

ImmPort Flow Cytometry Data Analysis/FLOCK

Access the ImmPort Flow Cytometry tools via the Tools menu.



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Flow Cytometry Analysis (Beta) / Overview

[Flow Cytometry](#) | [Data Management](#) ▾ | [FLOCK](#) ▾ | [View/Edit Results](#) ▾ | [Cross Sample](#) ▾ | [Help](#)

ImmPort's flow cytometry analysis component includes:

- ▶ [Single File Upload](#), [Multiple File Upload](#) and [Dataset Generation](#)
- ▶ Automated population identification using the [FLOCK](#) algorithm for individual sample or dataset
- ▶ Centroid-based result adjustment within our [Result Adjustment System \(RAS\)](#)
- ▶ Automated mapping populations across samples for [Cross-Sample Comparison](#)
- ▶ Result visualization and statistical analysis of population characteristics



FCSTrans (ImmPort FCS file converter), FLOCK and the Centroid Adjustment algorithms are sometimes refined in order to obtain optimal results. Please be sure to check the [release notes](#) to see if recent modifications would affect your analysis.

Flow Cytometry Analysis Workflow



The Procedure of flock

Flow cytometry clustering
without K

- 1) Generate initial clusters (yes, chicken first!)
 - Parameter selection
- 2) Normalize dimensions within clusters
- 3) Select dimensions for initial clusters
- 4) Partition and merge the initial clusters in their selected subspaces
- 5) Output the final clusters

*Details of each step in following slides

Complete slide presentation available at:
http://flowcap.flowsite.org/summit2010/slides/10_Qian.pdf



As presented at FlowCAP 2010

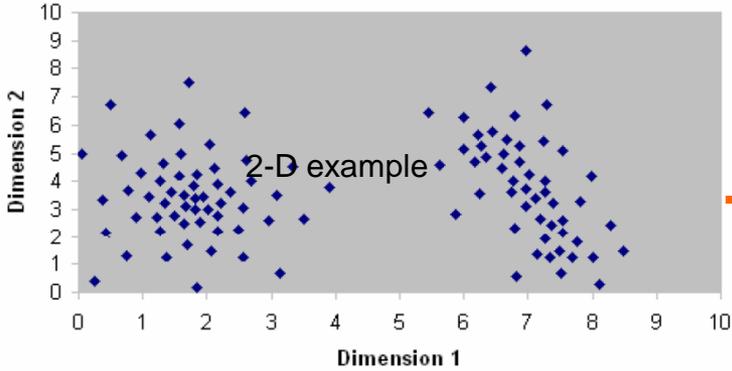
FLOCK: A Density-Based Clustering Method for Automated Identification and Comparison of Cell Populations in High-Dimensional Flow Cytometry Data

Max Yu Qian, Ph.D.

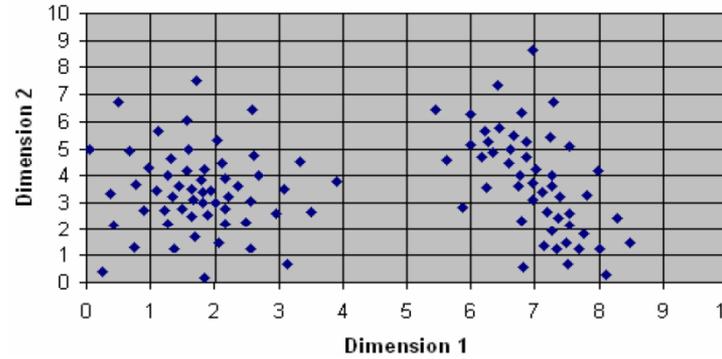
Division of Biomedical Informatics and Department of Pathology
University of Texas Southwestern Medical Center, Dallas, TX

