Single Cell Systems Biology: Measuring Cell Signaling by Flow Cytometry

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

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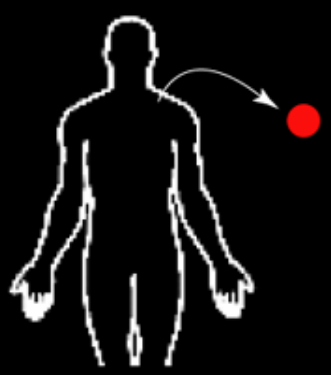
What's So Great About Flow?

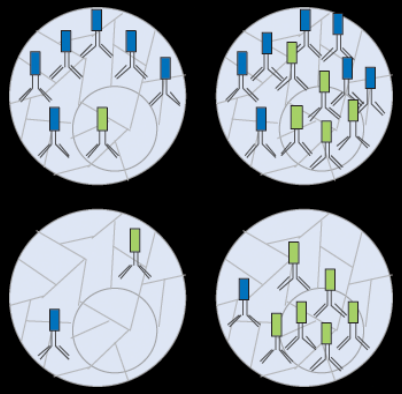
Challenge	Example Question
• Study heterogeneous primary tissues	Can we begin discovery in human samples?
• Pinpoint abnormal cell subsets	Can we spot pre-transformation cells?
• Identify and track cancer stem cells	Is there a rare, therapy-resistant subset?
• Look not just at 'pathways', but the broader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

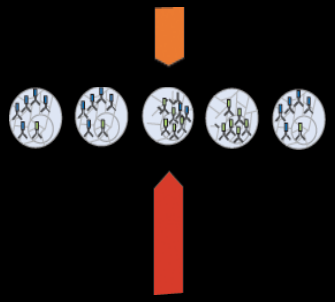
Irish, Kotecha, and Nolan, *Nat Rev Cancer* 2006

Flow cytometry allows tens of measurements *per cell*



Tumor Biopsy



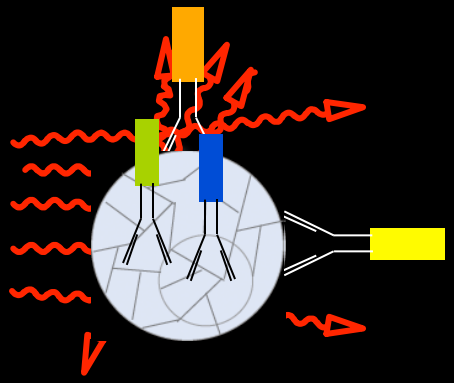


Cancer Patient

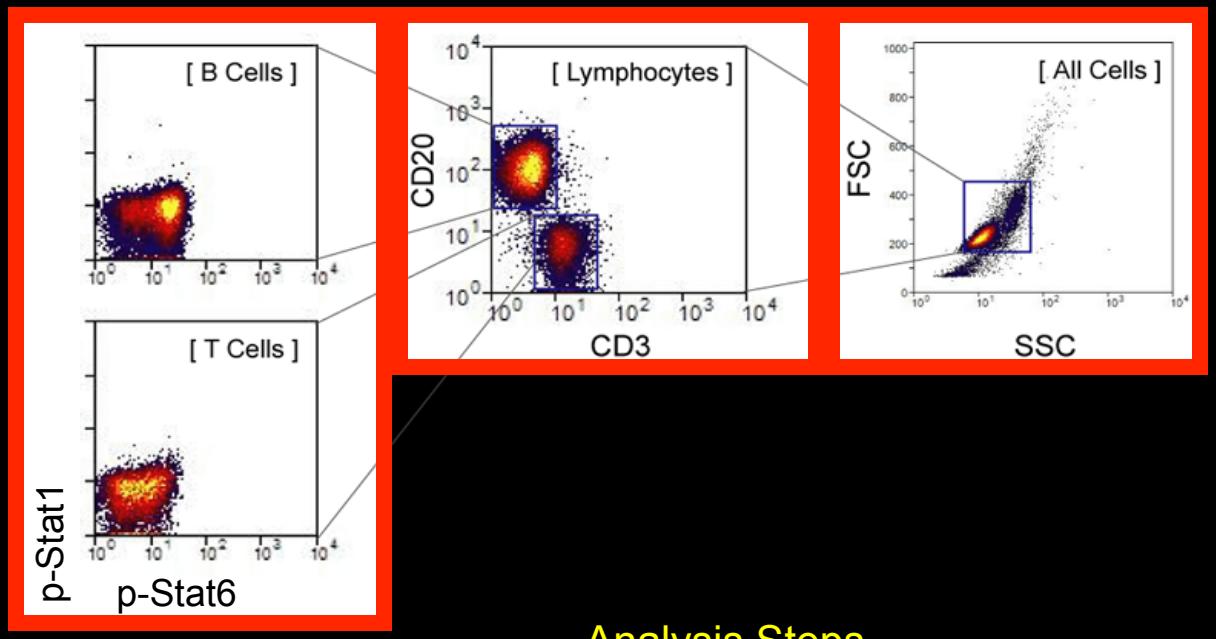
Expose to condition
Stain with antibodies of interest

Collect cells

Extract Cells

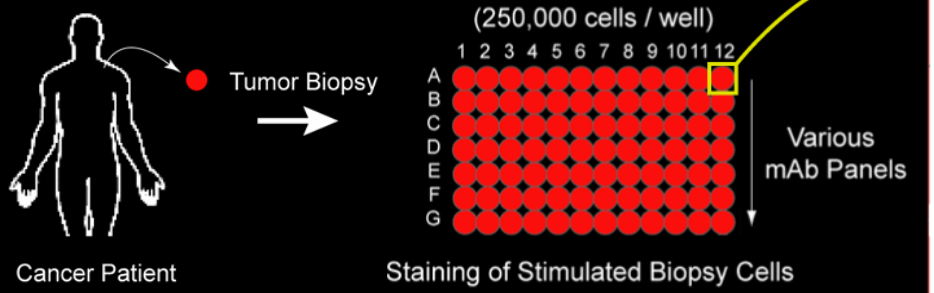


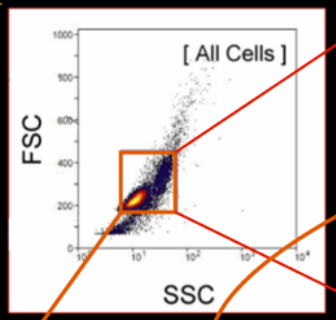
1. ***Identify live cells***
2. ***Identify cell type***
3. ***Evaluate cell signal***

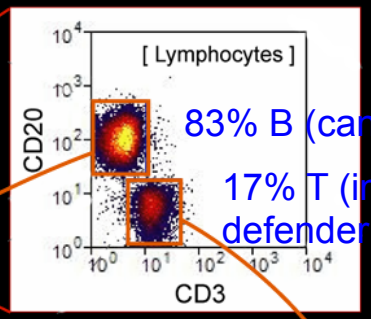


Analysis Steps

Dramatically Different Profile in Immune Defender Cells





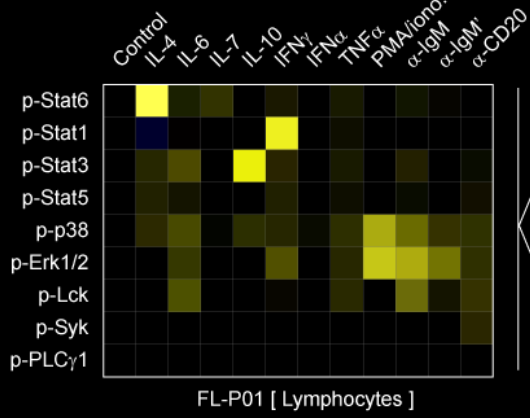


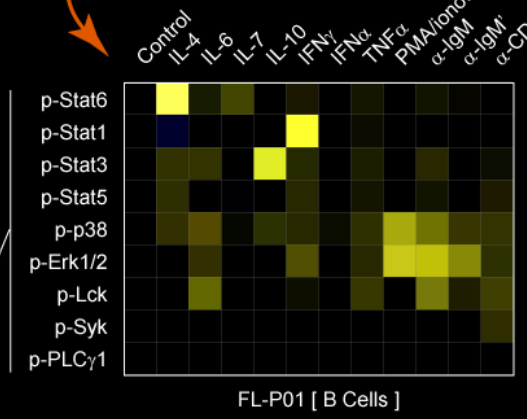
83% B (cancer)

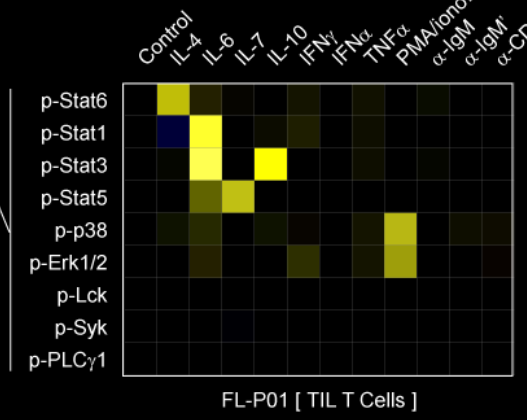
17% T (immune defender cells)

