Single Cell Systems Biology: Measuring Cell Signaling by Flow Cytometry

Cytobank: Manage, Analyze & Share your cytometry data from Anywhere

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### What’s So Great About Flow?

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Example Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study heterogeneous primary tissues</td>
<td>Can we begin discovery in human samples?</td>
</tr>
<tr>
<td>Pinpoint abnormal cell subsets</td>
<td>Can we spot pre-transformation cells?</td>
</tr>
<tr>
<td>Identify and track cancer stem cells</td>
<td>Is there a rare, therapy-resistant subset?</td>
</tr>
<tr>
<td>Look not just at ‘pathways’, but the broader signaling network</td>
<td>Are there off-target effects of a drug?</td>
</tr>
<tr>
<td>Identify targets for drug discovery</td>
<td>What (signaling) mechanisms enable cells to resist a particular therapy?</td>
</tr>
<tr>
<td>Choose, monitor, &amp; optimize therapies</td>
<td>Do patients that share responses share profiles?</td>
</tr>
<tr>
<td>Understand mechanisms of cell/cell and disease cell/host cell interactions</td>
<td>How do cancer cells interact with and alter the host microenvironment or immune system?</td>
</tr>
<tr>
<td>Detect disease earlier</td>
<td>Can we detect circulating cancer cells or immune cells that encountered tumor?</td>
</tr>
</tbody>
</table>

Flow cytometry allows tens of measurements per cell

**Analysis Steps**

1. **Identify live cells**
2. **Identify cell type**
3. **Evaluate cell signal**
Dramatically Different Profile in Immune Defender Cells

profiling a lymphoma patient

+2.5 fold
- no change
-2.5 fold

log$_2$ scale
Stimulated / Control

83% B (cancer)
17% T (immune defender cells)
**Mechanism**

Perez, et al.
Blood,

Krutzik, et al.
Nat Methods

**Technique**

Kotecha, et al.
Cancer Cell

**Screening**

Kiessling, et al.
Nat Chem Bio

**Diagnostics**

Irish, et al.
Cell
High dimensional cytometry is here

Cytobank cloud computing analysis of mass cytometry data

CD14 expression in cell populations (circles) identified by SPADE in one flow file

30+ Parameters hosted and analyzed on Cytobank

Bendall et al. Science 2011
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 = Collection of FCS files

Experiment Analysis

Experiment Details
Cytobank is available in multiple forms

- **Cytobank Community Version**— [www.cytobank.org](http://www.cytobank.org)
  - Hub for the Cytobank community & associated resources
  - Users can login to manage and analyze their data
  - Supported through vendor partnerships

- **Hosted Cytobank**— e.g. [labx.cytobank.org](http://labx.cytobank.org)
  - Cytobank version hosted, backed up, and maintained for lab or company X
  - Designated administrators regulate access and logins
  - **Dedicated Compute Resources**
  - **Access to premium modules and functionality** (e.g. SPADE)

  Maintenance, updates and support provided by Cytobank Inc. ([www.cytobankinc.com](http://www.cytobankinc.com))

A web-enabled device lets you get to your data from anywhere in the world...

User registered on www.cytobank.org
See http://blog.cytobank.org/category/user-stories/
What Researchers are Doing with Cytobank

- Search on “Cytobank” in Google Scholar:
  - Specific cellular signal-transduction responses to in vivo combination therapy with ATRA, valproic acid and theophylline in acute promyelocytic leukemia
    • Skavland et al (Norway) – Feb. 2011 Nature
  - CD137 stimulation enhances the antilymphoma activity of anti-CD20 antibodies
    • Kohrt et al (Stanford) – Sept 2010 Blood
  - Cell-to-Cell Communication of Cytokines Correlates PI3K-AKT Pathway Activity in Cell Populations
    • Yuan et al (Harvard) – Jan 2011 Current Biology
  - Oxidative Stress Induces Reactivation of Kaposi’s Sarcoma-Associated Herpesvirus in Primary Effusion Lymphoma Cells
    • Li et al (China) – Jan 2010 Journal of Virology
  - Poor cytokine-induced phosphorylation in chronic myeloid leukemia patients at diagnosis is effectively reversed by tyrosine kinases
    • Jalkanen et al (Finland) – Sep 2010 Experimental Hematology
  - Computational and informatics-scale data management and analyses
    • Schadt et al (California) – Sep 2010 Nature Reviews Genetics

More at http://blog.cytobank.org
Flow Cytometry Examples
(Lymphoma, Drug Discovery, Mass Cytometry)

Annotations using NCBO BioPortal

Cytobank: A cloud-computing platform for Cytometry

Cytobank Reports
A new way to publish data
Flow Cytometry Exampleless (Lymphoma, Drug Discovery, Mass Cytometry)