September 21, 2010

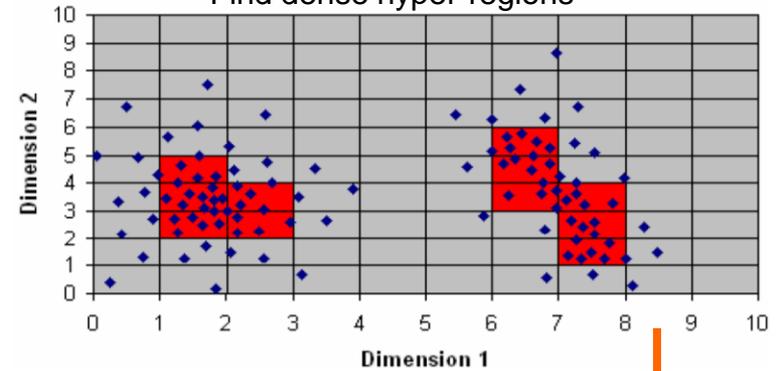
Generation of Initial Clusters



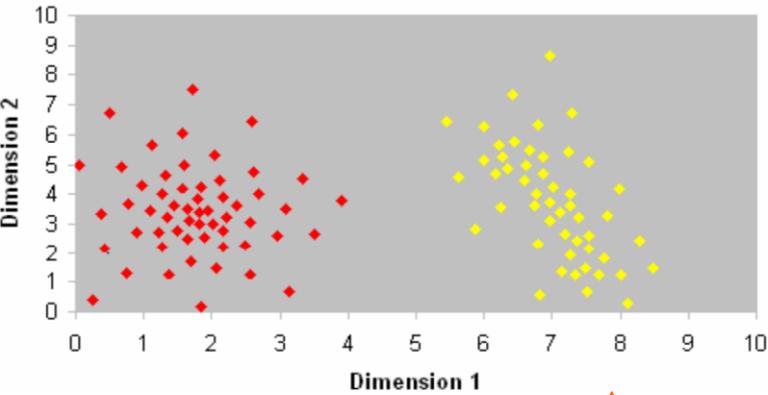
Divide with hypergrids



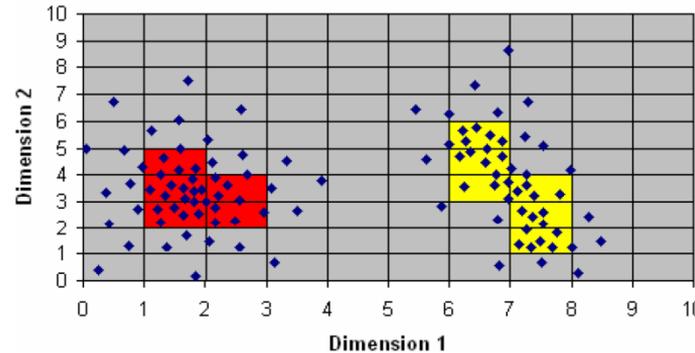
Find dense hyper-regions



Clustering based on region centers



Merge neighboring dense hyper-regions



Bin selection methods

Goal is to minimize the Mean Squared Error

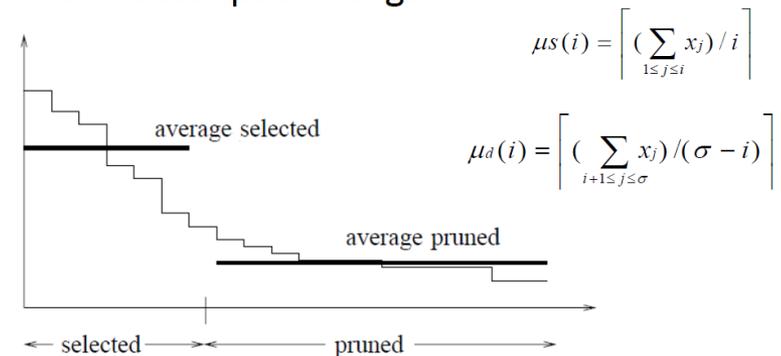
$$L(h(x), f(x)) = \int (h(x) - f(x))^2.$$

- Scott's method $v_{\text{scott}} = 3.49sN^{-1/3}$.
 - Stone's method $K(v, M) = \frac{1}{v} \left(\frac{2}{N-1} - \frac{N+1}{N-1} \sum_{m=1}^M \pi_m^2 \right)$
 - Knuth's method, to maximize
- $$N \log M + \log \Gamma\left(\frac{M}{2}\right) - M \log \Gamma\left(\frac{1}{2}\right) - \log \Gamma\left(N + \frac{M}{2}\right) + \sum_{k=1}^M \log \Gamma\left(n_k + \frac{1}{2}\right) + K$$



Density threshold selection

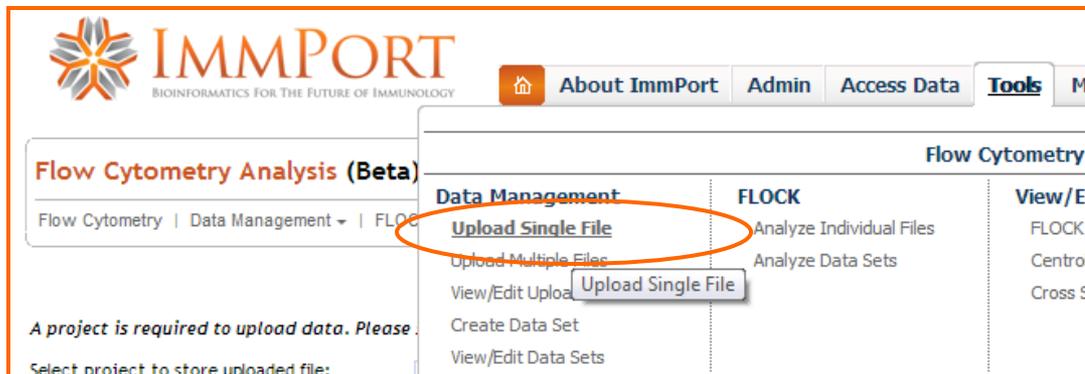
- Minimum description length



$$L(i) = \log_2(\mu_S(i)) + \sum_{1 \leq j \leq i} \log_2(|x_j - \mu_S(i)|) + \log_2(\mu_A(i)) + \sum_{i+1 \leq j \leq \sigma} \log_2(|x_j - \mu_A(i)|)$$

Flow Cytometry Data Analysis using FLOCK

Uploading single .fcs or .txt flow cytometry file



1. Select Upload Single File from Data Management dropdown menu
2. Select Research Project
3. Click Choose File to select flow cytometry file package to be loaded
4. Click 'Submit'

The screenshot shows the 'Flow Cytometry Analysis (Beta) / Data Management / File Upload' form. The breadcrumb trail is 'Flow Cytometry | Data Management | FLOCK | View/Edit Results | Cross Sample | Help'. A message states: 'A project is required to upload data. Please see the [User Guide](#) or contact the [help desk](#) for more information.' Below this is a dropdown menu for 'Select project to store uploaded file:' with '2.18 upload tests' selected. A note explains file formats: 'The uploaded data file can be in either .fcs or .txt format. The filename can't contain the following characters: ' ' \ and /. ImmPort automatically converts .fcs files to .txt files when the upload includes only .fcs files. The .txt files can be created using third party tools including Tree Star FlowJo™ on MacOS. For conversion details, please see the [help section](#).' The form has five rows: 'Name *' with a text input containing 'name of .fcs or .txt file to be uploaded'; 'Description' with a text input containing 'description of file to be uploaded'; 'Data File (.fcs)' with a 'Choose File' button and 'Test.001.fcs' text; 'Data File (.txt)' with a 'Choose File' button and 'No file chosen' text; and 'Marker File (.info)' with a 'Choose File' button and 'No file chosen' text. A red circle highlights the 'Choose File' button and 'Test.001.fcs' text for the .fcs file. A red bracket on the right side of the form groups the 'Name' and 'Description' fields. Below the form is an 'Upload' button and a note: 'NOTE: Clicking "Upload" will upload the above files into the private project workspace of your chosen project and create the data set.'

