



Polychromatic and Hyperspectral Flow Cytometry for Modeling Relationships in Heterogeneous Cell Populations

J. Paul Robinson
SVM Endowed Professor of Cytomics
Professor of Biomedical Engineering
Purdue University, West Lafayette, IN

PUCL

8:30 - 10:00 a.m. Plenary Session I

Outline

This presentation provides a background to past developments in single cell analysis with a view to showing how the technologies have matured into highly advanced approaches to systems analysis at the single cell level

- Overview of historical developments in flow cytometry
- Outline systems available for identifying properties of single cells
- Define how populations have been classified traditionally
- Identify emerging tools for single cell analysis
- Illustrate emerging applications that advance opportunities for using modeling approaches to data analysis

PUCL

Click to Return to Overall View

Cytometry 60 YEARS
VOLUME 10
THE CELL-SORTER INVENTION TIMELINE

1950: Robert C. Miller and Robert A. Linsley
1951: Walter C. Cline and Robert A. Linsley
1952: Robert C. Miller and Robert A. Linsley
1953: Robert C. Miller and Robert A. Linsley
1954: Robert C. Miller and Robert A. Linsley
1955: Robert C. Miller and Robert A. Linsley
1956: Robert C. Miller and Robert A. Linsley
1957: Robert C. Miller and Robert A. Linsley
1958: Robert C. Miller and Robert A. Linsley
1959: Robert C. Miller and Robert A. Linsley
1960: Robert C. Miller and Robert A. Linsley

1950 1960 Next

PUCL

"Cytometry- 60 Years of Innovation" ISBN 978-1-890473-10-5
DVD set, 2007, Purdue University Cytometry Laboratories

Cytometry 60 YEARS VOLUME 19 THE CELL-SORTER INVENTION TIMELINE

Click to Return to Overall View

1970 1980

Previous Next

PUCL "Cytometry- 60 Years of Innovation" ISBN 978-1-890473-10-5 DVD set, 2007, Purdue University Cytometry Laboratories

Cytometry 60 YEARS VOLUME 19 THE CELL-SORTER INVENTION TIMELINE

Click to Return to Overall View

1980 1990

Previous Next

18 color polychromatic cytometry Roederer
High content flow - Sklar
Hyperspectral cytometry Robinson
Causal networks based on machine learning Nolan

PUCL "Cytometry- 60 Years of Innovation" ISBN 978-1-890473-10-5 DVD set, 2007, Purdue University Cytometry Laboratories

The progression of cell Detection

- It's a cell
- It's a small cell or it's a big cell
- It's a small cell or it's a big cell and it has a DNA content of this
- It's a small cell or it's a big cell and it has a DNA content of this and we can identify this cell as a specific subset

PUCL

It's a small cell or it's a big cell
and we can identify this cell as a specific subset
within a subset of cells

It's a small cell or it's a big cell
and we can identify this cell as a specific subset
within a subset of cells

It's a small cell or it's a big cell
and we can identify each of these subsets within a
heterogeneous population simultaneously

It's a small cell or it's a big cell
and we can identify each of these subsets within a
heterogeneous population simultaneously
We can also evaluate cell function with several
simultaneous parameters. We can label cells with different
intensities of dyes to separate them into groups

PUCL

Progression of Cell Analysis

Preliminary draft of a talk presented before
the NATIONAL ELECTRONICS CONFERENCE
Chicago, October 3, 1956.

"HIGH SPEED AUTOMATIC BLOOD CELL COUNTER AND CELL SIZE ANALYZER"
BY
WALLACE H. COULTER, COULTER ELECTRONICS, CHICAGO, ILLINOIS

Wallace H. Coulter's only
Scientific publication

Cell Analysis – Circa 1956

FIGURE 4.
Oscilloscope pattern showing threshold
setting and relative cell size
and cell size distribution
of a typical sample.

