



# Publishing MIFlowCyt Compliant Data to ISAC's FlowRepository.org for Cytometry A and Other Journals

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June 27, 2012

Motivation Background

# Outline

### Introduction

- Motivation
- Background
- FlowRepository
  - Data review and download
  - Data upload and annotation
  - Data sharing
- Summary & Conclusions
  - Summary
  - Future Work
  - Acknowledgments



Motivation Background

# Why share your data?

- Promote open scientific inquiry and progress in the field
  - Allow for re-exploration of existing datasets to test new or alternative hypotheses and methods of analysis
  - Allow for independent validation and refutation of experimental findings
- Required or encouraged by many funding agencies and scientific journals

# What to share?

- A dump of FCS files is not enough
  - Data without context are not understandable to others
- Minimum Information about a Flow Cytometry Experiment
  - Outlines the minimum information required to report about flow cytometry experiments
  - Represents the community consensus
    - 33 coauthors from 19 institutions
    - ISAC Recommendation
  - Required/recommended by Cytometry A and Nature



Lee et al., MIFlowCyt: the Minimum Information about a Flow Cytometry Experiment. Cytometry A. 2008; 73(10): 926-930

Motivation Background

# MIFlowCyt components



Josef Špidlen, Ryan R. Brinkman FlowRepository.org – Resource of Flow Cytometry Data

# MIFlowCyt components

### Experiment overview

- Purpose
- Keywords
- Experiment variables
- Date(s)
- Organization(s)
- Primary contact
- Quality control measures

### Sample description

- Description
- Sample material
- Treatment
- Fluorescent reagents
- Source
- Biological samples: Organism with taxonomy, phenotype, genotype, age, gender, ...
- Location for environmental samples

### Data analysis

- FCS data files
- Compensation and other transformations
- Gating details including gate description, statistics and boundaries or images or gate membership details

### Instrumentation details

- Make
- Model
- User-adjustable components (e.g., detector voltages)
- Customized configurations

# How to share all these details?

- Manuscript, e.g., the methods section
- Manuscript's supplemental information
- Data repository



Data review and download Data upload and annotation Data sharing

# FlowRepository – What is it?

- A public online resource of annotated flow cytometry datasets
  - Primarily those associated with peer-reviewed publications
- Web-based application created by extending and adapting Cytobank
  - Mainly to incorporate MIFlowCyt
- Open source
  - Affero General Public License
- Supported by ISAC and generously hosted by Carnegie Mellon University

Data review and download Data upload and annotation Data sharing

# FlowRepository – What do you need to start?

- A computer with Internet connection
  - Fast connection is good, especially when uploading large datasets
- Web browser
  - Firefox or Chrome recommended
  - Safari or Internet Explorer also works but may not look great
- Ability to run Java Applets in the Web browser
  - Java version 1.5.1 or higher is required for data upload, download and online analysis
- A Google ID, Yahoo! ID, or another OpenID
  - Required for write access

Data review and download Data upload and annotation Data sharing

Accessing FlowRepository

- Navigate your Web browser to http://flowrepository.org
  - Or https://flowrepository.org if you prefer a secure protocol

Data review and download Data upload and annotation Data sharing

Accessing FlowRepository

- Navigate your Web browser to http://flowrepository.org
  - Or https://flowrepository.org if you prefer a secure protocol
- Demonstration (offline)
  - Access FlowRepository
  - Review a public dataset
  - Deposit, annotate and share a dataset

https://flowrepository.org

### **FL**WRepository

### 값 Login

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#### Did you know?

A guide to FlowRepository is available at the <u>documentation site for Cytobank and</u> <u>FlowRepository</u>.

We also have a Quick start guide.

You can contact us by filling out a support ticket.

#### Supporting journal



#### FlowRepository at <u>CYTO 2012</u>

» Sunday, June 24: State of the Art Lectures - Computational Analysis of High-Dimensional Data

» Tuesday, June 26: Parallel 8 - Cytometry Technology: Cytometry Software and Informatics

» Wednesday, June 27: Workshop 13 -Publishing MIFlowCyt Compliant Data to ISAC's FlowRepository.org for Cytometry A and Other Journals



#### FlowRepository

FlowRepository is a database of flow cytometry experiments where you can query and download data collected and annotated according to the MIFlowCyt standard.

Query		
Enter a term to search all publicly availa	ble experiments:	
[		Query
Hide query fields		
Experiment Names	Repository Identifiers	Keywords
Researcher Names	Reagents and Manufactu	urers 🗹 Instruments and Manufacturers
FCS Files (Headers)	Sample Annotations	✓ Pubmed IDs
Links		
Browse all public datasets	Quick start guide	Referencing Flow Repository and Cytobank
Browse community datasets	Submit data	FlowRepository Steering Committee & Advisory Board
Browse most popular datasets	Funding	

https://flowrepository.org

### **FL**WRepository

A guide to FlowRepository is available at

the documentation site for Cytobank and

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# FlowRepository.

Technology: Cytometry Software and

» Wednesday, June 27: Workshop 13 -Publishing MIFlowCyt Compliant Data to

Informatics

FlowRepository is a database of flow cytometry experiments where you can query and download data collected and annotated according to the MIFlowCyt standard.

We also have a Quick start guide.	Query		0				
You can contact us by filling out a <u>support</u> <u>ticket</u> .	Enter a term to search all publicly availa	nter a term to search all publicly available experiments:					
Supporting journal	Identification of B cells		Query				
Cytometry	Hide query fields	<ul> <li>✓ Repository Identifiers</li> <li>✓ Reagents and Manufactu</li> <li>✓ Sample Annotations</li> </ul>	✓ Keywords rers ✓ Instruments and Manufacturers ✓ Pubmed IDs				
	Links						
FlowRepository at <u>CYTO 2012</u>	Browse all public datasets	Quick start guide	Referencing Flow Repository and Cytobank				
» Sunday, June 24: State of the Art Lectures - Computational Analysis of High- Dimensional Data	Browse community datasets	Submit data	FlowRepository Steering Committee & Advisory Board				
Tuesday, June 26: Parallel 8 - Cytometry     Browse most popular datasets     Funding							

# • Type *Identification of B cells* in the Query field

Publishing MiFlowCyt Compliant Data to	
ISAC's FlowRepository.org for Cytometry A	



FlowRepository

https://flowrepository.org/public experiment representations/run/query

### **WRepository**

« Back to Start Page 1 matching experiment found. Search: Browse All Public Experiments » Experiment Primary MIFlowCyt Repository Pubmed . Filos Researcher Project Updated Namo ID(s) Illustrations Score Identification of B A guide to FlowRepository is available at FR-FCM-[20131398] 284 Karin Breuer 12:19 PM the documentation site for Cytobank and cells through negative gating We also have a Ouick start guide. Showing 1 to 1 of 1 entries

You can contact us by filling out a support ticket.



Did you know?

FlowRepository.

#### FlowRepository at CYTO 2012

» Sunday, June 24: State of the Art Lectures - Computational Analysis of High-Dimensional Data

» Tuesday, June 26: Parallel 8 - Cytometry Technology: Cytometry Software and Informatics

» Wednesday, June 27: Workshop 13 -Publishing MIFlowCvt Compliant Data to ISAC's FlowRepository.org for Cytometry A and Other Journals



- FR-FCM-ZZZ3 is the dataset repository identifier
- Each identifier is in the form of FR-FCM-xxxx
- A public view of an experiment can be accessed directly via

https://flowrepository.org/id/FR-FCM-xxxx



A https://flowrepository.org/id/FR-FCM-ZZZ3

### FL WRepository

Experiment: Identification of B cells through n	negative gating	ID: FR-FCM-ZZZ3 F	Primary Researc	her: Karin Breuer	1	MIFlowCyt Score	2: 89.83%	
« Back to All Public Experiments	- Experimer	nt Overview					(	
« Back to Start Page	Repository ID:	FR-FCM-ZZZ3		Experiment name:	Identification of B cells through negative gating	MIFlowCyt score:	89.83%	
Did you know?	Primary researcher:	Karin Breuer		Pl/manager:	Karin Breuer	Uploaded by:	Karin Breuer	
A guide to FlowRepository is available at the <u>documentation site for Cytobank and</u> <u>FlowRepository</u> .	Experiment dates:	2007-05-30 - 2007-08	1-21	Dataset uploaded:	Apr 2011	Last updated:	12:19 PM	
We also have a <u>Quick start guide</u> . You can contact us by filling out a support	Keywords:	[Innate Immune Respo cells] [MIFlowCyt]	onse] [Toll-like red	eptors] [Activation markers] [B	Pubmed IDs:	[20131398]		
ticket.	Organizations:	Child & Family Resea University of Washing	rch Institute, Depa ton Medical Cente	artment of Pediatrics, Vancouve er, Department of Immunology,	r, BC (Canada) Seattle, Washington (USA)			
Supporting journal	Purpose:	The purpose of the ex	periment presente	ed here was to test whether hur	nan B cells can be identified through a	negative-gating str	ategy.	
Cytometry	Conclusion:	B cells can be identifie CD123neg cells. Only all/most B cells respor our negative-gating st	ed through a nega on the extreme e nd but not monocy rategy would pote	tive-gating strategy. Specifical nds of a response spectrum (i.e rte, mDC or pDC) would this B entially be difficult to interpret if i	y, B lymphocytes are the MHC II+, CD1 , either none of the B cells respond to cell negative-gating strategy supply acc both B cell and non-B cell populations r	4neg, CD11cneg, a a given innate stim eptable data. In ott esponded in low fr	and ulation or ner words, equency.	
	Comments:	We set out to compare cell surface marker, C following TLR ligands between 30 May and	a negative-gatin D19. We also war : PAM3CSK4, LP 5 June 2007. The	g strategy to identify B cells with ted to assess the functional re 5, and CpG-A ODN 2336. The t flow cytometry analyses were p	n the usual positive identification of thes sponse of these B cells to Toll-like rece plood draws, PBMC isolation, and TLR performed between 8 June and 21 Aug	e cells using the c ptor stimulation usi stimulations were p ust 2007.	anonical B ng the performed	
FlowRepository at <u>CYTO 2012</u>	Quality control:	To standardize voltage settings across samples acquired on different days, single stained controls were included. Voltages were adjusted such that fluorescence intensity was identical for each antibody, regardless of date of acquisition.						
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» Tuesday, June 26: Parallel 8 - Cytometry Technology: Cytometry Software and Informatics	individuals	2006_01T2pb05i_A1_ 2006_01T2pb05i_A5	A01.fcs · a2006	D1T2pb05i_A2_A02.fcs · a200 D1T2pb05i_A6_A06.fcs · a200	6_01T2pb05i_A3_A03.fcs · a2006_01 6_01T2pb05i_A7_A07.fcs · a2006_01	T2pb05i_A4_A04.f T2pb05i_A8_A08.f	cs ·	
* Wednesday, June 27: Workshop 13 - Publishing MiFlowCyt Compliant Data to ISAC's FlowRepository.org for Cytometry A and Other Journals		12006_01T2pb05i_B1 12006_01T2pb05i_B5 12006_01T2pb05i_C5 12006_01T2pb05i_C5 12006_01T2pb05i_D1 12006_01T2pb05i_D5 12006_01T2pb05i_E1 12006_01T2pb05i_E5 12006_01T2pb05i_E5	B01.fcs a2006 B05.fcs a2006 C01.fcs a2006 C05.fcs a2006 D01.fcs a2006 D01.fcs a2006 E01.fcs a2006 E01.fcs a2006 E01.fcs a2006	D1T2pb05i_B2_B02.tcs · a200 D1T2pb05i_B6_B06.tcs · a200 O1T2pb05i_C2_C02.tcs · a200 O1T2pb05i_C2_C02.tcs · a200 O1T2pb05i_D2_D02.tcs · a200 O1T2pb05i_D6_D06.tcs · a200 O1T2pb05i_E6_E06.tcs · a200 D1T2pb05i_E6_E06.tcs · a200	5 O1T2pb05  B3 B03.ks. a2006 O1 5 O1T2pb05  B7 B07.ks. a2006 O1 6 O1T2pb05  C7 C07.ks. a2006 O1 6 O1T2pb05  C7 C07.ks. a2006 O1 6 O1T2pb05  D3 D03.ts. a2006 O1 6 O1T2pb05  D7 D07.ks. a2006 O1 5 O1T2pb05  E7 E07.ks. a2006 O1 5 O1T2pb05  E7 E07.ks. a2006 O1	T2pb05i_B4_B04.f T2pb05i_B8_B08.f T2pb05i_C4_C04 LT2pb05i_C8_C08 LT2pb05i_D4_D04 LT2pb05i_D4_D04 LT2pb05i_B8_D08 T2pb05i_E4_E04.f T2pb05i_E8_E08.f 2pb05i_E8_E08.f	cs cs fcs fcs fcs fcs fcs fcs fcs	

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Login

Use FlowRespository to access, review

Repository

	t Overview				
Repository ID:	FR-FCM-ZZZ3	Experiment name:	Identification of B cells through negative gating	MIFlowCyt score:	89.83%
Primary researcher:	Karin Breuer	PI/manager:	Karin Breuer	Uploaded by:	Karin Breuer
Experiment dates:	2007-05-30 - 2007-08-21	Dataset uploaded:	Apr 2011	Last updated:	12:19 PM
Keywords:	[Innate Immune Response] [Toll-like receptors] [Activation markers] [B cells] [MIFlowCyt]		Pubmed IDs:	[20131398]	
Organizations:	Child & Family Research Institute, Department of Pediatrics, Vancouver, BC (Canada) University of Washington Medical Center, Department of Immunology, Seattle, Washington (USA)				
Purpose:	The purpose of the experiment presented here was to test whether human B cells can be identified through a negative-gating strategy.				
Conclusion:	B cells can be identified through a negative-gating strategy. Specifically, B lymphocytes are the MHC II+, CD14neg, CD11cneg, and CD123neg cells. Only on the extreme ends of a response spectrum (i.e., either none of the B cells respond to a given innate stimulation or all/most B cells respond but not monocyte, mDC or pDC) would this B cell negative-gating strategy supply acceptable data. In other words, our negative-gating strategy would potentially be difficult to interpret if both B cell and non-B cell populations responded in low frequency.				
Comments:	We set out to compare a negative-gating strategy to identify B cells with the usual positive identification of these cells using the canonical B cell marker, CD19. We also wanted to assess the functional response of these B cells to Toll-like receptor stimulation using the following TLR ligands PAM3CSK4, LPS, and CpG-A ODN 2336. The blood draws, PBMC isolation, and TLR stimulations were performed between 30 May and 5 June at The flow cytometry analyses were performed between 8 June and 21 August 2007.				cell surface jands: June 2007.
Quality control:	To standardize voltage settings across sam fluorescence intensity was identical for eac	ples acquired on different days, sin h antibody, regardless of date of ac	gle stained controls were included. Voltages quisition.	s were adjusted su	ch that

