





Sharing and Annotating Data in Compliance with MIFlowCyt: the Minimum Information about a Flow Cytometry Experiment

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Why share your data?

Carrots are tasty

- Promote open scientific inquiry and progress in the field
- Re-exploration of existing datasets
- Reproducible research
- Get credited for sharing good quality data
 - Thomson Reuters Data Citation Index
- Sticks hurt
 - Required by funding agencies and journals

What to share?

A dump of FCS files is not enough Data without context are not understandable to others

<u>Cytometry A</u>, Author manuscript; available in PMC 2009 Nov 5. Published in final edited form as: <u>Cytometry A. 2008 Oct; 73(10): 926–930,</u> doi: 10.1002/cyto.a.20623 PMCID: PMC2773297 NIHMSID: NIHMS76346

MIFlowCyt: The Minimum Information about a Flow Cytometry Experiment

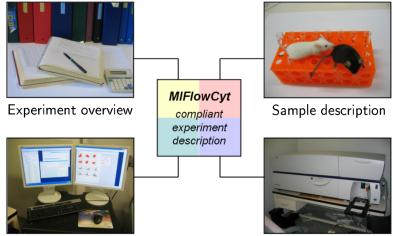
Jamie A. Lee, 'Josef Spidlen,[†] Keith Boyce,[‡] Jennifer Cai, 'Nicholas Crosbie,[§] Mark Dalphin,[†] Jeff Furlong,^{II} Maura Gaspareto,[†] Michael Goldberg,[#] Elizabeth M. Goralczyk,^{*} Bill Hyun,^{††} Kirstin Jansen,^{III} Tobias Kollmann,^{‡†} Megan Kong, 'Robert Left,^{§§} Shanon McWeeney,^{§†} Thomas D. Moloshok,^{**} Wayne Moore,^{III} Garty Nolan,^{##} John Nolan,^{***} Janko Nikolich-Zugich,^{†††} David Parrish,[‡] Barclay Purcell,^{‡‡†} Yu Qian, 'Biruntha Selvaraj,^{‡‡‡} Clayton Smith,[†] Olga Tchuzakina,[#] Anne Werthelmer,^{§§§} Peter Wilkinson,¹⁹⁷ Christopher Wilson,^{II} James Wood,^{IIIIII} Robert Zigon,^{###} the International Society for Analytical Cytology Data Standards Task Force, <u>Richard H. Scheuermann</u>,^{******}; and <u>Ryan R.</u> Brirkman[†]

Author information
Copyright and License information
JAL and JS contributed equally to the work in this article

- Outlines the minimum information required to report about flow cytometry experiments
- Represents the community consensus
 - ISAC Recommendation
- Required/recommended by Cytometry A and Nature



MIFlowCyt components



Instrumentation

Data analysis

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

MIFlowCyt summary

- Formalizes what to describe so that others understand
- Does not prescribe how to share
- Does not prescribe how to do what you do

How to share all these details? You are doing most of it already

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



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 and journal integration
- Open source
 - Affero General Public License
- Supported by ISAC, ICCS and ESCCA
- Hosted by Carnegie Mellon University

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 work but may not look great
- Ability to run Java Applets in the Web browser
 - Required for online analysis and FCS de-identification

Accessing FlowRepository

- Navigate your Web browser to https://flowrepository.org
- Demonstration (offline)
 - Access FlowRepository
 - Deposit, annotate and share a dataset

😣 😑 💿 🛛 FlowRepository - Mozilla Firefox FlowRepository ▼ C Q Search ☆ 自 ♣ 0 = https://flowrepository.org

FI WRepository

Login

The following open acces how to upload and annota data sets: Spidlen J. Breu R. 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), Current Protocols in Cytometry, UNIT 10.18, July 2012.

We also have a Quick start guide and a FAQ section.

You may download slides from our Workshop at CYTO 2012: Publishing MIFlowCyt Compliant Data to ISAC's FlowRepository.org for Cytometry A and Other Journals

Additional links and help options are listed in our support page.

You can contact us by filling out a support ticket.

Citing FlowRepository

Please reference us by citing: Spidlen J, Breuer K, Rosenberg C, Kotecha N and Brinkman RR. ElowRepository - A Resource of Annotated Flow Cytometry Datasets Associated with Peer-reviewed Publications. Cytometry A. 2012 Sep; 81(9):727-31.

Supporting Journal



s article describes te flow cytometry	FlowRe
er K and Brinkman	

pository is a database of flow cytometry experiments where you can guery and download data collected and annotated according to the MiFlowCyt standard.

Enter a term to search all publicly available experiments: Ouerv Show query fields Browse public datasets Browse OMIP datasets Referencing Flow Repository and Cytobank Browse community datasets Quick start guide FlowRepository Steering Committee & Advisory Board Browse most popular datasets Submit data Funding

Register/login in order to be deposit data

FlowRepository ×		
C Ahttps://flowrepository.org/lo	gin	
	Welcome to FlowRepository	
	Click an Icon to Login with OpenID	
	(Arhooi)	
	OR Enter your OpenID URL	
	http://	
	Or use Google+	
	g+ Sign in	
	Or Sign-in with Facebook	
	🖬 Log In	
	Or Login with your Email and Password Email: ohn.smith@yahoo.com	
	Password:	
	Log-in with email and password	
	New Users	
	You will need to create an account on FlowRepository before you	
	can login with your Open ID, Google+ or Facebook account.	
	Click Here to Register	
	Open ID is a way for users to use a single digital identity across the internet. You can use this identity to login to sites supporting openid.	
	How do you get one? Several sites are providing and supporting open ids. Google stopped supporting OpenID in April 2015. Google account	

