Historical Perspective of Cytometry: Past successes and Future Opportunities

J. Paul Robinson
SVM Professor of Cytomics
Professor of Biomedical Engineering
Director, Purdue University Cytometry Laboratories

"3rd Turkish-US Cytometry Wokshop"
Akdeniz University, Antalya, Turkey

www.cyto.purdue.edu (science link)
www.cyto.purdue.edu/trackpaul (fun link)

PUCL cytometry laboratories
PURDUE UNIVERSITY

Goals of this lecture

1. Give a historical background to cytometry
2. Show 3 developing technologies that will have a major impact on the field – both clinical and research

Changes in Technology

- Early History
  - Visual observation - Color and color correction (apochromatic) - dyes for staining
    - **2600 BC** Earliest written record of the use of dyestuffs in China
      715 BC Wool dyeing established as craft in Rome
    - **1327-1377** Edward III, "Royal Wool Merchant" offered protection to all foreigners living in England and to all who wanted to come to help improve the textile industry
    - **1646 Athanasius Kircher**, a German Jesuit priest, recorded an interesting observation of the wood extract of *Lignum nephriticum*. An aqueous infusion of this wood exhibited blue color by reflected light and yellow color by transmitted light
    - **1856 William Perkin,** an English chemist, synthesized a coal-tar dye, aniline purple
    - **1858-59** Magenta (fuchsin) discovered by Verguin the 2nd basic dye
    - **1861** Methyl violet, basic dye, by Lauth
    - **1862** Hofmann's Violet, Hofmann was one of the great dye chemists of all time
      1862 Bismarck Brown developed by Martius and Lightfoot, first soluble azo dye
      1863 Aniline Black, developed by Lightfoot, a black produced by oxidation of aniline on the cotton fiber.
      1866 Methyl Violet, basic dye
    - **1871 Adolph Von Baeyer**, a German chemist, synthesized a fluorescent dye, fluoresceine1871 Adolph Von Baeyer, a German chemist, synthesized a fluorescent dye, fluoresceine
    - **1872** Methyl Green by Lauth and Baubigny,
    - **1876** Caro a chemist, discovered Methyl Blue
    - **1877** Malachite Green, basic dye by Dobner and Fisher
    - **1882 Paul Erlich**, a German bacteriologist, employed the fluorescent dye uranin (sodium salt fluorescein) to track the pathway of secretion of aqueous humor in the eye. *This is the first case of the use of* in vivo *fluorochrome in animal physiology*.
    - **1884 The Gram stain**, gentian violet an essential component was developed by Hans Gram, a Danish physician.
    - **1887** Rhodamine B (brilliant red-violet) basic dye

Changes in Technology

Detection systems

- 1875, American, **G.R Carey** invented the phototube
- 1878, Englishman **Sir William Crookes** invented the 'Crookes tube', an early prototype of cathode-ray tube
- 1895, German, **Wilhelm Roengten** invented an early prototype Xray tube
- 1897, German, **Karl Ferdinand Braun** invents the cathode ray tube oscilloscope
- 1904, **John Ambrose Fleming** invented the first practical electron tube called the 'Fleming Valve'. Leming invents the vacuum tube diode
- 1922, **Philo T. Farnsworth** develops the first tube scanning system for television.
- 1923, **Vladimir K Zworykin** invented the iconoscope or the cathode-ray tube and the kinescope.
- 1926, **Hull and Williams** co-invented the tetrode electronic vacuum tube.
- 1938, Americans **Russell** and **Sigurd Varian** co-invented the klystron tube
- 1938 **Frits Zernike** built a microscope based on the principle in 1938 and received the Nobel Prize in 1953
- 1947 - The transistor was invented at Bell Telephone Laboratories by a team led by physicists **John Bardeen, Walter Brattain, and William Shockley**
- **1947 Gabor** invented the basic wave-front reconstruction technique of holography
- 1953 -Marvin Minsky invented (patented) the confocal microscope

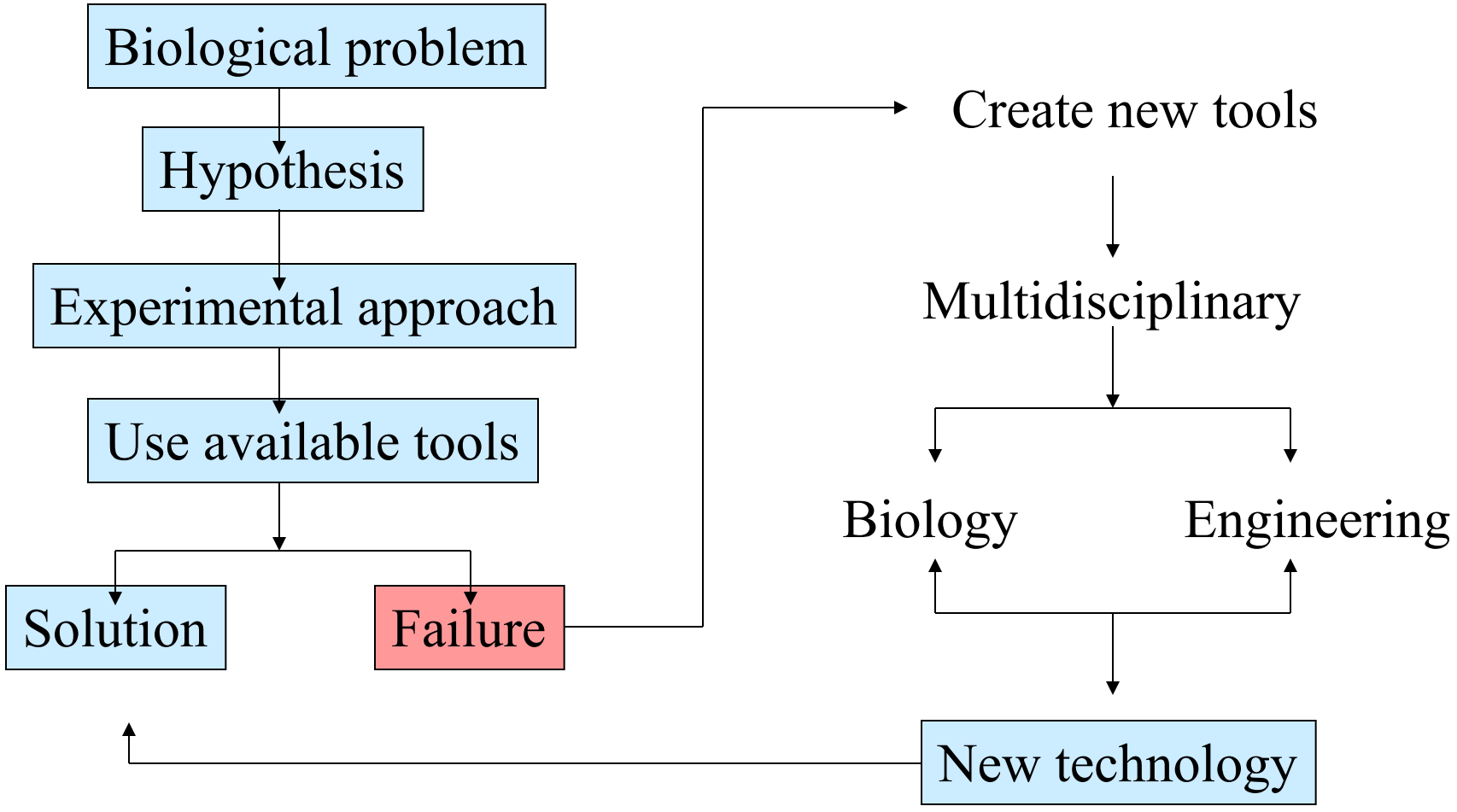
Cytometry has a history of over 100 years

- **Ehrlich** 1880s - *used acidic and basic dyes to identify acidophilic, eosinophilic, basophilic and neutrophilic leukocytes 1880's to study the dynamics of ocular fluids- used fluorescein for first time*
- **Robert Feulgen** (1925) - *demonstrated that DNA was present in both animal and plant cell nuclei - developed a stoichiometric procedure for staining DNA involving a derivatizing dye, (fuchsin) to a Schiff base*
- **Torbjorn Caspersson** - (1938-1998) - **1941** - *"demonstrated that nucleic acids, far from being waste products, were necessary prerequesites for the protein synthesis in the cell (published in Naturwissenschaften in January 1941) and that they actively participated in those processes."* ["*History of the Development of Cytophotometry from 1935 to the present" in Analytical and Quantitative Cytology and Histology, pp2-6, 1986*]
- Early interests in cancer diagnosis from the times of **Papanicolaou & Traut** (1941)
- **Wallace Coulter** (1953-56) – Coulter principle
- Tremendous technology developments in the 1960s – **Fulwyler** – Cell Sorter, **Sweet**, Inkjet printer
- Automation in the 1990s led to vast numbers of cytometry instruments
- 2000's – next generation technologies with advanced bioinformatics

Cell analysis technology *state-of-the-art*….?

• 1930-40s	• Cell cytochemistry & staining	Imaging
• 1950s	• Cell counting	
• 1960s	• Cell sorting	
• 1970s	• Cell detection	
• 1980s	• Cell separation/classification (MABs)	
• 1990s	• Polychromatic (multicolor) cytometry	
• 2000s	• Automated imaging, cytomics, metabolomics	Imaging Quantitative focus ?
• 2010s	• Technology Integration • Mass Cytometry	

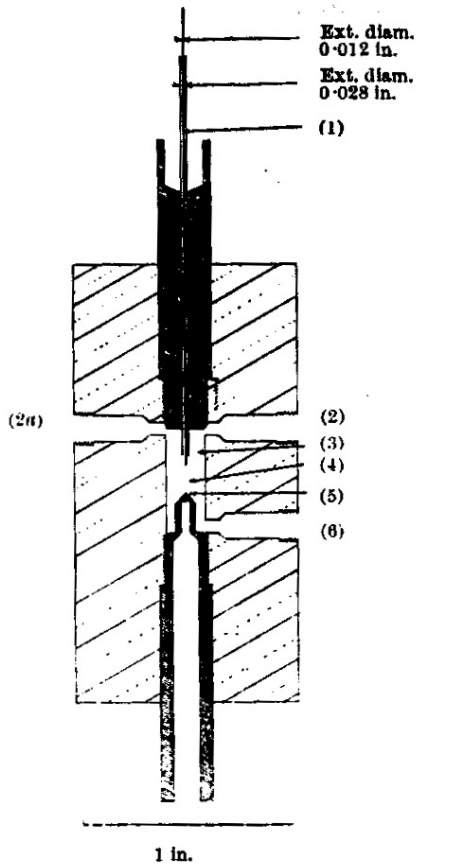
Pathways for Discovery



Maybe mass-cytometry fits here

P.J. Crosland-Taylor

Sheath Flow Principle – 1953



(1) Needle in holder; (2) and (2a) inflow tubes; (3) wide-bore tube; (4) observation area for (3); (5) vortex; (6) flushing tube

"Provided there is no turbulence, the wide column of particles will then be accelerated to form a narrow column surrounded by fluid of the same refractive index which in turn is enclosed in a tube which will not interfere with observation of its axial content."

A Device for Counting Small Particles Suspended in a Fluid through a Tube

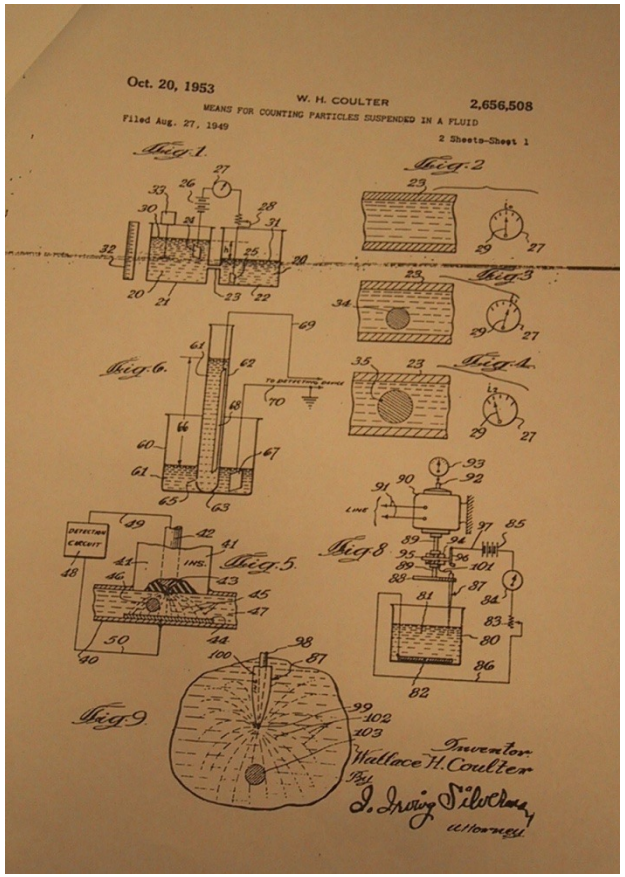
P.J. Crosland-Taylor
Bland-Sutton Institute of Pathology
Middlesex Hospital, London, W.1. **June 17, 1952**
Nature 171: 37-38, 1953

Wallace Coulter

Wallace Coulter - Coulter orifice - patent 1953

Commercialized in 1956 -

Measured changes in electrical conductance as cells suspended in saline passed through a small orifice



Coulter's Original 1953 Patent app'n

The first commercial version of the Coulter Counter was sold in 1956

1st Example of New Technology

1. **Invention of cell sorting** - flow cytometry

- **Problem:**
  - pathologist uses new technology (Coulter counter late 1950s)
  - Tries to use technology to interpret biology but does not understand it and makes fundamental error
- **Solution**:
  - New technology had to be developed to test hypothesis
  - Pathologist proved wrong
  - Others identify new uses for invented technology

The beginning of the "cell sorter"

H-4 Ju

Mr. R. G. Sweet
Applied Electronics Laboratory
Stanford University
Stanford, California

Dear Mr. Sweet:

I find your d
and fascinating de
whatever detailed information you can provide. I
of this instrument planned? If so, when and by w
you in advance.

Sincerely yo

Mack J. Fulv

MJF:ES

STANFORD ELECTRONICS LABORATORIES

STANFORD, CALIFORNI

August 14, 1964

Mr. Mack J. Fulwyler
Los Alamos Scientific Laboratory
P.O. Box 1663
Los Alamos, New Mexico 87544

Dear Mr. Fulwyler,

a most interesting and
your visit yesterday.
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The amplifier and transducer driving schematics are enclosed. If there is any other information that I have that would be of use to you, please let me know.

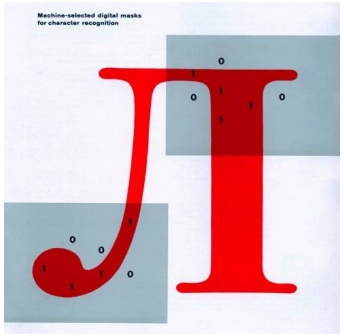
Best Regards,

Richard Sweet
Research Associate
Systems Techniques Lab

RS/kp
Encls.

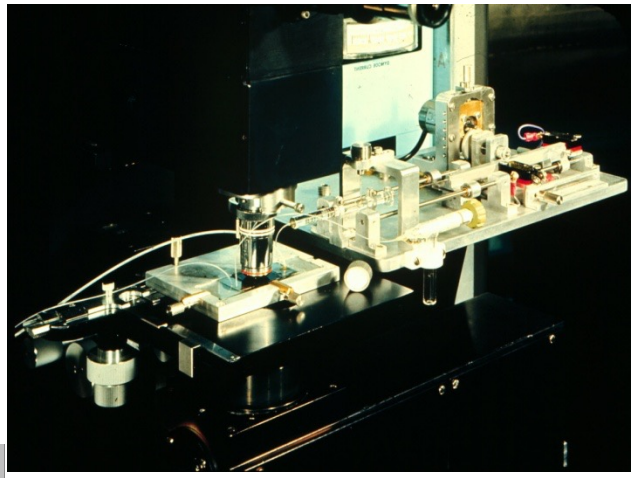
"After giving your ingenious cell-sorting scheme additional thought, my only conclusion is that it will surely work."

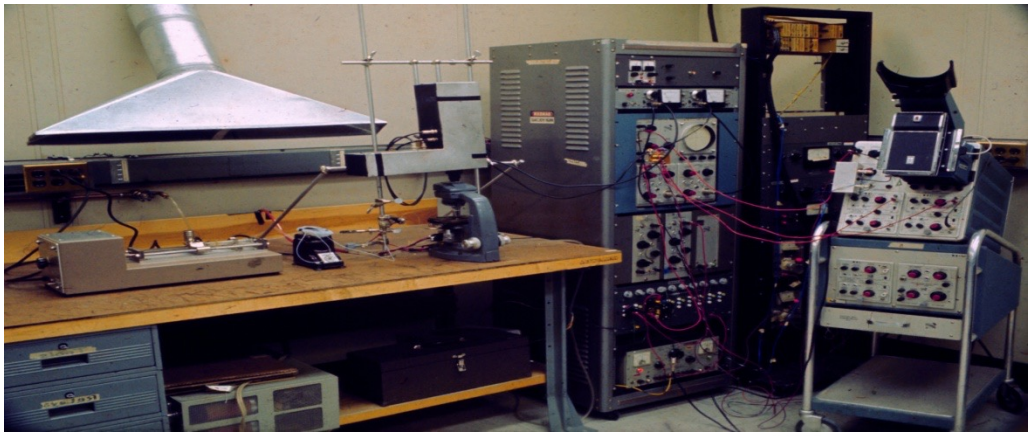
From Character recognition to automated cell recognition



High Content Screening

LA Kamentsky & CN Liu, Computer-automated design of multifont print recognition logic, IBM J. Research & Development 7, 1963





16 July

Question on Feasibility of Cell Separator

1.) Might it be better to vary the deflection plate voltage holding charge constant? This might lessen pickup by Coulter aperture system.

2.) Would RBC presence perturb formation of droplet? (I think not as the ratio of RBC vol/drop vol ~ 2000.) No prob.

3) Electrical pick-up of the deflection voltage pulse by the aperture system. (May be a problem)

4) How much flexibility exist in the following parameters:

a. Drop frequency 250kc → 10kc

b. Drop size

c. Driving pressure → 11psi and up to 60psi

d. Nozzle - paper separation distance

STANFORD ELECTRONICS LABORATORIES

STANFORD, CALIFORNIA

August 14, 1964

Mr. Mack J. Fulwyler
Los Alamos Scientific Laboratory
P.O. Box 1663
Los Alamos, New Mexico 87544

Dear Mr. Fulwyler,

I want to thank you for a most interesting and stimulating discussion during your visit yesterday. After giving your ingenious cell-sorting scheme additional thought, my only conclusion is that it will surely work. I certainly hope that you are successful in developing this and am looking forward to hearing more about it.

The amplifier and transducer driving schematics are enclosed. If there is any other information that I have that would be of use to you, please let me know.

Best Regards,

Richard Sweet

Richard Sweet
Research Associate
Systems Techniques Lab

RS/kp

H-4 January 24, 1966

Mr. R. A. Sweet
Stanford Electronics Laboratories
Stanford University
Stanford, California

Dear Mr. Sweet:

I am enclosing a reprint of the separator article which appeared in the November 12, 1965, issue of Science. If you are interested, I will be glad to give you more information on the device.

Dr. Leonard Herzenberg of the Genetics Department at the Stanford Medical School is considering building a separator for research involving the biology of cells.

Did you receive your ink drop gun in good condition? What about the droplet pictures?

Sincerely yours,

Mack J. Fulwyler

MJF:ES
Enc. 1 reprint

H-4 February 25, 1966

Mr. Richard Sweet
Stanford Electronics Laboratories
Stanford University
Stanford, California

Dear Mr. Sweet:

Dr. Leonard Herzenberg in the Department of Genetics of Stanford Medical School has a set of drawings of the droplet generator system. I understand that he is beginning to construct a separator.

I do not attempt to avoid formation of satellite droplets; as long as they recoalesce quickly, they do not affect the separation efficiency. Stabilizing the length of the fluid jet is of concern, but after a few minutes of operation this length is fairly stable.

As you will see from the blueprints, a good deal of effort has gone into features secondary to droplet formation such as the flushing system, removable apertures, etc. The latest design will, hopefully, withstand autoclave sterilization.

We are pressing biological applications of the device as quickly as time and personnel permit. We are also investigating optical particle sensors with the hope of measuring optical characteristics of cells and separating on this basis.

Unfortunately, I am unable to give you the drop-to-drop spacing, etc., which you requested. I fear I have lost the record of operating conditions at which these photographs were taken.

Perhaps I will be able to see you next time I am in Palo Alto. If you are ever in this area, please plan to visit us; I am sure you would find our work interesting.

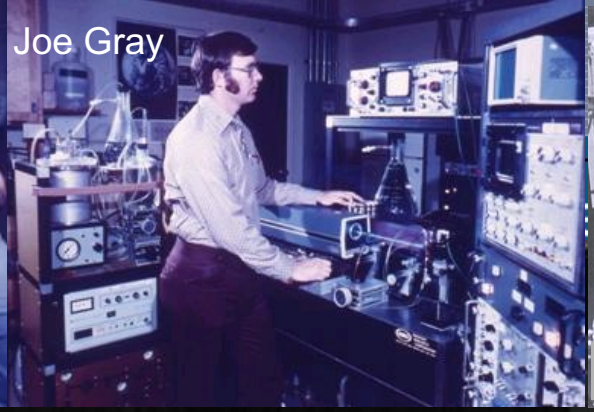
Is your ink writing oscillograph patented in such a way that this must be considered by a commercial company manufacturing the cell separator?

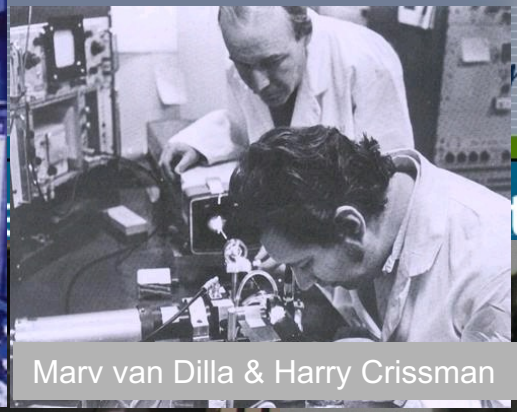
If, after talking to Dr. Herzenberg, you have questions, please feel free to write.

Sincerely yours,

Mack J. Fulwyler

MJF:ES
Enc. sketch





Fulwyler's original cell sorter – a 1967 model

Example of New Technology

2. Technology Integration Example

1. **Pap smear**: Situation:
   - Pathologist can read and interpret slide
- Problem:
   - Pathologist wants to automate technology
- Solution:
   - Engineer brings new imaging/cytometry technology to pathology problem

Four Sensors, Sorting, Auto Sampling and Computer Data Reduction 1966

Two analytic instruments were built a
LA Herzenberg at Stanford

J. Paul Robinson, Purdue University

PU
cytometry laboratories

Purdue University

PUCL
cytometry laboratories

Integration of Technologies

- Fundamental redesign of flow cytometry technology by integration of principles of chemical analysis, image analysis & informatics
  - Implement capabilities of instruments so that we get quantitative results
  - Integrating technologies to create new capabilities
  - Ability to separate populations and create multiparameter analysis
  - New computers and software tools

IMPACT:

Highly organized systems – particularly in the clinical domain

- Potential for creating automated systems
- Almost real time analytical tools now available
- High numbers of variables (parameters) in nicely designed instruments

Lets move to the future – what are some of the next-generation tools

1. Hyperspectral flow cytometry
2. High throughput Cytometry

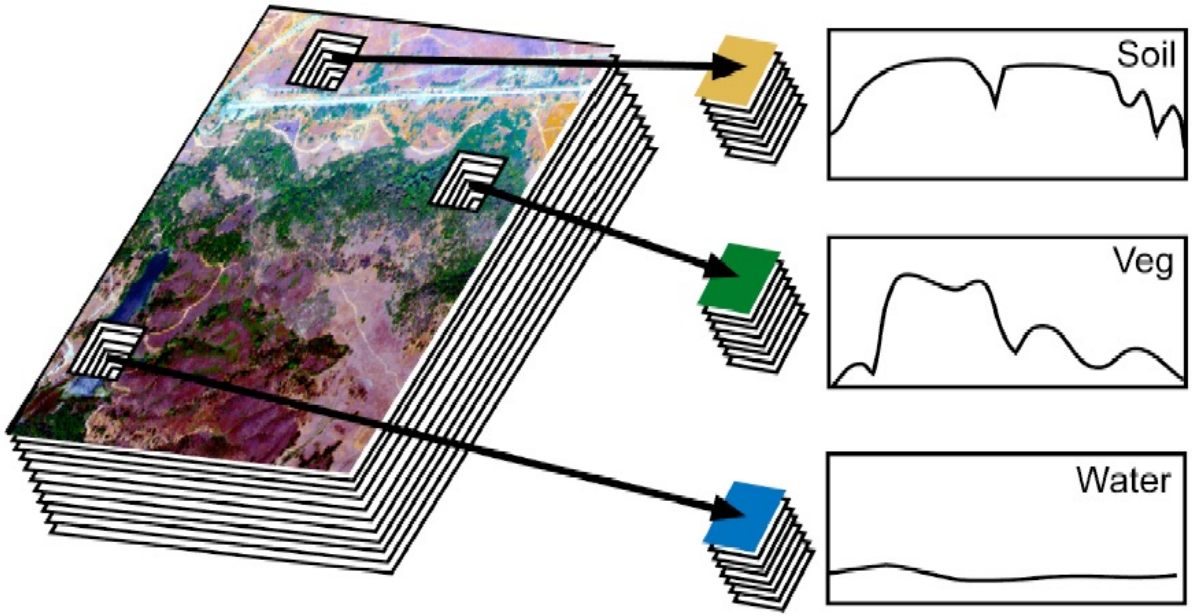
Both fundamentally quantitative technologies

3. Mass Spectroscopy – **CyTOF** – Very High Content
   20 to 100 parameters!!!

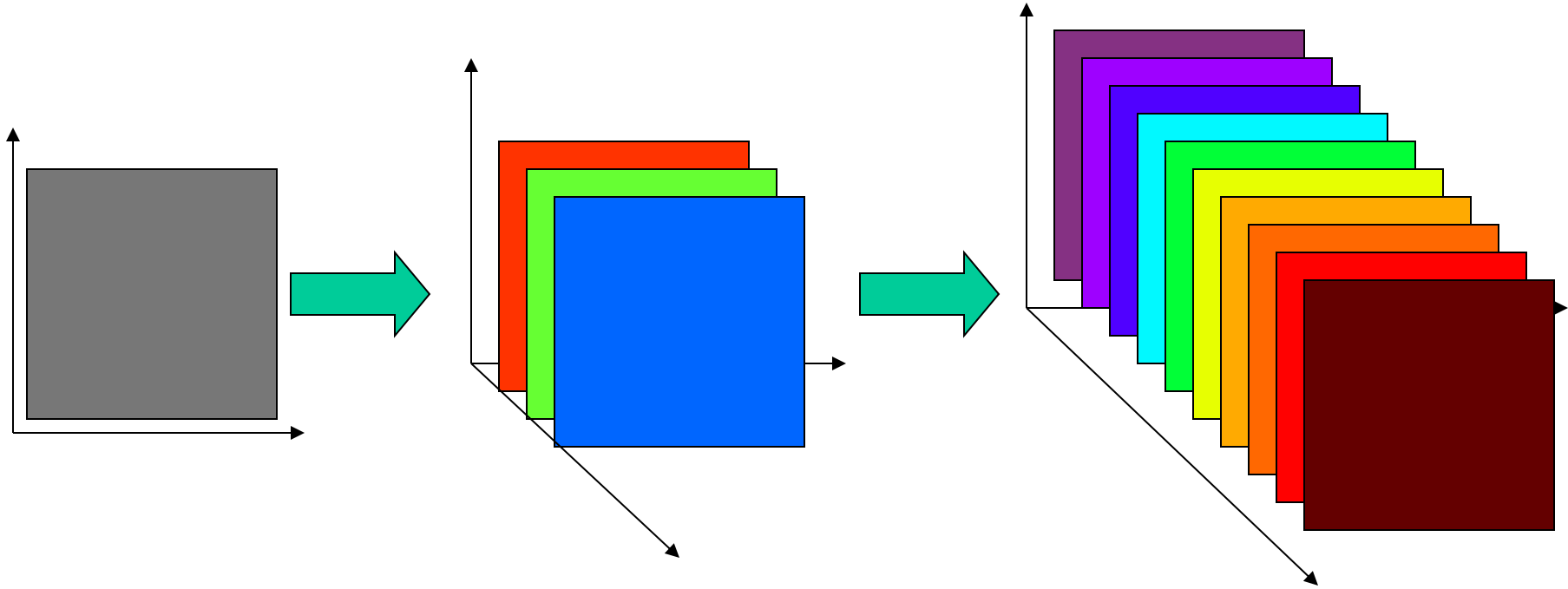
Introduction to Multispectral/hyperspectral Imaging