#### **Experiment variables**

#### Individuals

a2006\_O1T2pb05i\_A1\_A01.fcs · a2006\_O1T2pb05i\_A2\_A02.fcs · a2006\_O1T2pb05i\_A3\_A03.fcs · a2006\_O1T2pb05i\_A4\_A04.fcs · a2006\_O1T2pb05i\_A5\_A05.fcs · a2006\_O1T2pb05i\_A5\_A05.fcs · a2006\_O1T2pb05i\_A5\_A05.fcs · a2006\_O1T2pb05i\_B4\_B04.fcs · a2006\_O1T2pb05i\_

 Experiment description is organized into 4 boxes corresponding to the 4 sections of MIFlowCyt

#### Experiment Overview

Bonositony ID	ED ECM 7772	MiFlowCyt - Publicity available experiment - Overview
Repository ID	FR-FCW-ZZZ3	The Experiment Overview displays basis information shout the experiment including details
Primary researcher:	Karin Breuer	required by MIFlowCyt, section 1. Specifically, these details are displayed:
Experiment dates:	2007-05-30 - 2007-08-21	Repository ID - Each experiment (dataset) is assigned a unique repository identifier. The
Keywords:	[Innate Immune Response] [Toll-like rec [MIFlowCyt]	Identifier is typically the form of FR+FCM-xxxx where xxxx is a sequence of four alphanumeric characters (case sensitive). Knowing the repository identifier, a public view of a public experiment can be accessed directly by a LIRL in the form of
Organizations	Child & Family Research Institute, Depa University of Washington Medical Cente	https://flowrepository.org/id/ER-FCM-xxxx, e.g., https://flowrepository.org/id/ER-FCM-ZZZ3.
Purpose:	The purpose of the experiment presente	Experiment name - The name of the experiment as provided by the data uploader.
Conclusion:	B cells can be identified through a negation of a response	MIFlowCyt score - A value between 0% and 100% that reflects the compliance of provided annotation with MIFlowCyt.
	potentially be difficult to interpret if both	Primary researcher - The person doing the experiment.
Comments:	We set out to compare a negative-gating marker, CD19. We also wanted to asses	Pl/manager - The person responsible for the project.
	PAM3CSK4, LPS, and CpG-A ODN 233 The flow cytometry analyses were perfor	Uploaded by - The person who uploaded the dataset.
Quality control:	To standardize voltage settings across s fluorescence intensity was identical for e	Experiment dates - When was the experiment done, including preparation and data acquisition and analysis.
		Dataset uploaded - When was the dataset originally uploaded.
Experiment va	ariables	l ast undated - When was the annotation last undated
Individuals		and aparted the internation and updated.
	a2006_O1T2pb05i_A1_A01.fcs · a2006_O	Keywords - Key terms characterizing the experiment.
	a2006_O1T2pb05i_A5_A05.fcs · a2006_O a2006_O1T2pb05i_B1_B01.fcs · a2006_O	Pubmed IDs - Pubmed identifiers of any publications associated with this dataset.
	a2006_O1T2pb05i_B5_B05.fcs · a2006_O	Organizations - Organizations involved in this experiment

- Mousing over an "i" will display a related help topic
- The "x" closes the pop-up window
- Arrows on the left in title bars collapse and restore panels

Data review and download Data upload and annotation Data sharing

# Reviewing flow sample details

### • Scroll down to see additional information

Choose an FCS file to display details for: - all 284 FCS files -						
a2006_O1T2pb05	i_A1_A01.fcs :					
Description	Blood was drawn via sterile venipuncture into vacutainers containing 143 USP units of sodium-heparin (Becton Dickinson (BD): catalog no. 8019839). Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation as described in Jansen et al., J Immunol Methods 2008; 336(2): 183-192.					
Sample source:	[biological ] Human peripheral blood $\cdot$ Homo sapiens $\cdot$ 22 $\cdot$ years $\cdot$ unknown $\cdot$ healthy $\cdot$ None					
Sample characteristic:	Expected/analyzed types of cells: monocytes, myeloid dendritic cells, plasmacytoid dendritic cells, B lymphocytes. T lymphocytes will also be present but not analyzed. Red blood cells are not present as they are lysed when samples are frozen in FACSLyse solution.					
Sample treatment:	see attached file 'plate map'					
Staining:	Cell surface protein, CD11c, APC (BD Biosciences BD#340714) Intracellular Protein, TNFa, Alexa 700 (BD Biosciences BD#557996) Cell Surface Protein, CD14, PE-Cy7 (eBioscience selio#250149) Cell Surface Protein, MHCI, PerCPCy55 (BD Biosciences BD#custom) Intracellular Protein, IFNa, FITC/OG (Antigenix Antigenix#MC100133) Intracellular Protein, IL6, APC-Cy7 (BD Biosciences BD#custom) Cell Surface Protein, CD123, AmCyan (BD Biosciences BD#custom) Intracellular Protein, IL12p40/70, Pacific Blue (eBioscience eBio#577129)					

Data review and download Data upload and annotation Data sharing

### Instrumentation details and data analysis

#### BD FACSAria II, Becton Dickinson (BD Biosciences) used for: A02 details · 2nd Settings details · A04 details · A06 details · A07 details · A08 details · A09 details · B01 details · B02 details · B03 details · B04 details · B06 details · B07 details · B08 details · B09 details · B10 details · B11 details · C01 details · C02 details · C03 details · C04 details · C06 details · C06 details · C08 details · C09 details · C01 details · C02 details · C03 details D04 details · D06 details · D07 details · D08 d ils · E04 details · E06 details · E07 details · E08 details · 6 details -F07 details · F08 details · F09 details · F10 det s · G07 Instrument model: BD FACSAria II details · G08 details · G09 details · H01 details 108 Manufacturer: Becton Dickinson (BD Biosciences) details · H09 details · H10 details · A01 details Flow Cell Type: Ouartz cuvette Other flow fluidics details: 160-um x 250-um, 15-mm long Optical Paths: [FSC-H - FSC-Height] [SSC-H - SSC-Height ] [ FL1-H - CD45RA FITC ] [ FL2-H - CD45RO PE Choose which illustration to display: Panel ] [ FL2-A - FL2-A ] [ FL3-H - CD3 PerCP ] [ FL4-H - CD8 APC 1 Installation dates of filters in Optical Paths: When the instrument was bought in May 2007.

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# Download

- Panels on the left allow you to download
  - Data
  - Attachments
  - Saved illustrations
  - Gates (in Gating-ML)
  - MIFlowCyt reports

- Download 🔋 👔
FCS files Download FCS Files
Public illustrations
Case 1 APC vs. PerCP 🗾 <u>PDF</u>
Case 1 PE vs. PerCP 🔼 <u>PDF</u>
Case 1 FITC vs. PerCP 🔽 <u>PDF</u>
Attachments <u>CSI-</u> Portland_Tembhare_Case_1_Analysis.pdf Tembhare_Case_1_History.doc
Gating Export Gating-ML
➡ MIFlowCyt Report

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### Download FCS files - make sure Java is allowed to run

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Experiment: Identification of B cells through negative gating ID: FR-FCM-2223 Primary Researcher: Karin Breuer	IFlowCyt Score: 89.83%	
Back to Experiment Public View     Instructions		
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### Download FCS files - select download destination

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<b>FL</b> WRepository							Loj	gin
Experiment: Identification of B cells through ne	egative gating ID: FR-I	CM-ZZZ3 Primary Re	searcher: K	arin Breuer		MIFlowCyt S	core: 89.83	396
« Back to Experiment Public View	Instructions							
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		Browse For Folder		Download Sele	cted Files			
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1(1)	P FCS co	ellection for software testing	FR-FCM-ZZZ4	34	Ryan Brinkman	Community datasets	_	
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Sample Type	P study		FR-FCM-ZZZA	6	Francesca D'Alessio	full term cord blood		
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We also have a Quick start guide.								
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Experiment: Identification of B cells through	negative gating ID: <u>FR-FCM-</u>	2223 Labels: None Primar	y Researcher: <u>Karin B</u>	reuer Public:	Yes	MIFlowCyt Score: 89.83%	
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S Josef Spidlen [x]	Labels:	[Activation markers] [B cells] [MIFIC None	owCyt]		Organizations:	Child & Family Research Institute] [University of	
Share with a User (Full Access)	Pubmed IDs:	[20131398]			Start data	Washington Medical Center]	
					End date:	2007-08-21 Apr 2011	
→ Did you know?	Comments:	We set out to compare a negative-	-gating strategy to		Purpose:	The purpose of the experiment presented here was to	
You can request a one-on-one session to get started with your data by filling out a support licket		identify B cells with the usual posit these cells using the canonical B of	tive identification of cell surface marker,			test whether human B cells can be identified through a negative-gating strategy.	
A guide to Cytobank is available at <u>Current</u> Protocols in Cytometry		response of these B cells to Toll-lii stimulation using the following TLI	ke receptor R ligands:		Conclusion:	B cells can be identified through a negative-gating strategy. Specifically, B lymphocytes are the MHC II+,	
We also have a Quick start guide.		PAM3CSK4, LPS, and CpG-A OD draws_PBMC isolation_and TLR s	N 2336. The blood dimulations were			CD14neg, CD11cneg, and CD123neg cells. Only on the extreme ends of a response spectrum (i.e., either	
You can print/save your illustrations to PDF from the illustration view's left menu.		performed between 30 May and 5	June 2007. The			none of the B cells respond to a given innate stimulation or all/most B cells respond but not	
You can export your data to Excel from the Experiment Summary page.		June and 21 August 2007.				monocyte, mDC or pDC) would this B cell negative- gating strategy supply acceptable data. In other	
Give other users full control to modify your experiments through the "Sharing Permissions" box.						words, our negative-gating strategy would potentially be difficult to interpret if both B cell and non-B cell populations responded in low frequency.	
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File Name	Sample	Tube Name	Experiment Variables	Panel	Events	Size
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# Review MIFlowCyt compliance details

#### Approximated by MIFlowCyt score

• Automatically calculated value between 0% and 100%

Section	MIFlowCyt score contribution
Experiment Overview	30%
Flow Sample/Specimen Details	30%
Instrument Details	20%
Data Analysis Details	20%

• Click on the MIFlowCyt Score bar to review the details



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Experiment: Identification of B cells through i	negative gat	ing ID: <u>FR-FO</u>	CM-ZZZ3 Labels: No	one Primary Research	er: <u>Karin Breuer</u>	Public: Yes	MIFlowCyt Scot	re: 89.83%	
« Back to Experiment Summary	MIFlow	/Cyt Com	pliance Score	for Experiment	: Identifica	ation of B cells	s through negative g	gating -	
→ MIFlowCyt	Repos	itory ID: F	R-FCM-ZZZ3						
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Print View DPDE	▼ 1 · Ex	Ammillowry:t       Image: Construction Data       Invite a User       Support       Public View       Welcome, @ Josef       Logout         pring       ID: EE:ECM2ZZ2       Labels: None       Primary Researcher: Kain Breuxer       Public: Ves       MirFordCyt Score: 89.83%         wwCyt Compliance Score for Experiment: Identification of B cells through negative gating - sitory ID: FR-FCM-ZZZ3       owc/y compliance Score: 99.83%         Experiment Overview - 92.50% provided       O         mediered relatively based on importance, 30% contribution to total score.       O         intervariables       O         e       O         ords       O         ization       O         usions       O         y control measure       O							
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a2006_O1T2pb05i_A4_A04.fcs	0
a2006_O1T2pb05i_A5_A05.fcs	0
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# Upload and annotation of your own dataset

#### Typical steps

- Create a new experiment
- Opload data (FCS files)
- O Prepare annotation templates
  - Or prepare spreadsheets with annotations
- Annotate the experiment
  - Describe samples and sample sources
  - Provide experimental variables
  - Describe instrumentation settings
- Provide analysis details
  - Either analyze data online and create illustrations
  - Or upload third party analysis files (e.g., Flow Jo<sup>TM</sup> workspaces, FCS Express<sup>TM</sup> project files, FACS Diva<sup>TM</sup> files, etc.)
- Review (and improve) your MIFlowCyt compliance

# Upload and annotation of your own dataset

#### Typical steps

- O Create a new experiment
- Opload data (FCS files)
- O Prepare annotation templates
  - Or prepare spreadsheets with annotations
- Annotate the experiment
  - Describe samples and sample sources
  - Provide experimental variables
  - Describe instrumentation settings
- Provide analysis details
  - Either analyze data online and create illustrations
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- Review (and improve) your MIFlowCyt compliance

Data review and download Data upload and annotation Data sharing

#### Create a new experiment

- Bank New Experime	ent
* Experiment Name	IDCRP's HIV Natural History Study
Project	None •
* Primary Researcher	Nima Aghaeepour
* PI/Manager	Mario Roederer Invite a new user
	Allow PI/Manager to have full access to experiment <i>⊌</i>
* Starting Date	2007-07-01 (Vyyy-mm-dd)
End Date	2007-10-31 📑 (yyyy-mm-dd)
(optional)	
* Purpose	Identification of immunophenotypes correlated with AIDS free survival of HIV infected patients based on polychromatic flow cytometry analysis of 466 subjects enrolled in Infectious Disease Clinical Research Program's Natural History Study.