Complete name and description of single file

- [example Data File \(.fcs\)](#)
- [example Data File \(.txt\)](#)
- [example Marker File \(.info\)](#)

Flow Cytometry Data Analysis using FLOCK

Uploading multiple .fcs flow cytometry files

Flow Cytometry Analysis (Beta) / Data Management / Package Upload

Flow Cytometry | Data Management | FLOCK | View/Edit Results | Cross Sample | Help

Upload Single File
Upload Multiple Files
View/Edit Uploaded
Create Data Set
View/Edit Data Sets

Upload Multiple Files
Info File
ing the
Detected

Assemble and Submit a .zip Folder with Result Files, Spreadsheet and .info File

Review Data Submission

The ImmPort Flow Cytometry multiple file upload process supports uploading of FCS files (.fcs) and tab delimited text files (.txt) converted from FCS files. Uploaded .fcs files are automatically converted to .txt via FCSTrans, the ImmPort flow cytometry data converter. All uploaded files will be stored in a private project workspace. For more information please see the [User Guide](#) or conversion tutorials found in the menu bar under [Help](#).

Batch Upload:

1. Complete the [flowTextFiles.xls](#) template.
 - ▶ This template provides the opportunity to rename data files, allows for the addition of descriptive information about each individual file being uploaded and enables marker name changes via the inclusion of a marker information file.
 - ▶ The .fcs or fcs .txt data file names may NOT contain the following characters: " ' \ and /.
 - ▶ The fcs data files should be compensated before sending to ImmPort.
2. Save the Excel flowTextFiles.xls template as a tab delimited text file (flowTextFiles.txt). Please do not rename the flowTextFiles.txt file.
3. ZIP the completed template and the data files.
 - ▶ Create the ZIP file by selecting files from within a folder versus selecting the folder itself.
 - ▶ Add marker (channel) information files at this time if they are to be included (optional). [example Marker File \(.info\)](#)
 - ▶ Please do not include spaces in the .zip file name.
 - ▶ You may find it helpful to review an [example multiple fcs files upload package](#).
4. Select the project to which the data will be saved
5. Use the Browse feature to select the .zip file and click Submit. Time to complete the processing of submitted data is dependent on the size of the submission queue.
6. To review the uploaded data go to [Data Management, View / Edit Uploaded Data](#)

Research Project Title: 2.18 upload tests

Notes: notes about package upload

Upload Online

Choose File CAS_12Files.zip

Submit

1. Create multiple file upload package (see example data package in step 3.)
2. Select Upload Multiple File from Data Management dropdown menu
3. Select Research Project
4. Click Choose File to select flow cytometry file package to be loaded
5. Click 'Submit'

Flow Cytometry Data Analysis using FLOCK



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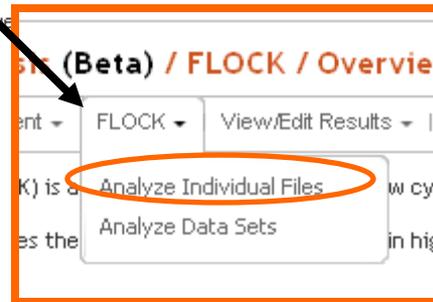
Analyze individual flow cytometry files

Flow Cytometry Analysis (Beta) / FLOCK / Overview

Flow Cytometry | Data Management | **FLOCK** | View/Edit Results | Cross Sample | Help

FLOCK (FLOW Clustering without K) is a computational approach to flow cytometry analysis which:

- ▶ Computationally determines the number of unique populations using rapid binning approach
- ▶ Can handle non-spherical hyper-shapes
- ▶ Maps populations across independent samples
- ▶ Calculates many useful summary statistics
- ▶ Finds the most informative parameters
- ▶ Reduces subjective factors in manual gating



ImmPort flow cytometry analysis module supports single or multiple file analyses. Click "FLOCK" to access analysis options or...

FCSTrans (ImmPort FCS file converter), FLOCK and the Centroid Adjustment algorithms are sometimes refined in order to obtain optimal results. Please be sure to check the [release notes](#) to see if recent modifications would affect your analysis.

Flow Cytometry Analysis Workflow



FLOCK Analysis: File selection



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..access uploaded files using Data Management dropdown menu

Flow Cytometry | Data Management ▾ | FLOCK ▾ | View/Edit Results ▾ | Cross Sample ▾ | Help

Use the 'Uploaded Files' menu to upload data files for FLOCK analysis, dataset creation or download of both .fcs files and .txt files.

The number of items to upload is limited by the size of the file and the number of files.

Select project: View/Edit Uploaded Data ▾ Include Semi-Public files in the Search **Select Project where FCS files are stored**

Upload Single File
Upload Multiple Files
Create Data Set
View/Edit Data Sets

Uploaded Flow Files Search

Search for Uploaded Files : _____ Results per page : 25 ▾

Advanced Search Attribute Tree

- Flow
 - Name
 - Text File Name
 - Description
 - Panel
 - Created By
 - Created By Last Name
 - Date Created

Flow: Name Like ▾

Flow: Description Like ▾

Use the Uploaded Flow Files Search to select search criteria for uploaded files. Clicking attributes from the Advanced Search Attribute Tree will open the search fields for input.

Select Files

Page 3 of 10 |

<input type="checkbox"/>	Name	Description	Panel	Exp Sample Acc	FCS Text File	Date Created	
<input type="checkbox"/>	CD4 purified		FSC SSC FL-HA CD25 CD		DD082009_012.txt	2010-10-28	
<input type="checkbox"/>	Spleen-pLN		FSC SSC FL-HA CD25 CD		DD082009_009.txt	2010-10-28	Thomson
<input type="checkbox"/>	Mfile		FSC-A FSC-H FSC-W SSC-A		d10_H3_H03.txt	2010-10-21	Thomson
<input type="checkbox"/>	Specimen_001_Tube_01		Forward Scatter Side Sc		Specimen_001_Tube_01	2010-10-06	Thomson
<input type="checkbox"/>	Specimen_001_Tube_01		Forward Scatter Side Sc		Specimen_001_Tube_01	2010-10-06	Thomson
<input type="checkbox"/>	Specimen_001_Tube_01		Forward Scatter Side Sc		Specimen_001_Tube_01	2010-10-06	Thomson
<input type="checkbox"/>	Specimen_001_Tube_00		Forward Scatter Side Sc		Specimen_001_Tube_00	2010-10-06	Thomson
<input type="checkbox"/>	Specimen 001 Tube 00		Forward Scatter Side Sc		Specimen 001 Tube 00	2010-10-06	Thomson