- 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



Basic imaging…

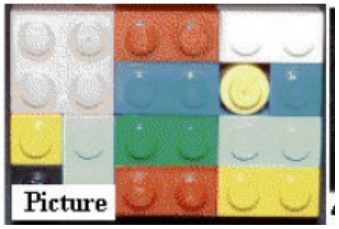


Greyscale image

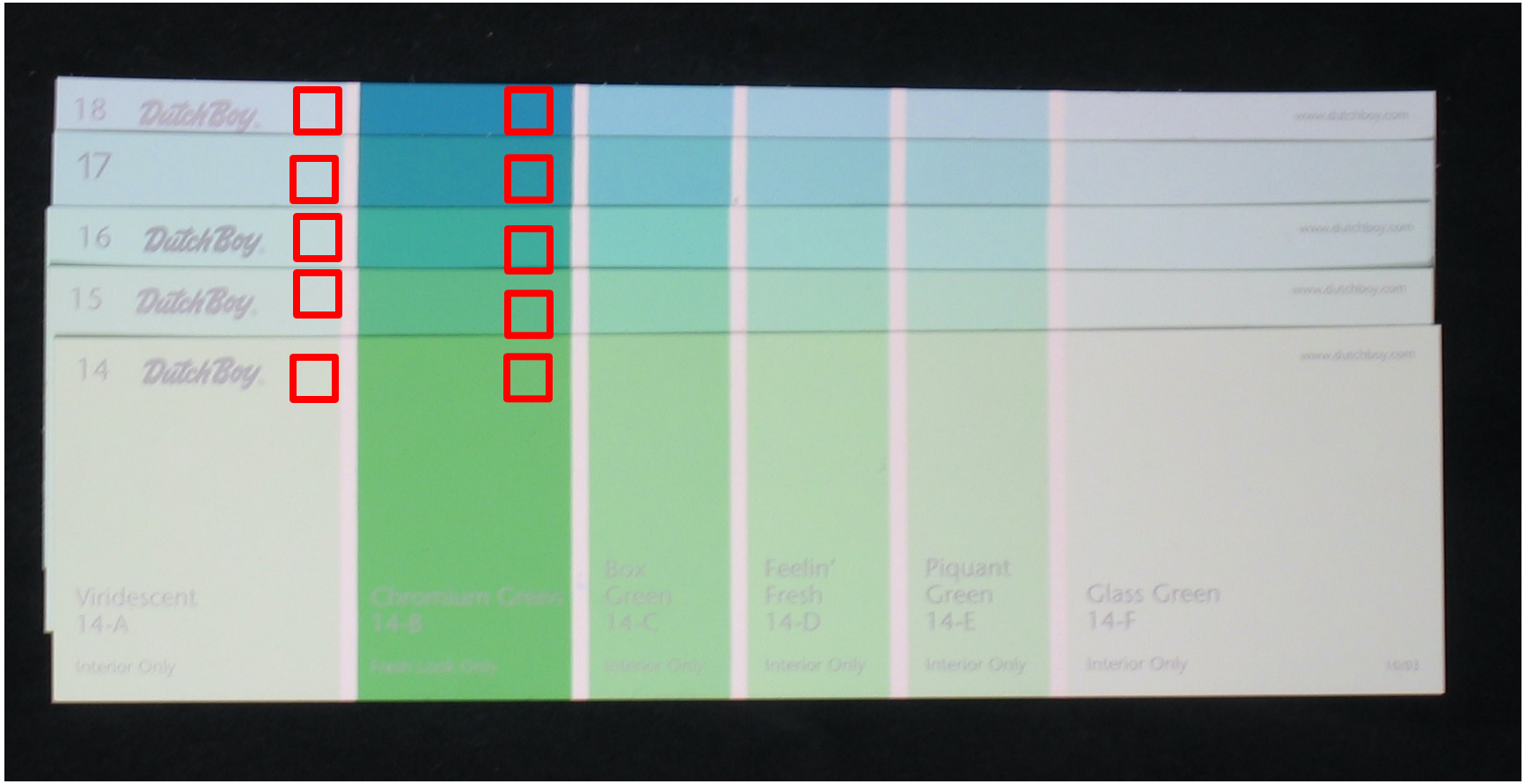
Color image

Multispectral image

Color composition is a mixture of spectral bands



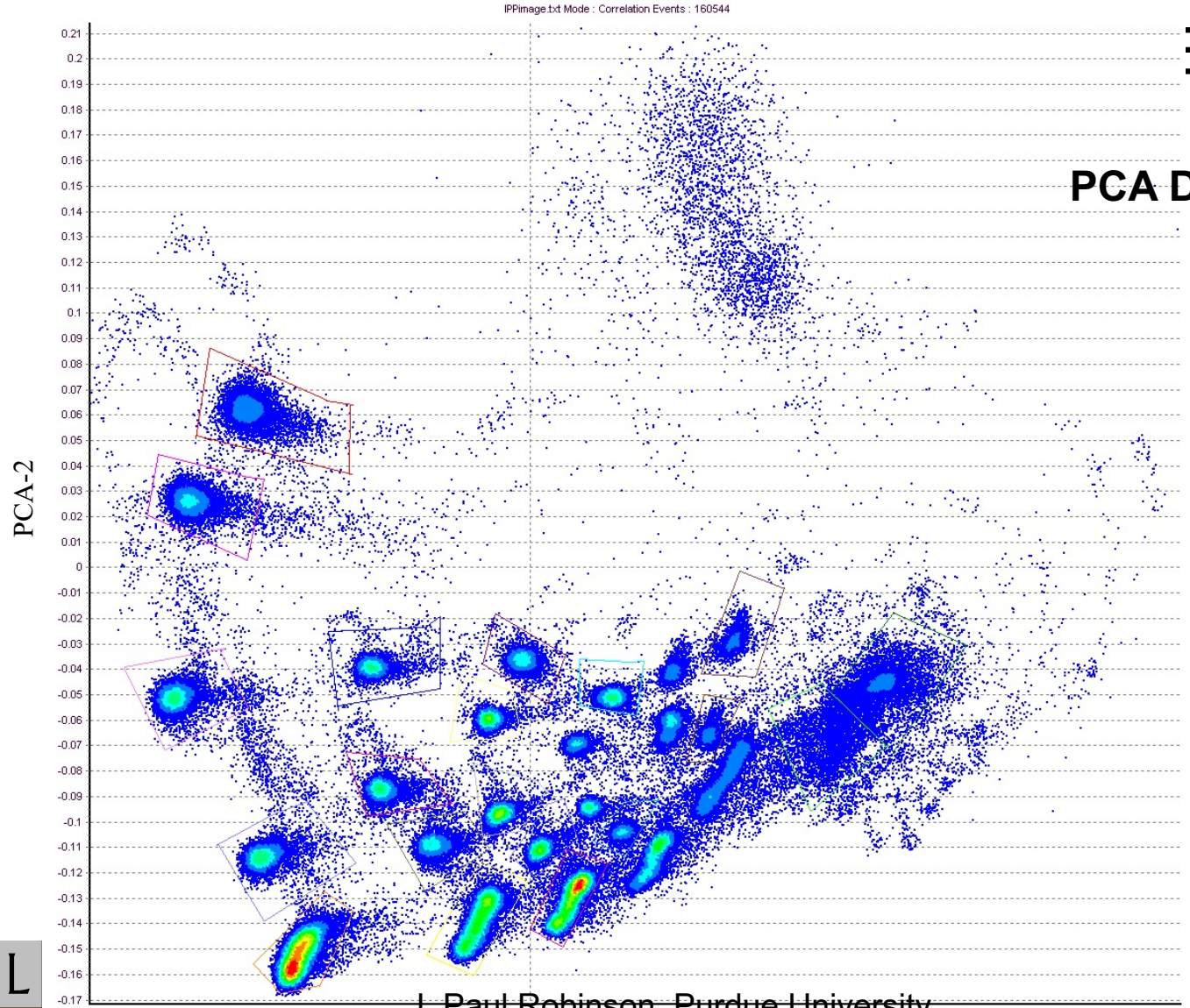
Absorption Example



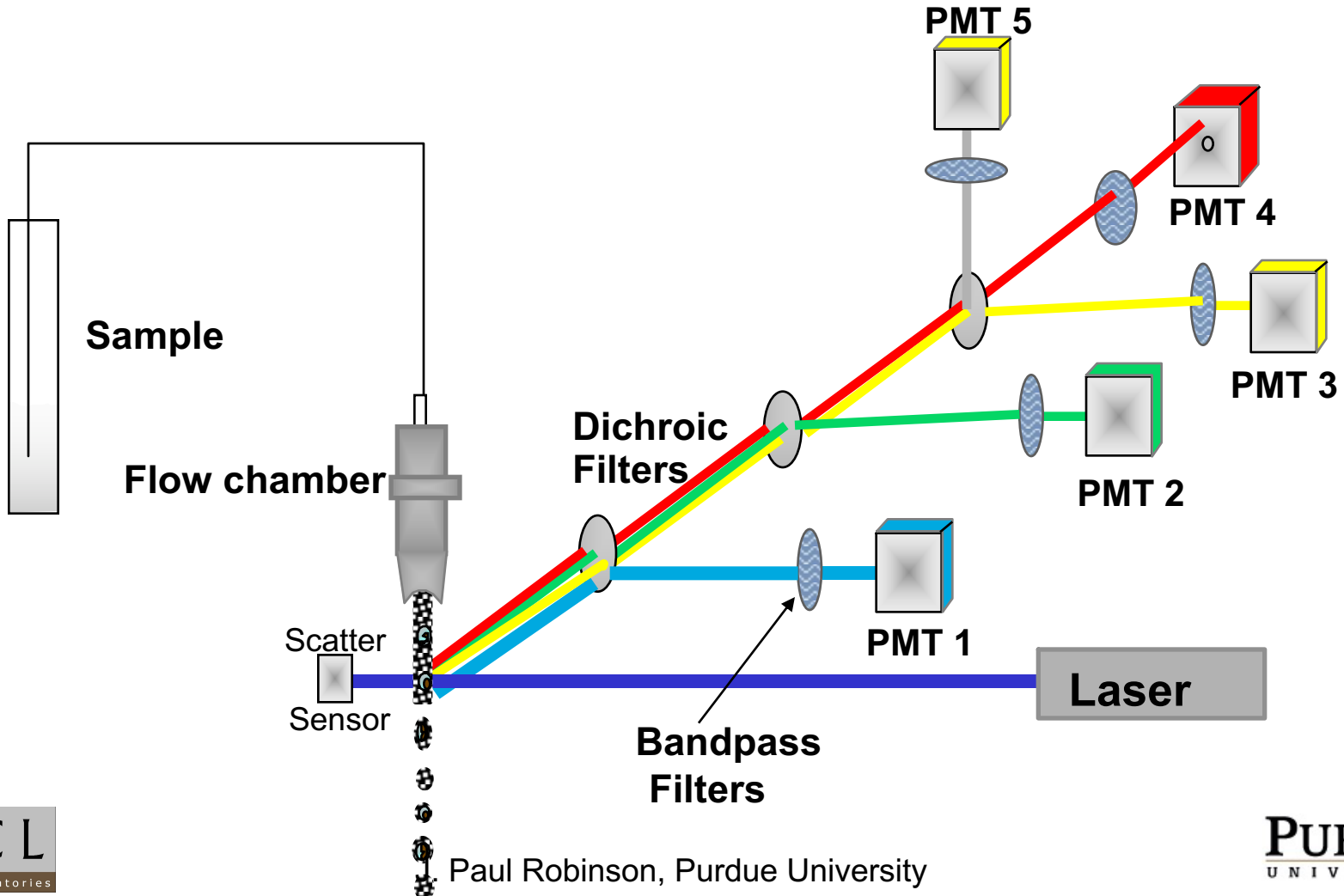
- Dutch Boy paint cards
- Colors difficult to distinguish by visual inspection

Example

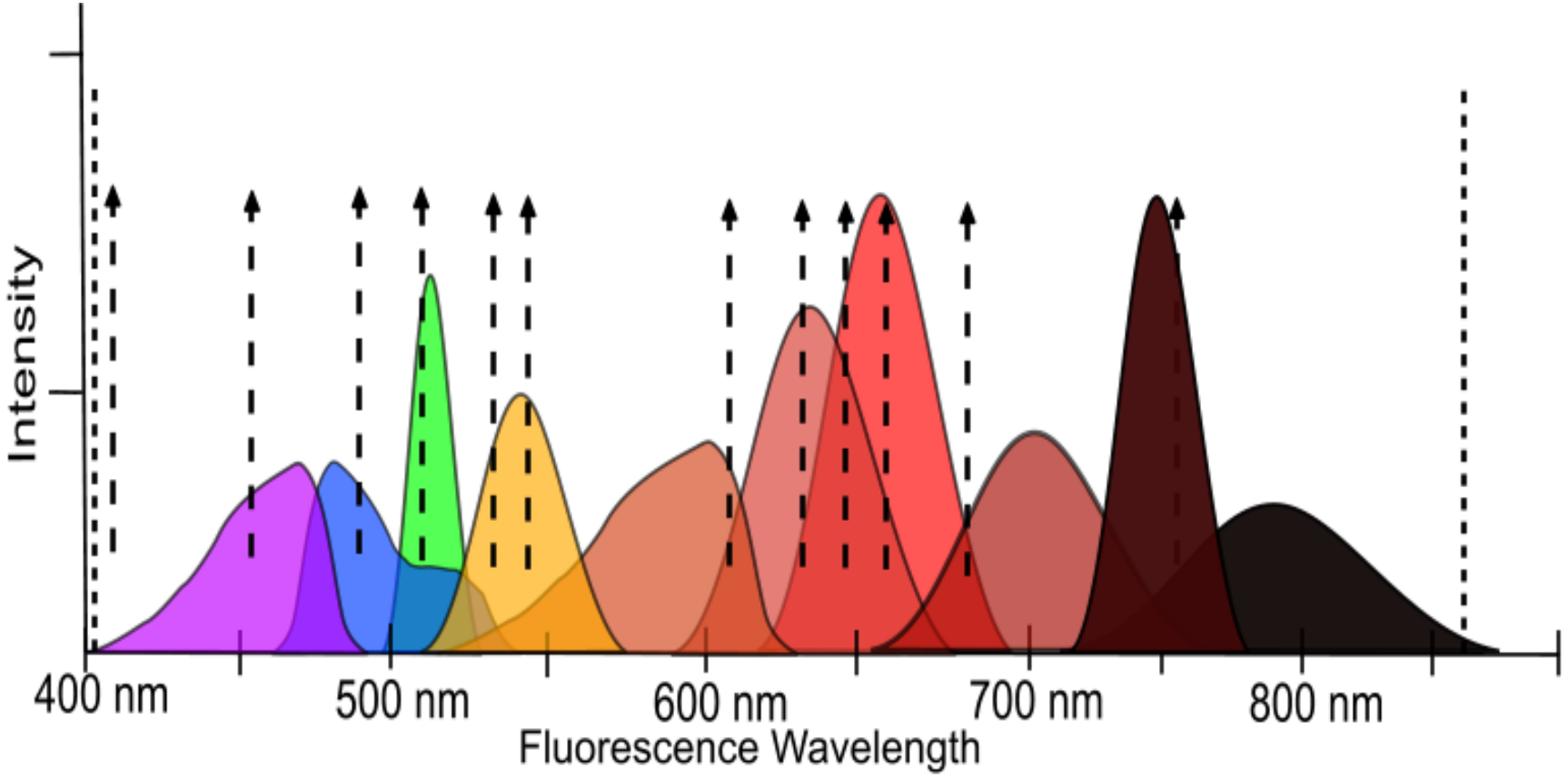
PCA Density Plot



Optical Design of a basic flow cytometer



Spectral Overlap makes for very complex analysis



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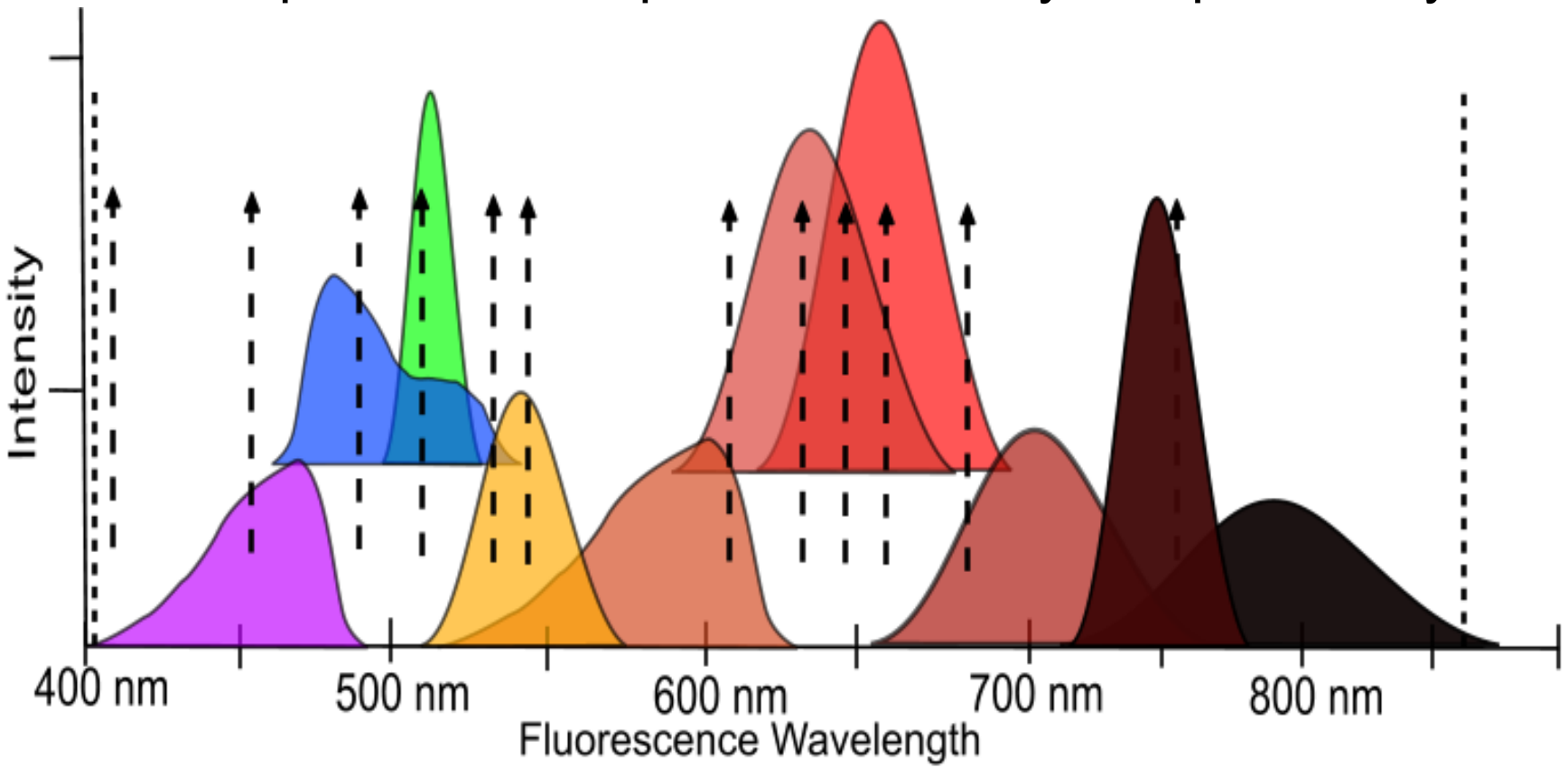
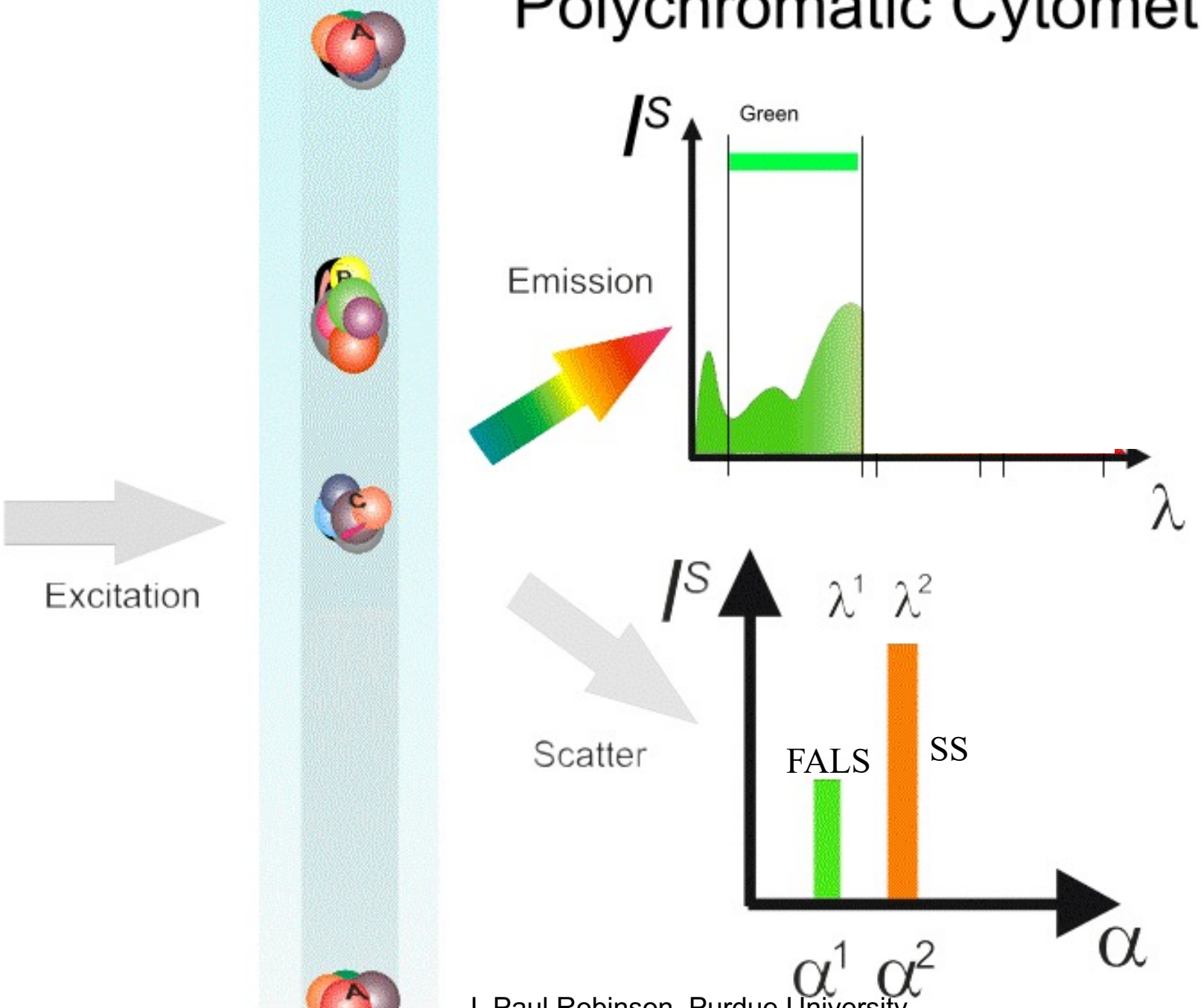
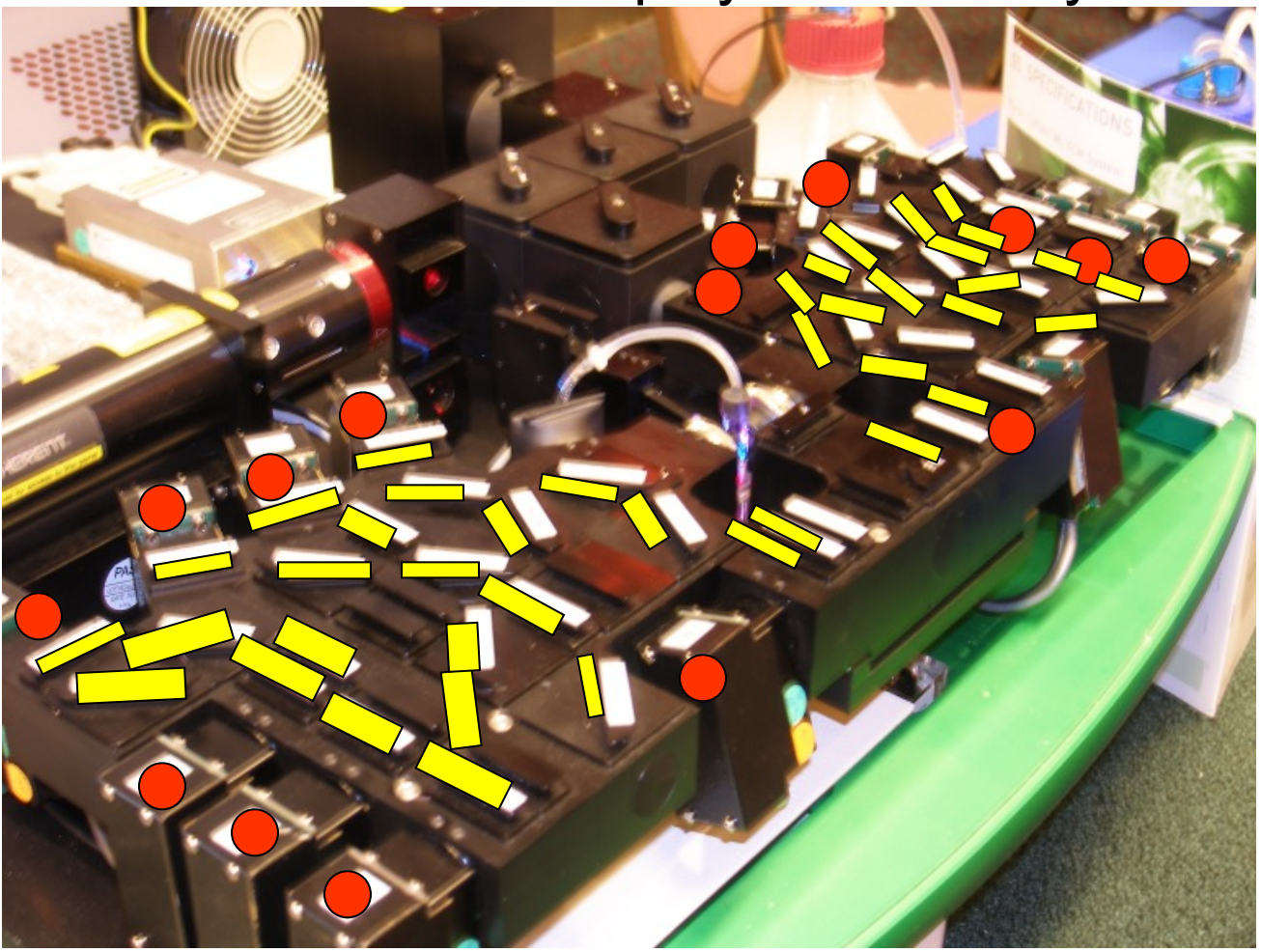


