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

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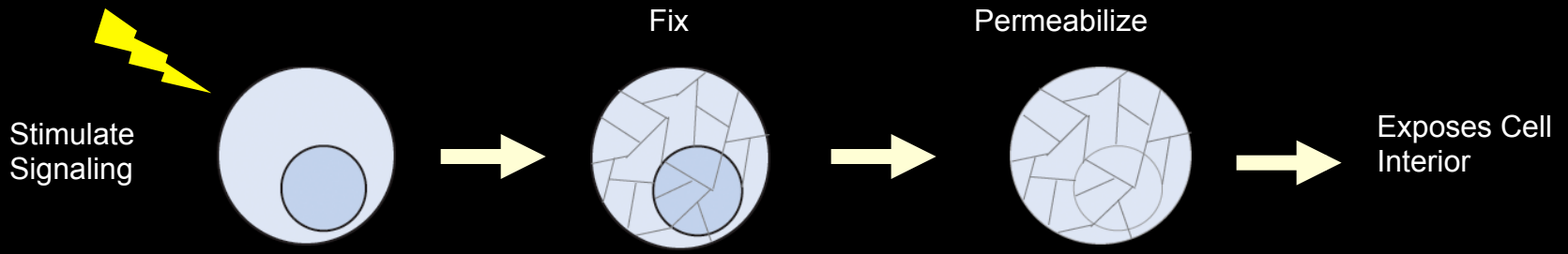
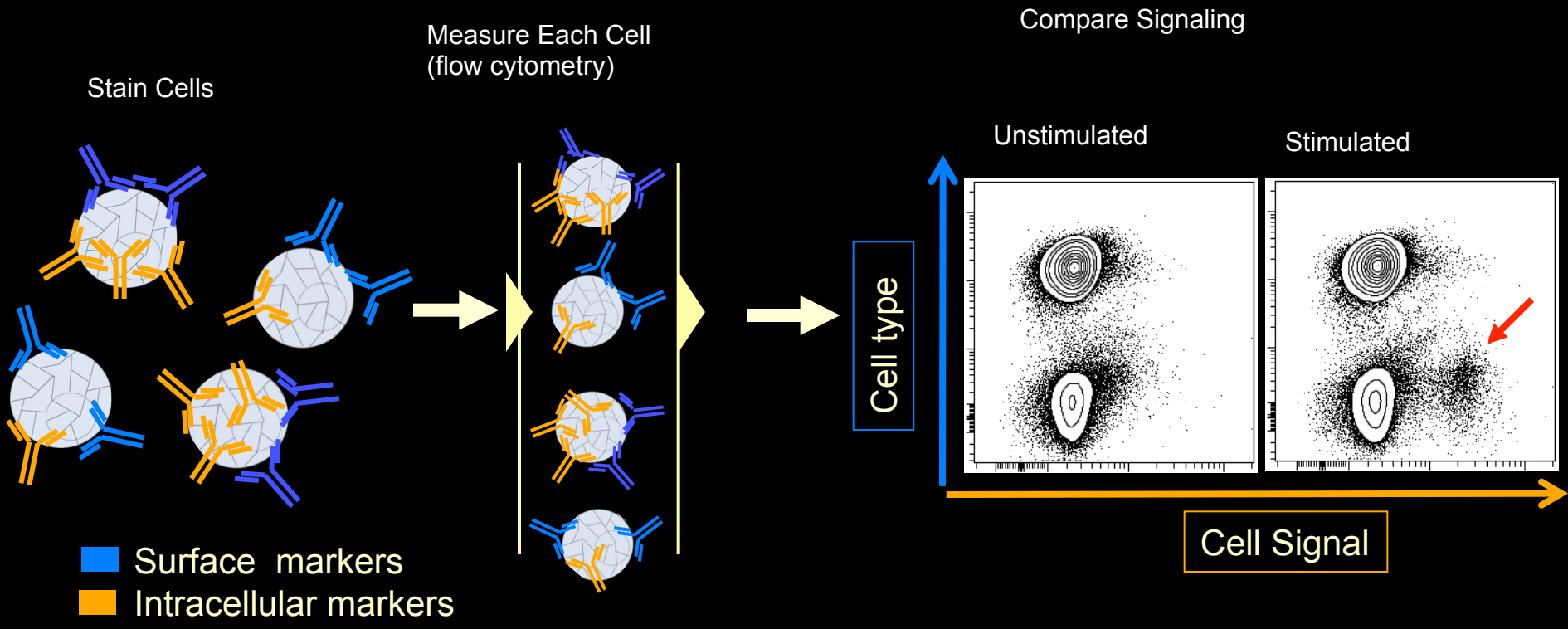
What's So Great About Cytometry?

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

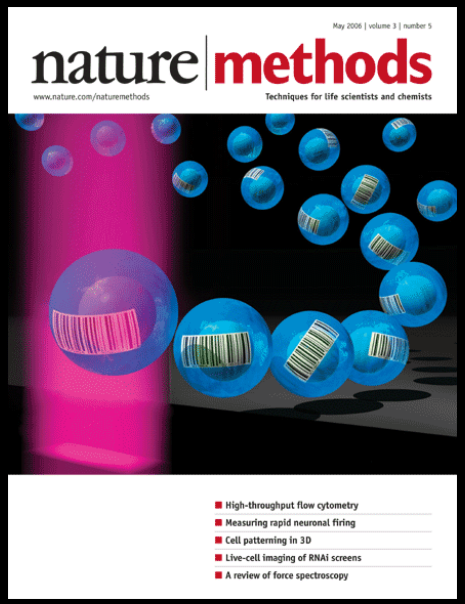
Overview: Compare cell signaling with Flow Cytometry



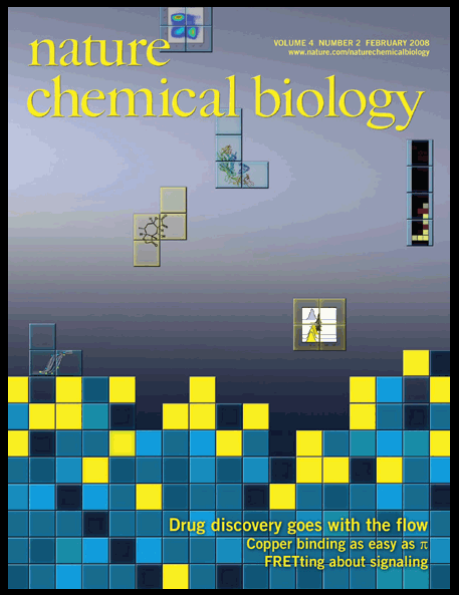


Mechanism Technique Screening Diagnostics

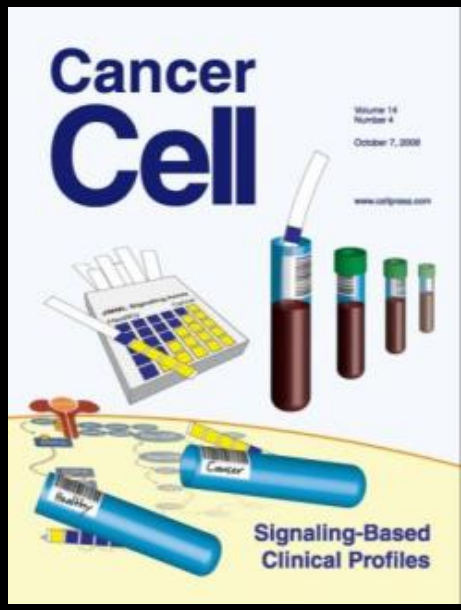
Perez, *et al*.
Blood,



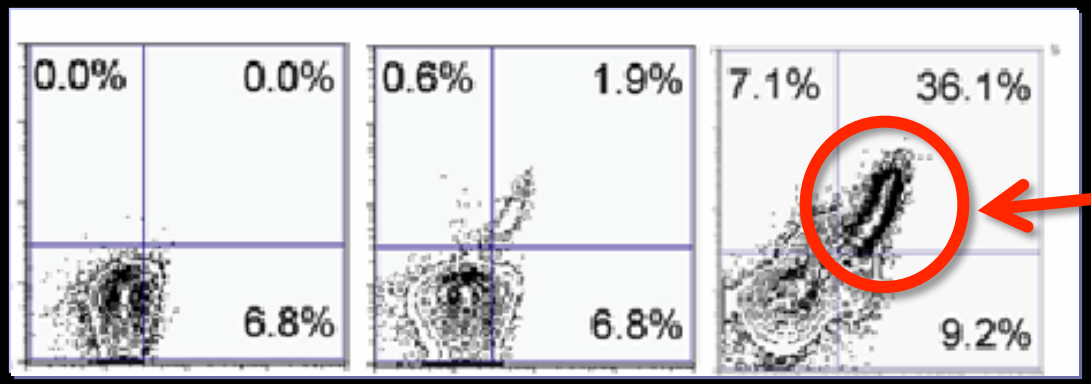
Krutzik, *et al*.
Nat Methods



Krutzik, *et al*.
Nat Chem Bio

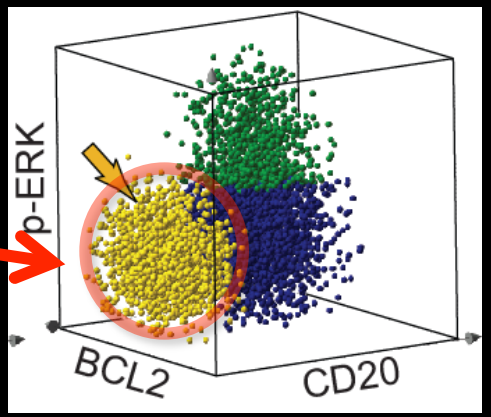


Kotecha, *et al*.
Cancer Cell



Irish, *et al*. Cell

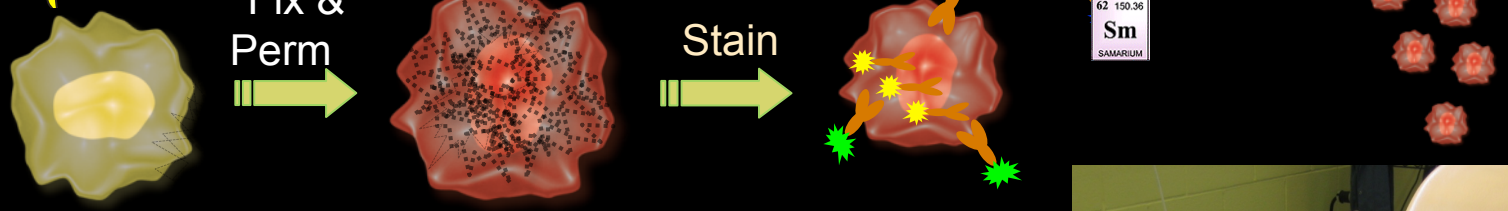
Bad Guy Cells

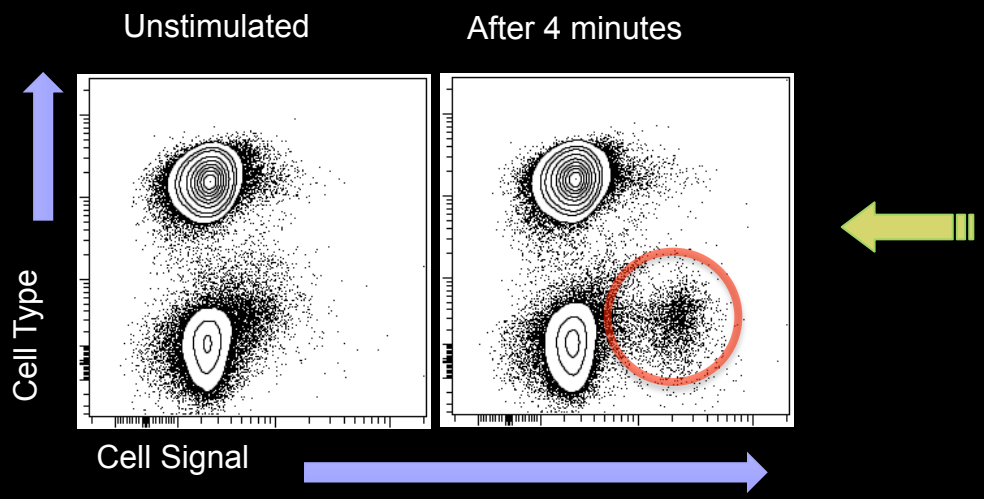


Mass Cytometry: 40+ parameters & no compensation

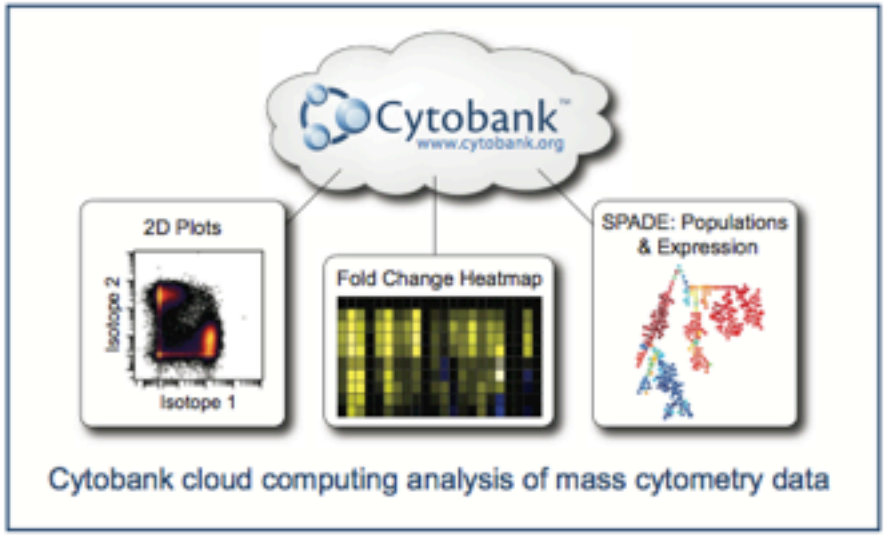
Assays to Relevant Biological Pathways:

