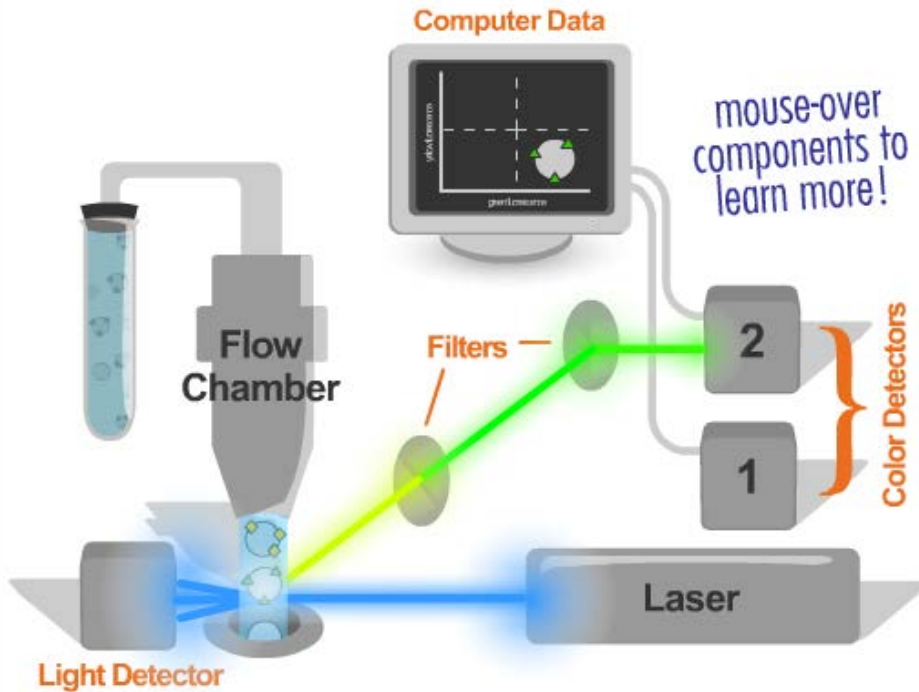
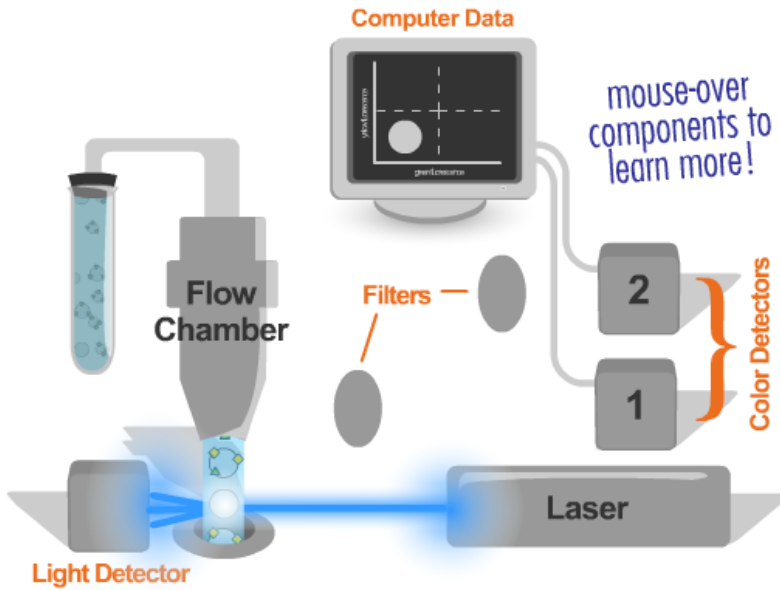
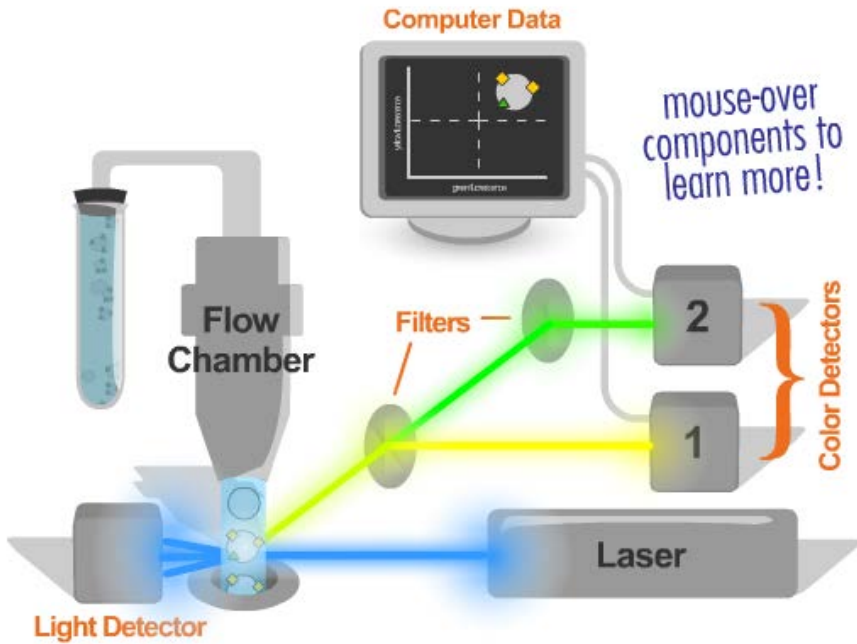


