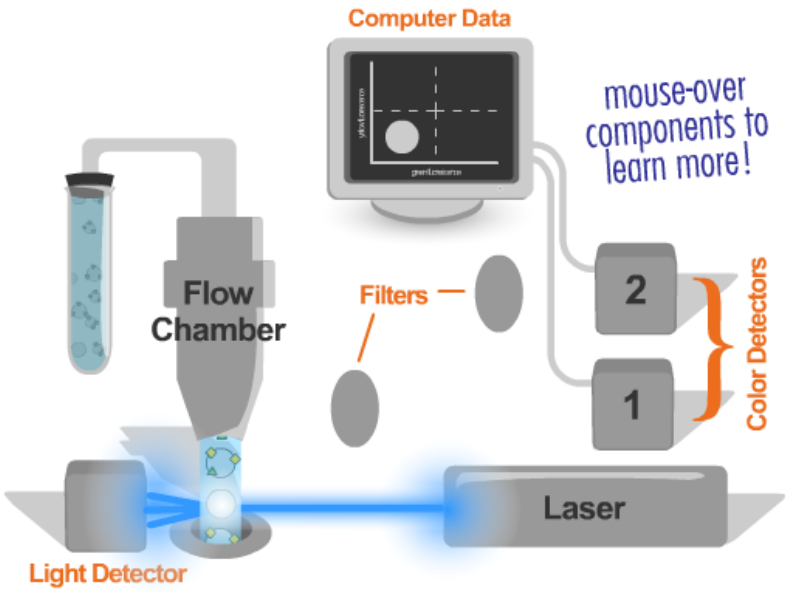
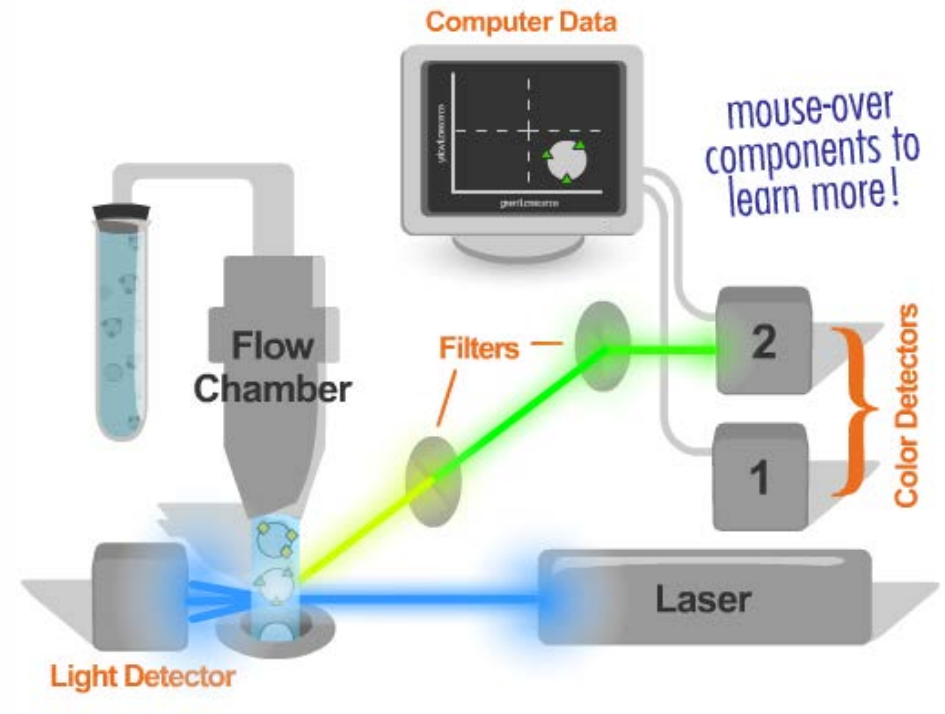
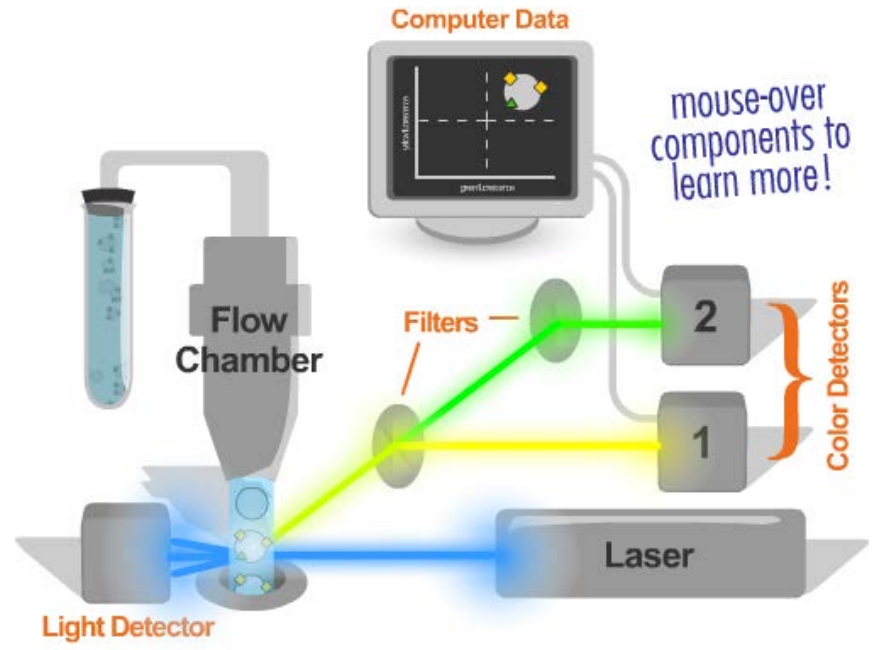
Flow Cytometry - How Does It Work?