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





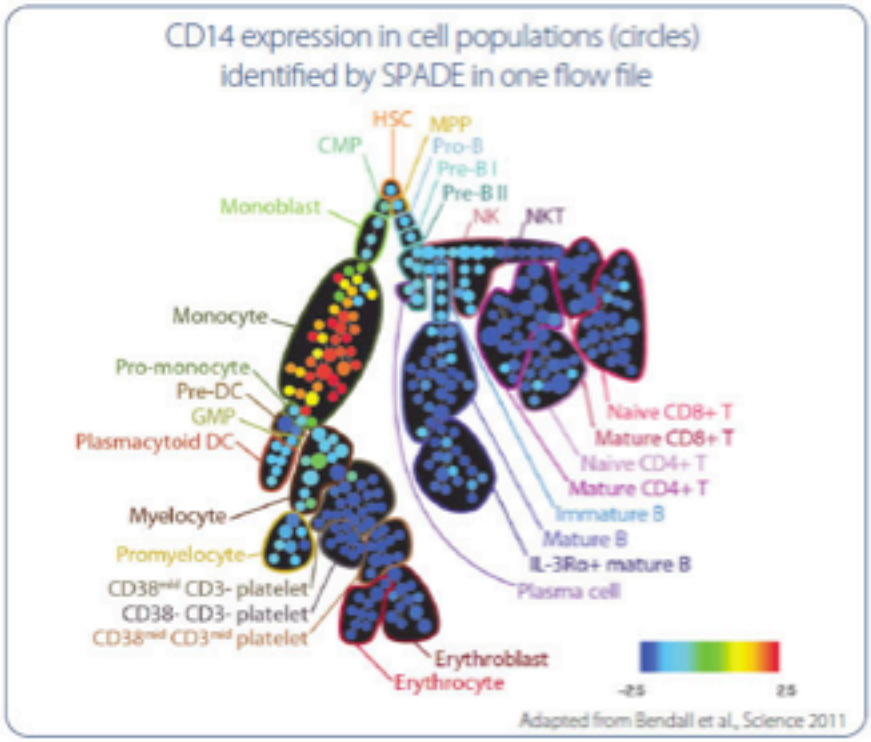
High Dimensional Cytometry is here



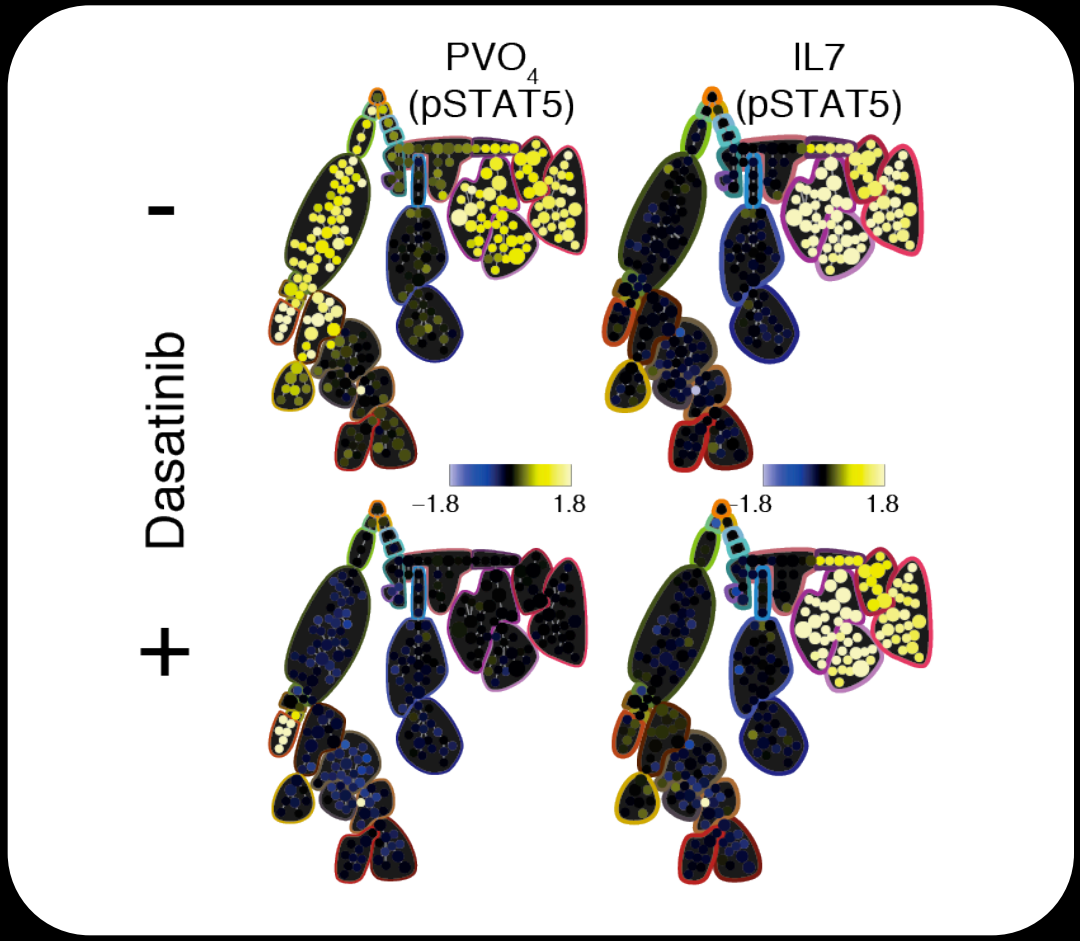
Cytobank cloud computing analysis of mass cytometry data

30+ parameters hosted and analyzed on Cytobank

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

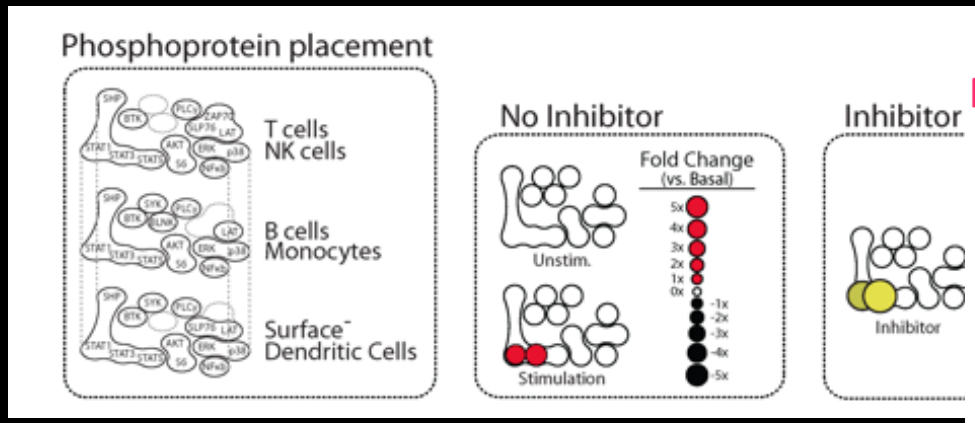


The Effect of a Small Molecule Kinase Inhibitor on Normal Immune Signaling – *Dasatinib Example*



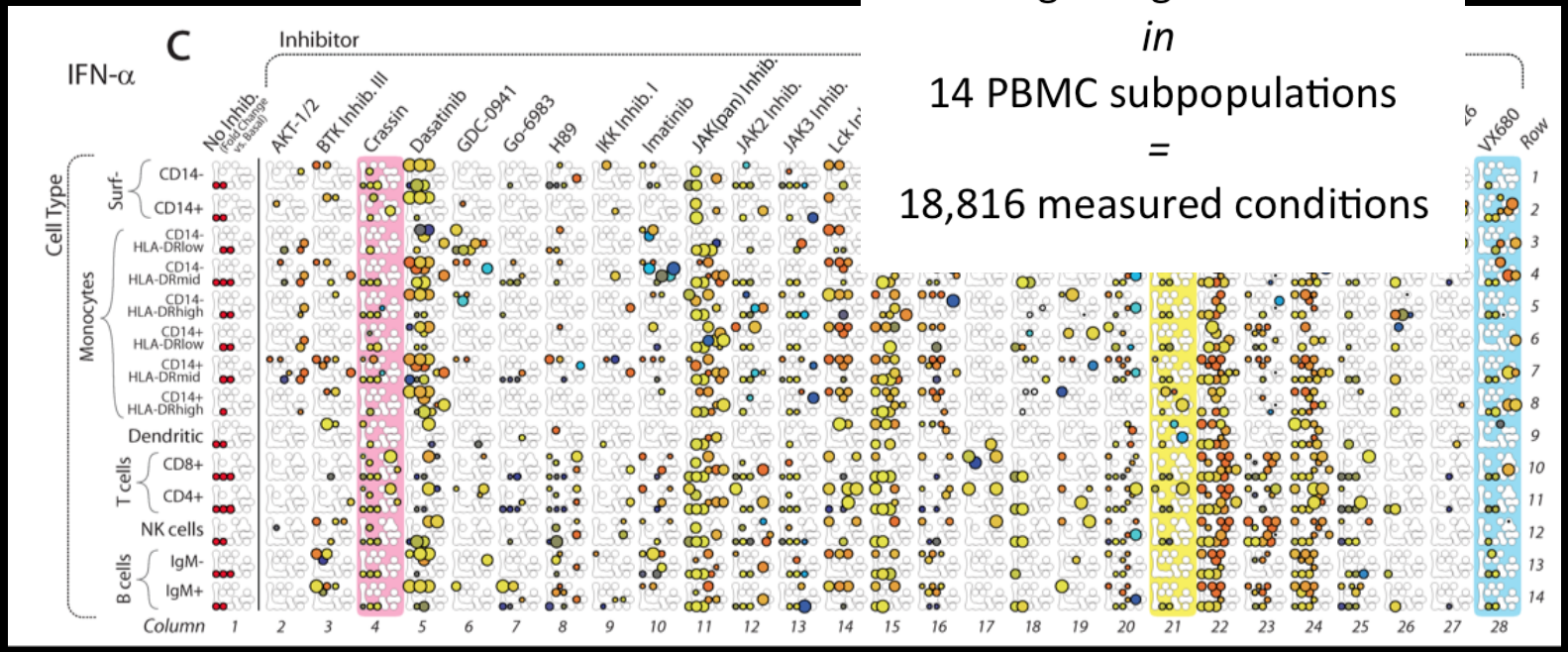
S. Bendall & E. Simonds. *et al*. *Science*, 2011

Deep Profiling of Immune and Drug Responses Across the Hematopoietic Continuum



For each of 27 kinase inhibitors:

8 inhibitor concentrations
on
12 stimuli
for
14 signaling molecules
in
14 PBMC subpopulations
=
18,816 measured conditions