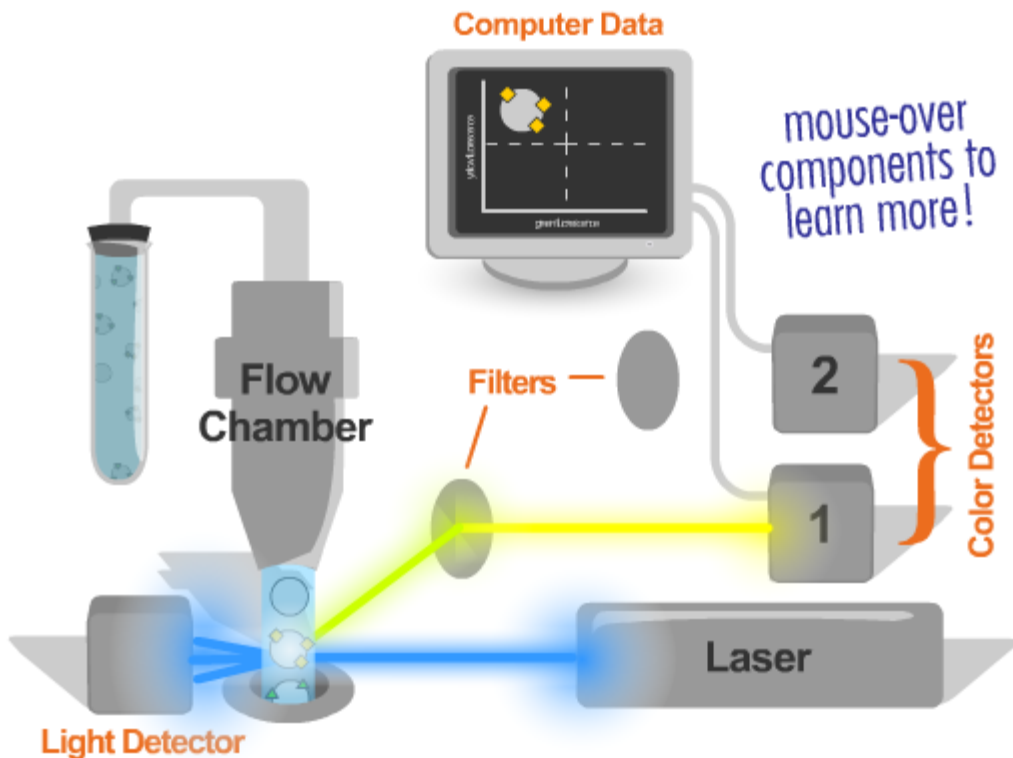
# Flow Cytometry - How Does It Work?