profiling a lymphoma patient

$\log_2$ scale
Stimulated / Control



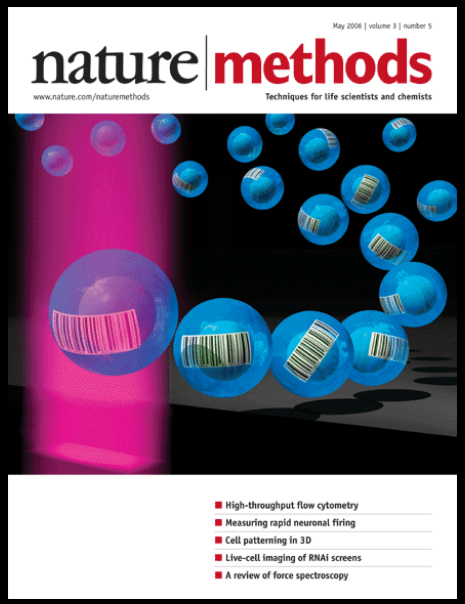




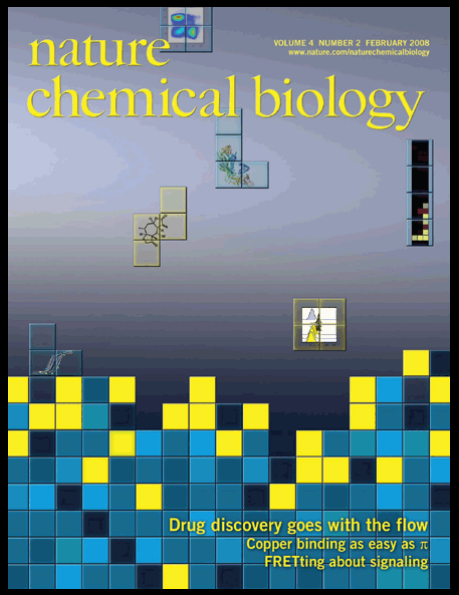
J. Irish

Mechanism Technique Screening Diagnostics

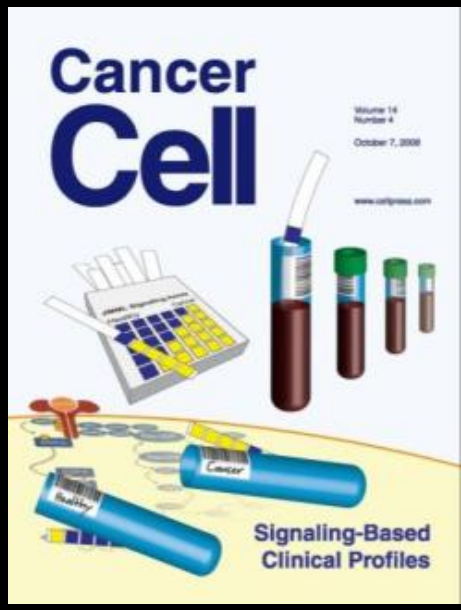
Perez, *et al*.
Blood,



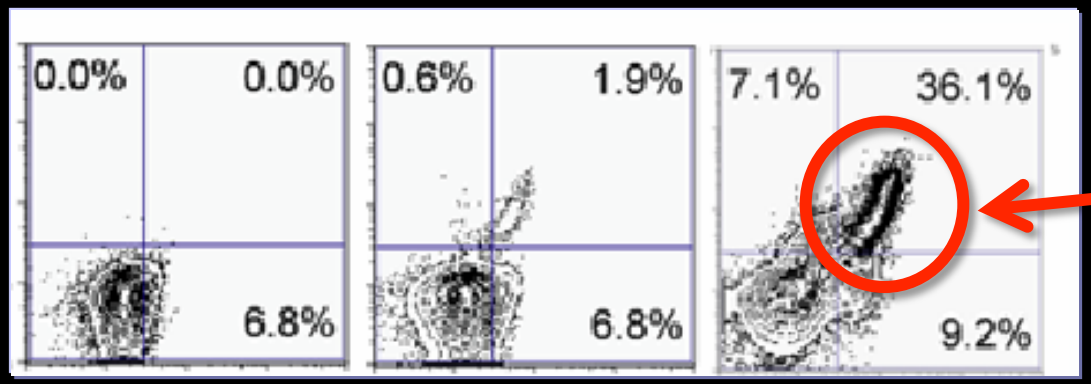
Krutzik, *et al*.
Nat Methods



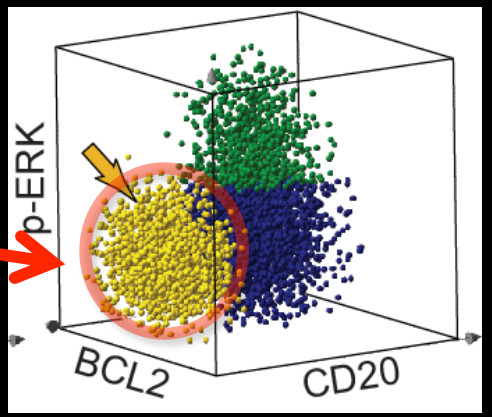
Krutzik, *et al*.
Nat Chem Bio



Kotecha, *et al*.
Cancer Cell

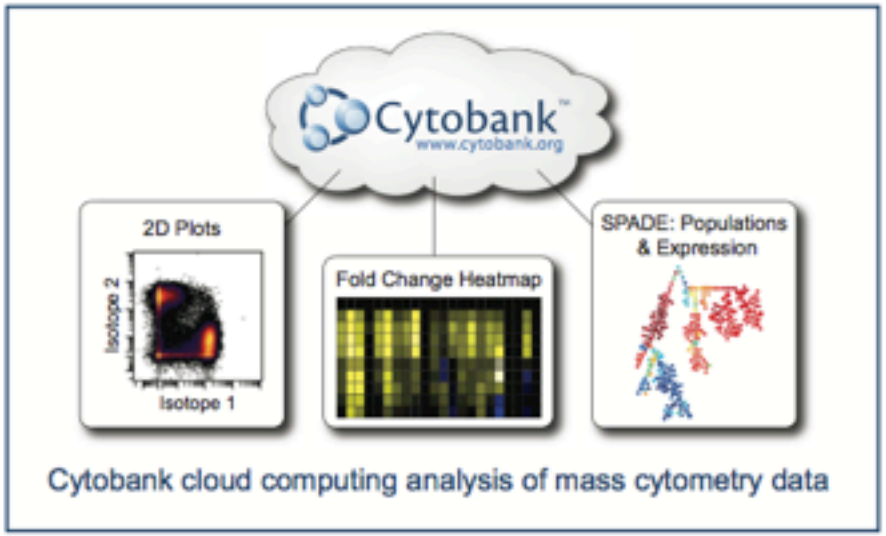


Bad Guy Cells



Irish, *et al*. Cell

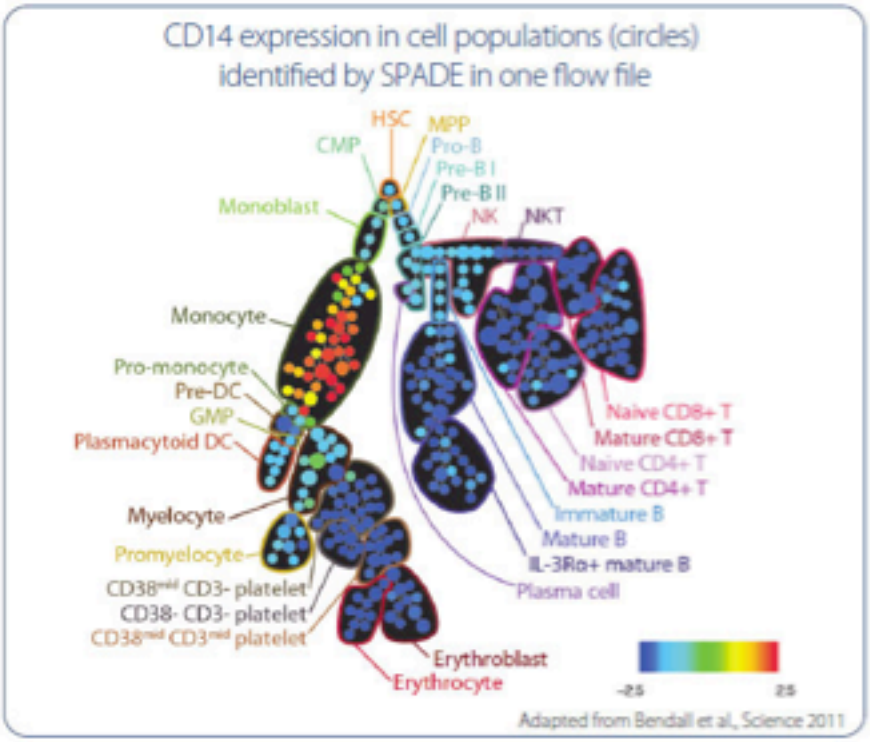
High dimensional cytometry is here



Cytobank cloud computing analysis of mass cytometry data

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

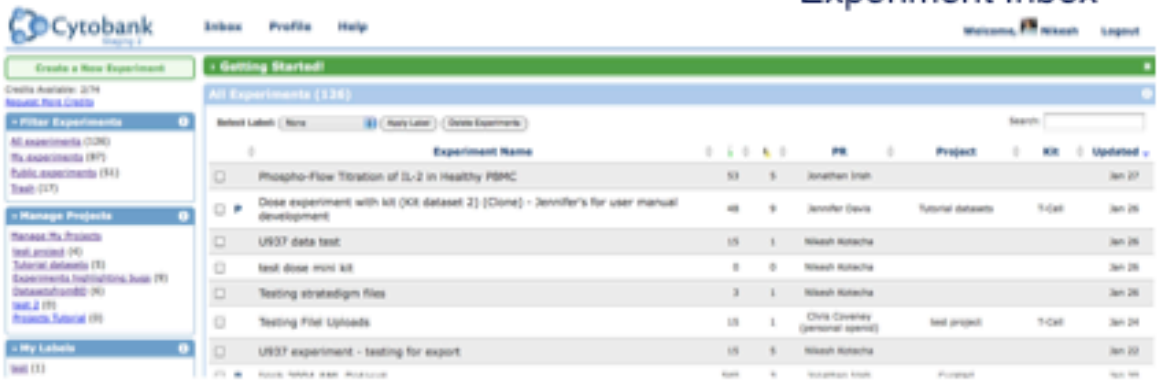
2) Flow cytometry & signaling network profiles
   - map signaling in every cell within a tumor specimen
   - markers for tumor, non-malignant, and cell subsets
   - cell sorting for follow up studies of genetics and epigenetics

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

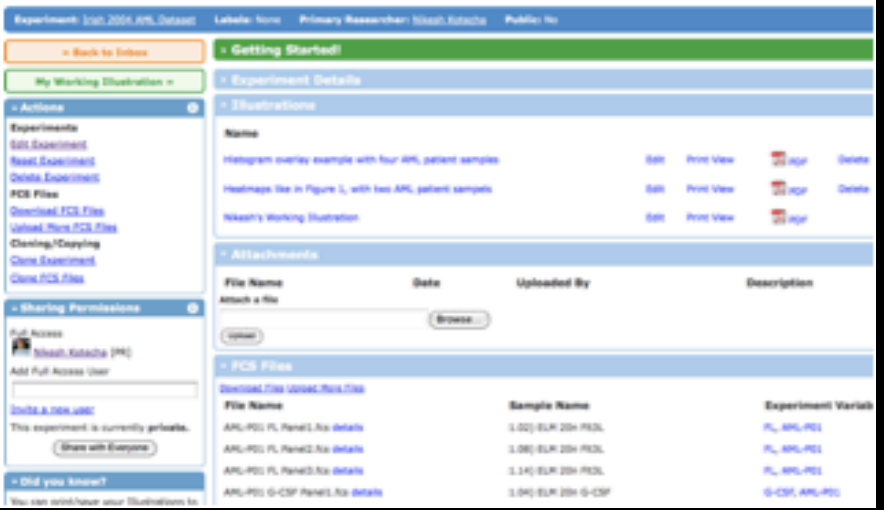
Experiment =
Collection of FCS files

Experiment Inbox



Experiment Analysis

Experiment Details



Cytobank is available in multiple forms

- Cytobank Community Version– 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
  - *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)

Upload. Analyze. Share. Anywhere.