Annotations using NCBO BioPortal

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Cytobank Reports A new way to publish data
References of Examples

• Lymphoma
  – Irish et. al PNAS 2010

• Drug Discovery
  – Krutzik et. al Nature Chemical Biology 2008

• Mass Cytometry
  – Bendall et. al Science 2011
Mapping Signaling in Every Cell using Flow Cytometry

Irish et al., PNAS 2010
The Subset Was Termed “Lymphoma Negative Prognostic” (LNP) Cells Because They Are Found in Patients with Poor Clinical Outcomes

Every 1% LNP cells in the tumor at diagnosis increased the patient’s risk of death in the next year by 2.5%

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Samples obtained prior to therapy; uniform initial therapy

Irish et al., *PNAS* 2010
Primary Cell Screening using Phosphoflow

- 4 natural products + 4 commercial inhibitors
- Titrate 6 concentrations of each compound
- Stimulate with IFNg, IL-4, IL-6, IL-7, IL-10, IL-15
- Analyze B cells, CD4+ and CD4- T cells, CD11b-hi neutrophils, CD11b-int macrophages
- Measure Stat1, Stat3, Stat5, Stat6 phosphorylation

Krutzik et al, Nature Chemical Biology, 2008
Blood

- Total cell measure from the blood indicates no drug effect.

- Looking at individual cell types shows B cells are potently inhibited.

Krutzik et al, Nature Chemical Biology, 2008
Drug Discovery Assays (linked to underlying data)
Mass Cytometry: 30+ parameters & no compensation

Assays to Relevant Biological Pathways:
- Cytokines & Growth Factors (G-CSF)
- Apoptosis Pathways
- Phosphatase Activity ($H_2O_2$)

Fix & Perm

Stain

Unstimulated

After 4 minutes

Cell Type

Cell Signal
SPADE allows for analysis of high dimensional cytometry

Figure 54b (Surface-only tube)

Expression of immunophenotype surface markers overlaid onto the SPADE plots of healthy human bone marrow: The expression of an additional 18 surface markers from the 31 surface marker analysis of the same sample was overlaid on the SPADE plot of the 31 core surface markers. These 18 surface markers were not used in the SPADE plot and their localized expression is based solely on the shared expression patterns of the 13 core surface markers.

Note: This figure was created in a flow cytometry analysis package called SPADE (not yet published) using data gated and exported from Cytobank. The link below will bring you to an illustration from which you can export the exact gated data used.

www.cytobank.org/nolanlab
Bendall et. al Science 2011
Qiu et al Nature Biotech 2011
Large Scale Computations (delivered to your browser)
Flow Cytometry Examples (Lymphoma, Drug Discovery, Mass Cytometry)

Annotations using NCBO BioPortal

Cytobank: A cloud-computing platform for Cytometry

Cytobank Reports
A new way to publish data
Analysis and annotation of flow cytometry data is fragmented

**Experimental Context**
- Stimulations, Inhibitors, Sample IDs
  - Source: Lab Notebook

**Machine settings**
- Raw values
  - Source: FCS Files

**FCS 3.0 File**
- Machine settings
- Raw values
- Collection of FCS files

**Data Preprocessing (Compensations, Transformations)**
- Data filtering (Gating)
- FCS file groupings
  - Source: Analysis Program

**Digested Figures + Results**
- Source: PPT Presentation

**Advanced analysis, Aggregate Views**
- Advanced Analysis, Aggregate Views
  - Source: Statistical Programs

**Figures to communicate results**
Trends in Cytometry & Cell Analysis (I)

Future of Flow
- New solution required
- Organize/manage experiments
- Communicate & collaborate
- Annotations & analyses linked
- Novel analyses & visualizations
- Scalable compute resources
- Platform to build on top of

- Majority of flow solutions built for this space
Cytobank is for managing, sharing & analyzing flow experiments over the web.

Experiment = Collection of FCS files

Experiment Analysis

Experiment Details
Create a new experiment and upload FCS files

Launch web browser & Login to Cytobank

Create a new Experiment

Upload FCS Files

Uploading to http://staging02.cytobank.org/cytobank/uploadServlet
Organize information around samples using Experimental Variables

**Variable Types**
- Individual Patients: AML Patient 4 (P04), AML Patient 5 (P05)
- Phospho-proteins: Phosphorylated Stat3 (p-Stat3), Phosphorylated Stat5 (p-Stat5)
- Conditions: Unstimulated, GM-CSF, IL-3, G-CSF, IFNγ, Flt3 Ligand (FL)
- Cell Populations: Intact Cells

**Experiment Variable Values**

**Assign samples to experimental variables**

**Identify populations of interest (gating)**
Use Experimental Variables to create and pivot figures.

Arrange figure using Experimental Variables.