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

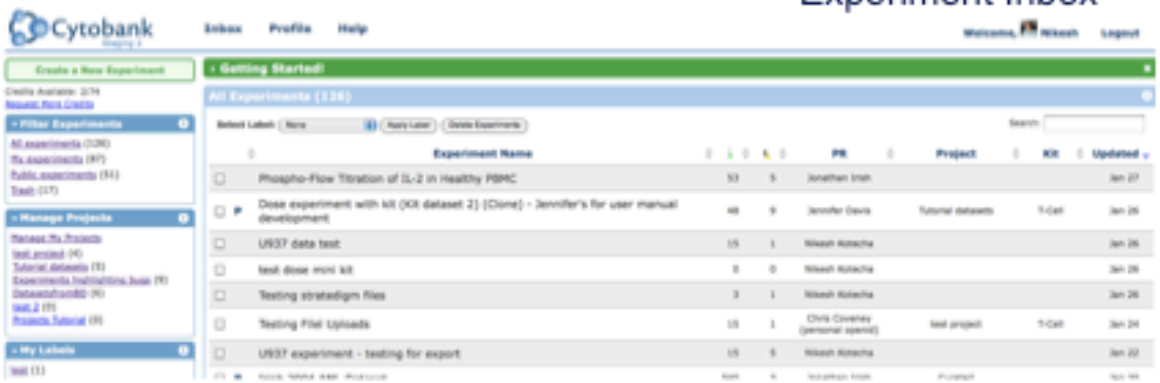
2) Flow cytometry & signaling network profiles
   - map signaling in every cell within a tumor specimen
   - markers for tumor, non-malignant, and cell subsets
   - cell sorting for follow up studies of genetics and epigenetics

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)

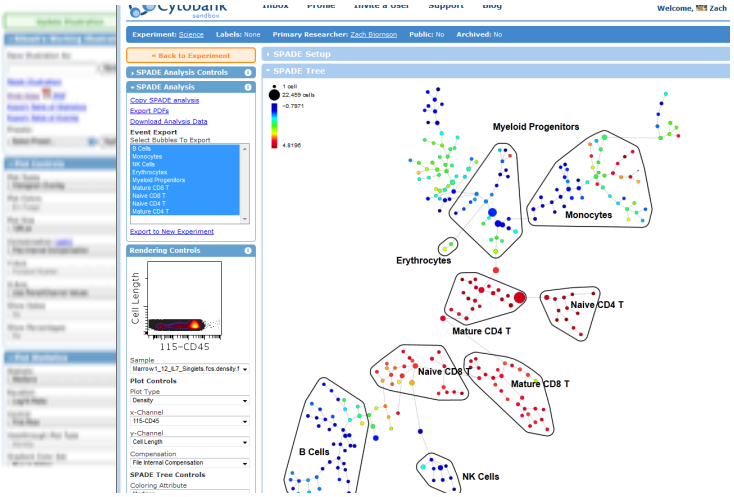
Cytobank is for ***managing, sharing*** &***analyzing*** flow experiments over the web

Experiment =
Collection of FCS files

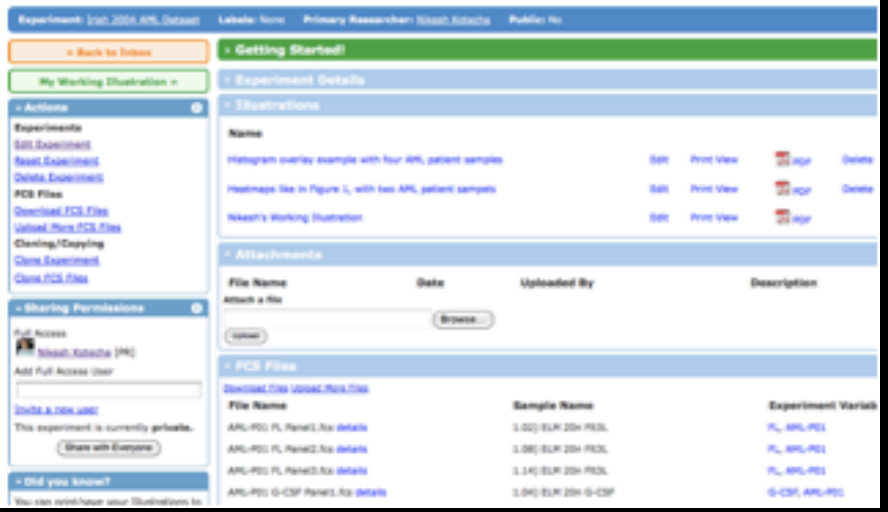
Experiment Inbox



Experiment Analysis

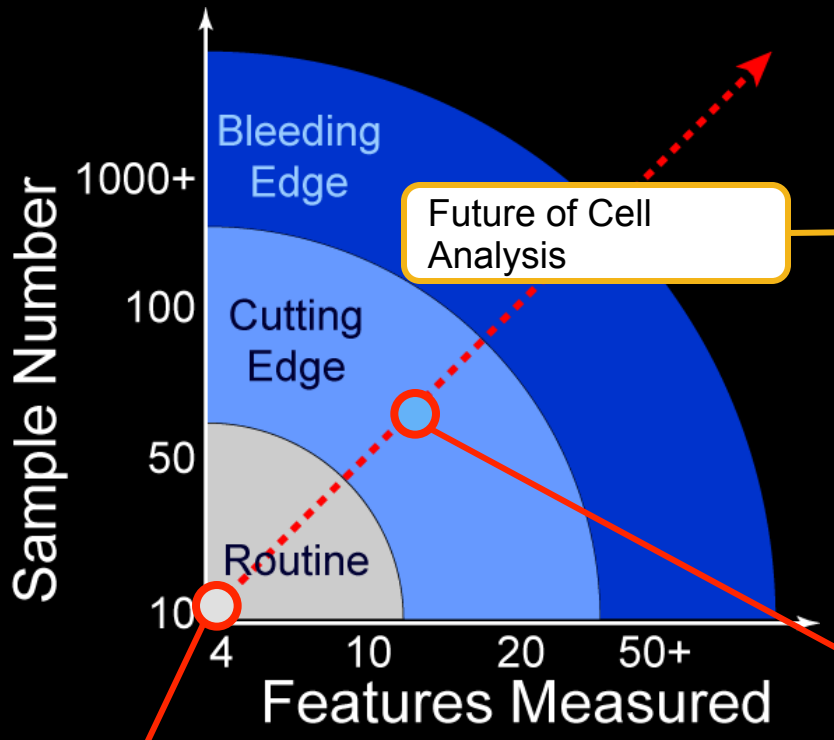


Experiment Details



Why did we build Cytobank?

The Future of Single Cell Analysis

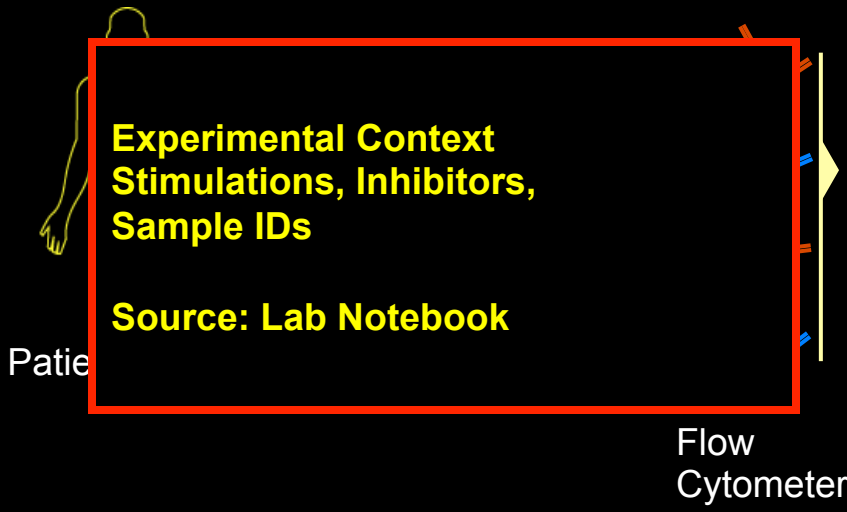


CyTOF

FACSCalibur

LSR ii

Analysis and annotation of flow cytometry data is fragmented



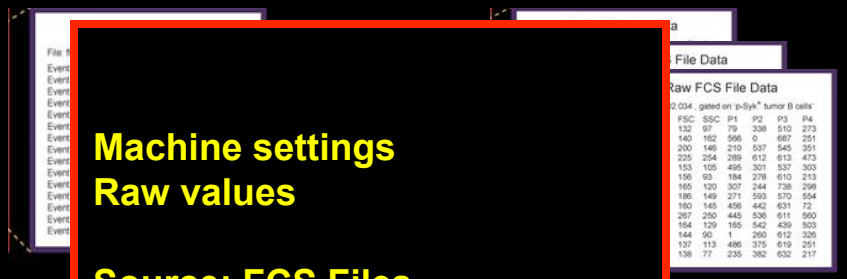
Experimental Context
Stimulations, Inhibitors,
Sample IDs