PUCL

Progression of Cell Analysis

400 word memory – Fulwyler's 1965 sorter

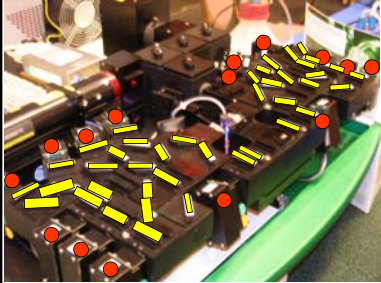

Equivalent list mode storage of about
200 cells with 1 parameter data

Over 30 years of technology development this has led to

PUCL

Hyperspectral cytometry

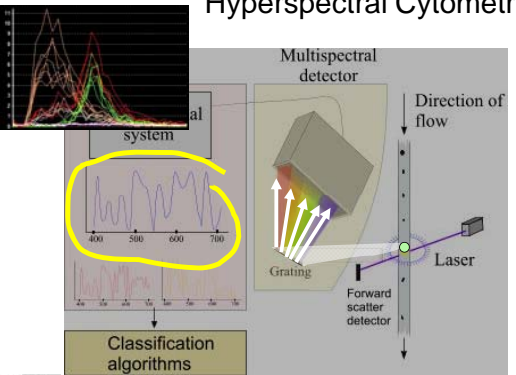
Advanced polychromatic cytometry

● 14-19 PMTs ■ 40-50 filters ■ 1 "PMT" ■ 1 "filter"

PUCL

Hyperspectral Cytometry



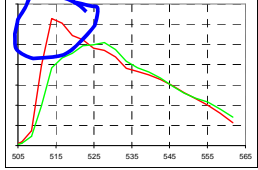
system
 Classification algorithms
 Multispectral detector
 Direction of flow
 Grating
 Laser
 Forward scatter detector

PUCL

Hyperspectral cytometry allows for spectral "unmixing"

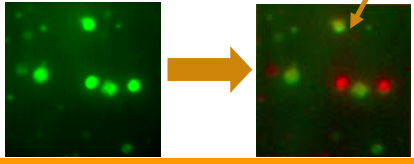
Dye 1: bis-(1,3-dibutylbarbituric acid)trimethine oxonol, DiBAC₆(3)
 Dye 2: 3,3'-dihexyloxacarbocyanine iodide, DiOC₆(3)

FITC filter block



Before: Green (DiBAC₆(3))
 After: Green (DiOC₆(3)) and Red (DiBAC₆(3))

Unmixed DiOC₆(3) And bis-oxonol



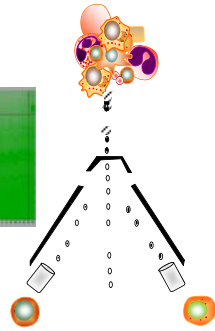
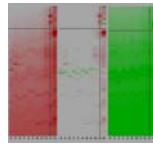
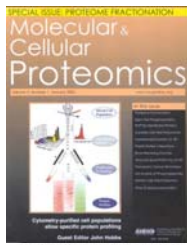
PUCL

Technology advances

- High speed sorting
- Advanced Polychromatic analysis
- Hyperspectral Analysis
- Hypercyte™ – High content Screening
- Multiparameter systems approach to pathways and cell signaling

PUCL

Proteomics profiling of heterogeneous populations ?



Integrating Cytomics and Proteomics*
Tylus Bernas¹, Gérald Grégoire¹, Eli K. Asent¹, and J. Paul Robinson¹
Molecular and Cellular Proteomics, 5:2-13, 2006

PUCL

You don't have to physically sort cells to apply a systems approach....

