

## FLOCK / Cross Sample Comparison: Create Dataset

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**Flow Cytometry  $\beta$**

Data Management	FLOCK	View/Edit Results	Cross Sample	Help
<a href="#">Upload Single File</a> <a href="#">Upload Multiple Files</a> <a href="#">View/Edit Uploaded Data</a> <a href="#">Create Data Set</a> <a href="#">View/Edit Data</a>	<a href="#">Analyze Individual Files</a> <a href="#">Analyze Data Sets</a>	<a href="#">FLOCK</a> <a href="#">Centroid Files</a> <a href="#">Cross Sample</a>	<a href="#">Start Comparison</a>	
iGenePattern (Gene expression analysis)	Genetic Analysis $\beta$	MHC Analysis $\beta$	tagSNP	
<a href="#">Data Management</a> <a href="#">Analysis Pipelines</a>	<a href="#">User Guide (PED)</a> <a href="#">Run .PED Generation</a> <a href="#">Generation Results</a>	<a href="#">Upload Data</a> <a href="#">Create Data Set</a> <a href="#">Analyze Data Set</a>	<a href="#">Run tagSNP</a> <a href="#">Results</a>	

Cross Sample Comparison automatically maps populations of cells across multiple flow samples and computes the summary statistics for downstream analysis.

Currently Cross Sample Comparison is available for FLOCK v1 output only—FLOCKv2 output Cross Sample Comparison will be available in a subsequent release.

To perform cross sample analysis the user is required to **define a data set** which contains the samples to be compared using the Data Management module. Click '**Create Data Set**' (see above)

## FLOCK / Cross Sample Comparison: Create Dataset

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

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

Use the 'Uploaded' data files for dataset creation.

The number of items to display is:   Include Semi-Public files in the Search

Select project:  [Create Data Set](#)

**Uploaded Flow Files**

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

**Use Advanced Search to find 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.*

Name	Description	FCS Text File	Date Created
<input checked="" type="checkbox"/> Specimen_001_...		Specimen_001_...	2013-02-19
<input checked="" type="checkbox"/> Specimen_001_...		Specimen_001_...	2013-02-19
<input checked="" type="checkbox"/> Specimen_001_...		Specimen_001_...	2013-02-19
<input type="checkbox"/> Specimen_001_...		Specimen_001_...	2013-02-19
<input type="checkbox"/> Specimen 001 ...		Specimen 001 ...	2013-02-19

**Select files and click arrow to move files to data set**

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

**Click 'Create Set'**



## FLOCK / Cross Sample Comparison: Create Dataset

**Save Set**

Name:  **Provide name and data set description**

Description:

**Marker Columns**

Show Assigned Name  Show Original Name

Name	Marker #	1	2	3	4	5	6	7	8	9	10
Specimen_00...	10	FSC-A	SSC-A	FITC-A	PerCP-A	Pacific Blu...	APC-A	APC-Cy7-A	PE-A	PE-Cy7-A	Time
Specimen_00...	10	FSC-A	SSC-A	FITC-A	PerCP-A	Pacific Blu...	APC-A	APC-Cy7-A	PE-A	PE-Cy7-A	Time
Specimen_00...	10	FSC-A	SSC-A	FITC-A	PerCP-A	Pacific Blu...	APC-A	APC-Cy7-A	PE-A	PE-Cy7-A	Time

Flow cytometry files being compared in Cross Sample comparison should contain the same markers (green shadowed).