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

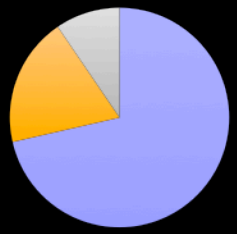
Advanced Analysis,
Aggregate Views

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

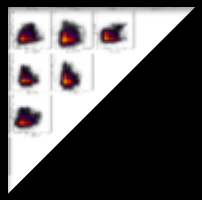
Source: Analysis Program

Gating

Current Analysis Methods are not a scalable solution



Design and Validation
Bench Time
Data Analysis



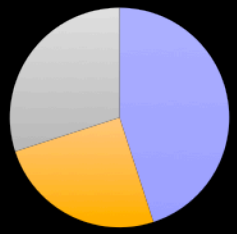
Parameters: 4

Plots: 6

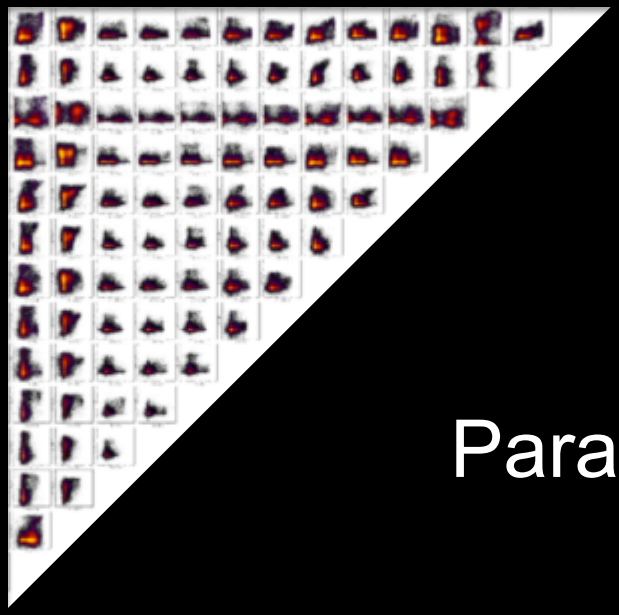
Individual Efforts

Bendall, Simonds, et al. *Science,* May 2011

Current Analysis Methods are not a scalable solution



- Design and Validation
- Bench Time
- Data Analysis



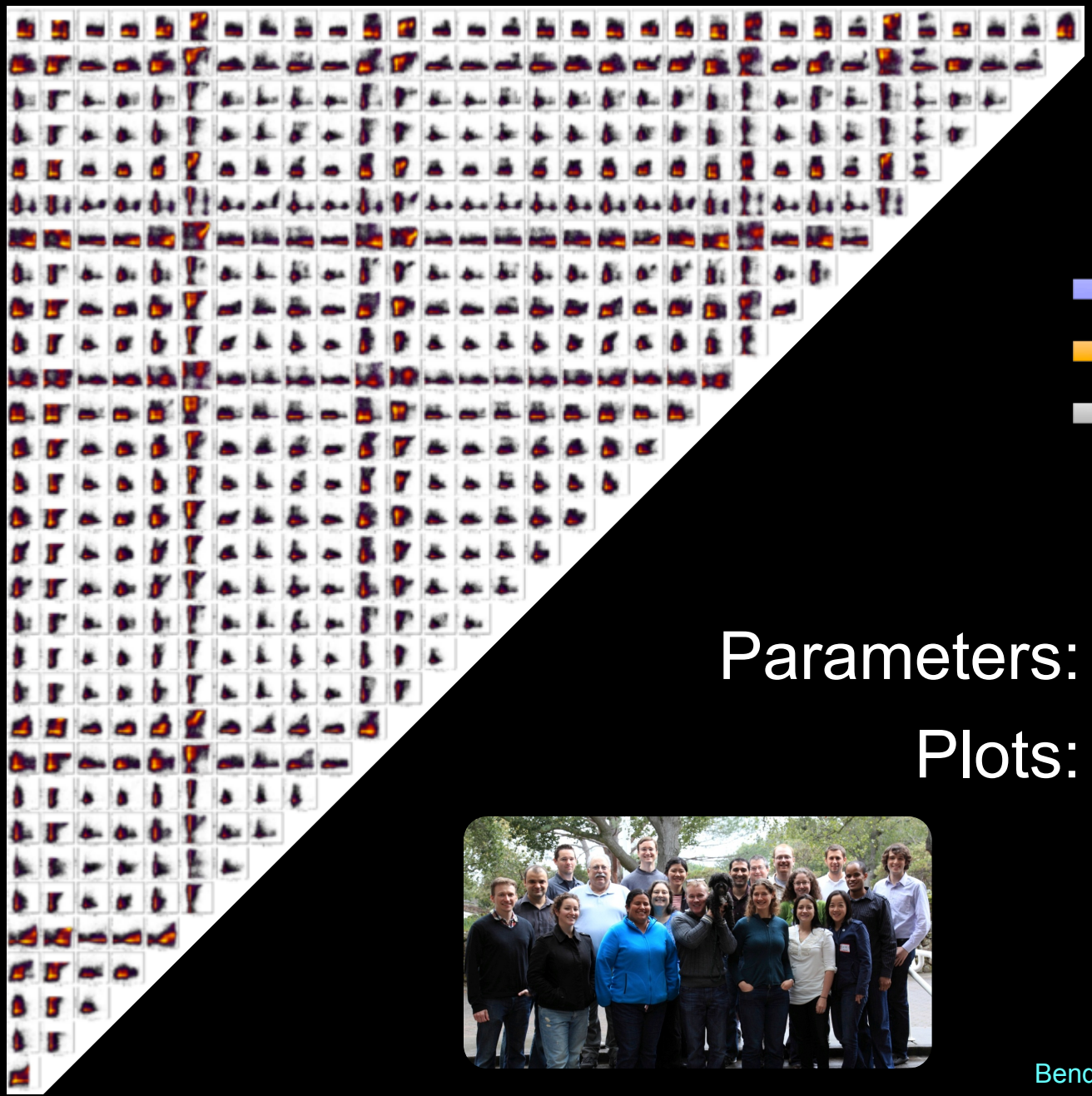
Parameters: 14

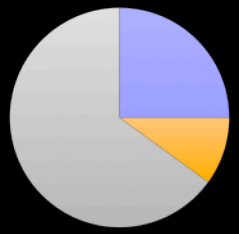
Plots: 91

Joint Efforts

Bendall, Simonds, et al. *Science,* May 2011

Current Analysis Methods are not a scalable solution

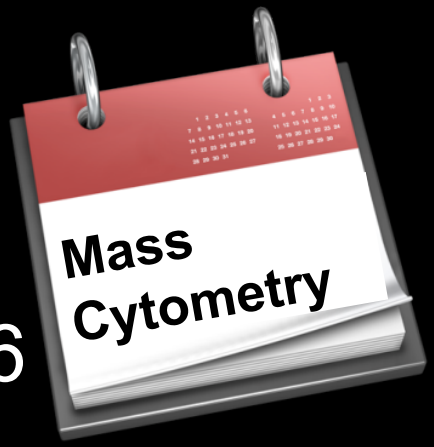




- Design and Validation
- Bench Time
- Data Analysis

Parameters: 32

Plots: 496



Team Efforts

Bendall, Simonds, et al. *Science,* May 2011

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



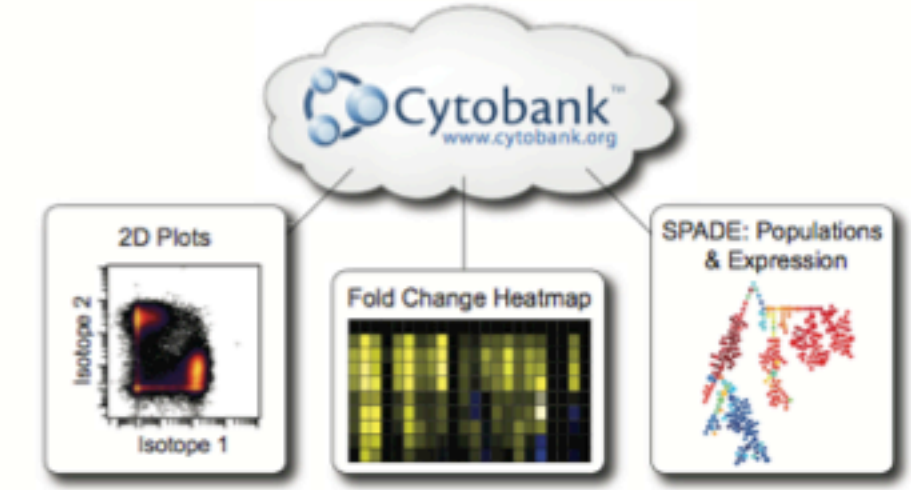



Cell Biology– Immunology- Informatics – Clinic
Cross Discipline/Institution Collaborations

Key Challenges

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

Cytobank enables single cell analysis



Clinically Actionable Finding

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

Cytobank is designed for:

Experiment Organization
Collaboration
Novel Analysis
High Dimensional Flow
Big Data
- Scalable Computation
- Platform Development

Reducing time to Results

Cytobank is available in multiple forms

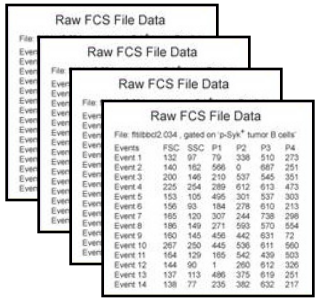
- Community Cytobank – www.cytobank.org
  - *Free access to **base functionality***
- DVS Cytobank– dvs.cytobank.org
  - For the mass cytometry community
  - ***Premium modules and functionality (e.g. SPADE)***
- Enterprise Cytobank – e.g. companyX.cytobank.org
  - *Hosted, backed up, and maintained 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. (www.cytobankinc.com)

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

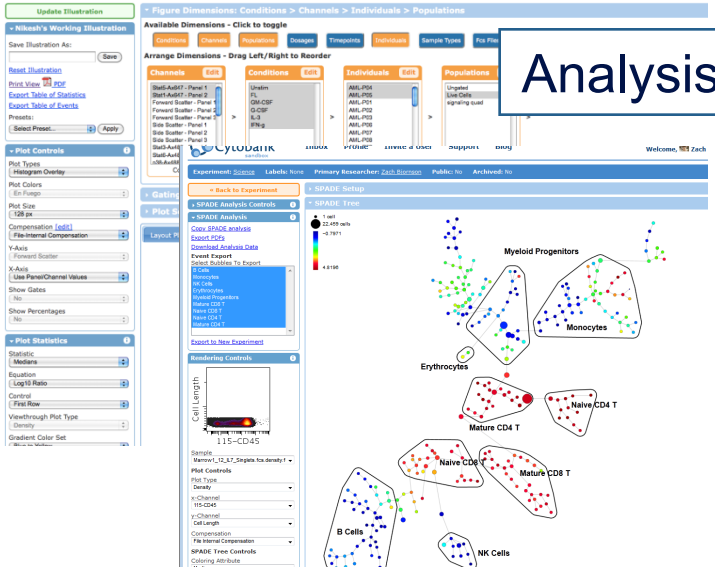
Lower barrier of entry for statisticians and computational biologists?

