Delivering advanced analyses to the broader community: a view from Cytometry

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#### What's So Great About Cytometry?

#### Challenge

- Study heterogeneous primary tissues
- Pinpoint abnormal cell subsets
- Identify and track cancer stem cells
- Look not just at 'pathways', but the broader signaling network
- Identify targets for drug discovery
- Choose, monitor, & optimize therapies
- Understand mechanisms of cell/cell and disease cell/host cell interactions
- Detect disease earlier

#### Example Question

Can we begin discovery in human samples?

Can we spot pre-transformation cells?

Is there a rare, therapy-resistant subset?

Are there off-target effects of a drug?

What (signaling) mechanisms enable cells to resist a particular therapy?

Do patients that share responses share profiles?

How do cancer cells interact with and alter the host microenvironment or immune system?

Can we detect circulating cancer cells or immune cells that encountered tumor?

Irish, Kotecha, and Nolan, Nat Rev Cancer 2006

### Overview: Compare cell signaling with Flow Cytometry



### Mechanism

### Technique

6.8%

### Screening

## Diagnostics

BCL2

CD20



9.2%

Irish, et al. Cell

6.8%

### Mass Cytometry: 40+ parameters & no compensation



## High Dimensional Cytometry is here



#### 30+ parameters hosted and analyzed on Cytobank

Bendall et al. Science 2011 Fienberg et al. Cytometry 2012 Behbehani et al. Cytometry 2012 Bodenmiller et al. Nature Biotech 2012



## The Effect of a Small Molecule Kinase Inhibitor on Normal Immune Signaling – *Dasatinib Example*



## Deep Profiling of Immune and Drug Responses Across the Hematopoetic Continuum

#### Phosphoprotein placement



Bodenmiller B. et al. Nature Biotech, 2012

Cytometry is growing more powerful ... ... in parallel the experiments, data sets, and analysis technique are also growing more complex

> How do we: Deliver advanced analysis & algorithms to biological and clinical communities?

Lower barrier of entry for statisticians and computational biologists?

(and meet technical needs of phospho-flow, fluorescent cell barcoding, and mass cytometry)

Cytometry is growing more powerful ... ... in parallel the experiments, data sets, and analysis technique are also growing more complex

## How do we: Enable Garry Nolan's next talk (Thu. Morning 9 am)

(and meet technical needs of phospho-flow, fluorescent cell barcoding, and mass cytometry)

## Key Tools

### 1) Access to samples

- ideally uniform initial therapy
- long term clinical outcomes or paired samples
- balanced training and testing sample sets
- 2) Flow cytometry & signaling network profiles
  - map signaling in every cell within a tumor specimen
  - markers for tumor, non-malignant, and cell subsets
  - cell sorting for follow up studies of genetics and epigenetics
- 3) Cloud computing to link all our knowledge & tools
  - data storage & annotation, data sharing
  - web based analysis tools for researchers
  - computational analysis & modeling tools (SPADE)
  - informatics (patient information, ontologies)

### Cytobank is for *managing*, *sharing* & *analyzing* flow experiments over the web Experiment Inbox



#### Experiment = Collection of FCS files

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## Why did we build Cytobank?

### The Future of Single Cell Analysis



## Analysis and annotation of flow cytometry data is fragmented



### Current Analysis Methods are not a scalable solution



Design and Validation

Bench Time

Data Analysis



## Parameters: 4 Plots: 6



### **Individual Efforts**

Routine

Bendall, Simonds, et al. Science, May 2011

### Current Analysis Methods are not a scalable solution



- Design and Validation
- Bench Time
- Data Analysis

## Parameters: 14 Plots: 91







### Joint Efforts

Bendall, Simonds, et al. Science, May 2011

### Current Analysis Methods are not a scalable solution



## Current approaches are not scalable ...

in an era of **big data**, **collaborative science** & **systems medicine** 

#### Multiple Tools for Data Collection and Reporting









Clinically Actionable Finding







Key Challenges Data Management Collaboration & Communication Scalable Computing Resources Novel Analyses & Visualizations Reduce Time to Results Platform to build on top of