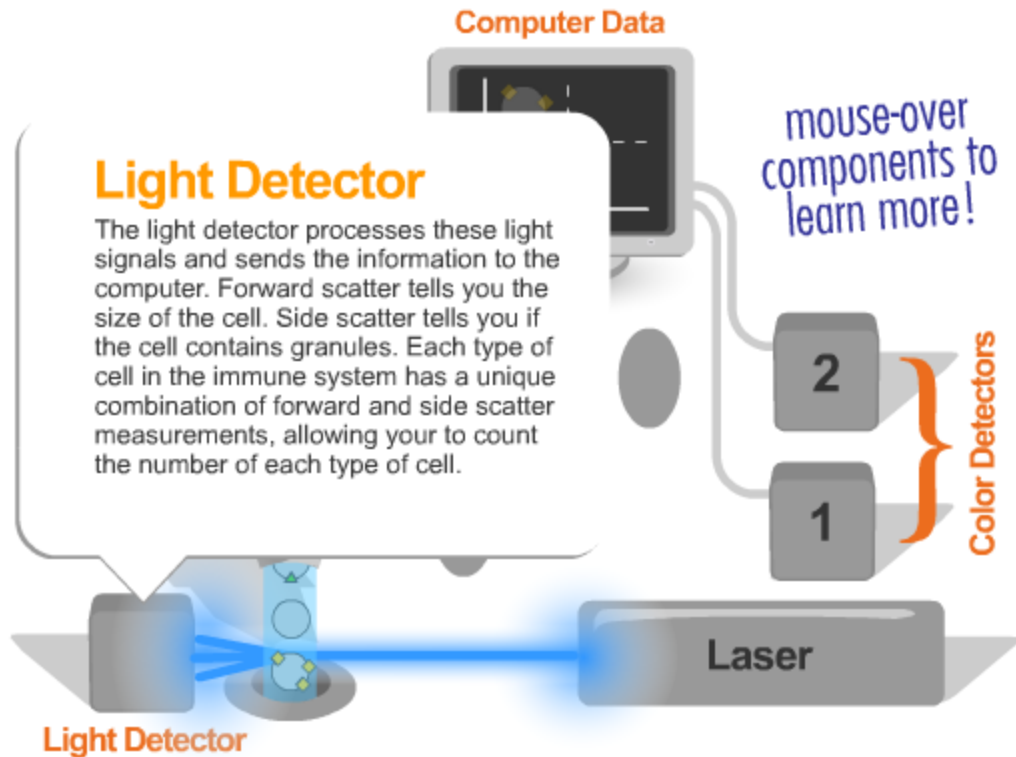
## Flow Cytometry - How Does It Work?



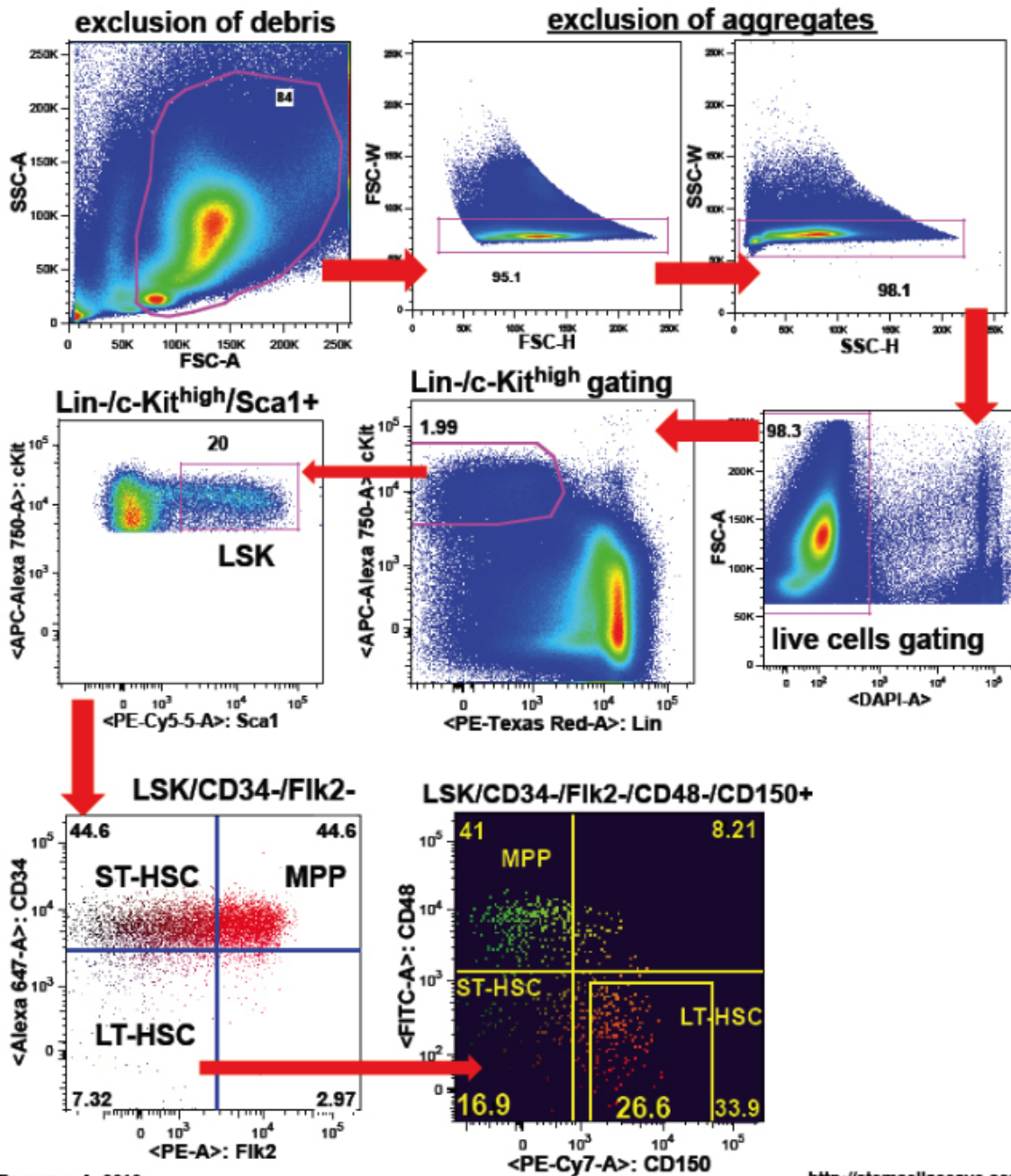
## Flow Cytometry - How Does It Work?