Registration

Register for FlowReposito							
Open ID is a way for users to use a single digital identity across the internet. You can use this identity to login to sites supporting openit. How do you get over 3 Several lites are providing and supporting open ids. Google stopped supporting Openity in April 2015. Google account users can use Google+ Sign-In instead. Facebook users may use Facebook. Click here if you need help with this form.							
First Name	John						
Last Name	Smith						
	Sign-in with Google+ or Facebook, or select your Open ID provider						
	Start Sign in						
	F Log In						
Open ID Provider	Yahoo 🔻						
Contact Email	john.smith@yahoo.com						
	You can also opt-out and use an email/password login only (note that email / password login is always available even if you register using one of the above).						
	Email / Password Sign-In Only						
Contact Email	john.smith@yahoo.com						
	This shall match your Open ID, Facebook or Google+ registered email.						
Privacy	Do not display my email address to other users						
	Optional Information						
Select Password							
Confirm Password							
	Passwords can be used instead of OpenID, Google+ or Facebook login. Please use 8 or more characters and include a number, an upper-case character and a lower-case character.						
ResearcherID	G-1234-2015						
(http://www.researcherid.com)							
ORCID (http://orcid.org/)	0001-0001-1234-1234						
Location	Vancouver, BC, Canada						
Company/Organization	BC Cancer Agency						
How did you find out about FlowRepository?	In an article/publication •						
	rant or assume any legal liability or responsibility for the accuracy, completeness, or						
	sefulness of any information displayed on this website. e to the FlowRepository Terms Of Service						
Tagre	A AN AND <u>EINTHANANAMANE EINTHANANAMANANA</u>						
	Register						

Share your data - 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
 - Or upload third party analysis files (e.g., FlowJo workspaces, FCS Express projects, FACS Diva files, etc.)
- Seview (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone

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Create a new experiment

* 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.

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 •

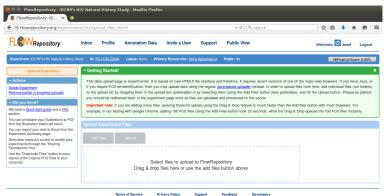
Create a new experiment (continued)

Keywords (optional)	HIV, AIDS Free Survival, Bioinformatics
Organizations (optional)	None 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

Upload and annotation of your own dataset

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Data upload (Option 1, HTML5-based)



Terms of Service Privacy Policy Feedback Developers

Data upload (Option 1, HTML5-based)

😝 💿 🕤 FlowRepository - IDCRP's H	HV Natura	l History St	udy - Mozilla Firef	x			
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FL WRepository	Inbox	Profile	Annotation Data	Invite a User	Support	Public View	Welcome, 🙆 Josef Logout
Experiment: IDCRP's HIV Natural History St	udy ID: <u>Fi</u>	R-FCM-ZZNW	Labels: None Pri	mary Researcher: <u>Nir</u>	ha Aghaeepour	Public: No	MIFlowCyt Score: 0.00%
* Back to Experiment	- Gettin	g Started!					×
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You can print/save your illustrations to PDF from the illustration view's left menu.	Upload	Experiment	Files				
You can export your data to Excel from the Experiment Summary page. Give other users thil 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.	x	167004.1cs 167005.1cs	Upload				
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	You can stil	Drag and Drop	fles here. Hit the Upload bu	tion when you are ready.			

Data upload (Option 1, HTML5-based)

Solution Constant Strength Str	HIV Natur	at History Si	udy - Mozilia Pilero	^			
e flowrepository.org/experime	nts/754/up	load_files_ht	:ml5			▼ C Q Search	☆ 自 ♣ 合 ❷ 〓
FL WRepository	Inbox	Profile	Annotation Data	Invite a User	Support	Public View	Welcome, 🕑 Josef Logout
Experiment: IDCRP's HIV Natural History 5	tudy ID:	R-FCM-ZZNW	Labels: None Prin	nary Researcher: <u>Nir</u>	na Aghaeepour	Public: No	MIFlowCyl Score: 0.00%
Back to Experiment	- Gett	ng Started!					×
Actions Detet Experiment Remove partial in-progress uploads Did you know? We have a Quick start guide and a EAQ section.	if you to the you si Impor	require FCS de upload list by a nould be redired tant note: If ye	Hentification, then you dropping them in the uple ted back to the experim ou are adding many files	may upload data usi ad box (preferable) o ent page once all file , queuing those for u	ng the regular <u>Ja</u> or by selecting th is are uploaded a pload using the I	wa-based uploader instead. In ord nem using the Add Files button (les and processed on the server. Drag & Drop feature is much faste	lons of one of the major web brevens. If you have Java, or er to upload little from here, add individual little (not tolders) as preferable), and it the Upload buffor. Please be patient, it than the Add files button with most browsers. For e the Drag & Drop queued the 100 FCS files instantly.
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	x	167209.1cs	5%				
	x	167297.1cs					
	x	167310.1cs					
	x	167311.fcs					
	Uploading						

Terms of Service Privacy Policy Support Feedback Developers

Data upload (Option 2, Java-based)

Experiment: IDCRP's HIV Natural History Stud	iy ID: FR-FCM-ZZZB	Labels: None	Primary Researcher: Nima Aghaeep	pur Public: No	MIFlowCyt Score: 0.00%
« Back to Experiment	This experiment does	not have any FC	S files unloaded vet		
+ Actions			o moo aproadoo you		
Delete Experiment	▶ Getting Started!				×
→ Did you know?	Upload Experimen	t Files			
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	1	Upload	Progress: 0%		

Terms of Service Privacy Policy Support Feedback

Data upload (Option 2, Java-based)

Experiment: IDCRP's HIV Natural History Stud	ly ID: FR-FCM-ZZZB Labels: None I	Primary Researcher: <u>Nima Aqhaee</u> j	xour Public: No M	FlowCyt Score:
« Back to Experiment	This experiment does not have any FCS	files unloaded vet		
- Actions	This experiment uses not have any FCS	nies 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 filling out a support ticket.	Browse For Fo	ilder Upload Selec	ted Files	
A guide to Cytobank is available at <u>Current</u> <u>Protocols in Cytometry</u>	Select <u>A</u> ll Deselect All	Select Flow Files	Deselect Flow Files	5
We also have a <u>Quick start guide</u> . You can print/save your Illustrations to PDF from the Illustration view's left menu.	De-identi	fy all FCS files before upl	oading	
You can export your data to Excel from the Experiment Summary page.	Files: Filename	FC	S Version Upload?	
Give other users full control to modify your	203037.fcs - 203037.fcs	FCS		-
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	294897.fcs - 294897.fcs	FCS	2.0	
	319267.fcs - 319267.fcs	FCS	2.0	
	251284.fcs - 251284.fcs	FCS		
	997430.fcs - 997430.fcs	FCS	2.0	
	122405.fcs - 122405.fcs	FCS	2.0	
	846228.fcs - 846228.fcs	FCS		
	130119.fcs - 130119.fcs	FCS	2.0	
	306870.fcs - 306870.fcs	FCS		
	978630.fcs - 978630.fcs	FCS	2.0	-
	Upload Pr	ogress: 0%		

