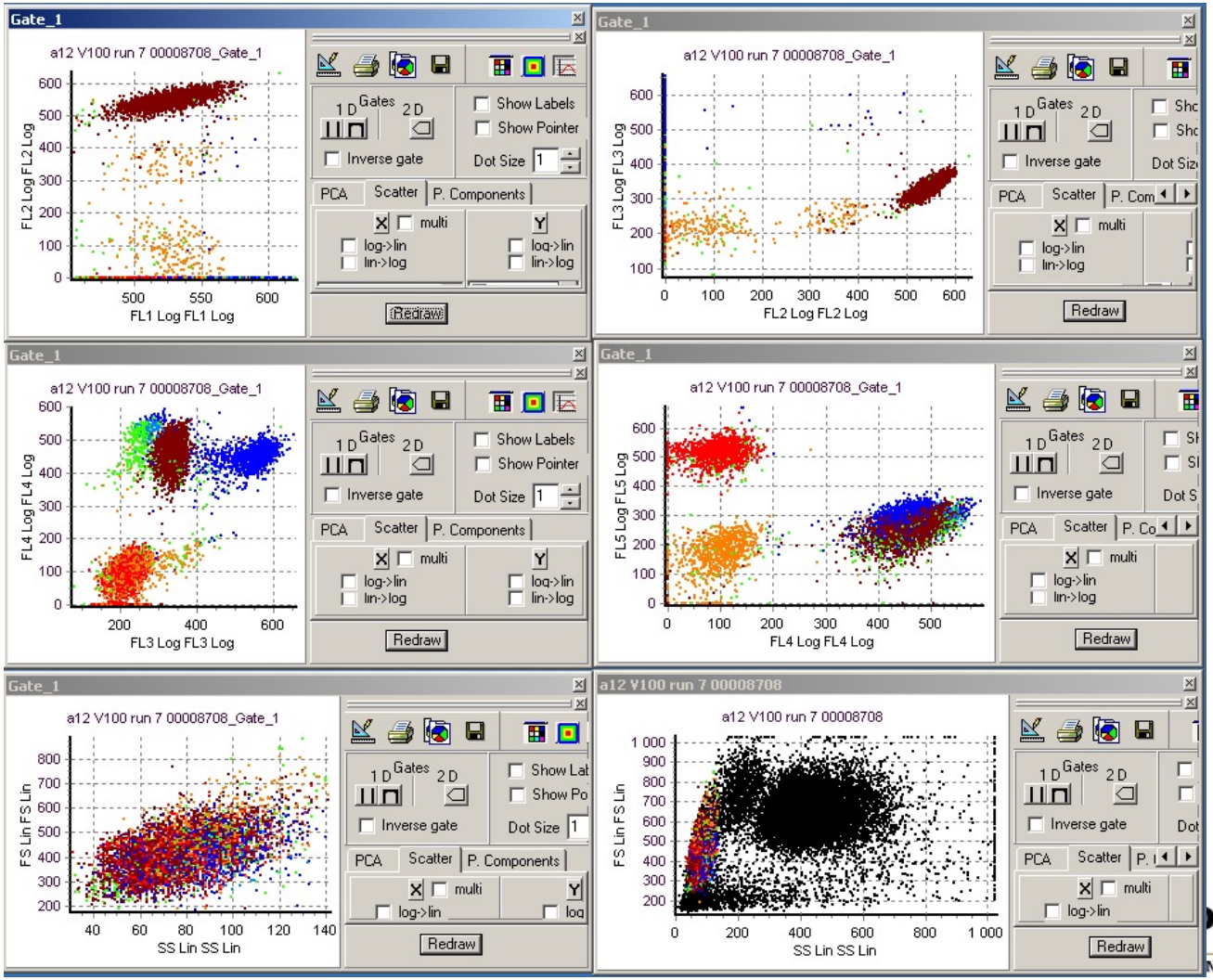


PCA using the 2 scatter channels

Now the brown cells are separated

Example: 5 color cell labeling

Traditional views of 2P dotplots but colors are derived from PCA populations

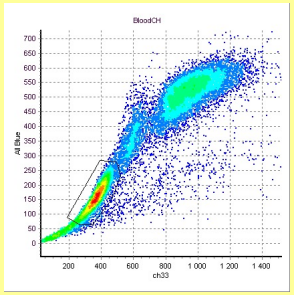


FL1=FITC
FL2=PE
FL3=ECD
FL4=Cy5
FL5=Cy7

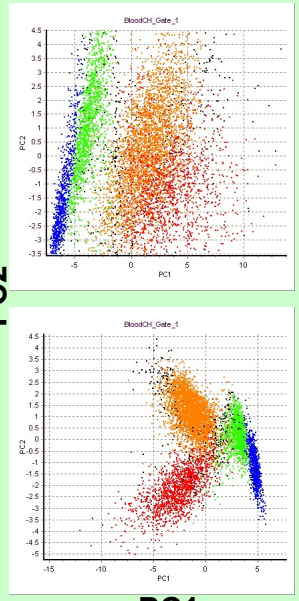
Comparisons of PCA results

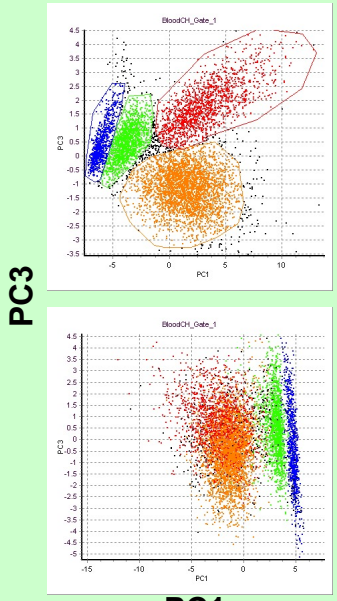
Purdue University

A blood sample incubated with :
-CD45 FITC
-CD4 PE
-CD8 ECD
-CD3 PC5
is analyzed both with the 32PMT and the 6PMTs. A PCA is run on both data. Lymphocytes are gated out from SSC vs FS.



32 channels PMT

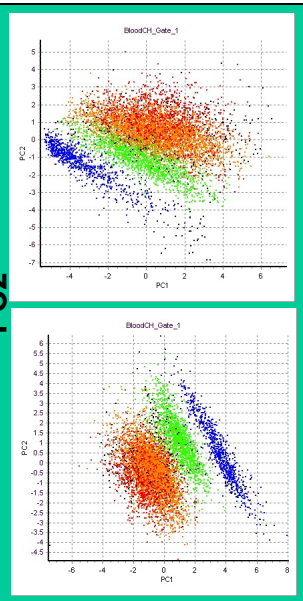


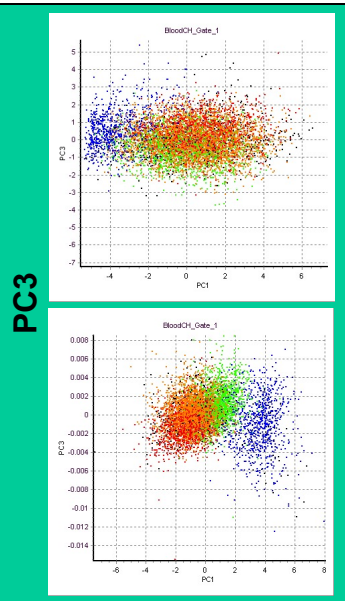


PCA (Correlation) on all the parameters :32channels + SSC+ FS(sum)+ RingB+ RingC+ RingD+ RingE,

PCA (Correlation, Hyperspherical) on all the parameters : 32channels + SSC+ FS(sum)+ RingB+ RingC+ RingD+ RingE,

6 PMTs device



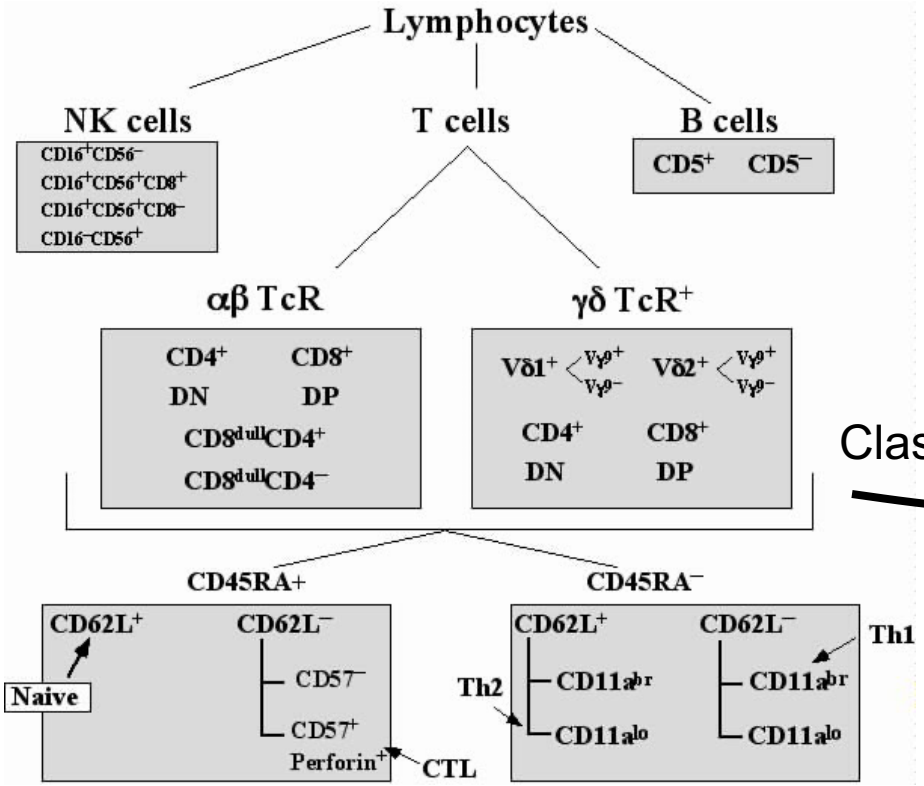


PCA (Correlation) on 4channels+SSC+ FS(sum)+ RingB+ RingC+ RingD+ RingE,

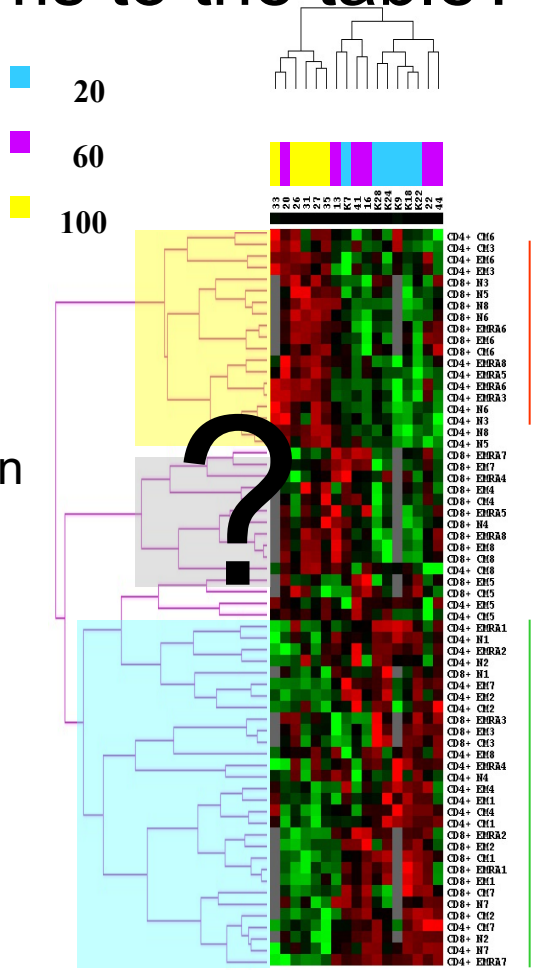
PCA (Correlation, Hyperspherical) on 4channels+SSC+ FS(sum)+ RingB+ RingC+ RingD+ RingE,

PUCL
cytometry laboratories

How do we bring clinical problems to the table?



Classification



From Roederer *et al*

Enrico Lugli et al, *Università di Modena e Reggio Emilia*
(Group of Andrea Cossarizza, University of Modena)

Cytometry 12:701–706 (1991)

Time Interval Gating for Analysis of Cell Function Using Flow Cytometry[1]

Gary Durack[2], Gretchen Lawler, Steve Kelley, Kathy Ragheb, R.A. Roth, P. Ganey, and J. Paul Robinson

Purdue University Cytometry Laboratories and Department of Veterinary Physiology and Pharmacology (G.D., G.L., S.K., K.R., J.P.R.) Purdue University, West Lafayette, IN, 47907 and Department of Pharmacology and Toxicology (R.A.R., P.G.), Michigan State University, E.Lansing, MI

Received for publication April 22, 1991; accepted July 5, 1991

Cytometry 12:82–90 (1991)

An Innovation in Flow Cytometry Data Collection and Analysis Producing a Correlated Multiple Sample Analysis in a Single File[1]

J. Paul Robinson,[2] Gary Durack, and Steve Kelley

Purdue University Cytometry Laboratories and Department of Veterinary Physiology and Pharmacology, Purdue University, West Lafayette, Indiana 47907

Received for publication April 17, 1990; accepted August 26, 1990

TIP System

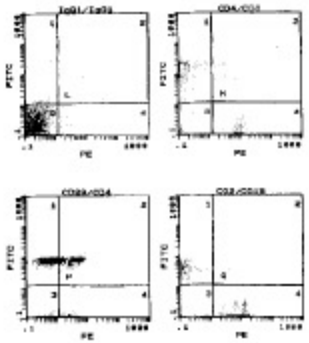
Flow Cytometry

[Real Time Analysis]

1 File

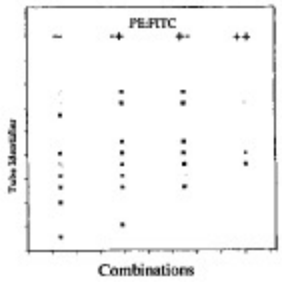
Option 1

Quadstat Statistics



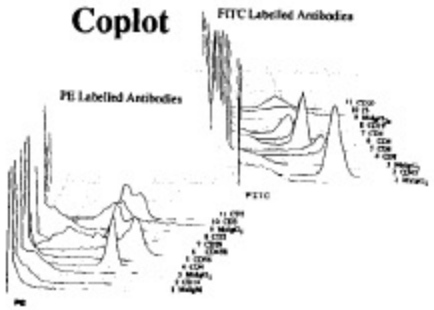
Option 2

"Phenogram"