Plot controls to specify plot type and display.

- Plot Controls
  - Plot Types
    - Heatmap
    - Histogram X
    - Histogram Y
    - Contour
    - Shaded Contour
    - Shadow
    - Density Dot
    - Dot
    - Heatmap
    - Histogram Overlay
    - Forward Scatter
  - X-Axis
    - Use Panel/Channel Values
    - Show Panel/Channel Values
    - Show Gates
      - No
    - Show Percentages
      - No
  - Illustration
    - Calculated Log10 Ratio of Medians by First Column using Panel/Channel Values
Share results and analyses with collaborators and the community.

Individual and project level sharing for collaborators.

Make experiments public to share with community.

Irish 2004 AML Dataset

Healthy PBMC Mini Profile - Comp Tester

Irish 2004 AML Dataset
Cytobank Benefits

- Easy way to manage, share and backup your .fcs files
- Share analyses and experiments with colleagues and collaborators
- 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?”
The Cytobank Platform

Data Collection Hooks Integration with Core Facilities (e.g. Stanford)

Translational Assays Drug Screening (Clinical) Reports

High Dimensional Cytometry, Mass Cytometry, (SPADE, Large Scale Computations)

Community Resources (BD FacsSelect) Public Cytometry Collections

Accessible from any web-enabled device anywhere
NCBO: Key activities - http://www.bioontology.org/

• We create and maintain a library of biomedical ontologies.

• We build tools and Web services to enable the use of ontologies and their derivatives.

• We collaborate with scientific communities that develop and use ontologies.
Ontology Services
  • Download
  • Traverse
  • Search
  • Comment

Mapping Services
  • Create
  • Download
  • Upload

Widgets
  • Tree-view
  • Auto-complete
  • Graph-view

Annotation
  Term recognition

Data Access
  Fetch “data” annotated with a given term

http://bioportal.bioontology.org
Integration with NCBO BioPortal

BCR Signaling in Follicular Lymphoma (Blood 2006)

An example of BCR signaling in human follicular lymphoma samples. When looking at the B cells within these human follicular lymphoma (FL) tumors, BCL2 marks the tumor cells (it is overexpressed due to the t14;19 translocation, a signature of FL). So in the illustrations, BCL2+ B cells are lymphoma cells, whereas BCL2- B cells are non-malignant B cells within the tumor. You can see that their signaling differs in response to BCR engagement. Also present within the tumor are T cells, which provide another control for these experiments comparing the kinetics of BCR signaling in tumor and non-malignant B cells within the same tumor sample.

- a-BCR, a-BCR + H2O2, 0m, 4m, 16m, 60m, 90m, FL Patient 10, FL Patient 9 bcl2-pe, cd20c-percpcy5.5, fl1-a, forward scatter, lambda-fitc, p-erk12-ax647, side scatter, p-syk-ax647
Ontologies (Initial Set) Used in Cytobank Keywords

- Gene Ontology, Gene Ontology Extension,
- ICD10, ICD10CM,
- Logical Observations Identifier Names and Codes (LNC),
- MedDRA (MDR),
- NCI Thesaurus (NCIt),
- RadLex (RID),
- SNOMED Clinical Terms (SNOMEDCT),
- Medical Subject Headings (MSH),
- Online Mendelian Inheritance in Man (OMIM)
- Molecule Role (INOH Protein name/family name ontology) (IMR)
Add Keywords To a Cytobank Experiment

tumor and non-malignant B cells within the same tumor sample: a-BCR, a-BCR + H202, 0m, 4m, 16m, 60m, 90m, FL Patient 10, FL Patient 9 bc12-pe, cd20c-percpcy5.5, fli-1-a, forward scatter, lambda-fitc, p-erk12-ax647, side scatter, p-syk-ax647

Keywords (74)

(lymphatic tissue carcinoma) or (lymphoma) 90m answer anterior apoptosis regulator bcl-2 b-cell cl/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 cell cells (3) chromosomal translocation process control (4) control group due to (2) example follicle follicular follicular lymphoma (3) forward (2) h202 homo sapiens (2) human (2) human - origin humans hydrogen peroxide kinetics (2) lymphoma (5) lymphoma cell count lymphoma cells (2) lymphoma, follicular malignant lymphoma mass mouse lymphoma murine t-lymphocytes murine tumor cells neoplasm (3) neoplasms non-malignant patient (4) patients pharmacokinetics positive present (3) presentation prevention & control protein domain provide (2) response (3) routine signature sample scientific control signal transduction signaling (2) signature specimen t-lymphocyte t-lymphocytes translocation tumor (2) tumor cells tumor cells, uncertain whether benign or malignant tumor tissue veterinary patient

Annotations (107)