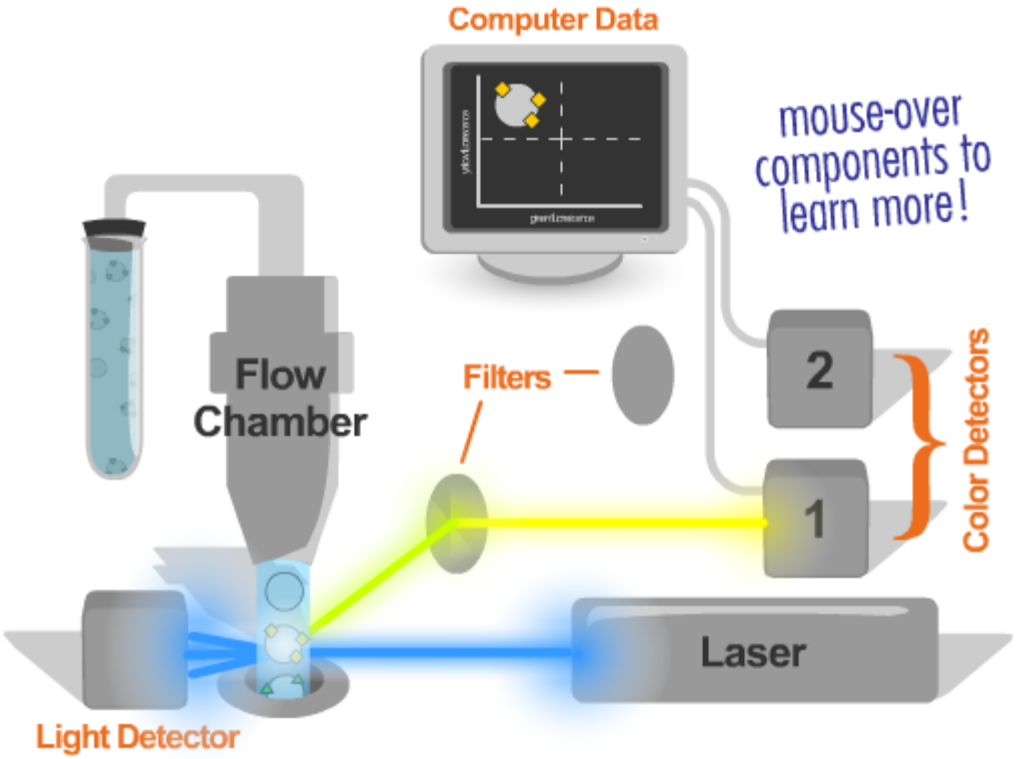


http://www.unsolvedmysteries.oregonstate.edu/flow_06

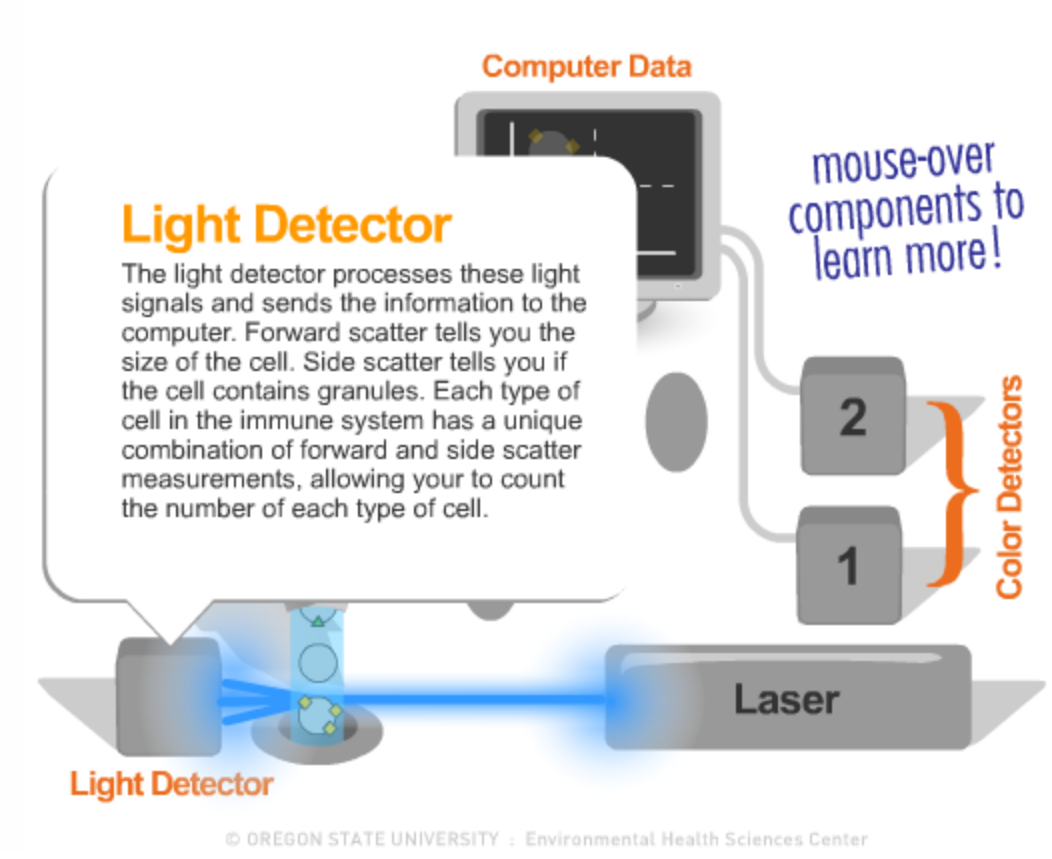
Flow Cytometry - How Does It Work?