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 m…*
    - Skavland et al (**Norway**) – **Feb. 2011** Nature…
  - *CD137 stimulation enhances the antilymphoma activity of anti-CD20 antibodies*
    - Kohrt et al (**Stanf…**
  - *Cell-to-C… …ates PI3K-AKT Pathway Activity in Cell Populations*
    - Yuan et al (**Harvard**) – **Jan 2011** Current Biology
  - *Oxidative Stress Induces Reactivation of Kaposi's Sarcoma …associated Herpesviru… …ry Effusion Lymphoma Cells*
    - Li et al (…urnal of Virology
  - *Poor cytokine-induced phosphorylation in chroni… …patients at diagnosis is effectively reversed by tyrosine ki…*
    - Jalkanen et al (**Finland**) – **Sep 2010** Experimental Hematology
  - *Com… …e-scale data management and analyses*
    - Schad… …**Sep 2010** Nature Reviews Genetics

Phosflow

Immunophenotyping

Biochemistry

Cancer

Apoptosis

Informatics

More at http://blog.cytobank.org

Flow Cytometry Exampless (Lymphoma, Drug Discovery, Mass Cytometry)

Cytobank: A cloud-computing platform for Cytometry

Annotations using NCBO BioPortal

Cytobank Reports

A new way to publish data

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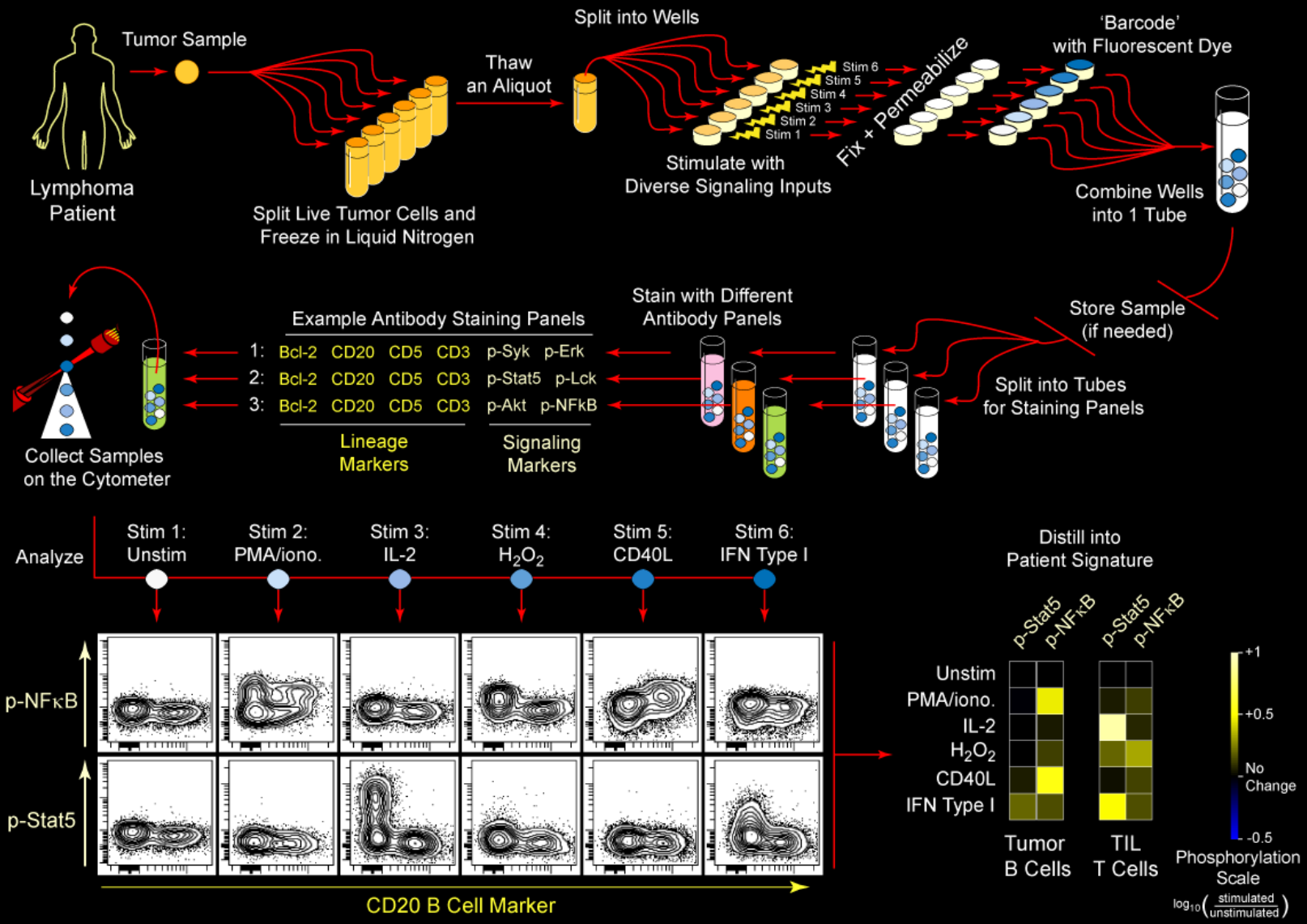
A new way to publish data

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

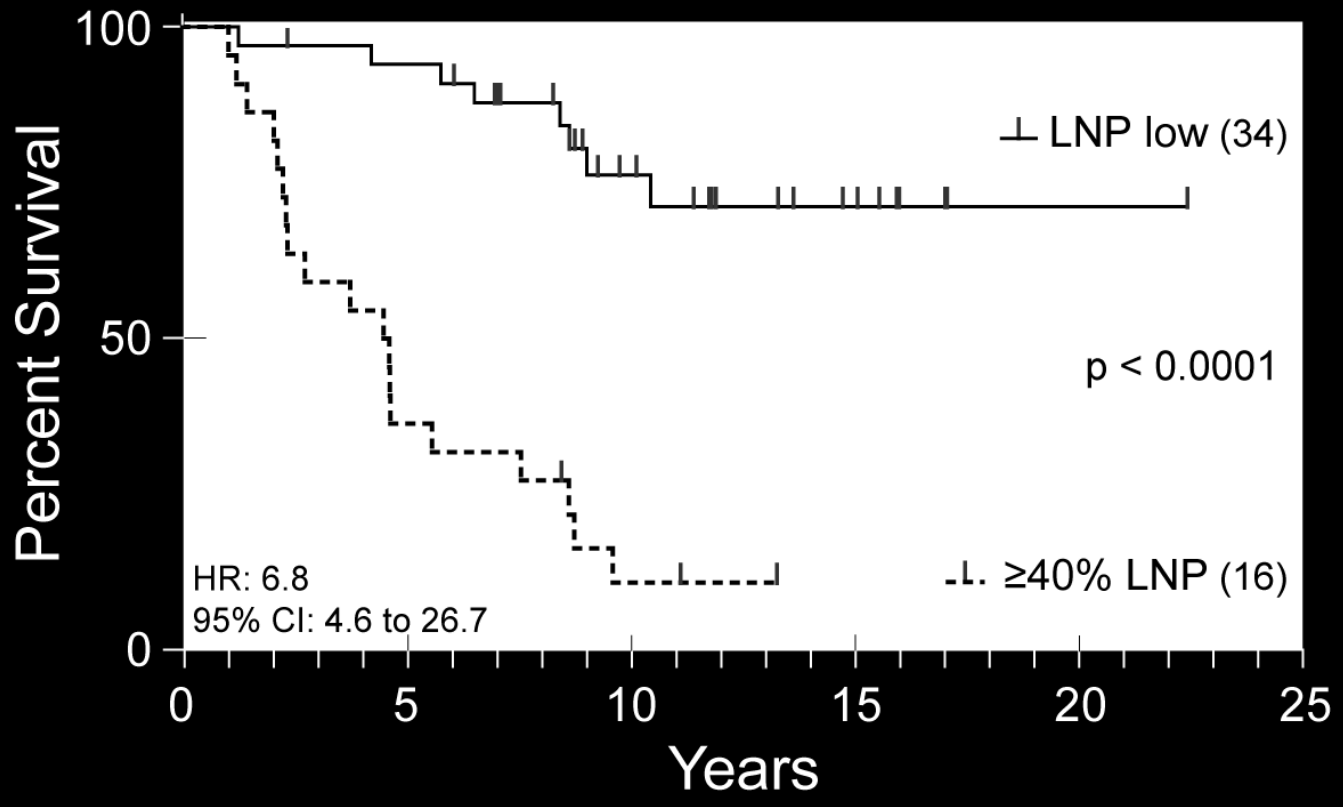
Mapping Signaling in Every Cell using Flow Cytometry



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%

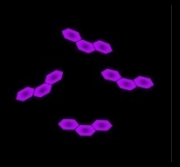


Samples obtained prior to therapy; uniform initial therapy

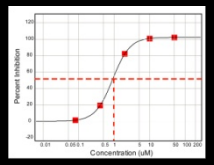
Irish et al., *PNAS* 2010

Primary Cell Screening using Phosphoflow

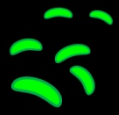
4 natural products + 4 commercial inhibitors