- Power of a systems approach to cell analysis is shown in work from Gary Nolan's Laboratory at Stanford

Cell, Vol. 118, 217-228, July 23, 2004, Copyright ©2004 by Cell Press

Single Cell Profiling of Potentiated Phospho-Protein Networks in Cancer Cells

Jonathan M. Irish,¹ Randi Hovland,^{2,3}
Peter O. Krutzik,¹ Omar D. Perez,¹
Oystein Brunserud,^{2,3} Bjorn T. Gjertsen,^{1,4}
and Garry P. Nolan^{1*}

PUCL

Mechanistic Insights from the Single Cell: Inference Engines for Clinically Predictive Indicators

Garry P. Nolan, Ph.D.
Stanford University
Dept. of Microbiology & Immunology

Flow Cytometry for Intracellular Staining

Drug Action, Perturbations, Induced Signaling

Antibodies (Fluorophore-conjugated)

Light Scatter Properties

Signaling

Cell Type

Analysis

Orbiter

Sample Specificities

- p38 MAPK
- JNK cJun
- AKT PIP2, PIP3
- PKC α / β / γ , Rsk
- Raf, Mek, ERK, ELK
- Rak, Creb,
- STATs, SRC
- CREB, cJUN, IKK α ,
- p53 s15, s20 s37, s392
- Pyk2, Shc, Fak, Src
- Sip1a, Zfp70, Syk, Lat, Vav,
- Lck, PLC γ
- Beta-integrins
- NF- κ B, p65
- Caveolin
- Paxillin
- FLN3
- MEK5

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.

>80 specificities

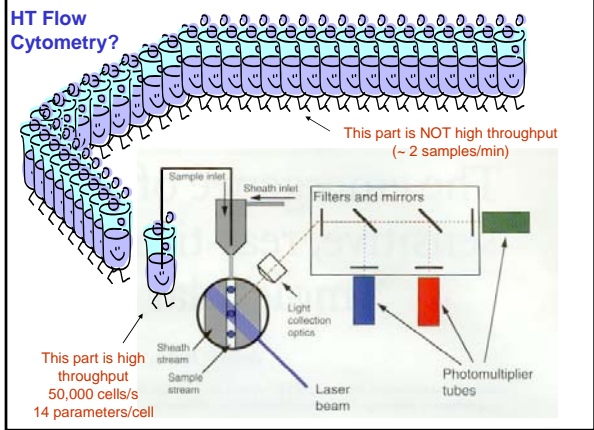
New Mexico Molecular Libraries Screening Center

Larry A. Sklar, PhD

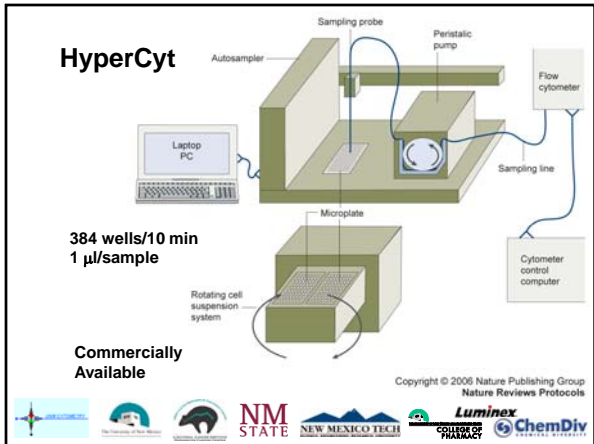
Regents Professor of Pathology
and Distinguished Professor of Pharmacy
Director of Basic Research, UNM Cancer Center
Director, New Mexico Molecular Libraries Screening Center

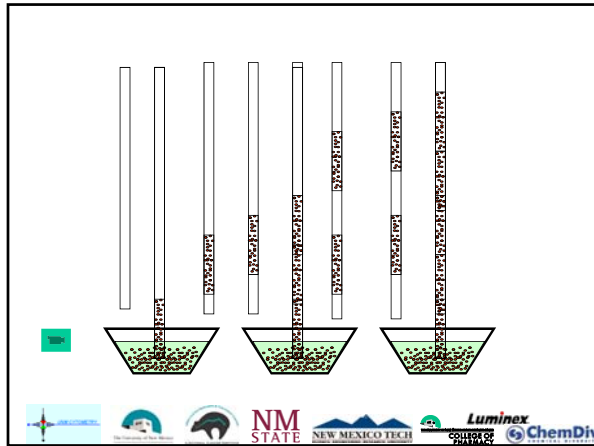


HT Flow Cytometry?