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Data review and download Data upload and annotation Data sharing

## Create a new experiment (continued)

Conclusion (optional)	Several immunophenotypes correlated with the survival times were identified. Details about this would typically be listed here but I am not at liberty to share this information during this talk.
Comments (optional)	For reagent and instrument details as well as the original manual gating strategy please see: Ganesan and Chattopadhyay et al., Immunologic and virologic events in early HIV infection predict subsequent rate of progression. Journal of Infectious Diseases, 2010:201:272–284.
Quality Control Measures (optional)	Per-channel empirical distribution comparison
Quality Control Experiment (optional)	None •

Data review and download Data upload and annotation Data sharing

# Create a new experiment (continued)

Keywords (optional)	HIV, AIDS Free Survival, Bioinformatics
Organizations	None
(optional)	BC Cancer Agency, Terry Fox Laboratory University of British Columbia, Faculty of Medica
	Add new organization
Pubmed IDs (optional)	20001854, 18667932
	* required field
	Create Experiment

Data review and download Data upload and annotation Data sharing

# Adding new organization

New organization details		
Name *	University of Toronto	
Department	Department of Cell & Systems Biology	
Zip (postal code)	M5S 3G5	
Address line #1		
Address line #2		
Address line #3		
City *		
State (province)		
Country*		
	Powered by Geonames	
	Cancel	Save

- Auto-complete based on ZIP supported for 60 countries
- Country is assumed based on your location unless specified in the Country field

Data review and download Data upload and annotation Data sharing

# Adding new organization

New organization details		
Name *	University of Toronto	
Department	Department of Cell & Systems Biology	
Zip (postal code)	M5S 3G5	
Address line #1		
Address line #2		
Address line #3		
City *	Toronto	
State (province)	Ontario	
Country*	Canada	
	Powered by Geonames	
	Cancel	Save

- Auto-complete based on ZIP supported for 60 countries
- Country is assumed based on your location unless specified in the Country field

# Upload and annotation of your own dataset

#### Typical steps

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- Review (and improve) your MIFlowCyt compliance

# Data upload

Experiment: IDCRP's HIV Natural History Study	ID: FR-FCM-ZZZB Labels: None Primary Researcher: <u>Nima Aghaeepour</u> Public: No MIFlowCyt Score: 0.00%
« Back to Experiment	This experiment does not have any FCS files uploaded yet.
- Actions	
Delete Experiment	• Getting Started!
→ Did you know?	Upload Experiment Files
You can request a one-on-one session to get started with your data by filling out a support ticket.	Browse For Folder Upload Selected Files
A guide to Cytobank is available at <u>Current</u> <u>Protocols in Cytometry</u>	Select All Deselect All Select Flow Files Deselect Flow Files
We also have a <u>Quick start quide</u> . You can print/save your Illustrations to PDF from the Illustration view's left menu.	De-identify all FCS files before uploading
You can export your data to Excel from the Experiment Summary page.	No folder selected. Click on "Browse for Folder" to select a folder. Filename FCS Version Upload?
Give other users full control to modify your experiments through the "Sharing Permissions" box.	
Use the "Download Files" button to save copies of the original FCS Files to your computer.	
)	
	Upload Progress: 0%
	Terms of Service Privacy Policy Support Feedback

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# Data upload

« Back to Experiment	This experiment does not have any ECS f	iles unloaded vet.			
+ Actions	,·,·				
Delete Experiment	Getting Started!				
- Did you know?	Upload Experiment Files				
ou can request a one-on-one session to					
support ticket.	Browse For Fo	Ider Upload Selee	ted Files		
A guide to Cytobank is available at Current Protocols in Cytometry	Select All Deselect All	Select Flow Files	Deselect Flo	w Files	
We also have a Quick start guide.					
You can print/save your Illustrations to PDF rom the Illustration view's left menu.	De-identif	y all FCS files before upl	oading		
You can export your data to Excel from the	Filename	FC	S Version U	pload?	
Sive other users full control to modify your	203037.fcs - 203037.fcs	FCS	2.0	× •	
xperiments through the "Sharing	797946.fcs - 797946.fcs	FCS	2.0	2	
ermissions" box.	922911.fcs - 922911.fcs	FCS	2.0	2	
Ise the "Download Files" button to save	802565.fcs - 802565.fcs	FCS	2.0	2	
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omputer.	351452.fcs - 351452.fcs	FCS	2.0	×	
	334791.fcs - 334791.fcs	FCS	2.0		
	294897.fcs - 294897.fcs	FCS	2.0	V	
	319267.fcs - 319267.fcs	FCS	2.0	2	
	251284.fcs - 251284.fcs	FCS	2.0	×	
	997430.fcs - 997430.fcs	FCS	2.0		
	122405.fcs - 122405.fcs	FCS	2.0	✓	
	846228.fcs - 846228.fcs	FCS	2.0	<b>V</b>	
	130119.fcs - 130119.fcs	FCS	2.0	2	
	306870.fcs - 306870.fcs	FCS	2.0	<b>V</b>	
	978630.fcs - 978630.fcs	FCS	2.0	✓	
	Upload Pre	ogress: 0%			
	opiouuri				

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Data review and download Data upload and annotation Data sharing

# Data upload - de-identification

☑ De-identify all FCS files before uploading

#### **De-identification**

- Remove identifiers that could be used to identify an individual
- Generally, privacy rules do not apply on de-identified data
  - $\rightarrow~$  Allows for sharing
    - Check with your regulatory authority as applicable, e.g., Health Insurance Portability and Accountability Act (HIPAA)

#### Implementation in FlowRepository

- Automated removal of all keyword values unless in our safe list
  - Safe list: Over 220 keywords identified from a few hundred FCS data files produced by dozens of instruments from several vendors
- Integrated in the upload process
  - $\bullet~$  Performed locally  $\rightarrow~$  no sensitive information leaves your computer

Data review and download Data upload and annotation Data sharing

# Data upload (large dataset)

# Batched Upload Due to the size of your upload, your files will be uploaded in 6 batches. The progress bar will reflect the progress of each batch. Please do not be alarmed if the progress bar restarts. At any point during the upload, please do not click away from this page. You will automatically be redirected to the next page when the upload is complete.

- In our example, we are uploading 11 GB of data
- This message is only shown for uploads larger than 2 GB

# Data upload

« Back to Experiment	This experiment does not have any ECS files upleaded upt			
- Actions	This experiment does not have any PCS lies uploaded yet.			
Delete Experiment	→ Getting Started!			
+ Did you know?	Upload Experiment Files			
You can request a one-on-one session to get started with your data by filing out a <u>support ticket</u> .	Browse For Folder	oad Selected Files	]	
A guide to Cytobank is available at <u>Current</u> Protocols in Cytometry	Select All Deselect All Select Flow	v Files Deselec	t Fl <u>o</u> w Files	
We also have a Quick start guide.				
You can print/save your Illustrations to PDF from the Illustration view's left menu.	De-identify all FCS files b	efore uploading		
You can export your data to Excel from the	Uploading batch 1 of 6	ECC Version	Linia ad 2	-
Experiment Summary page.	202027 fee 202027 fee	ECC2 0	opioadr	
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	997430.fcs - 997430.fcs	FCS2.0	V	
	122405.fcs - 122405.fcs	FCS2.0	V	
	846228.fcs - 846228.fcs	FCS2.0	V	
	130119.fcs - 130119.fcs	FCS2.0	2	
	306870.fcs - 306870.fcs	FCS2.0	2	
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	Unload Brogross	20%		_

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# Data upload

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2-bite Exercised       1 Cetting Started!         2-bite Exercised       1 Cetting Started!         2-bite Exercised       2-bite Exercised         2-bite Exercised Exercised Exercised       2-bite Exercised         2-bite Exercised Exercis	+ Actions				
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3192677.fs         FCS.20         W           2512847.6s         FCS.20         W           997430.fs         97430.fs         FCS.20         W           122405.fs         FCS.20         W         W           122405.fs         FCS.20         W         W           946228.fs         FCS.20         W         W           13011.916*         FCS.20         W         W           306870.fs         13011.916*         FCS.20         W           306870.fs         306870.fs         FCS.20         W		294897.fcs - 294897.fcs	FCS2.0	×	
251284.fcs         FC52.0         W           997430.fcs         FC52.0         W           122405.fcs         122405.fcs         FC52.0         W           84622.81cs         4622.81cs         FC52.0         W           13011.91cs         FC52.0         W           306870.fcs         150.50         W           306870.fcs         97680.01cs         FC52.0         W		319267.fcs - 319267.fcs	FCS2.0	2	
1997430.fcs         FCS2.0         P           122405.fcs         FCS2.0         P           132405.fcs         FCS2.0         P           13012.9fcs         FCS2.0         P           13012.9fcs         FCS2.0         P           306870.fcs         306870.fcs         FCS2.0         P           306870.fcs         306870.fcs         FCS2.0         P		251284.fcs - 251284.fcs	FCS2.0	<b>V</b>	
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Upload Progress: 100%		Upload Prog	ress: 100%	1	

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Data review and download Data upload and annotation Data sharing

# Data upload

					Welcome, 🕱 Josef	Logout
+ Did you know?						
You can request a one-on-one session to get started with your data by filling out a support ticket.	466 Files successfu	ly uploaded to experin	nent: IDCRP's Hi	V Natural History Study!		
A guide to Cytobank is available at Current	Processing Uploaded	Files				
We also have a <u>Quick start guide</u> .						
You can print/save your Illustrations to PDF from the Illustration view's left menu.	Current Task: Categorizing	FCS Files				
You can export your data to Excel from the Experiment Summary page.						
Give other users full control to modify your experiments through the "Sharing Permissions" box.						
Use the "Download Files" button to save copies of the original FCS Files to your computer.						
	Terms of Service	Privacy Policy	Support	Feedback		

• Data files will be automatically categorized and assigned to staining panels after the upload

# Data upload



FlowRepository - IDCRP': ×

🖕 🎃 😨 🔇 https://flowrepository.org/experiments/20



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# Upload and annotation of your own dataset

#### Typical steps

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- Review (and improve) your MIFlowCyt compliance

Data review and download Data upload and annotation Data sharing

### Prepare annotation data



- Follow the Annotation Data link
  - Set of *concepts* applicable to samples even from different datasets

Data upload and annotation

#### Prepare annotation data – reagents

- Act Add ne Add ne Add ne Add ne Add ne Add or Add ne Add ne 🗕 Did You ca get sta suppor A guid Protoc We als You ca

- Actions i	▶ Getting Starte	ed with MIFlow	Cyt Annotatior	is!				×
Add new keyword Add new organization Add new rendent Add new rendent Add new rendent Add organism Add organism Add new temptate for samples Add new temptate for sample sources	Keywords Orga New reagent Search: CD14	anizations Manu	facturers Reag	ents Instru	ments Organi	sms Templates		
→ Did you know?	Analyte 🔺	Analyte detector	Analyte reporter	Clone 🔶	Catalog 🝦	Manufacturer 🔶	\$	\$
You can request a one-on-one session to get started with your data by filling out a support ticket.	CD14	anti-CD14	Alexa 700	M5E2	BD#557923	BD Biosciences [website]	Edit	Remove
A guide to Cytobank is available at <u>Current</u> Protocols in Cytometry	CD14	anti-CD14	PE-Cy7	M5E2	eBio#25- 0149	eBioscience [website]	Edit	Remove
We also have a <u>Quick start guide</u> . You can print/save your Illustrations to PDF	CD14	-	FITC	RMO52	IM0645	Beckman Coulter [website]	<u>Edit</u>	Remove
You can export your data to Excel from the Experiment Summary page.	CD14	-	PE-Cy7	M5E2	557742 1:50	BD Biosciences [website]	<u>Edit</u>	Remove
Give other users full control to modify your experiments through the "Sharing	CD3/CD14	Anti- CD3/CD14	PECy7		-	Unknown	Edit	Remove
Permissions" box. Use the "Download Files" button to save copies of the original FCS Files to your computer.	VIVID/CD14 Showing 1 to 6 of 6	N/A 5 entries (filtered fro	V450 m 110 total entries)		N/A	Custom	<u>Edit</u>	Remove

#### Define reagents used in the dataset •

Data review and download Data upload and annotation Data sharing

#### Prepare annotation data – add reagents

▼ New Reagent	
Analyte *	CD4
Analyte detector *	Anti-CD4
Analyte reporter *	PE
Clone	13B8.2
Catalog nr *	IM0449
Manufacturer *	Beckman Coulter • New
	Create

• Provide details as required by MIFlowCyt

### Prepare annotation data – instruments

• Your instrument is most likely in the system already

Keywords Organ	izations Manufacturers Reagents	Instruments Orga	nisms	Templates
New instrument Search:				
Model 🔺	Manufacturer 🔶	Other 🍦	\$	\$
A10-Bryte	Apogee Flow Systems http://www.apogeeflow.com		Edit	Remove
A40-Military	Apogee Flow Systems http://www.apogeeflow.com		<u>Edit</u>	Remove
A50-Micro	Apogee Flow Systems http://www.apogeeflow.com		<u>Edit</u>	<u>Remove</u>
A50- Universal	Apogee Flow Systems http://www.apogeeflow.com		<u>Edit</u>	Remove
Accuri C6	Becton Dickinson (BD Biosciences) http://www.bdbiosciences.com		<u>Edit</u>	Remove
Attune	Applied Biosystems http://www.appliedbiosystems.com		<u>Edit</u>	Remove
Auto-A40	Apogee Flow Systems http://www.apogeeflow.com		<u>Edit</u>	Remove

Josef Špidlen, Ryan R. Brinkman

FlowRepository.org - Resource of Flow Cytometry Data

### Prepare annotation data – organisms

- The NCBI Taxonomy contains hundreds of thousands of organisms
- FlowRepository contains 20,000 of these
  - Selected based on either having a common English name or appearance in GeneBank
- But this is still a long list (for drop down selections)
  - ightarrow We only show what has been used or explicitly requested

Keywords Organizations Manufac	cturers Reagents Instrume	nts Organisms Templates		
Add organism				
Search:				
NCBI Taxonomy ID 💧 💧	Scientific name 🛛 🌲	Genbank common name 🛛 🍦		
9606 Homo sapiens		human		
10090	Mus musculus	house mouse		
10116 Rattus norvegicus Norway rat				

Data review and download Data upload and annotation Data sharing

# Prepare annotation data – add organism

- Follow the Add organism link
- Start typing either the Latin or the English name
- Auto-complete will show suggestions after the first 3 characters