Feedback

Data upload - de-identification (needs Java)

☑ 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

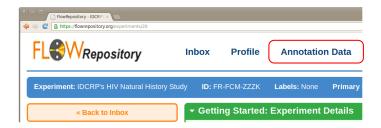
Implementation in FlowRepository

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

Upload and annotation of your own dataset

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Prepare annotation data



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

Prepare annotation data – reagents

	- Actions		Getting Starte	d with MIFlow	Cvt Annotation	hel																										
Add new keyword Add new cranization Add new cranization Add new reagent Add new reagent Add new restingent Add new remolate for samples				Ť	Ifacturers Reag		iments TOrgani	sms Templates																								
	Add new template for sample sources		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]	<u>Edit</u>	Remove																						
	A guide to Cytobank is available at Current Protocols in Cytometry		CD14	anti-CD14	PE-Cy7	M5E2	eBio#25- 0149	eBioscience [website]	Edit	Remove																						
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	computer.																															

• Define reagents used in the dataset

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	anisms	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>	Remove		
A50- Universal	Apogee Flow Systems http://www.apogeeflow.com		<u>Edit</u>	Remove		
Accuri C6	Becton Dickinson (BD Biosciences) http://www.bdbiosciences.com		Edit	Remove		
Attune	Applied Biosystems http://www.appliedbiosystems.com		Edit	Remove		
Auto-A40	Apogee Flow Systems http://www.apogeeflow.com		<u>Edit</u>	Remove		

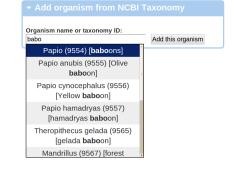
Prepare annotation data – organisms

- 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)
 - $\rightarrow\,$ We only show what has been used or explicitly requested

Keywords Organizations Manuf	acturers Reagents Instruments Orga	anisms Templates
Add organism		
Search:		
NCBI Taxonomy ID	Scientific name	Genbank common name
2	Bacteria	eubacteria
3055	Chlamydomonas reinhardtii	Chlamydomonas reinhardtii (green algae)
4890	Ascomycota	ascomycetes
4932	Saccharomyces cerevisiae	baker's yeast
9541	Macaca fascicularis	crab-eating macaque
9544	Macaca mulatta	Rhesus monkey
9606	Homo sapiens	human
9999	Spermophilus parryii	Arctic ground squirrel
10090	Mus musculus	house mouse
10116	Rattus norvegicus	Norway rat

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



Prepare annotation data - templates

• Start with sample sources

ions Manufacturers	Reagents	Instruments	Or	ganisms	Templates	
for samples for sample sources						
Template N	lame	User	\$	Public	\$	\$
Sample template Kollmann #1		Karin Breuer		yes	Show	<u>Remove</u>
JS Sample 1		Josef Spidlen		no	Show	<u>Remove</u>
sample source temp Kollmann #1	olate	Karin Breuer		yes	Show	<u>Remove</u>
12w MOLD/RkJ M mouse		Josef Spidlen		no	Show	<u>Remove</u>
	for sample sources Template N Sample template K JS Sample 1 sample source temp Kollmann #1	for sample sources Template Name Sample template Kollmann #1 JS Sample 1 sample source template Kollmann #1 12w MOLD/RkJ M mouse	Image: Sample sources User Sample template Name User Sample template Kollmann #1 Karin Breuer JS Sample 1 Josef Spidlen sample source template Kollmann #1 Breuer 12w MOLD/RkJ M mouse Josef Spidlen	Image: sources User Template Name User Sample template Kollmann #1 Karin Breuer JS Sample 1 Josef Spidlen sample source template Kollmann #1 Breuer 12w MOLD/RkJ M mouse Josef Spidlen	Image: sources User Public Template Name User Public Sample template Kollmann #1 Karin Breuer yes JS Sample 1 Josef Spidlen no sample source template Kollmann #1 Breuer yes 12w MOLD/RkJ M mouse Josef Spidlen no	Image: Sample sources User Public Public Sample template Kollmann #1 Karin Breuer yes Show JS Sample 1 Josef Spidlen no Show sample source template Kollmann #1 Karin Breuer yes Show 12w MOLD/RkJ M mouse Josef Spidlen no Show

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 *	environmental •	
Description *		-
Location *		4
Other		٦
	Can	cel 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] • ? years ?	
Phenotype * Genotype *	N/A N/A	
Treatment *	Carcel	Save
	Cancer	Rave

Prepare annotation data – create sample templates

Details for sample template		
Description * PBMC from HIV+ Subj	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 Save	

- 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
 - Or upload third party analysis files (e.g., FlowJo workspaces, FCS Express projects, FACS Diva files, etc.)
- Seview (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone

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

Use your favorite spreadsheet editor

eile	annotations.csv - Li Edit View Insert Format							
-	• 🛎 🔮 🖻 🔽 💈	1 🖴 💩	rec 🤒 😽 🗓	2 🔟 + 🏄 🔕 + 🤄 -	🗟 🏷 🛼 🧉 🖉 🗧	÷ 🖻 🖬 😫		
	Liberation Sans 1	10		E 🗉 🗉 📖 🤳	5 100 100 III III III	• 🔳 • 🏡 • 🕎		
P1	<i>f</i> (ω) Σ	=						
1	FCS File	Age	Gender	Condition	E	F	G	H A
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				
Sheel		1	Default			Sum=0	<i>\</i>	• • • • • • • • • • • • • • • • • • •

Save as a CSV (Comma Separated Values) file

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

- 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
 - Or upload third party analysis files (e.g., FlowJo workspaces, FCS Express projects, FACS Diva files, etc.)
- Review (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone

- 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
 - Or upload third party analysis files (e.g., FlowJo workspaces, FCS Express projects, FACS Diva files, etc.)
- Seview (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone



I Howepository - Iocki + X	/11							٩
« Back to Inbox	- Getting Start	ed: Experiment Details					×	
My Working Illustration »	This Experime	ent Details Page contain	s informatior	about the experiment	listed al	oove.		
MIFlowCyt Annotation »		ribing samples						
- Actions		ytometer information periment variables						
Experiment Edit Experiment Details	 Analyze y 	our data on-line						
Delete Experiment	 <u>Review M</u> <u>Download</u> 	FlowCyt annotation FCS files						
FCS Files Download FCS Files	For more tips	and guides please see:						
Upload More FCS Files De-identify FCS Files		<u>sitory Quick start guide</u> ation site for Cytobank a	nd FlowRep	ository				
Review Keywords in FCS files			·····					
- Sharing Permissions ()	▶ Experiment D)etails					Û	
Full Access Users	→ Illustrations						6	
O Nima Aghaeepour [PR]	▶ Attachments							
Josef Spidlen [x]	- FCS Files (46	6)					()	
O Ryan Brinkman [x]	Download Files Upload	More Files De-identify FCS Files Re	eview Keywords in	FCS files				
Invite a new user	File Name	Sample	Tube Name	Experiment Variables	Panel	Events	Size	
Share with a User (Full Access)	100715.fcs details	100715.fc describe sample	Tube_025		Panel 1	65016	4 MB	
	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 to share with reviewers.	109567.fcs details	109567.fcs describe sample	Tube_017		Panel 1	160074	9.8 MB	
Share with Reviewers	110539.fcs details	110539.fcs describe sample	Tube_022		Panel 1	364212	22.2 MB	
	113548.fcs details	113548.fcs describe sample	Tube_003		Panel 1	177102	10.8 MB	
➡ Did you know?	121069.fcs details	121069.fcs describe sample	Tube_001		Panel 1	542538	33.1 MB	
You can request a one-on-one session to get started with your data by filling out a	122405.fcs details	122405.fcs describe sample	Tube_010		Panel 1	476208	29.1 MB	

Describing samples

- 100715.fcs Sample	Information
Apply template:	Create templates
Description *	
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 BBi#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 Apply to undescribed FCS files in Panel 1

Describing samples – apply a template

▼ 100715.fcs Sample	Information
Apply template:	PBMC from HIV+ Subject Template Create templates
Description *	PBMC from HIV+ Subject
Sample source	• 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 I CD86, CD86, PE (eBioscience eBio#12-0869)
Staining cocktail(s)	None 🔺
	Cocktail no
	- New
	Save and proceed to next FCS file Apply to all undescribed FCS files Apply to undescribed FCS files in Panel 1

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)

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

Describing samples -3 options to save

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 I CD86, CD86, PE (eBioscience eBio#12-0869) Very
Staining cocktail(s)	None 🗅
	Cocktail no
	- New
C	Save and proceed to next FCS file Apply to all undescribed FCS files Apply to undescribed FCS files in Panel 1
C	and proceed to hear the report to an anacconsect the mes report to anacconsect the mes in trainer in

Samples and sample sources are now described

	ZB Labels: None Prima	ry Researcher: <u>Nin</u>	na Aghaeepour Public: N	lo <mark>MII</mark>	lowCyt Score	e: 61.50%
→ Getting Starte	ed: Experiment Details					×
► Experiment D)etails					i
→ Illustrations						i
→ Attachments						i
→ FCS Files (46	6)					i
Download Files Upload File Name	1 More Files <u>De-identify FCS Files</u> Sample	Review Keywords in Tube Name	FCS files Experiment Variables	Panel	Events	Size
100715.fcs details	show sample description	Tube_025		Panel 1	65016	4 MB
105696.fcs details	show sample description	Tube_009		Panel 1	455184	27.8 MB
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
109567.fcs details	show sample description	Tube_017		Panel 1	160074	9.8 MB
110539.fcs details	show sample description	Tube_022		Panel 1	364212	22.2 MB
113548.fcs details	show sample description	Tube_003		Panel 1	177102	10.8 MB
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
127225.fcs details	show sample description	Tube_021		Panel 1	257058	15.7 MB
129599.fcs details	show sample description	Tube_007		Panel 1	352314	21.5 MB
	Getting Startt Experiment D Experiment D Hustrations Hitustrations Attachments FCS Files (40 Dombad Eles Upton File Name 100715.fs.details 100905.fs.details 100905.fs.details 100905.fs.details 113548.fs.details 113548.fs.details 113548.fs.details 12406.fs.details 122205.fs.details 122205.fs.details 122205.fs.details 127225.fs.details	A Getting Started: Experiment Details Experiment Details Experiment Details Hustrations Hildstrations Attachments CS Files (466) Dombad Eles Upload More Elles De Identify ECS Eles File Name Sample 100715.fs.details show sample description 106905.fs.details show sample description 106905.fs.details show sample description 10595.fs.details show sample description 10595.fs.details show sample description 11539.fs.details show sample description 112608.fs.details show sample description 112543.fs.details show sample description 122405.fs.details show sample description 122405.fs.details show sample description 127225.fs.details show sample description	A Getting Started: Experiment Details Setting Started: Experiment Details Setting Started: Experiment Details Setting Started: Sta			

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

Upload the spreadsheet as attachment

FlowRepository - IDCRF: ×							
Experiment: IDCRP's HIV Natural History	Study ID: FR-FCM-ZZZ	B Labels: None Prim	ary Researcher: <u>Nir</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					Û
MIFlowCyt Annotation »	→ Illustrations						i
- Actions	• Attachments						i
Experiment Edit Experiment Details Delete Experiment FCS Files Download FCS Files	File Name Attach a file Choose File No f Upload	Date le chosen	Uploaded By	Size	m	15sum	
Jpload More FCS Files De-Identify FCS Files Review Keywords in FCS files	→ FCS Files (46) Download Files Upload	6) More Files De-identify FCS File	s Review Keywords in	1 FCS files			i
	File Name	Sample	Tube Name	Experiment Variables	Panel	Events	Size
- Sharing Permissions	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
nvite a new user	110539.fcs details	show sample description	Tube_022		Panel 1	364212	22.2 MB
Share with a User (Full Access)	113548.fcs details	show sample description	Tube_003		Panel 1	177102	10.8 MB
	121069.fcs details	show sample description	Tube_001		Panel 1	542538	33.1 MB
This experiment is currently private.	122405.fcs details	show sample description	Tube 010		Panel 1	476208	29.1 MB