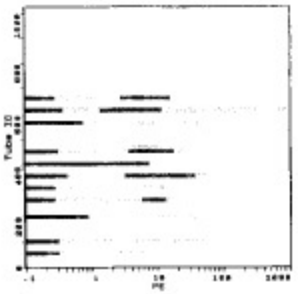
Option 3

Coplot



Option 4

"Overview" or "BOILERPLATE"



COMPOSITE QUADSTATS

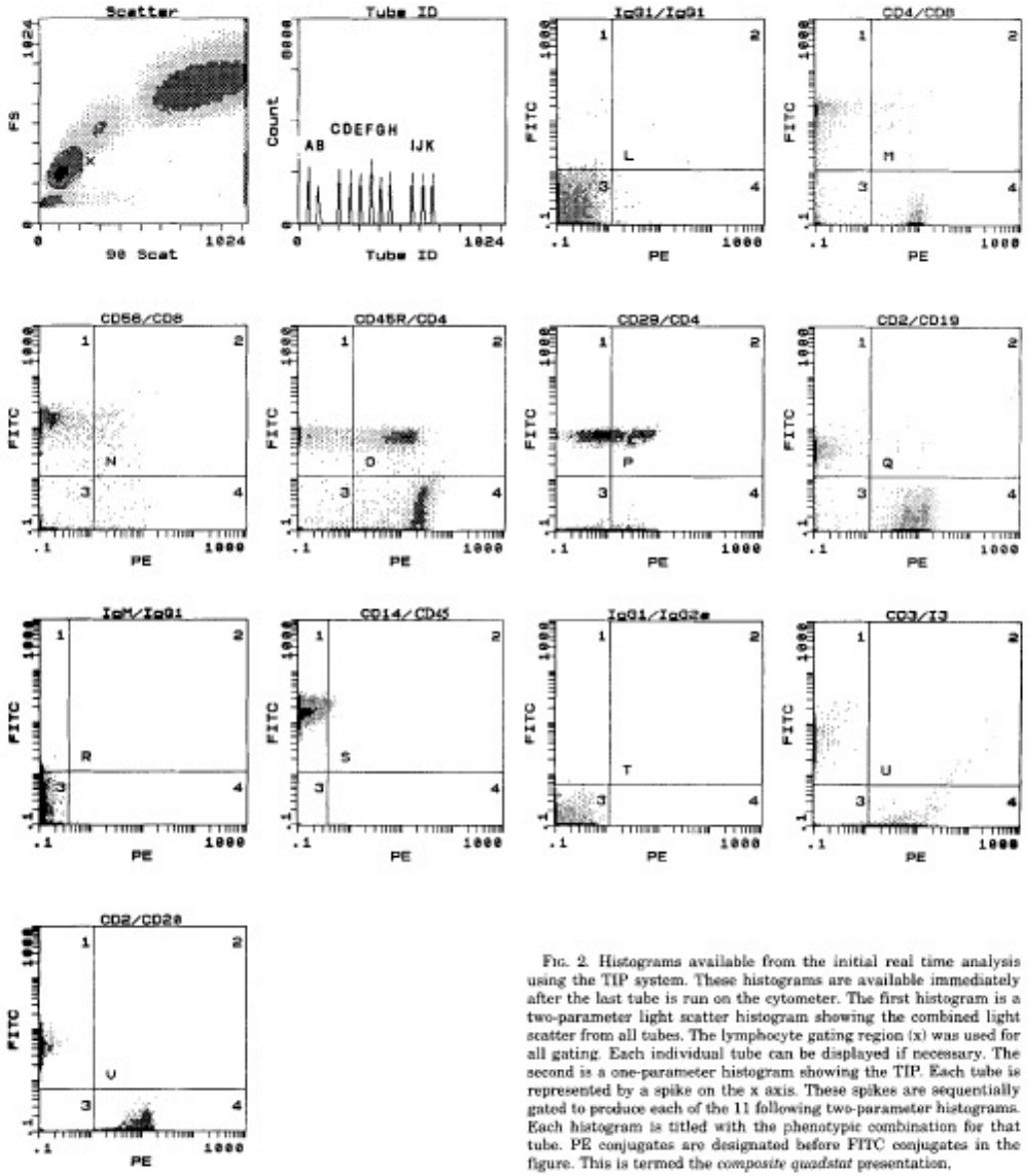
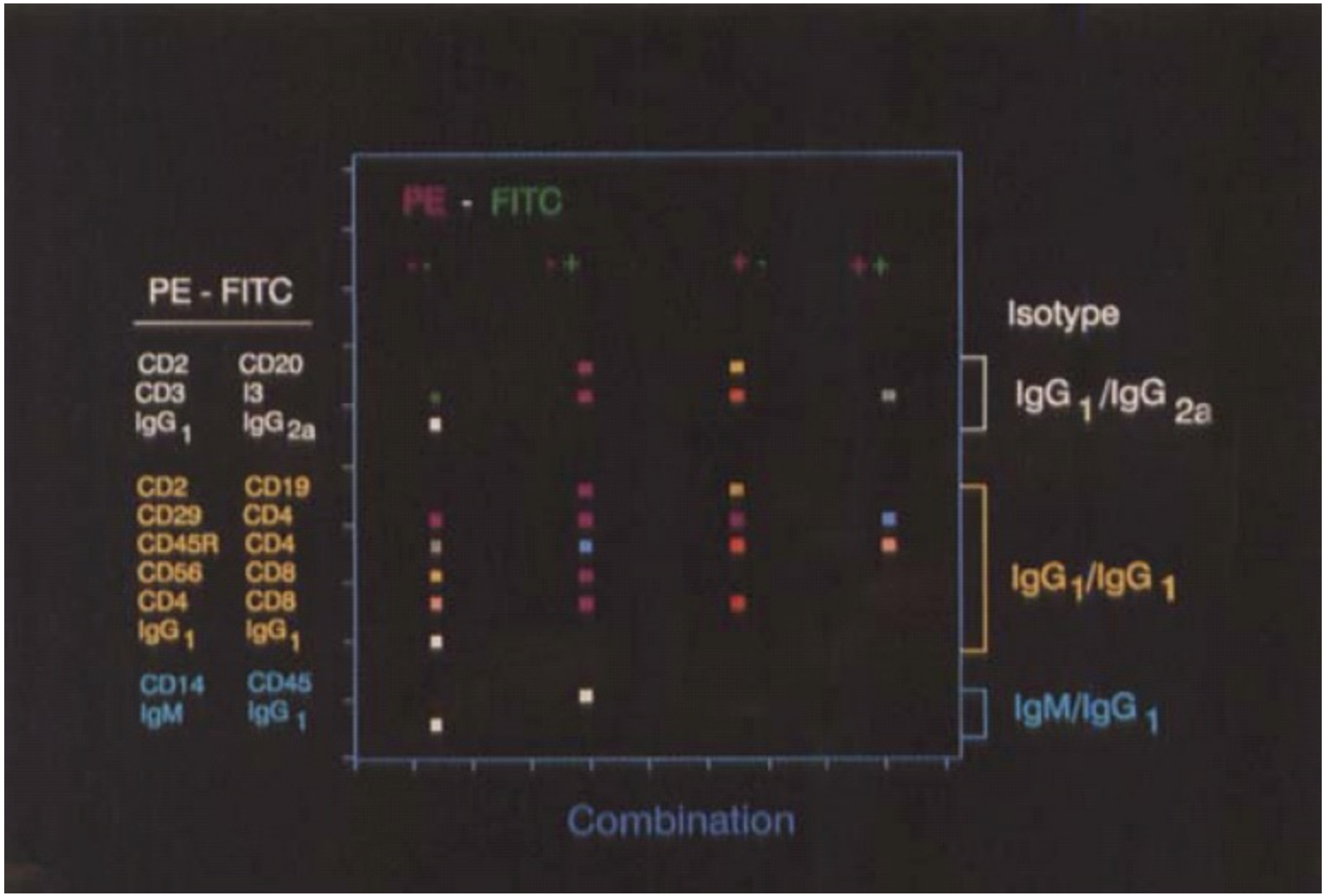
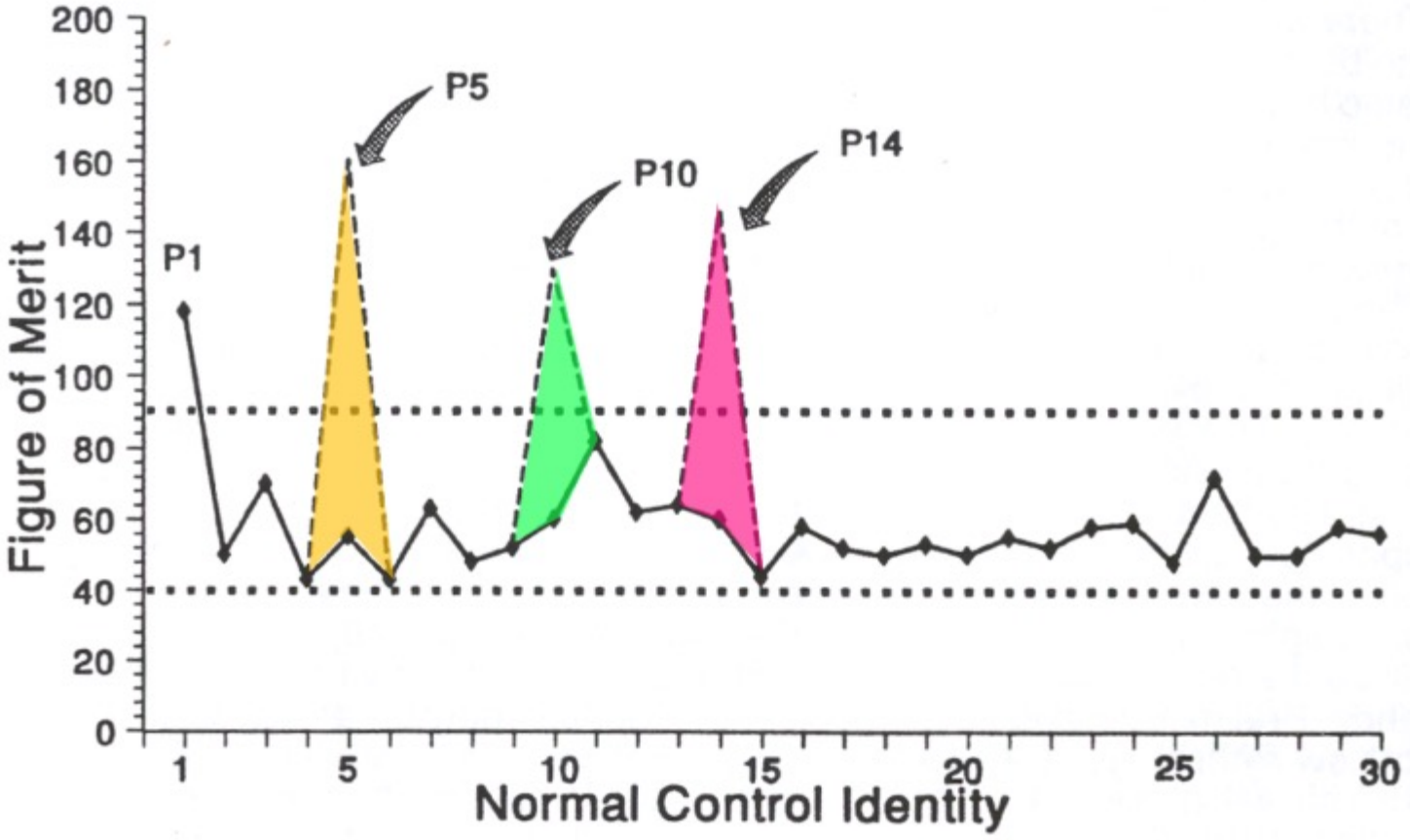


FIG. 2. Histograms available from the initial real time analysis using the TIP system. These histograms are available immediately after the last tube is run on the cytometer. The first histogram is a two-parameter light scatter histogram showing the combined light scatter from all tubes. The lymphocyte gating region (x) was used for all gating. Each individual tube can be displayed if necessary. The second is a one-parameter histogram showing the TIP. Each tube is represented by a spike on the x axis. These spikes are sequentially gated to produce each of the 11 following two-parameter histograms. Each histogram is titled with the phenotypic combination for that tube. PE conjugates are designated before FITC conjugates in the figure. This is termed the *composite quadstat* presentation.