Use buttons to access file details, Run FLOCK analysis, save files to datasets and download files

Search results appear in the table above. All files uploaded to the selected project will appear in this table if no search criteria is defined

FLOCK Detail

Project: TESTING: Bioinformatics Integration Support Proj ▾
 Name:
 Description:

FLOCK Version 📄: 2.0
 FLOCK Parameters: Auto

Provide analysis-specific descriptive information

Auto-detect is the default parameter setting. For more parameter selection information please review the User Guide

Use check boxes to select markers to be included in analysis. Markers to be excluded from analysis may include unused markers/channels, limiting multiple scatter parameters (FSC-A, FSC-H, FSC-W) to one parameter (e.g., FSC-H) or pre-gated markers. Please review [the Marker Selection tutorial](#) or [the User Guide](#) for more information.

Column Selection for 3 Files

Show Assigned Name
 Show Original Name
 Select All Markers
 Clear All Markers

Name	Marker #	<input checked="" type="checkbox"/>					
		1	2	3	4	5	6
FCS003	6	FSC-H	SSC-H	FL1-H	FL2-H	FL3-H	FL4-H
FCS001	6	FSC-H	SSC-H	FL1-H	FL2-H	FL3-H	FL4-H
FCS002	6	FSC-H	SSC-H	FL1-H	FL2-H	FL3-H	FL4-H

- Show assigned name:** select this button to display user-assigned marker names
- Show original name:** select this button to display marker names original to the uploaded .txt or .fcs file
- Select All, Clear All Markers:** Use buttons and column check boxes to select markers to be included in the FLOCK analysis
- Help:** pop-up provides marker column selection information. For more information please review the Marker selection tutorial found under Help in the Flow module menu

FLOCK Analysis: Results display for batch analysis

Status of FLOCK analysis

Flow Cytometry Analysis (Beta) / FLOCK / Analysis Status

Flow Cytometry | Data Management | FLOCK | View/Edit Results | Cross Sample | Help

Your FLOCK Analysis request was submitted successfully. Your task ID is **709**.

You will be sent an email when your task has been completed.

Click the "Show Analysis Results" button to proceed to the FLOCK Analysis History page.

Show Analysis Results

FLOCK analysis result

Flow Cytometry | Data Management | FLOCK | View/Edit Results | Cross Sample | Help

FLOCK Analysis Task Detail

Task Id	3053
Name	Analyze Sample Data 0010-0019
Description	Batch FLOCK analysis for 10 files
Start Date	05/03/2010 14:10:19
End Date	05/03/2010 14:12:01
Status	Completed

FLOCK Analysis Task Detail

FLOCK Analysis Result Files

Page 1 of 1

Displaying 1 - 10 of

Name	Description	Exp Sample ID	Exp Sample Name	FCS Text File	Bins	Dens	Status
10	FLOCK Analysis tests ample 10	ES_FCS10		FCS0010.txt	16	3	Results
11	FLOCK Analysis tests ample 11	ES_FCS11		FCS0011.txt	15	3	Results
12	FLOCK Analysis tests ample 12	ES_FCS12		FCS0012.txt	16	3	Results
13	FLOCK Analysis tests ample 13	ES_FCS13		FCS0013.txt	16	3	Results
14	FLOCK Analysis tests ample 14	ES_FCS14		FCS0014.txt	16	3	Results
15	FLOCK Analysis tests ample 15	ES_FCS15		FCS0015.txt	16	3	Results
16	FLOCK Analysis tests ample 16	ES_FCS16		FCS0016.txt	16	3	Results
17	FLOCK Analysis tests ample 17	ES_FCS17		FCS0017.txt	16	3	Results
18	FLOCK Analysis tests ample 18	ES_FCS18		FCS0018.txt	16	3	Results
19	FLOCK Analysis tests ample 19	ES_FCS19		FCS0019.txt	16	3	Results

Click 'Results'

Files run with FLOCK

FLOCK Analysis: Create Datasets

Flow Cytometry Analysis (Beta) / Data Management / Create Analysis Set

Flow Cytometry | Data Management | FLOCK | View/Edit Results | Cross Sample | Help

Use the 'Uploaded Flow Files Search' section to find data files for dataset creation.
The number of items in a dataset is limited to 300.

Select project: Include Semi-Public files in the Search

If files are in more than one project, select project from dropdown

Uploaded Flow Files Search

Search for Uploaded Files :

Results per page : 25

Submit

Cancel

Advanced Search Attribute Tree

- Flow
 - Name
 - Text File Name
 - Description
 - Panel
 - Created By
 - Created By Last Name
 - Date Created

Flow: Name

- Like
- Like
 - Exact Match
 - Starts With
 - Ends With
 - Not Null
 - Not Equal

Specimen

...

Remove

Use the Advanced Search Attribute Tree to search for uploaded files

Use arrow button to move files in or out of analysis set.

Mouse-over column titles, click down arrow to hide or show columns.