Manage, Analyze, Share Cytometry Data on the Web

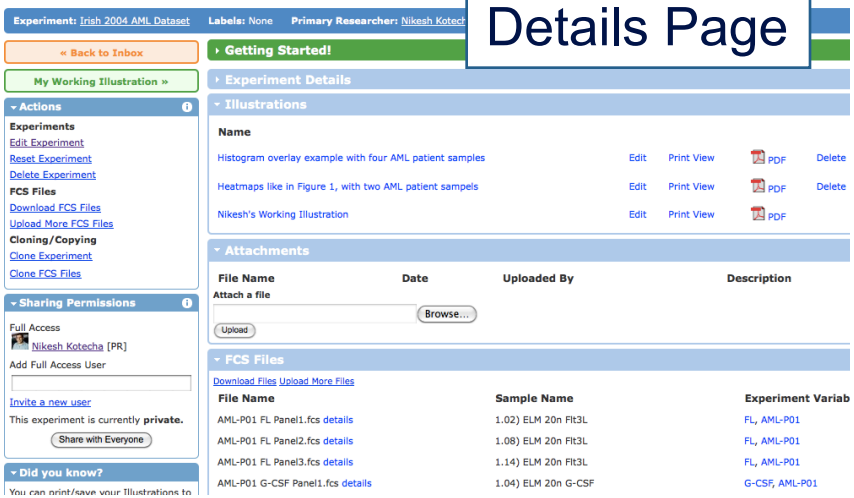


Experiment =
Collection of FCS files

Inbox

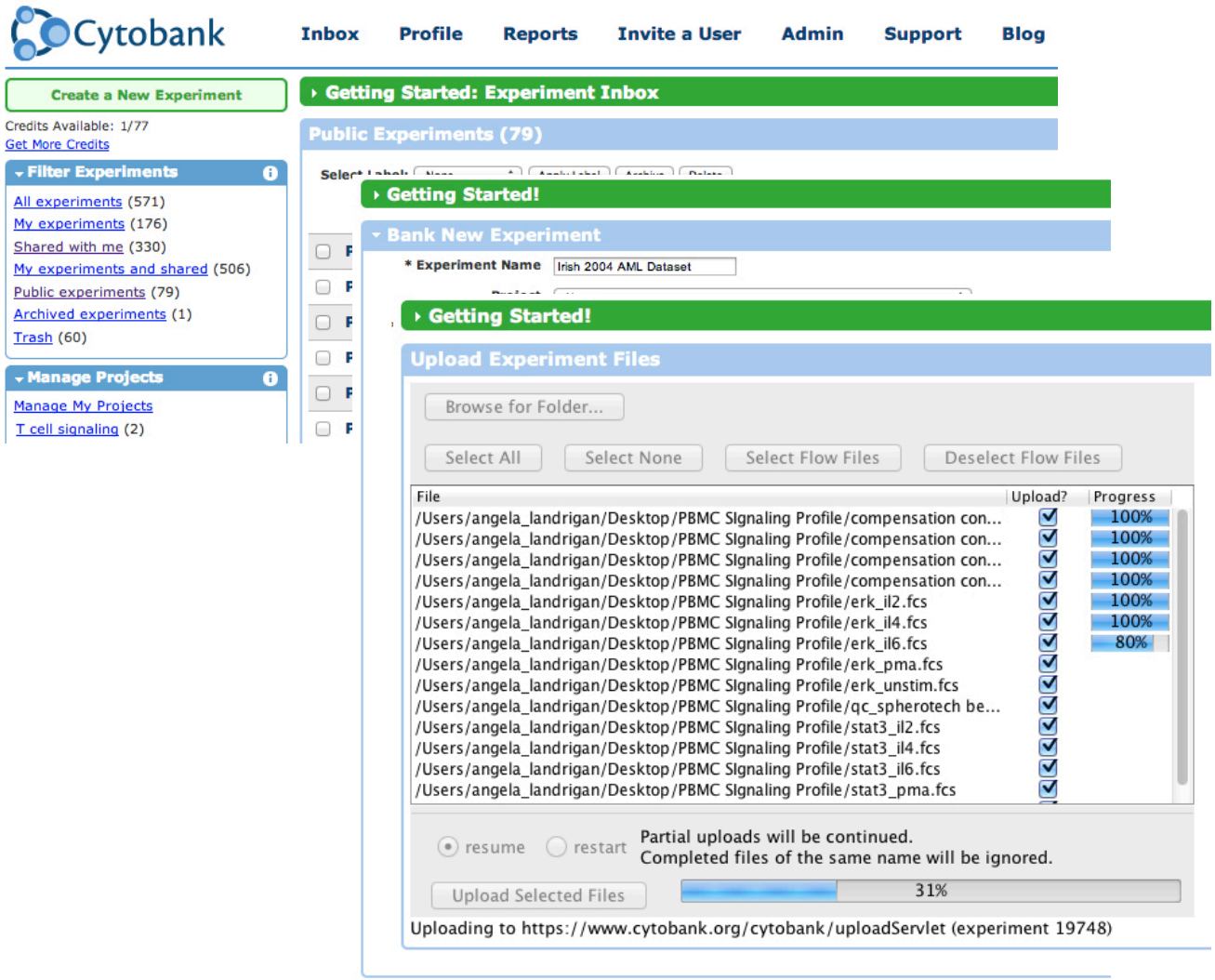


Analysis/Figures



Details Page

Create a new experiment and upload FCS files



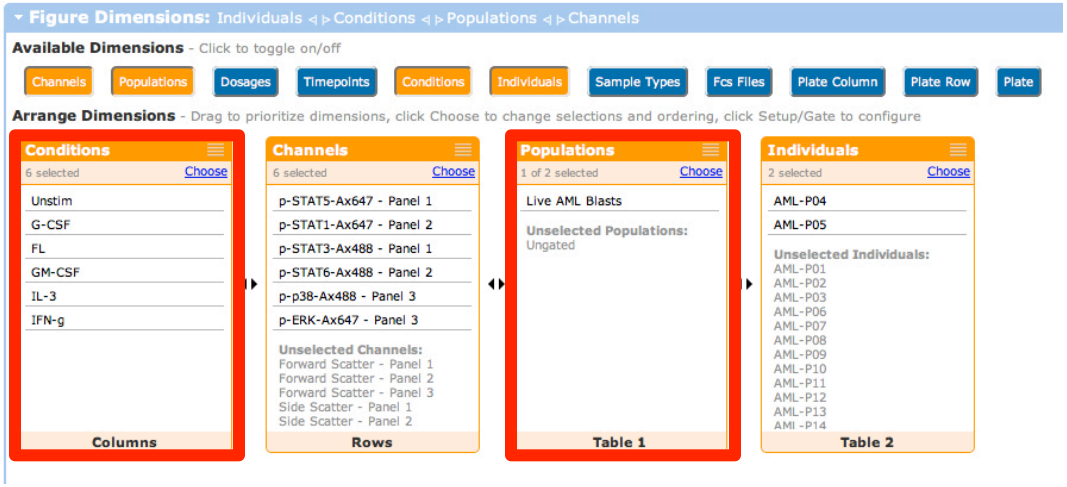
Launch web browser & Login to Cytobank

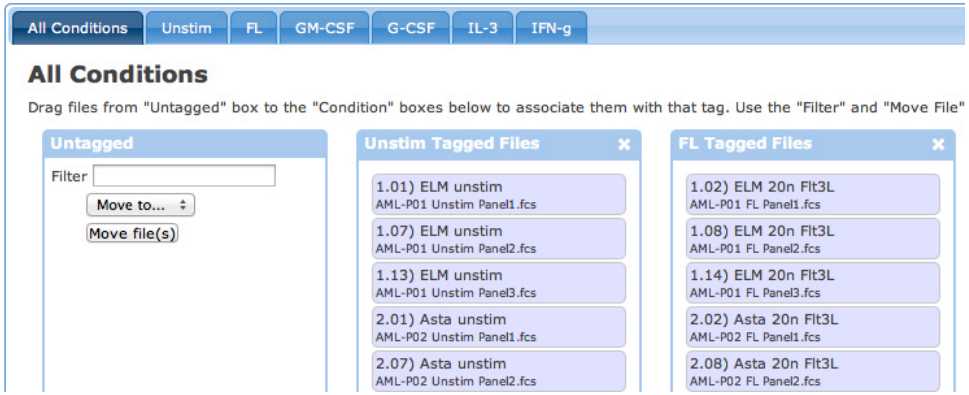
Create a new Experiment

Upload FCS Files

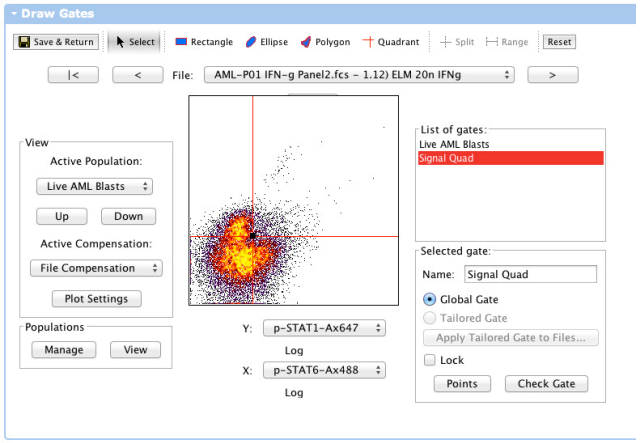
Organize information around samples using Experimental Variables

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



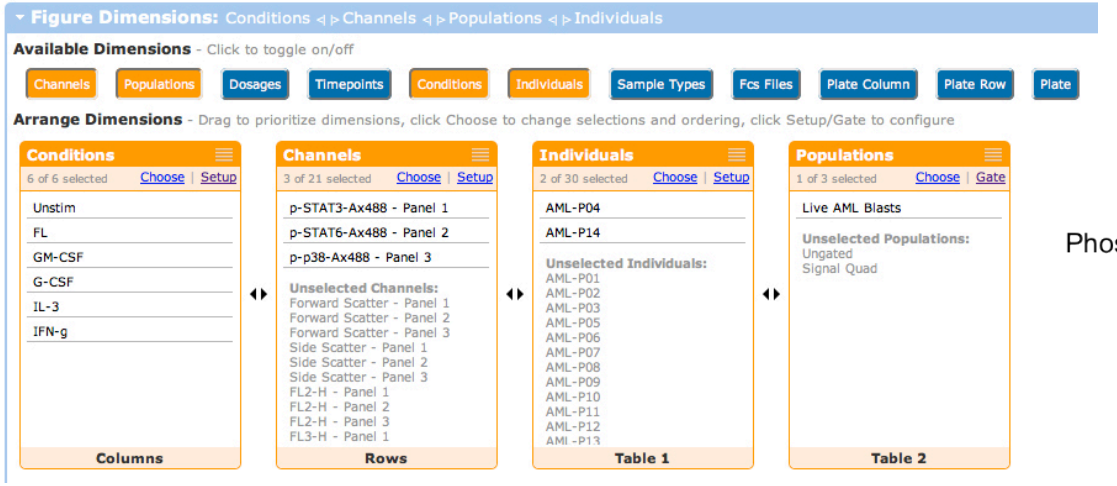


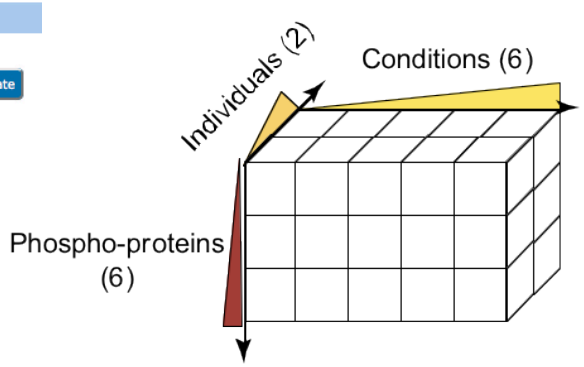
Assign samples to experimental variables



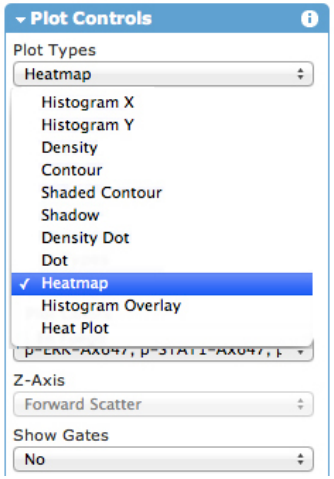
Identify populations of interest (gating)

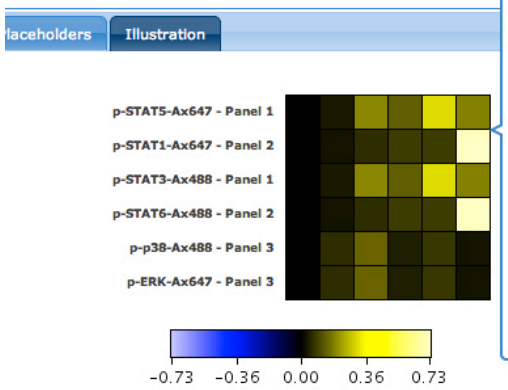
Use Experimental Variables to create and pivot figures

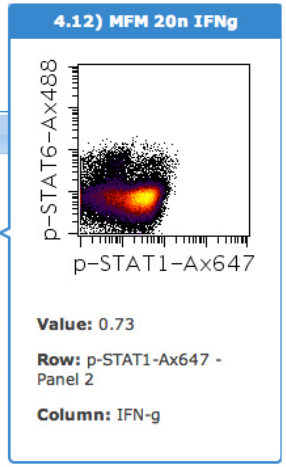




Arrange figure using Experimental Variables





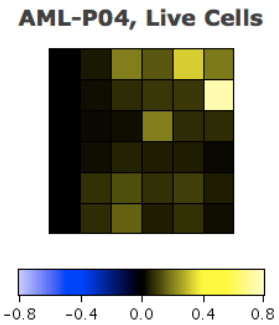


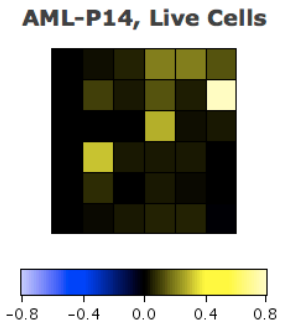
Plot controls to specify plot type and display

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
Created: Tue Mar 30 11:02:05 -0700 2010 | **Updated:** Tue Mar 30 11:15:32 -0700 2010

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)

Manage Projects

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

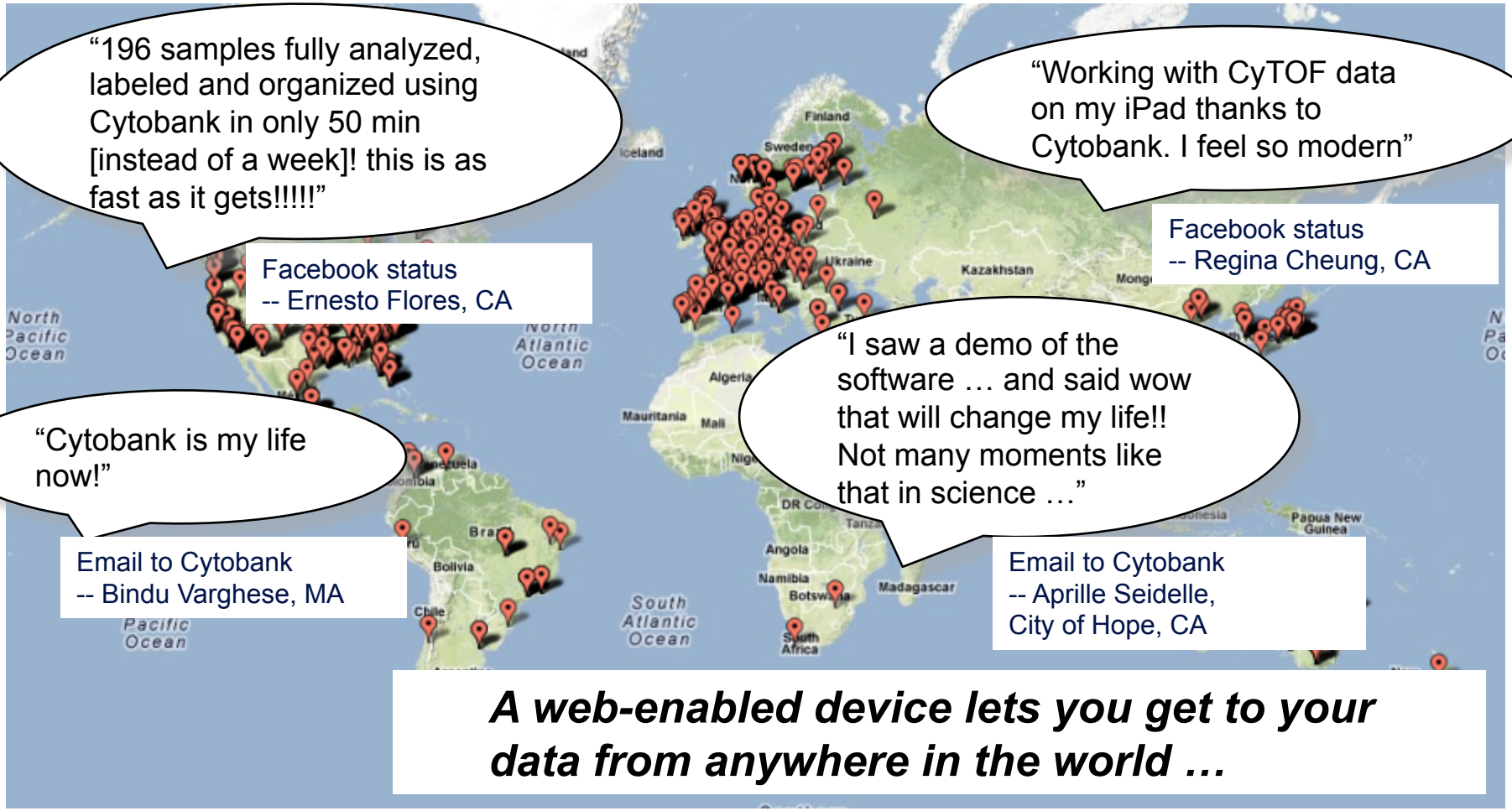
Make experiments public to share with community