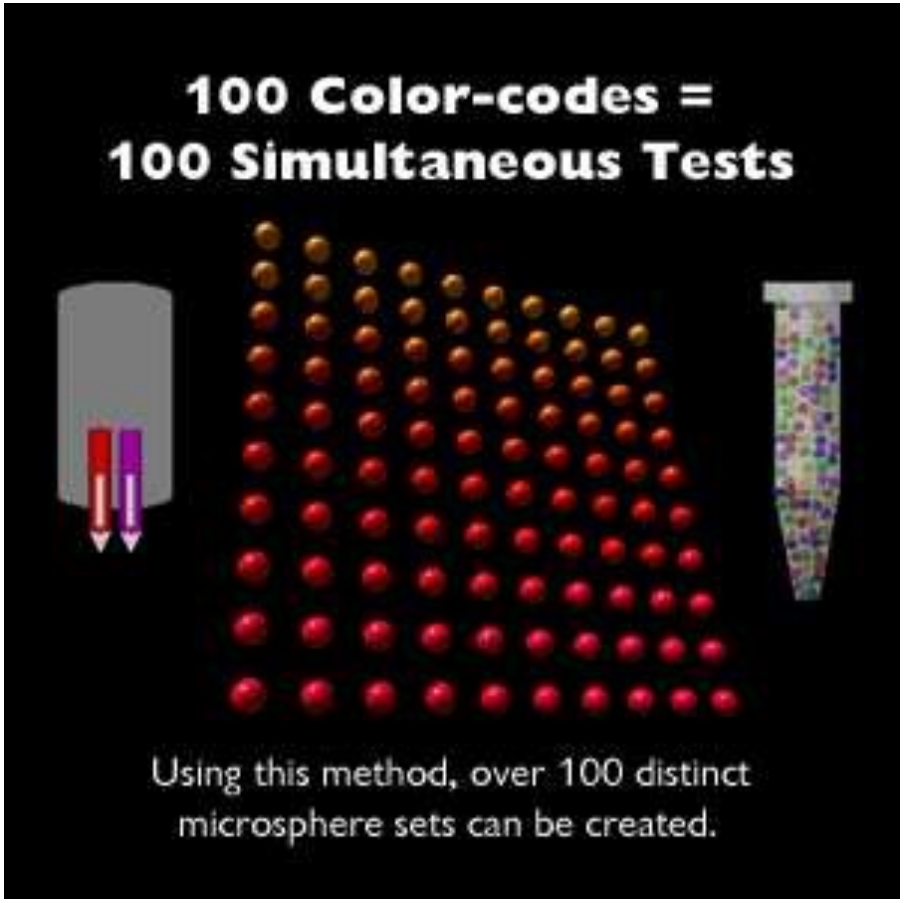
Phenogram


PE - FITC
PE - FITC
- -
- +
+ -
+ +
Isotype
CD2 CD20
CD3 I3
IgG 1 IgG 2a
IgG 1/IgG 2a
CD2 CD19
CD29 CD4
CD45R CD4
CD56 CD8
CD4 CD8
IgG 1 IgG 1
IgG 1/IgG 1
CD14 CD45
IgM IgG 1
IgM/IgG 1
Combination

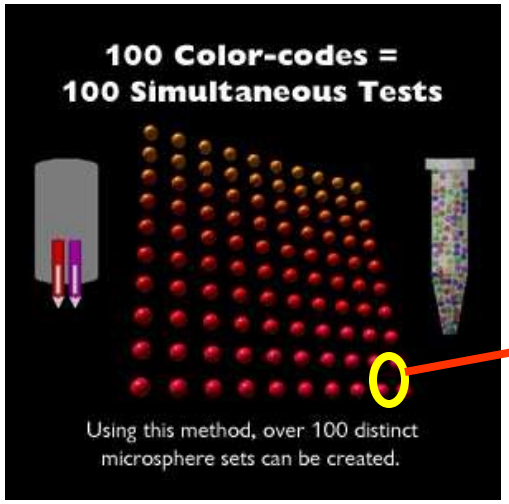
Automated analysis



Luminex


100 Color-codes =
100 Simultaneous Tests
Using this method, over 100 distinct
microsphere sets can be created.

Luminex


100 Color-codes =
100 Simultaneous Tests
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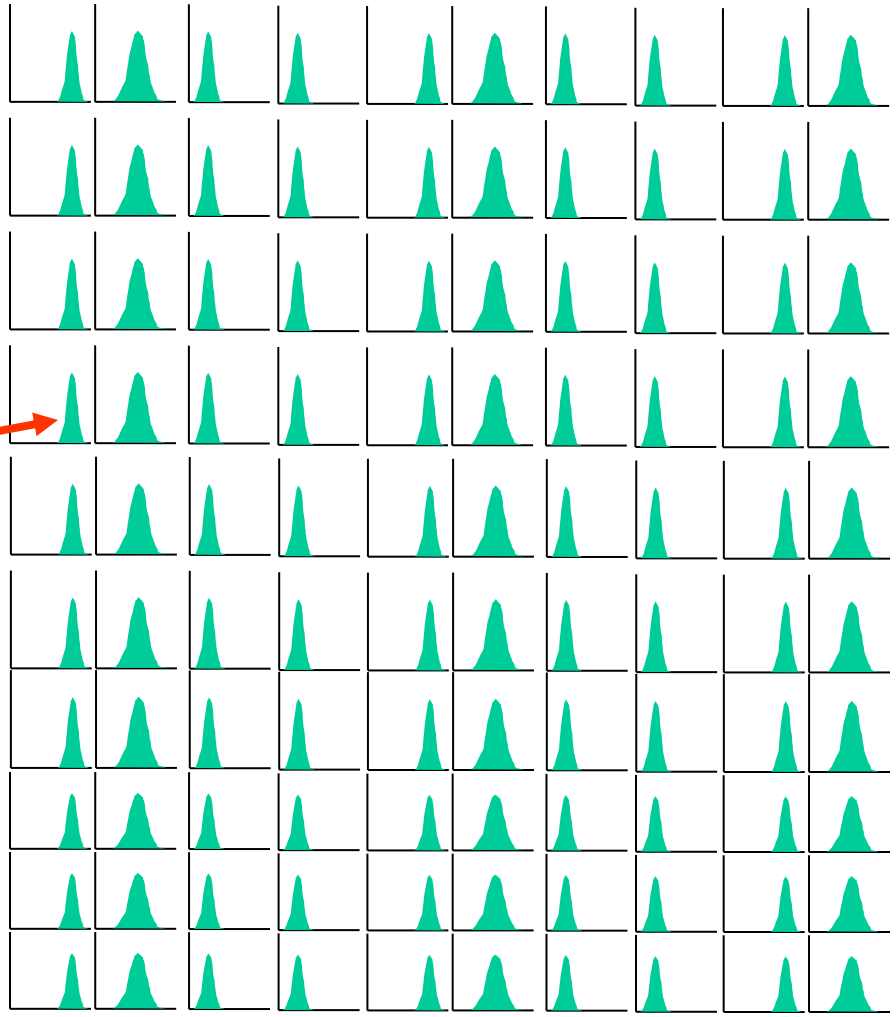
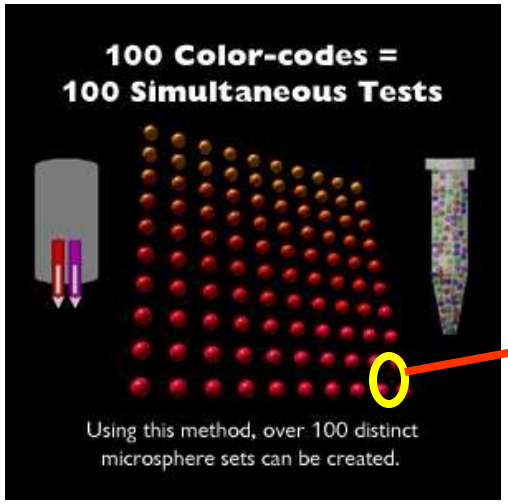


Luminex


100 Color-codes =
100 Simultaneous Tests
Using this method, over 100 distinct
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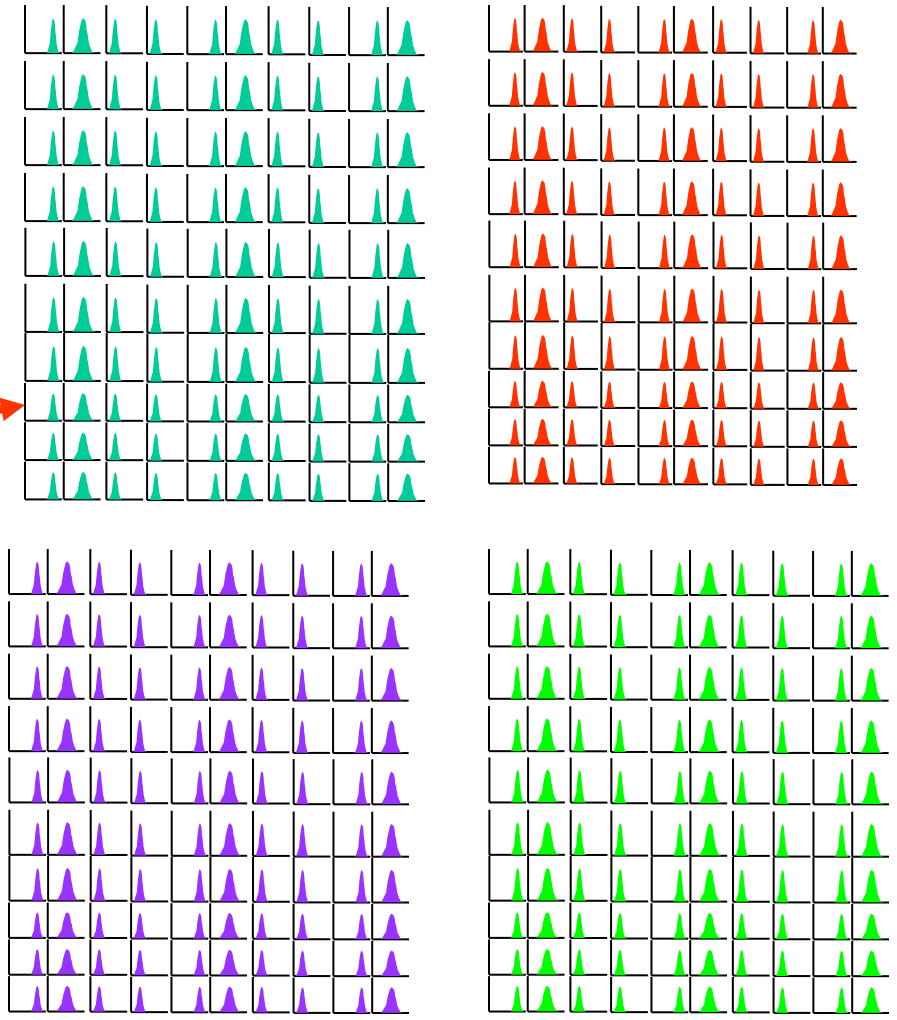