Parse the attachment – click on (P)

FlowRepository - IDCRP: ×							
By C (() https://flowrepository.org/experiments/11 Experiment: IDCRP's HIV Natural History 5	Study ID: FR-FCM-ZZZ	B Labels: None Prima	ry Researcher: <u>Nir</u>	na Aghaeepour Public: No	MIF	lowCyt Score	: 61.50%
« Back to Inbox	Attachment v	vas added to experiment.					
MIFlowCyt Annotation »	▶ Getting Starte	d: Experiment Details					×
✓ Actions	▶ Experiment D	etails					0
Experiment	→ Illustrations						Û
Edit Experiment Details Delete Experiment	- Attachments						Û
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	Attach a file	Parse the attachment to extract and	12:03 PM update annotations.	Josef Spidlen	15.5 KB	1de7	15
- Sharing Permissions							
Full Access Users	+ FCS Files (46	5)					6
O 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
alhost:8080/experiments/11/attachments/51/parse	109567.fcs details	show sample description	Tube 017		Panel 1	160074	9.8 MB

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	Μ	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	Μ	HIV Stage 3
130119.fcs	44	М	HIV Stage 1
132447.fcs	17	F	HIV Stage 1

- Create a new experiment
- Opload data (FCS files)
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- Seview (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone

Provide experimental variables

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

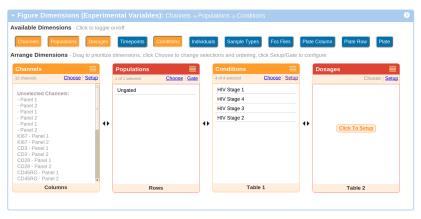


Provide experimental variables



• Example: patients treated by various dosages of LexivaTM

Provide experimental variables



- Example: patients treated by various dosages of LexivaTM
- Click on Dosages, than Setup

List doses

• Provide a comma-separated list of all doses

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

kiva 1400 bid │ Lexiva 1400 qd+Norvir 200 qd │ Lexiva 700 bid+Norvir 100	0 bid
--	-------

All Doses

Drag files from "Untagged" box to the "Dose" boxes below to associate them with that tag. Use the "Filter" and "Move File" controls to move groups of files.

Untagged	Lexiva 1400 bid Tagged Files	X Lexiva 1400 qd+Norvir 200 qd
Filter	100715.fcs (Tube_025)	Tagged Files
Move to	105696.fcs (Tube_009)	109025.fcs (Tube 009)
Move file(s)	108701.fcs (Tube_001)	110539.fcs (Tube_022)
134892.fcs (Tube_021)	127225.fcs (Tube_021)	121069.fcs (Tube_001)
140801.fcs (Tube_022) 145618.fcs (Tube_011)	130119.fcs (Tube_001)	122405.fcs (Tube_010)
158322.fcs (Tube 001)	132447.fcs (Tube_013)	
158483.fcs (Tube_012)	12276	9.fcs (Tube 002)
159665.fcs (Tube_004)	10210	9.rcs (1ube_002)
162173.fcs (Tube_005)		
162520.fcs (Tube_017)		
166139.fcs (Tube_010)		

- Create a new experiment
- Opload data (FCS files)
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- Seview (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone

• Navigate to the details of an FCS file

							_
C (O https://flowrepository.org/experiments/11 Experiment: IDCRP's HIV Natural History Stu-	dv ID: FR-FCM-ZZZ	ZB Labels: None P		her: <u>Nima Aqhaeepour</u> Public: No			
Experiment: IDCRP's HIV Natural History Stu	uy ID: FR-FCM-222	Labers: None P	nmary Researc	ner: <u>Nina Agnaeepour</u> Public: No	MIFlow	Cyt Score:	56.00%
« Back to Inbox	→ Getting Starte	ed: Experiment Deta	ails				×
My Working Illustration »	→ Experiment D	etails					i
MIFlowCyt Annotation »	→ Illustrations						i
- Actions	Attachments						6
Experiment	- FCS Files (46	6)					6
Edit Experiment Details Delete Experiment	Download Files Uploar File Name	I More Files De-identify FCS Sample	Files Review Ke Tube Name	ywords in FCS files Experiment Variables	Panel	Events	Size
FCS Files Download FCS Files	100715.fcs details	show sample description	Tube_025	HIV Stage 1, Lexiva 1400 bid	Panel 1	65016	4 MB
Upload More FCS Files	105696.fc details	show sample description	Tube_009	HIV Stage 4, Lexiva 1400 bid	Panel 1	455184	27.8 MB
De-identify FCS Files Review Keywords in FCS files	108701.fcs details	show sample description	Tube_001	HIV Stage 3, Lexiva 1400 bid	Panel 1	1000000	61 MB
- Sharing Permissions			-	HIV Stage 4, Lexiva 1400 qd+Norvir 200 qd	Panel 1		12.8 MB
Full Access Users		show sample description show sample description	-	HIV Stage 2, Lexiva 700 bid+Norvir 100 bid HIV Stage 1, Lexiva 1400 qd+Norvir 200 qd	Panel 1	160074 364212	9.8 MB 22.2 MB
O Nima Aghaeepour [PR]		show sample description	-	HIV Stage 2, Lexiva 700 bid+Norvir 100 bid		177102	10.8 MB
Josef Spidlen [x]		show sample description	-	HIV Stage 3, Lexiva 1400 qd+Norvir 200 qd	Panel 1	542538	33.1 MB
O 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 Share with a User (Full Access)		show sample description	-	HIV Stage 1, Lexiva 1400 bid	Panel 1	257058	15.7 MB
		show sample description	-	HIV Stage 1, Lexiva 700 bid+Norvir 100 bid	Panel 1	352314	21.5 MB
This experiment is currently private.		show sample description show sample description	-	HIV Stage 2, Lexiva 700 bid+Norvir 100 bid HIV Stage 3, Lexiva 700 bid+Norvir 100 bid	Panel 1 Panel 1	390528 230852	23.8 MB 14.1 MB