Page 1 of 1

Displaying 1 - 12 of 12

<input type="checkbox"/>	Name	Description	FCS Text File	Date Created
<input checked="" type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input checked="" type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input checked="" type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26
<input type="checkbox"/>	Specimen_001_...		Specimen_001_...	2013-02-26

Create Set Remove

<input type="checkbox"/>	Name	FCS Text File
<input type="checkbox"/>	Specimen_001_Tube_010	Specimen_001_Tube_010.txt
<input type="checkbox"/>	Specimen_001_Tube_011	Specimen_001_Tube_011.txt
<input type="checkbox"/>	Specimen_001_Tube_012	Specimen_001_Tube_012.txt

Select files from the left panel and click the center arrow to list files in right panel. Click 'Create Set'

FLOCK Analysis: Using Datasets

Analyze Data sets



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Flow Cytometry Analysis (Beta) / FLOCK / Overview

Flow Cytometry | Data Management | **FLOCK** | View/Edit Results | Cross Sample | Help

FLOCK (Flow Clustering without K) is a computational approach to flow cytometry analysis which:

- ▶ Computationally determines the number of unique populations in a dataset using a novel binning approach
- ▶ Can handle non-spherical hyper-shapes
- ▶ Maps populations across independent samples
- ▶ Calculates many useful summary statistics
- ▶ Finds the most informative parameters
- ▶ Reduces subjective factors in manual gating

The screenshot shows the 'FLOCK' dropdown menu with two options: 'Analyze Individual Files' and 'Analyze Data Sets'. The 'Analyze Data Sets' option is circled in orange. An arrow points from the 'FLOCK' menu item in the breadcrumb to this menu.

binning



FCSTrans (ImmPort FCS file converter), FLOCK and the Centroid Adjustment algorithms are sometimes refined in order to obtain optimal results. Please be sure to check the [release notes](#) to see if recent modifications would affect your analysis.

Flow Cytometry Analysis Workflow



FLOCK Analysis: Using Datasets



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Flow Cytometry Analysis (Beta) / Data Management / Flow Data Sets

Select a project

Flow Cytometry | Data Management ▾ | FLOCK ▾ | View/Edit Results ▾ | Cross Sample ▾ | Help

Select project: TESTING: Bioinformatics Integration Support Project ▾

Double click on a row to view detail screen. Mouse-over column titles, click down arrow to hide or show columns

<input type="checkbox"/>	Name	Description	File Count	Status	Project	Date Created ▾	Created By
<input type="checkbox"/>	Empty_10_files				TESTING: Bioinformatics	2010-05-06	Gian
<input type="checkbox"/>	Test 5 - Mar 3				TESTING: Bioinformatics	2010-03-03	Campbell
<input type="checkbox"/>	Create new data set te				TESTING: Bioinformatics	2009-12-15	Thomson
<input type="checkbox"/>	Data set for cross sampl	12/15/09	7	loaded	TESTING: Bioinformatics	2009-12-15	Thomson
<input type="checkbox"/>	test batch run FLOCK 15	12/15/09	15	loaded	TESTING: Bioinformatics	2009-12-15	Thomson

1. Select any Data Set and click **Detail** to view detailed data set information
2. Select any Data Set and click **Edit** to edit data set information from a detailed view
3. Click **Run FLOCK** to analyze the selected data set
4. Delete allows the deletion of selected data sets

FLOCK Data Set analysis: Marker selection



Flow Cytometry Analysis (Beta) / FLOCK / FLOCK Analysis Setting

Flow Cytometry | Data Management | FLOCK | View/Edit Results | Cross Sample | Help

FLOCK Detail

Project: FLOCK Parameters: Auto Manual

Name:

Description:

Provide a name and an informative description of the analysis

Auto-detect is the default parameter setting. For more parameter selection information please review the User Guide

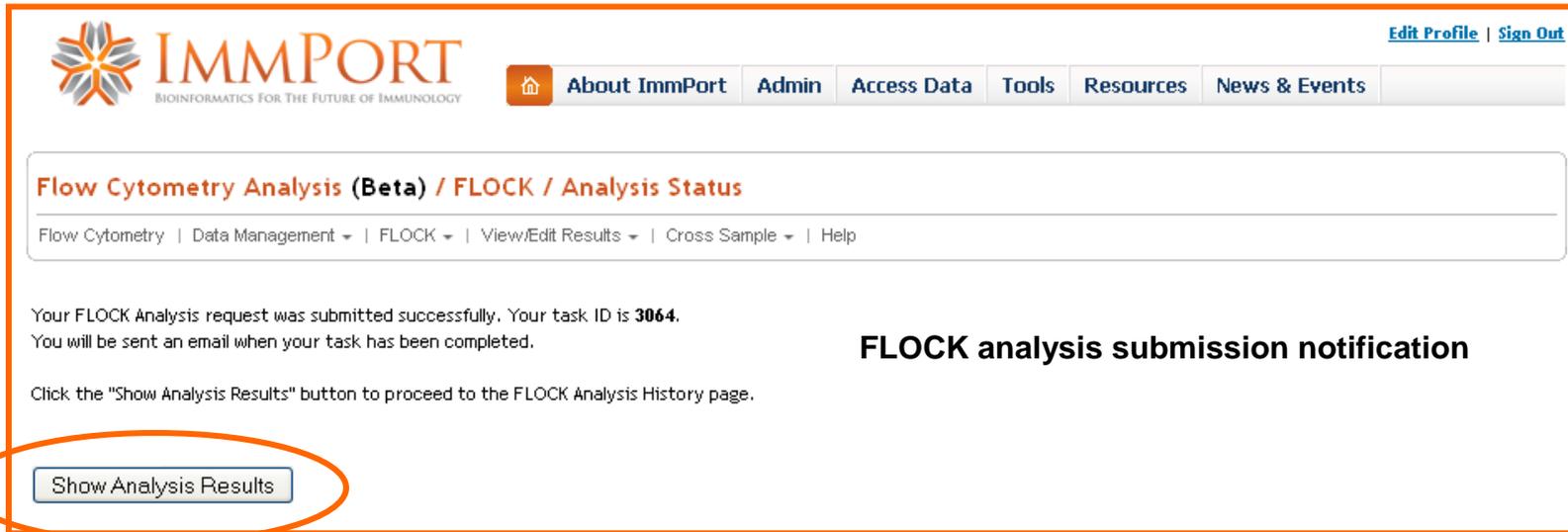
Use check boxes to select markers to be included in analysis. Markers to be excluded from analysis may include unused markers, limiting multiple scatter parameters (FSC-A, FSC-H, FSC-W) to one parameter (FSC-H) or pre-gating situation. Please review the Marker/Channel Selection tutorial or the User Guide for more information.