<table>
<thead>
<tr>
<th>Preferred Name</th>
<th>Term ID</th>
<th>Ontology</th>
<th>Semantic Type(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>D002477</td>
<td>Medical Subject Headings</td>
<td>T025: Cell</td>
</tr>
<tr>
<td>Cells</td>
<td>MTHU001933</td>
<td>Logical Observation Identifier Names and Codes</td>
<td>T025: Cell</td>
</tr>
<tr>
<td>Cells</td>
<td>LP14738-6</td>
<td>Logical Observation Identifier Names and Codes</td>
<td>T025: Cell</td>
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<tr>
<td>Cell</td>
<td>Cell</td>
<td>NCI Thesaurus</td>
<td>T999: NCBO BioPortal concept</td>
</tr>
<tr>
<td>tumor</td>
<td>np0:NPO_1573</td>
<td>NanoParticle Ontology</td>
<td>T999: NCBO BioPortal concept</td>
</tr>
<tr>
<td>Tumor</td>
<td>LP7564-8</td>
<td>Logical Observation Identifier Names and Codes</td>
<td>T191: Neoplastic Process</td>
</tr>
</tbody>
</table>
Flow Cytometry Examples
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Annotations using NCBO BioPortal

Cytobank: A cloud-computing platform for Cytometry

Cytobank Reports
A new way to publish data
Synopsis

We analyze cell signaling directly by next-generation mass cytometry 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, and follicular lymphoma. We have used this approach to distinguish predictive patterns of intracellular signaling to classify patient responses to chemotherapies and to determine how their signaling systems are altered in disease states. 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.

Autoimmune diseases in which we have particular interest include rheumatoid arthritis and systemic lupus erythematosus. In these diseases, we focus on understanding how the immune system becomes dysregulated as disease comes and goes. We can measure and determine the cellular network states in multiple cell subsets. In cancer, we are working in follicular lymphoma as well as acute myelogenous leukemia where we can look at disease progression as a measure of changes in disease states correlated to particular genetic changes in the genome of human cancer cells. Also, we have made determined efforts in understanding how the cancerous microenvironment modulates immune signaling.

Published Experiments

<table>
<thead>
<tr>
<th>Article</th>
<th>Journal</th>
<th>Date</th>
<th>Cytobank Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum</td>
<td>Science</td>
<td>May 2011</td>
<td>View Data</td>
</tr>
</tbody>
</table>

Providing published data to the computational biology and cytometry communities
IL7 → pStat5 in T cells

IL-7 is a canonical activator of T cell proliferation. Here, IL-7 mediated activation of pSTAT5 in T cells is shown as an example of the cell-type specific signaling responses that can be detected by minutes was detected using an antibody against STAT5 phosphorylated at the Y694 residue.

This same data is summarized in 4 squares of the heatmap highlighted in Figure 3b of the paper.
Targeting the Ideal Reagent Quickly with FACSelect

[Image of the FACSelect Buffer Compatibility Resource]

**www.cytobank.org/facselect**
Conclusions: Analysis & Presentation

Annotate at the cytometer – it will save time and help find files later

For figures based on large datasets or new statistics / analysis tools, always show representative primary data

Organization, experiment design, and annotation are critical

- computational analysis (e.g. SPADE)
- collaborations & long term projects
- sharing data with publications
Cytobank Links/Emails

- Cytobank Documentation – [http://support.cytobank.org](http://support.cytobank.org)
  - Documentation, Tutorials and Walkthroughs for Cytobank
  - Actively updated. Send comments!!!
- Cytobank Blog – [http://blog.cytobank.org](http://blog.cytobank.org)
- Support, questions, feedback – [helpdesk@cytobank.org](mailto:helpdesk@cytobank.org)
  - Quickest way to ask any questions/get support on Cytobank
  - Can also fill out a support ticket via Help in Cytobank
- Cytobank website – [www.cytobank.org](http://www.cytobank.org)
- Cytobank Inc. – [www.cytobankinc.com](http://www.cytobankinc.com)
- Referencing Cytobank:
Thank you!

nikesh@cytobank.org
Data Security & Privacy

• All connections to the Cytobank app through SSL / HTTPS
  – The same that financial institutions use
• Cytobank's servers are firewalled and hardened to restrict access and prevent attacks.
  – Only members of Cytobank's operations team have root access to the servers.
• All data on any Cytobank is *always* private by default until you choose to share it with someone
• Each Cytobank installation is self-contained and independent from the others -- no accounts are shared and no data from one instance is visible in another.
  – Customer administrators can choose to require validation of all accounts before they can be used.
Data Backup & Server Maintenance

- All servers are kept up to date with the latest security patches according to common industry practice
- Cytobank's servers are monitored 24x7.
  - Using services like AlertSite and Nagios
- Cytobank maintains local and remote backups of each user's data.
- All access to the servers and applications are logged.