• Press the Describe instrument settings button

FlowRepository - IDCRP1 ×	Sloc.2042	Þ									
Experiment: IDCRP's HIV Natural History St		R-FCM-Z	ZZZB	Labels: None	Primary	Resea	rcher: <u>1</u>	Nima Aghaee	pour Pu	iblic: No	MIFlowCyt Score: 66.00%
« Back to Experiment Summary	▶ 1007	15.fcs	- FCS	File Informatic	n						
	~ 1007										
Download Tab-Separated Events File Show Sample Details De-Identify the ECS file Review Keywords in the ECS file	Describe	instrume	nt setting	9							
→ Did you know?	~ 1007	'15.fcs	- FCS	File Laser Info	rmatio	on					
You can request a one-on-one session to get started with your data by filling out a support ticket.	ASF	Name	Delay								
A guide to Cytobank is available at <u>Current</u> Protocols in Cytometry	Red	0.55	-59.80								
We also have a <u>Quick start guide</u> . You can print/save your Illustrations to PDF	Violet	0.48	-24.40	_							
from the Illustration view's left menu. You can export your data to Excel from the Experiment Summary page.	Green		-82.60	File Channel I	nform	ation					
Give other users full control to modify your experiments through the "Sharing Permissions" box.		el Short		Channel Name	Gain	Bits	Amp	Range	Voltage	Amp Value	
Use the "Download Files" button to save	FSC-A				1	32		262207.0		0.0	
copies of the original FCS Files to your computer.	FSC-H				1	32		262207.0		0.0	
	SSC-A	SSC-A			1	32		261588.0		0.0	
	B515-A			KI67	1	32		261588.0		0.0	
	R780-4			CD3	1	32		261588.0		0.0	
	0710			0000	4	20		201500.0		0.0	-

• Select the make and model of the instrument used

FlowRepository - IDCRP: × FlowRepository.org/experiments/11/cs.fl										
Experiment: IDCRP's HIV Natural History Stu	idy ID: FR-FCM-ZZZB	Labels: None	Primary	Resea	ircher: <u>I</u>	Nima Aghaee	pour Pu	iblic: No	MIFlowCyt Score: 66.00%	
« Back to Experiment Summary										
→ Actions										
Download Tab-Separated Events File Show Sample Details De-identify the FCS file Review Keywords in the FCS file	File-specific instrument set	-	n provid	ed!						
🗕 Did you know?										
You can request a one-on-one sest get started with your data by filling of support ticket.	e instrument settings Instrument *							×		
A guide to Cytobank is available at Protocols in Cytometry							•			
We also have a Quick start guide.										
You can print/save your Illustrations from the Illustration view's left menu							_	_		
You can export your data to Excel f Experiment Summary page.	100/13.105 - FCS						Cancel	Save		
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	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		
	R780-A	CD3	1	32		261588.0		0.0		

• New instruments may be added in the annotation data section

FlowRepository - IDCRP1 ×								
🔶 🔮 🔇 https://flowrepository.org/experiments/11/fcs_f								
Experiment: IDCRP's HIV Natural History St	udy ID: FR-FCM-ZZZB	Labels: None	Primary R	esearchei	: <u>Nima Aghaeepou</u>	r Pu	iblic: No	MIFlowCyt Score: 66.00%
« Back to Experiment Summary								
+ Actions								
Download Tab-Separated Events File	File-specific instrument	settings have not be	en provided!					
Show Sample Details De-identify the FCS file Review Keywords in the FCS file	Describe instrument sett	ungs						
- Did you know?								
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experiments through the "Sharing Permissions" box.	BD FACSCount, Becto BD FACSScan, Becto	n Dickinson (BD Bios	ciences)			age	Amp Valu	e
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copies of the original FCS Files to your computer.	BD LSR II, Becton Dicl BD LSRFortessa, Bec	kinson (BD Bigsciend ton Dickinson (BD Bi	ces) osciences)				0.0	-
	- Bentley 150, Bentley In	nstruments	0001011003)					_
	Bentley Combi 150:, B Bentley FTS, Bentley I	Instruments					0.0	
	Bentley FTS/FCM, Ber Cell Lab Ouanta MPL.						0.0	

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

Describe instru	ument settings	
	Instrument *	
BD	D LSR II, Becton Dickinson (BD Biosciences)	•
Use defa	ault instrument	
Installation dat	tes for filters in optical paths	
	instrument has been purchased new on July 1, 2011; all optical filters and with the instrument.	are original and
	Other	
РМТ	T voltages specified within the FCS data files.	
	$\ensuremath{\boldsymbol{\mathscr{S}}}$ Use these settings for all FCS files in this experiment	
		Cancel Save

- Advanced case: Describe all details
 - Required by MIFlowCyt for customized instruments only
 - See http://flowrepository.org/quick_start_guide

Describe instrum	ent settings		
	Instrument *		-
BD LS	SR II, Becton Dickinson (BD Bios	ciences)	
Use defau	lt instrument settings		
F	low cell type	Quartz cuvette • Add new flow cell type	
Other	flow fluidics		
c	Optical paths	None Add new optical path	

- Create a new experiment
- Opload data (FCS files)
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 - Either analyze data online
 - Or upload third party analysis files (e.g., FlowJo workspaces, FCS Express projects, FACS Diva files, etc.)
- Seview (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone

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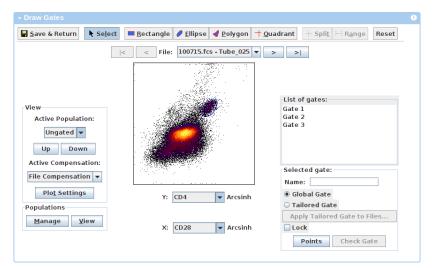
Analyze data online