Cell Biology– Immunology- Informatics – Clinic Cross Discipline/Institution Collaborations

## Cytobank enables single cell analysis



Cytobank is designed for: Experiment Organization Collaboration Novel Analysis High Dimensional Flow Big Data Scalable Computation Platform Development Reducing time to Results

Cell Biology– Immunology- Informatics – Clinic Cross Discipline/Institution Collaborations

## Cytobank is available in multiple forms

- Community Cytobank <u>www.cytobank.org</u>
  - Free access to base functionality
- DVS Cytobank– <u>dvs.cytobank.org</u>
  - For the mass cytometry community
  - Premium modules and functionality (e.g. SPADE)
- Enterprise Cytobank e.g. <u>companyX.cytobank.org</u>
  - Hosted, backed up, and maintained for lab or company X
  - Designated administrators regulate access and logins
  - Premium modules and functionality (e.g. SPADE)
  - Dedicated Compute Resources

Maintenance, updates and support provided by Cytobank Inc. (<u>www.cytobankinc.com</u>) How do we: Deliver advanced analysis & algorithms to biological and clinical communities?

Lower barrier of entry for statisticians and computational biologists?

## Manage, Analyze, Share Cytometry Data on the Web



#### Experiment = Collection of FCS files

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### Create a new experiment and upload FCS files

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## Organize information around samples using Experimental Variables



#### All Conditions Unstim FL GM-CSF G-CSF IL-3 IFN-g

#### All Conditions

Drag files from "Untagged" box to the "Condition" boxes below to associate them with that tag. Use the "Filter" and "Move File"



Assign samples to experimental variables



Identify populations of interest (gating)

### Use Experimental Variables to create and pivot figures



# Share results and analyses with collaborators and the community









Individual and project level sharing for collaborators

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## Cytobank Benefits (End Users)

- Manage, share and backup your raw data (fcs files)
- Share analyses and experiments with colleagues and collaborators
  - Publication quality figures
- Managers and PIs eliminate fear about "losing your data" when people leave their lab
- Core facilities/Service labs can use Cytobank for value added services
  - Data management and backup
  - Remote analysis and support
- Capture experiment information while creating figures
  - "What did I measure in that experiment from 6 months ago again?"

## So... How is this working?

## Upload. Analyze. Share. Anywhere.



http://blog.cytobank.org/category/user-stories/

User registered on <u>www.cytobank.org</u> as of December 2011

## What Researchers are Doing with Cytobank

- Search "Cytobank" in Google Scholar for 50+ citations (in < 2yrs):</li>
  - Specific cellular signal-transduction respons on therapy with **Phosflow** ATRA, valproic acid and theophylline in acute Skavland et al (Norway) – Feb. 2011 Nature Blood Cancer ctivity of anti-CD20 antibodies CD137 🕬 Immunophenotyping Kohrte Cell-to-Cell Variability in PI3K Protein Level Regulat ctivity **Biochemistry** in Cell Populations Yuan et al (Harvard) Lan 2011 Current Biology Oxidative ation of Kaposi's Sarcoma-Associated Cancer Herpesvirus and common numary Effusion Lymphoma Cells • Li et al (UCLA) – Jan 2011 Journal of Virology Apoptosis Poor cytokine-induced phosphorylation in chronic a patients at *timely reversed by tyrosine kinase inhibitor therapy* diagnosis is Informatics 2010 Experimental Hematology Computational solutions to large-scale data management and analyses Schadt et al (California) – Sep 2010 Nature Reviews Genetics

#### More at http://blog.cytobank.org/publications

## Cytobank is the platform for CyTOF Analysis



Cytobank cloud computing analysis of mass cytometry data

30+ parameters hosted and analyzed on Cytobank

Bendall et al. Science 2011 Fienberg et al. Cytometry 2012 Behbehani et al. Cytometry 2012 Bodenmiller et al. Nature Biotech 2012



## Published Data (and Protocols) for the community

### Nolan Lab

Signaling-Based (Flow & Mass) Cytometry Resource

Powered by Cytobank

#### www.cytobank.org/nolanlab

#### home



Principal Investigator: Garry P. Nolan, Ph.D. Affiliation: Curator: Website: Research:

Stanford University, Stanford, CA Garry Nolan http://www.stanford.edu/group/nolan

cancer, cell signaling, immunology, leukemia, intracellular cytometry, mass cytometry, systems biology

#### About the lab

We analyze cell signaling directly by next-generation mass and traditional flow cytometry, focusing on following multiple phosphoproteins in complex populations of primary cells such as mouse cells and human clinical samples. Using mass cytometry, up to 34 simultaneous protein parameters can be measured in single cells including multiple kinases, phosphoproteins, cell cycle proteins, and other parameters, enabling resolution of cellular activation states.

We are using these techniques to study healthy biochemical signaling in the immune system and dysfunctional signaling in hematological malignancies including AML, ALL, JMML, MDS, follicular lymphoma and autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus. We are also using the technique for drug screening in primary cells to truly select for drugs with efficacies in certain cell subsets but not others.

#### About this resource

This resource was developed to facilitate dissemination of protocols and materials used in the lab as well as provide access to published articles linked to the underlying data and analysis via Cytobank Reports



Fienberg H, Simonds EF, Fantl WJ, Nolan GP, Bodenmiller B

Cytometry, May 2012

Other Resources Nolan Lab Homepage

Stanford Proteomics Cytobank Blog

Contact Cytobank.org Cytobank Twitter (@cytobank) Cytobank Support

**Experiment Protocols** for flow and mass cytometry

**Conjugation protocols** for mass cytometry

Publications linked to underlying data and analysis

www.cytobank.org/nolanlab

### Publish Data & Annotations with Manuscript



Flow cytometry is an essential tool for dissecting the functional complexity of hematopoiesis...

#### Experiment Overvie

#### Purpose:

We sought to use mass cytometry to obtain a system-wide view of immune signaling in healthy human hematopoiesis...





Canonical, cell type-specific signaling functions. Stimulation by IL-7, B cell receptor cross-linking (BCR), or lipopolysaccharide (LPS)...

Nolan lab mass cytometry dataset www.cytobank.org/nolanlab



#### Bendall et al., Science 2011 data hosted by Cytobank

## What's Next?

## The Cytobank Platform



## Integration with Core Facilities (Stanford)

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## The Cytobank Platform



## Drug Discovery Assays (linked to underlying data)



#### Cytobank Premium

## Signaling Diagrams (linked to underlying data)

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## Add Ontological Keywords To a Cytobank Experiment

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#### Keywords (74)

(lymphatic tissue carcinoma) or (lymphoma) 90m answer anterior apoptosis regulator bcl-2 b-cell cll/lymphoma 2 b-lymphocyte b-lymphocytes bcl2 bcl2 gene bcl2\_human bcl2\_mouse bcl2\_rat bcr (4) bcr gene bcr protein biospecimen biospecimen core resource breakpoint cluster region

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#### - Annotations (107)

Preferred Name	Term ID	Ontology	Semantic Type(s)
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Cells	MTHU001933	Logical Observation Identifier Names and Codes	T025: Cell
Cells	LP14738-6	Logical Observation Identifier Names and Codes	T025: Cell
Cell	<u>Cell</u>	NCI Thesaurus	T999: NCBO BioPortal concept
tumor	npo:NPO 1573	NanoParticle Ontology	T999: NCBO BioPortal concept
Tumor	<u>LP7664-8</u>	Logical Observation Identifier Names and Codes	T191: Neoplastic Process

## The Cytobank Platform





## Large Scale Computations (delivered to your browser)

**Cytobank Premium** 

## The Cytobank Platform



## Published Data (and Protocols) for the community

### Nolan Lab

Signaling-Based (Flow & Mass) Cytometry Resource

Powered by Cytobank

#### www.cytobank.org/nolanlab

#### home



Principal Investigator: Garry P. Nolan, Ph.D. Affiliation: Curator: Website: Research:

Stanford University, Stanford, CA Garry Nolan http://www.stanford.edu/group/nolan

cancer, cell signaling, immunology, leukemia, intracellular cytometry, mass cytometry, systems biology

#### About the lab

We analyze cell signaling directly by next-generation mass and traditional flow cytometry, focusing on following multiple phosphoproteins in complex populations of primary cells such as mouse cells and human clinical samples. Using mass cytometry, up to 34 simultaneous protein parameters can be measured in single cells including multiple kinases, phosphoproteins, cell cycle proteins, and other parameters, enabling resolution of cellular activation states.