# Flow Cytometry - How Does It Work?



## Example of a gating strategy.



Bersenev A, 2010

<http://stemcellassays.com>

Manual gating, is a labor-intensive process and dependent on user experience.

Demarcation achieved through successive 2/3 dimensions. That hinders the identification of higher-dimensional features.

# Standardized .FCS format

FCS Extract 1.02

Text Segment | Data Segment | Batch Extraction | Setup |

Read Write C:\Users\sqqz\Desktop\FACS\_Multivariate\SMALL\_phospho.lymphgated.fcs\111306C\_5minLymphocytes.fcs

File Header

```
Version: FCS2.0
TextFirst: 58
TextLast: 2501
DataFirst: 2560
DataLast: 323423
AnalysisFirst: 0
AnalysisLast: 0
```

Text Segment  AutoSave to .TXT

Keyword	Value
\$TOT	20054
\$PAR	8
\$MODE	L
\$BYTEORD	4,3,2,1
\$FIL	111306C_5minLymphocytes.fcs
\$NEXTDATA	0
\$DATATYPE	I
\$BTIM	22:29:36
\$CYT	FACSCalibur
\$DATE	13-Nov-06
\$ETIM	22:30:12
\$DP	Lisa Maier
\$SYS	Macintosh System Software 10.2.8
\$TIMESTEP	0.10