#### Add organism from NCBI Taxonomy



Data review and download Data upload and annotation Data sharing

### Prepare annotation data – templates

#### • Start with sample sources

Keywords Organizati	ons Manufacturers	Reagents	Instruments	Or	ganisms	Femplates	
New annotation template New annotation template Search:	for samples for sample sources						
Annotation 🔺 Type	Template N	lame 🔶	User	*	Public	\$	\$
Sample	Sample template Kollmann #1		Karin Breuer		yes	Show	<u>Remove</u>
Sample	JS Sample 1		Josef Spidlen		no	Show	Remove
Sample source	sample source template Kollmann #1		Karin Breuer		yes	Show	Remove
Sample source	12w MOLD/RkJ M r	nouse	Josef Spidlen		no	Show	<u>Remove</u>

Josef Špidlen, Ryan R. Brinkman FlowRepository.org - Resource of Flow Cytometry Data

## Prepare annotation data – create sample source templates

- Different items required based on the sample source type
- Form changes accordingly
- Use ? for variable fields

Details for	sample source template		×
Sa	mple source type *	environmental •	
	Description *		
	Location *		
	Other		
		Cancel Sav	ve

## Prepare annotation data – create sample source templates

- Different items required based on the sample source type
- Form changes accordingly
- Use ? for variable fields

Details for	sample source template		
Sa	mple source type *	other •	
	Description *		
	Other		
		A	
		Cancel	Save

## Prepare annotation data – create sample source templates

- Different items required based on the sample source type
- Form changes accordingly
- Use ? for variable fields

Details for sample source template	
Sample source type * Description * HIV+ subject	biological •
Organism * Age * Age unit * Gender *	Homo sapiens (9606) [human] • 2 years
Phenotype * Genotype *	N/A N/A
Treatment *	
	Cancel Save

Data review and download Data upload and annotation Data sharing

## Prepare annotation data – create sample source templates

• Finally, name and save your Sample source template

<ul> <li>New Annotation Ter</li> </ul>	nplate
Template type	Sample Source
Template	HIV+ subject (ID 220)
Template name	HIV+ subject template
Public	no •
	Create

## Prepare annotation data – create sample templates

Details for sample template	
Description * PBMC from HIV+ Sub	ect
Sample characteristic	HIV+ • New
Sample treatment	New
Staining	None B cells, MHCII, PerCPCy5.5 (BD Biosciences BD#custom) T cells, CD40, FITC/OG (eBioscience eBio#11-0409) CD14 positive cells, CD14, Alexa 700 (BD Biosciences BD#557923) CD86, CD86, PE (eBioscience eBio#12-0869)
Staining cocktail(s)	None Cocktail no. 1
	Cancel

Data review and download Data upload and annotation Data sharing

### Prepare annotation data – create sample templates

#### • Describe sample treatment



#### Prepare annotation data – create sample templates

- Define reagents including what characteristics they are measuring, especially if there may be ambiguity, e.g.,
  - Propidium lodide (PI) with permeabilized cells  $\rightarrow$  DNA content (cell cycle)
  - $\bullet\,$  PI with non-permeabilized cells  $\rightarrow$  cell viability

New reage	nt details		*
Charac	teristic measured *	Monocytes idenitfication	
	Other		
	Used reagent *	CD14, PE-Cy7 (eBioscience eBio#25-0149)	۲
			Cancel Save
Data review and download Data upload and annotation Data sharing

#### Prepare annotation data – create sample templates

Double-click on a reagent to see details



Data review and download Data upload and annotation Data sharing

#### Prepare annotation data – create sample templates

- Select reagents (and/or cocktails) used
- Hold Ctrl (or Shift) to select multiple items

Details for sample template	
Description * PBMC from HIV+ Sub	ject
Sample characteristic	HIV+ New
Sample treatment	PBMCs were thawed in warm • New
Staining	B cells, MHCII, PerCPCy5.5 (BD Biosciences BD#custom) T cells, CD40, FITC/OG (eBioscience eBio#11-0409) CD14 positive cells, CD14, Alexa 700 (BD Biosciences BD#557923) CD86, CD86, PE (eBioscience eBio#12-0869) Monocytes idenitification, CD14, PE-Cy7 (eBioscience eBio#25-0149) New
Staining cocktail(s)	None Cocktail no. 1
	Cancel Save

#### Prepare annotation data – create sample templates

• Finally, name and save your Sample template

<ul> <li>New Annotation Template</li> </ul>				
Sample				
PBMC from HIV+ Subject (ID 3581)				
PBMC from HIV+ Subject Template				
no •				
Create				

# Upload and annotation of your own dataset

#### Typical steps

- Create a new experiment
- Opload data (FCS files)
- Prepare annotation templates
  - Or prepare spreadsheets with annotations
- Annotate the experiment
  - Describe samples and sample sources
  - Provide experimental variables
  - Describe instrumentation settings
- Provide analysis details
  - Either analyze data online and create illustrations
  - Or upload third party analysis files (e.g., Flow Jo<sup>TM</sup> workspaces, FCS Express<sup>TM</sup> project files, FACS Diva<sup>TM</sup> files, etc.)
- Review (and improve) your MIFlowCyt compliance

### Prepare spreadsheets with annotations

- Use your favorite spreadsheet editor
  - MS Excel, GoogleDoc Spreadsheet, OpenOffice Calc, etc.
- Name one column as **FCS File**; values should correspond to file names in your dataset
- Other "understandable" columns:
  - Samples: Sample Description, Sample Characteristic, Sample Treatment, Sample Source Description, Sample Source Treatment, Age, Age unit, Gender, Phenotype, Genotype, Location, Other Sample Source Information
  - Experiment Variables: Condition, Dose, Timepoint, Individual, Experimental variable sample type
  - Instrumentation Details: Instrument, Default Instrument Settings, Optical Filters Installation Dates, Other Flow Fluidics Information, Other Instrument Settings Information, Flow Cell Type

Data review and download Data upload and annotation Data sharing

# Use your favorite spreadsheet editor

X	HIV Annotation:	;	×						
4 . ·	C 🔒 https://docs.ç	pogle.co	m/spreadshee	t/ccc?key=0AucGnK-E	YsuQdEtWeDZJYzZveHlMaE51cH	R2eXdrUGc#gid=0			\$ <b>%</b>
								Josef Spidlen	-
	HIV Annotations 🔅 🖿 🔒 Share								
	File Edit View Insert Format Data Tools Help All changes saved								
		<b>-</b>	s s	% 123 -	10pt   B Abc A	• 🖽 • 🖽 • 🔳	· 22 - 🚍 > - 🛄	$\overline{\mathbf{Y}}$	
f×	158483.fcs							Show all formulas	$\times$
	А	в	С	D	E	F	G	н	
1	FCS File	Age	Gender	Condition					
2	100715.fcs	51	F	HIV Stage 1					
3	105696.fcs	25	F	HIV Stage 4					
4	108701.fcs	21	М	HIV Stage 3					
5	109025.fcs	20	М	HIV Stage 4					
6	109567.fcs	36	F	HIV Stage 2					
7	110539.fcs	43	М	HIV Stage 1					
8	113548.fcs	38	F	HIV Stage 2					
9	121069.fcs	33	М	HIV Stage 3					
10	122405.fcs	43	М	HIV Stage 2					
11	127225.fcs	21	F	HIV Stage 1					
12	129599.fcs	40	М	HIV Stage 1					
13	129730.fcs	20	F	HIV Stage 2					
14	129869.fcs	21	М	HIV Stage 3					-
15	130119.fcs	44	М	HIV Stage 1					¥
	+ = 5	Sheet	1 -				1	L58483.fcs	

Data review and download Data upload and annotation Data sharing

# Use your favorite spreadsheet editor

<mark>eile</mark>	©⊙⊙ © annotations.csv - LibreOffice Calc File Edit Ulew (nsert Format Tools Data Window Help & ★ ×								
	- E - E - A - E - E - E - E - E - E - E								
	🚯 Liberation Sans 📷 10 📷 🙈 🕖 🛓 🗊 🗮 📰 🗰 🖶 🤳 🦇 🛄 🛱 🐖 🐖 🐖 👘 💮 🗄 - 🗮								
P1	$\mathbb{P}_1 \qquad \mathbb{P}  \mathbb{P}_2 = \mathbb{P}_2$								
1	FCS File	Ade	Gender	Condition	E	F	G	Ha.	
2	100715.fcs	51	F	HIV Stage 1					
3	105696.fcs	25	F	HIV Stage 4					
4	108701.fcs	21	М	HIV Stage 3					
5	109025.fcs	20	М	HIV Stage 4					
6	109567.fcs	36	F	HIV Stage 2					
7	110539.fcs	43	М	HIV Stage 1					
8	113548.fcs	38	F	HIV Stage 2					
9	121069.fcs	33	М	HIV Stage 3					
10	122405.fcs	43	М	HIV Stage 2					
11	127225.fcs	21	F	HIV Stage 1					
12	129599.fcs	40	М	HIV Stage 1					
13	129730.fcs	20	F	HIV Stage 2					
14	129869.fcs	21	М	HIV Stage 3					
15	130119.fcs	44	М	HIV Stage 1					
Shee	t1/1		Default		STD [3]	Sum=0	Θ	· · · · · · · · · · · · · · · · · · ·	

Data review and download Data upload and annotation Data sharing

# Save as CSV (Comma Separated Values)

• Look in the File menu for Save As, Download, or Export



# Upload and annotation of your own dataset

#### Typical steps

- Create a new experiment
- Opload data (FCS files)
- O Prepare annotation templates
  - Or prepare spreadsheets with annotations
- Annotate the experiment
  - Describe samples and sample sources
  - Provide experimental variables
  - Describe instrumentation settings
- Provide analysis details
  - Either analyze data online and create illustrations
  - Or upload third party analysis files (e.g., Flow Jo<sup>TM</sup> workspaces, FCS Express<sup>TM</sup> project files, FACS Diva<sup>TM</sup> files, etc.)
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- Review (and improve) your MIFlowCyt compliance



C https://flowrepository.org/experiments/11 ٩. × - Getting Started: Experiment Details « Back to Inbox This Experiment Details Page contains information about the experiment listed above. My Working Illustration » Start describing samples MIFlowCyt Annotation » Add flow cytometer information Actions A Review experiment variables Experiment Analyze your data on-line Edit Experiment Details Review MIFlowCyt annotation Delete Experiment Download FCS files ECS Files For more tips and guides please see: Download FCS Files Upload More FCS Files FlowRepository Quick start guide De-identify FCS Files Documentation site for Cytobank and FlowRepository Review Keywords in FCS files 6 - Sharing Permissions Full Access Users 0 Nima Aghaeepour [PR] Josef Spidlen [x] O Ryan Brinkman [x] Download Files Upload More Files De-identify FCS Files Review Keywords in FCS files File Name Sample Tube Name Experiment Variables Panel Events Size Invite a new user Share with a User (Full Access) 100715.fcs describe sample 100715 fcs details Tube 025 4 MB Panel 1 65016 105696 fcs details 105696.fcs describe sample Tube 009 Panel 1 455184 27.8 MB This experiment is currently private. 108701 fcs details 108701.fcs describe sample Tube 001 Panel 1 1000000 61 MB Share with Everyone 109025 fcs details 109025.fcs describe sample Tube 009 Panel 1 210186 12.8 MB You can also create a secret access code 109567.fcs details 109567.fcs describe sample Tube 017 160074 9.8 MB Panel 1 to share with reviewers. 110539.fcs details 110539.fcs describe sample Tube\_022 364212 22.2 MB Share with Reviewers Panel 1

113548.fcs describe sample

121069 fcs describe sample

122405.fcs describe sample

Tube 003

Tube 001

Tube 010

10.8 MB

33.1 MB

29.1 MB

Panel 1 177102

Panel 1 542538

Panel 1 476208

#### - Did you know?

You can request a one-on-one session to get started with your data by filling out a

113548.fcs details

121069 fcs details

122405.fcs details

Data review and download Data upload and annotation Data sharing

# Describing samples

Apply template:	Create templates
Description *	
Sample source	New
Sample characteristic	New
Sample treatment	New
Staining	None B cells, MHCII, PerCPCy5.5 (BD Biosciences BD#custo T cells, CD40, FITC/OG (eBioscience eBio#11-0409) CD14 positive cells, CD14, Alexa 700 (BB Biosciences L CD86, CD86, PE (eBioscience eBio#12-0869)
Staining cocktail(s)	None Cocktail no
	Save and proceed to next FCS file Apply to all undescribed FCS files Apply to undescribed FCS files in Panel 1

Data review and download Data upload and annotation Data sharing

### Describing samples – apply a template

➡ 100715.fcs Sample	
Apply template:	PBMC from HIV+ Subject Template  Create templates
Description *	PBMC from HIV+ Subject
Sample source	New New
Sample characteristic	HIV+ • New
Sample treatment	PBMCs were thawed in warm • New
Staining	None
	T cells, CD40, FITC/OG (eBioscience eBio#11-0409)
	CD14 positive cells, CD14, Alexa 700 (BD Biosciences ) CD86, CD86, PE (eBioscience eBio#12-0869)
Staining cocktail(s)	None Coolitii aa
	- New
	Save and proceed to next FCS file Apply to all undescribed FCS files Apply to undescribed FCS files in Panel 1

Data review and download Data upload and annotation Data sharing

#### Describing samples – create a sample source

- Use templates again
- Adjust accordingly for each sample
- Or just leave it (we can fix it later using spreadsheets)

New sample source details		
Create from template:	Create new template(s)	
Sample source type *	•	
Description *		1
Organism *	•	
Age *		
Age unit *		
Gender*		
Phenotype *		
Genotype *		
Treatment *		,
	Cance	el Save

Data review and download Data upload and annotation Data sharing

#### Describing samples – create a sample source

Ne

- Use templates again
- Adjust accordingly for each sample
- Or just leave it (we can fix it later using spreadsheets)

imple source details		
Create from template: HIV+ subject	template  • Create new template(s)	
Sample source type *	biological	
	biologicui	
Description *		_
HIV+ subject		
Organism *	Homo sapiens (9606) [human] ·	
Are *	0	
Age	0	
Age unit *	years	
0		
Gender*	?	
Phenotype *	N/A	
Genotype *	N/A	
Treatment *		
None		٦