New Mexico Molecular Libraries Screening Center

Larry A. Sklar, PhD

Regents Professor of Pathology

and Distinguished Professor of Pharmacy

Director of Basic Research, UNM Cancer Center

Director, New Mexico Molecular Libraries Screening Center

UNM CYTOMETRY

The University of New Mexico

A National Cancer Institute Designated Cancer Center

NM STATE

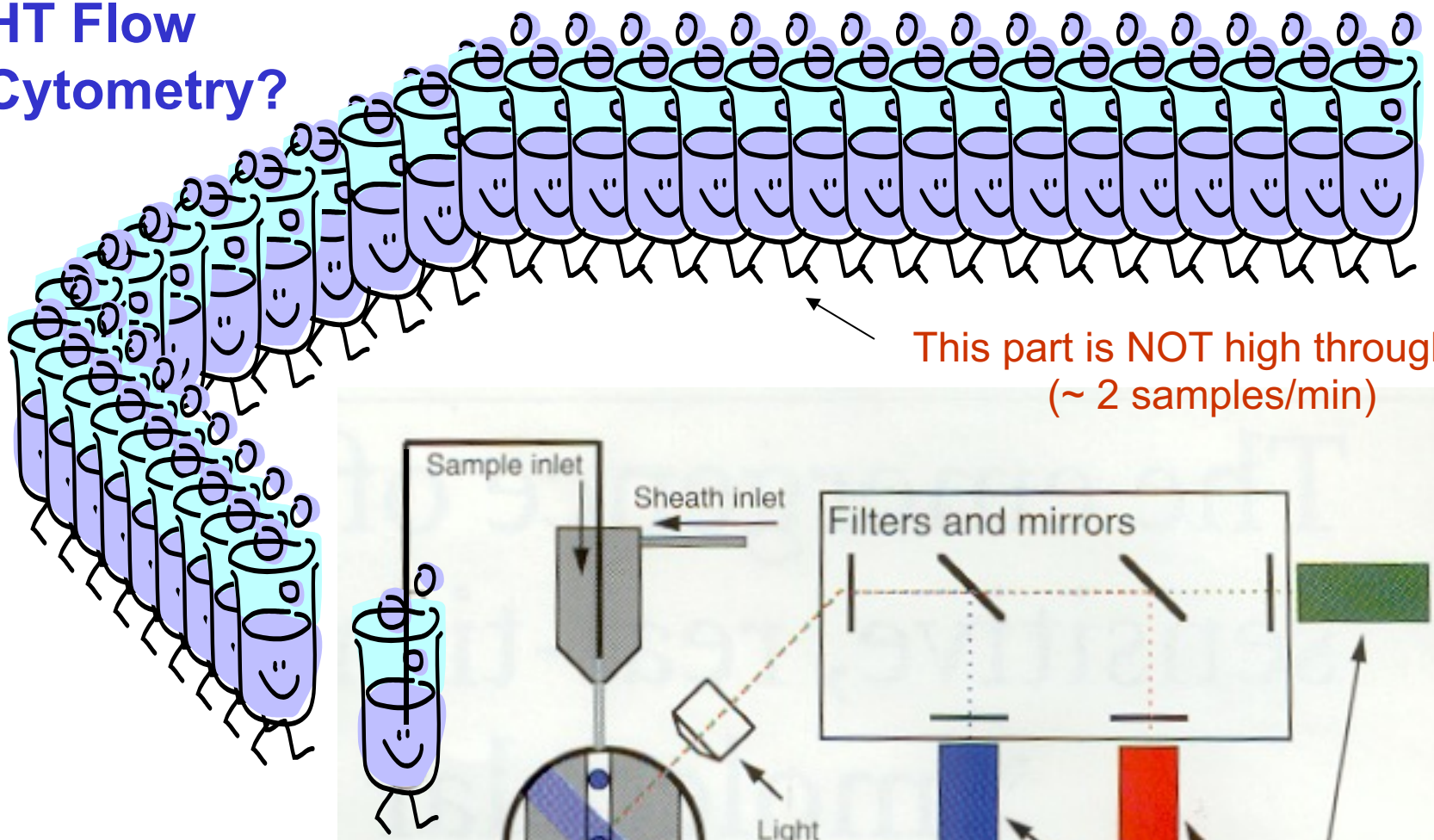
NEW MEXICO TECH
SCIENCE · ENGINEERING · RESEARCH · UNIVERSITY

THE UNIVERSITY OF NEW MEXICO HEALTH SCIENCES CENTER
COLLEGE OF PHARMACY

Luminex

ChemDiv
CHEMICAL DIVERSITY

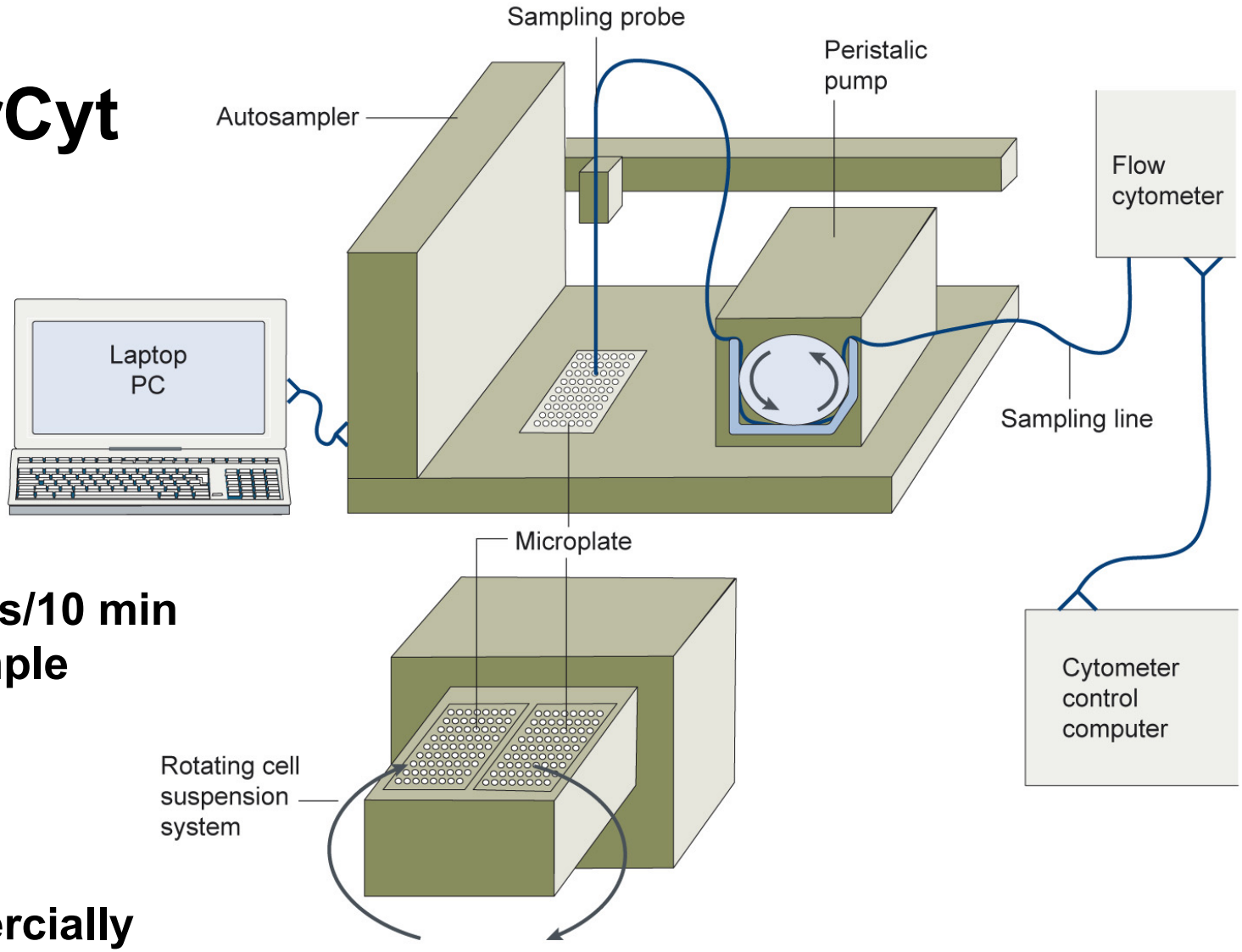
HT Flow Cytometry?



This part is NOT high throughput
(~ 2 samples/min)

This part is high throughput
50,000 cells/s
14 parameters/cell

HyperCyt



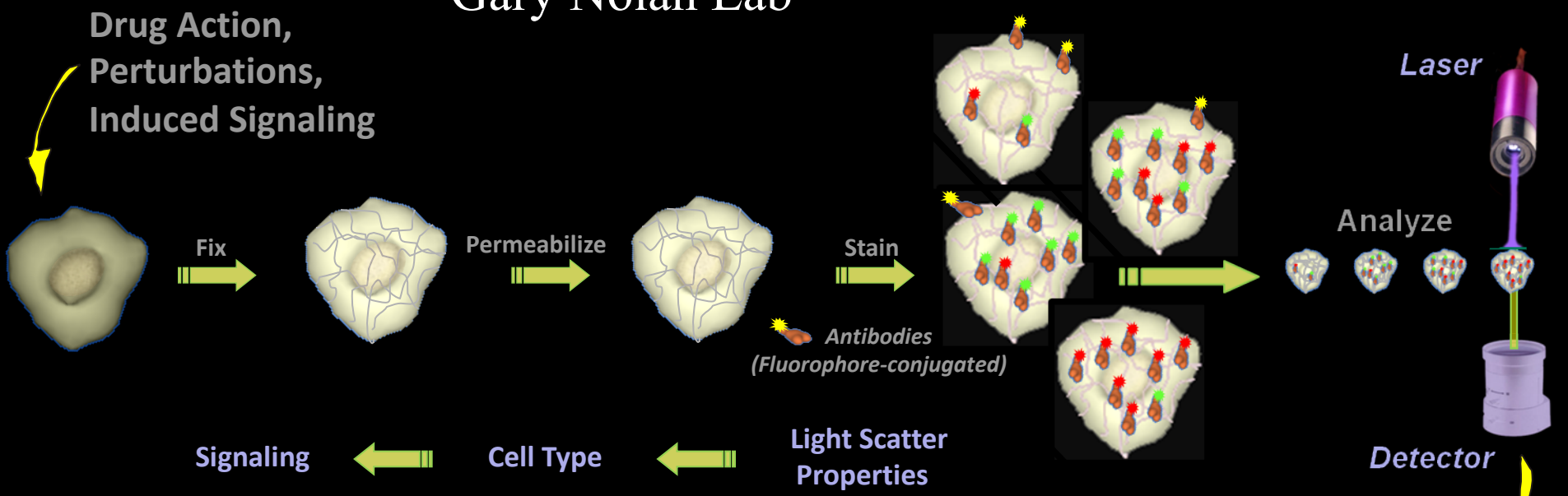
384 wells/10 min
1 μl/sample

Commercially Available

UNM CYTOMETRY
The University of New Mexico
UNM Cancer Research & Treatment Center — A National Cancer Institute Designated Cancer Center
NM STATE
NEW MEXICO TECH — SCIENCE · ENGINEERING · RESEARCH · UNIVERSITY
The University of New Mexico Health Sciences Center — College of Pharmacy
Luminex
ChemDiv — Chemical Diversity

Flow Cytometry for Intracellular Staining

Gary Nolan Lab

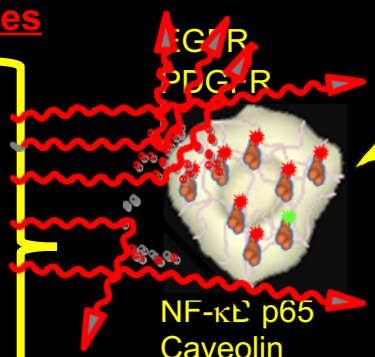


Thorough development of fixation protocols for cell lines and whole-blood (immediately out of patient).

1. State specific antibodies: phospho-specific antibodies and others
2. Adopting entirely new fluorophores….
3. Generation of efficient conjugation, purification, and testing protocols.

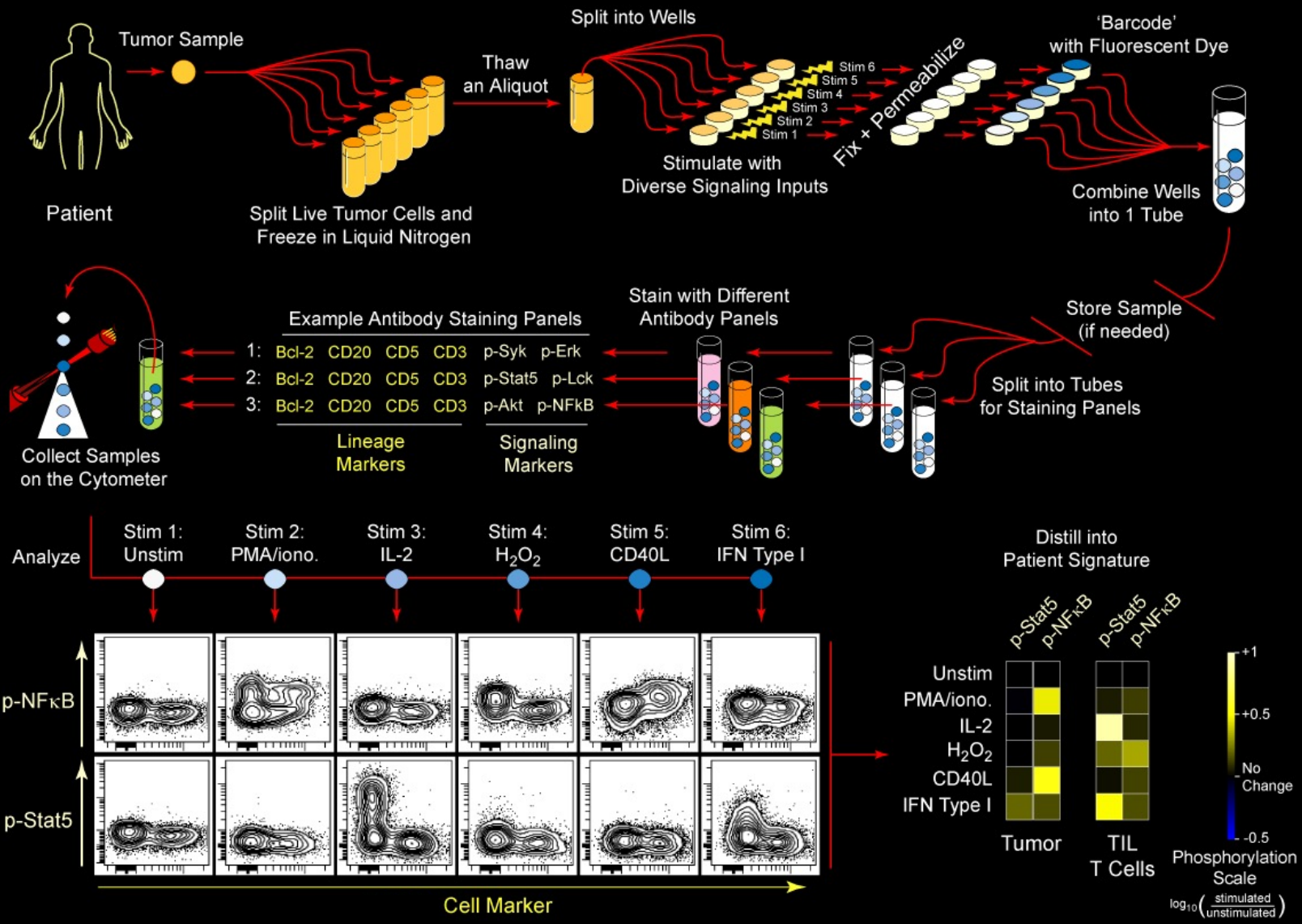
Sample Specificities

p38 MAPK
JNK, cJun
AKT, PIP2, PIP3,
PKCα/β/θ/δ, Rsk
Raf, Mek, ERK, ELK
Rsk, Creb,
STATs, SRC
CREB, cJUN, IKKα
p53 s15, s20 s37, s392
Pyk2, Shc, Fak, Src
Slp76, Zap70, Syk, Lat, Vav,
Lck, PLCγ
Beta-integrins



>80 specificities

Mapping Altered Signaling in Every Tumor Sample Cell



J. Irish, Levy / Nolan labs

Advanced approaches to modeling based on single cell data - Nolan Lab

- Question: can you predict a signaling network based on network connectivity knowledge from single cell analysis?

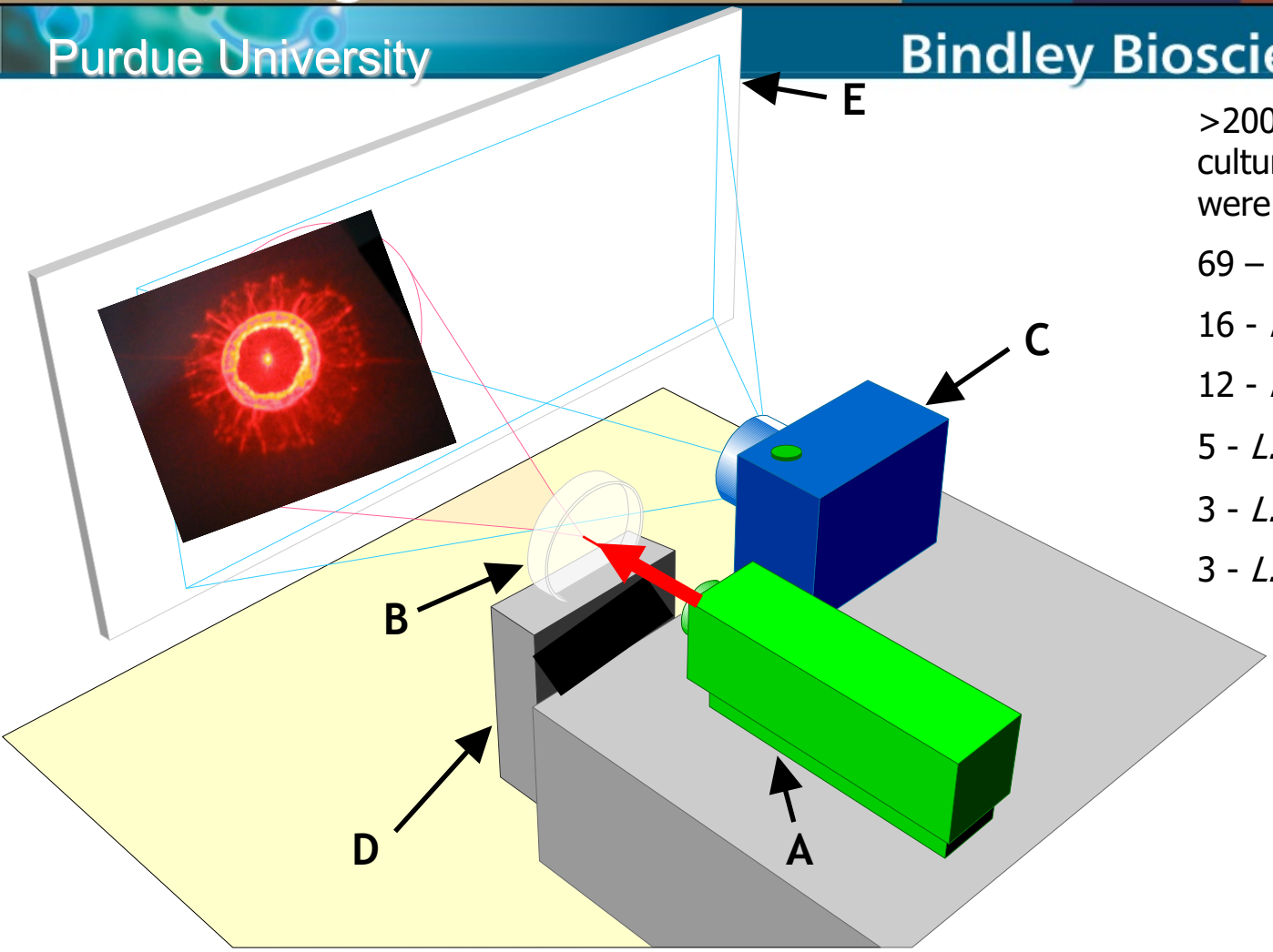
SCIENCE VOL 308 22 APRIL 2005, 523

Science

Causal Protein-Signaling Networks Derived from Multiparameter Single-Cell Data

Karen Sachs,[1]* Omar Perez,[2]* Dana Pe'er,[3]*
Douglas A. Lauffenburger,[1]† Garry P. Nolan[2]†

Machine learning was applied for the automated derivation of causal influences in cellular signaling networks. This derivation relied on the simultaneous measurement of multiple phosphorylated protein and phospholipid components in thousands of individual primary human immune system cells. Perturbing these cells with molecular interventions drove the ordering of connections between pathway components, wherein Bayesian network computational methods automatically elucidated most of the traditionally reported signaling relationships and predicted novel interpathway network causalities, which we verified experimentally. Reconstruction of network models from physiologically relevant primary single cells might be applied to understanding native-state tissue signaling biology, complex drug actions, and dysfunctional signaling in diseased cells.