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

Available Dimensions - Click to toggle on/off Channels Populations Dosages Timepoints Conditions Individuals Sample Types Fcs Files Plate Column Plate Row Plate Arrange Dimensions - Drag to prioritize dimensions, click Choose to change selections and ordering, click Setup/Gate to configure I a selected Choose Sature I a selected Choose Sature Hiv Stage 1 Hiv Stage 1 Lexiva 1400 bid Hiv Stage 2 Hiv Stage 2 Invisite 2 Kows Table 1									0
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Analyze data online

- Online data analysis is not our focus
- Only basic analysis supported



Analyze data online

More on analyzing data online in Cytobank or FlowRepository:

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

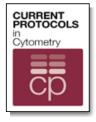
Nikesh Kotecha 1,2,3 , Peter O. Krutzik 1,2 , Jonathan M. Irish 1

Published Online: 1 JUL 2010

DOI: 10.1002/0471142956.cy1017s53

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Lab Protocol Title



Current Protocols in Cytometry

- Create a new experiment
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- Seview (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone

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

• Upload these as attachments

				i
File Name	Date	Uploaded By	Size	md5sum
annotations.csv 🐼 🕑 File specific sample source details	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 Overview figure	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 😵 Tables and stats	12:07 PM	Josef Spidlen	253.6 KB	95641c1
Extended_description.docx 😒 More details on experimental design	12:07 PM	Josef Spidlen	208.2 KB	76f301b
Attach a file				
Choose File No file chosen				

- Create a new experiment
- Opload data (FCS files)
- Prepare annotation templates
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- Share with everyone

FlowRepository - IDCRP': ×										
🔶 😋 🔇 https://flowrepository.org/exp	eriments/11									ર
Experiment: IDCRP's HIV Natural History Str	udy ID: FR-FCM-ZZZB	Labels: None Primar	y Researcher: <u>Nima</u>	a Aghaeepo	ur Public: No		M	FlowCyt Scor	e: 100.00%	
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→ Did you know?	+ FCS Files (466)								6	
You can request a one-on-one session to get started with your data by filling out a		re Files De-identify FCS Files R	eview Keywords in FC	S files						
support ticket.	File Name	Sample	Tube Name	Experim	nent Variables		Panel	Events	Size	
A guide to Cytobank is available at <u>Current</u> <u>Protocols in Cytometry</u>	100715.fcs details	show sample description	Tube_025	HIV Stage	e 1, Lexiva 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 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 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 1400 qd+Norv	/ir 200 qc	i 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%

I - Experiment Overview - 100.00% provide

Labels: None

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

ltem	Compliance [+ -]	Improve
Purpose	Provided purpose	Fully provided
Keywords	Provided keywords	Fully provided
Experiment variables	Provided experiment variables	Fully provided
Organization	Provided organization name Provided organization address	Fully provided
Contact	First 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 would show direct links to pages/forms in case some information was 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	
	Provided sample source description	

6

MIFlowCyt Score: 100.00%

٩

Upload and annotation of your own dataset

- Create a new experiment
- Opload data (FCS files)
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- Seview (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone

Data sharing

- 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 Everyone You can also create a secret access code to share with reviewers.

θ

Share with Reviewers

Share with reviewers



Share with reviewers





Experiment was successfully updated. This experiment has been locked for reviewers' access and may be accessed via the following URL:

https://flowrepository.org/id/RvFrFI5UsYaDgWZoVC6bxPrNUjMMcjlgxYxyXW5jXy62tFXyij1uHrxHvllL9nLL. Please share this URL with your reviewers.



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

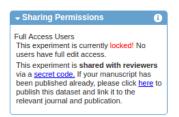
Terms of Service Privacy Policy Support Feedback
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Share your data - typical steps

- 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
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 - Or upload third party analysis files (e.g., FlowJo workspaces, FCS Express projects, FACS Diva files, etc.)
- Seview (and improve) your MIFlowCyt compliance
- Share with reviewers
- Share with everyone

Making the data public upon manuscript publication

- Depending on the journal,
 - The editor may contact FlowRepository administrators and arrange for the dataset to be published
 - Or, you may have to do this



Making the data public upon manuscript publication

Depending on the journal,

- The editor may contact FlowRepository administrators and arrange for the dataset to be published
- Or, you may have to do this

- Publish Your Experi	ment				
	xperiment public. Are you publishing your dataset because a related manuscript has been published? If so, please tell us which the manuscript identifier(s) (PubMed and/or PMC) if available already.				
PubMed ID(s):	A comma-separated list of PubMed identifiers (optional).				
PMC ID(s):	A comma-separated list of PMC identifiers (optional).				
Journal:	Nature Methods Select a related journal if available in the list (optional).				
Other journal:	Type in the name of a journal (optional, use only if journal isn't present in the list above).				
	Publish Experiment Clicking on this button will publish the experiment and also link it to a journal or publication if specified.				
Why two fields for journals?					
Journals that have been as	sociated with FlowRepository experiments in the past are included in the provided list. If a journal hasn't been linked to any				
experiment before, please just write down the name and our administratve stuff will do the rest. Specifically, we will lookup the journal, obtain its logo and URL,					
add it to the list, and link your experiment "properly".					
My PubMed ID or PMC ID is not available yet, but it will be; what do I do?					
Sometimes, PubMed IDs or PMC IDs are not available right after the publication. For now, you may leave it blank and publish the experiment. Once the PubMed					
ID and/or PMC ID is available, you can come back and link the manuscript to your experiment.					

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 and
- A web browser
 - With Java support if you want online analysis or de-identification

How can other access the data you just shared?