Data review and download Data upload and annotation Data sharing

# Describing samples – 3 options to save

	Information
Apply template:	PBMC from HIV+ Subject Template  Create templates
Description *	PBMC from HIV+ Subject
Sample source	HIV+ subject v New
Sample characteristic	HIV+ • New
Sample treatment	PBMCs were thawed in warm • New
Staining	None B cells, MHCII, PerCPCy5.5 (BD Biosciences BD#custo T cells, CD40, FITC/OG (eBioscience eBio#11-0409) CD14 positive cells, CD14, Alexa 700 (BD Biosciences L CD86, CD86, PE (eBioscience eBio#12-0869)
Staining cocktail(s)	None Cocktail no
(	Save and proceed to next FCS file apply to all undescribed FCS files Apply to undescribed FCS files in Panel 1

Data review and download Data upload and annotation Data sharing

## Describing samples – 3 options to save

	Information
Apply template:	PBMC from HIV+ Subject Template  Create templates
Description *	PBMC from HIV+ Subject
Sample source	HIV+ subject   New
Sample characteristic	HIV+ • New
Sample treatment	PBMCs were thawed in warm • New
Staining	None B cells, MHCII, PerCPCy5.5 (BD Biosciences BD#custo T cells, CD40, FITC/OG (eBioscience eBio#11-0409) CD14 positive cells, CD14, Alexa 700 (BD Biosciences CD86, CD86, PE (eBioscience eBio#12-0869)
Staining cocktail(s)	None Cocktail no
	Save and proceed to next FCS file Apply to all undescribed FCS files pply to undescribed FCS files in Panel 1

Data review and download Data upload and annotation Data sharing

## Describing samples – 3 options to save

	Information
Apply template:	PBMC from HIV+ Subject Template  Create templates
Description *	PBMC from HIV+ Subject
	<b></b> _
Sample source	HIV+ subject • New
Sample characteristic	HIV+ • New
Sample treatment	PBMCs were thawed in warm • New
Staining	None
	T cells, CD40, FITC/OG (eBioscience eBio#11-0409)
	CD14 positive cells, CD14, Alexa 700 (BD Biosciences L CD86, CD86, PE (eBioscience eBio#12-0869) • (New
Staining cocktail(s)	None 🔺
	Cocktail no
	. New
	Save and proceed to next FCS file Apply to all undescribed FCS file Apply to undescribed FCS files in Panel 1

Data review and download Data upload and annotation Data sharing

#### Samples and sample sources are now described

Forkpatty: 100%     Forkpatty: 100%     C this:/forecostrumpioneriment.il								4
Experiment: IDCRP's HIV Natural History Stu	dy ID: FR-FCM-ZZZI	3 Labels: None Primar	y Researcher: <u>Nin</u>	na Aghaeepour Public: No	MIF	lowCyt Score	e: 61.50%	
« Back to Inbox	▶ Getting Starte	d: Experiment Details					×	
My Working Illustration »	Experiment D	etails					0	
MIFlowCyt Annotation »	→ Illustrations						Û	
- Actions	Attachments						Û	
Experiment	→ FCS Files (466							
Edit Experiment Details Delete Experiment	Download Files Upload File Name	More Files De-identify FCS Files Sample	Review Keywords in Tube Name	<u>FCS files</u> Experiment Variables	Panel	Events	Size	]
Download FCS Files	100715.fcs details	show sample description	Tube_025		Panel 1	65016	4 MB	
Upload More FCS Files	105696.fcs details	show sample description	Tube_009		Panel 1	455184	27.8 MB	
De-identity FCS Files Review Keywords in FCS files	108701.fcs details	show sample description	Tube_001		Panel 1	1000000	61 MB	
	109025.fcs details	show sample description	Tube_009		Panel 1	210186	12.8 MB	
- Sharing Permissions	109567.fcs details	show sample description	Tube_017		Panel 1	160074	9.8 MB	
Full Access Users	110539.fcs details	show sample description	Tube_022		Panel 1	364212	22.2 MB	
Nima Aghaeepour [PR]	113548.fcs details	show sample description	Tube_003		Panel 1	177102	10.8 MB	
Josef Spidlen [x]	121069.fcs details	show sample description	Tube_001		Panel 1	542538	33.1 MB	
Ryan Brinkman [x]	122405.fcs details	show sample description	Tube_010		Panel 1	476208	29.1 MB	
Invite a new user Share with a Llogr (Full Assess)	127225.fcs details	show sample description	Tube_021		Panel 1	257058	15.7 MB	
Share with a Oser (Full Access)	129599.fcs details	show sample description	Tube_007		Panel 1	352314	21.5 MB	
This experiment is currently private.	129730.fcs details	show sample description	Tube_017		Panel 1	390528	23.8 MB	
	129869 fcs details	show sample description	Tube 002		Panel 1	230852	14.1 MB	

Data review and download Data upload and annotation Data sharing

#### But not everything is correct!

- Our Sample source organisms vary in age and gender
- We left this out from our template
- Time to fix this
  - We can now use the spreadsheet created earlier

Data review and download Data upload and annotation Data sharing

### Upload the spreadsheet as attachment

FlowRepository - IDCRP: ×								
Experiment: IDCRP's HIV Natural History Stu	iy ID: FR-FCM-ZZZE	3 Labels: None Prim	ary Researcher: <u>Nin</u>	na Aghaeepour Public: No	MIE	lowCyt Score	: 61.50%	
« Back to Inbox	• Getting Starte	d: Experiment Details	;				×	
My Working Illustration »	• Experiment De	etails					i	
MIFlowCyt Annotation »	→ Illustrations						6	
- Actions	- Attachments						i	
Experiment Edit Experiment Details Delete Experiment FCS Files Download ECS Files	File Name Attach a file Choose File No fil Upload	Date le chosen	Uploaded By	Size	mo	l5sum		
Upload More FCS Files	▼ FCS Files (466	5)					6	Í
De-identify FCS Files Review Keywords in FCS files	Download Files Upload	More Files De-identify FCS File	s Review Keywords in	FCS files				11
- Sharing Permissions A	File Name	Sample	Tube Name	Experiment Variables	Panel	Events	Size	
Full Access Users	105696.fcs details	show sample description	Tube_023		Panel 1	455184	27.8 MB	
Nima Aghaeepour [PR]	108701.fcs details	show sample description	Tube_001		Panel 1	1000000	61 MB	
S Josef Spidlen [x]	109025.fcs details	show sample description	Tube_009		Panel 1	210186	12.8 MB	
O Ryan Brinkman [x]	109567.fcs details	show sample description	Tube_017		Panel 1	160074	9.8 MB	
Invite a new user	110539.fcs details	show sample description	Tube_022		Panel 1	364212	22.2 MB	
Snare with a User (Full Access)	113548.fcs details	show sample description	Tube_003		Panel 1	177102	10.8 MB	
This experiment is currently private.	121069.fcs details	show sample description	Tube_001		Panel 1	542538	33.1 MB	
	122405.fcs details	show sample description	Tube 010		Panel 1	476208	29.1 MB	

Data review and download Data upload and annotation Data sharing

### Upload the spreadsheet as attachment

FlowRepository - IDCRP: ×								
Image: Control of the state								4
Experiment: IDCRP's HIV Natural History Stu	idy ID: FR-FCM-ZZZI	B Labels: None Prin	ary Researcher: <u>Nin</u>	na Aghaeepour Public: No	MIE	lowCyt Score	e: 61.50%	
« Back to Inbox	▶ Getting Starte	d: Experiment Detail	5				×	J
My Working Illustration »	→ Experiment D	etails					i)	
MIFlowCyt Annotation »	→ Illustrations						6	
- Actions	✓ Attachments						6	
Experiment Edit Experiment Details Detete Experiment FCS Files Download ECS Files	File Name Attach a file Choose File anno Upload	Date otations.csv	Uploaded By	Size	ma	15sum		
Upload More FCS Files De-identify FCS Files	➡ FCS Files (466)	5)					i	Í
Review Keywords in FCS files	File Name	More Files De-identify FCS Fil Sample	es <u>Review Keywords in</u> Tube Name	Experiment Variables	Panel	Events	Size	
→ Sharing Permissions     (i)	100715.fcs details	show sample description	Tube_025		Panel 1	65016	4 MB	
Full Access Users	105696.fcs details	show sample description	Tube_009		Panel 1	455184	27.8 MB	
Nima Aghaeepour (PR)	108701.fcs details	show sample description	Tube_001		Panel 1	1000000	61 MB	
Josef Spidlen [x]	109025.fcs details	show sample description	Tube_009		Panel 1	210186	12.8 MB	
O Ryan Brinkman [x]	109567.fcs details	show sample description	Tube_017		Panel 1	160074	9.8 MB	
Invite a new user	110539.fcs details	show sample description	Tube_022		Panel 1	364212	22.2 MB	
Snare with a User (Full Access)	113548.fcs details	show sample description	Tube_003		Panel 1	177102	10.8 MB	
This experiment is currently private.	121069.fcs details	show sample description	Tube_001		Panel 1	542538	33.1 MB	
	122405.fcs details	show sample description	Tube 010		Panel 1	476208	29.1 MB	

Data review and download Data upload and annotation Data sharing

### Upload the spreadsheet as attachment

FlowRepository - IDCRP: ×							
🔶 🌼 🖉 🔇 https://flowrepository.org/experiments/11							4
Experiment: IDCRP's HIV Natural History Stud	ly ID: FR-FCM-ZZZB	3 Labels: None	Primary Researcher.	Nima Aghaeepour	Public: No Mi	FlowCyt Score	: 61.50%
« Back to Inbox My Working Illustration »	Attachment w	as added to experim	ent.				
MIFlowCyt Annotation »	• Getting Started	a: Experiment De	talis				*
→ Actions ()	▶ Experiment De	etails					0
Experiment	► Illustrations						0
Edit Experiment Details Delete Experiment	- Attachments						0
FCS Files	File Name		Date	Uploaded By	Size	md5su	im
Download FCS Files Upload More FCS Files De-identify FCS Files Review Keywords in FCS files	annotations.csv 😒 🤄 Click to add a descript Attach a file Choose File No fil	tion e chosen	12:03 PM	Josef Spidlen	15.5 KB	1de7e1	5
- Sharing Permissions	Upload						
Full Access Users	➡ FCS Files (466)	)					6
Nima Aghaeepour (PR)	Download Files Upload N	More Files De-identify FC	S Files Review Keywor	ds in FCS files			
Josef Spidlen [x]	File Name	Sample	Tube Nam	e Experiment Va	ariables Panel	Events	Size
O Ryan Brinkman [x]	100715.fcs details	show sample descrip	tion Tube_025		Panel 1	65016	4 MB
Invite a new user	105696.fcs details	show sample descrip	tion Tube_009		Panel 1	455184	27.8 MB
Share with a User (Full Access)	108701.fcs details	show sample descrip	tion Tube_001		Panel 1	1000000	61 MB
This experiment is currently private.	109025.fcs details	show sample descrip	tion Tube_009		Panel 1	210186	12.8 MB
	109567.fcs details	show sample descrip	tion Tube 017		Panel 1	160074	9.8 MB

Data review and download Data upload and annotation Data sharing

# Describe the attachment (optional)

FlowRepository - IDCRP: ×							
🔶 🕼 https://flowrepository.org/experiments/11							4
Experiment: IDCRP's HIV Natural History Stur	dy ID: FR-FCM-ZZZE	B Labels: None Prima	ary Researcher: <u>Nin</u>	na Aghaeepour Public: No	MIE	lowCyt Score	: 61.50%
« Back to Inbox My Working Illustration »	Attachment w	as added to experiment.					
MIFlowCyt Annotation »	Getting Started	d: Experiment Details					×
- Actions	► Experiment De	tails					6
Experiment	→ Illustrations						0
Edit Experiment Details Delete Experiment	- Attachments						i
FCS Files	File Name		Date	Uploaded By	Size	md5	sum
Download FCS Files Upload More FCS Files De-identify FCS Files Review Keywords in FCS files	annotations.csv 🕑 🤅 File specific sample s Attach a file	ource details	12:03 PM	Josef Spidlen	15.5 KB	1de7	15
→ Sharing Permissions	Upload	e chosen					
Full Access Users	→ FCS Files (466	)					0
Nima Aghaeepour [PR]	Download Files Upload	More Files De-identify FCS File	s Review Keywords in	FCS files			
Josef Spidlen [X]	File Name	Sample	Tube Name	Experiment Variables	Panel	Events	Size
O Ryan Brinkman [x]	100715.fcs details	show sample description	Tube_025		Panel 1	65016	4 MB
Invite a new user	105696.fcs details	show sample description	Tube_009		Panel 1	455184	27.8 MB
Share with a User (Full Access)	108701.fcs details	show sample description	Tube_001		Panel 1	1000000	61 MB
This experiment is currently private	109025.fcs details	show sample description	Tube_009		Panel 1	210186	12.8 MB
This experiment is currently private.	109567.fcs details	show sample description	Tube 017		Panel 1	160074	9.8 MB

Data review and download Data upload and annotation Data sharing

#### Parse the attachment – click on (P)

FlowRepository - IDCRP: ×							
< 🔅 🕲 https://flowrepository.org/experiments/11							4
Experiment: IDCRP's HIV Natural History Stud	dy ID: FR-FCM-ZZZE	3 Labels: None Prima	ry Researcher: <u>Nin</u>	na Aghaeepour Public: No	MIE	lowCyt Score	2: 61.50%
« Back to Inbox My Working Illustration »	Attachment w	as added to experiment.					
MIFlowCyt Annotation »	• Getting Started	u. Experiment Details					•
- Actions	► Experiment De	etails					0
Experiment	► Illustrations						0
Edit Experiment Details Delete Experiment	- Attachments						()
FCS Files	File Name		Date	Uploaded By	Size	md5	sum
Download FCS Files Upload More FCS Files De-identify FCS Files Review Keywords in FCS files	annotations.csv 🕄 🕼 File specific sample s Attach a file	Parse the attachment to extract and	12:03 PM update annotations.	Josef Spidlen	15.5 KB	1de7	15
- Sharing Permissions	Upload						
Full Access Users	- FCS Files (466	i)					6
Nima Aghaeepour [PR]	Download Files Upload	More Files De-identify FCS Files	Review Keywords in	FCS files			
Josef Spidlen [x]	File Name	Sample	Tube Name	Experiment Variables	Panel	Events	Size
O Ryan Brinkman [x]	100715.fcs details	show sample description	Tube_025		Panel 1	65016	4 MB
Invite a new user	105696.fcs details	show sample description	Tube_009		Panel 1	455184	27.8 MB
Share with a User (Full Access)	108701.fcs details	show sample description	Tube_001		Panel 1	1000000	61 MB
This experiment is currently private	109025.fcs details	show sample description	Tube_009		Panel 1	210186	12.8 MB
localhost:8080/experiments/11/attachments/51/parse	109567.fcs details	show sample description	Tube 017		Panel 1	160074	9.8 MB