We are using these techniques to study healthy biochemical signaling in the immune system and dysfunctional signaling in hematological malignancies including AML, ALL, JMML, MDS, follicular lymphoma and autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus. We are also using the technique for drug screening in primary cells to truly select for drugs with efficacies in certain cell subsets but not others.

#### About this resource

This resource was developed to facilitate dissemination of protocols and materials used in the lab as well as provide access to published articles linked to the underlying data and analysis via Cytobank Reports



Fienberg H, Simonds EF, Fantl WJ, Nolan GP, Bodenmiller B

Cytometry, May 2012

Other Resources Nolan Lab Homepage

Stanford Proteomics Cytobank Blog

Contact Cytobank.org Cytobank Twitter (@cytobank) Cytobank Support

**Experiment Protocols** for flow and mass cytometry

**Conjugation protocols** for mass cytometry

Publications linked to underlying data and analysis

www.cytobank.org/nolanlab

### **BD FACSelect: Link Interactive Data to Reagents**

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http://www.cytobank.org/facselect

### **BD FACSelect: Link Primary Flow Data to Reagents**

🔇 Cytobank - CD3\_UCHT1\_Ht × 🔽

←

→ C (S) reports.cytobank.org/21/v1

CD3 PE-Cy5



Calculated Raw values of statistic using X-Axis channel(s): Use Panel/Channel Values

	LIVE	Ferm 1	Ferm II	Ferm III	Fermity (0.5x)	Fermine (1.0x)
1x	6.8	5.79	4.48	4.56	4.72	4.2
1/4x	X	5.16	4.15	3.83	4.23	3.67
1/16x	X	4.19	3.28	2.82	3.28	2.66

View in Cytobank Jump to Gating Hierarchy

Hierarchy Back to Top

http://www.cytobank.org/facselect

☆ 🤱 🔧

### **DVS Cytobank Resource**



http://dvs.cytobank.org

Cytometry is growing more powerful ...

... in parallel the experiments, data sets, and analysis technique are also growing more complex

## We designed Cytobank to simplify data sharing and analysis for larger flow cytometry experiments

(and to meet technical needs of phospho-flow, fluorescent cell barcoding, mass cytometry, and computational analysis)

Cytometry is growing more powerful ...

... in parallel the experiments, data sets, and analysis technique are also growing more complex

## We designed Cytobank to enable Garry Nolan's next talk (Thu. Morning 9 am)

(and to meet technical needs of phospho-flow, fluorescent cell barcoding, mass cytometry, and computational analysis)

#### **Garry Nolan**

#### Projects:

Jonathan Irish Erin Simonds Matt Clutter Michael Linderman Zach Bjornson Peter Krutzik Sean Bendall Tiffany Chen Rob Bruggner

#### Cytobank Inc:

Chad Rosenberg Geoffrey Kraker Angela Landrigan Amy Lee Chris Coveney Jennifer Davis Stu Blair Robin Powell

#### Rest of Nolan & Irish Lab

UCSF: Mignon Loh/Nikki Flores Kevin Shannon <u>Stanford:</u> *Peng Qui/Sylvia Plevritis* <u>U Toronto & DVS</u> *Scott Tanner et. al.* 

<u>Becton Dickenson</u> Erika O'Donnell et. al Bob Balderas



Join the Community – www.cytobank.org

More at blog.cytobank.org

nikesh@cytobank.org

Other references: dvs.cytobank.org www.cytobank.org/facselect www.cytobank.org/nolanlab DVS Sciences Becton Dickenson Thank you!

## nikesh@cytobank.org