Titrate 6 concentrations of each compound

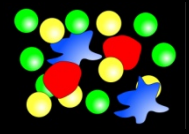


Stimulate with IFNg, IL-4, IL-6, IL-7, IL-10, IL-15

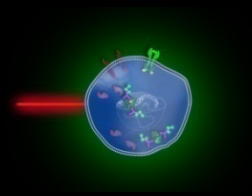


Phospho Flow

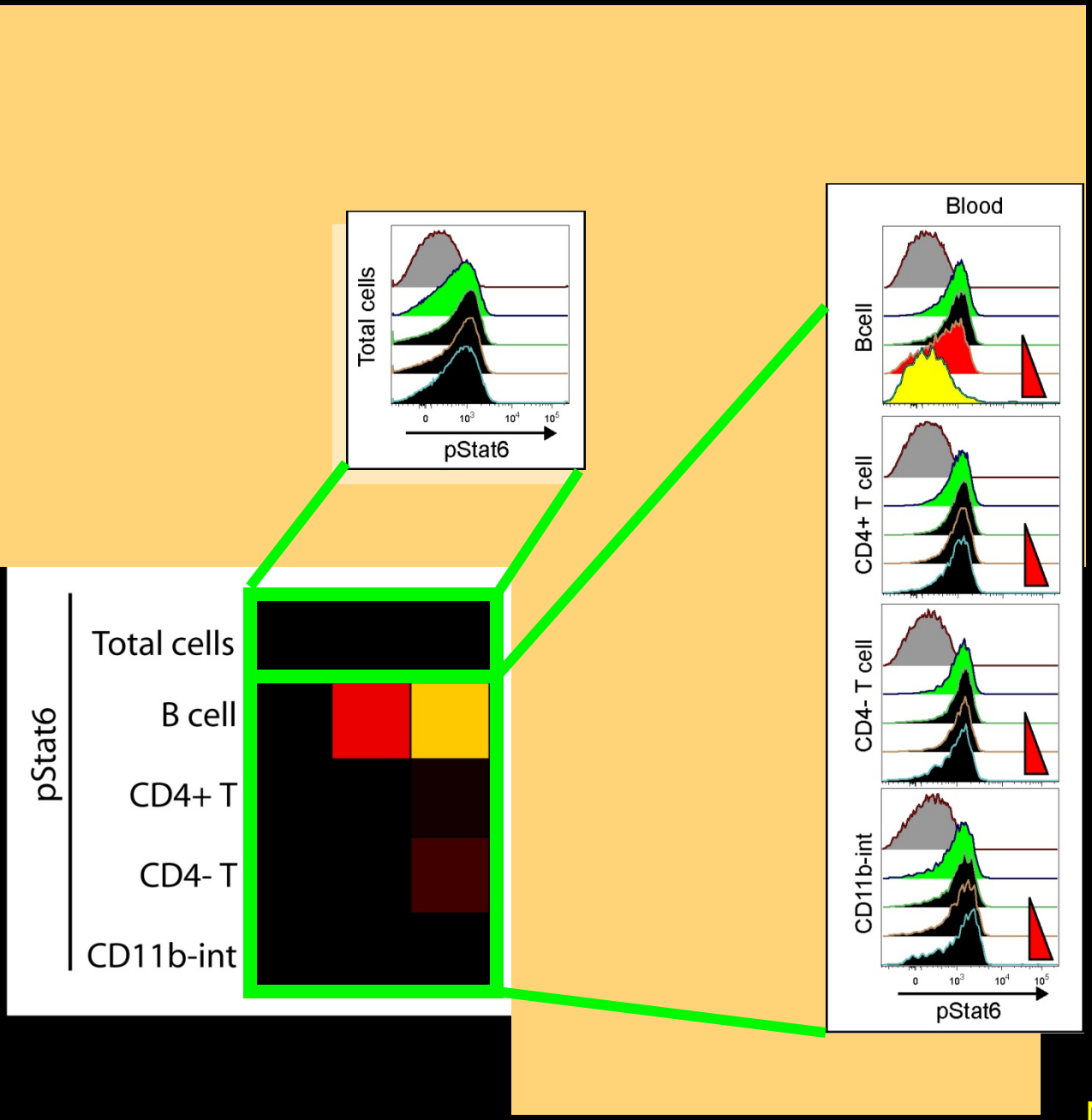
Analyze B cells, CD4+ and CD4- T cells,
CD11b-hi neutrophils, CD11b-int macrophages



Measure Stat1, Stat3, Stat5, Stat6 phosphorylation



Krutzik et al, Nature Chemical Biology, 2008

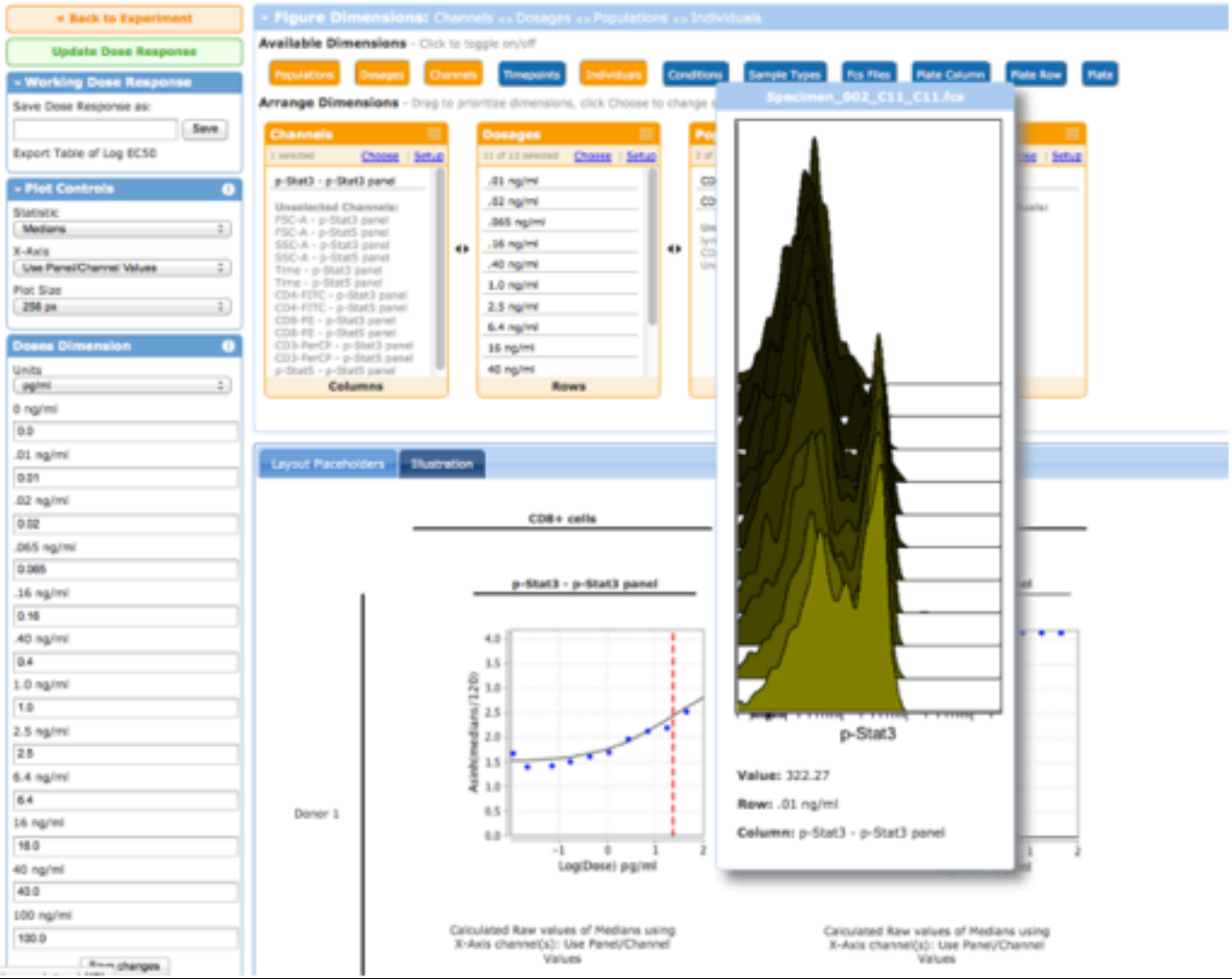


Blood

- 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)

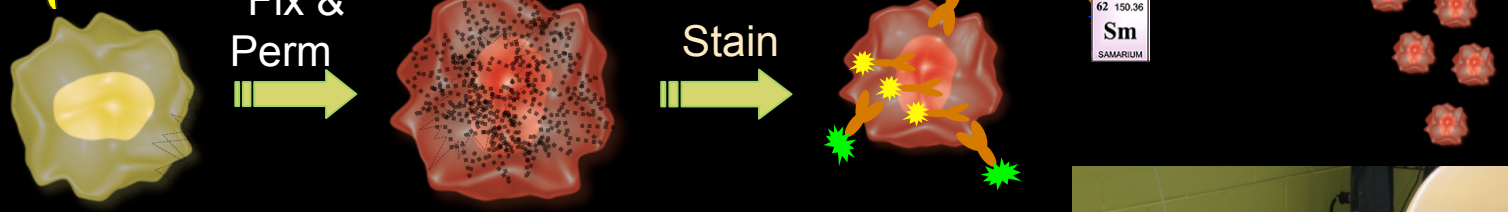


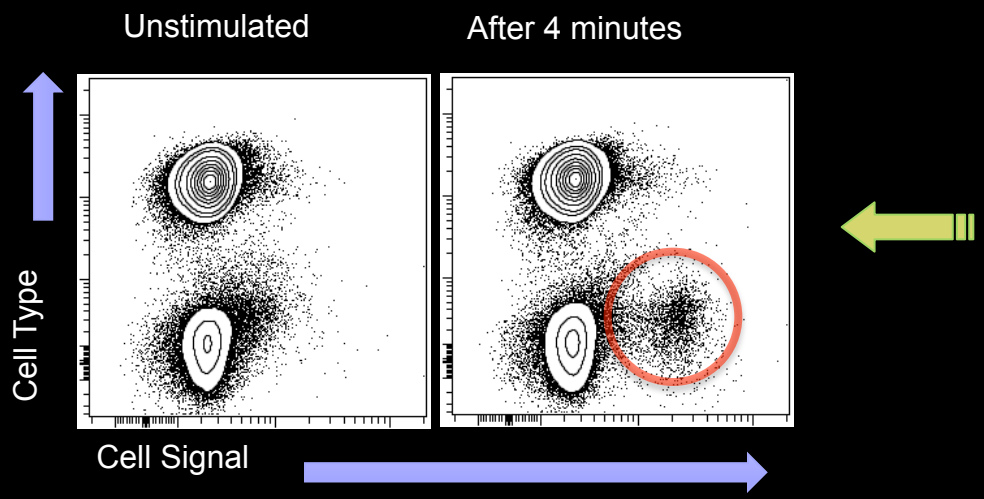
Cytobank Premium

Mass Cytometry: 30+ parameters & no compensation

Assays to Relevant Biological Pathways:

Cytokines & Growth Factors (G-CSF)
Apoptosis Pathways
Phosphatase Activity (H_20_2)



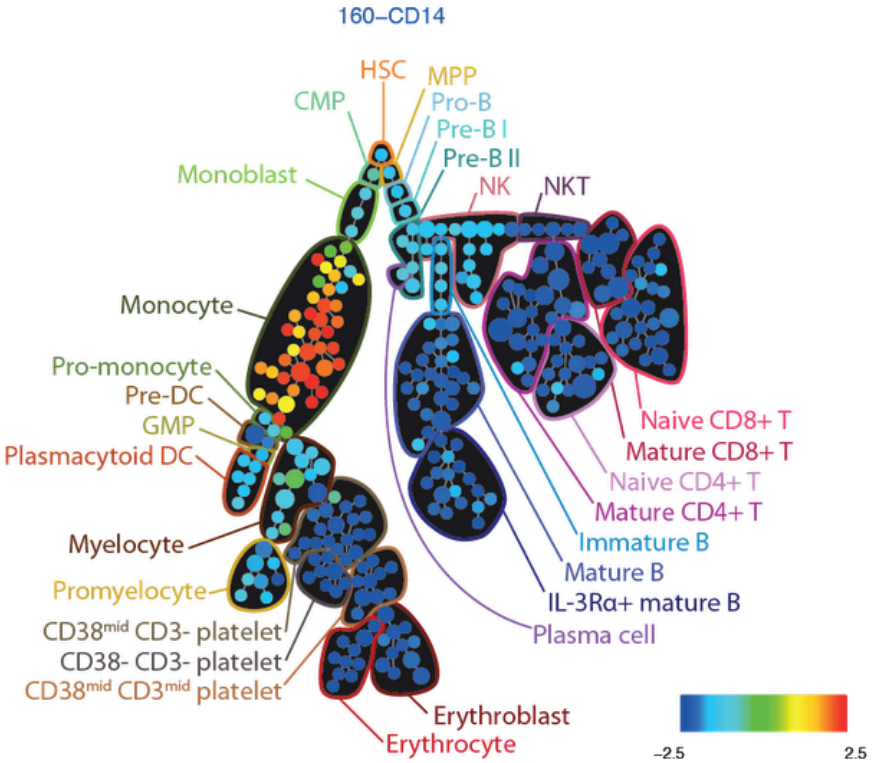


SPADE allows for analysis of high dimensional cytometry

gure S4b (Surface-only tube)

xpression 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 SPA rom 13 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.

lote: 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 us igure.