Polychromatic Cytometry



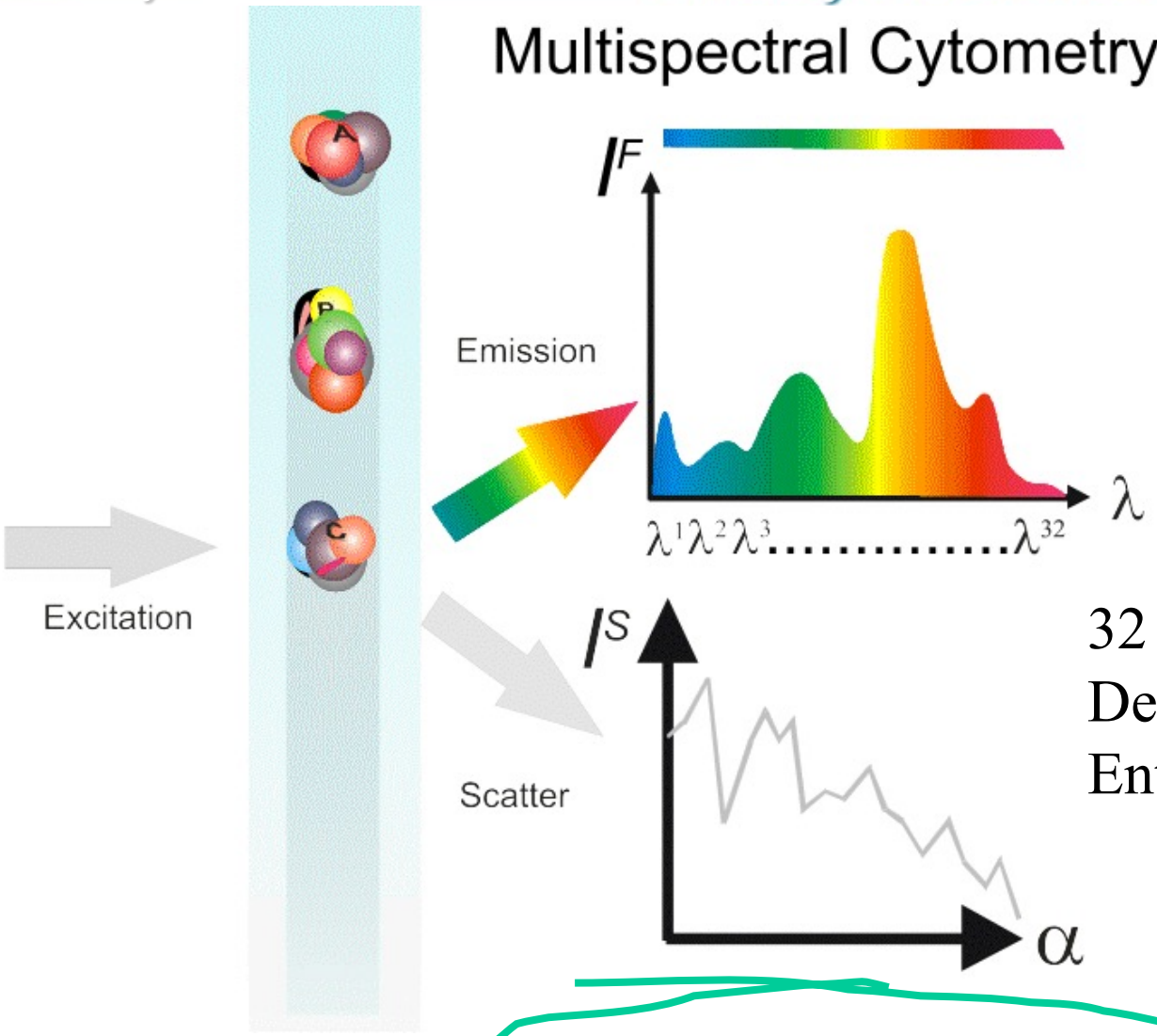
Advanced polychromatic cytometry



14 PMTs

41 filters

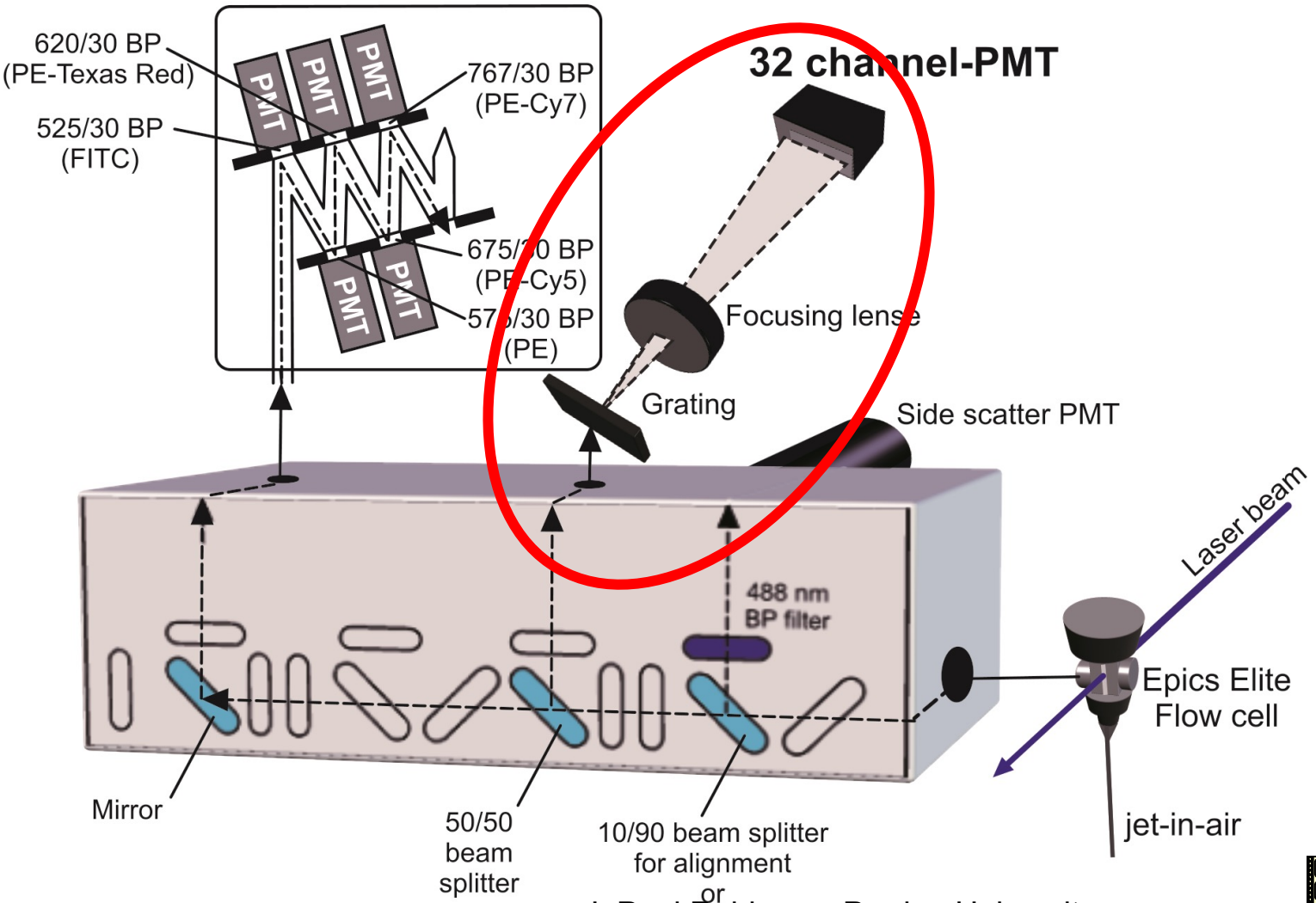
Multispectral Cytometry

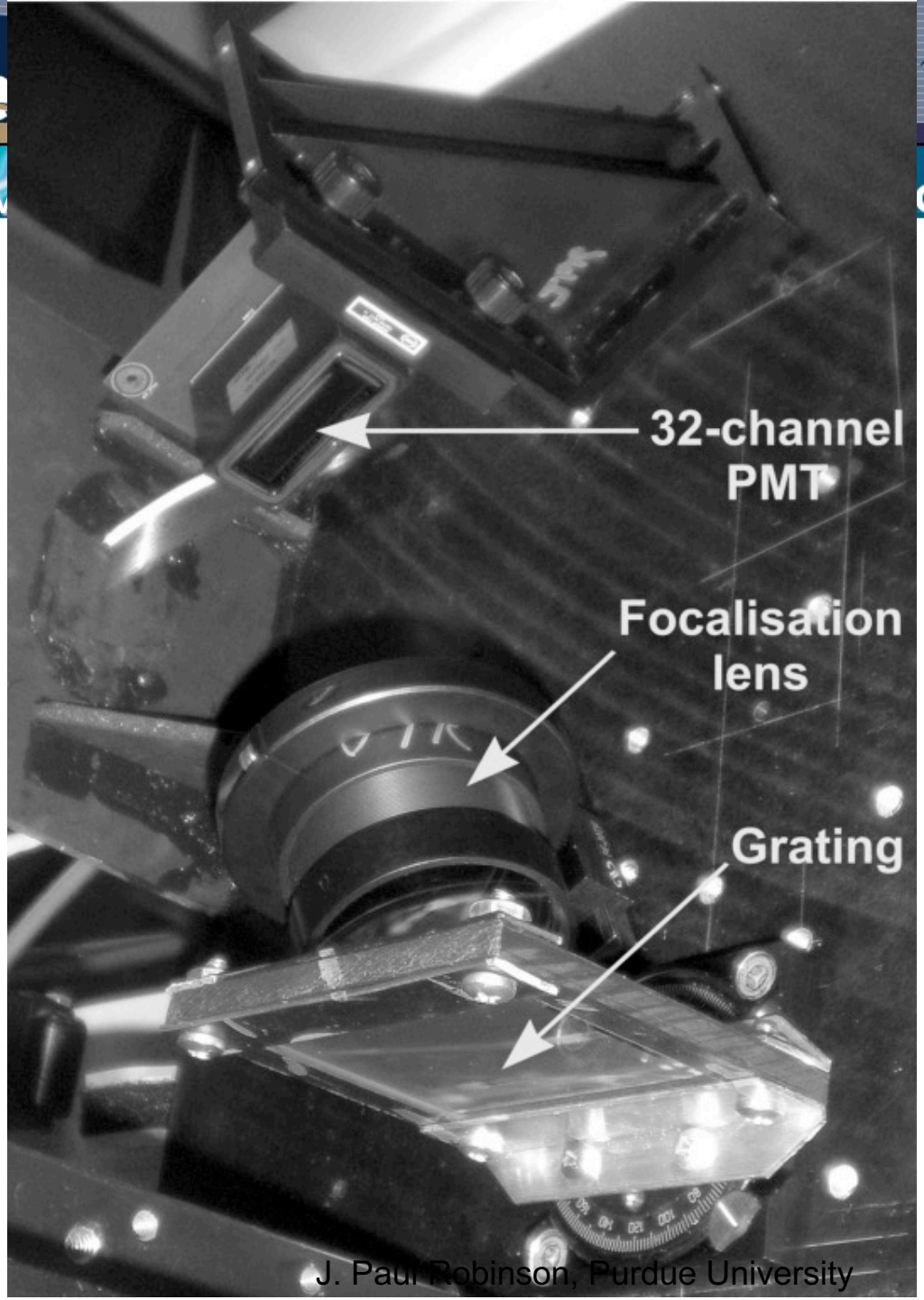


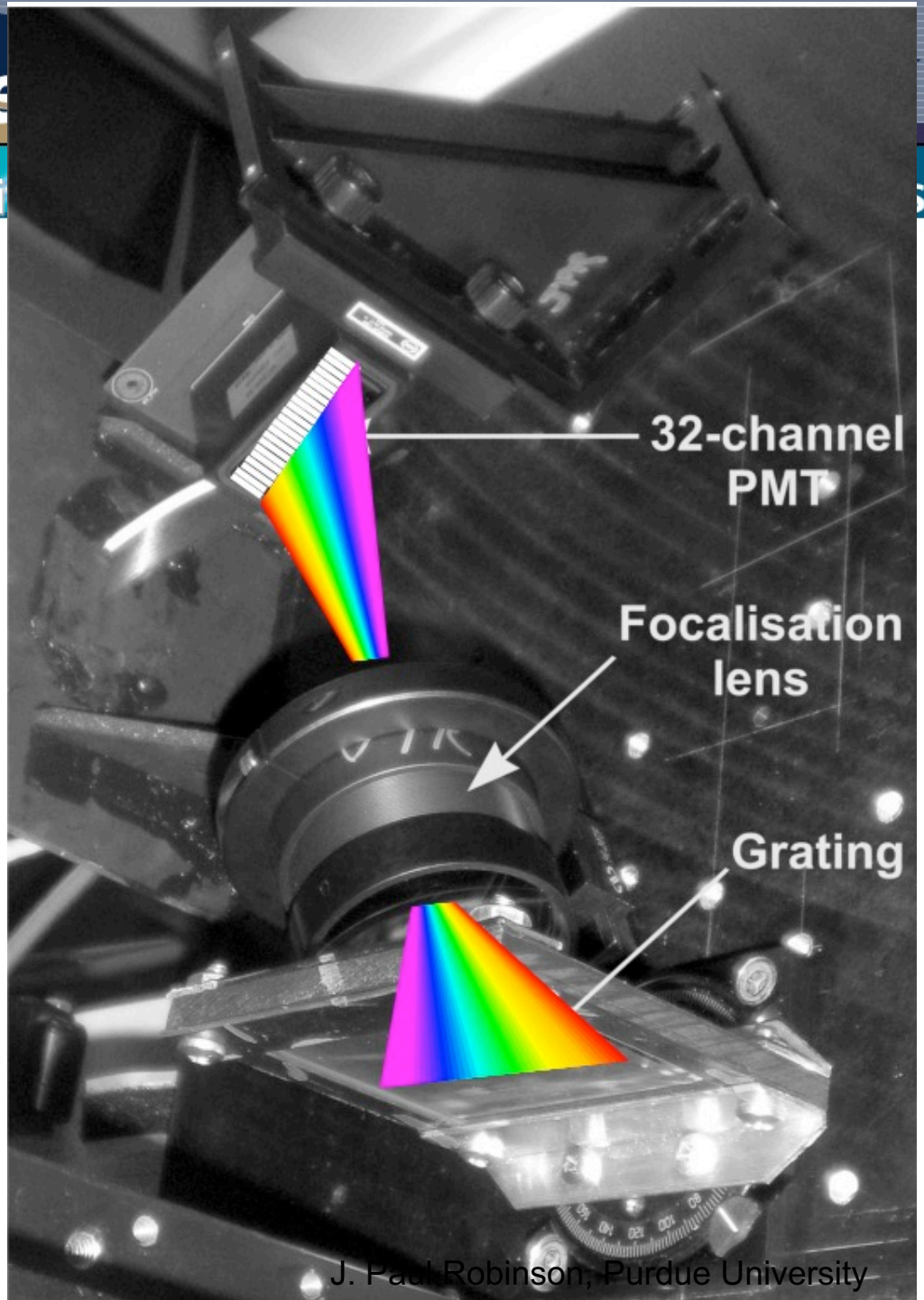
32 Channel Pulse Describes the Entire spectrum

Spectrum becomes a parameter

Asahi Spectra USA inc.
6-channel detection unit




32-channel
PMT
Focalisation
lens
Grating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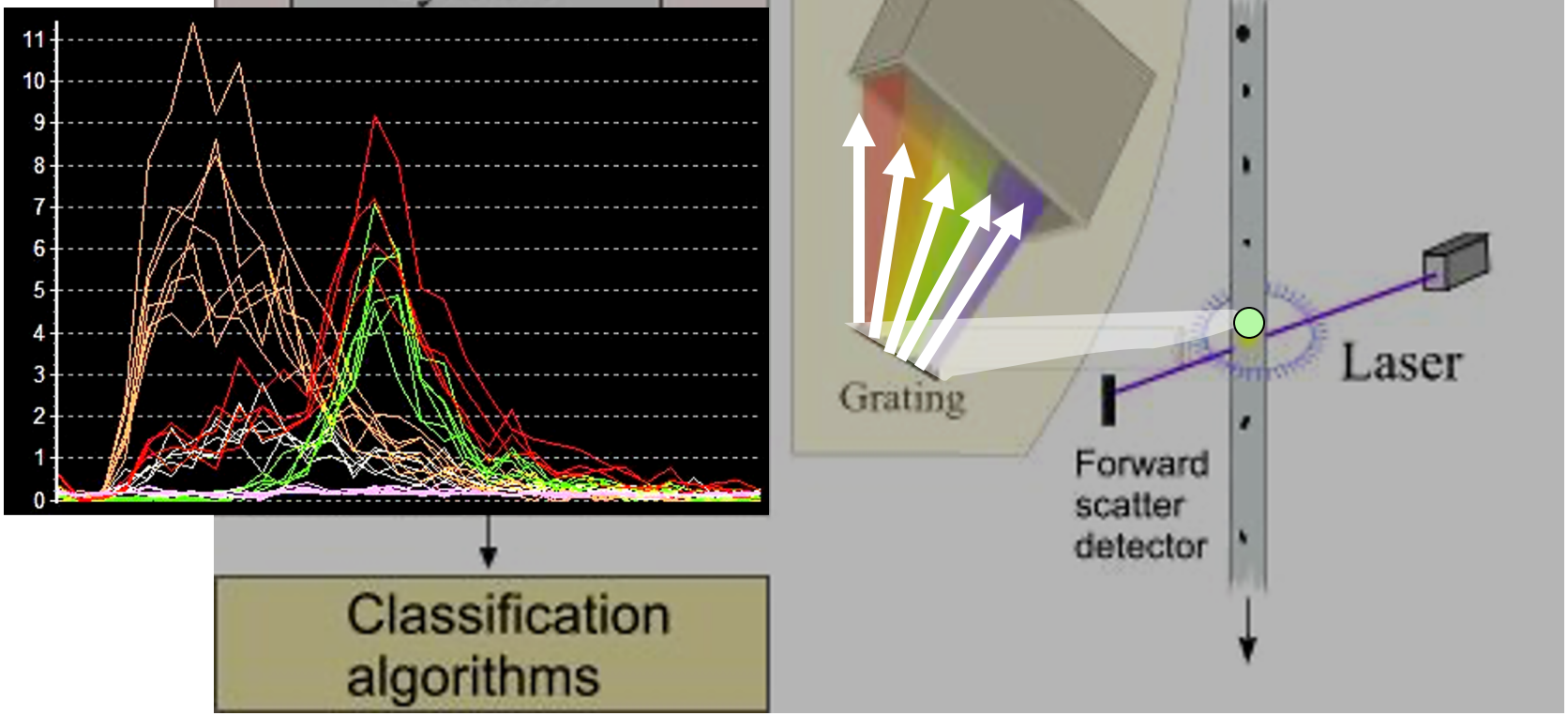
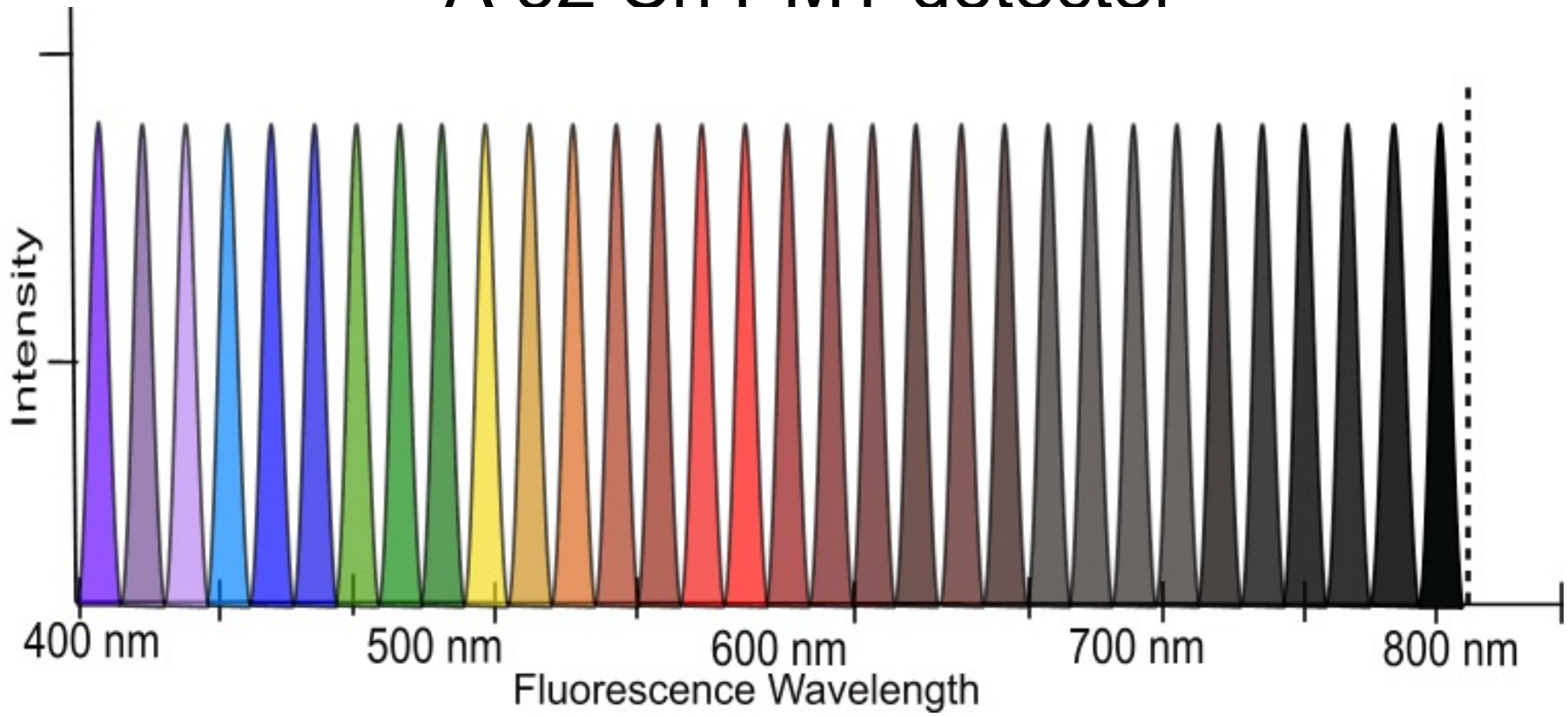
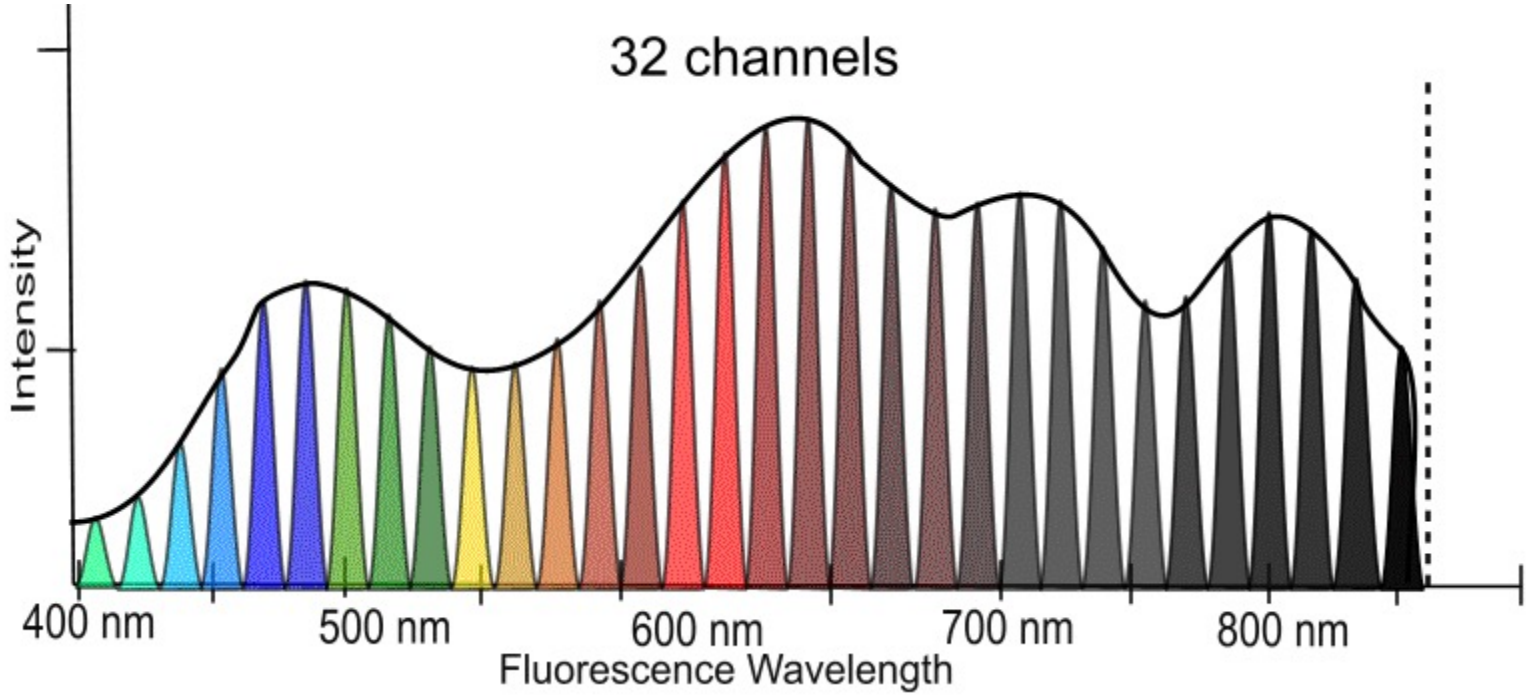
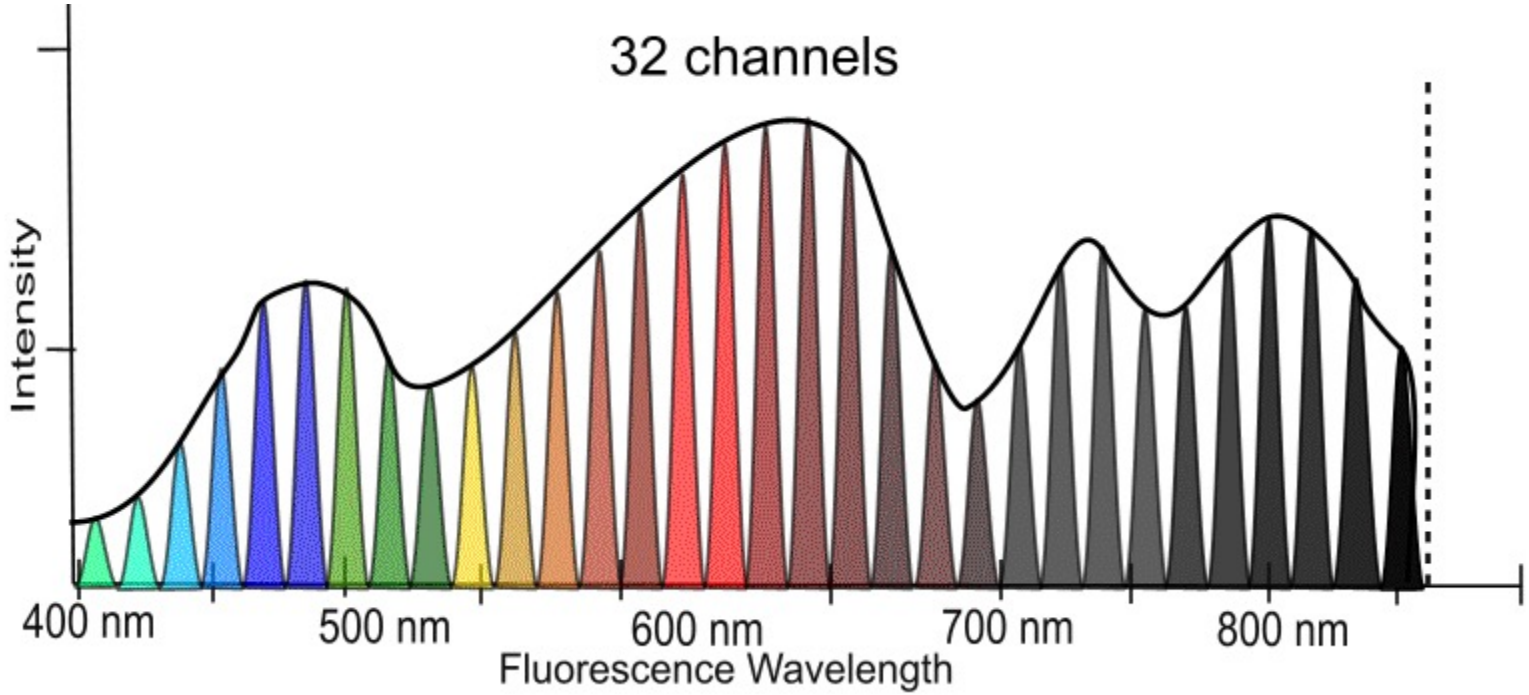

Multispectral detector
Computational system
Direction of flow
Grating
Laser
Forward scatter detector
Classification algorithms
11
10
9
8
7
6
5
4
3
2
1
0

A 32 Ch PMT detector




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


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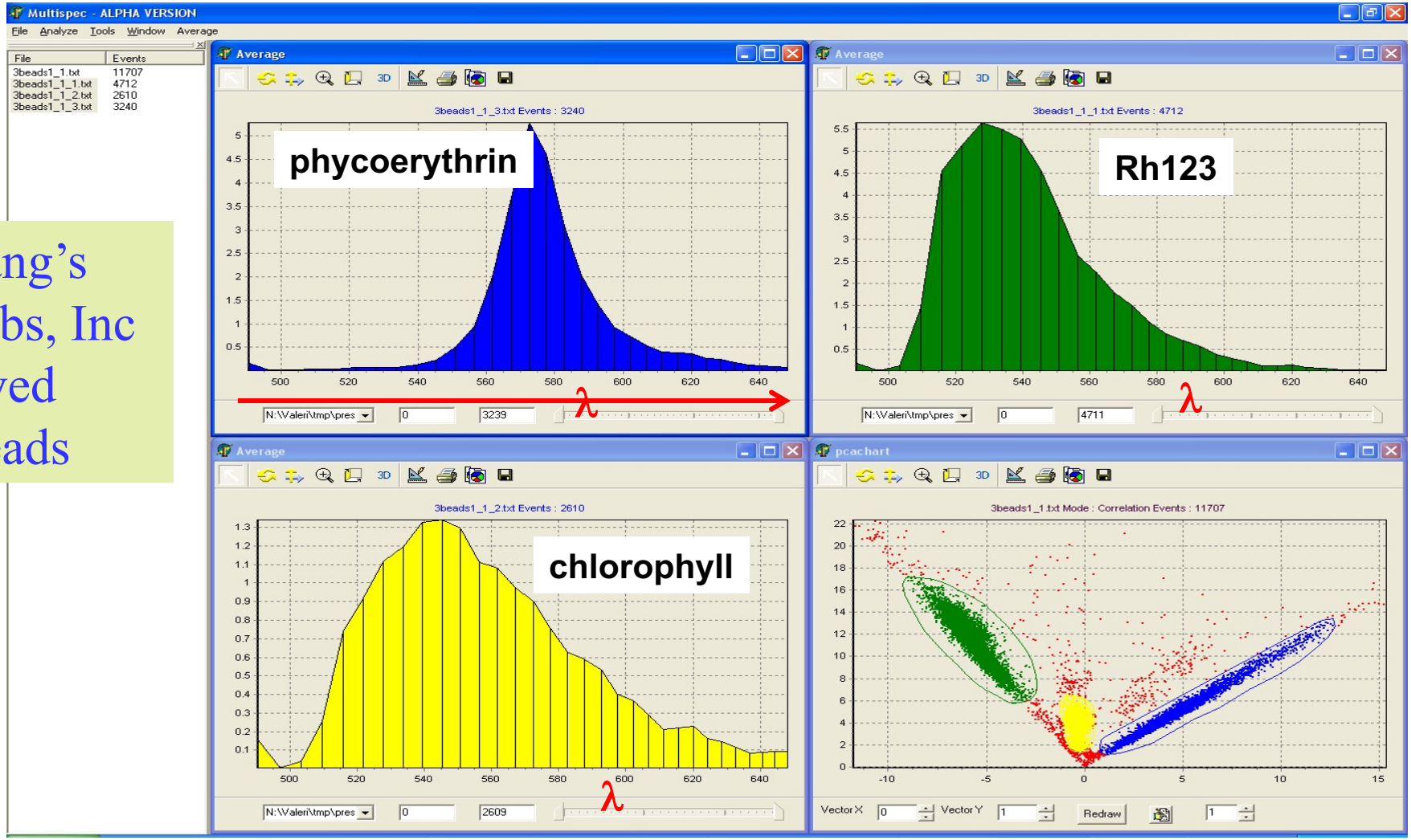
500 nm
600 nm
700 nm
800 nm
Fluorescence Wavelength

Bang's Labs, Inc Dyed Beads



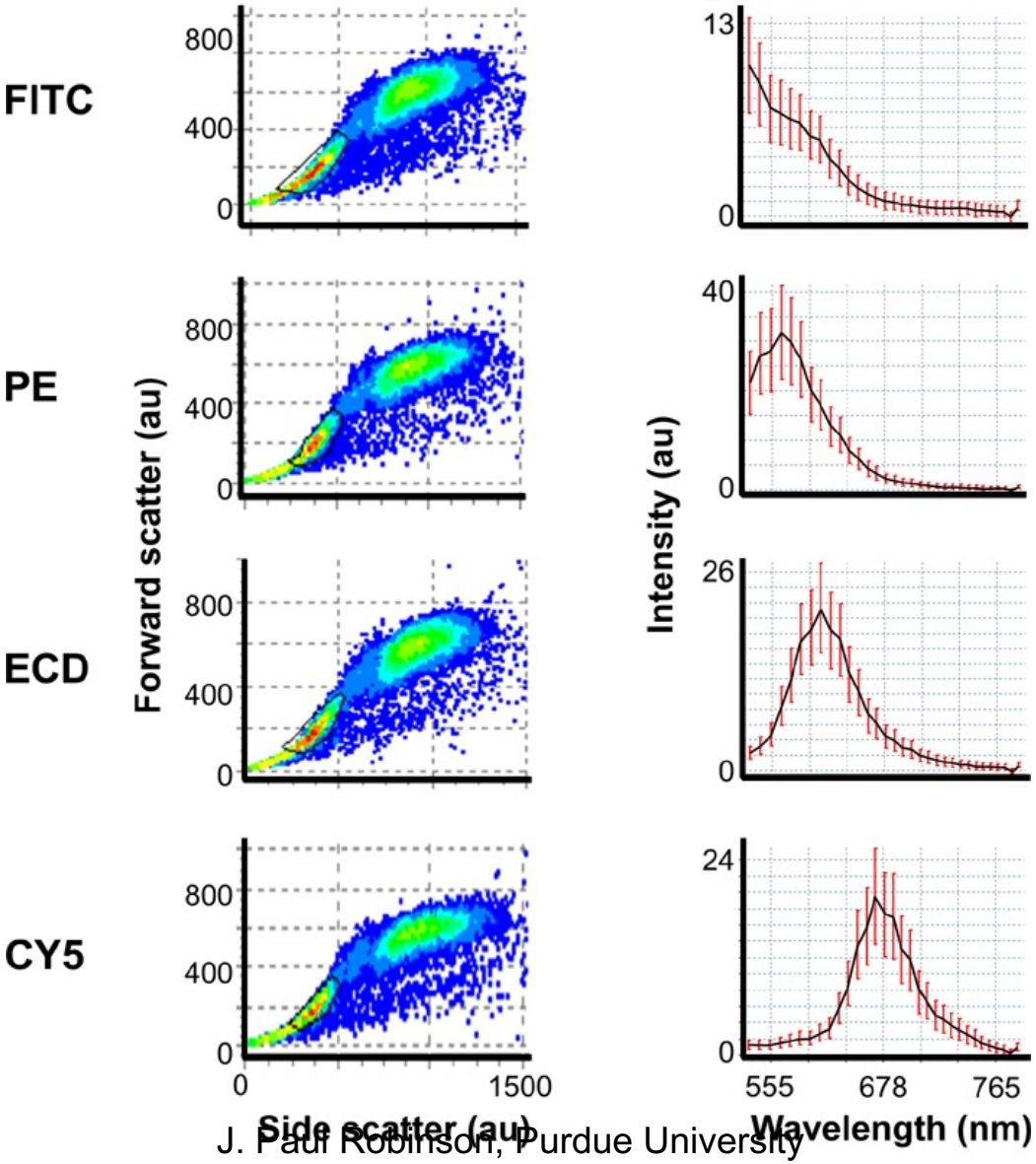
PUCL cytometry laboratories

What!! No need for gates???