- From their web browser by going http://flowrepository.org
- Directly from R
 - See http://bioconductor.org/packages/FlowRepositoryR

```
jspidlen@BIOINFO17L: ~
 library(FlowRepositoryR)
 flowRep.search('OMIP')
[1] "FR-FCM-ZZ2L" "FR-FCM-ZZ2T" "FR-FCM-ZZ2V" "FR-FCM-ZZ3Z" "FR-FCM-ZZ3Y"
[6] "FR-FCM-ZZ36" "FR-FCM-ZZ74" "FR-FCM-ZZ9H"
                                               "FR-FCM-ZZAZ"
                                                             "FR-FCM-ZZEB"
[11] "FR-FCM-ZZEC" "FR-FCM-ZZWU" "FR-FCM-ZZK4" "FR-FCM-ZZX9"
[16] "FR-FCM-ZZOP" "FR-FCM-ZZSC"
ds <- flowRep.get('FR-FCM-ZZAZ')
> summary(ds)
flowRepData object (FlowRepository dataset) OMIP-022: Exemplary Data for compr
ehensive assessment of antigen-specific human t-cell functionality and memory
3 FCS files. 1 attachments. NOT downloaded
> ds <- download(ds)</p>
Downloading to /home/jspidlen/FR-FCM-ZZAZ
File AFW002244 A6 A06.fcs downloaded.
File AFW002244 B6 B06.fcs downloaded.
File AFW002244 G6 G06.fcs downloaded.
File OMIPExampleData downloaded.
Download finished.
> librarv(flowCore)
> myFCS <- read.FCS(ds@fcs.files[[1]]@localpath)</p>
 library(flowViz)
 plot(mvFCS)
```

How can other access the data you just shared?

- From their web browser by going http://flowrepository.org
- Directly from R
 - See http://bioconductor.org/packages/FlowRepositoryR
- From certain third-party software
 - Such as FlowJo :-)

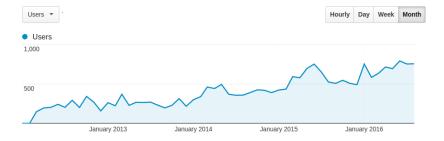
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Role	Size		<u>_</u>]				Group
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Accessing FlowRepository data from FlowJo

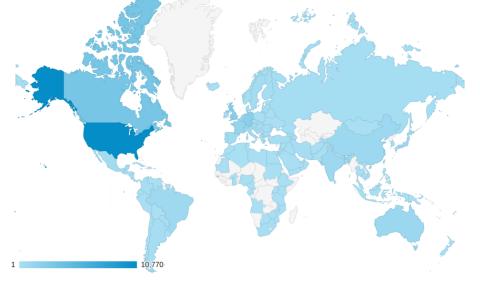




Number of distinct users monthly

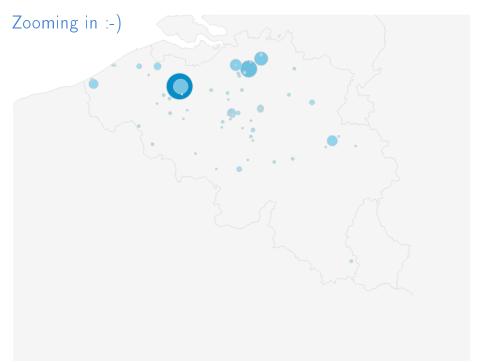


FlowRepository Visits by Country (2012 – 2016) Guess who's in the top 10:-)



FlowRepository Visits by Country (2012 – 2016) Guess who's in the top 10:-)

		At Sta				
		Country	Visits		Country	Visits
	1.	United States	10,770	11.	Japan	756
	2.	Canada 💦	3,316	12.	Switzerland	723
	3.	United Kingdom	2,176	13.	Italy	664
	74.	Germany	2,069 🌋 🥇	14.	Spain	611
	5.	France	1,382	15.	Netherlands	539
	6.	Australia	988	16.	Mexico 🦂	443
	7.	China	927	17.	Czech Republic	414
	8.	India	856	18.	Sweden	409
	9.	Brazil 🖉 🖉	793	19.	🗸 Russia 🔪 🍊	327
	10.	Belgium	756	20.	Singapore 💦	317
		1-52		J-J		
		34,500 visi	ts from 144	count	ries worldwide	× >
1		10,770				



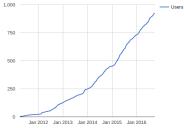
Zooming in :-)

	City	Visits
1.	Ghent	296
2.	Ranst	87
3.	Zoersel	64
4.	Antwerp	45
5.	Leuven	36
6.	Liege	33
7.	Veurne	28
8.	Brussels	23
9.	Maldegem	18
10.	lxelles	14

	· · · · · · · · · · · · · · · · · · ·	
•	City	Visits
11.	Hasselt	8
12. <	Bruges	8
13.	Charleroi	7
14.	Woluwe-Saint-Lambert	4
15.	Wavre	4
16.	Boechout	3
17.	Malle	3
18.	Dendermonde	3
19.	Nazareth	3
20.	Oudenaarde	3

Some more stats... (as of September 2016)

- 927 Registered users
- 709 Datasets
 - 349 of these public
- 84,853 FCS files (640 GB)
- 20,346 dataset downloads



Registered FlowRepository Users over Time

Please share your data.

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.
- Spidlen J, Breuer K, Rosenberg C, Kotecha N and Brinkman RR. FlowRepository – A Resource of Annotated Flow Cytometry Datasets Associated with Peer-reviewed Publications. *Cytometry A*. 2012 Sep;81(9):727-31
- Spidlen J and Brinkman RR. Use FlowRepository to share your clinical data upon study publication. *Cytometry B Clin Cytom*. 2016 Jun; doi: 10.1002/cyto.b.21393. [Epub ahead of print]
- FlowRepository quick start guide: https://flowrepository.org/quick_start_guide

Acknowledgments

BC Cancer Agency	Ryan Brinkman, Karin Breuer, Patrick Tan
Cytobank, Inc.	Nikesh Kotecha, Chad Rosenberg, Jennifer Davis, Chris Coveney, Christina Dong, Robin Powell, Jonathan Irish, Amy Lee, TJ Chen
Carnegie Mellon University	Bob Murphy, Thom Gulish, Mark Held, Kimble Marshall, William Love
NIH NIAID VRC	Mario Roederer
Cytometry A	Attila Tárnok
Wiley	Larry Graup
ISAC	Michelle Butler

ISAC Terry Fox Foundation Terry Fox Research Institute Wallace H. Coulter Foundation Michael Smith Foundation for Health Research