>2000 scatter patterns from cultures of 108 *Listeria* strains were measured and analyzed

69 – *L. monocytogenes*

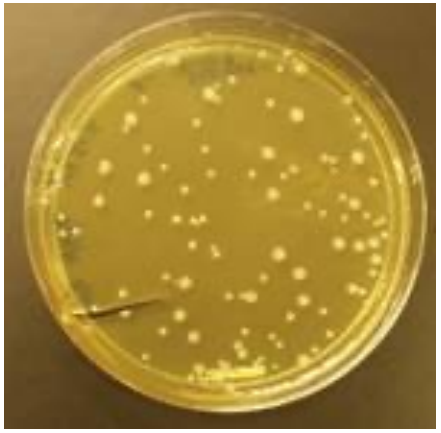
16 - *L. innocua*

12 - *L. ivanovii*

5 - *L. seeligeri*

3 - *L. welshimeri*

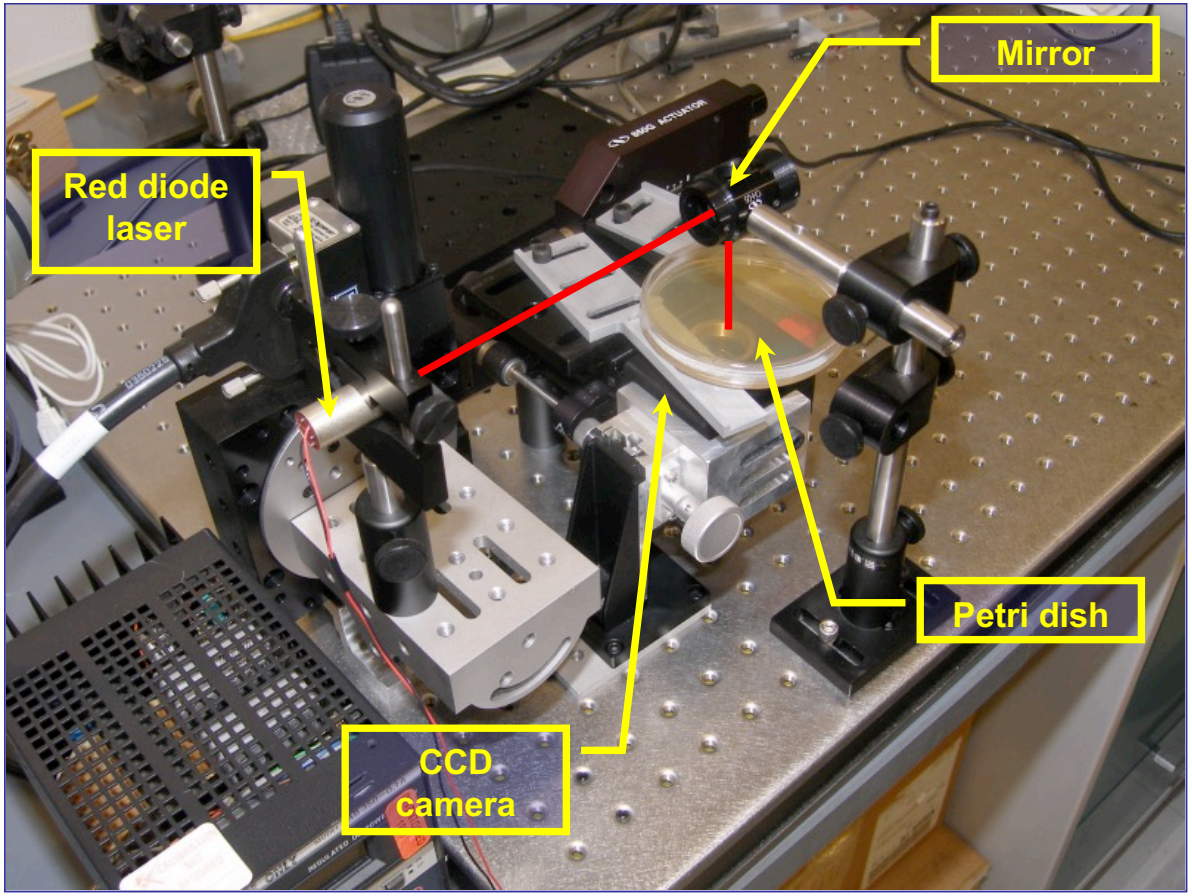
3 - *L. grayi*



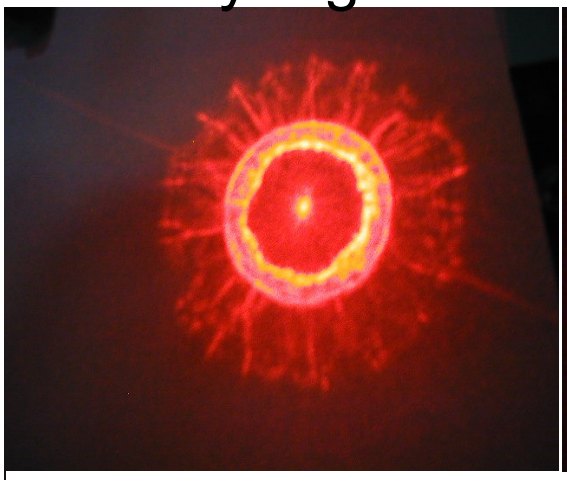
Schematic representation of the laser scatterometer used to perform analysis of bacterial colonies. A – 635-nm diode laser, B – Petri dish containing bacterial colonies, C – CCD camera, D – Petri-dish holder, and E – detection screen.

Bacteria Rapid Detection using Optical Scattering Technology (BARDOT) – the new system

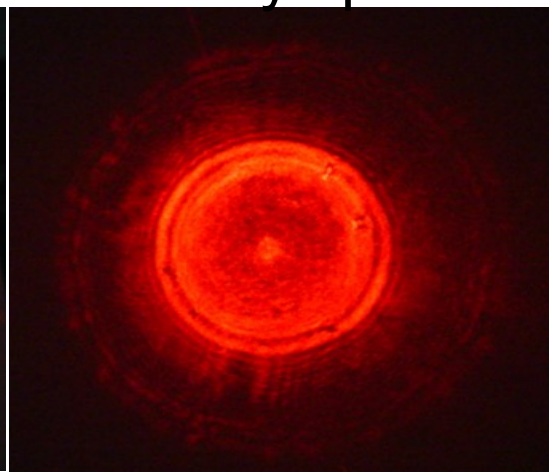
- BARDOT (Bacteria Rapid Detection using Optical Scattering Technology) designed by Hirleman group.
- Broaden the library of scatter images for additional bacterial colonies (Bhunia group)
- New technology for features extraction (Rajwa/Robinson group)



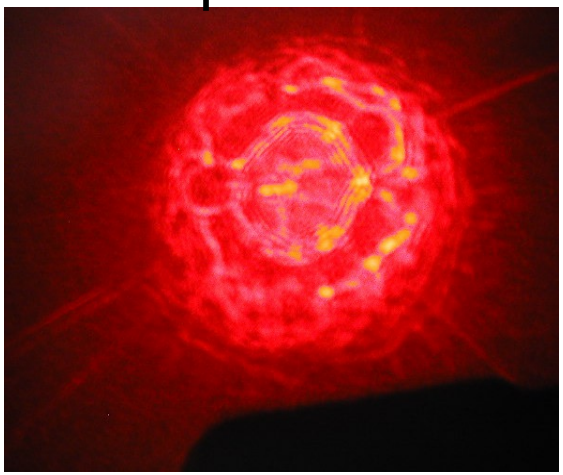
Every organism has a very specific scatter pattern