Cytobank Benefits (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 www.cytobank.org as of December 2011

What Researchers are Doing with Cytobank

- Search “Cytobank” in Google Scholar for 50+ citations (in < 2yrs):
  - *Specific cellular signal-transduction respons… …on therapy with ATRA, valproic acid and theophylline in acute …*
    - Skavland et al (**Norway**) – **Feb. 2011** Nature Blood Cancer
  - *CD137 sti… …ctivity of anti-CD20 antibodies*
    - Kohrt et al (…
  - *Cell-to-Cell Variability in PI3K Protein Level Regulat… …Activity in Cell Populations*
    - Yuan et al (**Harvard**) … **Jan 2011** Current Biology
  - *Oxidative … …ation of Kaposi's Sarcoma-Associated Herpesvirus and Death of Primary Effusion Lymphoma Cells*
    - Li et al (**UCLA**) – **Jan 2011** Journal of Virology
  - *Poor cytokine-induced phosphorylation in chronic … …mia patients at diagnosis is effectively reversed by tyrosine kinase inhibitor therapy*
    - … **2010** Experimental Hematology
  - *Computational solutions to large-scale data management and analyses*
    - Schadt et al (**California**) – **Sep 2010** Nature Reviews Genetics

Phosflow

Immunophenotyping

Biochemistry

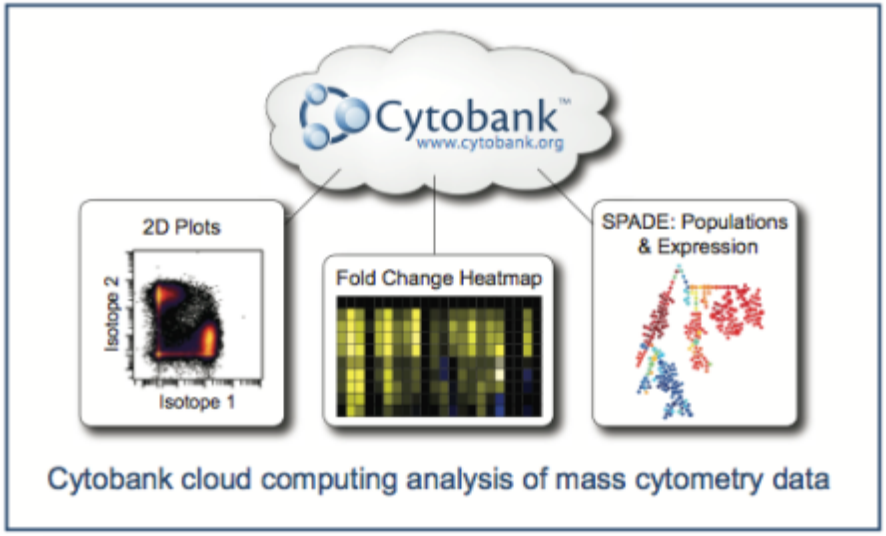
Cancer

Apoptosis

Informatics

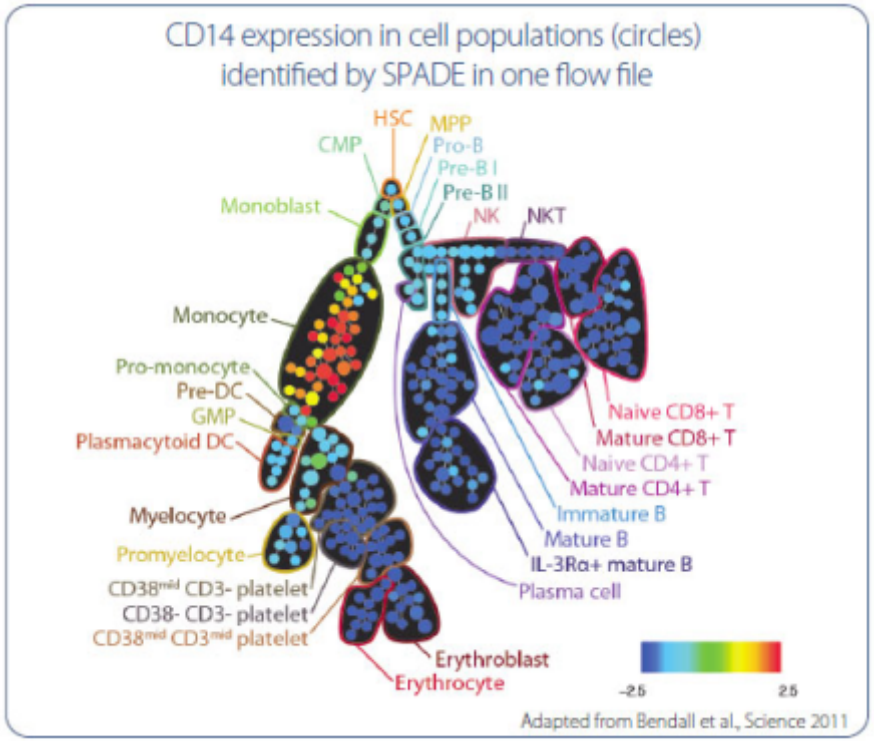
More at http://blog.cytobank.org/publications

Cytobank is the platform for CyTOF Analysis



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

www.cytobank.org/nolanlab

Nolan Lab | Signaling-Based (Flow & Mass) Cytometry Resource

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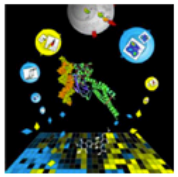
home



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

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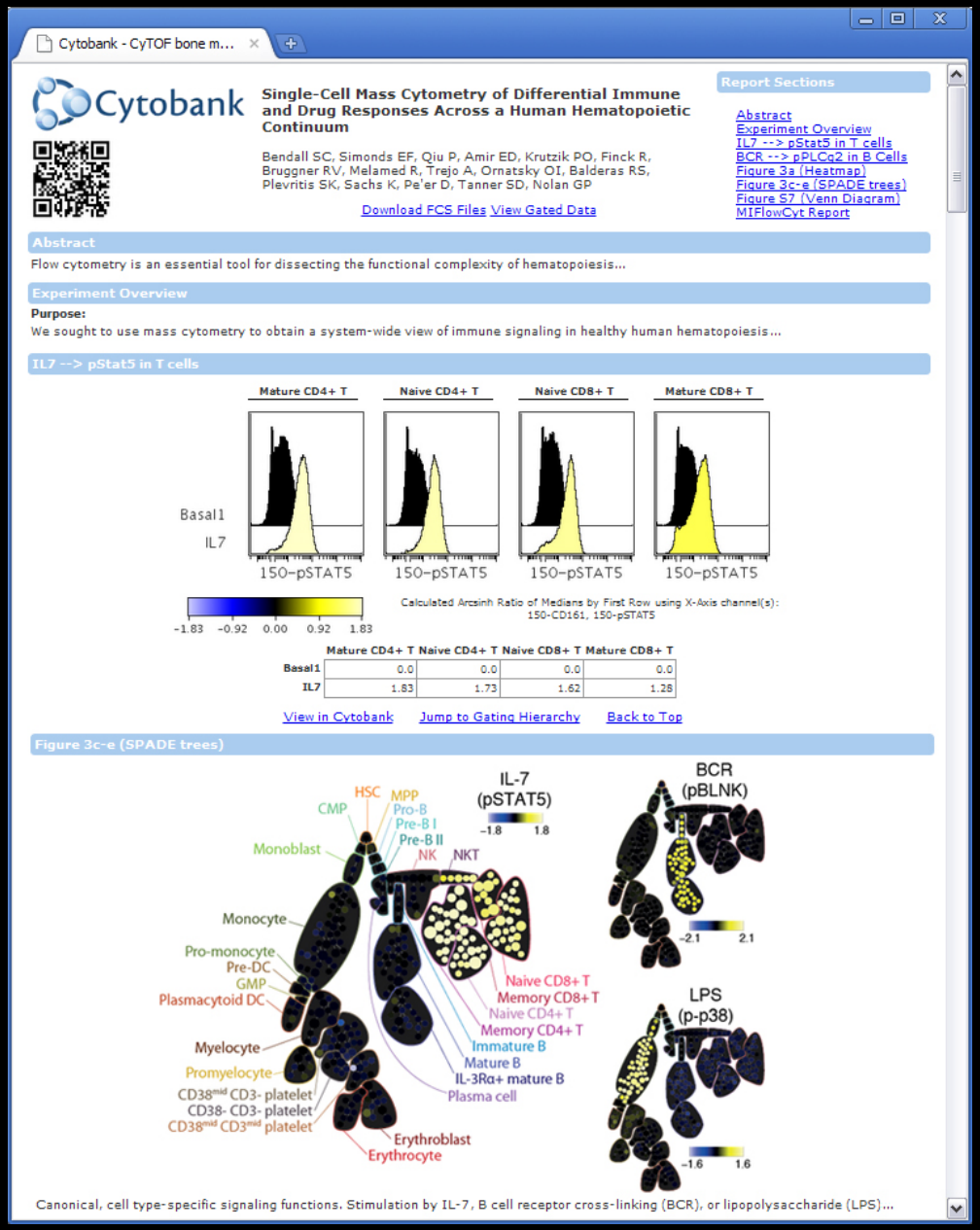
Publish Data & Annotations with Manuscript



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

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

What’s Next?

The Cytobank Platform

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

Translational
Assays
Drug Screening
(Clinical) Reports

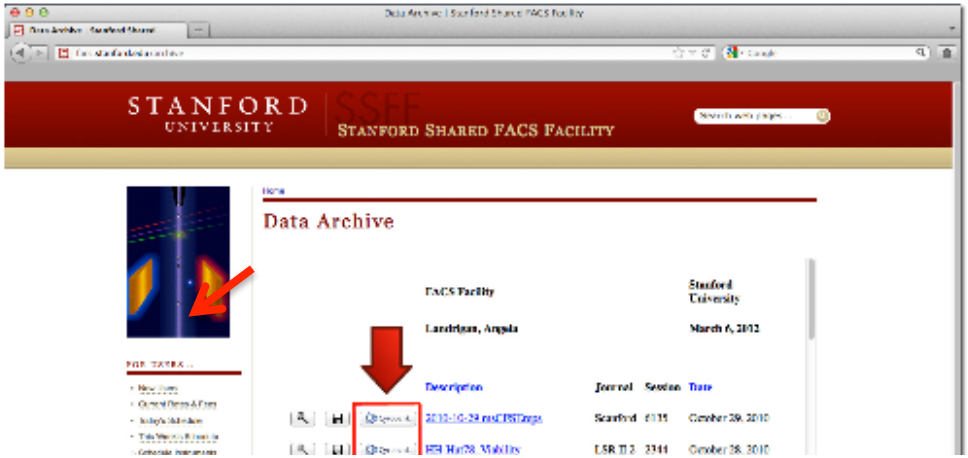
High Dimensional Cytometry,
Mass Cytometry,
(SPADE, Large Scale Computations)