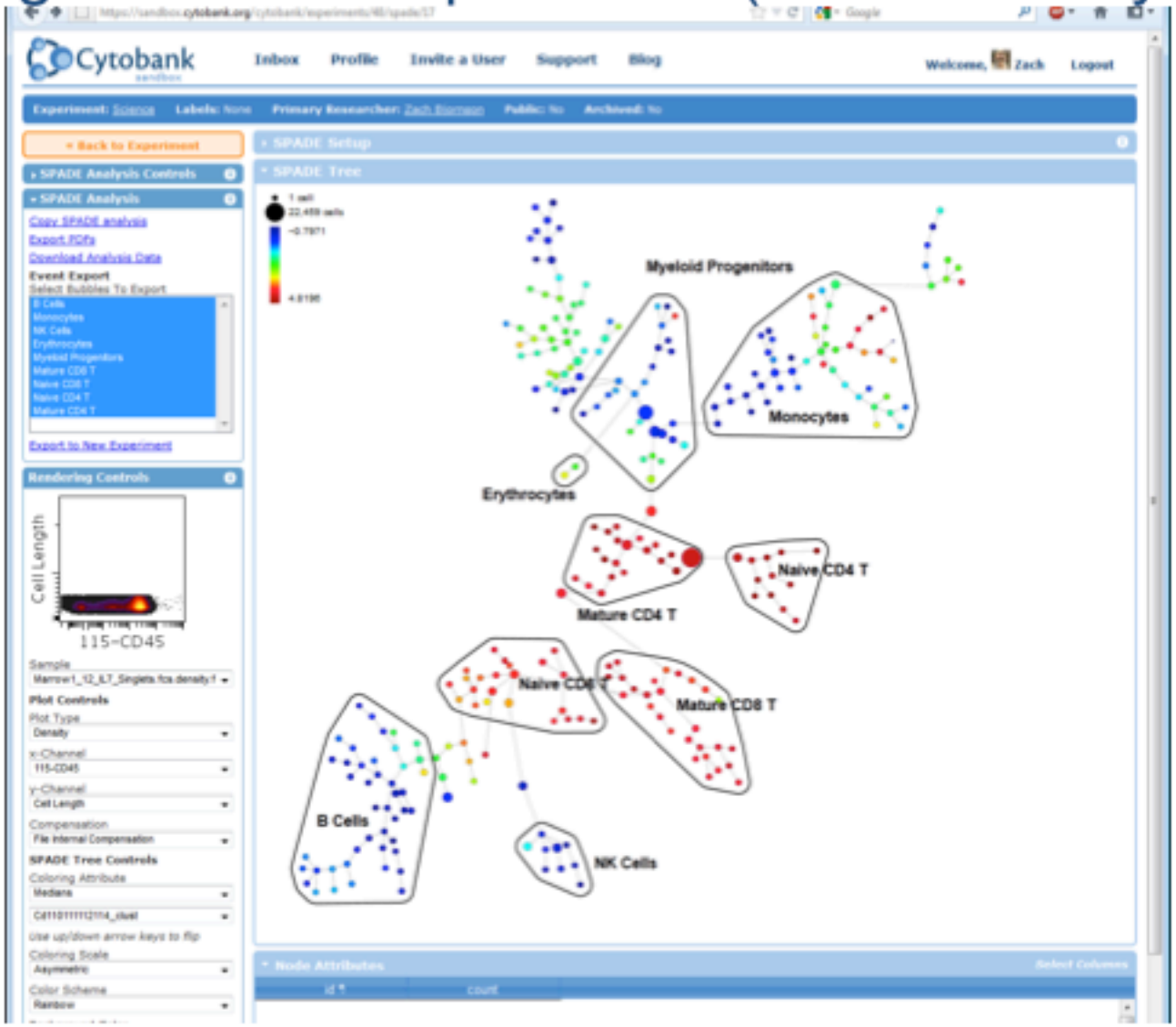


www.cytobank.org/nolanlab

Bendall et. al Science 2011

Qiu et al Nature Biotech 2011

Large Scale Computations (delivered to your browser)



Cytobank Premium

Flow Cytometry Exampless (Lymphoma, Drug Discovery, Mass Cytometry)

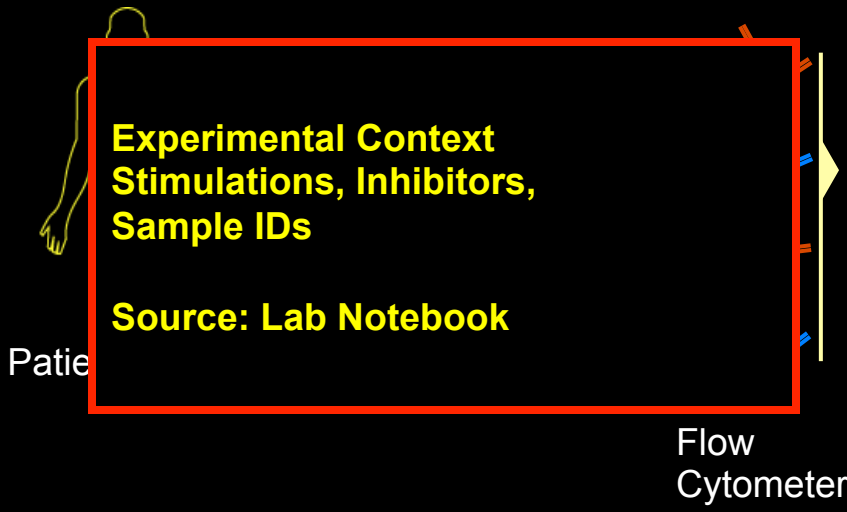
Cytobank: A cloud-computing platform for Cytometry

Annotations using NCBO BioPortal

Cytobank Reports

A new way to publish data

Analysis and annotation of flow cytometry data is fragmented



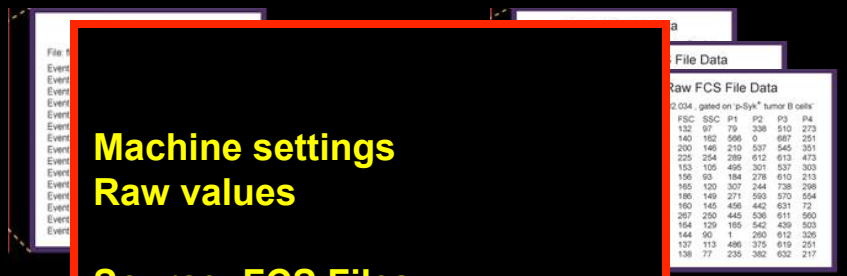
Experimental Context
Stimulations, Inhibitors,
Sample IDs

Source: Lab Notebook

Patie

Flow
Cytometer

FCS 3.0 File



Machine settings
Raw values

Source: FCS Files

Raw
+
Machine settings
+
Keyword/Value pairs
(Vendor determined)

t =
Collection of FCS files

Digested Figures
+ Results

Source: PPT Presentation

Figures to communicate results

Advanced analysis,
Aggregate Views

Source: Statistical Programs

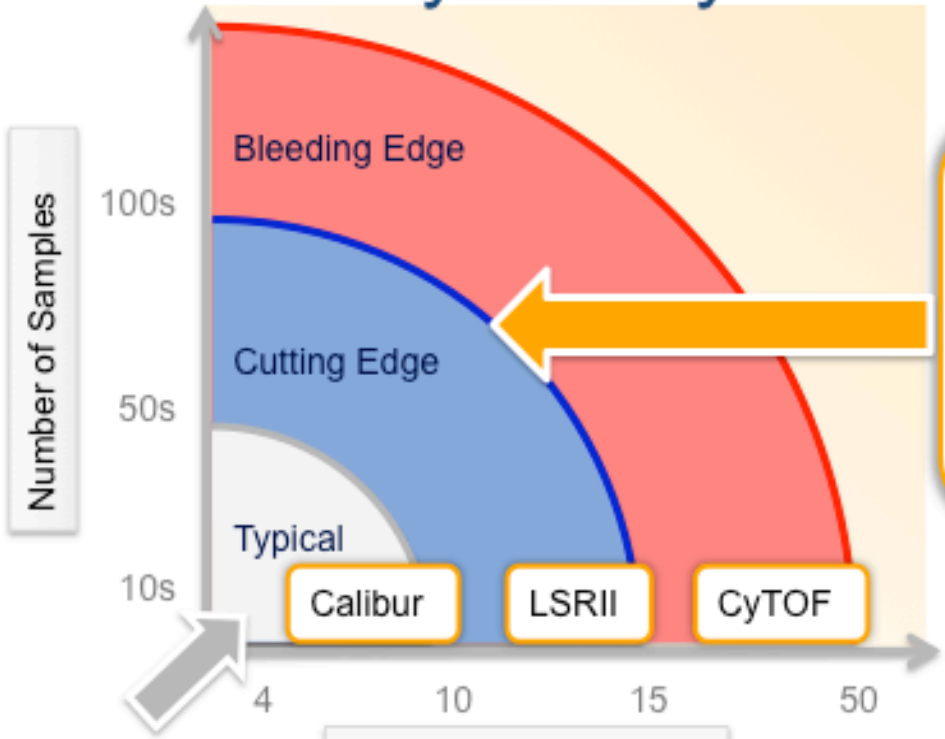
Advanced Analysis,
Aggregate Views

Data Preprocessing (Compensations,
Transformations)
Data filtering (Gating)
FCS file groupings

Source: Analysis Program

Gating

Trends in Cytometry & Cell Analysis (I)