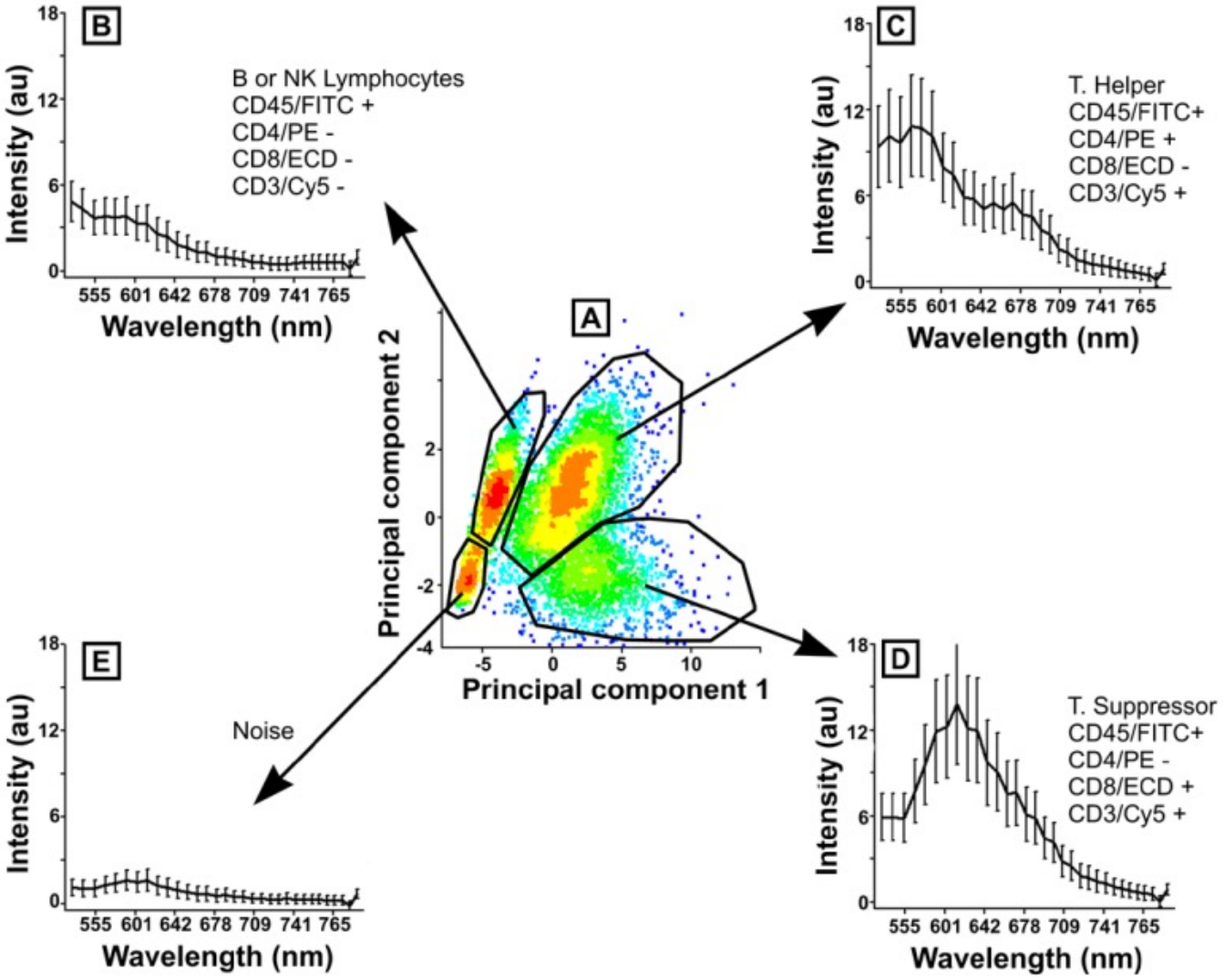
J. Paul Robinson, Purdue University

PURDUE UNIVERSITY

White Blood Cells



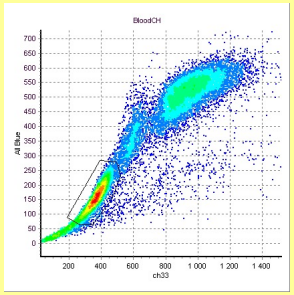
White Blood Cells



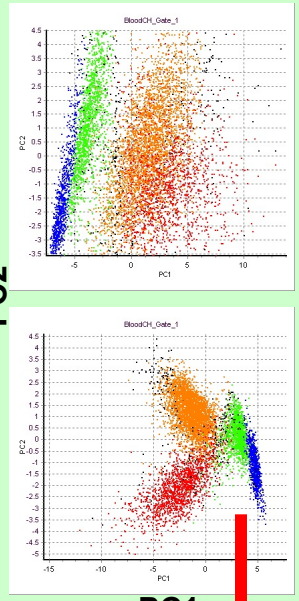
Comparisons of PCA results

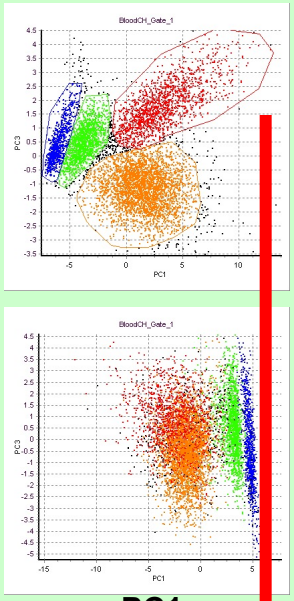
Purdue University

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

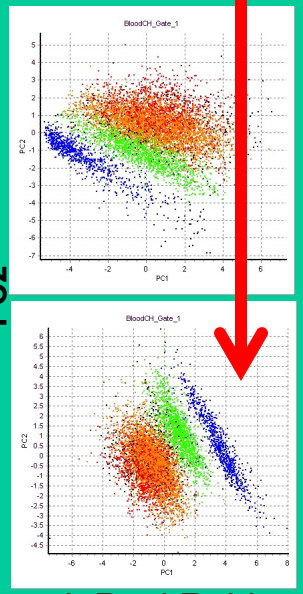


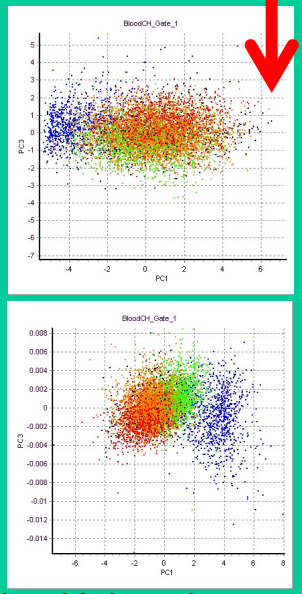


PCA (Correlation) on all the parameters :32channels + SSC+ FS(sum)+ RingB+ RingC+ RingD+ RingE,

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

6 PMTs device

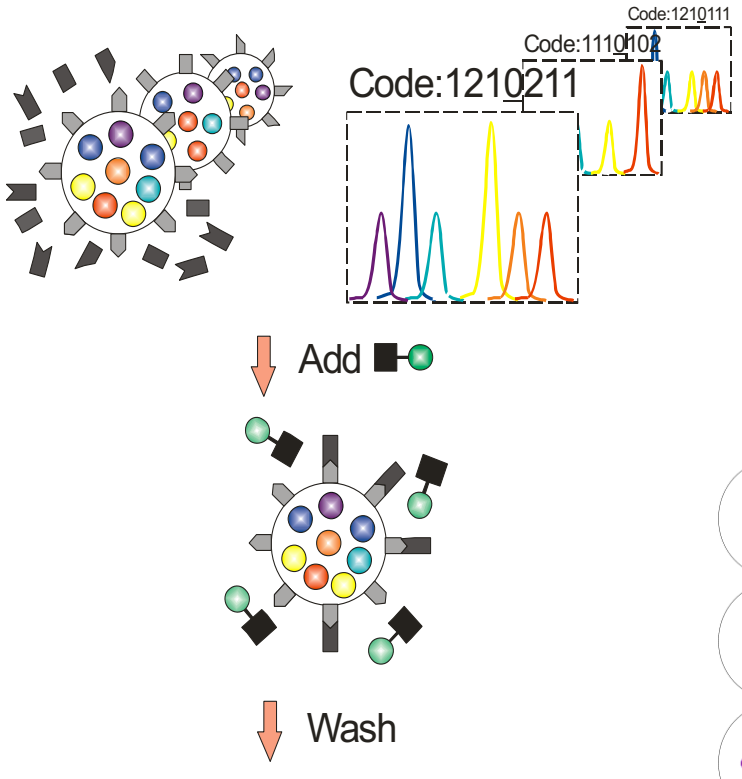




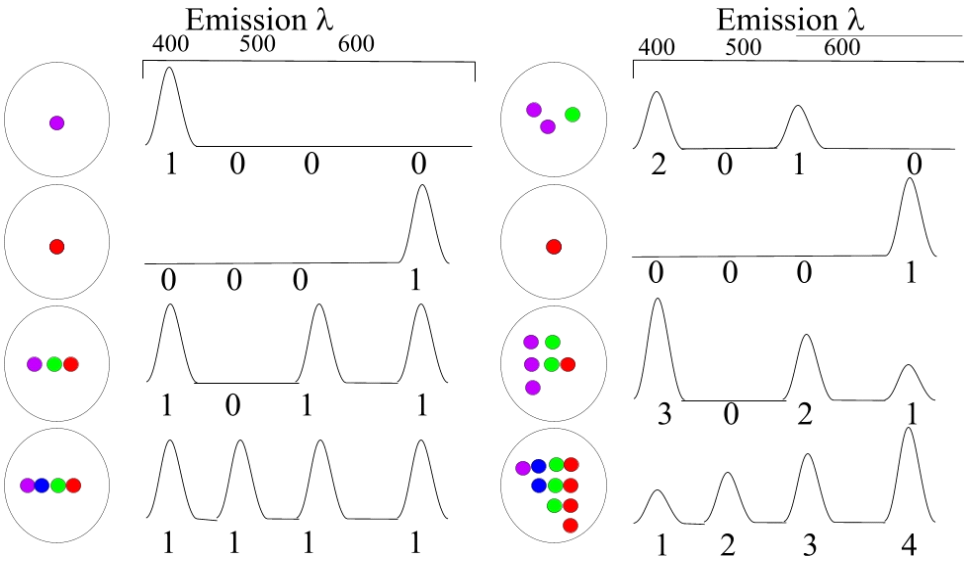
PCA (Correlation) on 4channels+SSC+ FS(sum)+ RingB+ RingC+ RingD+ RingE,

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

Nanocrystals/Micro-Dots multiplexed systems



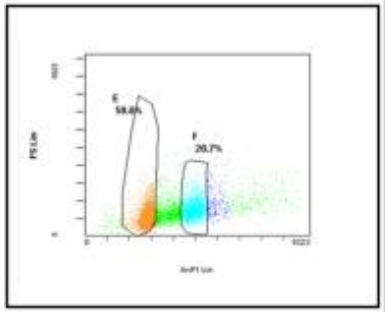
- New probes
- Potentially 1000's of combinations
- Sensitive, long lived, less bleaching
- Difficult to make
- Will require some advanced classification

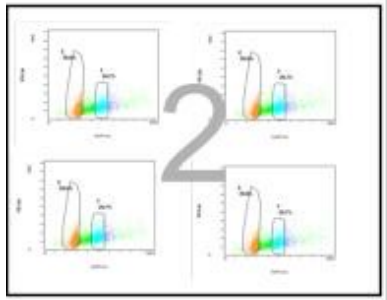


2. Automation & Automated Analysis

- Will improve accuracy because of standardization
- Will decrease time to analysis
- Will identify subsets otherwise missed
- Current software cannot analyze huge data sets
- Concept lends itself to more automation
- Only way to make flow cytometry a systems biology tool

So here is the problem….

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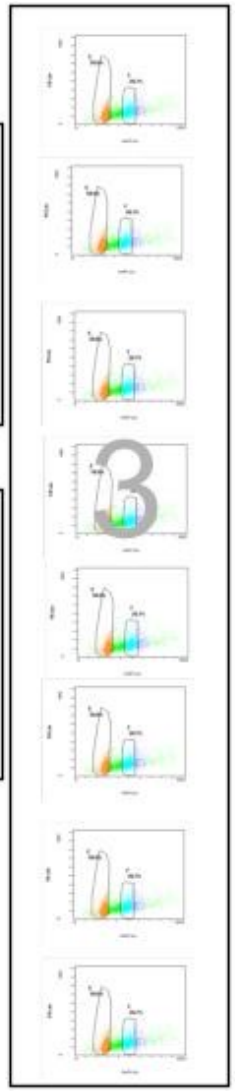
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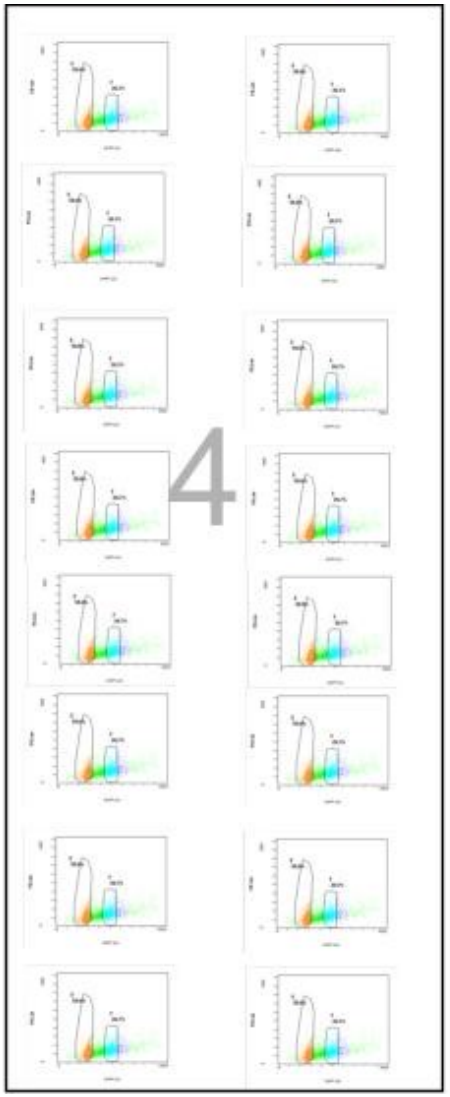
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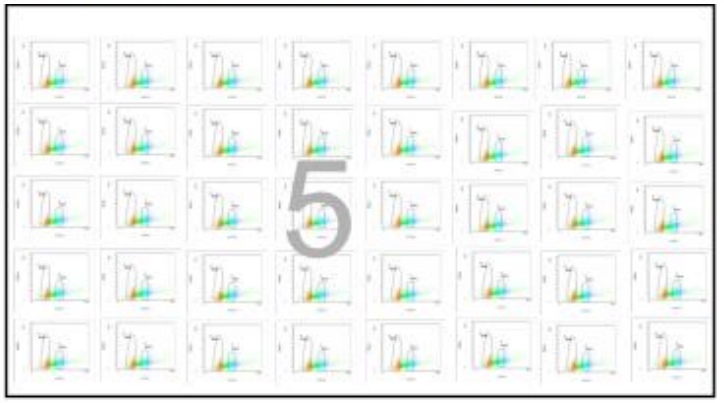
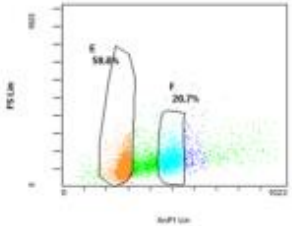

5


6


7

A complicated mess….
And perhaps hundreds of files

So several questions arise…



So several questions arise…

- What kinds of analytical tools do you need and how do you efficiently achieve an analytical solution?
- How do you handle huge data sets?
- What is the place of automation?
- Is it better to collect more variables/parameters on fewer cells..
- Or less variables/parameters and lots of cells….

and most people answer …

and most people answer …

- We want a lot of parameters and a lot of cells…..fast…..and easy…..

So 2 examples of very large data sets….

Discovery Park

Purdue University

It will

Bindley Bioscienc

HT Analysis Assays
* Range of redox assays
* Mitochondrial MP
* Glutathione
* Oxidative metabolism
* Viability
* Superoxide production

HT Analysis tools
* Automated data processing
* Whole plate analysis
* Parallel processing of data
* Multiple gating capabilities
* Multiple parameter data
* Ungated analysis options
* Statistical data processing

HT Screening tools
* Automated assay preparation
* Rapid high content sampling
* Automated Assay collection
* Very low volume samples
* 20,000 samples per day
* High precision and reproducibiligy

Assay Development for Integration of Systems
* Combining flow and image data by running identical assays
* develop models that define phenotypes

J. Paul Robinson, Purdue University

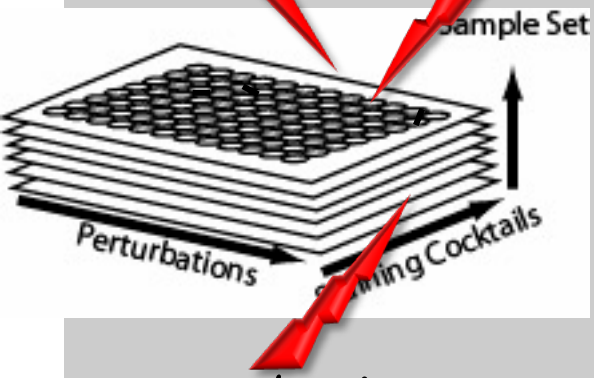
PUCL
cytometry laboratories

Primary T-Lymphocyte Data

Conditions (96 well format)

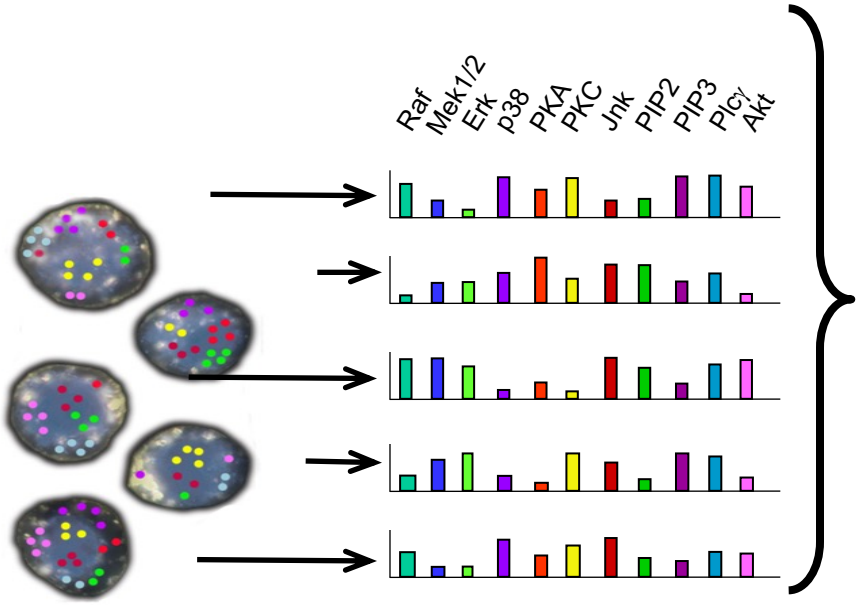
perturbation a

perturbation b



perturbation n

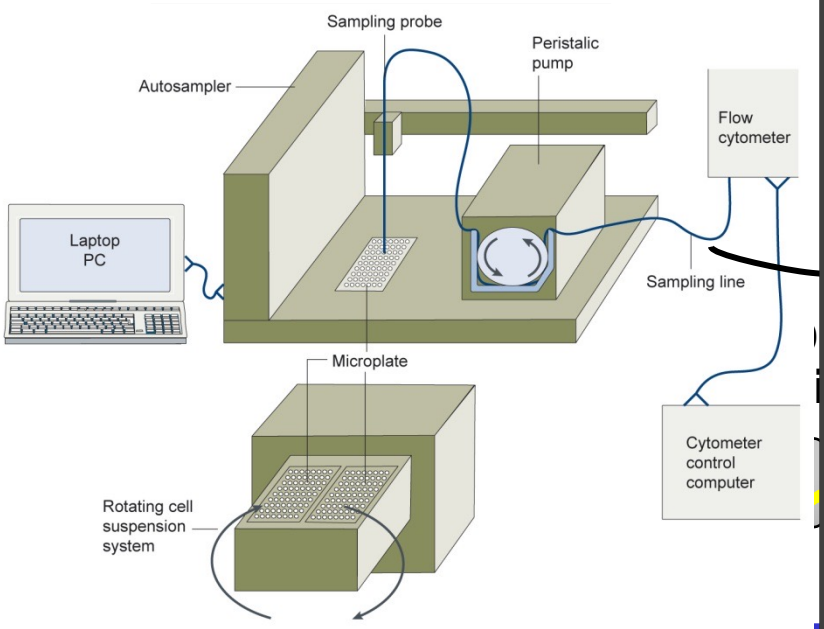
11 Color Flow Cytometry



- 9 phosphoproteins, 2 phospholipids
- 600 cells per condition
  - 5400 data-points

- Primary human T-Cells
- 9 conditions
  - (6 **Specific** interventions)

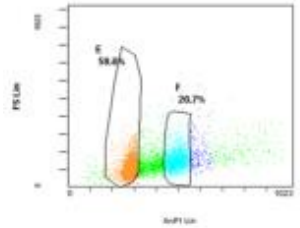
HyperCyt system added to flow cytometer



HyperCyte diagram kindly supplied by Larry Sklar

384 wells/10 min
1 µl/sample 5000 cells/µl

Too much information to comprehend as small pieces….

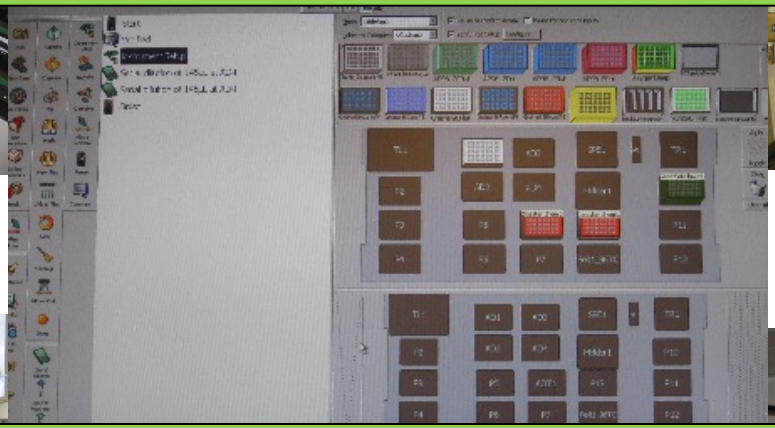


Systems biology is looking at the big picture…

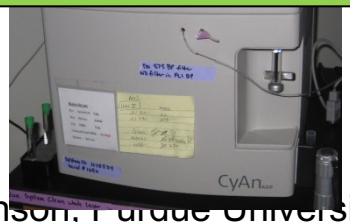
References: **Robinson, J. Paul**, Durack, Gary & Kelley, Stephen: "*An innovation in flow cytometry data collection & analysis producing a correlated multiple sample analysis in a single file*". *Cytometry* 12:82-90,1991.

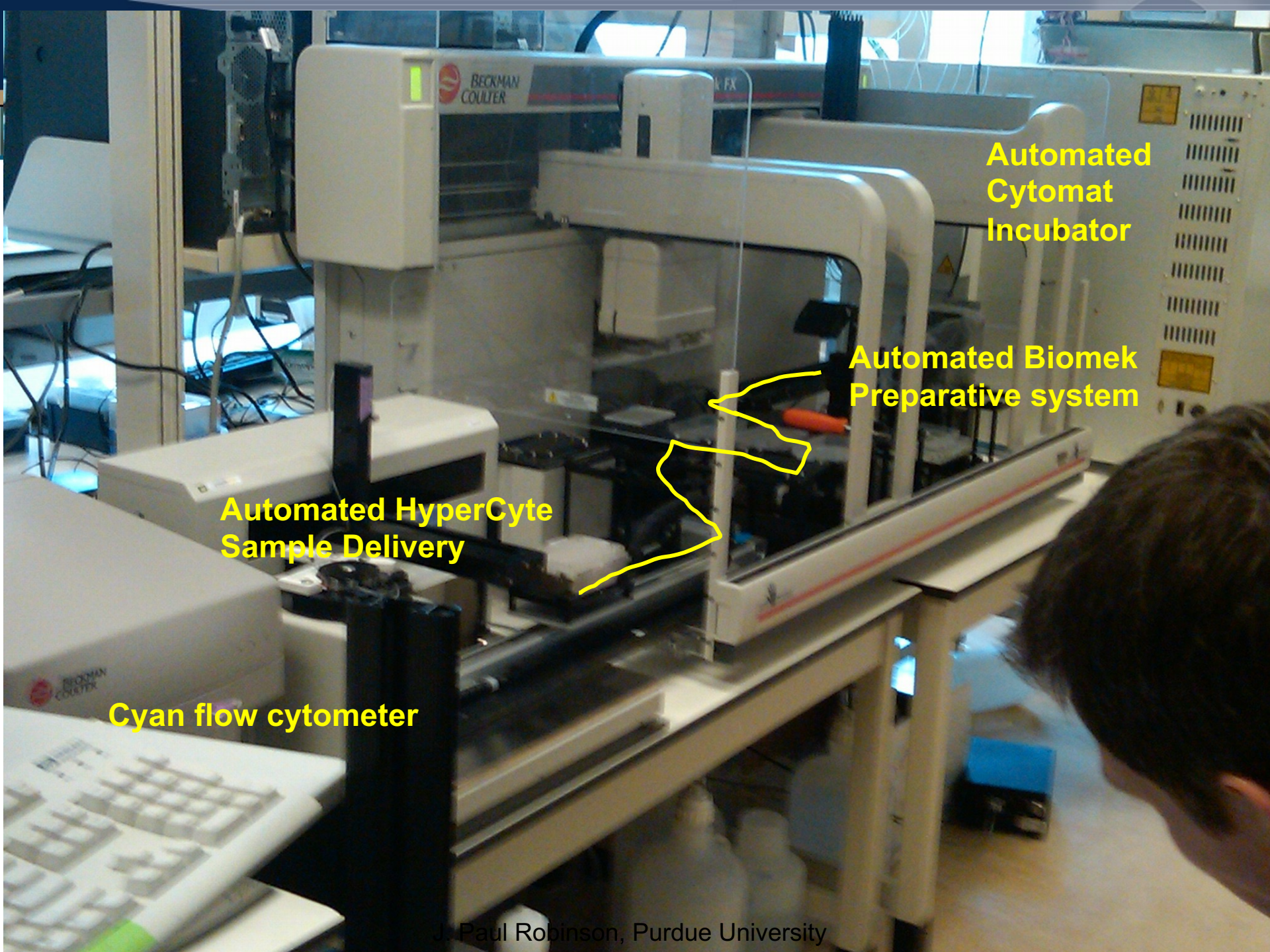
Durack, Gary, Lawler, Gretchen, Kelley, Stephen, Ragheb, Kathy, **Robinson, J. Paul**: "*Time Interval Gating for analysis of cell function using flow cytometry*" *Cytometry*, 12:701-706, 1991

Automation is not the process of having everyone in the lab work really fast!

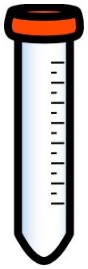


Automation that is slow and painful has little to no real value!