Column Selection for 5 Files																			
Name	Marker #	<input checked="" type="checkbox"/>	1	<input checked="" type="checkbox"/>	2	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	4	<input checked="" type="checkbox"/>	5	<input checked="" type="checkbox"/>	6	<input checked="" type="checkbox"/>	7	<input checked="" type="checkbox"/>	8	<input checked="" type="checkbox"/>	9
Specimen_001_Tube_003	9	<input checked="" type="checkbox"/>	FSC-A	<input checked="" type="checkbox"/>	SSC-A	<input checked="" type="checkbox"/>	FITC-A	<input checked="" type="checkbox"/>	PerCP-A	<input checked="" type="checkbox"/>	Pacific Blue-A	<input checked="" type="checkbox"/>	APC-A	<input checked="" type="checkbox"/>	APC-Cy7-A	<input checked="" type="checkbox"/>	PE-A	<input checked="" type="checkbox"/>	PE-Cy7-A
Specimen_001_Tube_001	9	<input checked="" type="checkbox"/>	FSC-A	<input checked="" type="checkbox"/>	SSC-A	<input checked="" type="checkbox"/>	FITC-A	<input checked="" type="checkbox"/>	PerCP-A	<input checked="" type="checkbox"/>	Pacific Blue-A	<input checked="" type="checkbox"/>	APC-A	<input checked="" type="checkbox"/>	APC-Cy7-A	<input checked="" type="checkbox"/>	PE-A	<input checked="" type="checkbox"/>	PE-Cy7-A
Specimen_001_Tube_005	9	<input checked="" type="checkbox"/>	FSC-A	<input checked="" type="checkbox"/>	SSC-A	<input checked="" type="checkbox"/>	FITC-A	<input checked="" type="checkbox"/>	PerCP-A	<input checked="" type="checkbox"/>	Pacific Blue-A	<input checked="" type="checkbox"/>	APC-A	<input checked="" type="checkbox"/>	APC-Cy7-A	<input checked="" type="checkbox"/>	PE-A	<input checked="" type="checkbox"/>	PE-Cy7-A
Specimen_001_Tube_002	9	<input checked="" type="checkbox"/>	FSC-A	<input checked="" type="checkbox"/>	SSC-A	<input checked="" type="checkbox"/>	FITC-A	<input checked="" type="checkbox"/>	PerCP-A	<input checked="" type="checkbox"/>	Pacific Blue-A	<input checked="" type="checkbox"/>	APC-A	<input checked="" type="checkbox"/>	APC-Cy7-A	<input checked="" type="checkbox"/>	PE-A	<input checked="" type="checkbox"/>	PE-Cy7-A
Specimen_001_Tube_004	9	<input checked="" type="checkbox"/>	FSC-A	<input checked="" type="checkbox"/>	SSC-A	<input checked="" type="checkbox"/>	FITC-A	<input checked="" type="checkbox"/>	PerCP-A	<input checked="" type="checkbox"/>	Pacific Blue-A	<input checked="" type="checkbox"/>	APC-A	<input checked="" type="checkbox"/>	APC-Cy7-A	<input checked="" type="checkbox"/>	PE-A	<input checked="" type="checkbox"/>	PE-Cy7-A

- Show assigned name:** select this button to display user-assigned marker names
- Show original name:** select this button to display marker names original to the uploaded .txt or .fcs file
- Select All, Clear All Markers:** Use buttons and column check boxes to select markers to be included in the FLOCK analysis
- Help:** pop-up provides marker column selection information. For more information please review the Marker selection tutorial found under Help in the Flow module menu

Run FLOCK

FLOCK Analysis: Data Set Analysis Status



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Flow Cytometry Analysis (Beta) / FLOCK / Analysis Status

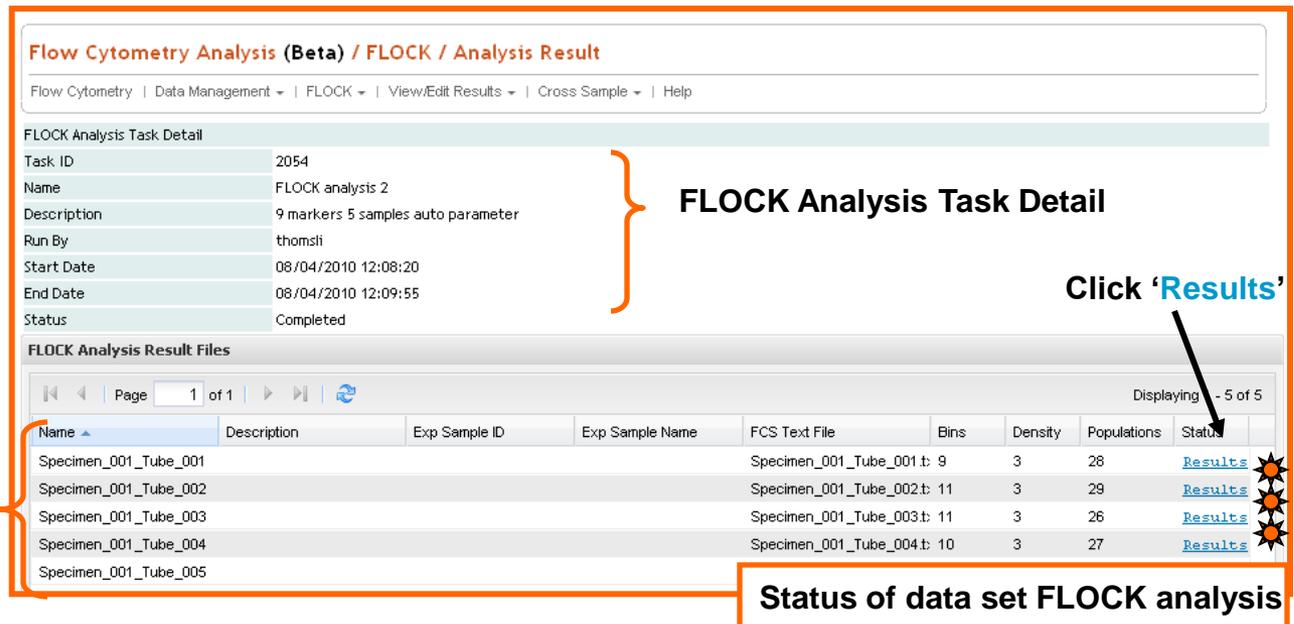
Flow Cytometry | Data Management ▾ | FLOCK ▾ | View/Edit Results ▾ | Cross Sample ▾ | Help

Your FLOCK Analysis request was submitted successfully. Your task ID is **3064**.
You will be sent an email when your task has been completed.