Future of Flow

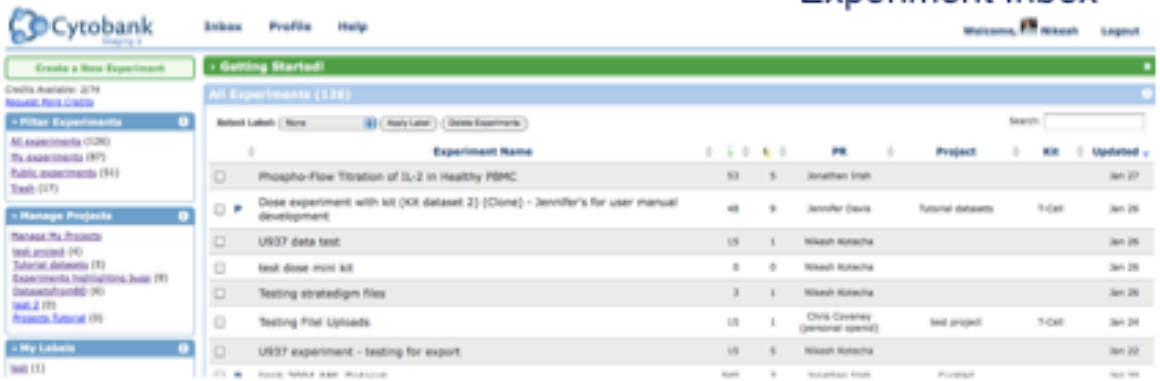
- New solution required
- Organize/manage experiments
- Communicate & collaborate
- Annotations & analyses linked
- Novel analyses & visualizations
- Scalable compute resources
- Platform to build on top of

- Majority of flow solutions built for this space

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

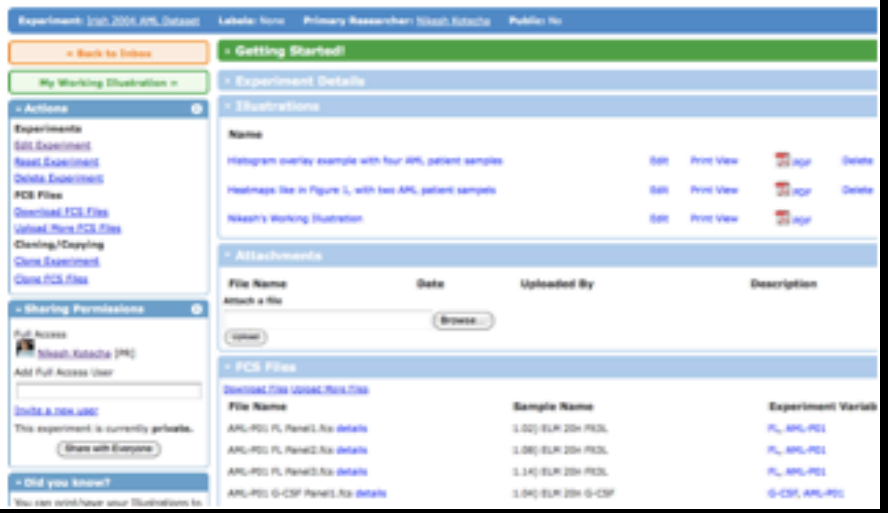
Experiment =
Collection of FCS files

Experiment Inbox

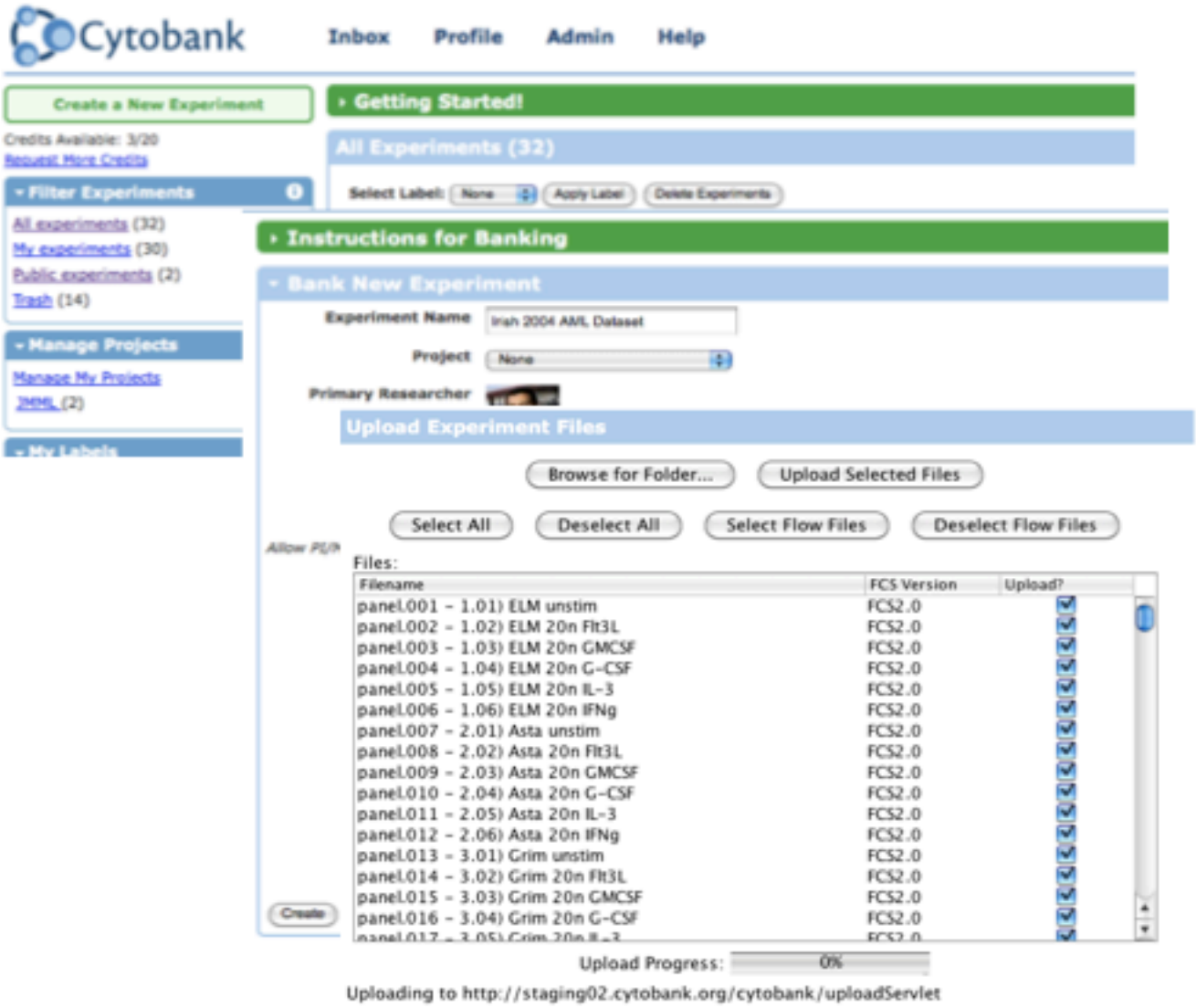


Experiment Analysis

Experiment Details



Create a new experiment and upload FCS files



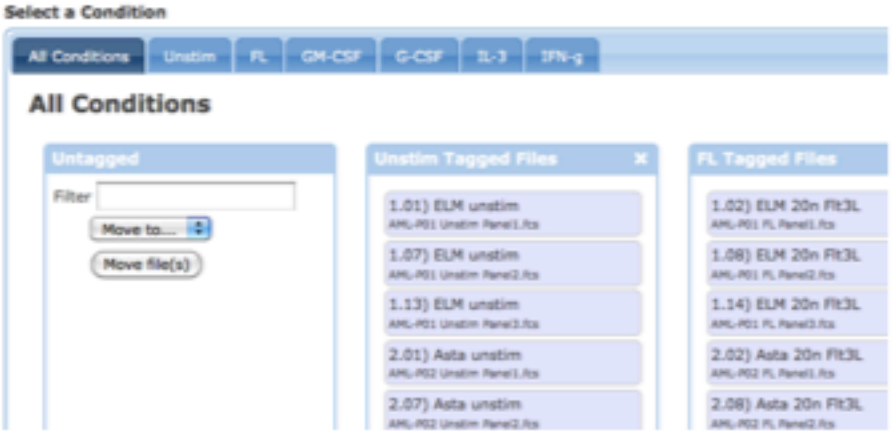
Launch web browser & Login to Cytobank

Create a new Experiment

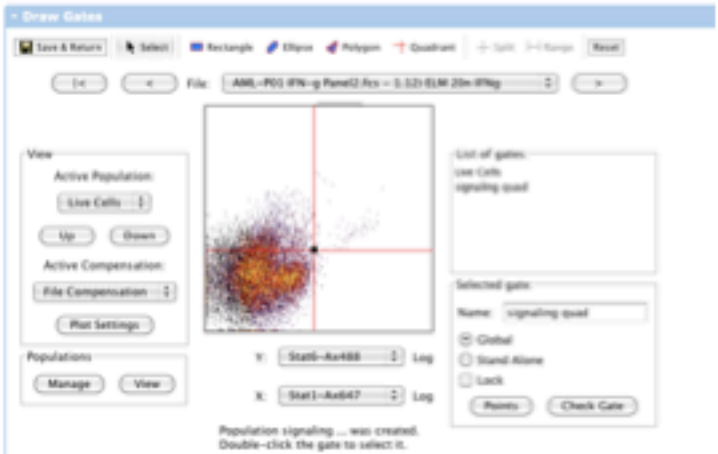
Upload FCS Files

Organize information around samples using Experimental Variables

Variable Types	Experiment Variable Values
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 IFNg Flt3 Ligand (FL)
Cell Populations:	Intact Cells

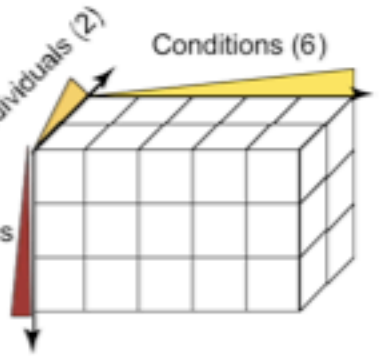


Assign samples to experimental variables

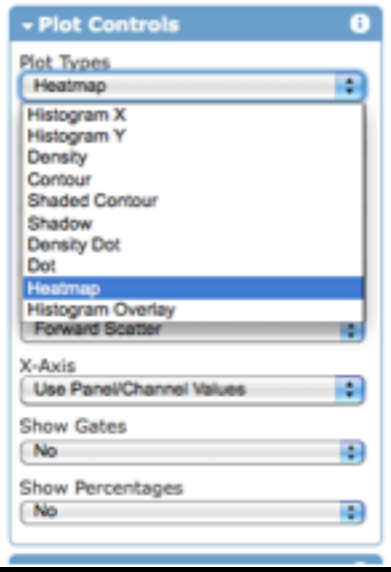


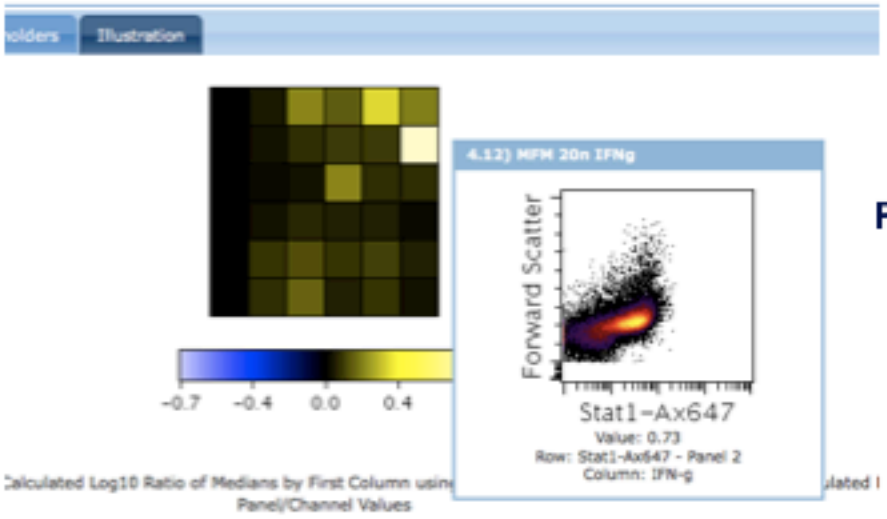
Identify populations of interest (gating)