Automated
Cytomat
Incubator
Automated Biomek
Preparative system
Automated HyperCyte
Sample Delivery
Cyan flow cytometer

Multiparametric cytometry and multifactorial HTS



$$c_1 = [m_1, m_2, m_3, \ldots m_n]$$
$$c_2 = [m_1, m_2, m_3, \ldots m_n]$$
$$c_3 = [m_1, m_2, m_3, \ldots m_n]$$
$$\vdots$$
$$c_n = [m_1, m_2, m_3, \ldots m_n]$$

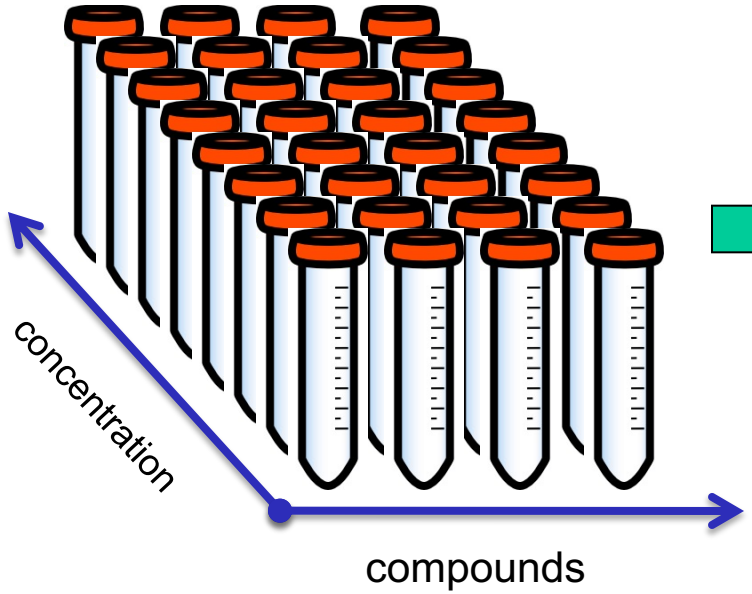
Number of cells is measured,
Fluorescence intensity is a feature

$g(c)$

Define multiple populations in the feature space

Find the features describing the populations $f_1, \ldots, f_n$

Flow cytometry



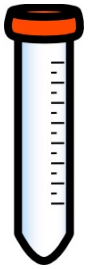
$$r = \begin{pmatrix} m_{11} & \cdots & m_{1n} \\ \vdots & \ddots & \vdots \\ m_{m1} & \cdots & m_{mn} \end{pmatrix}$$

Perform $m \times n$ single measurements

Find the features (such as IC_{50})

HTS

Multiparametric and multifactorial HT cytometry

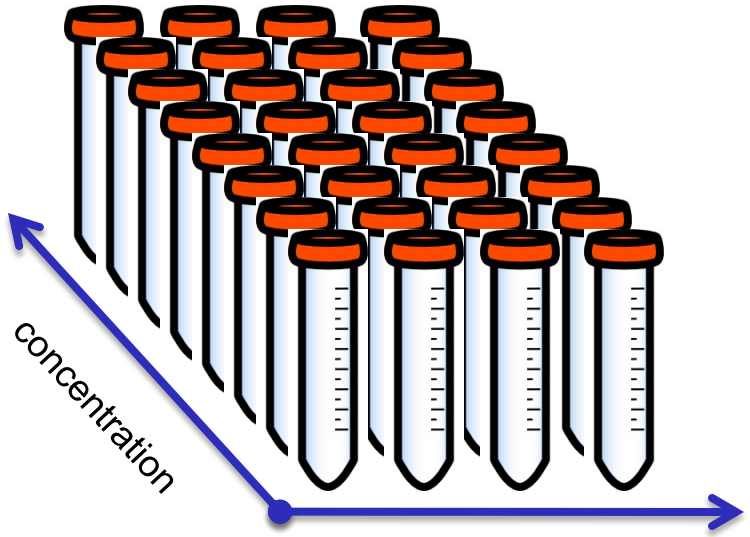


$$c_1 = [m_1, m_2, m_3, \ldots m_n]$$
$$c_2 = [m_1, m_2, m_3, \ldots m_n]$$
$$c_3 = [m_1, m_2, m_3, \ldots m_n]$$
$$\vdots$$
$$c_n = [m_1, m_2, m_3, \ldots m_n]$$

$g(c)$

Find the features describing the populations f_1,.. f_n

Define multiple populations in the feature space



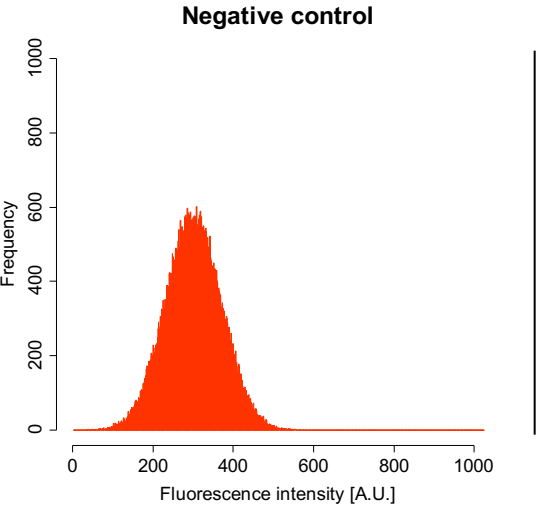
$$r = \begin{pmatrix} m_{11} & \cdots & m_{1n} \\ \vdots & \ddots & \vdots \\ m_{m1} & \cdots & m_{mn} \end{pmatrix}$$

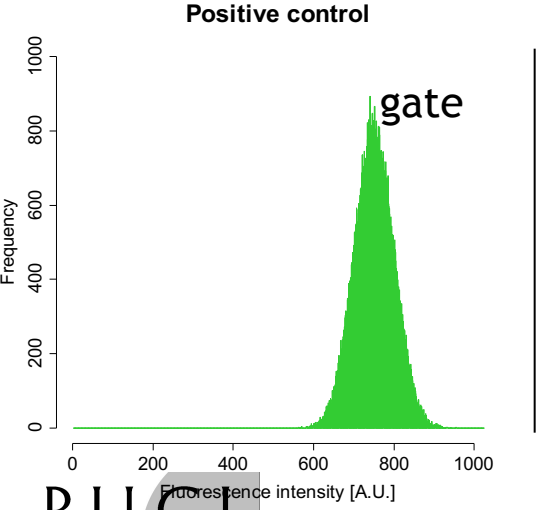
Find the features (such as IC_{50})

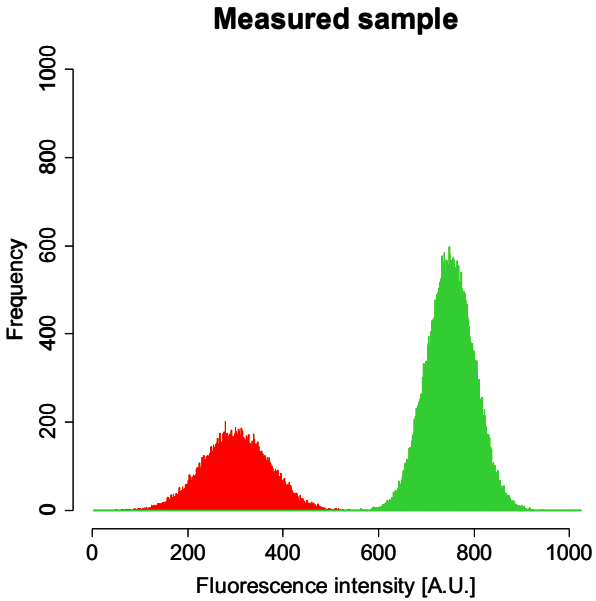
Create $n \times m$ array of features, where every m could be a vector $[f_1,\ldots,f_n]$

HT flow cytometry

Problem 1: eliminate operator's input in analysis







Calculate fraction of "positive" and "negative" cells

Problems?

Well… plenty actually

J. Paul Robinson, Purdue University

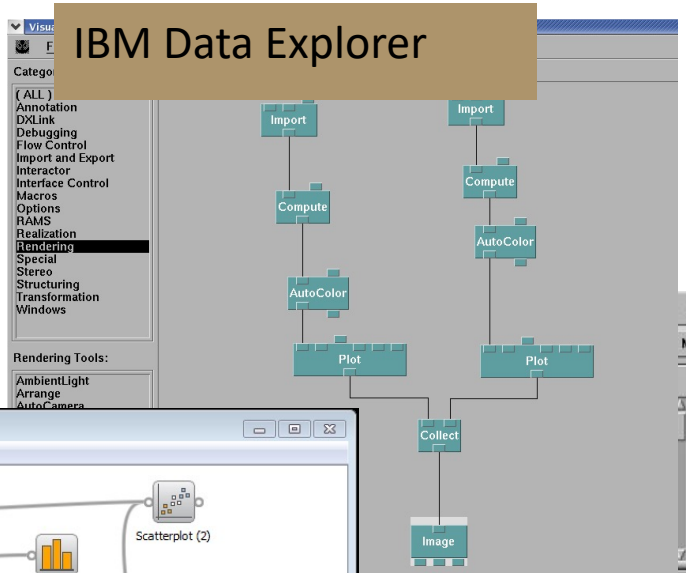
- **Method I**
  - Sort the apples ("gate" the green and red populations, or red and non-red)
  - Count red and green (non-red)
  - Compare the counts
- **Method II**
  - Find a bucket full of red apples only
  - Find the distribution
  - Take a bucket with red and green apples and find the distribution
  - Compare the distribution

Alternative?

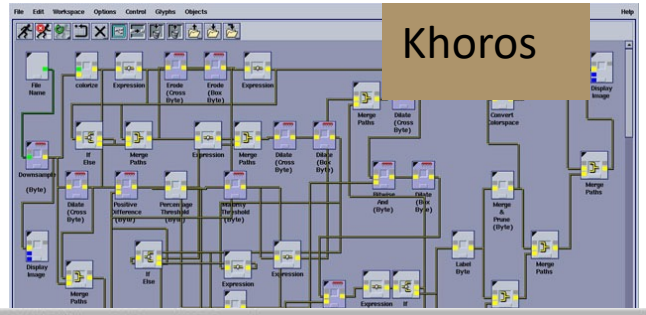
- Heuristic methods
  - Minkowski-form
  - Weighted-Mean-Variance (WMV)
- Distance functions used in nonparametric tests
  - χ^2 (Chi Square)
  - Kolmogorov-Smirnov (KS)
  - Cramer/von Mises (CvM)
- Information-theory divergences
  - **Kullback-Liebler (KL)**
  - **Jeffrey divergence (JD)**
- Spectral measures
  - Spectral angle mapper
  - Bhatacharyya distance
- Ground distance measures
  - Histogram intersection (Overton)
  - **Quadratic form distance (QF)**
  - **Wasserstein-Rubinstein-Mallows distance (Earth Movers Distance)**

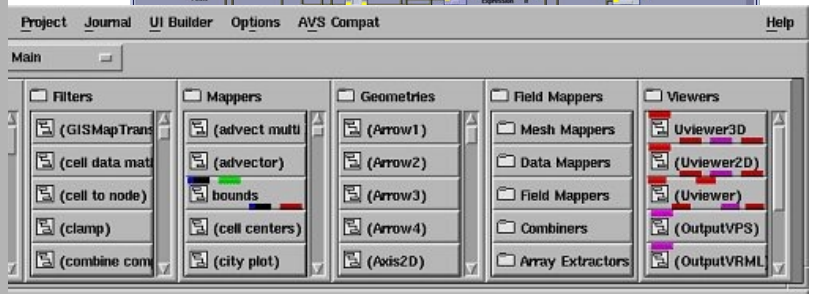
Graphical data processing environments

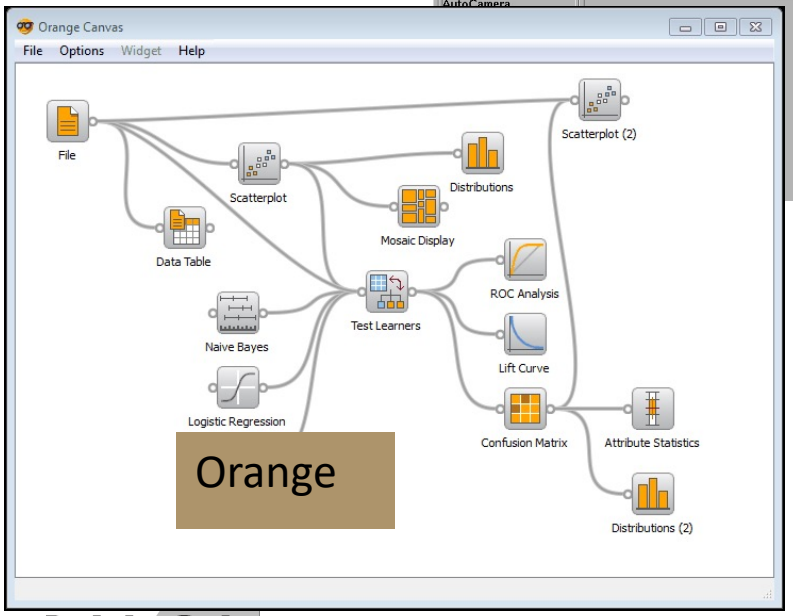
IBM Data Explorer



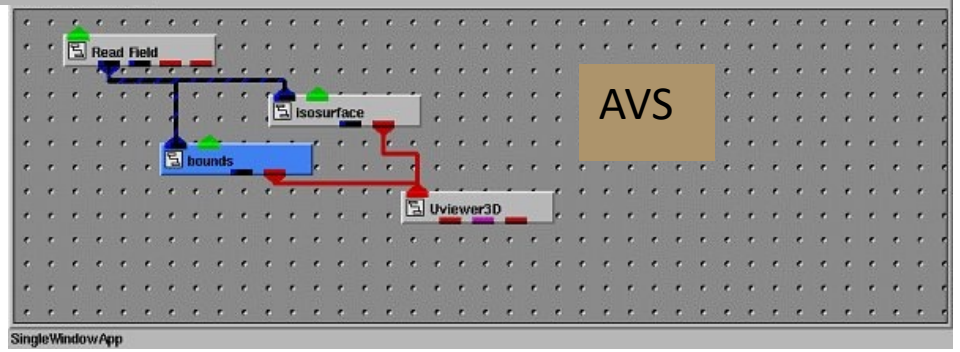
Khoros





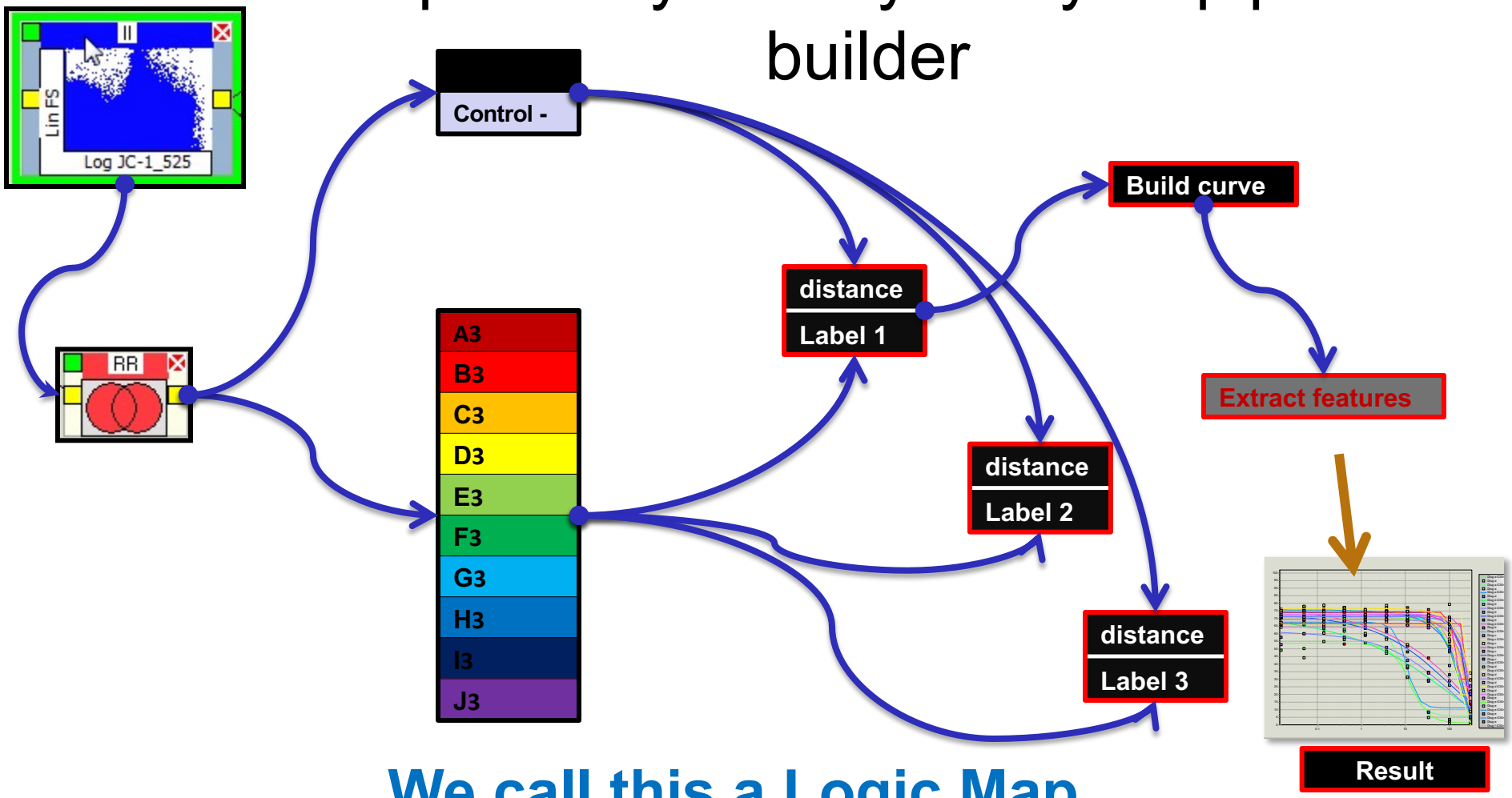


Orange



AVS

Graphical cytometry analysis pipeline builder



We call this a Logic Map

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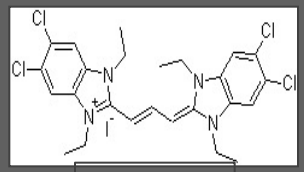
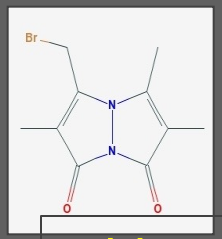
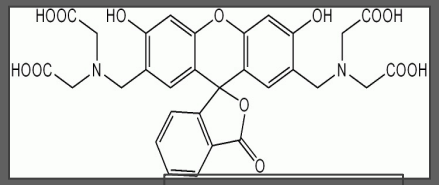
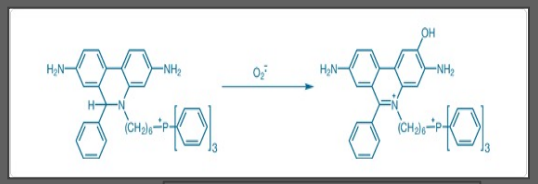
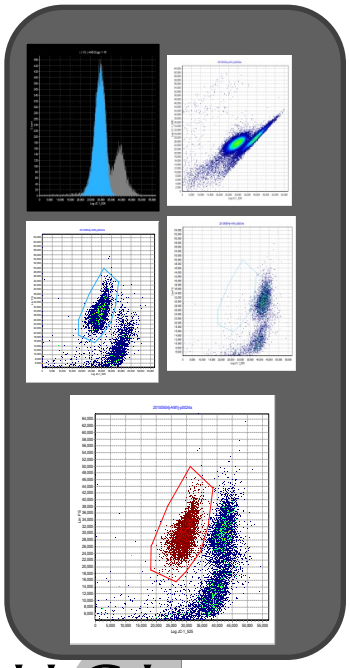
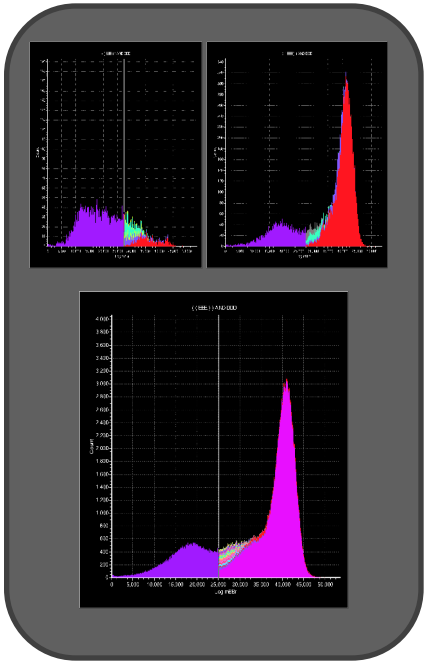

JC-1

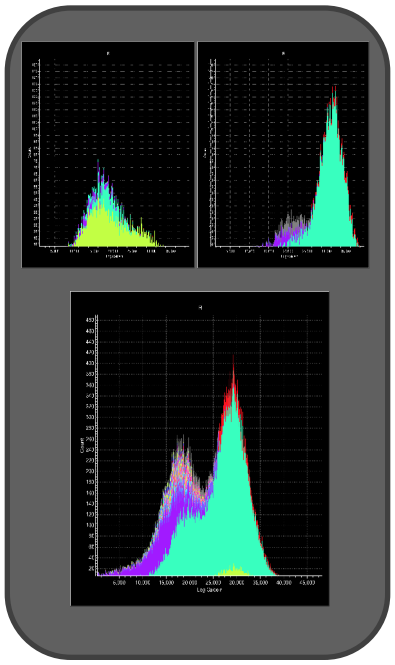

mbbr

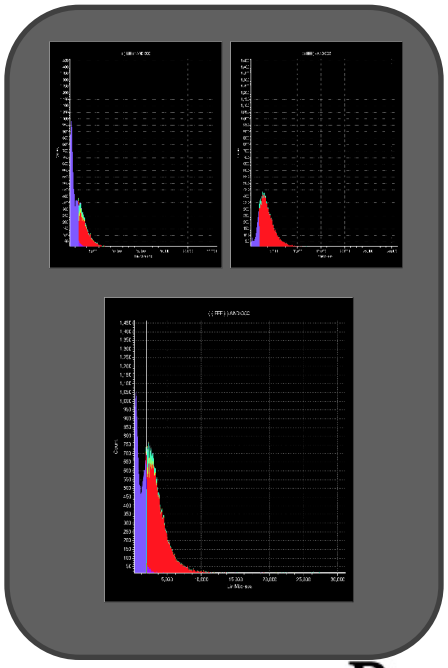

Calcein


Mitosox [1]





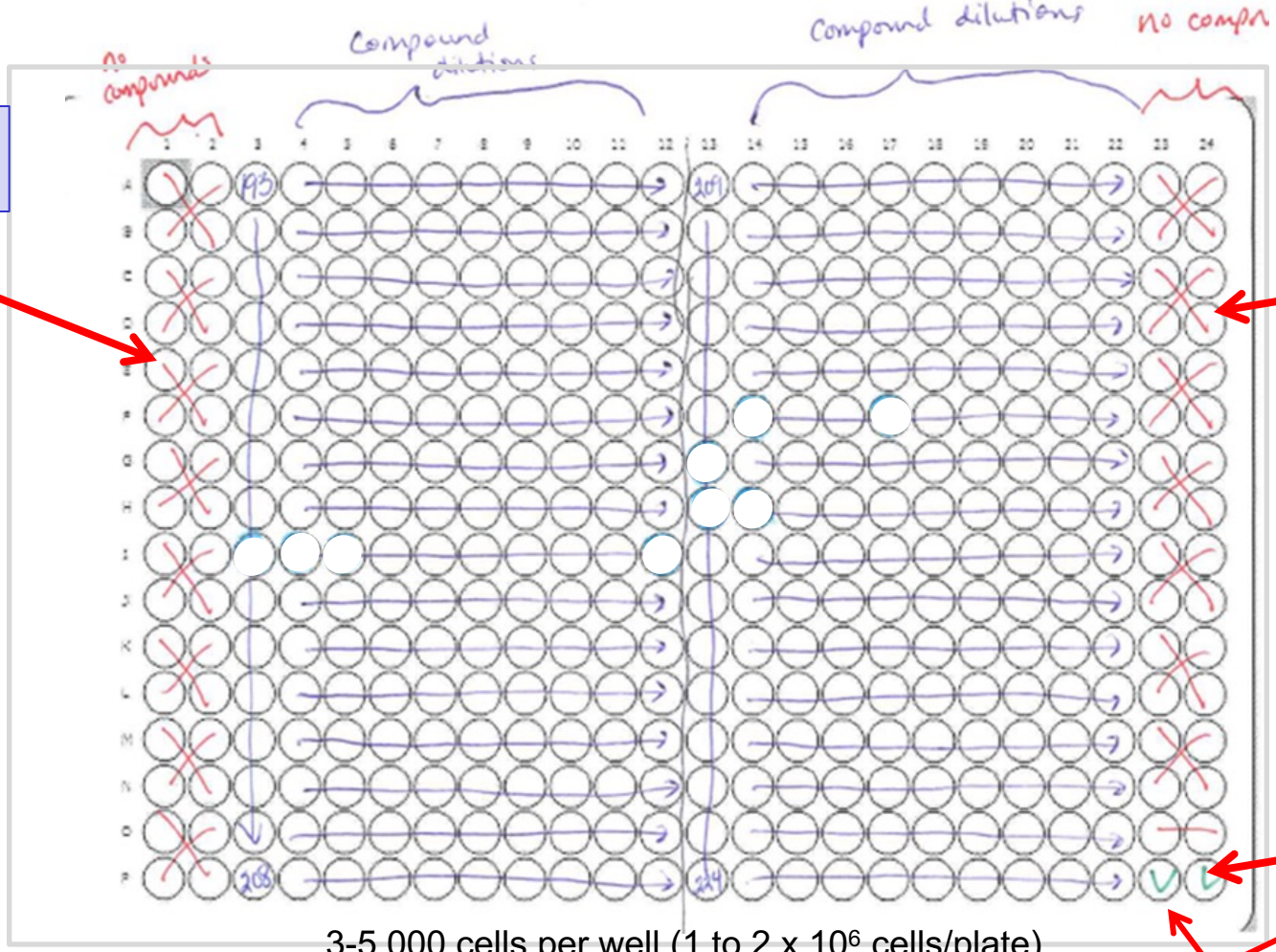




384 Well Plate Format For drug screen - Redox

Redox
3 color assay

Negative control wells

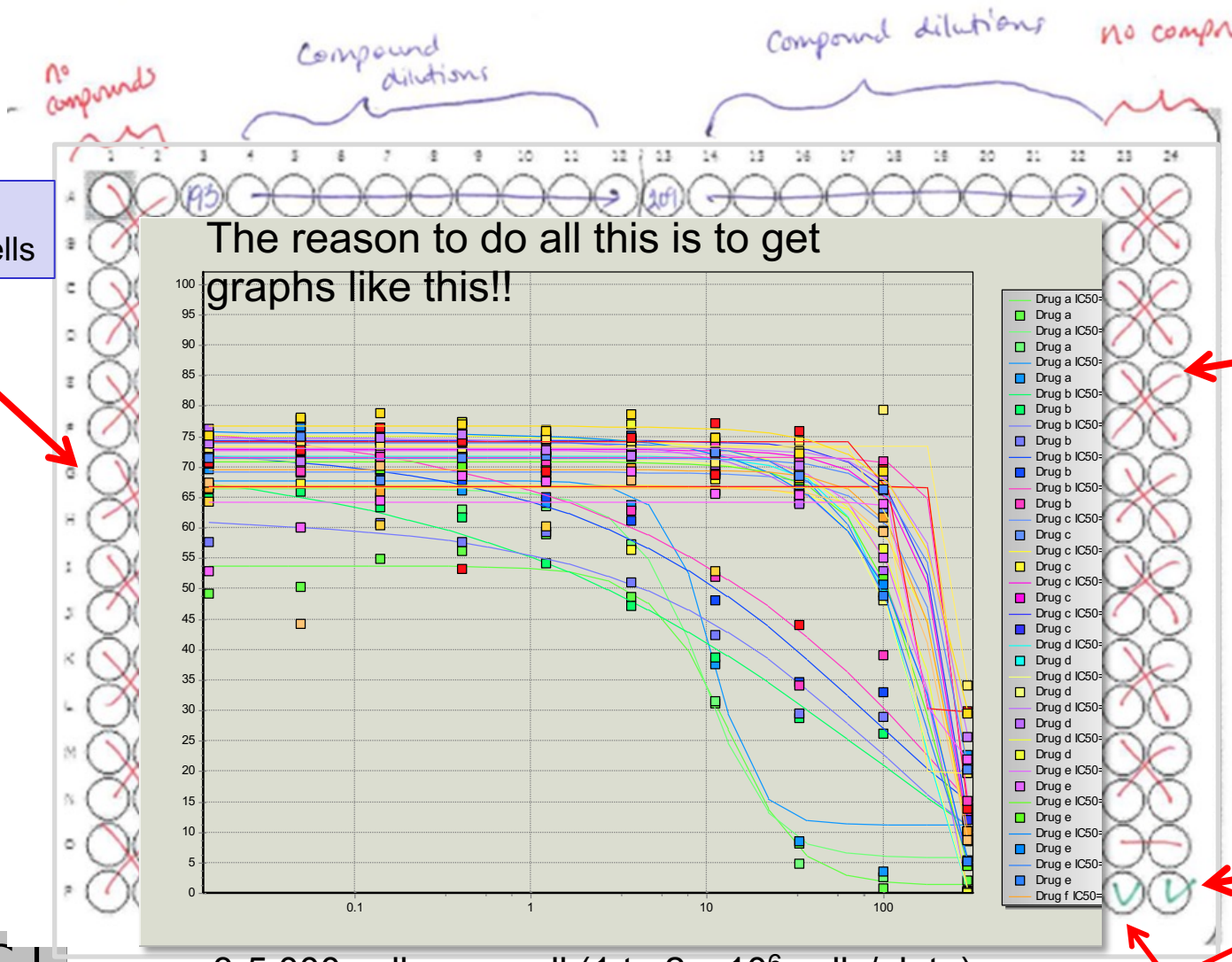


Negative control wells

Ethanol positive controls

3-5,000 cells per well (1 to 2 x 10^6 cells/plate)

JC-1
2 color assay



Negative control wells

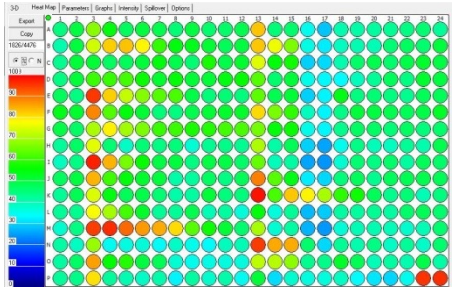
Negative control wells

Valinomycin positive controls

3-5,000 cells per well (1 to 2 x 10^6 cells/plate)