Click the "Show Analysis Results" button to proceed to the FLOCK Analysis History page.

[Show Analysis Results](#)

FLOCK analysis submission notification



Flow Cytometry Analysis (Beta) / FLOCK / Analysis Result

Flow Cytometry | Data Management ▾ | FLOCK ▾ | View/Edit Results ▾ | Cross Sample ▾ | Help

FLOCK Analysis Task Detail

Task ID	2054
Name	FLOCK analysis 2
Description	9 markers 5 samples auto parameter
Run By	thomsl
Start Date	08/04/2010 12:08:20
End Date	08/04/2010 12:09:55
Status	Completed

FLOCK Analysis Result Files

Page 1 of 1 | Refresh | Displaying 5 of 5

Name	Description	Exp Sample ID	Exp Sample Name	FCS Text File	Bins	Density	Populations	Status
Specimen_001_Tube_001				Specimen_001_Tube_001.t	9	3	28	Results
Specimen_001_Tube_002				Specimen_001_Tube_002.t	11	3	29	Results
Specimen_001_Tube_003				Specimen_001_Tube_003.t	11	3	26	Results
Specimen_001_Tube_004				Specimen_001_Tube_004.t	10	3	27	Results
Specimen_001_Tube_005								

Results for individual files within the dataset

Click 'Results'

Status of data set FLOCK analysis

FLOCK Analysis: Status

FLOCK results from batch run

Flow Cytometry Analysis (Beta) / FLOCK / Analysis Result

Flow Cytometry | Data Management | FLOCK | View/Edit Results | Cross Sample | Help

FLOCK Analysis Task Detail

Task Id: 740
Name: FLOCK Analysis w/ Data Set
Description: 10/20/09
Start Date: 10/22/2009 08:42:42
End Date: n/a
Status: In Progress

Flock Analysis Result Files

Name	Description	FCS Test File	Bin	Density	Status
healthy2084	healthy subject	02084004_001.tst	19	3	Detail
healthy2114	healthy subject	02114004_001.tst	23	3	Detail
healthy2134	healthy subject	02134004_001.tst	20	3	Detail
healthy2164	healthy subject	02164002_001.tst	20	3	Detail
healthy2204	healthy subject	02204002_001.tst	15	3	Detail

Viewing individual FLOCK results from a batch run:

Click on any 'Detail' and a pop-up will appear which contains the thumbnail overview display for centroid editing (RAS, Result Adjustment System).

From this screen it is possible to:

- 1) Edit centroid
- 2) View select populations in 2-D matrix, view MFI, Score and Centroid values
- 3) Download results
- 4) Access help documentation

FLOCK result detail

Result Adjustment System (RAS) - Task Name: Planned_visit_search_1 File Name: 11283151.003.tcs

3-D view

Population Selection table detail on next slide

#	color	name	%	self%
1	Red		5.92	5.92
2	Yellow		3.78	3.78
3	Green		3.26	3.26
4	Blue		0.73	0.73
5	Orange		5.53	5.53
6	Purple		6.98	6.98
7	Light Green		6.08	6.08
8	Cyan		3.09	3.09
9	Magenta		1.85	1.85
10	Light Blue		6.39	6.39
11	Dark Blue		3.75	3.75
12	Red-Orange		4.99	4.99
13	Dark Green		3.47	3.47
14	Yellow-Orange		4.99	4.99
15	Light Purple		4.45	4.45
16	Black		1.5	1.5
17	Dark Purple		7.83	7.83
18	Light Purple		4.9	4.9
19	Light Blue		3.4	3.4
20	Light Green		5.06	5.06
21	Light Purple		1.5	1.5
22	Red-Orange		3.06	3.06
23	Dark Green		2.6	2.6

Result Adjustment System (RAS) - Task Name: Planned_visit_search_1 File Name: 11283151.003.tcs

Reset Spin Centroid

Axis Selection

X Axis: FSC
Y Axis: FITC CD4
Z Axis: PP CD8

Population Selection

#	color	name	%	self%
1	Red		5.92	5.92
2	Yellow		3.78	3.78
3	Green		3.26	3.26
4	Blue		0.73	0.73
5	Orange		5.53	5.53
6	Purple		6.98	6.98
7	Light Green		6.08	6.08
8	Cyan		3.09	3.09
9	Magenta		1.85	1.85
10	Light Blue		6.39	6.39
11	Dark Blue		3.75	3.75
12	Red-Orange		4.99	4.99

Overview | Summary Tables | Download Tables | **Download Results** | Centroid | 3D View | * 3D View works with results generated after July 21, 2011 |

Result Adjustment System(RAS) - Task | **Download Results** | **Download All** | **Task: centroid_search_1** | **File Name: 11283151.003.fcs**

	FSC	SSC	FITC CD4	PE CCR3	PP CD8	APC CCR4
FSC						
SSC						
FITC CD4						
PE CCR3						
PP CD8						
APC CCR4						

Output Files:
 Flock_results.txt
 MFI.txt
 Population_center.txt
 props.txt
 See next slide for details