Use Experimental Variables to create and pivot figures



Arrange figure using Experimental Variables



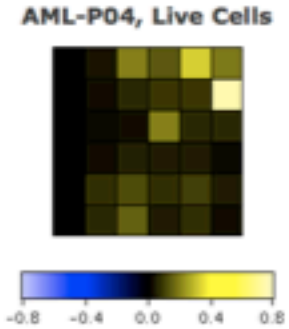


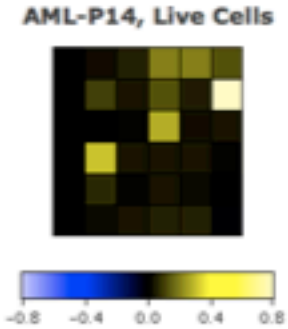
Plot controls to specify plot type and display

Share results and analyses with collaborators and the community

Irish 2004 AML Dataset > Nikesh's Working Illustration
Illustration Author: Nikesh Kotecha
Primary Researcher: Jonathan Irish

Display as PDF
Jump to Gating Hierarchy
Share Illustration





Individual and project level sharing for collaborators

Cytobank
Staging 2

Inbox Profile Admin Help

Create a New Experiment

Credits Available: 4/79
Request More Credits

Filter Experiments
All experiments (131)
My experiments (92)
Public experiments (51)
Trash (17)

Getting Started!

All Experiments (131)

Select Label: None Apply Label Delete Experiments

		Experiment Name
☐	P	Healthy PBMC Mini Profile - Comp Tester
☐	P	Irish 2004 AML Dataset

Make experiments public to share with community

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

Cytobank

Accessible from any web-enabled device anywhere

Flow Cytometry Exampless (Lymphoma, Drug Discovery, Mass Cytometry)

Cytobank: A cloud-computing platform for Cytometry

Annotations using NCBO BioPortal

Cytobank Reports

A new way to publish data

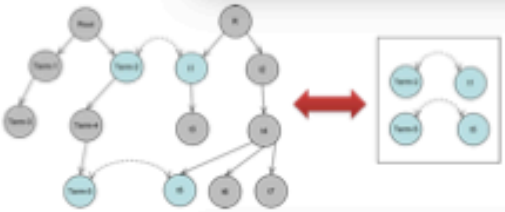
NCBO: Key activities - http://www.bioontology.org/

- 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.

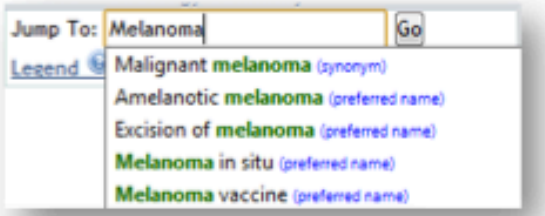
http://rest.bioontology.org

Ontology Services
Views
- Download
- Traverse
- Search
- Comment

Mapping Services
- Create
- Download
- Upload



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



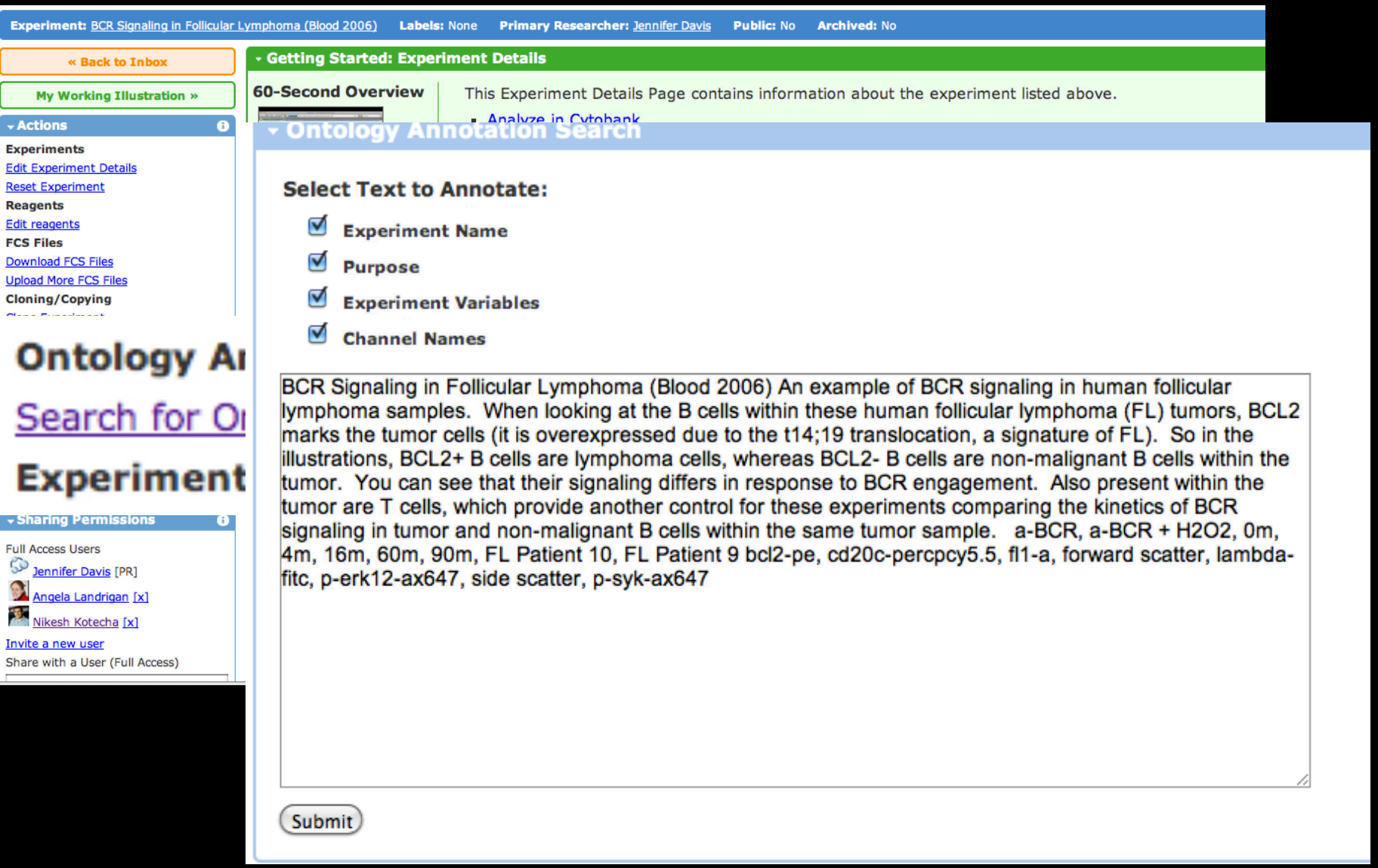
Annotation → **Term recognition**

Expression, Expression of bladder, bladder, smooth, bladder muscle, muscle, smooth muscle, cells, mechanical, mechanical stimulation, stimulation, Chronic, results, bladder overdistension, associated, associated with, with, loss, genes, altered

Data Access → Fetch "data" annotated with a given term

http://bioportal.bioontology.org

Integration with NCBO BioPortal



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 + 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"

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
cell
cells (3)
chromosomal translocation
process
control (4)
control group
due to (2)
example
follicle
follicular
follicular lymphoma (3)
forward (2)
h2o2
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 (3)
tumor cells
tumor cells, uncertain whether benign or malignant
tumor tissue
veterinary patient

Annotations (107)

Preferred Name	Term ID	Ontology	Semantic Type(s)
Cells	D002477	Medical Subject Headings	T025: Cell
Cells	MTHU001933	Logical Observation Identifier Names and Codes	T025: Cell
Cells	LP14738-6	Logical Observation Identifier Names and Codes	T025: Cell
Cell	Cell	NCI Thesaurus	T999: NCBO BioPortal concept
tumor	npo:NPO_1573	NanoParticle Ontology	T999: NCBO BioPortal concept
Tumor	LP7664-8	Logical Observation Identifier Names and Codes	T191: Neoplastic Process

Flow Cytometry Exampless (Lymphoma, Drug Discovery, Mass Cytometry)

Cytobank: A cloud-computing platform for Cytometry

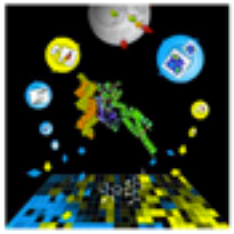
Annotations using NCBO BioPortal

Cytobank Reports

A new way to publish data

Cytobank Public Cytometry Collection

Cytobank.org



Nolan Lab

Principal Investigator: Garry P. Nolan, Ph.D.
Affiliation: Stanford University, Stanford, CA
Curator: Garry Nolan
Website: http://www.stanford.edu/group/nolan
Research: cancer, cell signaling, immunology, leukemia, intracellular cytometry, mass cytometry, systems biology

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 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

Article	Journal	Date	Cytobank Report
Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum Bendall SC, Simonds EF, Qiu P, Amir ED, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI, Balderas RS, Plevritis SK, Sachs K, Pe'er D, Tanner SD, Nolan GP	Science	May 2011	View Data

Providing published data to the computational biology and cytometry communities

Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum

Bendall SC, Simonds EF, Qiu P, Amir ED, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI, Balderas RS, Plevritis SK, Sachs K, Pe'er D, Tanner SD, Nolan GP

Report Sections

Abstract
Experiment Overview
IL7 --> pStat5 in T cells
BCR --> pPLCg2 in B Cells
Figure 3a (Heatmap)
Figure 3c-e (SPADE trees)
Figure S7 (Venn Diagram)
Figure S4b (Surface-only tube)

Powered By Cytobank

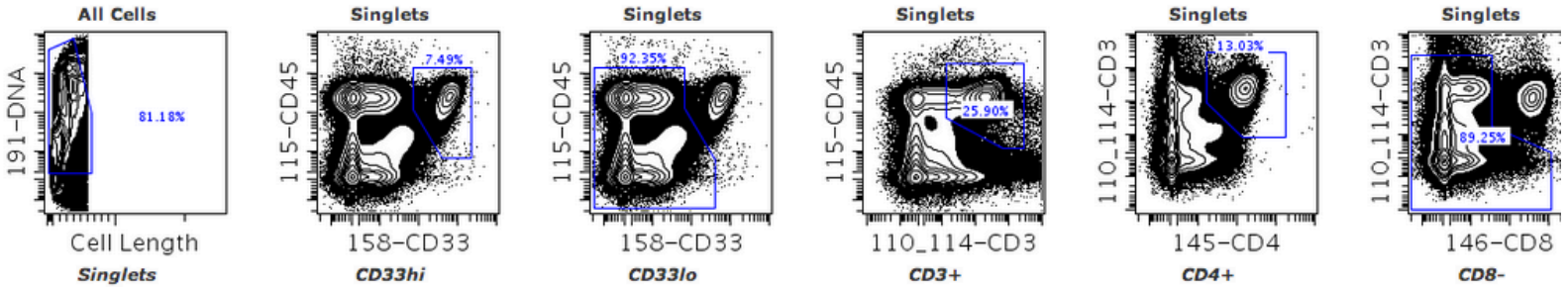
IL7 --> pStat5 in T cells

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.