J. Paul Robinson, Purdue University

Logic Process



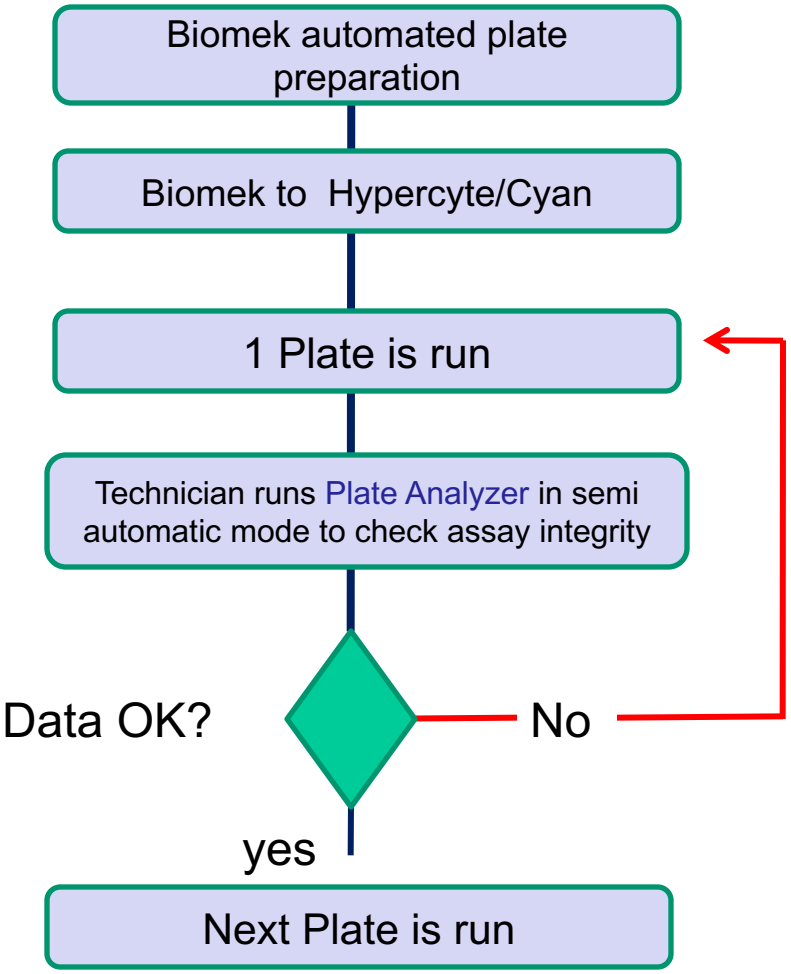
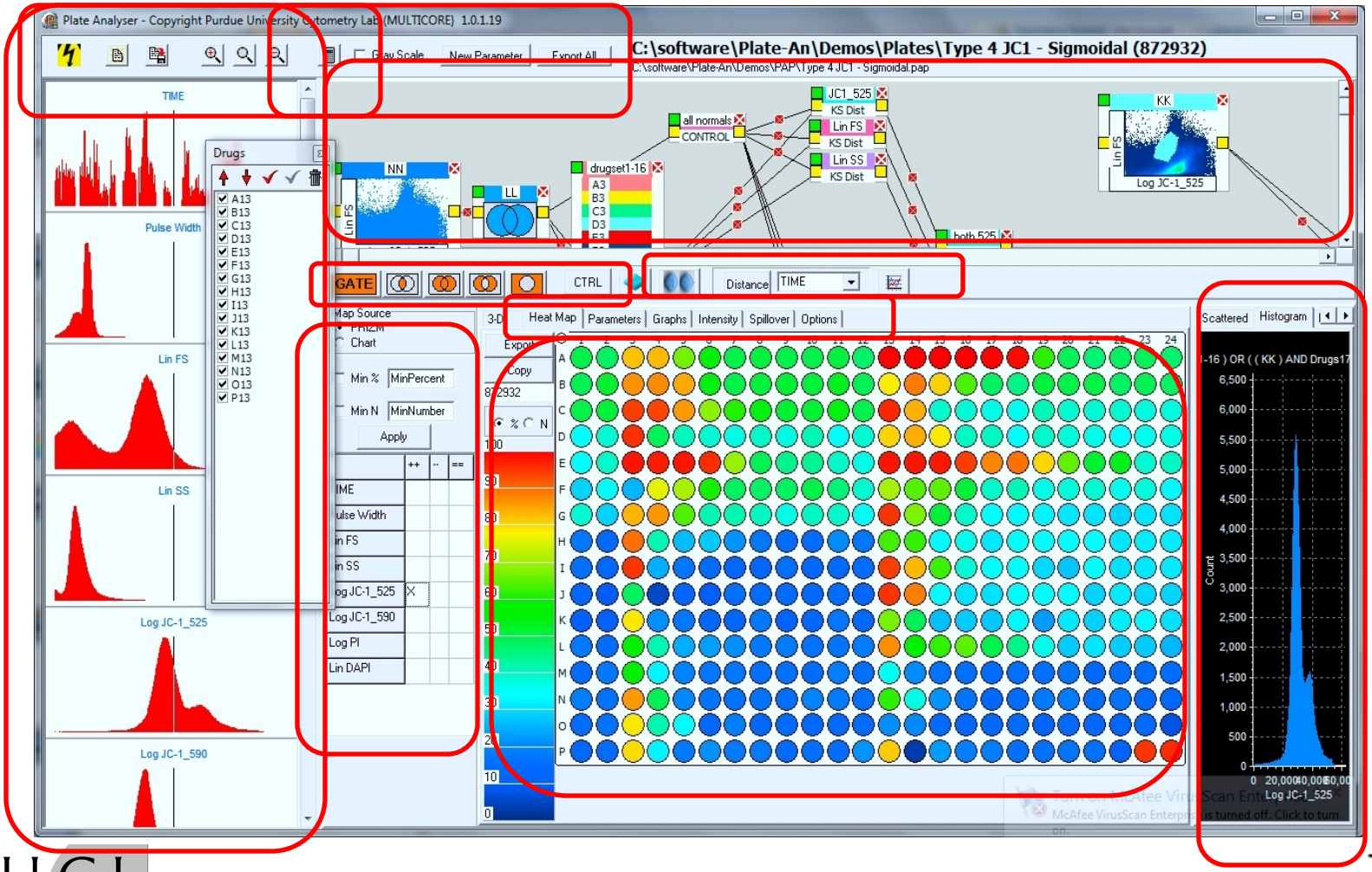


Plate Analyzer – all the parts



nstantly see any well or combination of wells with any parameter or combination of parameters

Purdue University

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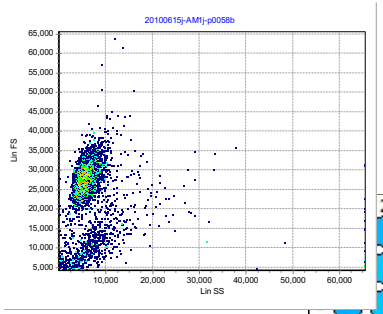

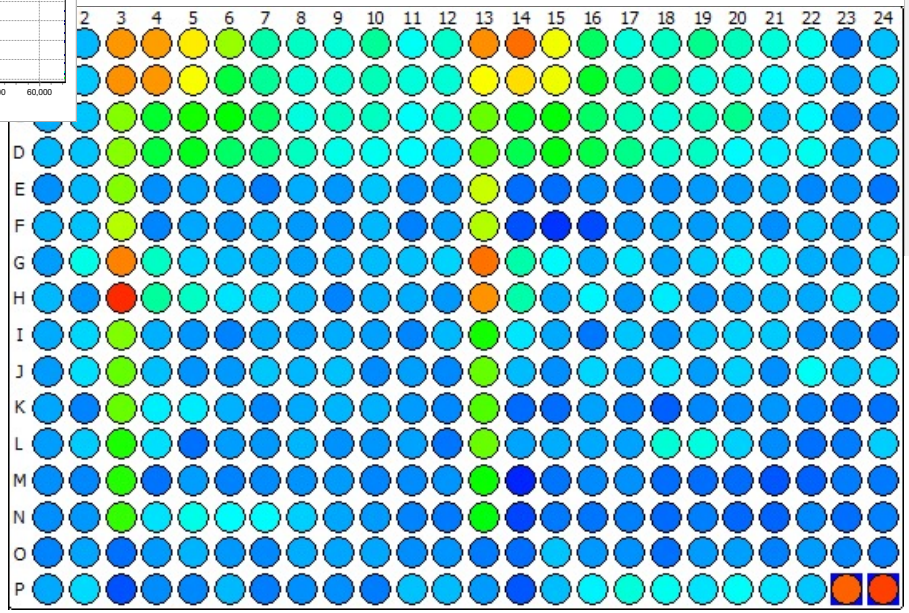

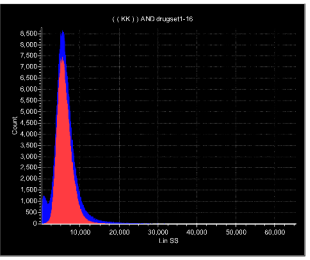

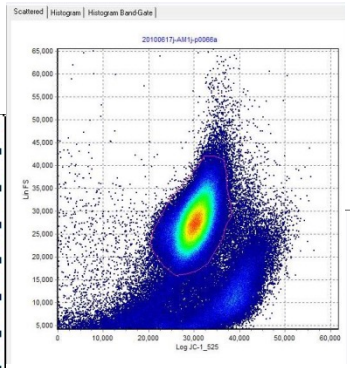

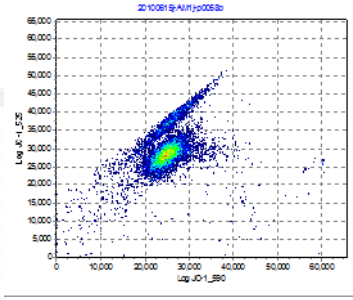

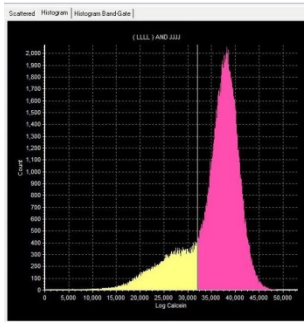

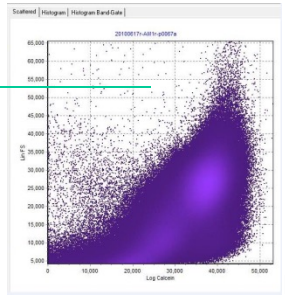

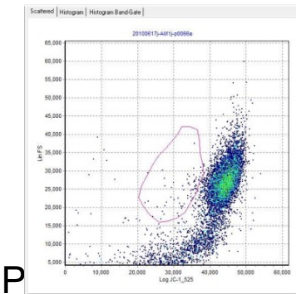

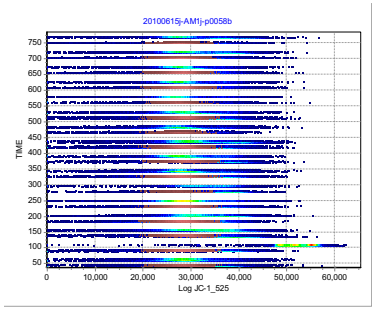

PUCL cytometry laboratories

nson, P

PURDUE UNIVERSITY

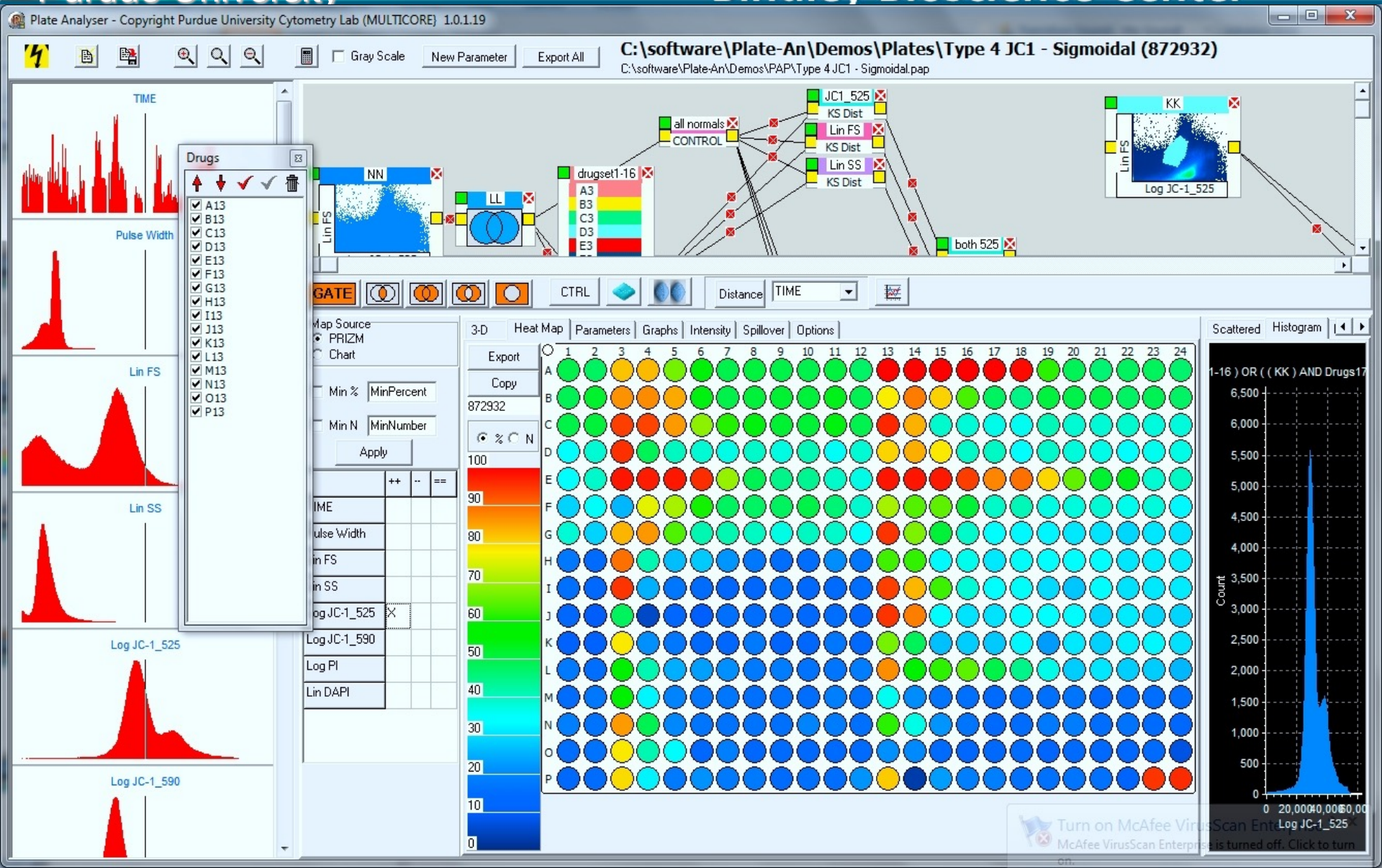
Plate Analyser - Copyright Purdue University Cytometry Lab (MULTICORE) 1.0.1.19
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C:\software\Plate-An\Demos\PAP\Type 4 JC1 - Sigmoidal.pap
TIME
Pulse Width
Lin FS
Lin SS
Log JC-1_525
Log JC-1_590
Drugs
A13
B13
C13
D13
E13
F13
G13
H13
I13
J13
K13
L13
M13
N13
O13
P13
NN
LL
drugset1-16
A3
B3
C3
D3
E3
all normals
CONTROL
JC1_525
KS Dist
Lin FS
KS Dist
Lin SS
KS Dist
both 525
KK
Lin FS
Log JC-1_525
GATE
CTRL
Distance
TIME
Map Source
PRIZM
Chart
Min %
MinPercent
Min N
MinNumber
Apply
Log JC-1_525
Log JC-1_590
Log PI
Lin DAPI
3-D
Heat Map
Parameters
Graphs
Intensity
Spillover
Options
Export
Copy
872932
Scattered
Histogram
1-16) OR ((KK) AND Drugs17
Count
Log JC-1_525

Discovery Park

It will happen here.

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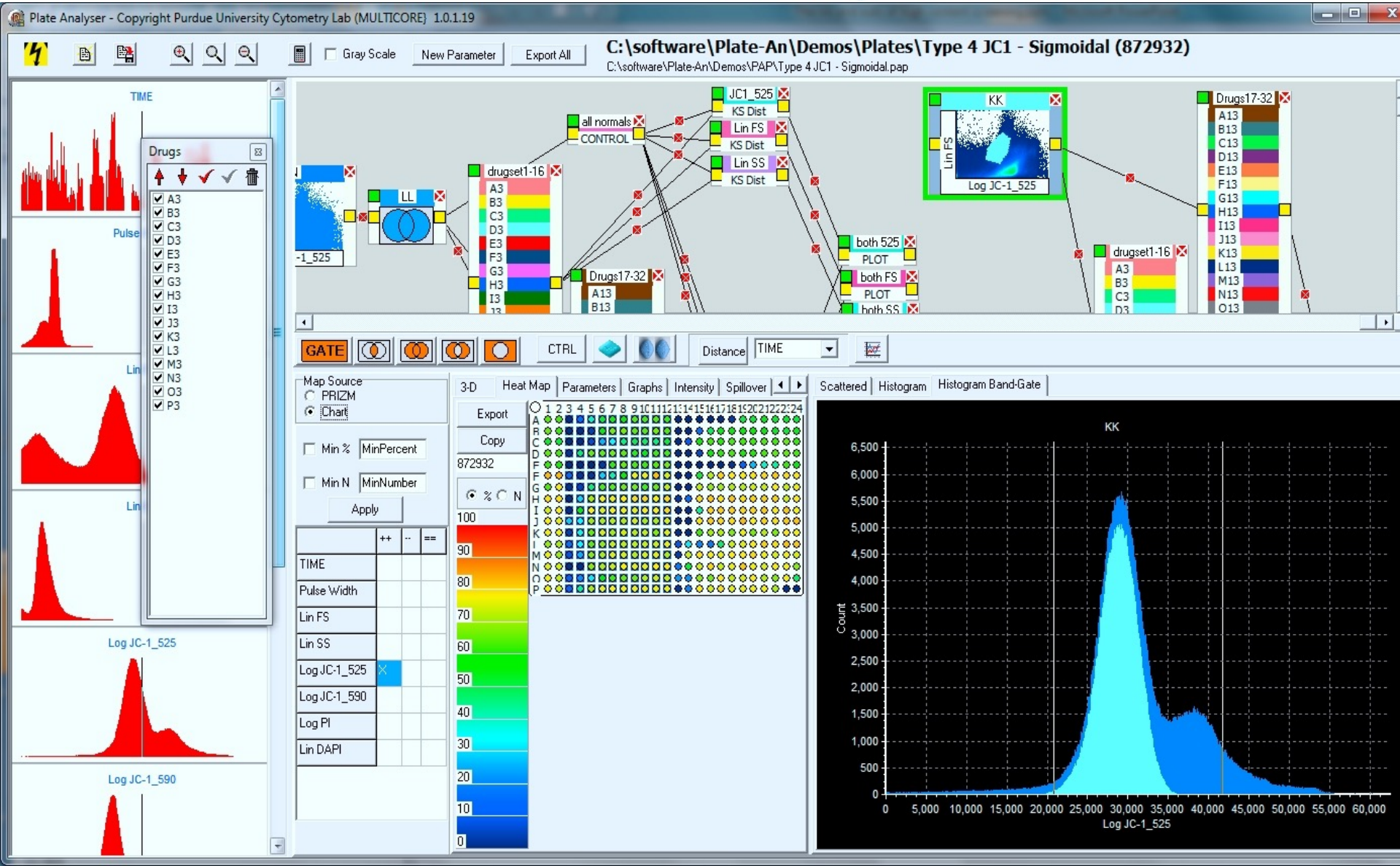

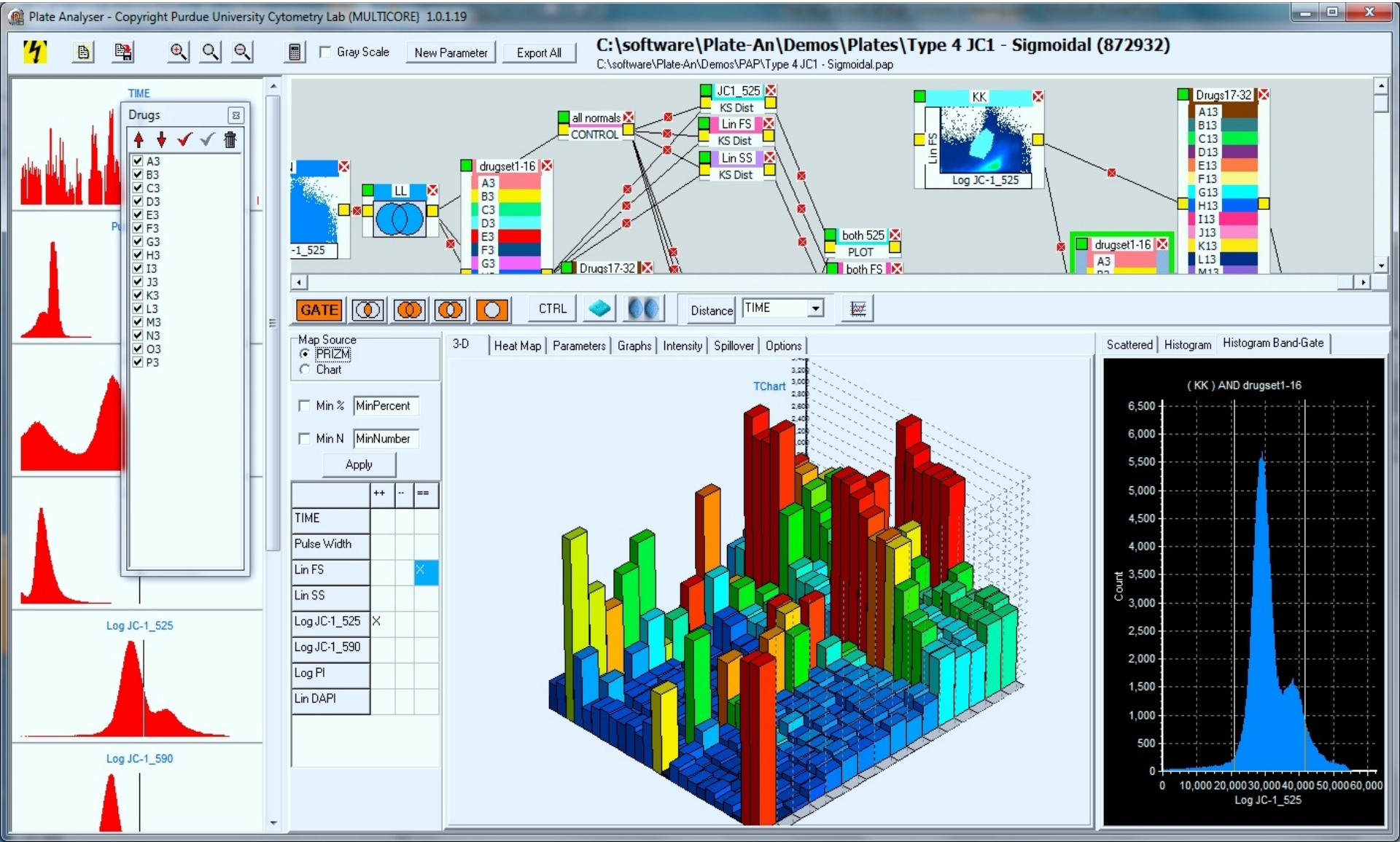
Plate Analyser - Copyright Purdue University Cytometry Lab (MULTICORE) 1.0.1.19
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New Parameter
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C:\software\Plate-An\Demos\PAP\Type 4 JC1 - Sigmoidal.pap
TIME
Drugs
A3
B3
C3
D3
E3
F3
G3
H3
I3
J3
K3
L3
M3
N3
O3
P3
Log JC-1_525
Log JC-1_590
all normals
CONTROL
drugset1-16
LL
JC1_525
KS Dist
Lin FS
Lin SS
both 525
PLOT
both FS
KK
Log JC-1_525
Drugs17-32
GATE
CTRL
Distance
TIME
Map Source
PRIZM
Chart
Min %
MinPercent
Min N
MinNumber
Apply
TIME
Pulse Width
Lin FS
Lin SS
Log JC-1_525
Log JC-1_590
Log PI
Lin DAPI
3-D
Heat Map
Parameters
Graphs
Intensity
Spillover
Options
TChart
Scattered
Histogram
Histogram Band-Gate
(KK) AND drugset1-16
Count
Log JC-1_525

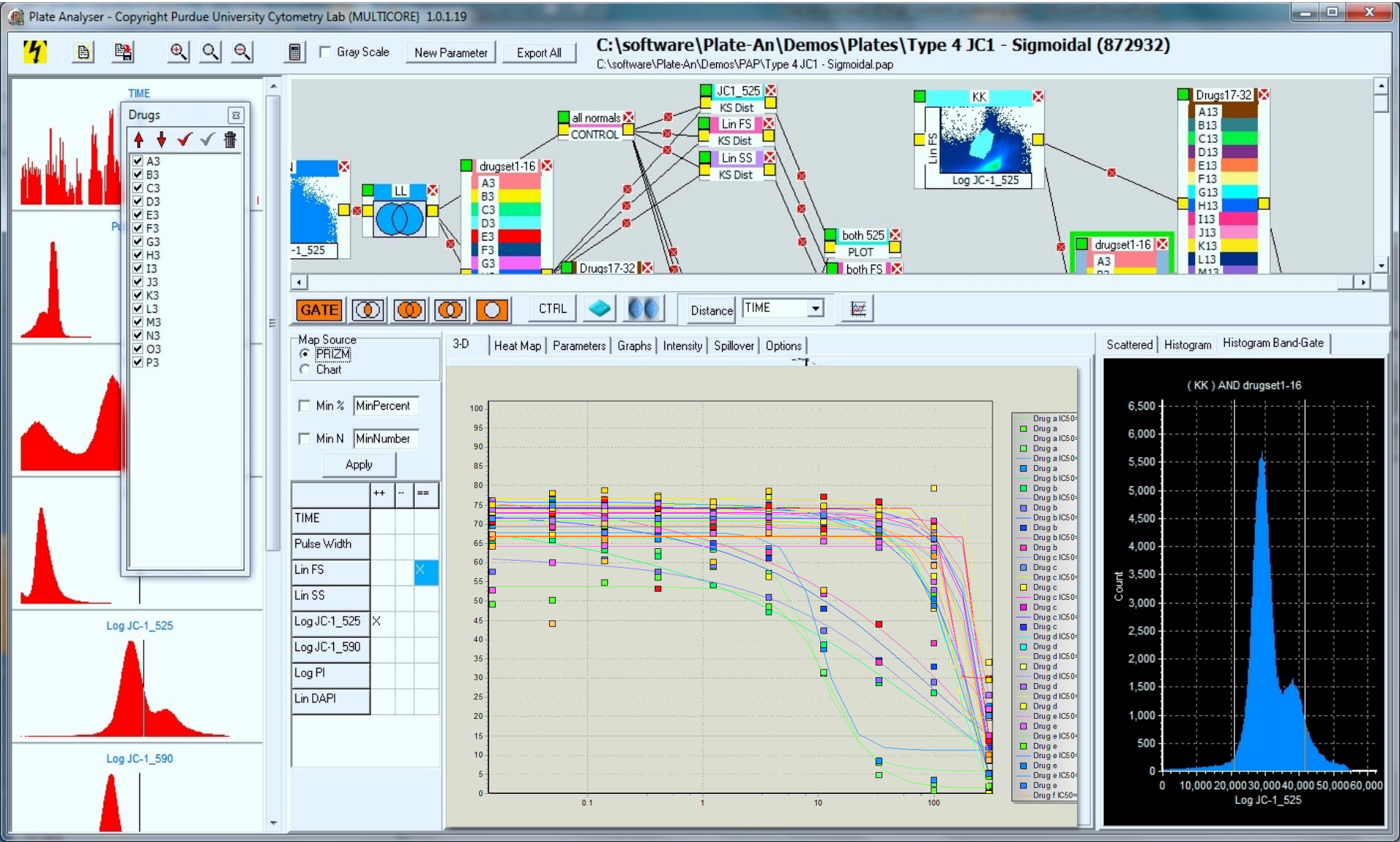
Plate Analyser - Copyright Purdue University Cytometry Lab (MULTICORE) 1.0.1.19
Gray Scale
New Parameter
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C:\software\Plate-An\Demos\PAP\Type 4 JC1 - Sigmoidal.pap
TIME
Drugs
A3
B3
C3
D3
E3
F3
G3
H3
I3
J3
K3
L3
M3
N3
O3
P3
Log JC-1_525
Log JC-1_590
all normals
CONTROL
drugset1-16
LL
JC1_525
KS Dist
Lin FS
Lin SS
both 525
PLOT
both FS
KK
Log JC-1_525
Drugs17-32
GATE
CTRL
Distance
TIME
Map Source
PRIZM
Chart
Min %
MinPercent
Min N
MinNumber
Apply
TIME
Pulse Width
Lin FS
Lin SS
Log JC-1_525
Log JC-1_590
Log PI
Lin DAPI
3-D
Heat Map
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Graphs
Intensity
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Histogram
Histogram Band-Gate
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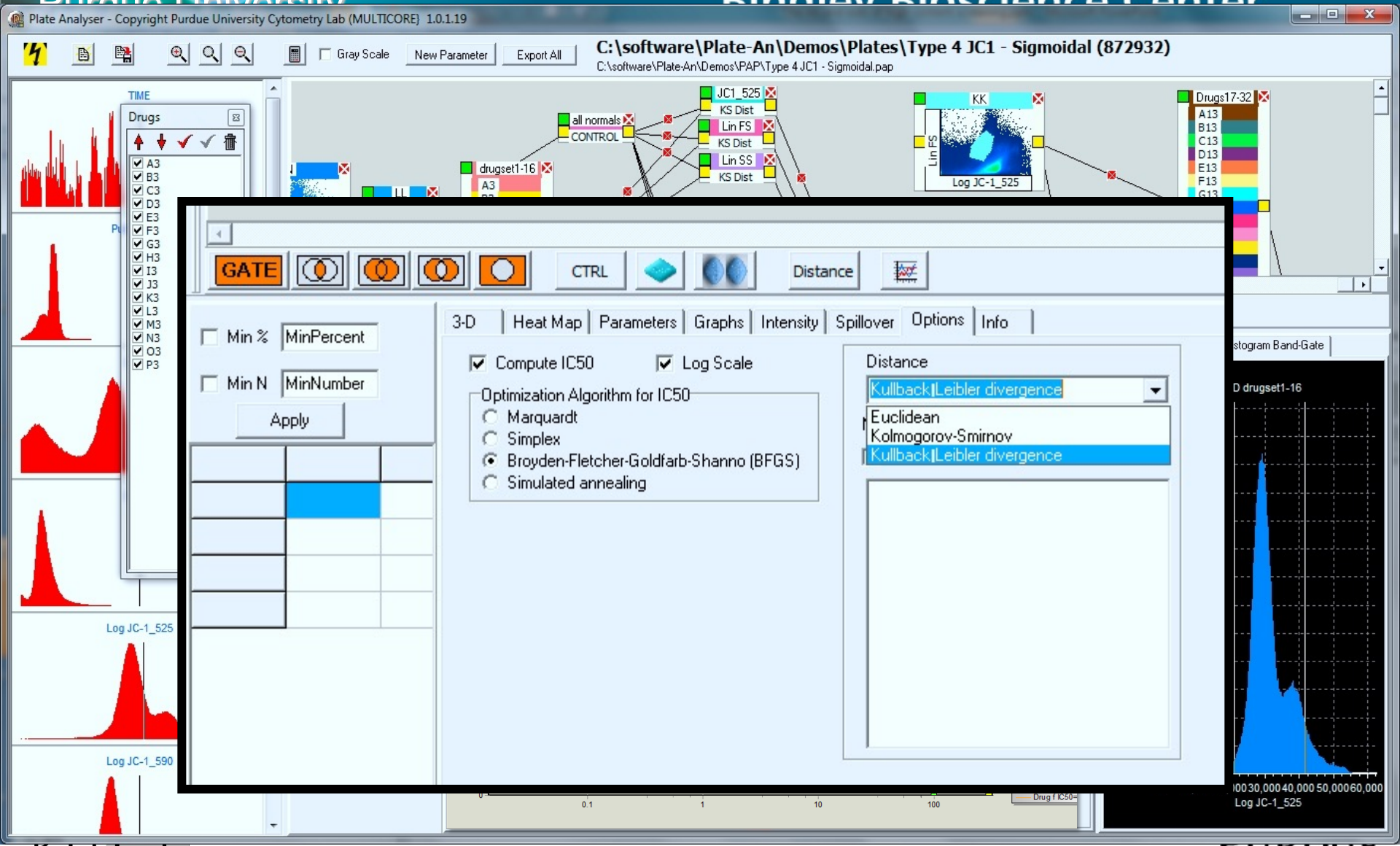
Plate Analyser - Copyright Purdue University Cytometry Lab (MULTICORE) 1.0.1.19
Gray Scale
New Parameter
Export All
C:\software\Plate-An\Demos\Plates\Type 4 JC1 - Sigmoidal (872932)
C:\software\Plate-An\Demos\PAP\Type 4 JC1 - Sigmoidal.pap
TIME
Drugs
A3
B3
C3
D3
E3
F3
G3
H3
I3
J3
K3
L3
M3
N3
O3
P3
all normals
CONTROL
drugset1-16
A3
JC1_525
KS Dist
Lin FS
KS Dist
Lin SS
KS Dist
KK
Lin FS
Log JC-1_525
Drugs17-32
A13
B13
C13
D13
E13
F13
G13
GATE
CTRL
Distance
Min %
MinPercent
Min N
MinNumber
Apply
3-D
Heat Map
Parameters
Graphs
Intensity
Spillover
Options
Info
Compute IC50
Log Scale
Optimization Algorithm for IC50
Marquardt
Simplex
Broyden-Fletcher-Goldfarb-Shanno (BFGS)
Simulated annealing
Distance
Kullback|Leibler divergence
Euclidean
Kolmogorov-Smirnov
Kullback|Leibler divergence
stogram Band-Gate
D drugset1-16
Log JC-1_525
Log JC-1_590
Drug f IC50=
0.1
1
10
100

Discovery Park

It will happen here.