***L. monocytogenes* ATCC19113**

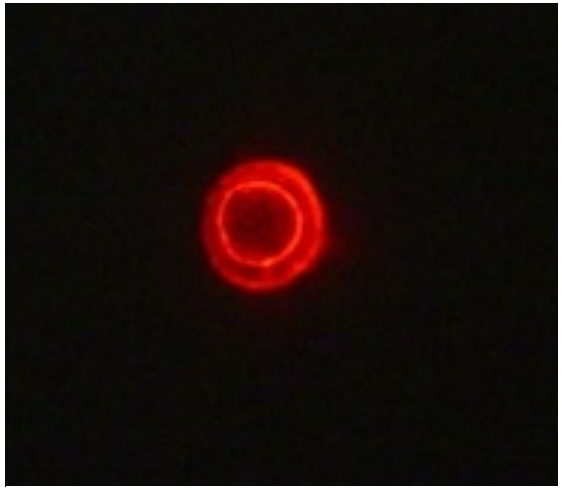


***L. innocua* F4248**

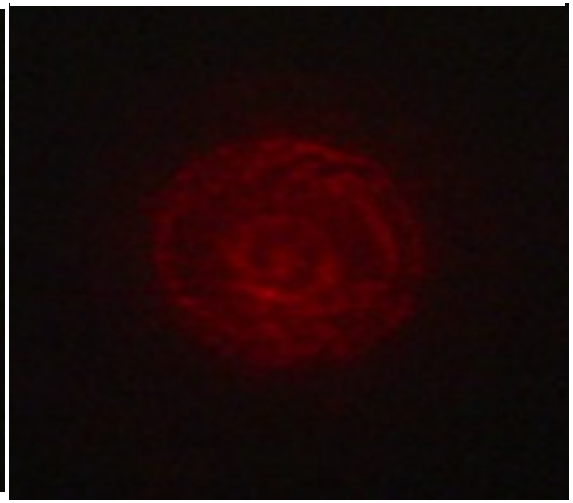


***L. ivanovii* ATCC19119**

***L. seeligeri* LA 15**



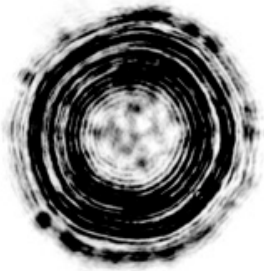
***L. welshimeri* ATCC35897**



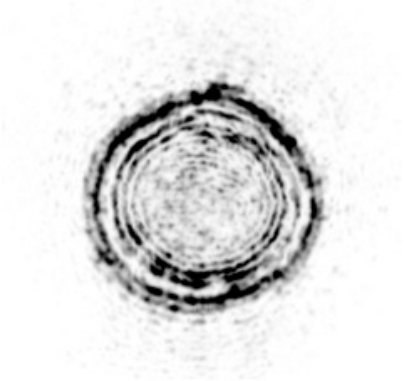
***L. grayi* LM37**

Listeria scatter patterns

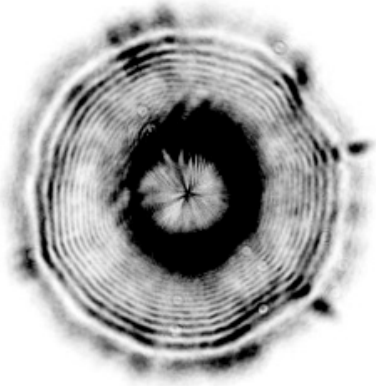
L. welshimeri ATCC35897



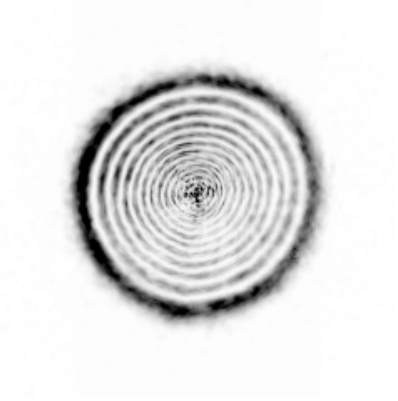
L. innocua V58



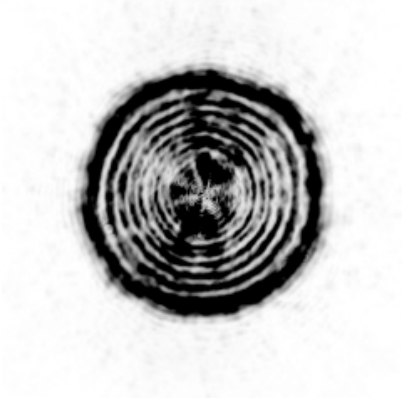
L. ivanovi ATCC19119



L. ivanovi SE98

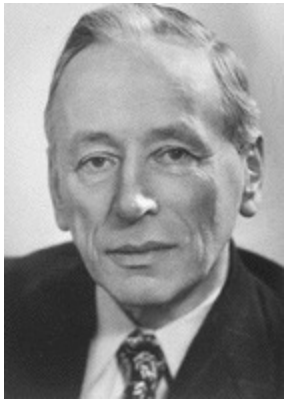


L. monocytogenes ATCC19113



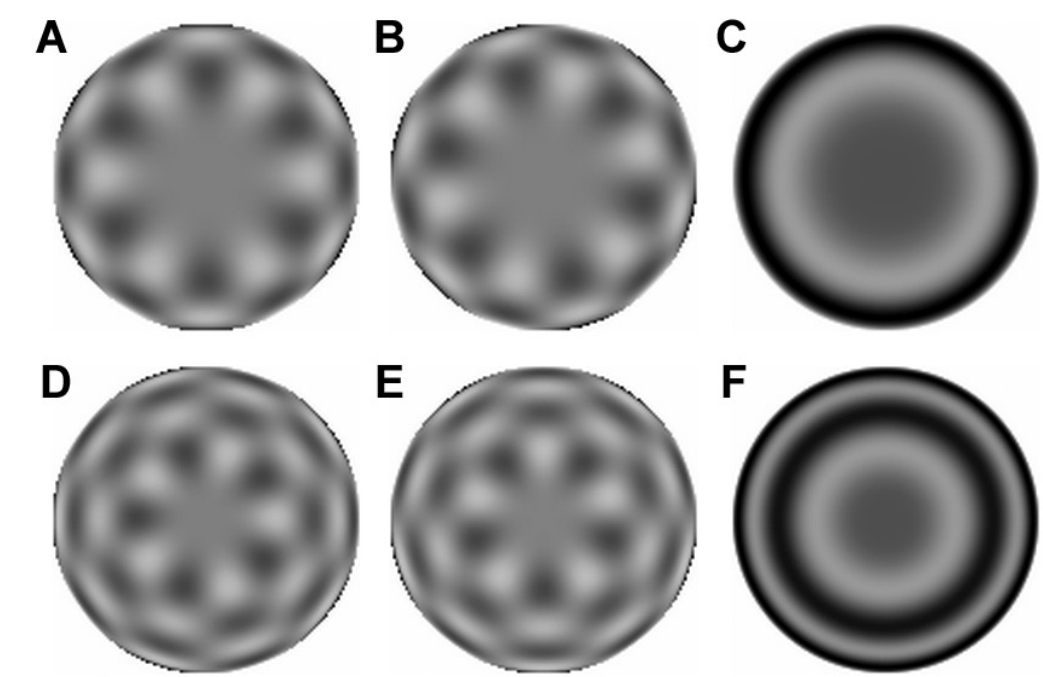
L. monocytogenes V7

Image analysis using 2D radial Zernike polynomials



Frits Zernike
The Nobel Prize in Physics 1953

The Zernike polynomials are a set of orthogonal polynomials that arise in the expansion of a wavefront function for optical systems with circular pupils. They were introduced by F. Zernike in 1934: Zernike, F. "Beugungstheorie des Schneidenverfahrens und seiner verbesserten Form, der Phasenkontrastmethode." Physica 1, 689-704, 1934.

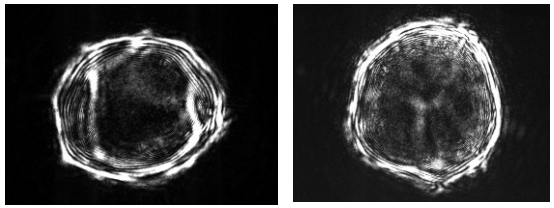


Graphical representation of radial Zernike polynomials $Z_{n,m}$ in 2D (image size 128 x 128 pixels), and their magnitudes: A – real part $Z_{10,6}$; B – imaginary part $Z_{10,6}$; C – magnitude $Z_{10,6}$; D – real part $Z_{13,5}$; E – imaginary part $Z_{13,5}$; F – magnitude $Z_{13,5}$. The larger the n-$|m|$ difference, the more oscillations are present in the shape. Features used in this study are the magnitudes of Zernike polynomials. One may note that the values of the magnitudes do not change when arbitrary rotations are applied.

Based on scatter patterns, we can identify everything we have attempted so far. All of the organisms of interest have been pathogens – mostly food borne in nature