## FLOCK / Cross Sample Comparison: Create Centroid

**Flow Cytometry Analysis (Beta) / FLOCK / Analysis History**

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

Select project: 0 revised exams **FLOCK**  
Centroid Files  
Cross Sample

**Select FLOCK result to identify centroid for comparison**

**FLOCK Analysis History**

Details	Name	Description
<a href="#">Detail</a>	batch run1	
<a href="#">Detail</a>	tst run 3	
<a href="#">Detail</a>	tst run2	

**FLOCK Analysis Task Detail**

Task ID: 3987  
Name: batch run1  
Description:  
Algorithm Version: 1.0  
Run By: Thomson  
Start Date: 02/18/2013 13:06:02  
End Date: 02/18/2013 13:09:45  
Status: Completed

**FLOCK Analysis Result Files**

Name	Description	Panel	FCS Text File	Bins	Density	Populations	Status
R2	tst file	FSC-A SSC-A FITC-A PE-A FL3-A PE-Cy7-...	R2.txt	34	3	21	<a href="#">Results</a>
R4	tst file	FSC-A SSC-A CD11C FITC-A CD21 PE-A CD...	R4.txt	30	3	15	<a href="#">Results</a>
tst file	adding test post upload	FSC-A SSC-A CD11C FITC-A CD21 PE-A CD...	R1.txt	32	3	27	<a href="#">Results</a>

**Click 'Results' to view thumbnail matrix and select centroid**

A centroid file is required for comparison and is generated by running FLOCK on one or more files of the data set or on a file external to the data set to which the data set is being compared

Note: the number of markers (columns in each file) must be consistent within the data set and the saved centroid file in order to use Cross Sample comparison

## 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 / Cross Sample Comparison: Create Centroid

Flow Cytometry Analysis (Beta) / RAS / Result Overview

Overview | Summary Tables | Download Tables | Download Results | Centroid | 3D View | \* 3D View

Result Adjustment System(RAS) - Task Name: batch run1 File Name: R2.txt

Centroid | 3D View | \* 3D View

Undo Changes

**Save Centroid**

Population Selection

Update Data Display

<input checked="" type="checkbox"/>	#	color	name	%	sel%	centroid FSC-A
<input checked="" type="checkbox"/>	1	■	test n...	0.47	0.47	3137
<input checked="" type="checkbox"/>	2	■	test n...	7.47	7.47	1768
<input checked="" type="checkbox"/>	3	■		5.25	5.25	1855
<input checked="" type="checkbox"/>	4	■		12.48	12.48	1754
<input checked="" type="checkbox"/>	5	■		2.87	2.87	1873
<input checked="" type="checkbox"/>	6	■		2.85	2.85	3173

Flow Cytometry Analysis (Beta) / RAS / Result Overview

Overview | Summary Tables | Download Tables | Download Results | Centroid | 3D View | \* 3D View

Result Adjustment System(RAS) - Task Name: batch run1 File Name: R2.txt

**Save Centroid**

Name: centroid name

Description: centroid description

Save Centroid Reset

FLOCK result thumbnail matrix  
Select 'Save Centroid' from Centroid dropdown menu  
Provide name and description for the new centroid

## FLOCK / Cross Sample Comparison: Access

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### Flow Cytometry $\beta$

Data Management	FLOCK	View/Edit Results	Cross Sample	Help
<ul style="list-style-type: none"> <li>Upload Single File</li> <li>Upload Multiple Files</li> <li>View/Edit Uploaded Data</li> <li>Create Data Set</li> <li>View/Edit Data Sets</li> </ul>	<ul style="list-style-type: none"> <li>Analyze Individual Files</li> <li>Analyze Data Sets</li> </ul>	<ul style="list-style-type: none"> <li>FLOCK</li> <li>Centroid Files</li> <li>Cross Sample</li> </ul>	<ul style="list-style-type: none"> <li>Start Comparison</li> </ul>	
iGenePattern (Gene expression analysis)	Genetic Analysis $\beta$	MHC Analysis $\beta$	tagSNP	
<ul style="list-style-type: none"> <li>Data Management</li> <li>Analysis Pipelines</li> <li>Analysis Modules</li> <li>View Results</li> <li>Help</li> </ul>	<ul style="list-style-type: none"> <li>User Guide (PED)</li> <li>Run .PED Generation</li> <li>Generation Results</li> <li>User Guide (IGAT)</li> <li>Run Genetic Analysis</li> <li>Analysis Results</li> </ul>	<ul style="list-style-type: none"> <li>Upload Data</li> <li>Create Data Set</li> <li>Analyze Data Set</li> <li>Analysis Results</li> <li>User Guide</li> </ul>	<ul style="list-style-type: none"> <li>Run tagSNP</li> <li>Results</li> </ul>	

Access Cross Sample Comparison through the ImmPort Tools menu.