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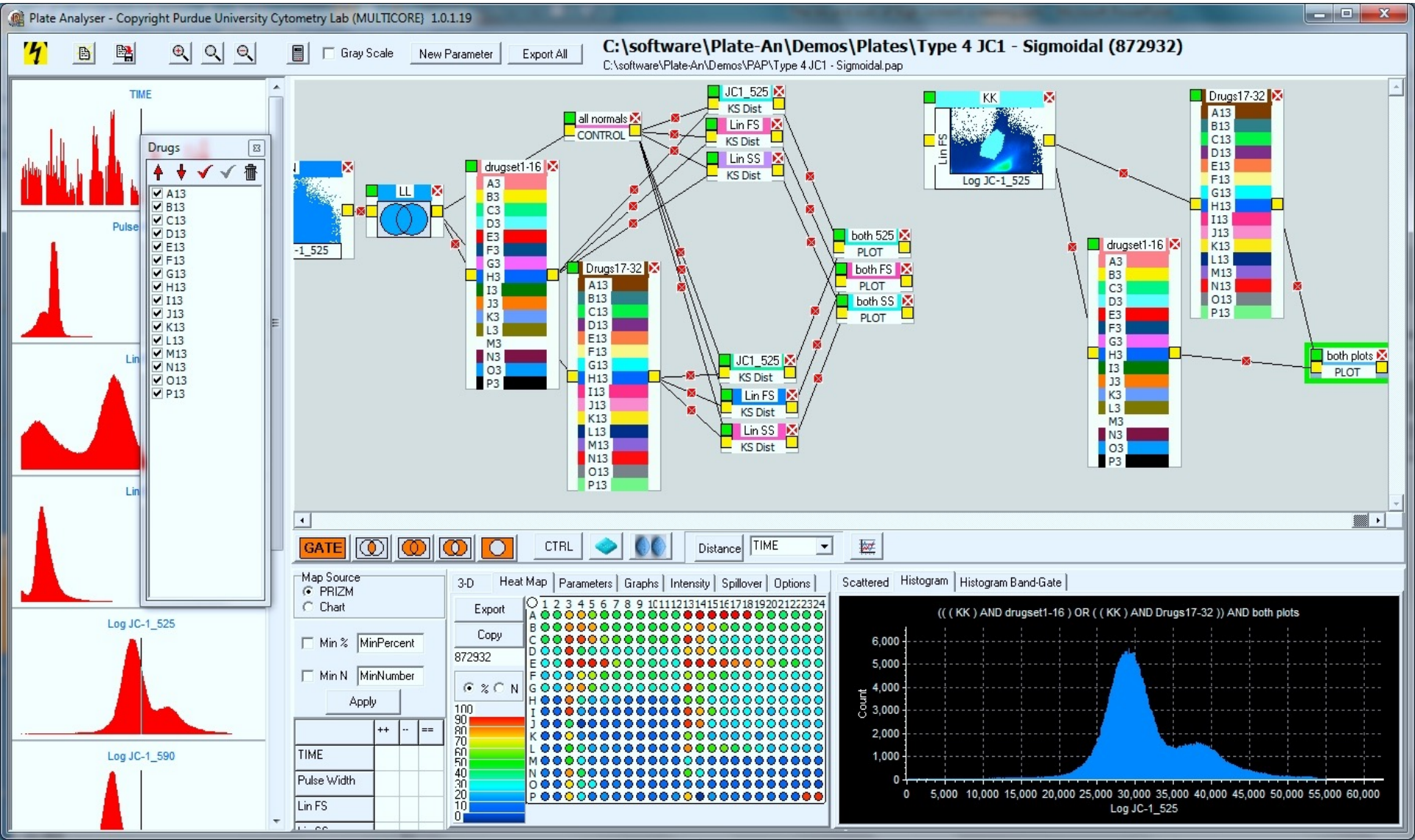
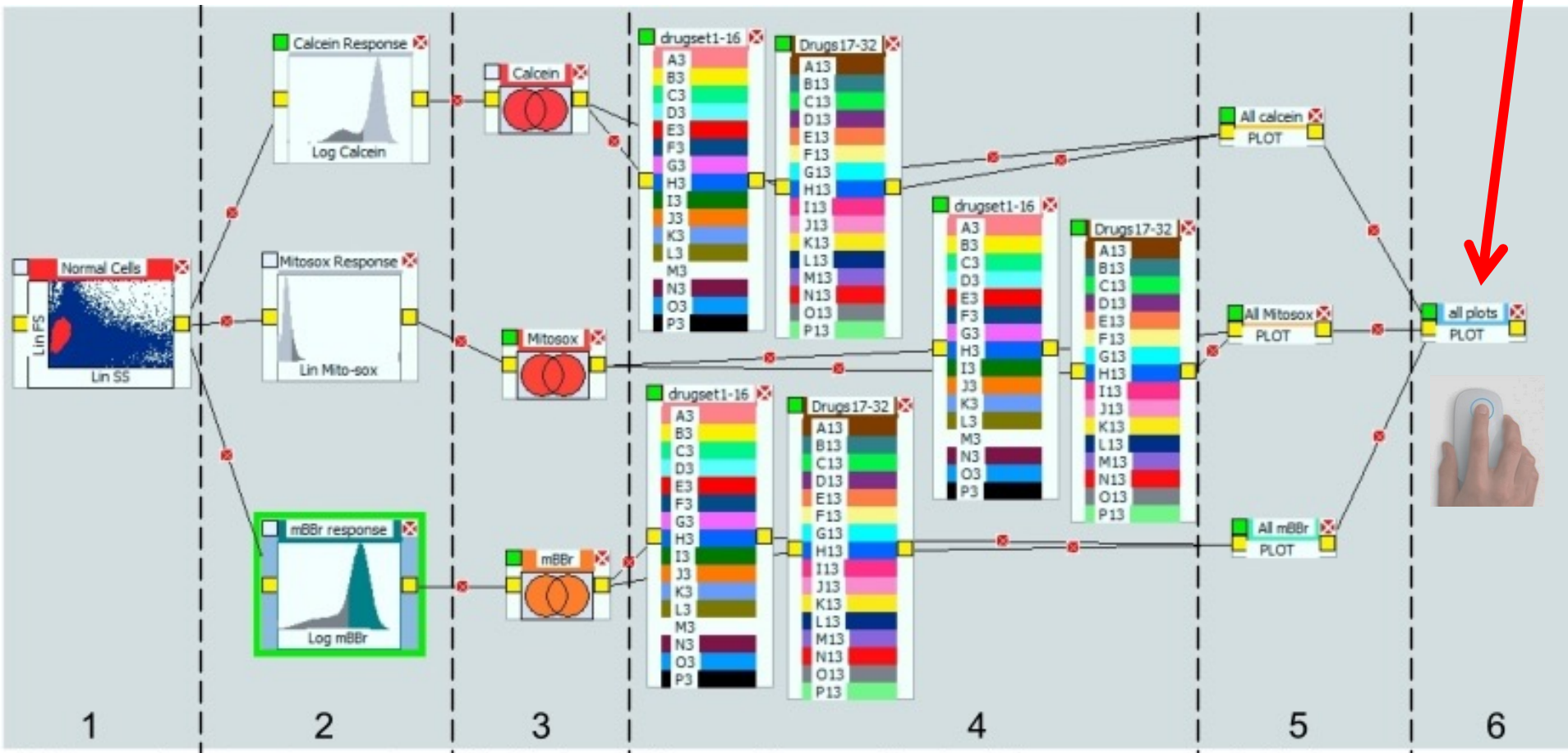
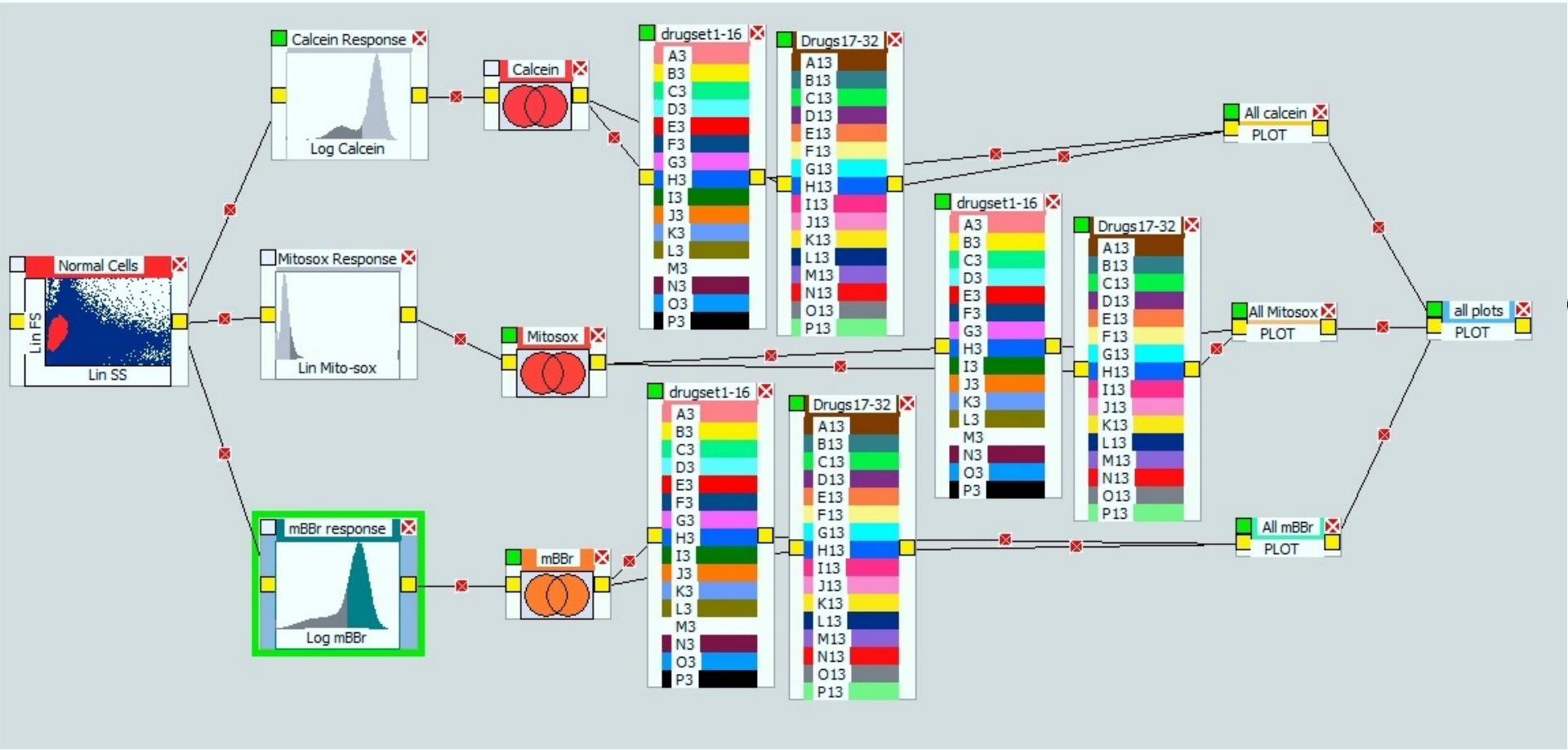


Logic Map for 3 color Redox assay in 384 well plate – 96 dose response curves in 1 click



3 functional assays, 32 drugs, 96 curves, 10 points/drug

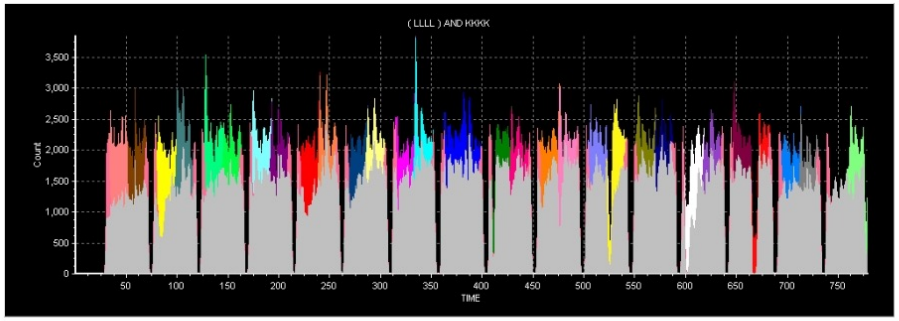
One 384 well plate, 8 minutes collection time



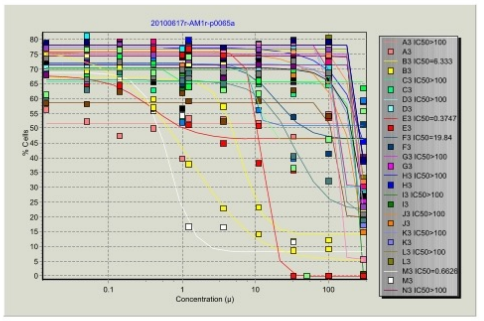
← Collection time – 8 min →

Each Drug is a different color

mBBr

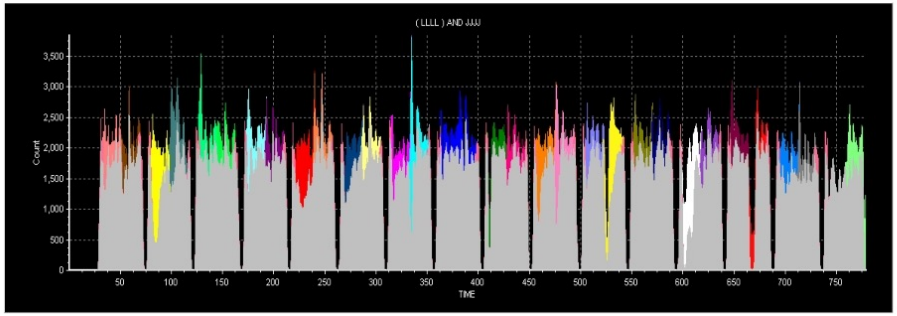


Gray represts a dose response of **mbbr** with each color representing a different drug

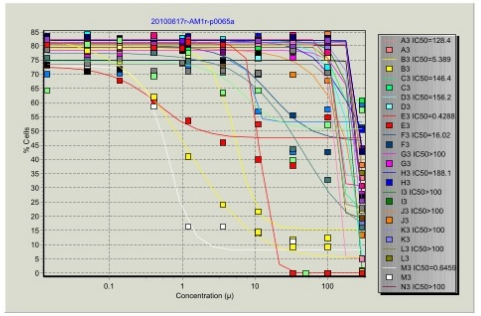


mbbr IC50 curves (32 drugs, 10-point curves)

Calcein

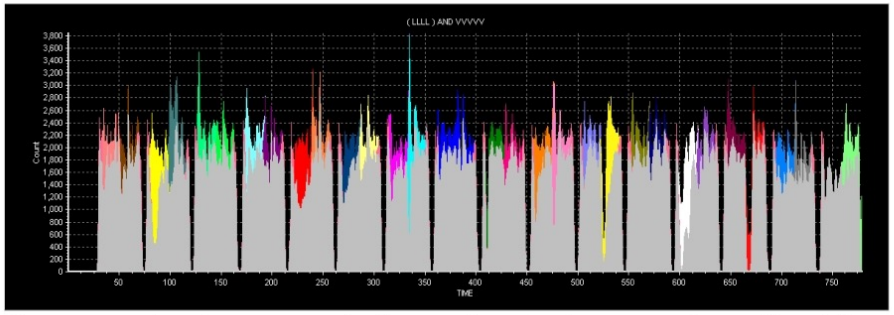


Gray represents a dose respose of **calcein** with each color representing a different drug

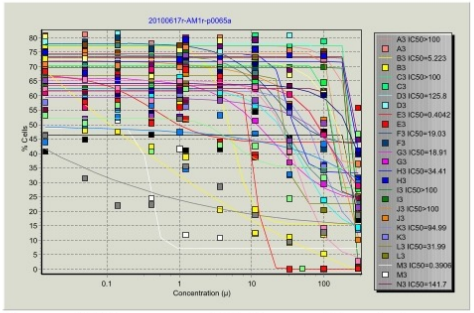


Calcein IC50 curves (32 drugs, 10-point curves)

Mitosox



Gray represents a dose respose of **mitosox** with each color representing a different drug



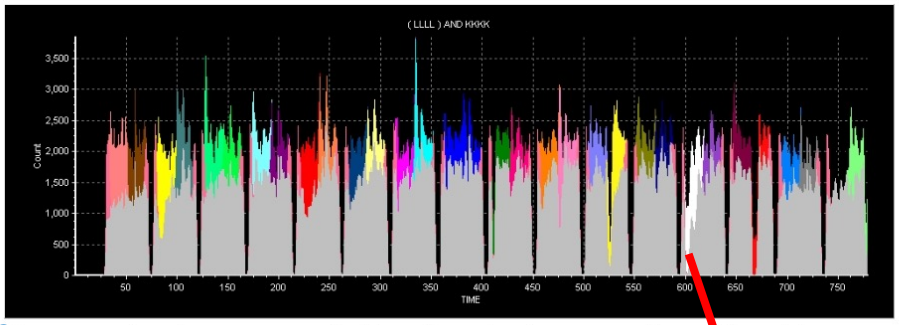
Mitosox IC50 curves (32 drugs, 10-point curves)

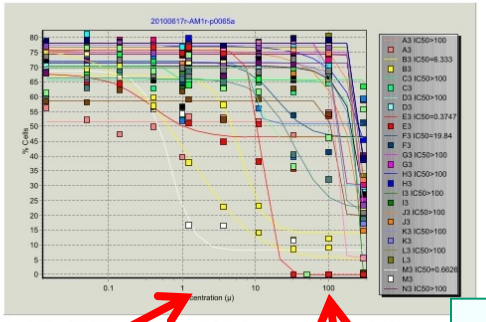
time plot 2.xar

96 Curves

‘Things don’t change. You change your way of looking, that’s all.’ *Carlos Castaneda*

Time ⟶





Circa 2011

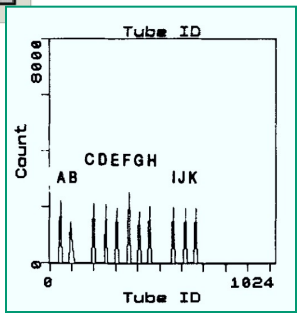
Circa 1990

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



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

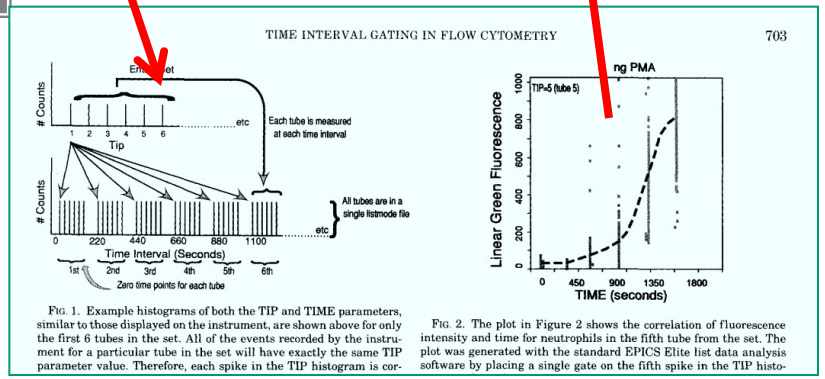
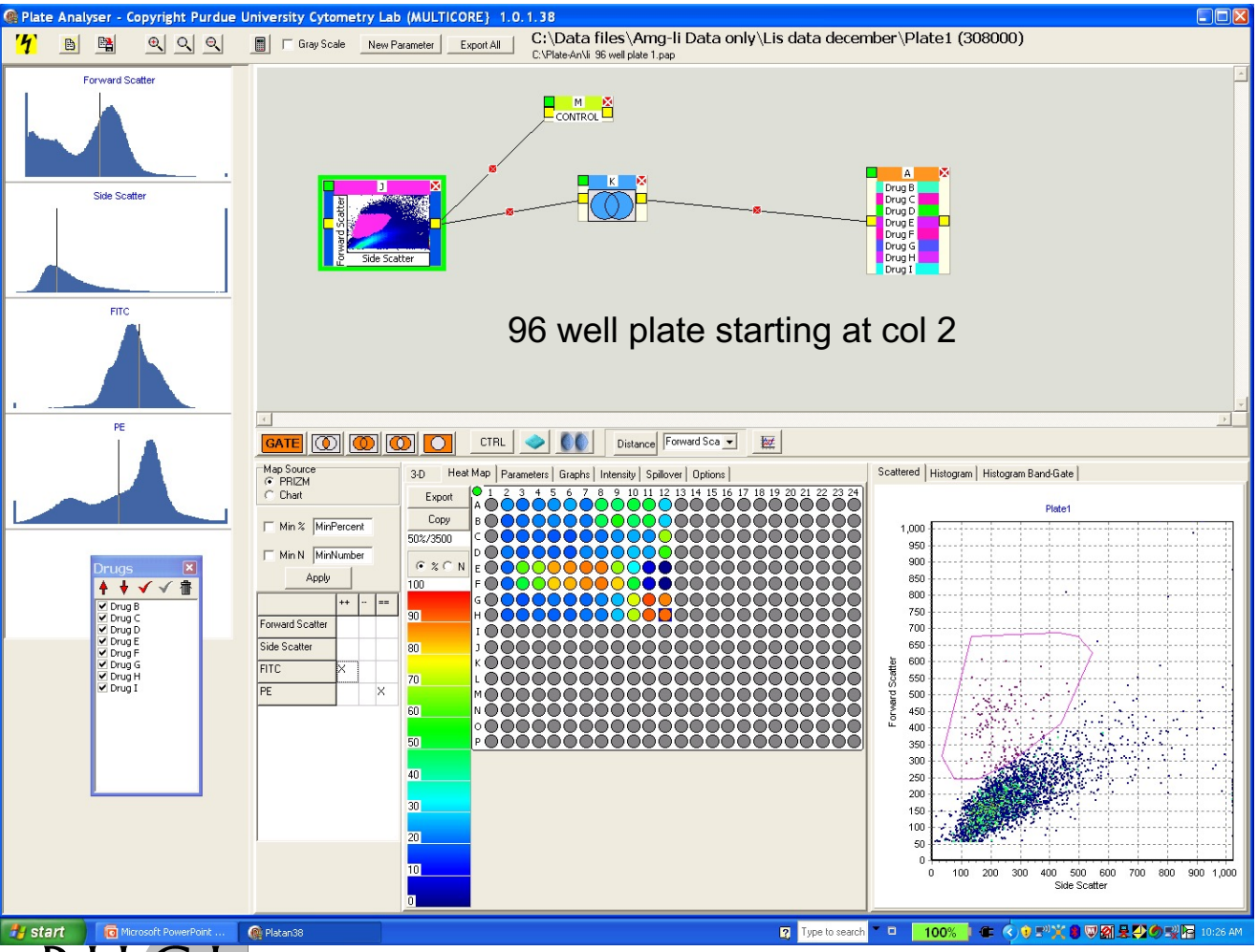
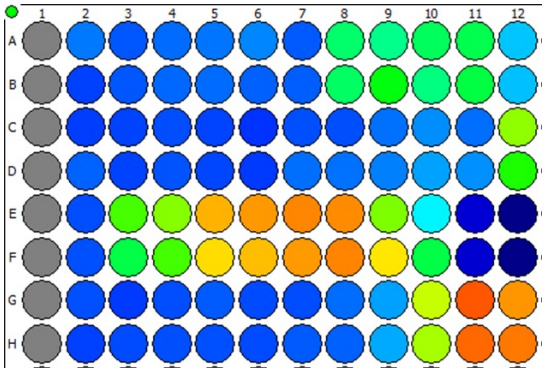


FIG. 1. Example histograms of both the TIP and TIME parameters, similar to those displayed on the instrument, are shown above for only the first 6 tubes in the set. All of the events recorded by the instrument for a particular tube in the set will have exactly the same TIP parameter value. Therefore, each spike in the TIP histogram is cor-

FIG. 2. The plot in Figure 2 shows the correlation of fluorescence intensity and time for neutrophils in the fifth tube from the set. The plot was generated with the standard EPICS Elite list data analysis software by placing a single gate on the fifth spike in the TIP histo-

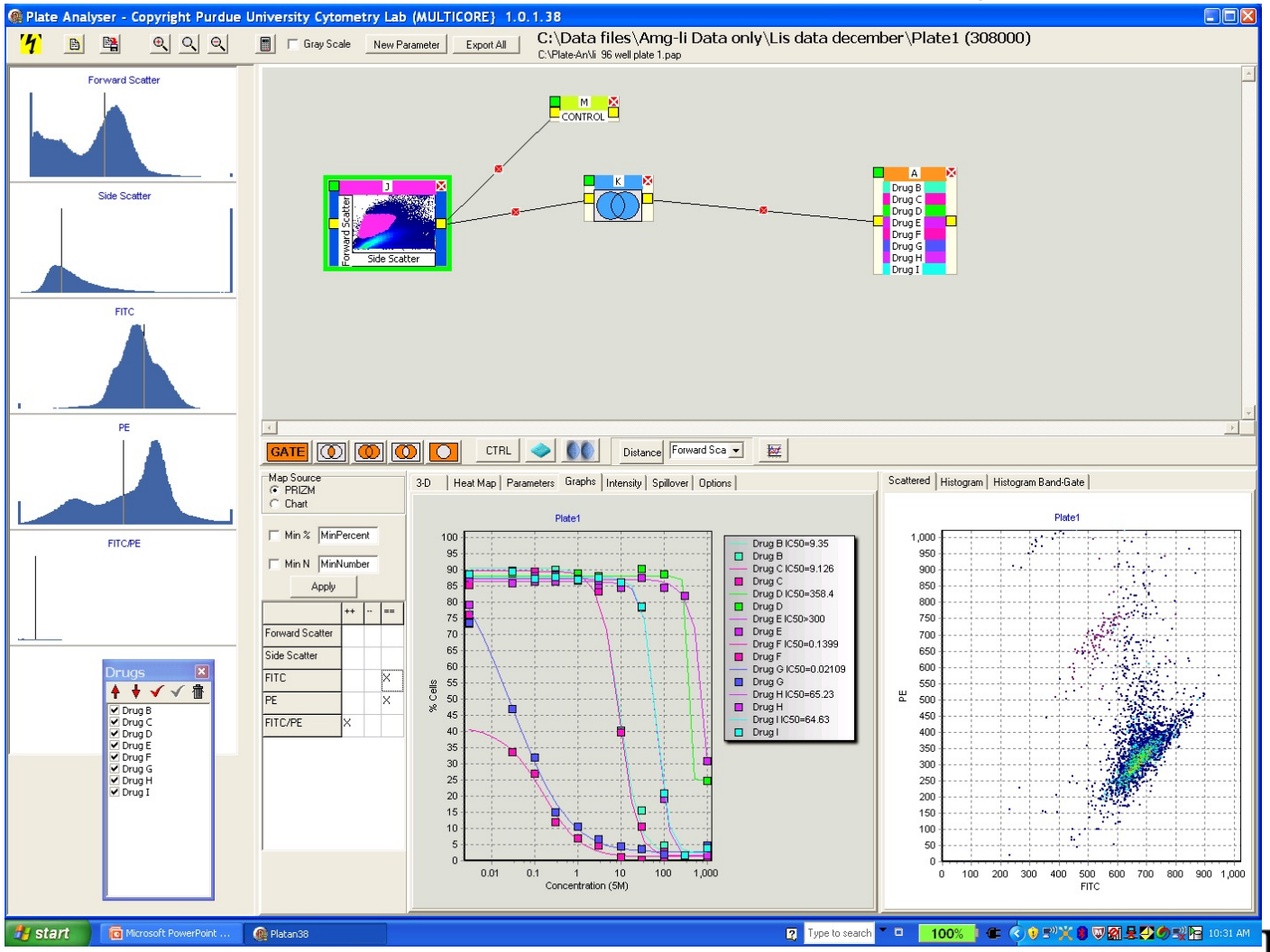
96 well data works fine too (FACSCanto, LSRII, Acuri, Cyan)