20,054 rows Read time[sec] = 0.9

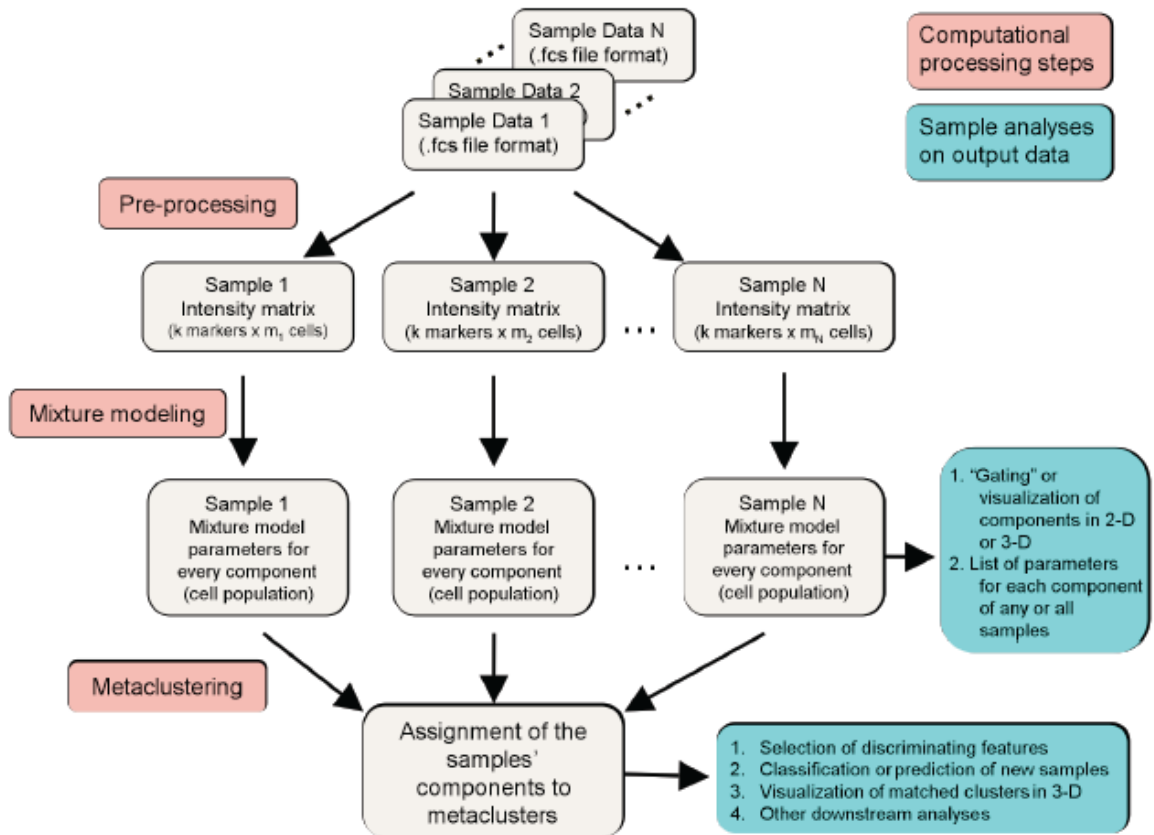
FCS Extract 1.02

Text Segment | Data Segment | Batch Extraction | Setup |

Parameter	1	2	3	4	5	6	7	8
Name	FSC-H	SSC-H	FL1-H	FL2-H	FL3-H	FL2-A	FL4-H	Time
String			SLP76-A488	ZAP70Y292-F	CD4-PerCP		CD45RA-APC	Time (102.40)
Range	1024	1024	1024	1024	1024	1024	1024	512
Bits	16	16	16	16	16	16	16	16
1	484	289	137	154	11	0	743	0
2	412	164	222	288	462	0	562	0
3	388	164	124	74	144	0	726	0
4	361	140	109	98	428	0	212	0
5	424	237	139	306	489	0	278	1
6	414	109	210	204	479	0	679	1
7	443	181	191	162	290	0	118	1
8	388	176	212	322	246	0	381	1
9	418	128	108	95	168	0	753	1
10	459	221	181	249	320	0	804	1
11	360	110	117	233	484	0	671	1
12	443	208	142	179	135	0	783	1
13	442	143	130	138	441	0	191	1
14	468	246	186	188	246	0	790	1
15	504	197	143	153	37	0	719	2
16	383	153	218	335	225	0	776	2
17	380	189	255	382	311	0	793	2

20,054 rows Read time[sec] = 0.9

# FLAME Workflow



**Supplementary Figure S1. Schematic representation of FLAME dataflow.** In this flowchart, we outline the dataflow for FLAME's computational pipeline beginning with the raw flow cytometric data files (in .fcs format) for all the samples and ending with the assignment of their components to metaclusters. At each stage of the process we indicate what external analyses or visualizations can be done with the intermediate data (output files). FLAME processing steps are noted in salmon, external functions are noted in turquoise.

Pyne et al. Automated high-dimensional flow cytometric data analysis. Proc Natl Acad Sci U S A. 2009 May 26; 106(21): 8519–8524.