Data review and download Data upload and annotation Data sharing

#### Information extracted from attachment

#### • Review the result

Note: We also provided one experimental variable (the condition)

# The following information extracted from attachment annotations.csv

FCS file	age	gender	condition
100715.fcs	51	F	HIV Stage 1
105696.fcs	25	F	HIV Stage 4
108701.fcs	21	М	HIV Stage 3
109025.fcs	20	М	HIV Stage 4
109567.fcs	36	F	HIV Stage 2
110539.fcs	43	М	HIV Stage 1
113548.fcs	38	F	HIV Stage 2
121069.fcs	33	М	HIV Stage 3
122405.fcs	43	М	HIV Stage 2
127225.fcs	21	F	HIV Stage 1
129599.fcs	40	М	HIV Stage 1
129730.fcs	20	F	HIV Stage 2
129869.fcs	21	М	HIV Stage 3
130119.fcs	44	М	HIV Stage 1
132447.fcs	17	F	HIV Stage 1

# Upload and annotation of your own dataset

#### Typical steps

- Create a new experiment
- Opload data (FCS files)
- O Prepare annotation templates
  - Or prepare spreadsheets with annotations
- Annotate the experiment
  - Describe samples and sample sources
  - Provide experimental variables
  - Describe instrumentation settings
- Provide analysis details
  - Either analyze data online and create illustrations
  - Or upload third party analysis files (e.g., Flow Jo<sup>TM</sup> workspaces, FCS Express<sup>TM</sup> project files, FACS Diva<sup>TM</sup> files, etc.)
- Review (and improve) your MIFlowCyt compliance

#### Provide experimental variables

- Follow Review experiment variables in the Getting Started panel
- Or navigate to your Working Illustration



Reset Working Illustration

Josef Špidlen, Ryan R. Brinkman

Data review and download Data upload and annotation Data sharing

### Provide experimental variables

- Figure Dimensions (Experime				
Available Dimensions - Click to toggle of	on/off			
Channels Populations Dosages Arrange Dimensions - Drag to prioritize	Timepoints Conditions Ind dimensions, click Choose to change	ividual selec	Is Sample Types Fcs Files Plate Column Plate Row Column and ordering, click Setup/Gate to configure	Plate
Channels	Populations		Conditions	
Unselected Channels: - Panel 1 - Panel 2 - Panel 2 - Panel 2 - Panel 2 - Panel 2 - Panel 1 - Panel 2 - Panel 1 KI67 - Panel 1 CD3 - Panel 1 CD3 - Panel 2 CD3	Ungated	••	HIV Stage 1 HIV Stage 4 HIV Stage 3 HIV Stage 2	
Columns	Rows	ļ	Table 1	

• Example: patients treated by various dosages of Lexiva<sup>TM</sup>

Data review and download Data upload and annotation Data sharing

## Provide experimental variables

ailable Dimens	ione - Cli	ck to to	aalo (	on/off	,							
Channels Pop	pulations ons - Drag	Dosa g to pric	ages oritize	Timepoints dimensions, click C	Conditions	Individ ange se	uals lectio	Sample Types	Fcs Files	Plat e to cor	te Column Plate Row P	late
Channels 2 channels	<u>Choose</u>	Setup		Populations	Choose	Gate	4	of 4 selected	Choose   Setu	p	Dosages	Setu
Unselected Chan - Panel 1 - Panel 2 - Panel 2 - Panel 2 - Panel 2 - Panel 2 - Panel 2 CD3 - Panel 1 CD3 - Panel 2 CD3 - Panel 1 CD28 - Panel 1 CD28 - Panel 1 CD28 - Panel 2 CD45RO - Panel 2 CD45RO - Panel 2	nels:		4	Ungated		•		HIV Stage 1 HIV Stage 4 HIV Stage 3 HIV Stage 2		•	(Click To Setup)	
								Table	1		Table 2	

- Example: patients treated by various dosages of Lexiva<sup>TM</sup>
- Click on Dosages, than Setup

Data review and download Data upload and annotation Data sharing

### List doses

#### • Provide a comma-separated list of all doses

		i
Enter a comma separated list of Doses to add:		
Lexiva 1400 bid, Lexiva 1400 qd+Norvir 200 qd, Lexiva 700 bid+Norvir 100 bid	Add Doses	

# Assign FCS files to the right doses

- Drag & Drop files into the appropriate boxes
- Or use the *Filter* with *Move to*

All Doses	Lexiva 1400 bid	Lexiva 1400 qd	+Norvir 200 qd	Lexiva 700 bid+Norv	rir 100 bid				
All Dos Drag files fr	SES om "Untagged" box to	the "Dose" boxes	s below to associa	ate them with that tag. L	Jse the "Fi	Iter" and "Move File" controls to move	e groups	s of fil	les.
Untagg	Jed		Lexiva 1400 I	bid Tagged Files Tube_025)	×	Lexiva 1400 qd+Norvir 200 qd Tagged Files	×		Le: Ta
M	ove to ove file(s)	•	105696.fcs (T 108701.fcs (T	Tube_009) Tube_001)		109025.fcs (Tube_009)			1
13489	2.fcs (Tube_021) 1.fcs (Tube_022)		127225.fcs (T 130119.fcs (T	ube_021) ube_001)		121069.fcs (Tube_001) 122405.fcs (Tube_010)			1
14561	2.fcs (Tube_011) 2.fcs (Tube_001)		132447.fcs (T	Tube_013)					1
15966	5.fcs (Tube_004) 3.fcs (Tube_005)			13276	9.fcs (Tub	e_002)			
16252	0.fcs (Tube_017) 9.fcs (Tube_010)								

# Upload and annotation of your own dataset

#### Typical steps

- Create a new experiment
- Opload data (FCS files)
- Prepare annotation templates
  - Or prepare spreadsheets with annotations
- Annotate the experiment
  - Describe samples and sample sources
  - Provide experimental variables
  - Describe instrumentation settings
- Provide analysis details
  - Either analyze data online and create illustrations
  - Or upload third party analysis files (e.g., Flow Jo<sup>TM</sup> workspaces, FCS Express<sup>TM</sup> project files, FACS Diva<sup>TM</sup> files, etc.)
- Review (and improve) your MIFlowCyt compliance

#### Instrumentation description

#### • Navigate to the details of an FCS file

FlowRepository - IDCRP: ×								
< 🔅 🥸 https://flowrepository.org/experiments/11								2
Experiment: IDCRP's HIV Natural History Stud	ly ID: FR-FCM-ZZ	ZB Labels: None P	rimary Researc	her: <u>Nima Aghaeepour</u> Public: No	MIFlow	Cyt Score: (	36.00%	ן י
« Back to Inbox	▶ Getting Start	ed: Experiment Deta	ails				×	J
My Working Illustration »	→ Experiment D	Details					Ü	
MIFlowCyt Annotation »	→ Illustrations						Û	J
- Actions	→ Attachments						i)	]
Experiment								
Edit Experiment Details	Download Files Uploa	d More Files De-identify FCS	Files Review Ke	words in FCS files				
Delete Experiment	File Name	Sample	Tube Name	Experiment Variables	Panel	Events	Size	H
Download FCS Files	100715.fc details	show sample description	Tube_025	HIV Stage 1, Lexiva 1400 bid	Panel 1	65016	4 MB	
Upload More FCS Files	105696.fcs details	show sample description	Tube 009	HIV Stage 4, Lexiva 1400 bid	Panel 1	455184	27.8 MB	
De-identify FCS Files	108701 fcs details	show sample description	Tube 001	HIV Stage 3, Lexiva 1400 bid	Panel 1	1000000	61 MB	
Review Keywords in FCS files	109025 fee dataile	ehow eample description	Tube 009	HIV Stage 4 Levius 1400 ad+Nondr 200 ad	Danel 1	210186	12.8 MD	
- Sharing Permissions	1005023.455 details	show sumple description	Tube_003	Hit Clage 4, Lexing 1400 duritoria 200 du	Denella	100074	0.0 MD	
Full Access Lisers	109567.ics details	show sample description	Tube_017	HIV Stage 2, Lexiva 700 bid+Norvir 100 bid	Panel 1	160074	9.0 MB	
	110539.fcs details	show sample description	Tube_022	HIV Stage 1, Lexiva 1400 qd+Norvir 200 qd	Panel 1	364212	22.2 MB	
	113548.fcs details	show sample description	Tube_003	HIV Stage 2, Lexiva 700 bid+Norvir 100 bid	Panel 1	177102	10.8 MB	
Josef Spidlen [x]	121069.fcs details	show sample description	Tube_001	HIV Stage 3, Lexiva 1400 qd+Norvir 200 qd	Panel 1	542538	33.1 MB	
Ryan Brinkman [x]	122405.fcs details	show sample description	Tube_010	HIV Stage 2, Lexiva 1400 qd+Norvir 200 qd	Panel 1	476208	29.1 MB	
Invite a new user	127225.fcs details	show sample description	Tube_021	HIV Stage 1, Lexiva 1400 bid	Panel 1	257058	15.7 MB	
Share with a User (Full Access)	129599.fcs details	show sample description	Tube_007	HIV Stage 1, Lexiva 700 bid+Norvir 100 bid	Panel 1	352314	21.5 MB	
This experiment is currently private.	129730.fcs details	show sample description	Tube_017	HIV Stage 2, Lexiva 700 bid+Norvir 100 bid	Panel 1	390528	23.8 MB	
Ohmen with Deserver	129869.fcs details	show sample description	Tube 002	HIV Stage 3, Lexiva 700 bid+Norvir 100 bid	Panel 1	230852	14.1 MB	

Josef Špidlen, Ryan R. Brinkman Flow

#### Instrumentation description

#### • Press the Describe instrument settings button

FlowRepository - IDCRP: ×		Þ									
🔶 🕒 🔮 🕼 https://flowrepository.org/experiments/11/fcs_file	5/3043										
Experiment: IDCRP's HIV Natural History Stud	y ID: F	R-FCM-Z	ZZZB	Labels: None	Primary	Resea	ircher: <u>N</u>	lima Aghaee	pour Pu	iblic: No	MIFlowCyt Score: 66.00%
« Back to Experiment Summary	▶ 1007	15.fcs	- FCS	File Informatic	n						
- Actions											
Download Tab-Separated Events File Show Sample Details De-Identify the ECS file Review Keywords in the ECS file	File-spec	ific instru instrume	iment sel	tings have not beer	n provid	ed!					
→ Did you know?	<b>~</b> 1007	'15.fcs	- FCS	File Laser Info	rmatio	on					
You can request a one-on-one session to	ASF	Name	Delay								
get started with your data by filling out a support ticket.	Blue	0.66	0.00								
A guide to Cytobank is available at <u>Current</u> Protocols in Cytometry	Red	0.55	-59.80								
We also have a Quick start guide.	Violet	0.48	-24.40								
You can print/save your Illustrations to PDF from the Illustration view's left menu.	Green	0.53	-82.60								
You can export your data to Excel from the Experiment Summary page.	<b>-</b> 1007	'15.fcs	- FCS	J File Channel II	nform	ation					
Give other users full control to modify your experiments through the "Sharing Permissions" box.	Chann	el Short	Name	Channel Name	Gain	Bits	Amp	Range	Voltage	Amp Value	
Use the "Download Files" button to save	FSC-A				1	32		262207.0		0.0	
computer.	FSC-H				1	32		262207.0		0.0	
	SSC-A				1	32		261588.0		0.0	
	B515-A			KI67	1	32		261588.0		0.0	
	D790 /			CD2	1	22		261599.0		0.0	
	1.100-4	`		003	-	32		201300.0		0.0	

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#### Instrumentation description

#### • Select the make and model of the instrument used

romspacey - 10.00* *      C      D https://owyperimens/11./fs_files/3043									٩	
Experiment: IDCRP's HIV Natural History Stu	dy ID: FR-FCM-ZZZB	Labels: None	Primary	Resea	ircher: <u>1</u>	lima Aghaee	pour Pu	blic: No	MIFlowCyt Score: 66.00%	
« Back to Experiment Summary										
Download Tab-Separated Events File Show Sample Details De-identify the ECS file Review Keywords in the ECS file	Segurated Exercise File specific instrument settings have not been provided!     Details     Deta									
→ Did you know?										
You can request a one-on-one ses Describe instrument settings X get stande with your data by filling c supporticities instrument *										
A guide to Cytobank is available at <u>Protocols in Cytometry</u>										
We also have a <u>Quick start guide</u> . You can print/save your Illustrations from the Illustration view's left menu							Cancel	Save		
Experiment Summary page.	* 100/10.1CS - FCS	File Gilaillei I		autom						
Give other users full control to modify your experiments through the "Sharing Permissions" box.	Channel Short Name	Channel Name	Gain	Bits	Amp	Range	Voltage	Amp Value		
Use the "Download Files" button to save copies of the original FCS Files to your computer.	FSC-A		1	32		262207.0		0.0		
	FSC-H		1	32		262207.0		0.0		
	SSC-A		1	32		261588.0		0.0		
	B515-A	KI67	1	32		261588.0		0.0		
	R780-A	CD3	1	32		261588.0		0.0		