HyperCyt



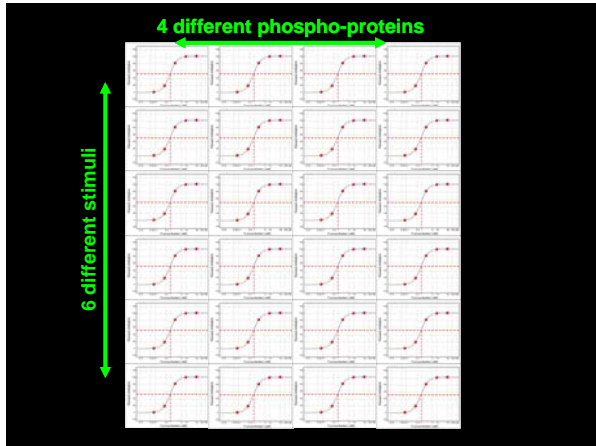


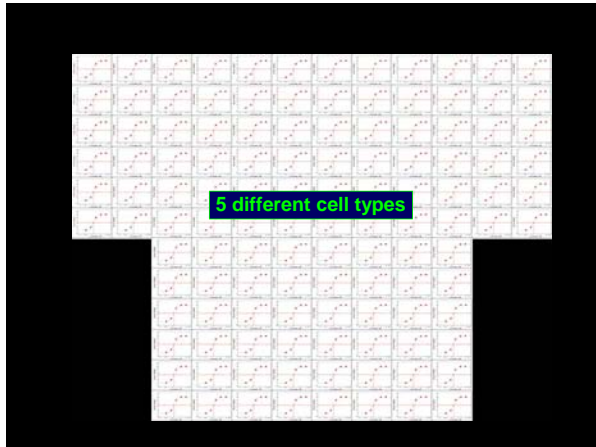
HT Flow Cytometry Opportunities

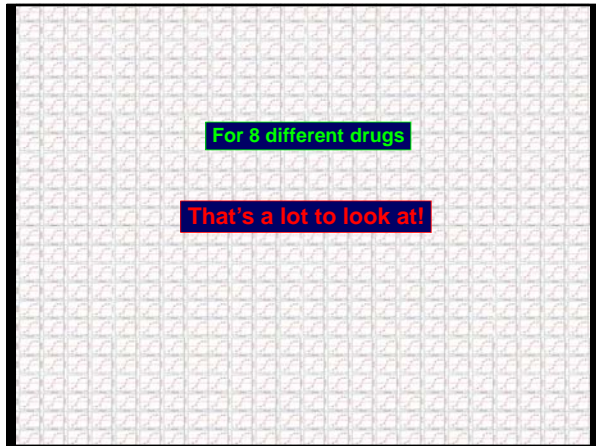
- Thousands of cell-based assays
- Growing number of bead-based assays for analytes and assemblies
- Multiparameter Platform
 - Homogeneous resolution of free and bound for ligand binding and protein assemblies to 500 nM
 - High Content
 - Multiplexing (384 x 20 plexes per 10 min)
 - Platform Opportunities: 1536 wells in 10 min
- NM MLSC Regional Partnerships for imaging agents, PET, SPECT-CT, LANL Isotopes

HyperPlex = HyperCyt and Luminex

Theoretical potential for 50 plex in 1536 well format, 10 min
(20M per day per detector)
Selectivity

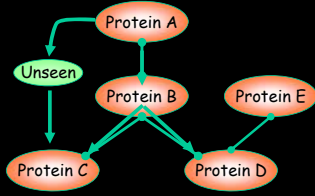






Bayesian Networks...

A statistic representation of reality: Machine Learning



A Mathematical (probabilistic) description of the connections in network ...