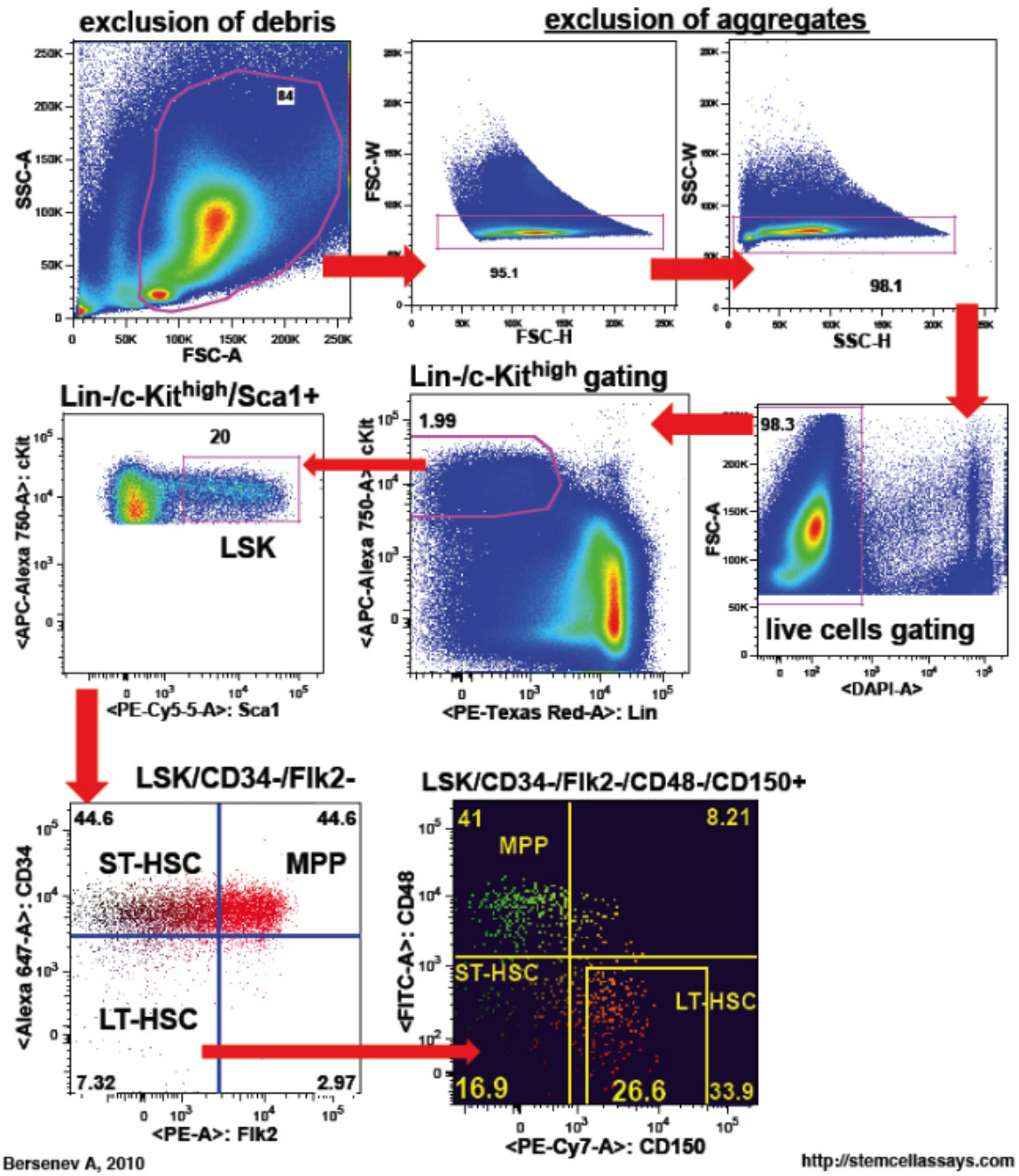
Flow Cytometry - How Does It Work?



Flow Cytometry - How Does It Work?