Josef Špidlen, Ryan R. Brinkman
## Instrumentation description

#### • New instruments may be added in the annotation data section

FlowRepository - IDCRP: ×				
🔶 🌼 😅 🔇 https://flowrepository.org/experiments/11/fcs_file	5/3043			4
Experiment: IDCRP's HIV Natural History Stud	iy ID: FR-FCM-ZZZB Labels: None Primary Researcher: <u>Nima Aghaeepour</u>	Ρι	ublic: No	MIFlowCyt Score: 66.00%
« Back to Experiment Summary				
- Actions				
Download Tab-Separated Events File	File-specific instrument settings have not been provided!			
Show Sample Details De-Identify the FCS file Review Keywords in the FCS file	Describe instrument settings			
- Did you know?				
You can request a one-on-one ses: Describe	instrument settings			
get started with your data by filling c support ticket.	Instrument *			
A guide to Cytobank is available at Protocols in Cytometry	BactoCount IBC, Bentley Instruments			
We also have a Quick start guide.	BD FACSAria, Becton Dickinson (BD Biosciences)			
You can print/save your Illustrations	BD FACSAria II, Becton Dickinson (BD Biosciences) BD FACSAria III, Becton Dickinson (BD Biosciences)			
from the illustration view's left menu	BD FACSArray, Becton Dickinson (BD Biosciences)	el	Save	
Experiment Summary page.	BD FACSCalibur, Becton Dickinson (BD Biosciences) BD FACSCanto, Becton Dickinson (BD Biosciences)			
Give other users full control to modify your	BD FACSCanto II, Becton Dickinson (BD Biosciences)			
experiments through the "Sharing	BD FACSCount, Becton Dickinson (BD Biosciences) BD FACSScan, Becton Dickinson (BD Biosciences)	age	Amp Value	
Permissions box.	BD Influx, Becton Dickinson (BD Biosciences)		0.0	
copies of the original FCS Files to your	BD LSR, Becton Dickinson (BD Biosciences) BD LSR II. Becton Dickinson (BD Biosciences)		0.0	-
computer.	BD LSRFortessa, Becton Dickinson (BD Biosciences)		0.0	
	Bentley 150, Bentley Instruments Bentley Combi 150:, Bentley Instruments		0.0	
	Bentley FTS, Bentley Instruments			-
	Cell Lab Quanta MPL, Dako Cytomation		0.0	
	R780-A CD3 1 32 261588.0		0.0	
				-

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FlowRepository.org - Resource of Flow Cytometry Data

### Instrumentation description

#### • Simple case: Same instrument with default settings for all FCS files

Describe instrument settings	
Instrument * BD LSR II, Becton Dickinson (BD Biosciences)	¥
Use default instrument ≤ settings	
Installation dates for filters in optical paths	
The instrument has been purchased new on July 1, 2011; all optical filters are original came with the instrument.	and
Other	
PMT voltages specified within the FCS data files.	
$\ensuremath{\mathscr{U}}$ Use these settings for all FCS files in this experiment	
С	ancel Save

## Instrumentation description

#### • Advanced case: Describe all details

• Required by MIFlowCyt for customized instruments only

Describe instrur	nent settings		
	Instrument *		-
BD L	SR II, Becton Dickinson (BD	Biosciences)	
Use defai	ult instrument settings		
I	Flow cell type	Quartz cuvette • Add new flow cell type	
Othe	r flow fluidics		
	Optical paths	None - Add new optical path	

Data review and download Data upload and annotation Data sharing

## Instrumentation description



Data review and download Data upload and annotation Data sharing

## Instrumentation description

- Double click on each of component
- Either select an existing component or create a new description with all the details as required by MIFlowCyt

Light Source component X
Select existing light source: Laser - 488nm - 13mW -
Create new light source
Cancel Add component

## Instrumentation description

• New light source description:

Light Source component	×
Select	t existing light source
Light source type *	Y
Excitatory wavelength [nm] *	
Power*	
Polarization	
Beam	
Other	
	Cancel Add component

## Instrumentation description

#### • New optical filter description:

Optical Filter component	*
	Select existing filter
Filter type *	Y
Transmitted wavelengths *	
Model	
Manufacturer	• New
Other	
	Cancel Add component

Data review and download Data upload and annotation Data sharing

## Instrumentation description

#### • New optical detector description:

Optical Detector component	
	Select existing detector
Optical detector type *	•
Name *	
Amplification type	
Other	
	Cancel Add component

## Instrumentation description

• The MIFlowCyt score finally reached the "green area" once the instrumentation description is provided

FlowRepository - IDCRP: ×										
🔶 📦 📽 🔕 https://flowrepository.org/experiments/11/fcs_fil	🛊 🥪 📽 🔕 https://fowmpository.org/scoperiments/11/rds_Piles/3043									
Experiment: IDCRP's HIV Natural History Stu	dy <b>ID</b> : FR	-FCM-ZZ	ZB La	bels: None	Primary Researcher	r: <u>Nima Aghaeepour</u>	Public: No	MIFlowCyt Score: 86.00%	]	
« Back to Experiment Summary	→ 10071	.5.fcs -	FCS Fil	le Informat	tion					
✓ Actions	<b>~</b> 10071									
Download Tab-Separated Events File Show Sample Details De-identify the FCS file Review Keywords in the FCS file	Instrument mode: BD U.S.B. (BB Biosciences) Manufacturum: Eacon Dickinson (BD Biosciences) Folvo Call Thype: Using default settings for BD LSR II Optical Thats: Using default settings for BD LSR II									
→ Did you know?	Installation (	dates of fil	ters in Opt	ical Paths: The	instrument has been pur	chased new on July 1,	2011; all optical f	Iters are original and came with th	a	
You can request a one-on-one session to get started with your data by filling out a support ticket.	Other: PMT voltages specified within the FCS data files. Change instrument seeings v 100715/fcs - FCS File Laser Information									
A guide to Cytobank is available at <u>Current</u> Protocols in Cytometry									Ĕ I	
We also have a <u>Quick start guide</u> .	ASE	Name	alay						71	
from the Illustration view's left menu.	Dhu									
You can export your data to Excel from the	Blue	0.00	0.00							
Give other users full control to modify your	difv your									
experiments through the "Sharing Permissions" box.	Violet	0.48 ·	24.40							
Use the "Download Files" button to save	Green	0.53	82.60							
computer.	+ 10071	.5.fcs -	FCS Fil	le Channel	Information				Í.	

# Upload and annotation of your own dataset

### Typical steps

- Create a new experiment
- Opload data (FCS files)
- O Prepare annotation templates
  - Or prepare spreadsheets with annotations
- Annotate the experiment
  - Describe samples and sample sources
  - Provide experimental variables
  - Describe instrumentation settings
- Provide analysis details
  - Either analyze data online and create illustrations
  - Or upload third party analysis files (e.g., Flow Jo<sup>TM</sup> workspaces, FCS Express<sup>TM</sup> project files, FACS Diva<sup>TM</sup> files, etc.)
- Review (and improve) your MIFlowCyt compliance

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### Typical steps

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- Opload data (FCS files)
- Prepare annotation templates
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  - Or upload third party analysis files (e.g., Flow Jo<sup>TM</sup> workspaces, FCS Express<sup>TM</sup> project files, FACS Diva<sup>TM</sup> files, etc.)
- Review (and improve) your MIFlowCyt compliance

Data review and download Data upload and annotation Data sharing

## Analyze data online

- Navigate to your *Working illustration*
- Click on Gate in the Populations panel

Available Dimensions - Click to to	ggle o	on/off						
Channels Populations Dosa	iges	Timepoints	Conditions	Indi	vidual	s Sample Type	s Fcs Files	
Plate Column Plate Row Plate	ate							
Arrange Dimensions - Drag to price	ritize	dimensions, clic	k Choose to c	hange	selec	tions and ordering,	click Setup/Gate t	o configure
Populations		Conditions				Dosages		
1 of 1 selected Choose Gate	)	4 of 4 selected	<u>Choose</u>	<u>Setup</u>		3 of 3 selected	Choose   Setup	
Ungated		HIV Stage 1				Lexiva 1400 bid		
		HIV Stage 4				Lexiva 1400 qd+N	lorvir 200 qd	
		HIV Stage 3				Lexiva 700 bid+No	orvir 100 bid	
		HIV Stage 2						
Calumna			2014/2			Tab	lo 1	
Columns		, r	10115			Tab	IC T	

Data review and download Data upload and annotation Data sharing

## Analyze data online

#### • Draw your gates

Select	🗖 <u>R</u> ectangle 🥒 <u>E</u> llipse 🚽 <u>P</u> olygon 🕇 <u>Q</u> u	adrant + Split H Range Reset				
<	< File: 100715.fcs - Tube_025 V :	> >				
		List of gates:				
View		Gate 1				
Active Population:		Gate 3				
Ungated 💌		duce 5				
Up Down						
Active Compensation:		Felested aster				
File Compensation 👻		Selected gate.				
		Name:				
Plo <u>t</u> Settings	Y: CD4 🔽 Arcsinh	Global Gate				
Populations		Tailored Gate				
Manage View		Apply Tailored Gate to Files				
Tienade Tien	X: CD28 🗸 Arcsinh	Lock				
		Points Check Gate				

## Analyze data online

### • Select what to include and organize figure dimensions

Figure Dimensions (Exp	erime	ental Variables): Pop	ulations 👍	Co	nditions 👍 Dosa	ges		
vailable Dimensions - Click to	toggle o	on/off						
Channels Populations Do	sages	Timepoints Conditio	Indivi	idual	s Sample Typ	es Fcs Fil	es Plat	e Column
Plate Row Plate								
rrange Dimensions - Drag to p	noritize	dimensions, click Choose	to change s	elec	tions and ordering	g, click Setup/	Gate to co	ntigure
Channels 🗧		Fcs Files			Populations			
3 selected Choose   Setu	Þ	2 selected	Choose		3 selected	Choose	Gate	
KI67 - Panel 1	*	100715.fcs (Tube_025)			Ungated			
CD3 - Panel 1		399676.fcs (Tube_025)			Gate 1			
CD28 - Panel 1	=	Unselected Fcs Files:			Gate 2			
Unselected Channels: - Panel 1 - Panel 2 - Panel 2 - Panel 1 - Panel 2 - Panel 2 - Panel 2 CD3 - Panel 2 CD3 - Panel 2 CD3 - Panel 2 CD3 - Panel 2 CD4 - Panel	••	105696.tcs (Tube_009) 108701.tcs (Tube_001) 109025.tcs (Tube_017) 109567.tcs (Tube_017) 110539.tcs (Tube_002) 113548.tcs (Tube_003) 121069.tcs (Tube_001) 122405.tcs (Tube_0107) 129739.tcs (Tube_017)		••	Unselected Pop Gate 3	ulations:		
Columns		Rows			Tal	ble 1		

Data review and download Data upload and annotation Data sharing

## Analyze data online

#### • Configure plot and stats settings

→ Plot Controls (i)
Plot Types
Density Dot ·
Plot Colors
En Fuego 🔹
Plot Size
128 px •
Compensation [edit]
File-Internal Compensation
Y-Axis
CD4 ·
X-Axis
Use Panel/Channel Values •
Z-Axis
Use Panel/Channel Values
Show Gates
No •
Show Gate Statistic
None
Show Plot Statistics
Yes •

- Plots: Style i
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Percent per Contour
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Outliers Start At
10.0
Smoothing
1.0
Aspect Ratio
1.0

<del>-</del> Plots: Scale Display	•
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Power (10^2)	۲
Show Tickmarks	
Yes	•
Show Scale Numbers	
No	•
Show Axis Labels	
Yes	•

→ Plot Statistics (i)
Statistic
Medians •
Percentile (1-99)
50
Selected Gate
Gate 1
Equation
Raw
Control
First Row •
Viewthrough Plot Type
Density •
Gradient Color Set
Blue to Yellow •
Gradient Scale Type
Global (Automatic)
Min
0.0
Inflection
Max
1.0

Data review and download Data upload and annotation Data sharing

## Analyze data online

- Review the illustration
- Name it and save it

🗕 Josef's Working Illustrati	on	6
Save Illustration as:		
Illustration 1	Save	
Split Illustration by channels		
Reset Illustration		
Print View DPDE		
Export Table of Statistics		
Export Table of Events		
Export Table of Scaled Events		
Export Gated FCS Files		
Export Gates in Gating-ML		



# Analyze data online

More on analyzing data online in Cytobank or FlowRepository:

# UNIT 10.17 Web-Based Analysis and Publication of Flow Cytometry Experiments

Nikesh Kotecha $^{1,2,3},$  Peter O. Krutzik $^{1,2},$  Jonathan M. Irish $^1$ 

Published Online: 1 JUL 2010

DOI: 10.1002/0471142956.cy1017s53

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Lab Protocol Title



Current Protocols in Cytometry

# Upload and annotation of your own dataset

### Typical steps

- Create a new experiment
- Opload data (FCS files)
- O Prepare annotation templates
  - Or prepare spreadsheets with annotations
- Annotate the experiment
  - Describe samples and sample sources
  - Provide experimental variables
  - Describe instrumentation settings
- Provide analysis details
  - Either analyze data online and create illustrations
  - Or upload third party analysis files (e.g., Flow Jo<sup>TM</sup> workspaces, FCS Express<sup>TM</sup> project files, FACS Diva<sup>TM</sup> files, etc.)
- Seview (and improve) your MIFlowCyt compliance

Data review and download Data upload and annotation Data sharing

## Provide third party analysis files, figures, tables, etc.

#### • Upload these as attachments

				i
File Name	Date	Uploaded By	Size	md5sum
annotations.csv  () () () () () () () () () () () () ()	Jun 07	Josef Spidlen	15.5 KB	1de7e15
HIV_Analysis.jo Complete analysis in FlowJo	11:57 AM	Josef Spidlen	14.4 MB	d8a8ab5
HIV_Analysis_Overview.png	12:06 PM	Josef Spidlen	169.3 KB	4958a88
HIV_Analysis_Class_Comparison.jpg 😣 HIV class comparison figure	12:06 PM	Josef Spidlen	201.9 KB	5795d5e
All_Statistics.xlsx	12:07 PM	Josef Spidlen	253.6 KB	95641c1
Extended_description.docx  One details on experimental design	12:07 PM	Josef Spidlen	208.2 KB	76f301b
Attach a file				
Choose File No file chosen				
Upload				

# Upload and annotation of your own dataset

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- O Review (and improve) your MIFlowCyt compliance