If Protein A is **Off**, Protein B is **On** with probability 0.8

$$P(B=1|A=0) = 0.8$$

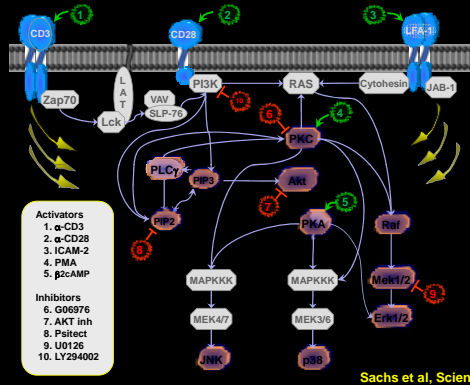
$$P(B=1|A=1) = 0.3$$

A Network..

• Bayesian Networks find correlations across signaling nodes by building inference map one connection at a time, providing a statistical score for each potential link.

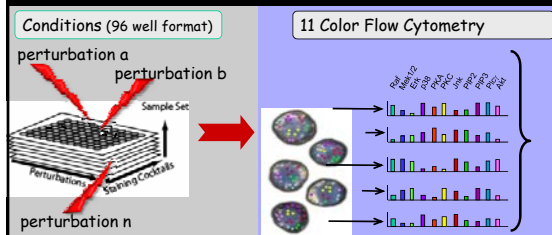
Sachs et al, Science, 2005

Interventions on a known map...



Sachs et al, Science, 2005

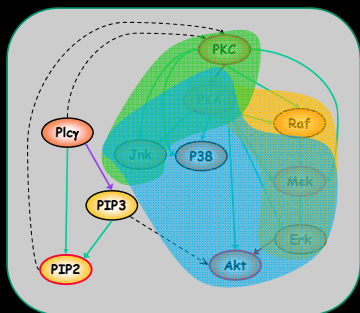
Primary T-Lymphocyte Data



- 9 phosphoproteins, 2 phospholipids
- 600 cells per condition
 - 5400 data-points
- Primary human T-Cells
- 9 conditions
 - (6 Specific interventions)

Sachs et al, Science, 2005

Deep Correlations in the data: A T cell signaling map *ab initio* from multiparameter data by Bayesian Inference.



T Cells	15/15
All Cells	17/17
Reversed	1
Missed	3

Sachs et al, Science, 2005

Result...



- “..we correctly reverse-engineered and rapidly inferred the basic structure of a classically understood signaling network that connects a number of key proteins in human T cell signaling, a map built by classical biochemistry and genetic analysis over the past 2 decades.”

PUCL

Science 308: 527, 2005

Summary and Conclusions

- Technologies such as flow cytometry are often assumed to have a narrow phenotypic or cell cycle application
- New technologies are emerging creating even more detection opportunities
- High throughput sampling is now a reality
- The power of multiparameter detection and powerful analytic capability is evident
- Systems modeling approaches are clearly the next implementation in cytometry
- Innovative assay design and software approaches have created a new paradigm for single cell analysis
- It is now possible to implement informatics approaches to cytometry data

PUCL

Acknowledgements

Thanks to collaborators on this presentation:
Gary Nolan, Larry Sklar, Bruce Edwards

Senior Scientists: Bartek Rajwa, Yanan Jiang**
Postdocs: Valery Patsekina, Tytus Bernas, Sang Youp Lee, Lova Rakotomalala

Graduate Students: Wamiq Ahmed, Bulent Bayraktar, Silas Leavesley, Connie Snyder, Muru Venkatapathi, Jia Liu

Faculty: Kinam Park, V.J.Davison, Arun Bhunia, Dan Hirleman

PUCL Staff

- Jennie Sturgis (Imaging)
- Kathy Ragheb (Flow)
- Cheryl Holdman (Flow)
- Gretchen Lawler (CPC)
- Steve Kelley (Network)
- Hildred Rochon (C4L)

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



PUCL

Bindley Bioscience Center, Purdue University



PUCL, Purdue University