- Modules & Pipelines
- Category  suite  all
- all | close all
- Recently Used
- sToCsv
- AMEChooseOptimalClusterNumber
- AMEMetacluster
- AMEMixtureModel
- AMEPreprocess
- reviewFCS
- Annotation
- GeneCruiser
- Clustering
- ConsensusClustering
- HierarchicalClustering
- MeansClustering
- MFConsensus
- OMClustering
- ParseHierarchicalClustering
- ubMap
- Format Conversion
- amToSam
- edToGif
- ivToGif
- prToGct
- stqToSam
- sToCsv
- km\_trackingToGct
- ctToPcl
- itoGct
- ard\_SamToFastq
- amToBam
- napToGct
- Flow Cytometry
- IdFCSEventIndex
- IdFCSParameter
- IdNoiseToFCS
- mplyGatingML
- mpensateFCS
- IdentifyFCS
- tractFCSdataset
- tractFCSkeywords
- tractFCSparameters
- OMFeatureExtraction
- CMSinglePanelQC
- SNormalization
- AMEChooseOptimalClusterNumber
- AMEContourDataGenerator
- AMEMetacluster
- AMEMixtureModel
- AMEPreprocess
- AMEPreviewTransformation
- owClusterClassifyFCS

8/15/12 **QuaSAR** now available: *A tool for QC, analysis and visualization of data from MRM-MS experiments.*

8/9/12 **GenePattern-BCCRC Flow Cytometry Suite** now available. Select from Suites in Modules & Pipelines Panel on the left or click [here](#) for more information.

**FLAMEMetacluster** version 5  Show parameter descriptions

\* required field   [properties](#) | [export](#) | [help](#)

optimal g mixture model\*   
Select a single file under 2GB to upload.  
 Specify URL  Basic Upload  
A .zip file containing the optimal mixture modeling result of each sample from the output of the FLAMEChooseOptimalClusterNumber module.

sample class   
Select a single file under 2GB to upload.  
 Specify URL  Basic Upload

estimate mode\*   
Used only for skew distributions. Whether to estimate the mode for each cluster. Must be the same value used in FLAMEMixtureModel.

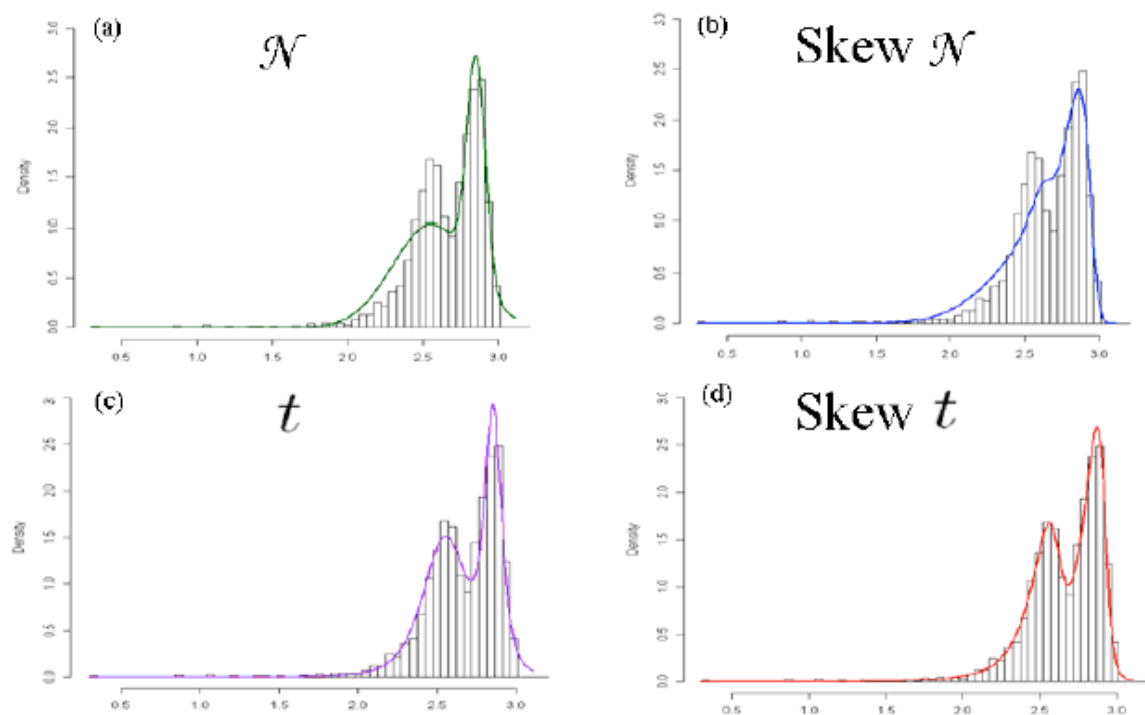
estimation increment\*   
Used only for skew distributions and when estimate mode is set to yes. The smaller the increment, the more accurate the estimation, but the slower the estimation step. Must be the same value used in FLAMEMixtureModel.

output intermediate results\*   
Choose whether to output the intermediate metaclustering results, such as within-class matching results.

output prefix\*   
A prefix for output files.