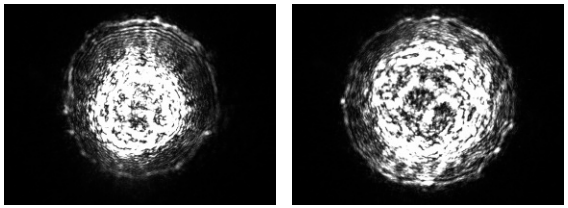
Escherichia coli

Nonpathogenic

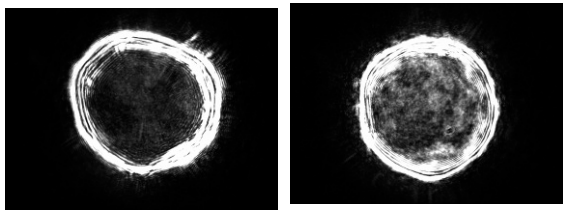


E. coli K12

EPEC

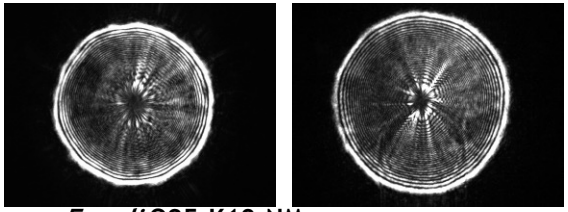


E. coli O142:H6 E851171

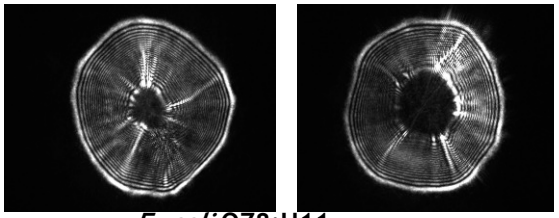


E. coli E2348169 O127:H6

ETEC



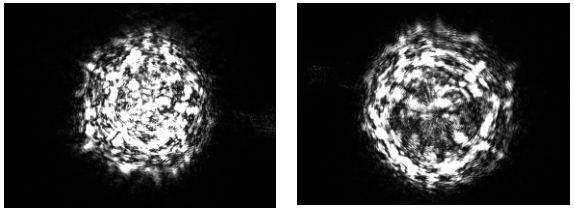
E. coli O25:K19:NM



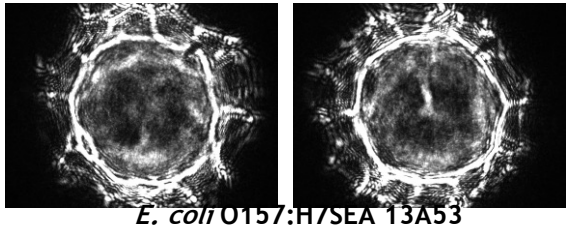
E. coli O78:H11

EHEC

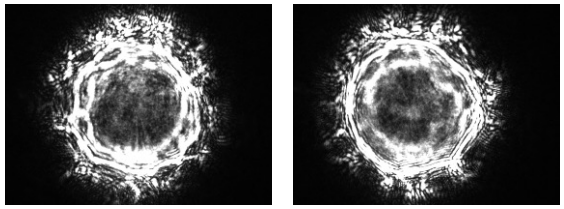
Pattern I



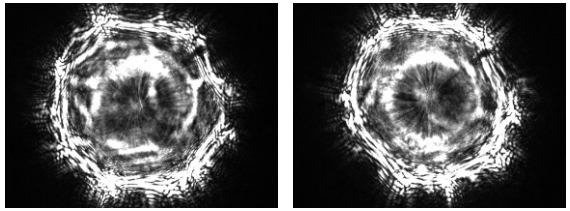
E. coli O157:H7 01



E. coli O157:H7SEA 13A53

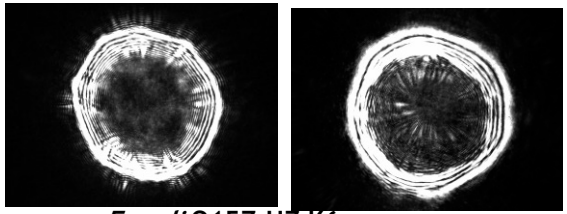


E. coli O157:H7 505B

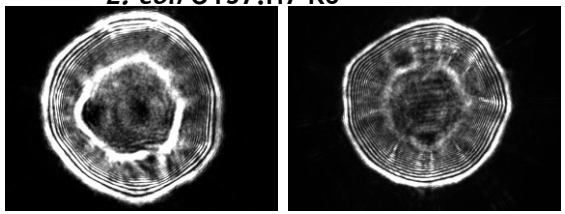


E. coli O157:H7 K1

Pattern II

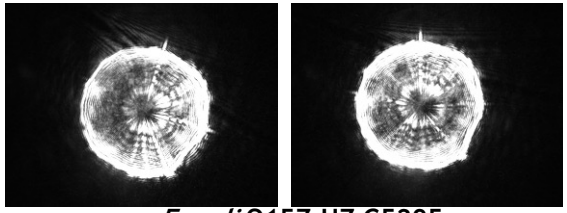


E. coli O157:H7 K6

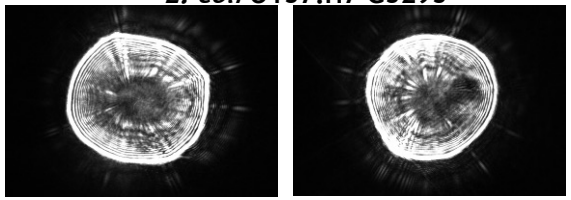


E. coli O157:H7 EDL933

Pattern III

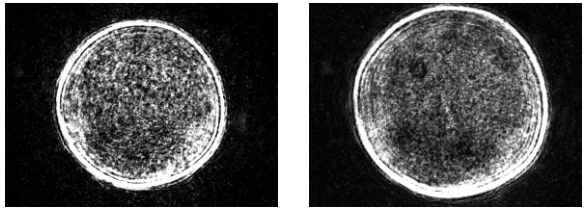


E. coli O157:H7 G5295

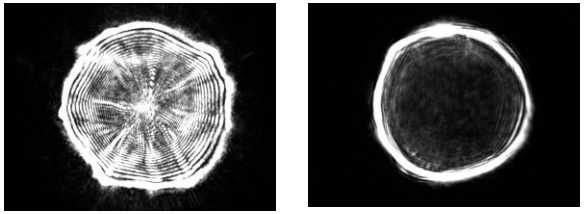


E. coli O157:H7 G458

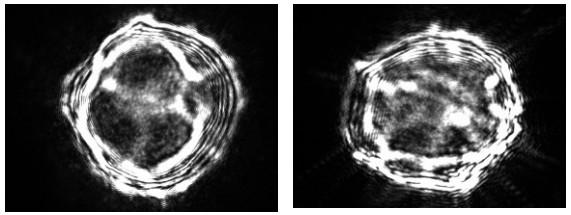
Salmonella



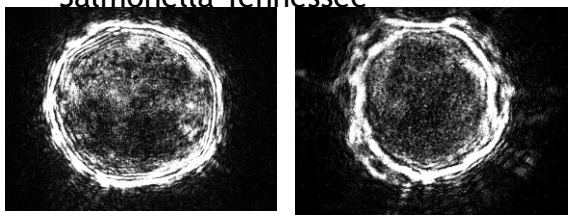
Salmonella Typhimurium



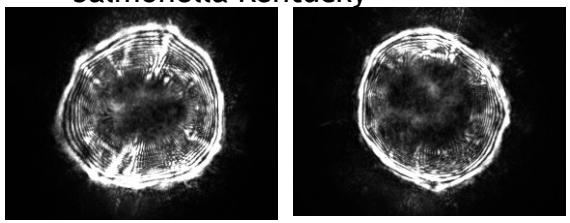
Sal. Typhimurium copenhagen



Salmonella Tennessee

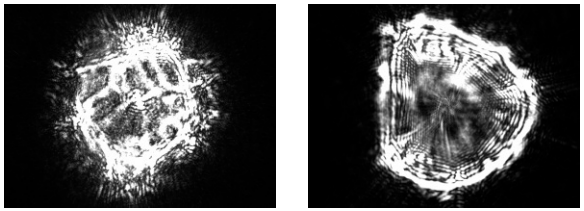


Salmonella Kentucky

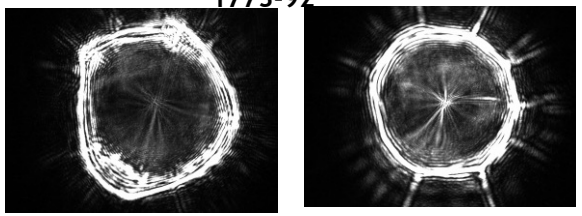


Salmonella Agona

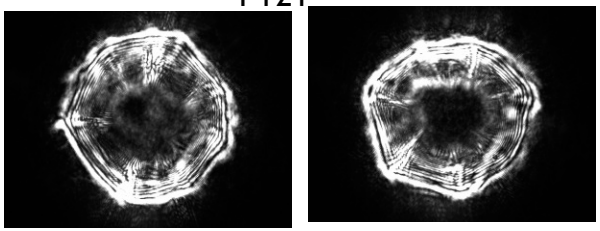
Sal. Enteritidis



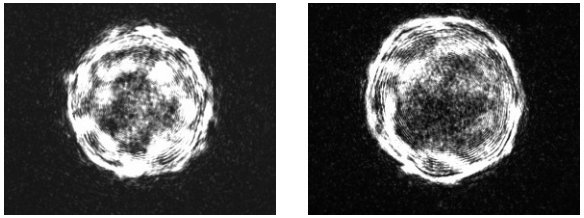
1773-92



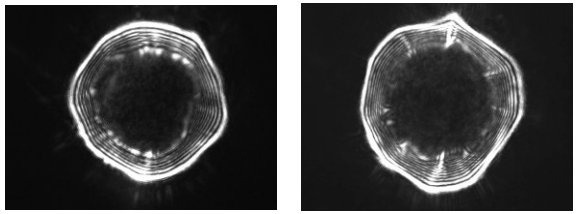
PT21



PT4



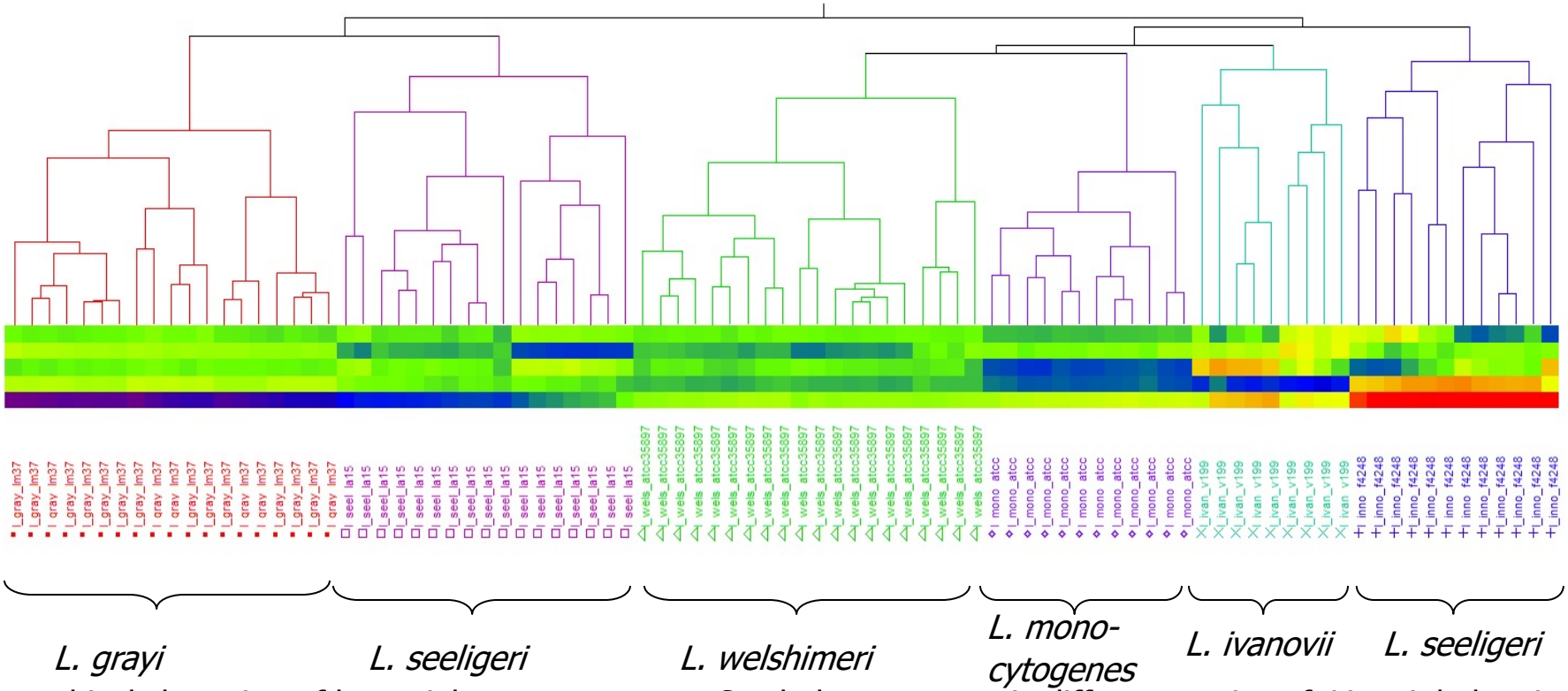
13096



PT28

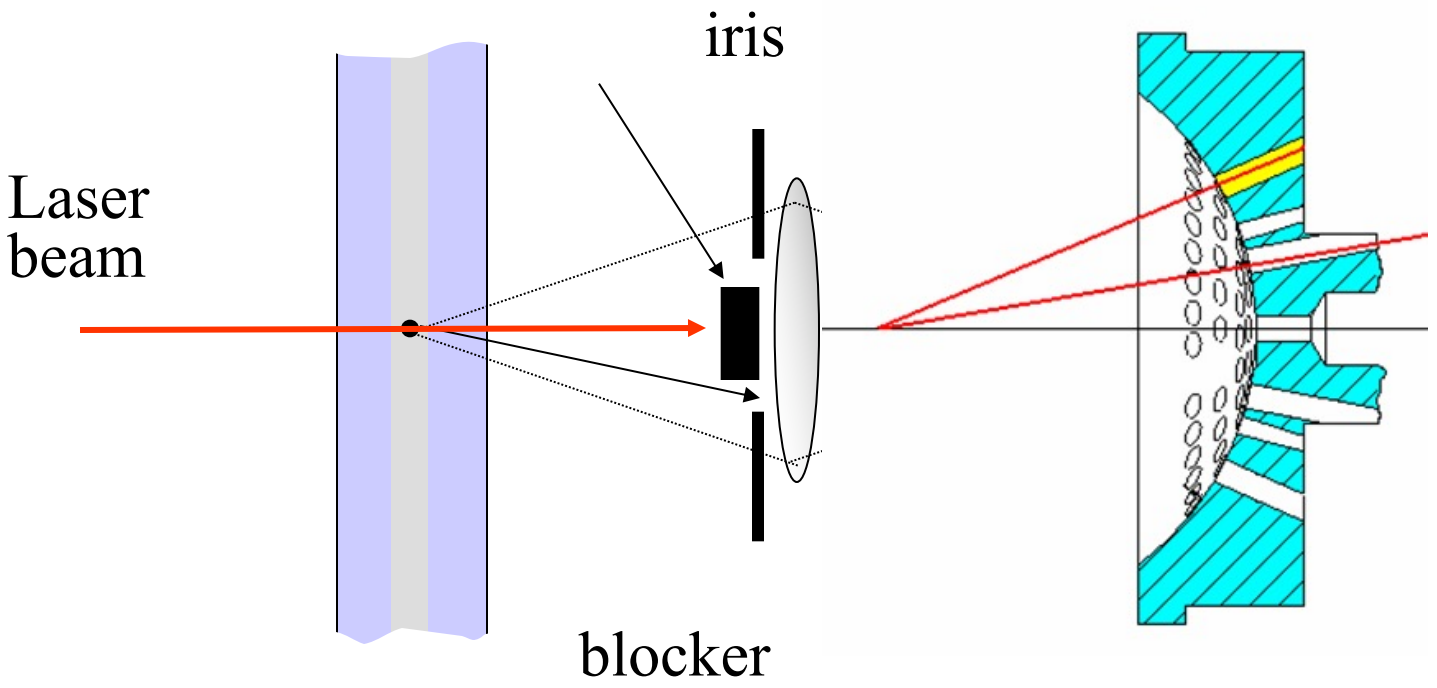
Color map visualizing PC values

Hierarchical clustering based on Zernike moment invariants

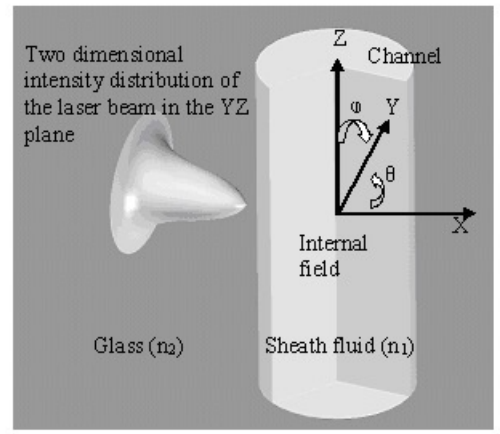


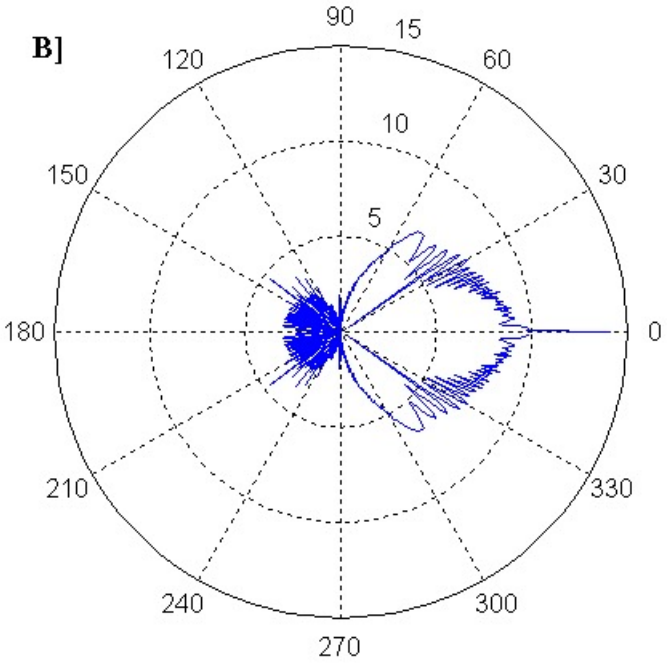
Hierarchical clustering of bacterial scatter patterns. Symbols represent six different strains of *Listeria* belonging to six species: ■ *L. grayi* LM37, □ *L. seeligeri* LA15, r *L. welshimeri* ATCC35897, ◊ *L. monocytogenes* ATCC19113, + *L. innocua* F4248, *L. ivanovii* V199. Numbers represent identified clusters of patterns. Note that identified clusters coincide with the groups of colonies from different strains.

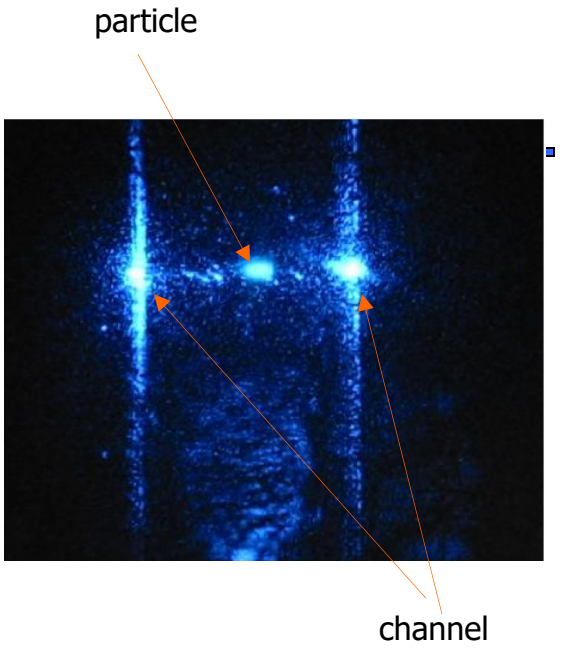
Adding advanced light scatter to traditional systems
Optics for forward scatter



Not to scale



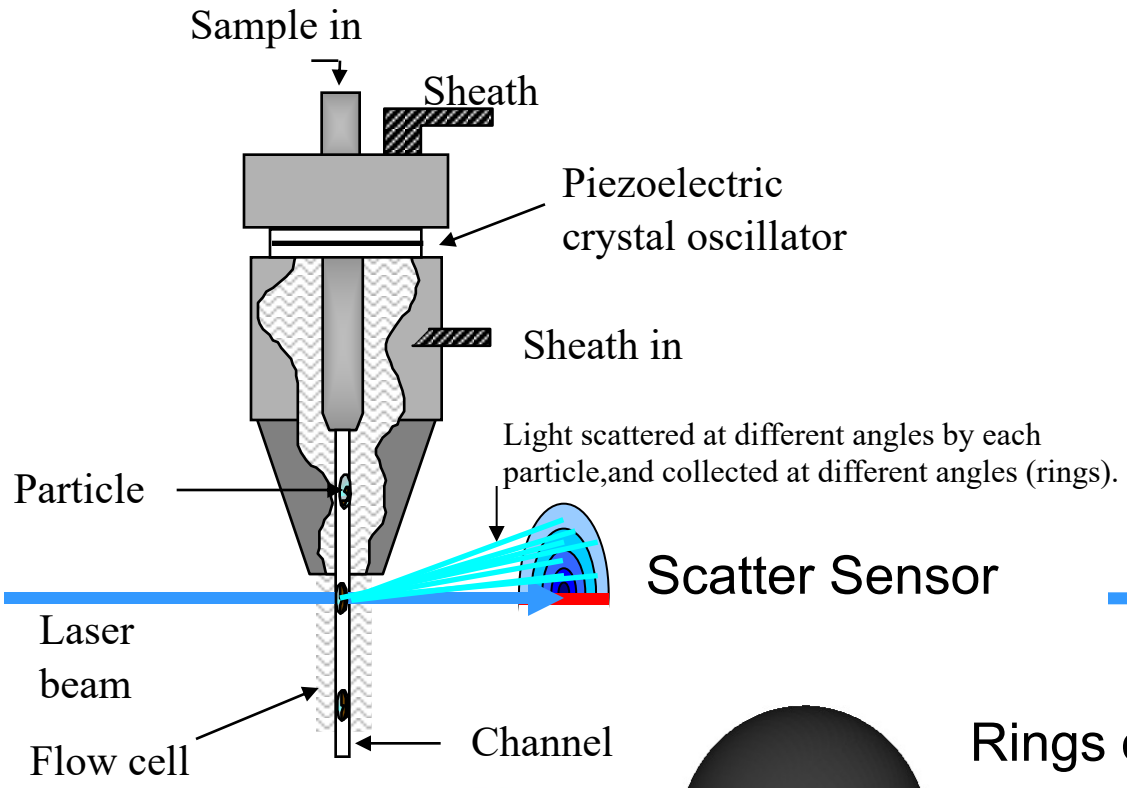




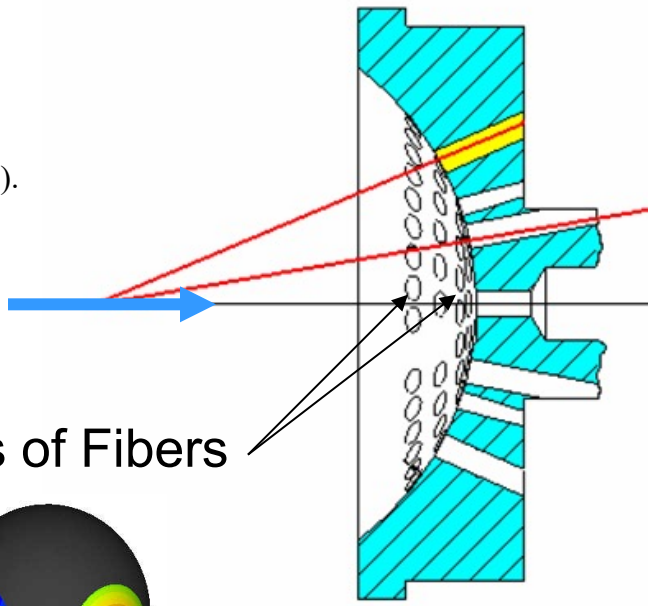
Scatter from a particle / cylindrical flow channel interactive system