**NOTE: Cross Sample Comparison is currently limited to FLOCK 1 output**

## FLOCK / Cross Sample Comparison: Start Comparison

### Flow Cytometry Analysis (Beta) / Cross Sample / Overview

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

FLOCK Cross Sample Comparison automatically maps populations of cells and generates the summary statistics for downstream analysis. To perform cross sample analysis:

- ▶ Define a dataset that contains all samples to be compared
- ▶ Generate a centroid file based on FLOCK results
- ▶ Apply the centroid file to all the samples in the dataset

Start Comparison

- Go to 'Cross Sample' from the menu bar and click to see overview.
- Click 'Start Comparison' to begin Cross Sample Analysis

### Flow Cytometry Analysis Workflow



# FLOCK / Cross Sample Comparison: Analysis Set-up



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## Flow Cytometry Analysis (Beta) / Cross Sample / Run Analysis

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

Select project:

### Choose Centroid File

Page 1 of 1

<input type="checkbox"/>	Name	Description	Status	Project	Date Created
<input type="checkbox"/>	Cent_population_24	FLOCK output auto 24 populations	loaded	Research project 1	2010-08-03
<input type="checkbox"/>	Cent_auto_edit_5	single file FLOCK analysis auto outp	loaded	Research project 1	2010-08-03
<input type="checkbox"/>	Centroid 3	auto FLOCK output	loaded	Research project 1	2010-08-03
<input type="checkbox"/>	Centroid 2	auto parameter edited	loaded	Research project 1	2010-08-03
<input type="checkbox"/>	Centroid 1	Edited auto parameter output	loaded	Research project 1	2010-08-03

### Choose One or More Analysis Sets

Page 1 of 1

<input type="checkbox"/>	Name	Description	Status	Project	Date Created
<input type="checkbox"/>	Data set 3	12 files 7 markers	loaded	Research project 1	2010-08-03
<input type="checkbox"/>	Data set 2	5 files 7 markers	loaded	Research project 1	2010-08-03
<input type="checkbox"/>	Data set 1	3 files 4 markers	loaded	Research project 1	2010-08-03
<input type="checkbox"/>	Data Set3	5 files 6 markers	loaded	Research project 1	2010-07-27
<input type="checkbox"/>	Dataset2_Jul_1				
<input type="checkbox"/>	Dataset_Jul_15				
<input type="checkbox"/>	save set test				

Select:

- Centroid from the top table
- Data set from the bottom table
- Click 'Continue'

**Note:** Comparison requirement--centroid and dataset files must have the same markers in the same order for a valid analysis

# Cross Sample Comparison: Column Comparison



## Flow Cytometry Analysis (Beta) / Cross Sample / Cross Sample Analysis Setting

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

### Cross Sample

Project:  ▼  
Name:   
Description:



- Project is pre-selected—cross sample analysis is restricted to files within a selected project.
- Provide Name and Description for the analysis

### Marker Selection

Show Assigned Name Show Original Name Help

Type	Name	Marker #	1	2	3	4	5	6
Centroid	Centroid 1	6	FSC	SSC	FL1	FL2	FL3	FL4
Flow File	TXT001	6	FSC	SSC	FL1	FL2	FL3	FL4
Flow File	TXT002	6	FSC	SSC	FL1	FL2	FL3	FL4
Flow File	TXT003	6	FSC	SSC	FL1	FL2	FL3	FL4

**Column Selection Help**

In FLOCK run with dataset and cross sample run, column's background colors are used to indicate data consistency:

- \* green = identical
- \* red = non-identical
- \* gray = unavailable in centroid file

Please review the Marker/Channel Selection tutorial or the User Guide for more information.