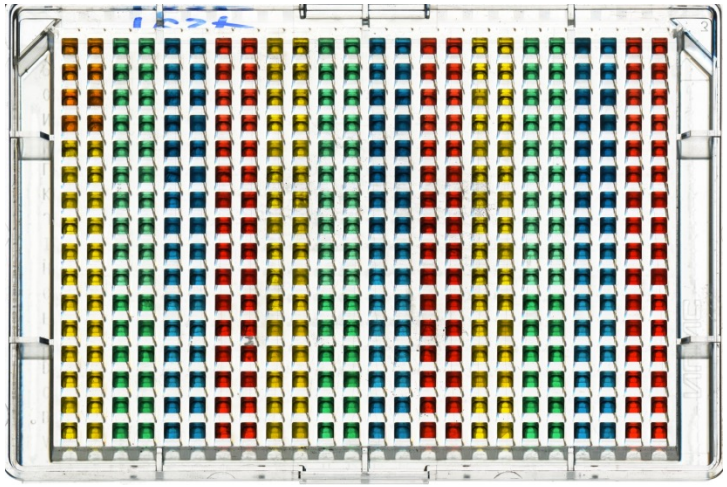
96 well plate starting at col 2

96 well data functional assay

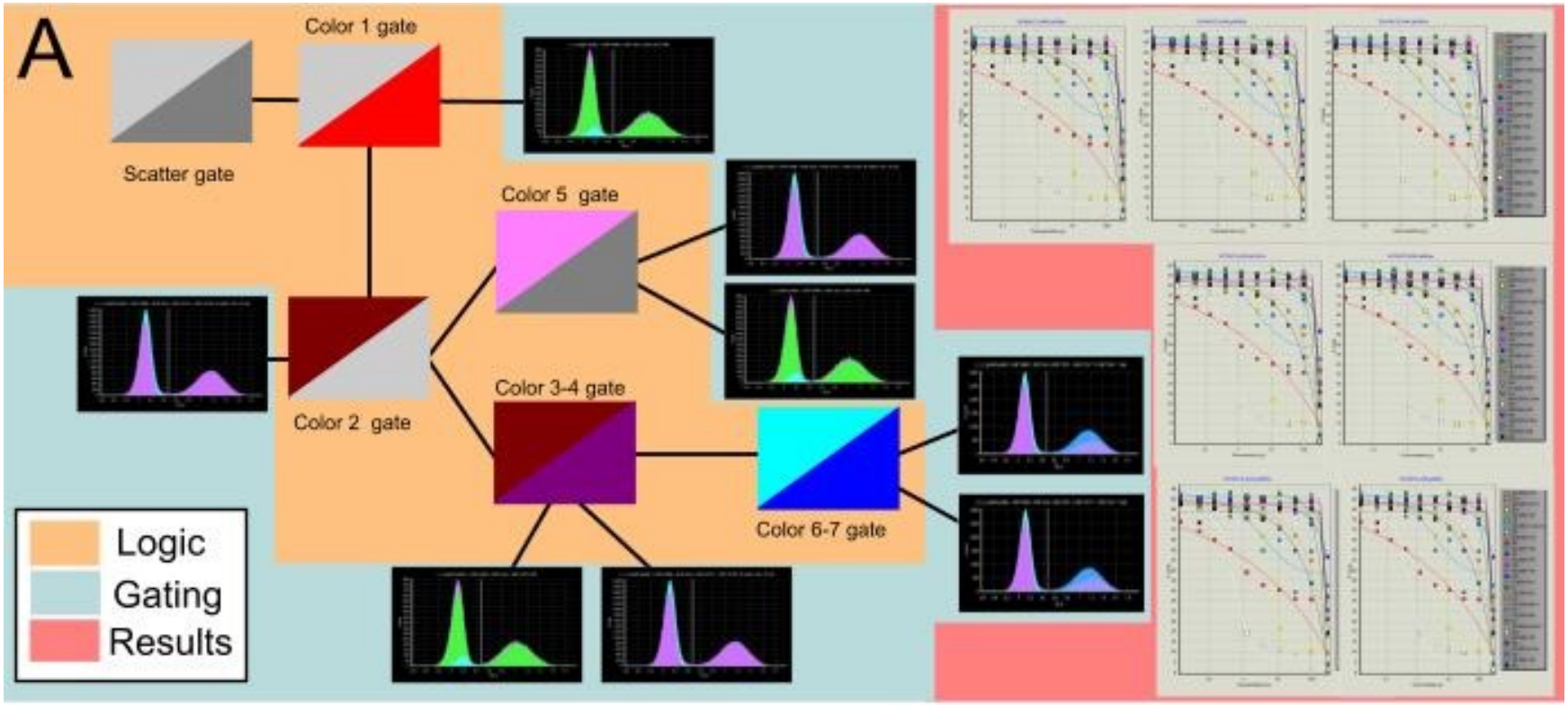


Complex assay: 288 tubes, 7 color, 9 variables, 4 pop'n, 5 x10^6 cells

- Stimulate PBMC with IL6, IL10, and LPS. Measure phosphorylation of Stat3
- and p38 after 15 minutes of stimulation. Stain for CD4+ T cells, CD4- T cells,
- monocytes, and B cells.
- Pstat3 – ALEXA647
- Pstat1 - ALEXA488
- CD33 – PE-CY7
- CD4 – PacBlu
- CD20 – PE-Cy55
- CD3 – PE



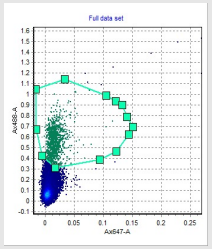
Assay data from Dr. Peter Krutchik


A
Color 1 gate
Scatter gate
Color 5 gate
Color 2 gate
Color 3-4 gate
Color 6-7 gate
Logic
Gating
Results
B

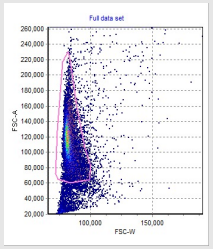
Initial Gate Criteria

7 color, 9 parameter Simultaneous Analysis, 80 Dose Response curves on 4 simultaneous populations

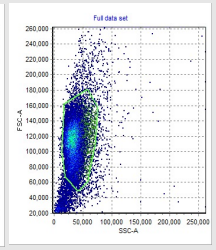
Assay data from Dr. Peter Krutchik



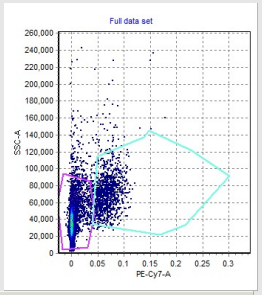
Stat $^{+}$-ve



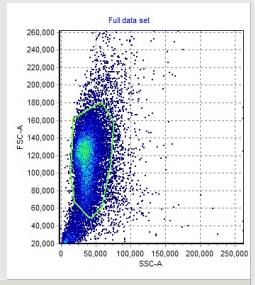
Width



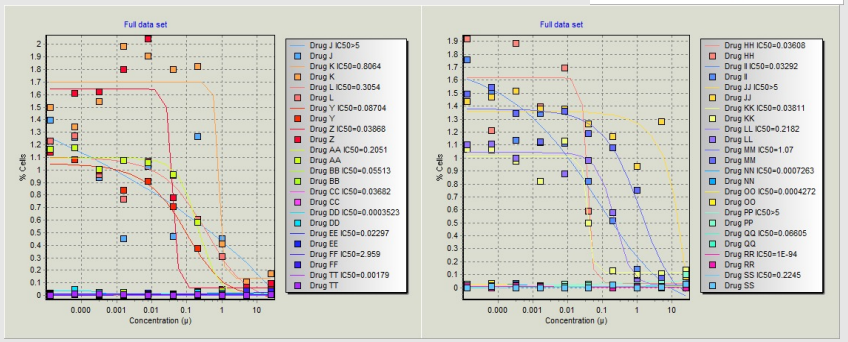
Size

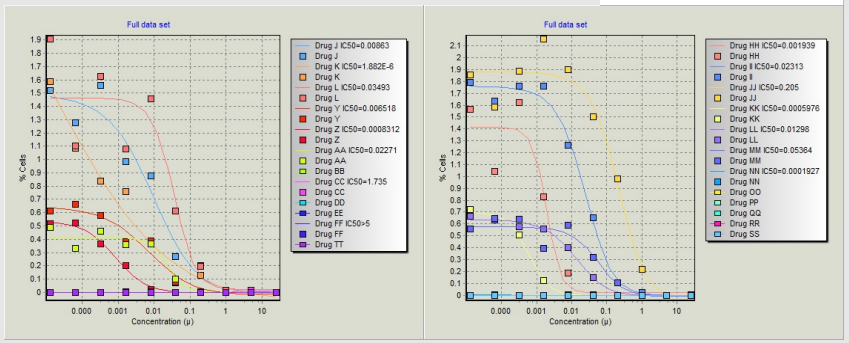


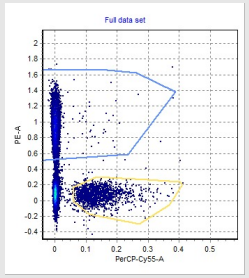
CD33^{+} MN



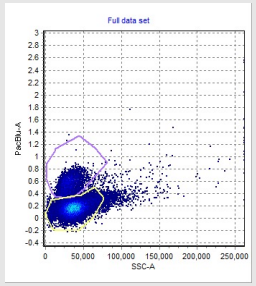
CD20^{+} B Cells



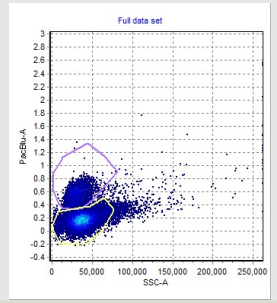




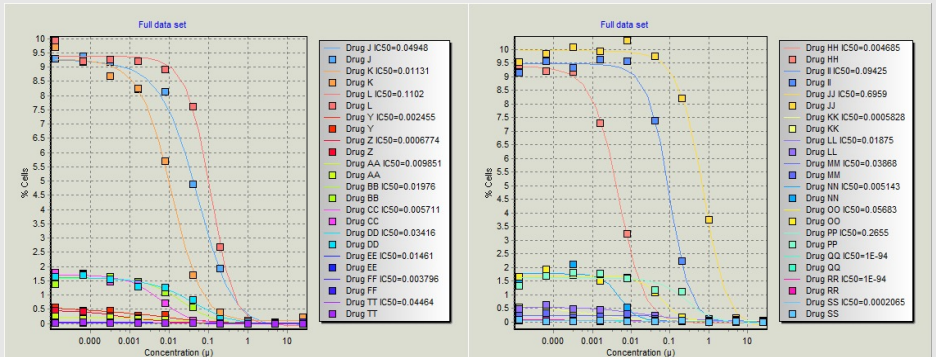
CD3^{+} T cells

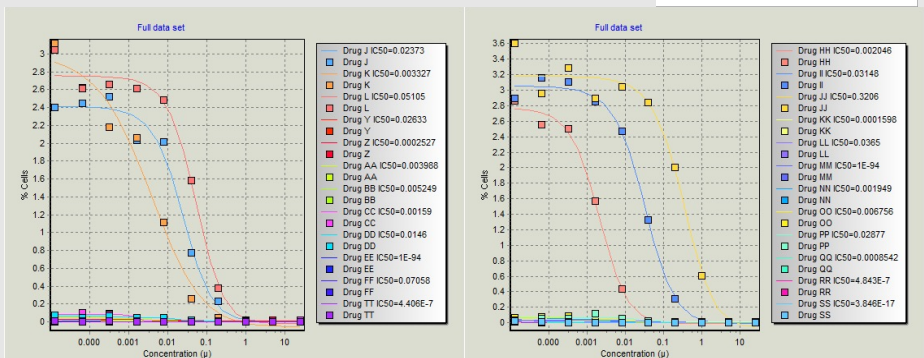


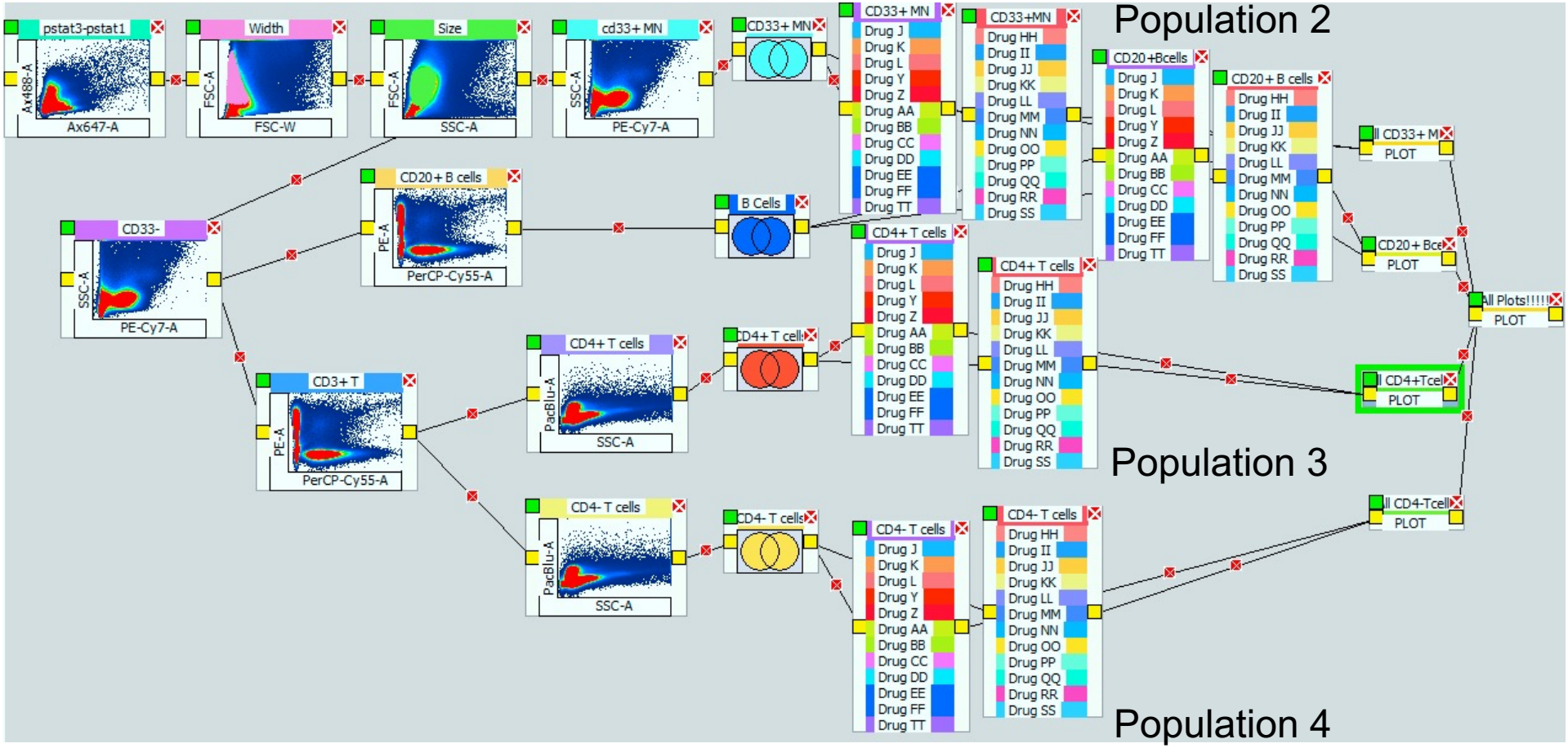
CD4^{+} T cells



CD4^{-} T cells







Assay data from Dr. Peter Krutchik

3. The next flow cytometer

29 different variables (parameters)

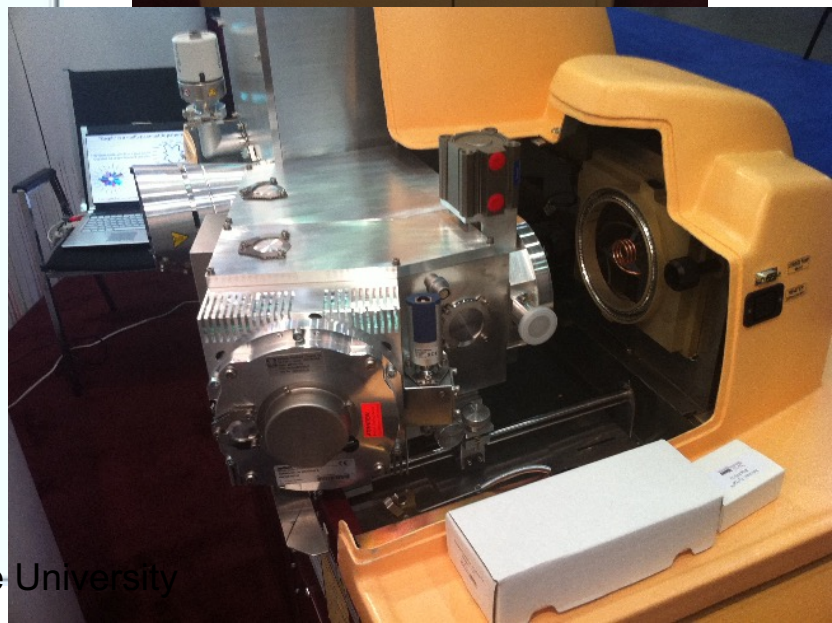
14 different populations of cells

12 activation molecules

14 distinct pathways

8 point dose response curves

- [ ] Time
- [ ] Cell_length
- [x] DNA(Ir191)D
- [x] DNA(Ir193)D
- [x] CD3(Cd110)D
- [x] CD3(Cd111)D
- [x] CD3(Cd112)D
- [x] CD3(Cd113)D
- [x] CD3(Cd114)D
- [x] CD45(In115)D
- [x] pNFkb(Nd142)D
- [x] pp38(Nd144)D
- [x] CD4(Nd145)D
- [x] CD33(Nd148)D
- [x] pStat5(Nd150)D
- [x] CD20(Sm147)D
- [x] pAkt(Sm152)D
- [x] pSHP2(Sm154)D
- [x] CD123(Eu151)D
- [x] pStat1(Eu153)D
- [x] pZap70(Gd156)D
- [x] pStat3(Gd158)D
- [x] CD14(Gd160)D
- [x] pSlp76(Dy164)D
- [x] pBtk(Er166)D
- [x] pPLCg2(Er167)D
- [x] pErk12(Er168)D
- [x] pLat(Er170)D
- [x] IgM(Yb171)D
- [x] pS6(Yb172)D
- [x] HLA_DR(Yb174)D
- [x] CD7(Yb176)D
- [x] 110:114(Merged)

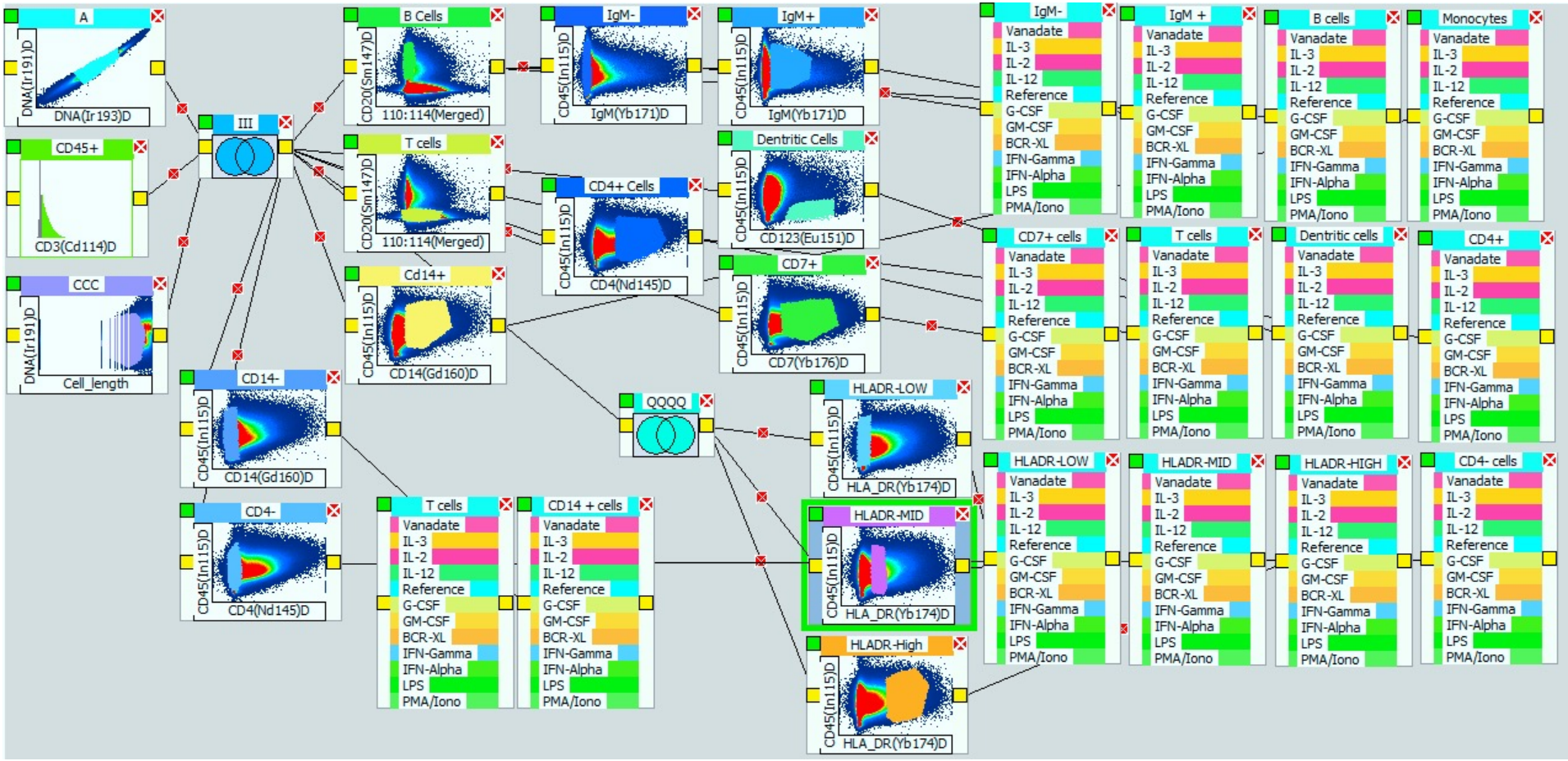


PUCL cytometry laboratories

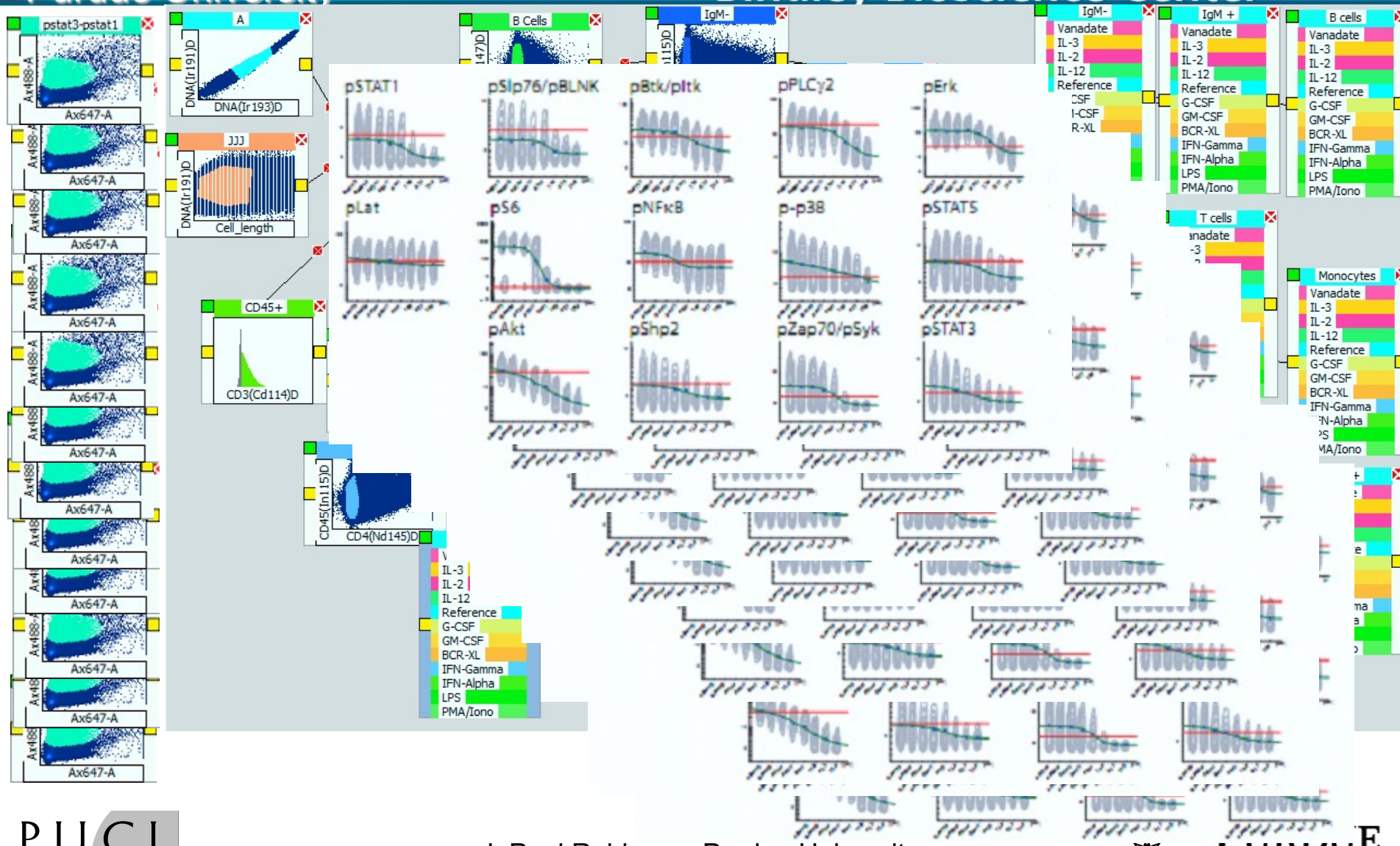
Initial gating

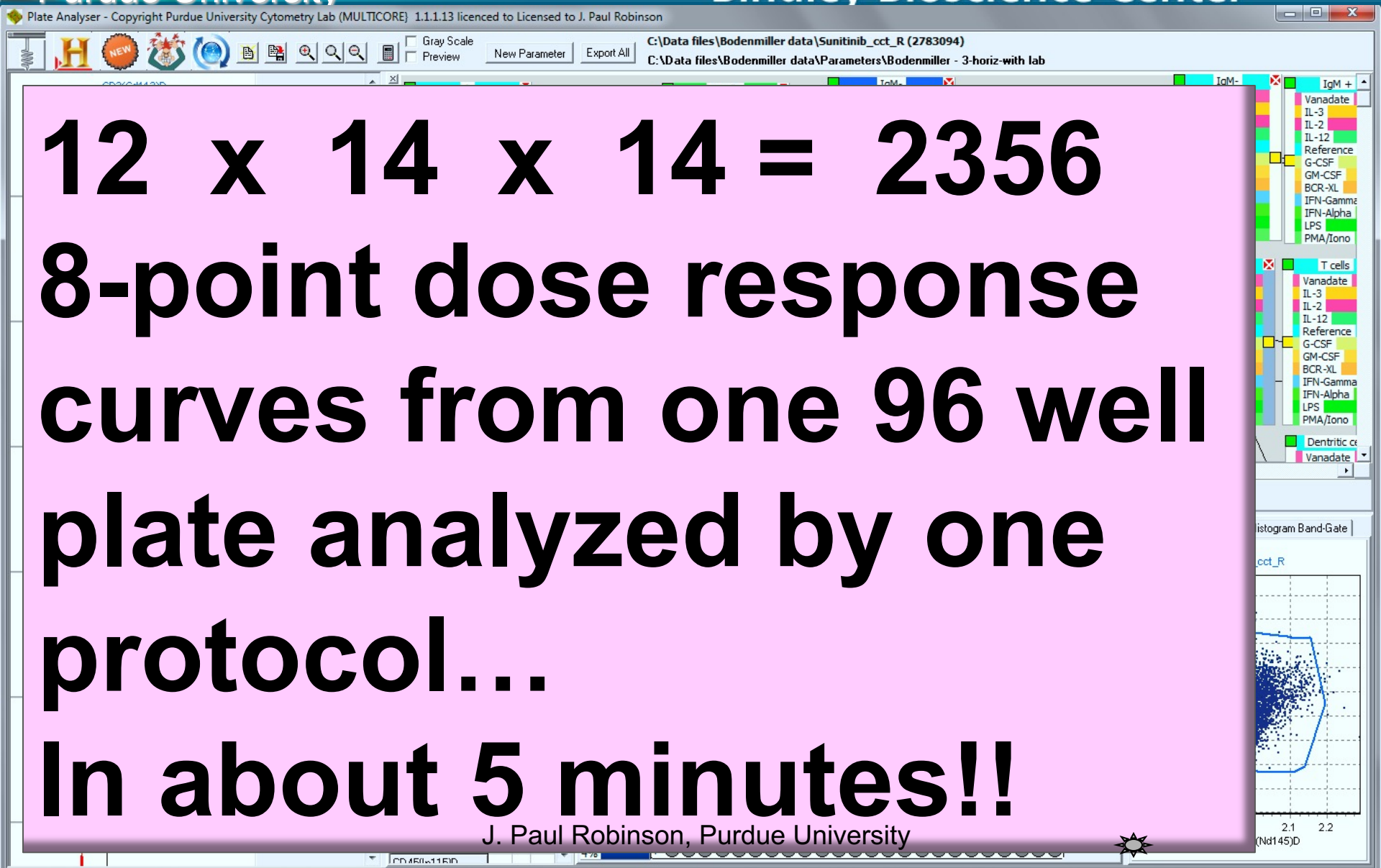
14 populations

14 sets of dose response curves



Purdue University Bindley Bioscience Center





12 x 14 x 14 = 2356 8-point dose response curves from one 96 well plate analyzed by one protocol…

In about 5 minutes!!

Summary & Conclusions

- The first 40 years took 40 years to get us to today!
- The next 10 years will expand by 10 times today's common lab assay styles
- Automation will be efficient and cost effective and more accurate than the best analyst today
- We will stop talking about intensity values, % of change, we will talk in probability functions
- We will all have to go back to school if we are to understand these new powerful tools
- But – these are the tools that will bring systems biology into the flow cytometry laboratory

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Contributors

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