Community Resources
(BD FacsSelect)
Public Cytometry Collections

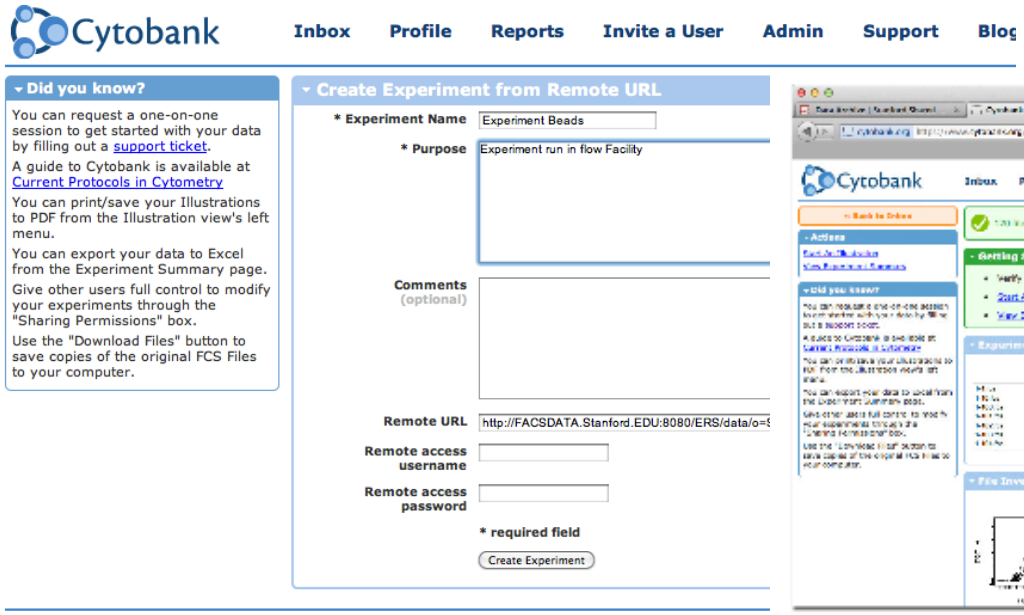
Cytobank

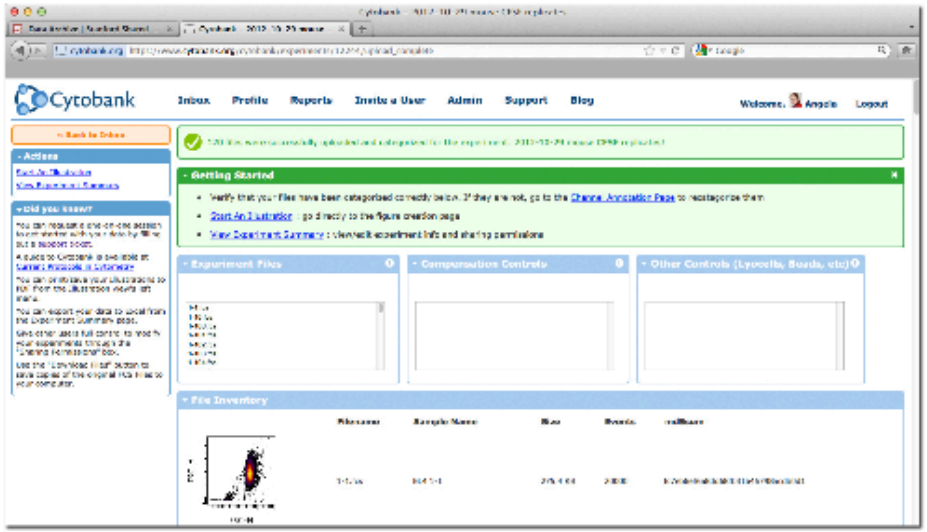
Accessible from any web-enabled device anywhere

Integration with Core Facilities (Stanford)



Retrieve experiment by URL





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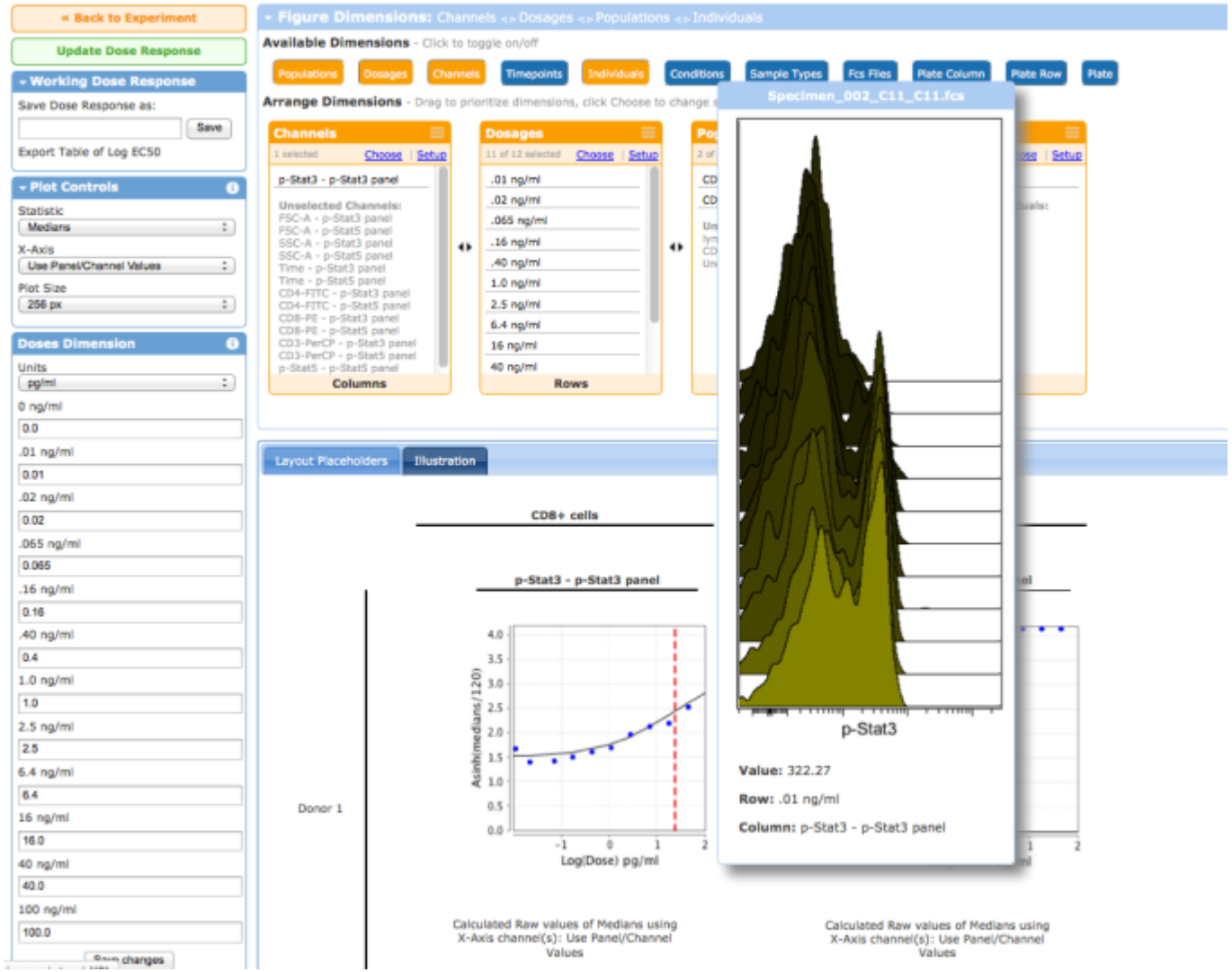
Drug Discovery Assays (linked to underlying data)



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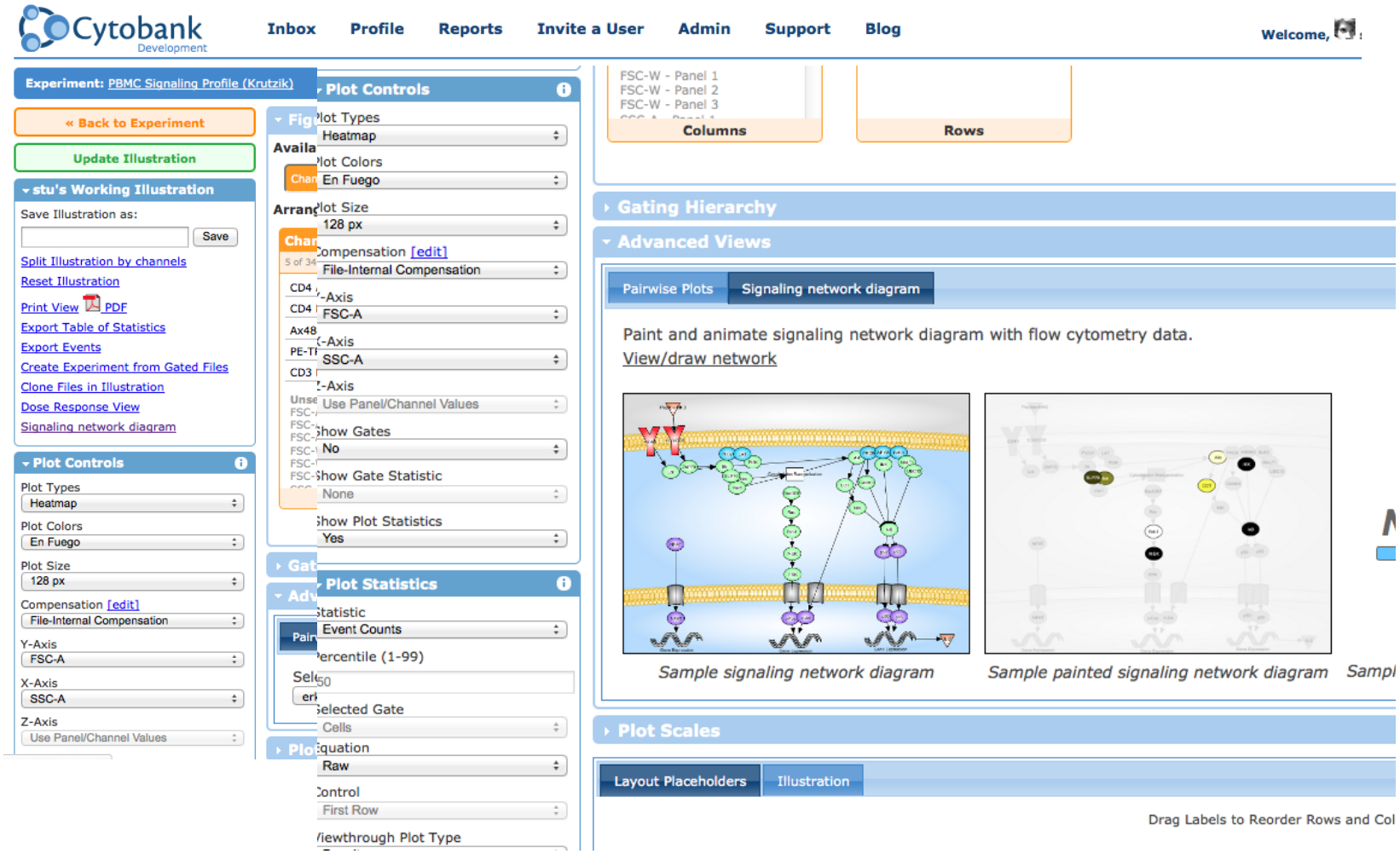
Signaling Diagrams (linked to underlying data)



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Add Ontological 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

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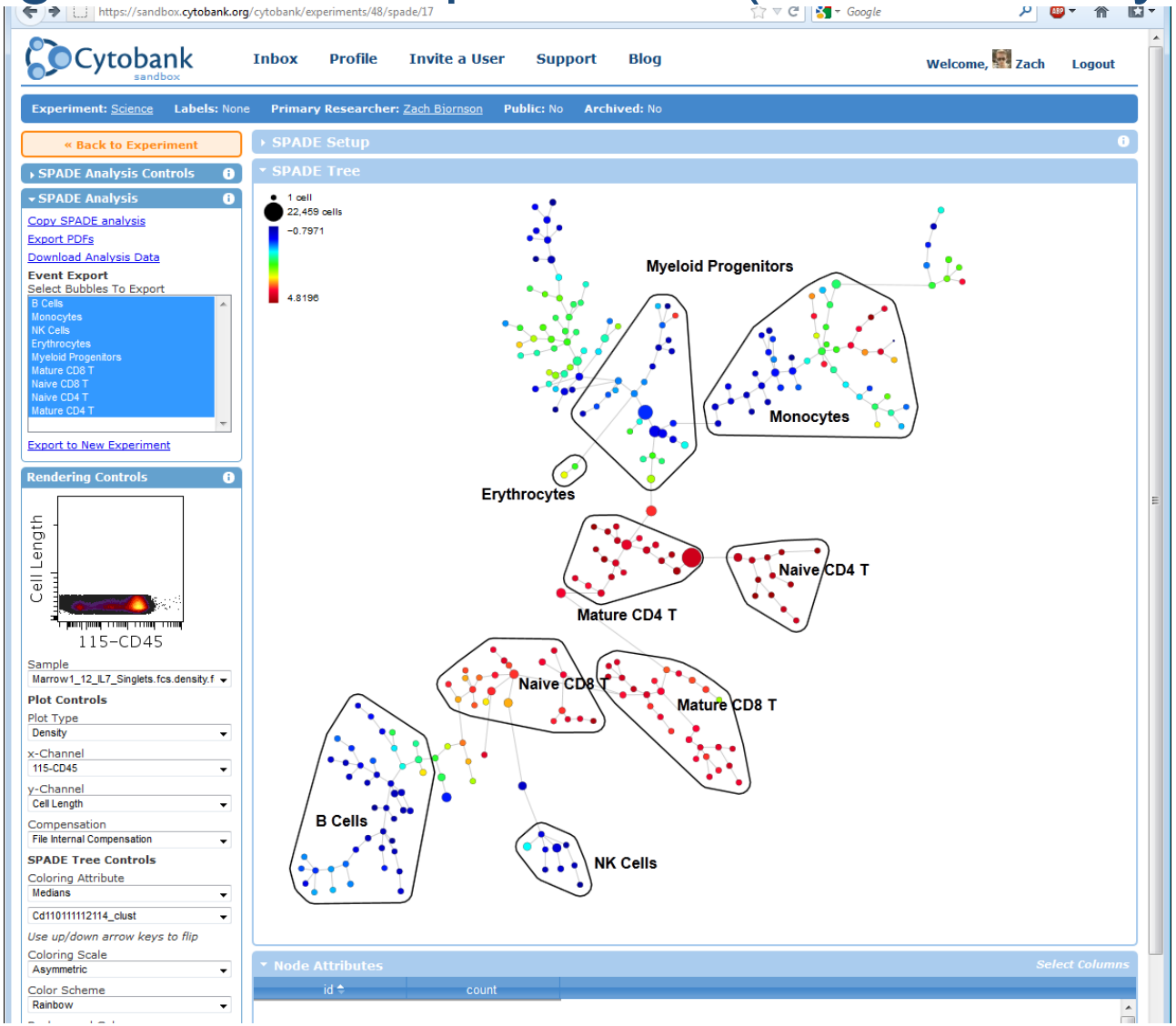