Population Selection

Update | Data Display

<input checked="" type="checkbox"/>	#	color	<input checked="" type="checkbox"/> hide data	sel%
<input checked="" type="checkbox"/>	1	red	<input checked="" type="checkbox"/>	3.2%
<input checked="" type="checkbox"/>	2	yellow	<input checked="" type="checkbox"/>	0.7%
<input checked="" type="checkbox"/>	3	green	<input checked="" type="checkbox"/>	
<input checked="" type="checkbox"/>	4	blue	<input checked="" type="checkbox"/>	
<input checked="" type="checkbox"/>	5	orange	<input checked="" type="checkbox"/>	5.53 5.53
<input checked="" type="checkbox"/>	6	purple	<input checked="" type="checkbox"/>	6.98 6.98
<input checked="" type="checkbox"/>	7	olive	<input checked="" type="checkbox"/>	6.08 6.08
<input checked="" type="checkbox"/>	8	cyan	<input checked="" type="checkbox"/>	3.09 3.09
<input checked="" type="checkbox"/>	9		<input checked="" type="checkbox"/>	
<input checked="" type="checkbox"/>	10		<input checked="" type="checkbox"/>	
<input checked="" type="checkbox"/>	11		<input checked="" type="checkbox"/>	
<input checked="" type="checkbox"/>	12		<input checked="" type="checkbox"/>	
<input checked="" type="checkbox"/>	13		<input checked="" type="checkbox"/>	
<input checked="" type="checkbox"/>	14		<input checked="" type="checkbox"/>	
<input checked="" type="checkbox"/>	15	light green	<input checked="" type="checkbox"/>	4.45 4.45
<input checked="" type="checkbox"/>	16	black	<input checked="" type="checkbox"/>	1.5 1.5
<input checked="" type="checkbox"/>	17	teal	<input checked="" type="checkbox"/>	7.83 7.83
<input checked="" type="checkbox"/>	18	purple	<input checked="" type="checkbox"/>	4.91 4.91
<input checked="" type="checkbox"/>	19	orange	<input checked="" type="checkbox"/>	3.41 3.41
<input checked="" type="checkbox"/>	20	yellow	<input checked="" type="checkbox"/>	5.06 5.06
<input checked="" type="checkbox"/>	21	teal	<input checked="" type="checkbox"/>	1.51 1.51
<input checked="" type="checkbox"/>	22	red	<input checked="" type="checkbox"/>	5.86 5.86
<input checked="" type="checkbox"/>	23	teal	<input checked="" type="checkbox"/>	2.6 2.6

Select which values to view

Use boxes to select individual populations to view in 2-D matrix, click update when selection has been completed

FLOCK Analysis: Output files

.txt output files converted to .xls for viewing

	A	B	C	D	E	F	G	H	I	J	K	L
1	FSC-A	SSC-A	FITC-A	PerCP-A	Pacific Blue-A	APC-A	APC-Cy7-A	PE-A	PE-Cy7-A	Event	Population	
2	37	3	210	0	23	76	0	0	53	1	1	
3	637	128	269	241	236	187	466	447	279	2	25	
4	462										20	
5	697										25	
6	280										18	
7	493										21	
8	171										14	
9	554										20	

flock_results.txt—columns displayed include event ID, each event's marker/parameter FLOCK values and assigned population ID

	A	B	C	D	E	F	G	H	I	J	
1	Population	FSC-A	SSC-A	FITC-A	PerCP-A	Pacific Blue-A	APC-A	APC-Cy7-A	PE-A	PE-Cy7-A	
2	1	73	59	82	53	20	45	17	42	26	
3	2	278	878	486	459	413	304	169	311	319	
4	3	80	85	655	196	87	75	101	27	135	
5									58	330	106
6									173	506	200
7									142	357	217
8									231	293	258
9	8	293	176	756	174	167	102	256	48	267	

MFI.txt—provides mean fluorescence intensity for each population for each marker/parameter

	A	B	C	D	E	F	G	H	I	J
1	Population	FSC-A	SSC-A	FITC-A	PerCP-A	Pacific Blue-A	APC-A	APC-Cy7-A	PE-A	PE-Cy7-A
2	1	98	67	48	45	18	63	17	37	27
3	2	529	807	451	314	407	327	259	473	243
4	3	96	79	580	136	51	47	56	79	129
5									365	149
6									567	208
7									363	257
8									343	282
9									128	253

population_center.txt—contains centroid coordinates for each population ID

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Populatio	Cell Type	Description	FSC-A	SSC-A	FITC-A	PerCP-A	Pacific Blue-A	APC-A	APC-Cy7-A	PE-A	PE-Cy7-A	Percentage
2	1		FSC-A-SSC-A-FITC-A-PerCP-A-Pacific Blue-A-APC-A-APC-Cy7-A-PE-A-PE-Cy7-A	1	1	1	1	1	1	1	1	1	2.2
3	2		FSC-A-SSC-A-FITC-A-PerCP-A-Pacific Blue-A-APC-A-APC-Cy7-A-PE-A-PE-Cy7-A	1	4	2	2	2	2	1	1	2	0.45
4	3		FSC-A-SSC-A										3.42
5	4		FSC-A-SSC-A										2.17
6	5		FSC-A-SSC-A										1.8
7	6		FSC-A-SSC-A										1.39
8	7		FSC-A-SSC-A										4.23
9	8		FSC-A-SSC-A										2.23

props.txt—includes population identifier, population description where markers are annotated with approximate expression displayed as hi, lo, + or - and also assigned corresponding numeric values from 1-4

- Name

 -  flock_results.txt
 -  MFI.txt
 -  population_center.txt
 -  props.txt



FLOCK Analysis: Concatenated FCS files For Centroid File

One of the challenging aspects of the Cross Sample analysis module is choosing a FCS file that includes all of the cell populations.

One option is to run a FLOCK analysis on a concatenated set of files.

The FlowJo manual on concatenation can be found here: <http://www.flowjo.com/v765/en/export.html>

FLOCK source code is available at sourceforge: <http://importflock.sourceforge.net/>

Access ImmPort flow cytometry module user guide

https://import.niaid.nih.gov/help/FLOCK_Analysis_User_Guide.pdf

Related publications:

FCSTrans: An open source software system for FCS file conversion and data transformation

Yu Qian, et al., Cytometry A. 2012 May;81(5):353-6. doi: 10.1002/cyto.a.22037. Epub 2012 Mar 19.

Elucidation of seventeen human peripheral blood B-cell subsets and quantification of the tetanus response using a density-based method for the automated identification of cell populations in multidimensional flow cytometry data. Yu Qian, et al., Cytometry B Clin Cytom. 2010;78 Suppl 1:S69-82. doi: 10.1002/cyto.b.20554.