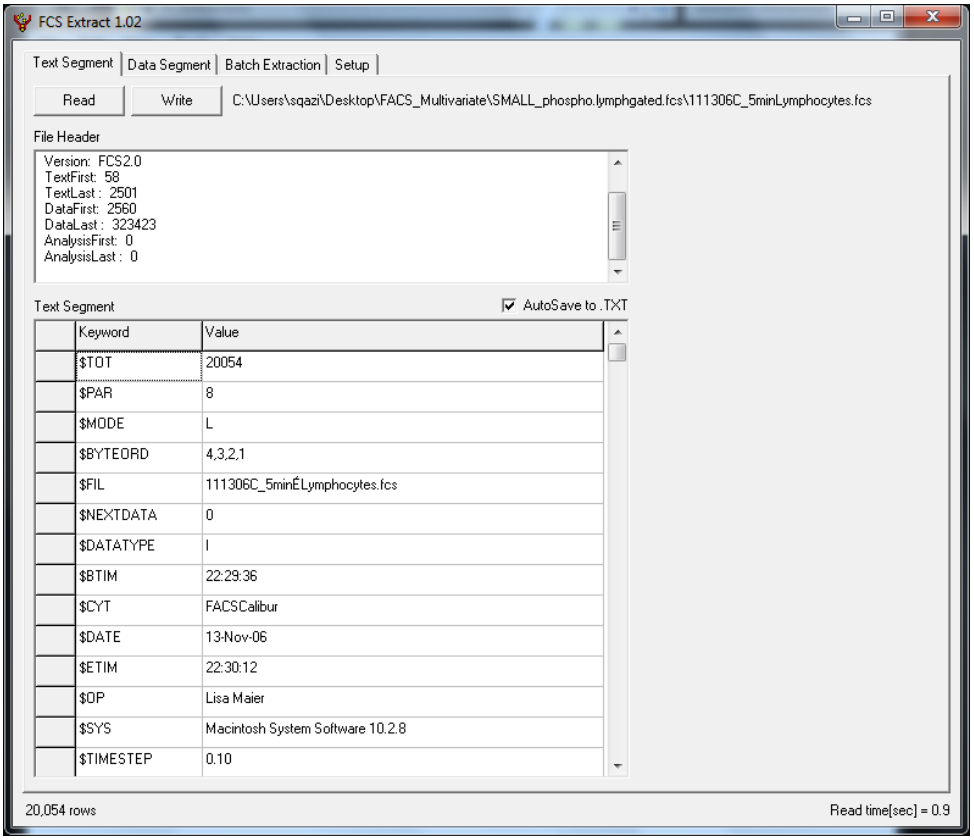
Example of a gating strategy.