M. Venkatapathi, G. Grégori, K. Ragheb, J. P. Robinson, E. D. Hirleman, "Measurement and Analysis of Angle-resolved Scatter from Small Particles in a Cylindrical Microchannel", ***Applied Optics, Vol. 45, No. 10, pp. 2222-2232, (2006).***

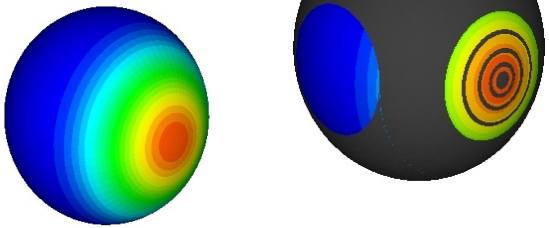
Advanced Detection System



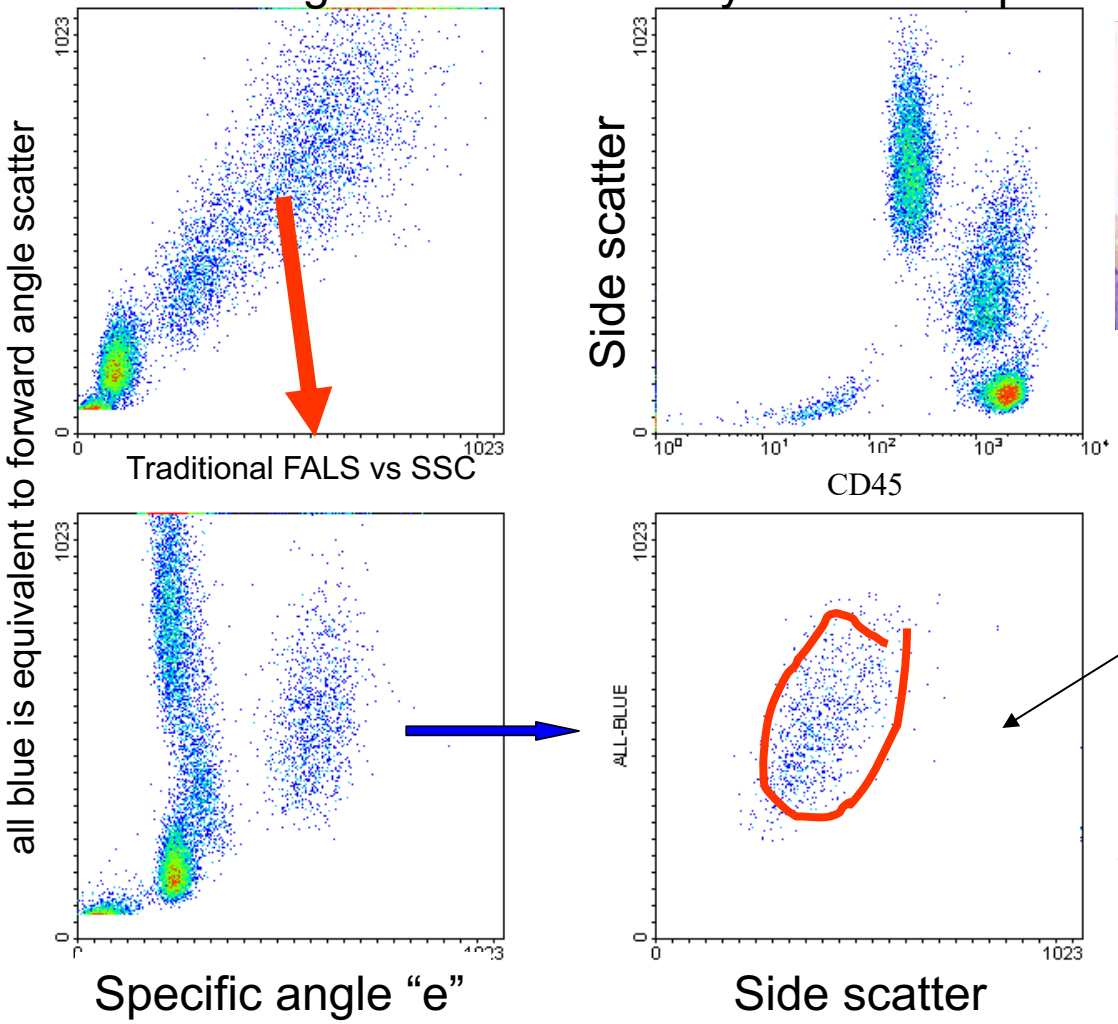
Proprietary Optical Head
from Beckman-Coulter

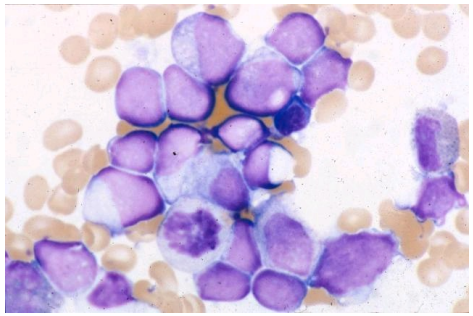


Rings of Fibers



Application of multiangle scatter to identify AML cells spiked into normal blood





These are identified entirely from the special scatter profile

Image from: http://www.thecrookstoncollection.com/v/diseased/neoplasms/myeloid/acute/AML-zoom.jpg.html

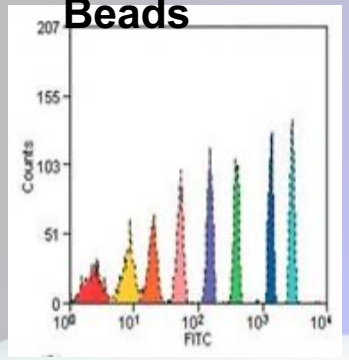
Ring e =angle e
All blue =combined rings

AML cells were spiked into normal whole blood. FALS (standard forward scatter) versus SS (side scatter) is unremarkable. CD45 vs SS indicates a larger than expected population of Mono-myelocytic cells. FOA clearly resolves AML cell population (lower left panel). Gating on this population demonstrates light scatter (FALS vs SS) characteristic of Monocytes and Granulocytes.

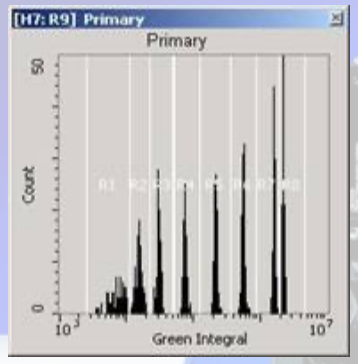
Some advanced analysis in laser Scanning Cytometry

Development and Validation of the LSC

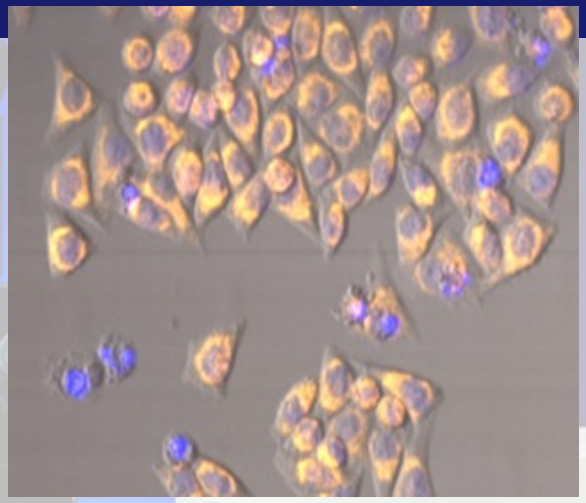
Spherotech 8-peak Rainbow Beads



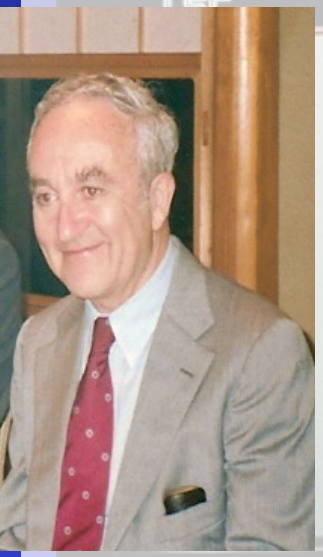
Flow



LSC



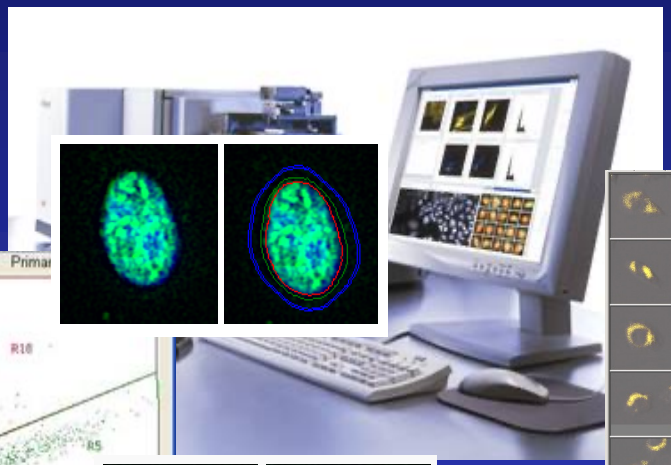
LSC image combining fluorescence and forward scatter

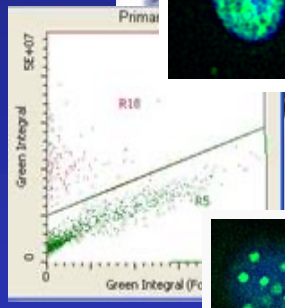


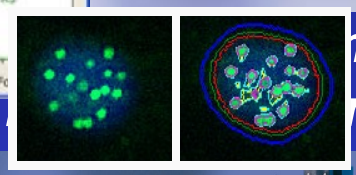
Kamentsky

Slide kindly supplied by Elena Holden, Compucyte

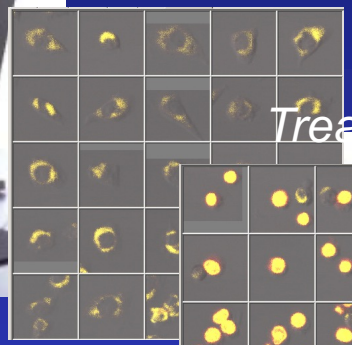
Next Generation



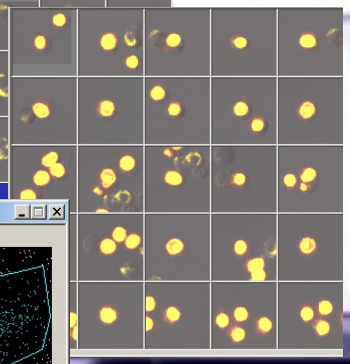


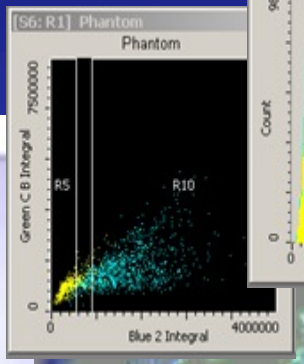


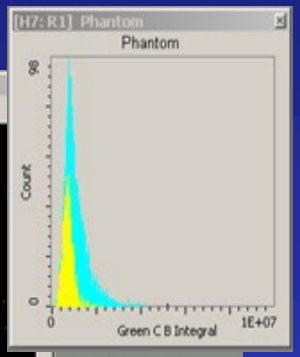
Untreated

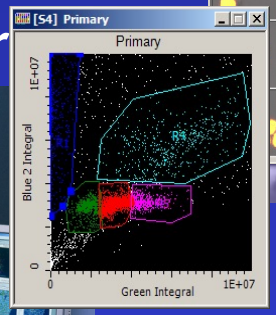


Treated









Quantification of total γH2AX expression & foci count

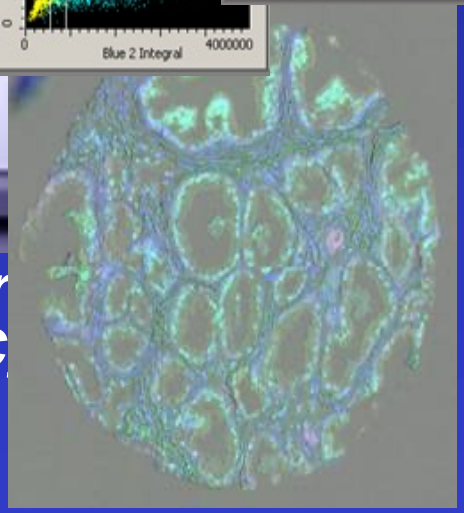
Drug Discovery

iCys® Research Imaging Cytometer

iColor® Fluor Imaging C

Drug-induced apoptosis results in changes to cell morphology

Basic Research



P27 in Prostate Tissue

Research Pathology

Slide kindly supplied by Elena Holden, Compucyte

Enhanced Scatter – what does it offer?

- Very low cost – plenty of signal
- Each type of cell, or organelle, has a unique scatter characteristic
- By adding in multiple scatter tools in regular flow cytometers, we can probably discriminate many different cells in populations that just "look broad"
- Morphologically modified cells may be identified more easily
- Advanced scatter properties in imaging are also very powerful signals that are not frequently used
- Combining scatter, fluorescence and imaging techniques opens new opportunities in cellular and tissue imaging

Conclusions

- Cytometry has many different implementations across many fields of science
- Hyperspectral analysis may fundamentally change the current concepts for detection in cell analysis
- Spectral cytometry may be a far better alternative for specific applications requiring advanced classification such as diagnostics
- Spectral cytometry can separate probes of very similar emission and also extract autofluorescence
- Multiplexing of systems creates opportunities for using functional outputs
- High throughput systems are possible at the single cell level
- Regardless of the technology, advanced modeling and classification tools are going to play much more importance in cytomics

Acknowledgements

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Staff

- Jennie Sturgis (Imaging)
- Kathy Ragheb (Flow)
- Cheryl Holdman (Flow)
- Hildred Rochon (C4L)

Funding:
NIH, NSF, USDA, Purdue University
Corporate: Beckman-Coulter, Point-Source, Parker-Hannifin, Polysciences, Bangs labs, MediaCybernetics, Q-Imaging, Edmund Optics, Kodak Medical Systems, Crystalplex, Becton-Dickinson, eBioscience

cytometry laboratories

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