OK

Run Cross Sample

# FLOCK / Cross Sample Comparison: Task confirmation, Analysis Detail

## Cross Sample Analysis Request

Flow Cytometry Analysis (Beta) / Cross Sample / Analysis Status

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

Your Cross Sample Analysis request was submitted successfully. Your task ID is **714**.  
You will be sent an email when your task has been completed.

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

Show Analysis History

### From Cross Sample Analysis Request screen

- Click 'Show Analysis History' to view Cross Sample results

### From the Analysis Detail screen

- Click Centroid Information Detail to view the centroid file
- Click Analysis Results to view Cross Sample results
- Click 'Results' from Cross Sample Analysis Result Files table to view individual file results

## Cross Sample Analysis Detail

Flow Cytometry Analysis (Beta) / Cross Sample / Analysis Detail

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

Cross Sample Analysis

Task ID	714
Name	Add analysis name here
Description	Add description here
Start Time	10/21/2009 15:04:49
End Time	10/21/2009 15:05:34
Status	
Centroid Information <a href="#">Detail</a>	
Name	save day 6 centroid
Description	day 6 centroid

Click Analysis Results to view Cross Sample results

Click to view centroid file details

Flow Analysis Sets

Page 1 of 1		
Name	Description	Status
tetanus_demo		loaded

Cross Sample Analysis Result Files

Page 1 of 1		
Name	Description	FCS Text File
day1		tetvac3-d1(CD19+)_6D.txt
day12		tetvac3-d12(CD19+)_6D.txt
day6		tetvac3-d6(CD19+)_6D.txt
day7		tetvac3-d7(CD19+)_6D.txt
day8		tetvac3-d8(CD19+)_6D.txt

Click to view single file results

Results

Results

Results

Results

Results

# Cross Sample Comparison: Breakdown of Analysis Detail

## Centroid Details

## Cross Sample Analysis Detail

## Cross Sample Analysis Results

**Flow Cytometry Analysis (Beta) / RAS / Centroid File Data**

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

Project: TESTING: Bioinformatics Integration Support Project  
 Centroid Id: 26  
 Task Id: 713  
 Flock Input Id: 65  
 Name: save day 6 centroid  
 Description: day 6 centroid  
 Status: loaded  
 Created By: yqian  
 Date Created: 2009-10-21

1	1040	1947	2248	981	50	1095
2	743	1755	2159	996	75	779
3	1051	1972	1974	801	854	1413
4	856	1720	1885	967	335	700
5	858	1772	2103	986	1150	595
6	1037	1907	2087	1499	902	729
7	1035	1928	2153	1642	66	834

**Flow Cytometry Analysis (Beta) / Cross Sample / Analysis Detail**

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

sample Analysis

714

Add analysis name here

Add description here

10/21/2009 15:04:49

10/21/2009 15:05:34

[Analysis Results](#)

id information [Detail](#)

save day 6 centroid

day 6 centroid

analysis Sets

Page 1 of 1

Name	Description	Status
js_demo		loaded

Cross Sample Analysis Result Files

Page 1 of 5

Name	Description	FCS Text File	Status
day1		tetvac3-df(CD19)-_8D.txt	<a href="#">Results</a>
day12		tetvac3-df(CD19)-_8D.txt	<a href="#">Results</a>
day6		tetvac3-df(CD19)-_8D.txt	<a href="#">Results</a>
day7		tetvac3-df(CD19)-_8D.txt	<a href="#">Results</a>
day8		tetvac3-df(CD19)-_8D.txt	<a href="#">Results</a>