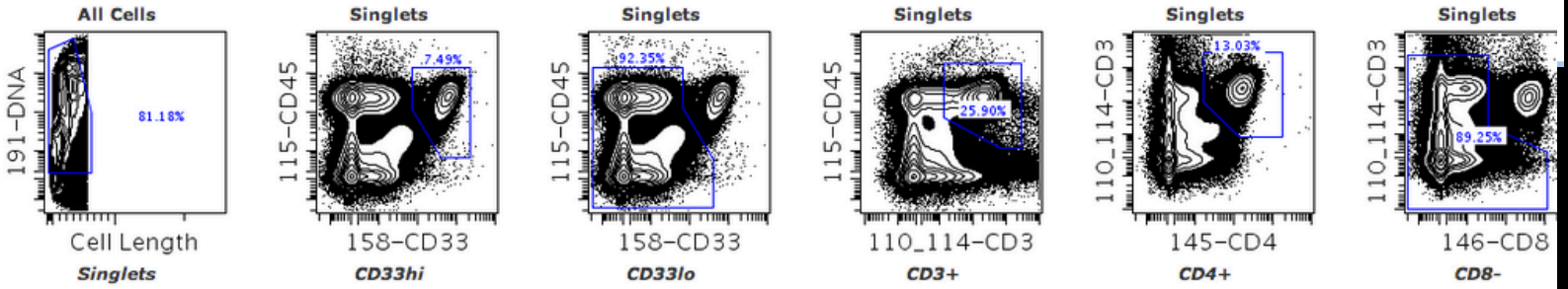
This same data is summarized in 4 squares of the heatmap highlighted in Figure 3B of the paper.

IL7 --> pStat5 in T cells (Gating Hierarchy)

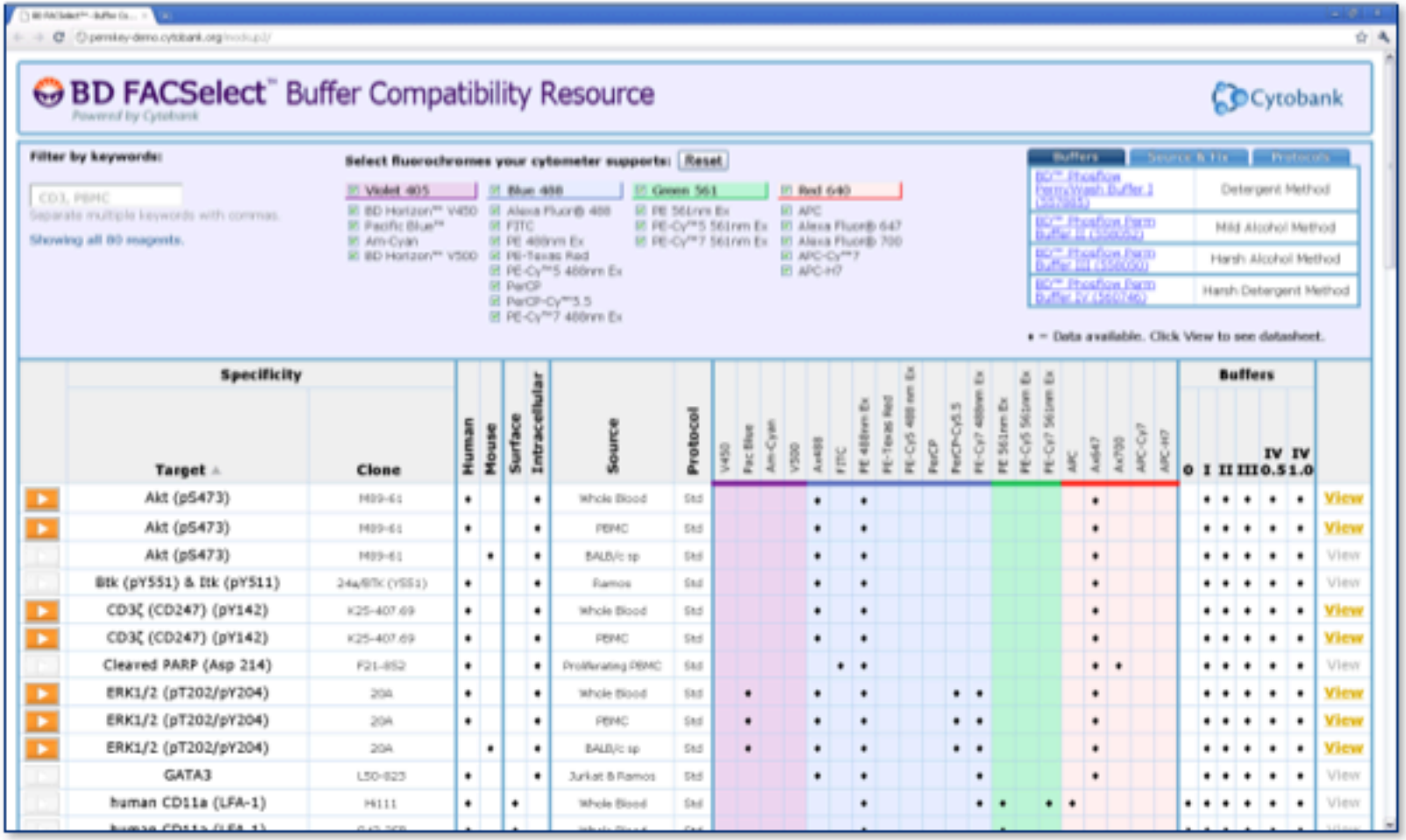
Mature CD4+ T



Naive CD4+ T



Targeting the Ideal Reagent Quickly with FACSelect



www.cytobank.org/facselect

CD3e (145-2C11)

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[Back to FACSelect](#)

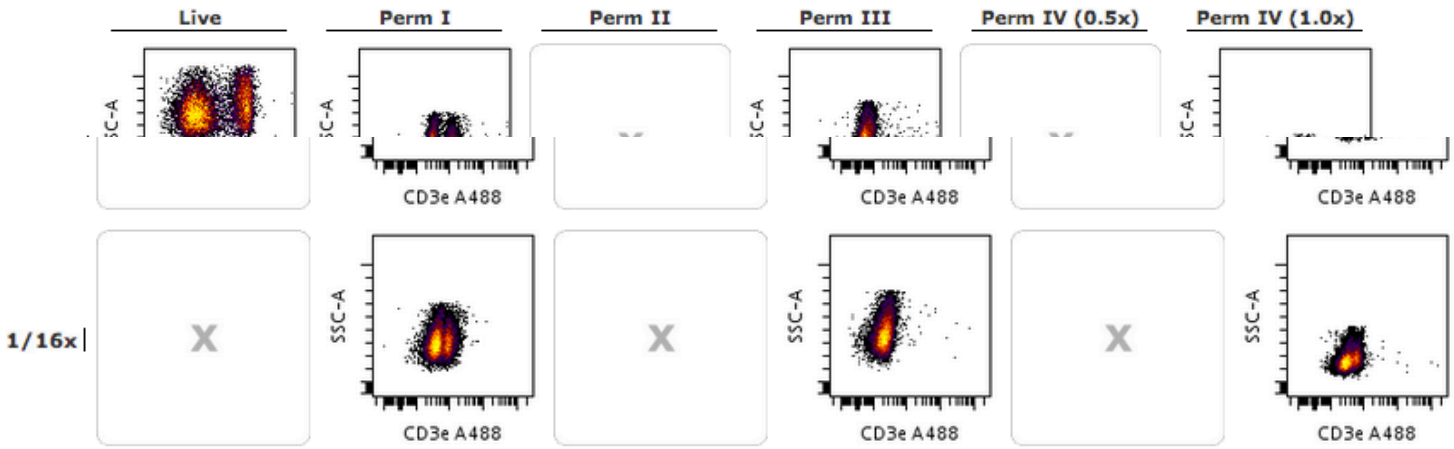
About CD3e (145-2C11)

Protein Name: CD3e
Clone: 145-2C11
Isotype: IgG1, k
Reactive species: Mouse
Host species: Armenian Hamster
Protocol: details
Experiment Cell Source: BALB/c Spleen
Cytometer used: FACSCantoII

Conjugates Shown

Alexa Fluor® 488 [BD]
FITC [BD]
PE [BD]
PerCP [BD]
PerCP-Cy5.5 [BD]
PE-Cy™7 [BD]
APC [BD]
APC-Cy™7 [BD]

CD3e A488



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

	Live	Perm I	Perm II	Perm III	Perm IV (0.5x)	Perm IV (1.0x)
1x	4.92	2.07	X	1.18	X	1.66
1/4x	X	2.14	X	1.25	X	1.82
				1.36	X	1.46

View in Cytobank

o Gating Hierarchy Back to Top

CD3e FITC

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
  - *Documentation, Tutorials and Walkthroughs for Cytobank*
  - *Actively updated. Send comments!!!*
- Cytobank Blog – http://blog.cytobank.org
- Support, questions, feedback – 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
- Cytobank Inc. – www.cytobankinc.com
- Referencing Cytobank:
  - *Kotecha et al.* ***Web based analysis and publication of flow cytometry experiments****. Current Protocols in Cytometry. July 2010.*

Thank you!

nikesh@cytobank.org

Extras

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.