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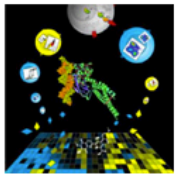

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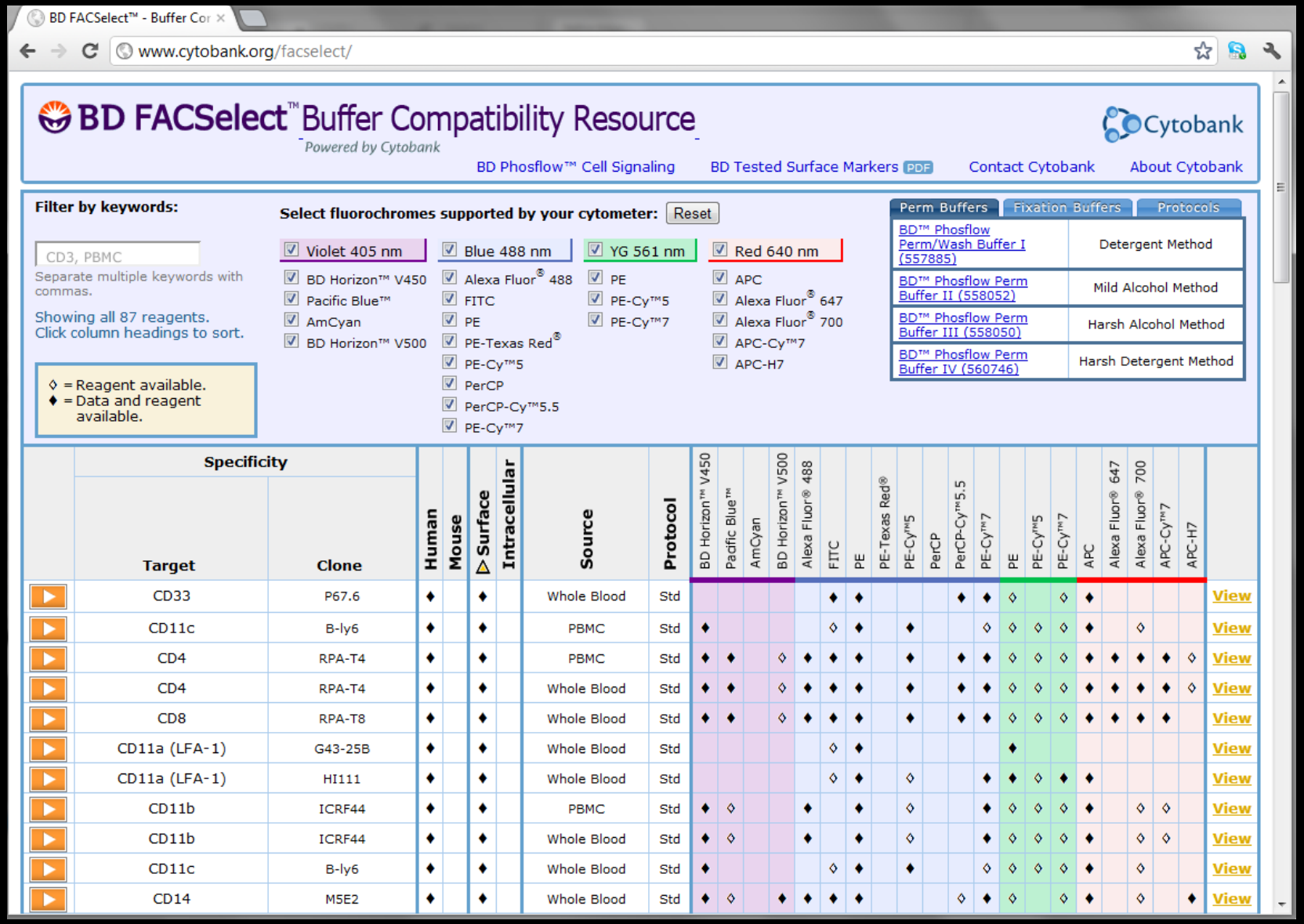
BD FACSelect: Link Interactive Data to Reagents



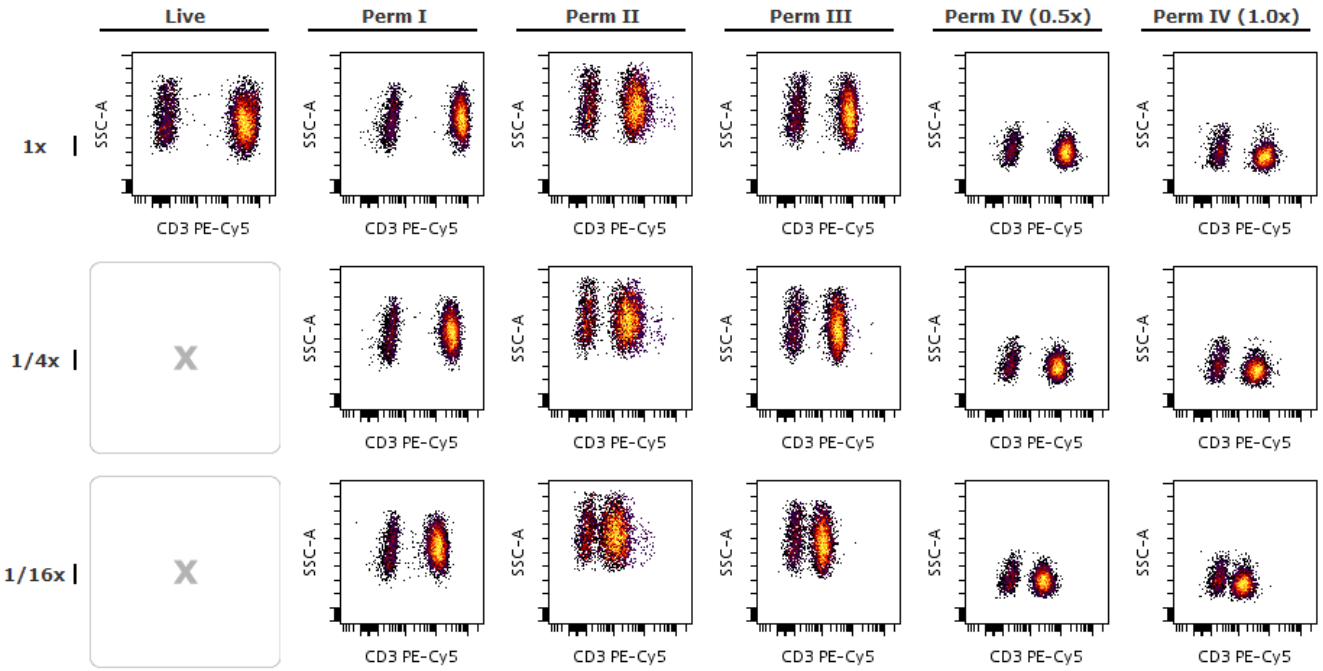
http://www.cytobank.org/facselect

BD FACSelect: Link Primary Flow Data to Reagents

Cytobank - CD3_UCHT1_Hu

reports.cytobank.org/21/v1

CD3 PE-Cy5



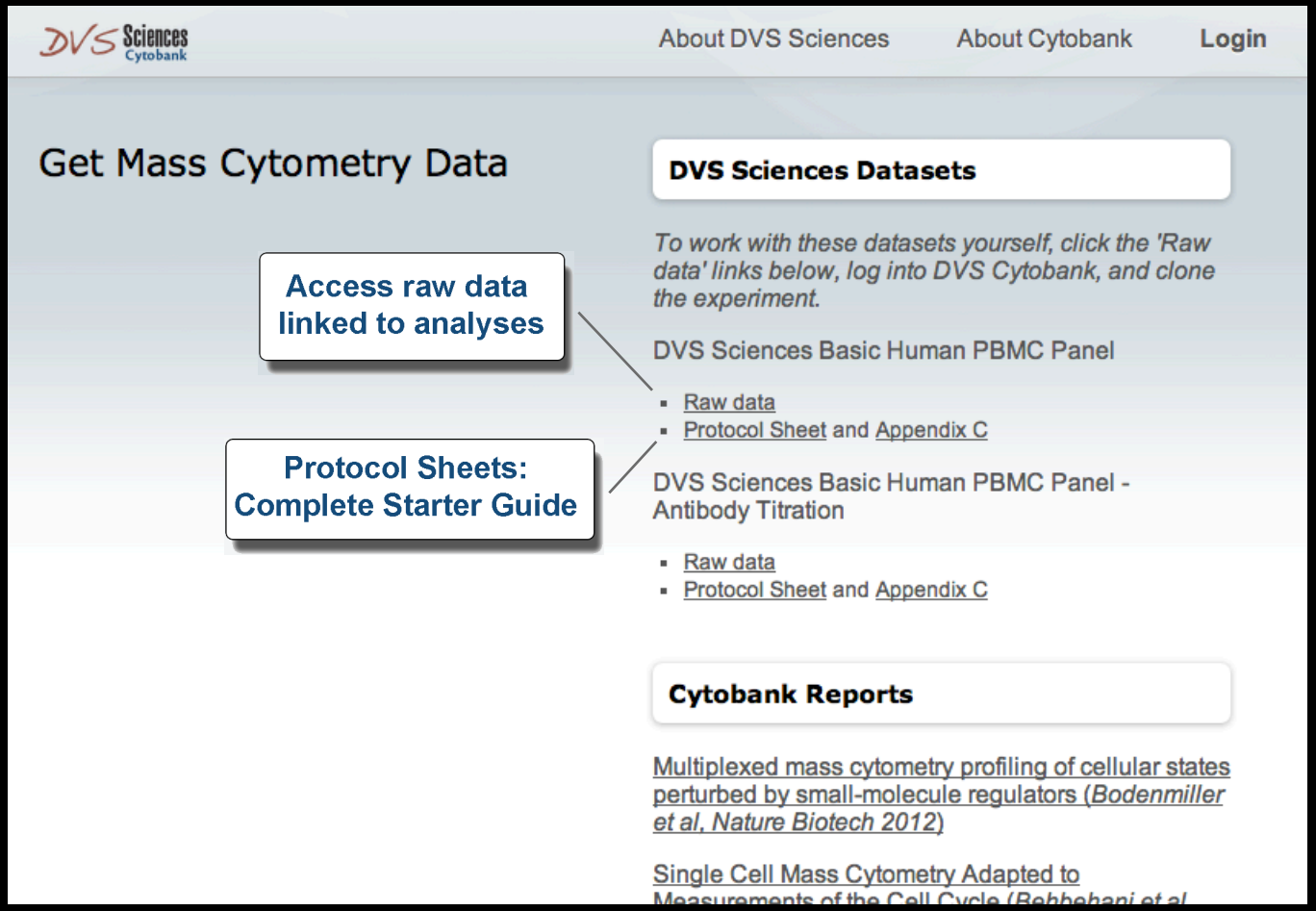
Calculated Raw values of statistic using X-Axis channel(s): Use Panel/Channel Values

	Live	Perm I	Perm II	Perm III	Perm IV (0.5x)	Perm IV (1.0x)
1x	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 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

Stanford:

Peng Qui/Sylvia Plevritis

U Toronto & DVS

Scott Tanner et. al.

Becton Dickenson

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