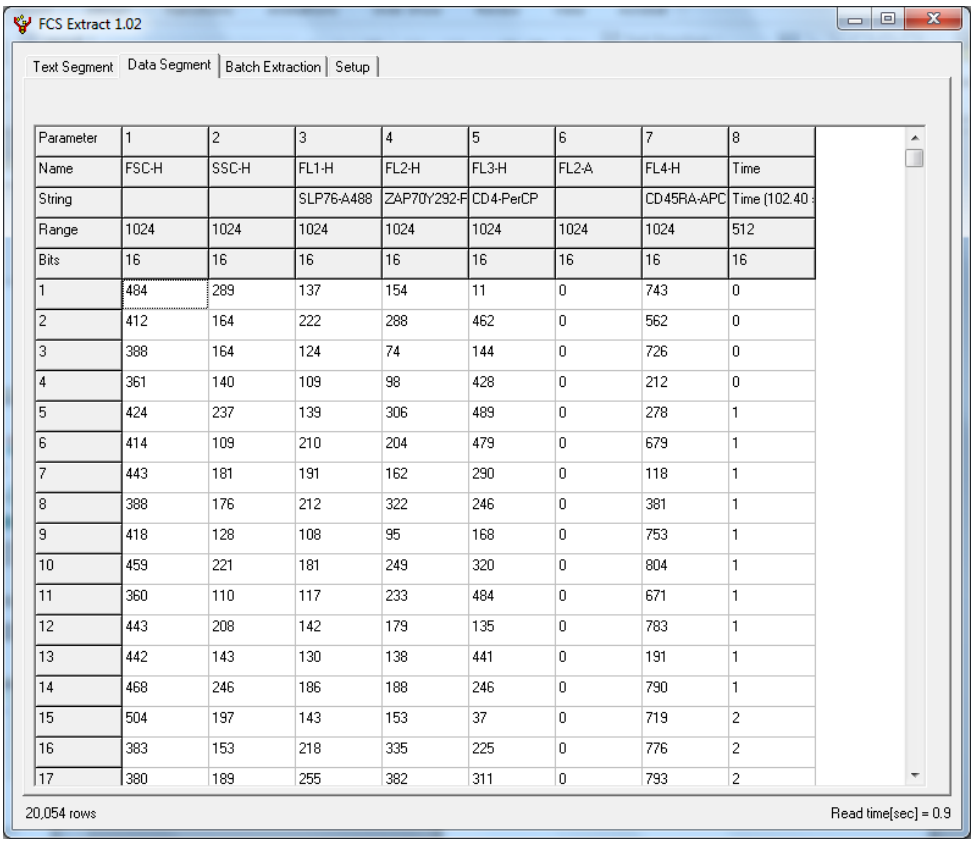


Manual gating, is a labor-intensive process and dependent on user experience.

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