FlowRepository - IDCRP': ×										
🔶 🗼 🧲 🔇 https://flowrepository.org/ex	periments/11									
Experiment: IDCRP's HIV Natural History S	tudy ID: FR-FCM-ZZZE	B Labels: None Primar	y Researcher: <u>Nima</u>	a Aghaeepo	<u>ur</u> Publi	ic: No		M	FlowCyt Scor	e: 100.00%
« Back to Inbox	→ Getting Started	I: Experiment Details								×
My Working Illustration »	► Experiment De	tails								6
MIFlowCyt Annotation »	- Illustrations									0
- Actions	Name	Active Dimension	IS					Author	Created	Updated
Experiment Edit Experiment Details	Illustration 1	Channels (3) x Fcs F	iles (2) x Population	ns (3)	Print View	🔁 PDF	Delete	S Josef Spidlen	11:37 AM	
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Review Keywords in FCS files	File Name		1	Date	Uple	oaded By		Size	md5s	um
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Josef Spidlen [x]	HIV_Analysis_Overvie Overview figure	ew.png 🕲		12:08 PM	3	Josef Spidle	n	169.3 KB	4958a	88
Invite a new user Share with a User (Full Access)	HIV_Analysis_Class_ HIV class comparison	Comparison.jpg 😒 figure		12:08 PM	2	Josef Spidle	n	201.9 KB	5795d	5e
This experiment is currently private.	All_Statistics.xlsx 🟵 Tables and stats			12:09 PM	3	Josef Spidle	n	253.6 KB	95641	c <b>1</b>
Share with Everyone	Extended_description More details on exper	.docx 🕲 imental design		12:09 PM	3	Josef Spidle	n	208.2 KB	76f301	b
You can also create a secret access code to share with reviewers.	Attach a file									
Share with Reviewers	Choose File No file Upload	chosen								
✓ Did you know?	- FCS Files (466)									i
You can request a one-on-one session to get started with your data by filling out a	Download Files Upload M	tore Files De-identify FCS Files R	teview Keywords in FC	S files						
support ticket.	File Name	Sample	Tube Name	Experim	ent Variat	bles		Panel	Events	Size
A guide to Cytobank is available at <u>Current</u> Protocols in Cytometry	100715.fcs details	show sample description	Tube_025	HIV Stage	e 1, Lexiva 1	1400 bid		Panel 1	65016	4 MB
We also have a Quick start guide.	105696.fcs details	show sample description	Tube_009	HIV Stage	e 4, Lexiva 1	1400 bid		Panel 1	455184	27.8 MB
You can print/save your Illustrations to PDF from the Illustration view's left menu	108701.fcs details	show sample description	Tube_001	HIV Stage	e 3, Lexiva 1	1400 bid		Panel 1	1000000	61 MB
You can export your data to Excel from the	109025.fcs details	show sample description	Tube_009	HIV Stage	e 4, Lexiva 1	1400 qd+Nor	vir 200 d	qd Panel 1	210186	12.8 MB



#### FlowRepository - IDCRP': ×

C https://flowrepository.org/experiments/11/miflowcyt

#### Experiment: IDCRP's HIV Natural History Study

#### « Back to Experiment Summary

#### - MIFlowCyt

Show MIFlowCyt score details Report Suspicious Score...

Print View DPDE

#### Did you know?

You can request a one-on-one session to get started with your data by filling out a support ticket.

A guide to Cytobank is available at <u>Current</u> <u>Protocols in Cytometry</u>

We also have a Quick start guide.

You can print/save your Illustrations to PDF from the Illustration view's left menu.

You can export your data to Excel from the Experiment Summary page.

Give other users full control to modify your experiments through the "Sharing Permissions" box.

Use the "Download Files" button to save copies of the original FCS Files to your computer.

#### MIFlowCyt Compliance Score for Experiment: IDCRP's HIV Natural History Study - Repository ID: FR-FCM-ZZZB

Public: No

Primary Researcher: Nima Aghaeepour

Total MIFlowCyt compliance score: 100.00%

#### - 1 - Experiment Overview - 100.00% provide

Labels: None

Items considered relatively based on importance, 30% contribution to total score.

em	Compliance [ + - ]	Improve
urpose	Provided purpose	Fully provided
eywords	Provided keywords	Fully provided
xperiment variables	Provided experiment variables	Fully provided
Organization	Provided organization name Provided organization address	Fully provided
Contact	Sirst name of primary researcher provided Last name of primary researcher provided Email of primary researcher provided	Fully provided
Date	Provided experiment starting date Provided experiment end date	Fully provided
conclusions	Provided conclusions	Fully provided
Quality control measures	Provided quality control description	Fully provided

 The "Improve" column will show direct links to pages/forms in case some information is still missing

#### 2 - Flow Sample/Specimen Details - 100.00% provided

Items considered relatively based on importance, 30% contribution to total score.

FCS file	Compliance [+•]	Improve
	0	
	Provided sample description	

MIFlowCyt Score: 100.00%

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Data review and download Data upload and annotation Data sharing

# Data sharing

- You can share with other FlowRepository users
  - This will grant full access
- You can make your experiment public
  - This will grant read access to everyone, including anonymous visitors
- You can share with reviewers

Sharing Permissions
Full Access Users
Nima Aghaeepour [PR]
Josef Spidlen [x]
O Rvan Brinkman [x]
Invite a new user
Share with a User (Full Access)
This experiment is currently private.
Share with Everyone
You can also <b>create a secret access code</b> to share with reviewers.
Share with Reviewers

Data review and download Data upload and annotation Data sharing

# Data sharing

- You can share with other FlowRepository users
  - This will grant full access
- You can make your experiment public
  - This will grant read access to everyone, including anonymous visitors
- You can share with reviewers
  - This will lock your experiment and create a secret access code

✓ Sharing Permissions
Full Access Users
Nima Aghaeepour [PR]
Josef Spidlen [x]
O Ryan Brinkman [x]
Invite a new user
Share with a User (Full Access)
This experiment is currently private
Share with Evenyone
Share with Everyone
You can also <b>create a secret access code</b> to share with reviewers.
Share with Reviewers

Data review and download Data upload and annotation Data sharing

## Data sharing



Data review and download Data upload and annotation Data sharing

## Data sharing



Experiment was successfully updated. This experiment has been locked for reviewers' access and may be accessed via the following URL: https://flowrepository.org/id/RvFrEI5USYaDgWZoVC6bxPrNUjMMcjlgxYxyXW5jXy62tFXyij1uHrxHvllL9nLL. Please share this URL with your reviewers.

#### - Sharing Permissions

Full Access Users This experiment is currently locked! No users have edit access to this experiment. This experiment is shared with reviewers via a secret code.

A state

# What to do with the secret code?

- Share the "secret code" with the editor in your cover letter
- The editor will pass it to reviewers
- Reviewers will use it to obtain read-only access to your dataset
  - By navigating directly to https://flowrepository.org/id/RvFrFI5UsYaDgWZ....
  - Or entering RvFrFI5UsYaDgWZ.... in the "Query" field



# What to do if editor/reviewer requires changes?

- Depending on the journal,
  - The editor may contact FlowRepository administrators and arrange for the dataset to be unlocked
  - Or, you may have to fill out a FlowRepository support ticket and ask for the dataset to be unlocked

s of Service Privacy Policy Support Feedback
--

Summary Future Work Acknowledgments

# Summary

### FlowRepository can be used to

- Access
- Review
- Download
- Deposit
- Annotate
- Share
- Analyze

flow cytometry datasets.

### All you need is

- A computer
  - With Internet connection
- A web browser
  - With Java support
- An OpenID (e.g., Google Account)
  - Required for write access only

Summary Future Work Acknowledgments

## Site visits in the last 3 months



609 people visited this site

Additional Visits: 991

Summary Future Work Acknowledgments

## FlowRepository Visits by Country, March – June 2012



Summary Future Work Acknowledgments

## FlowRepository Visits by Country, March – June 2012



Summary Future Work Acknowledgments

# Some more stats... (as of June 13, 2012)

### But "only"

- 52 Registered users
- 31 Datasets
  - Only 8 of these public
- 4,850 FCS files (28 GB)

Summary Future Work Acknowledgments

## Some more stats... (as of June 13, 2012)

### But "only"

- 52 Registered users
- 31 Datasets
  - Only 8 of these public
- 4,850 FCS files (28 GB)

 $\rightarrow$  Please share your data.

Summary Future Work Acknowledgments

# Additional resources

- Spidlen J, Breuer K and Brinkman RR. Preparing a Minimum Information about a Flow Cytometry Experiment (MIFlowCyt) Compliant Manuscript Using the International Society for Advancement of Cytometry (ISAC) FCS File Repository (FlowRepository.org). *Curr Protoc Cytom*. 2012 Jul; Chapter 10: Unit 10.18.
- Kotecha N, Krutzik PO and Irish JM. Web-based analysis and publication of flow cytometry experiments. *Curr Protoc Cytom.* 2010 Jul; Chapter 10: Unit 10.17.
- Spidlen J, Breuer K, Rosenberg C, Kotecha N and Brinkman RR. FlowRepository – A Resource of Annotated Flow Cytometry Datasets Associated with Peer-reviewed Publications. (submitted)
- FlowRepository quick start guide: https://flowrepository.org/quick\_start\_guide
- Cytobank documentation site: http://docs.cytobank.org
## What new features to expect... (development in progress)

- FlowRepository Application Programming Interface
- ightarrow Third party software will be able to
  - Directly work with datasets saved in FlowRepository
  - Deposit data to FlowRepository on your behalf
  - In fact, initial implementation already exists in FlowJo<sup>TM</sup> (for now, only read access to FlowRepository is provided and only basic data and annotations shared)
  - If you are a Flow Cytometry Software vendor, please talk to us!

Introduction FlowRepository Summary & Conclusions Summary Future Work Acknowledgments

What new features to expect... (development in progress)

#### MIFlowCyt Reports



< i> 🕲 🕼 localhost:8080/public\_experiment\_representations/1/print\_cyt\_a

#### Cytometry Part A Author Checklist - MIFlowCyt-Compliant Items for Experiment Test 01 (Repository ID: FR-FCM-ZZZY)

Requirement	Requested Information	
1.1. Purpose	Test, new clean install	
1.2. Keywords	test	
1.3. Experiment variables	Dottion Soling of A. A02. A07. B03. B08. B08. B10. B11. D01. D03   - 200 up of A. A02. A08. B03. B04. B05. C01. C04. D06 D06   - 200 up of A. A02. A09. B02. C03. C06. C09. D02. D07. D08 D06   Endodualitie - A04. A06. A07. B08. C02. C01. D04. D06. D06 D06   - A08. A06. A07. B08. C02. D01. D04. D06. E04 - A06. A09. B07. B09. B10. D02. D03. D09. E01 E06. E07. E09. F02   - Mocare J. B07. B09. B10. D02. D03. D09. E01 E06. E07. E09. F02 - A06. A09. A07. A08. B03. B08. D02. C03. D04. B07. E09. F02   - Mocare J. B07. B09. B10. D02. D03. D09. E01 E06. E07. E09. F02 - A06. B03. E09. B11. C04. C07. D01   - Mocare J. A07. A08. B03. B08. B08. B08. B08. B03. E09. F02. C03 - antionmalismed in for 6 hours: A07. A08. B01. B08. D07. D01 D01. D01. D01. D01. D01. D01. D01.	
1.4. Organization name and address	None	
1.5. Primary contact name and email address	Josef Spidlen, jspidlen@gmail.com	
1.6. Date or time period of experiment	2011-01-01	
1.7. Conclusions	None	
1.8. Quality control measures	None	
2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description	All FCS files: Sample template 1	
2.1.1.2. (2.1.2.1, 2.1.3.1) Sample source description	Description Applicable ID   Additionable ID Additionable ID Additionable ID   Additionable ID Additionable ID Additionable ID Additionable ID   Mouse Additionable ID Additionable ID Additionable ID Additionable ID   Mouse Additionable ID Additionable ID Additionable ID Additionable ID   Mouse Additionable ID Additionable ID Additionable ID Additionable ID   Mouse Additionable ID Additionable ID Additionable ID Additionable ID   Mouse Additionable ID Additionable ID Additionable ID Additionable ID Additionable ID   Mouse Additionable ID Additionable ID Additionable ID Additionable ID Additionable ID Additionable ID   Mouse Additionable ID Additionable ID Additionable ID Additionable ID Additionable ID Additionable ID   Mouse Additionable ID Additionable ID Additionable ID Additionable ID Additionable ID Additionable ID   Mouse AdditiD Addi	
2.1.1.3.1 Source organism taxonomy	Taxonomy Applicable to Mars Traucular Section 2010 Control 1000 Control 10000 Control 10000 <th< th=""></th<>	
	Age Applicable to 21 weeks 2nd Settings	

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### What new features and changes to expect...

- Relaxing some of the required annotations
- Annotation export in spreadsheet form
- User configurable inbox view
- Complete one click dataset download (with all attachments, illustrations, etc.)
- Better instrumentation support based on defaults obtained from manufactures
- Better performance for datasets with thousands of FCS files
- Better support for experiment variables (including continuous variables)
- Additional sanity checks, e.g., annotations vs. contents of the data files, possibly with the use of ontologies

### What new features and changes to expect...

- Better import, export and merging
- Improved interface (e.g., more "smart" auto-complete)
- Better support for CyTOF
- Support for the Human Immunology Project Consortium (HIPC) Lyoplates
- Continuous improvements and fixes

### What new features and changes to expect...

- Better import, export and merging
- Improved interface (e.g., more "smart" auto-complete)
- Better support for CyTOF
- Support for the Human Immunology Project Consortium (HIPC) Lyoplates
- Continuous improvements and fixes
- Also, whatever the users ask for
  - As long as it is feasible to implement within our budget
  - This may be a good time to suggest additional features and improvements

#### Acknowledgments

BC Cancer Agency	Ryan Brinkman, Karin Breuer, Patrick Tan, Nima Aghaeepour, Mehrnoush Malekesmaeili
Cytobank, Inc.	Nikesh Kotecha, Chad Rosenberg, Jennifer Davis, Chris Coveney, Christina Dong, Robin Powell, Jonathan Irish, Amy Lee
Carnegie Mellon University	Bob Murphy, Thom Gulish, Mark Held, Kimble Marshall, William Love
NIH NIAID VRC	Mario Roederer
Cytometry A	Attila Tarnok
ISAC	Todd Philbrick

ISAC Terry Fox Foundation Terry Fox Research Institute Wallace H. Coulter Foundation Michael Smith Foundation for Health Research Introduction FlowRepository Summary & Conclusions Summary Future Work Acknowledgments

#### The End

# *Thank you! Questions and Comments?*



Josef Špidlen, Ryan R. Brinkman

FlowRepository.org - Resource of Flow Cytometry Data