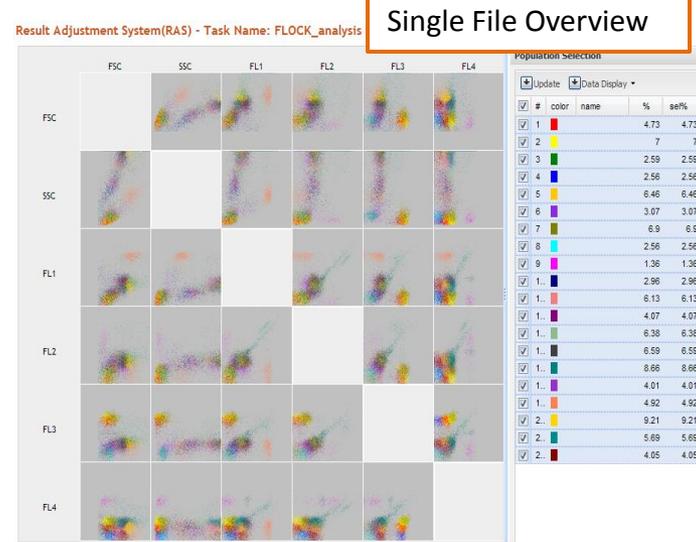
**Flow Cytometry Analysis (Beta) / Cross Sample / Analysis Result**

Percentage Table | Marker by Population | Download

**Cross Sample Analysis - Task Name: Add analysis name here**

File	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	Pop 10	Pop 11
day1	15.05	8.05	9.77	4.96	9.32	4.31	4.63	7.72	4.05	2.48	2.78
dayE											3.9
day7											1.69
dayC											2.93
day1											2.61

**Detail View for this table is on the next slide**



- Cross Sample Analysis Detail screen links:
- Centroid Information [Detail](#) link displays details pertaining to the centroid applied in the cross sample comparison
  - The [Analysis Results](#) link displays the Cross Sample Analysis Results table from which the Population Percentage table, Marker by Population visualization and downloads can be accessed
  - Cross Sample Analysis Result Files table [Results](#) link displays individual file cross sample results

The **Cross Sample /Analysis Results** table displays the Cross Sample results from which the Population Percentage table, Marker by Population visualization, results statistics and downloads may be accessed

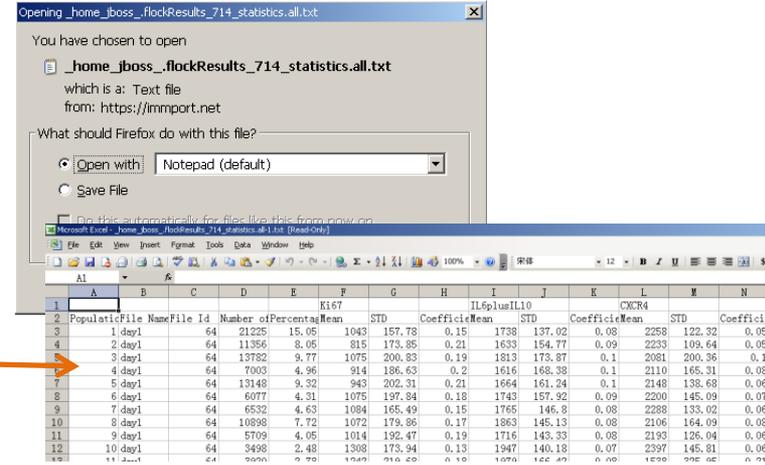
## Cross Sample Analysis for Percentage Table

### Flow Cytometry Analysis (Beta) / Cross Sample / Analysis Result

Percentage Table | Marker by Population | **Download**

### Cross Sample Analysis - Task Name: Add analysis name here

File	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	Pop 10	Pop 11
<a href="#">day1</a>	15.05	8.05	9.77	4.96	9.32	4.31	4.63	7.72	4.05	2.48	
<a href="#">day6</a>	10.55	7.25	3.87	3.42	5.14	3.23	5.43	6.77	5.31	5.51	
<a href="#">day7</a>	14.48	5.42	2.02	1.06	2.35	1.48	5.03	5.53	3.65	9.31	
<a href="#">day8</a>	8.67	11.4	5.98	4.61	7.34	3.89	6.13	8.16	5.92	3.8	
<a href="#">day12</a>	8.31	13.01	5.87	5.85	8.67	3.87	5.56	7.37	6.36	3.24	