[properties](#) | [export](#) | [help](#)

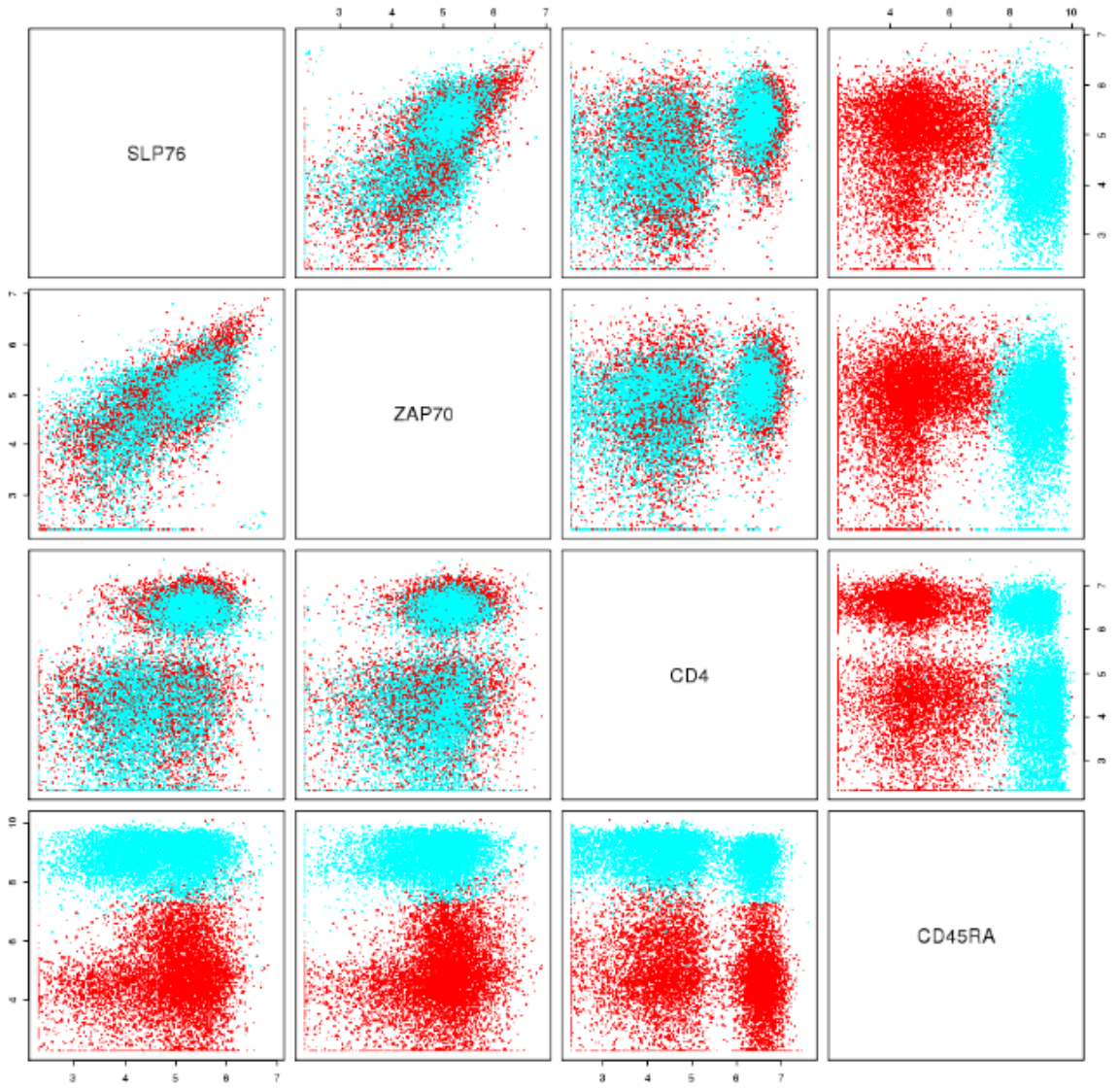
- Recent Jobs Uploads
- FLAMEMetacluster (582989) Aug 28 09:50:02 AM  
MetaCluster.SmallPhospho.FinalAlignedClusters.zip  
stdout.txt
  - FLAMEChooseOptimalClusterNumber (582987) Aug 28 09:40:03 AM  
SmallPhospho.ClusterNumber.OptimalG.zip  
stdout.txt
  - FLAMEMixtureModel (582888) Aug 28 12:48:19 AM  
SmallPhospho.PreprocessedData.MixtureModel.zip  
stdout.txt
  - FLAMEPreprocess (582882) Aug 27 10:11:58 PM  
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stdout.txt
  - PreviewFCS (582862) Aug 27 09:01:42 PM  
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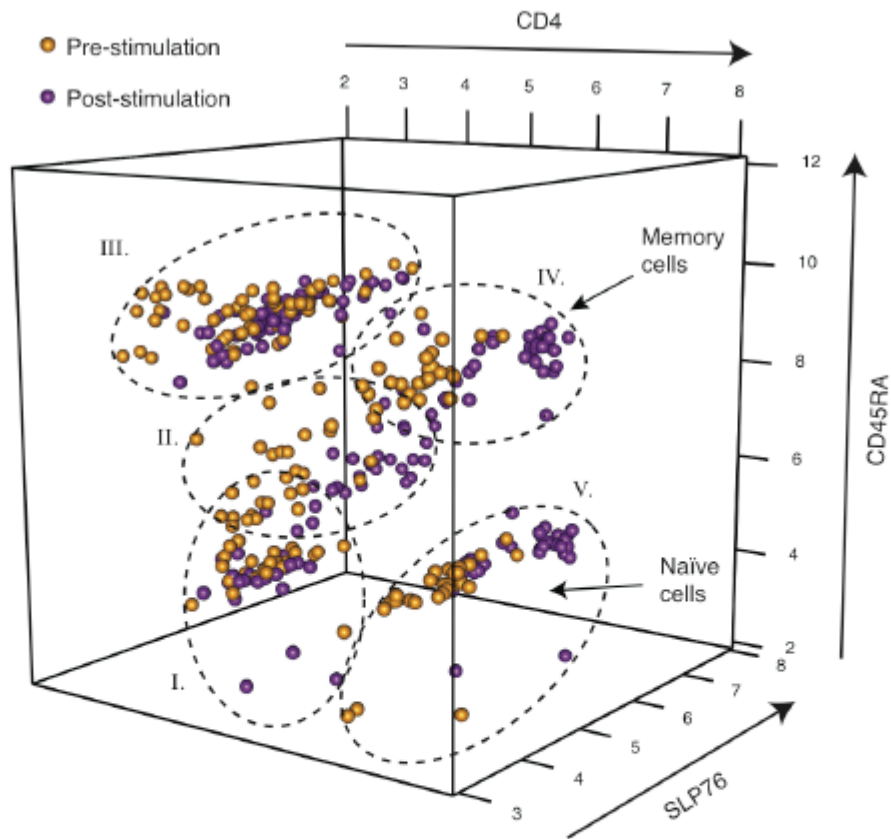


**Supplementary Figure S6. Mixture modeling with different distributions.** Here we fit a skewed one-dimensional intensity distribution (unpublished data, from M.G. Kharas and D.G. Gilliland) with (a) normal, (b) skew normal, (c)  $t$  and (d) skew  $t$  mixture models plotted in green, blue, violet and red respectively. While all four distributions yield 2-component univariate models, skew  $t$  provides the best fit to the actual distribution.



matched.111306C\_5minLymphocytes.mst.2.aligned





**Supplementary Figure S3. Meta-clustering of cell populations across 29 subjects measured before and after T cell receptor stimulation.** Results of FLAME's use of PAM to match subjects' clusters across the cohort. First, the pre- (yellow) and post- (purple) stimulation modes for each cohort were metaclustered independently – each cohort yielding five populations. Next, the corresponding metaclusters between the two classes were identified. In the figure, all modes from all subjects are overlaid to illustrate the five subpopulations and the difference in phosphorylation after stimulation.