### Output Files:

- **coordinates.txt**—output is the intensity values for each marker and event
- **flock\_results.txt**—a combination of input file, event identifiers and population identifiers
- **MFI.txt**—provides mean fluorescence intensity for each population for each marker/parameter
- **population\_id.txt**—contains population identifiers
- **population\_center.txt**—contains centroid coordinates for each population ID
- **percentage.txt**—includes population identifiers and percentage of events within that population relative to the whole data file
- **profile.txt**—displays an expression profile where the approximate expression level for each marker is assigned a numeric value from 1-4 for each population
- **fcs\_properties.txt**—contains the # of events, # of populations and # of markers as well as the algorithm parameters used in the analysis
- **percentage.all.txt**—population percentages
- **statistics.all.txt**—contains Event count, percentage of events for each flow cytometry file and the mean, standard deviation and coefficient of variation for each marker of each flow file
- **summary.all.txt**—list of files in analysis

### Flow Cytometry Analysis (Beta) / Cross Sample / Analysis Result

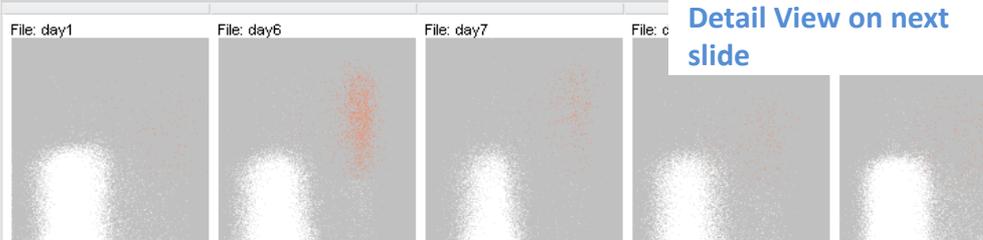
Percentage Table | Marker by Population | **Download**

### Marker by Population

### Cross Sample Analysis - Task Name: Add analysis name here

Population: 19 | Marker 1: Ki67 | Marker 2: CD138

Population: 19 Marker 1: Ki67 Marker 2: CD138



## Cross Sample Comparison: Analysis Result, Marker by Population viewing

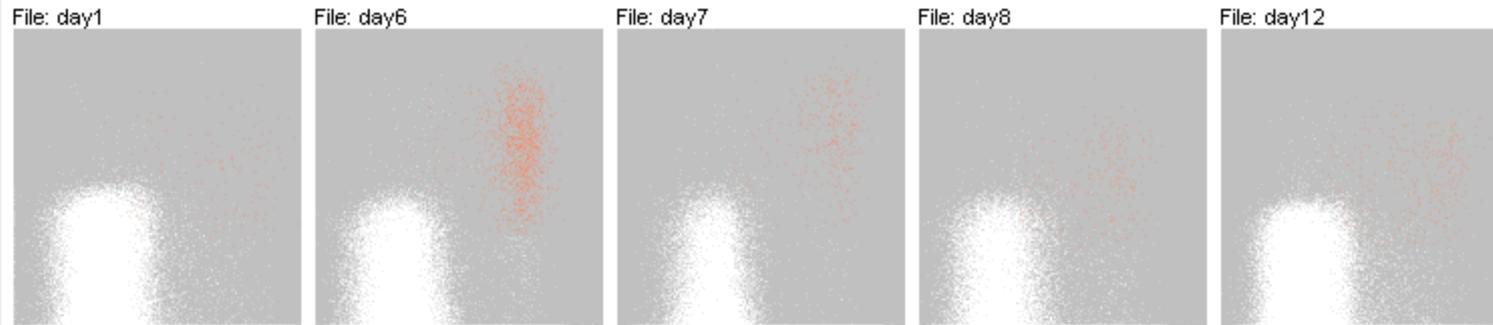
### Flow Cytometry Analysis (Beta) / Cross Sample / Analysis Result

Percentage Table | Marker by Population | Download ▾ |

### Cross Sample Analysis - Task Name: Add analysis name here

Population: 19 ▾ Marker 1: Ki67 ▾ Marker 2: CD138 ▾

Population: 19 Marker 1: Ki67 Marker 2: CD138



Use Population and Marker drop-down menus to assist with Cross-Sample viewing for multiple files and marker combinations