Standardized .FCS format





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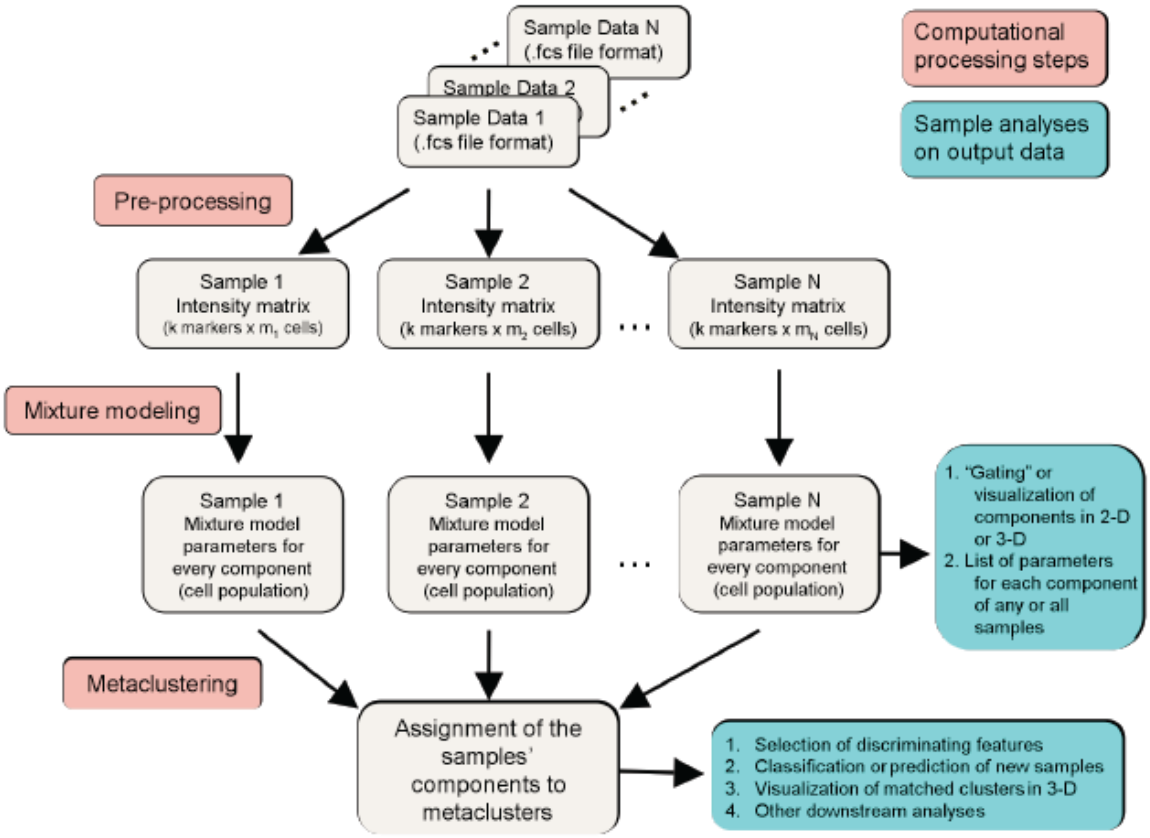
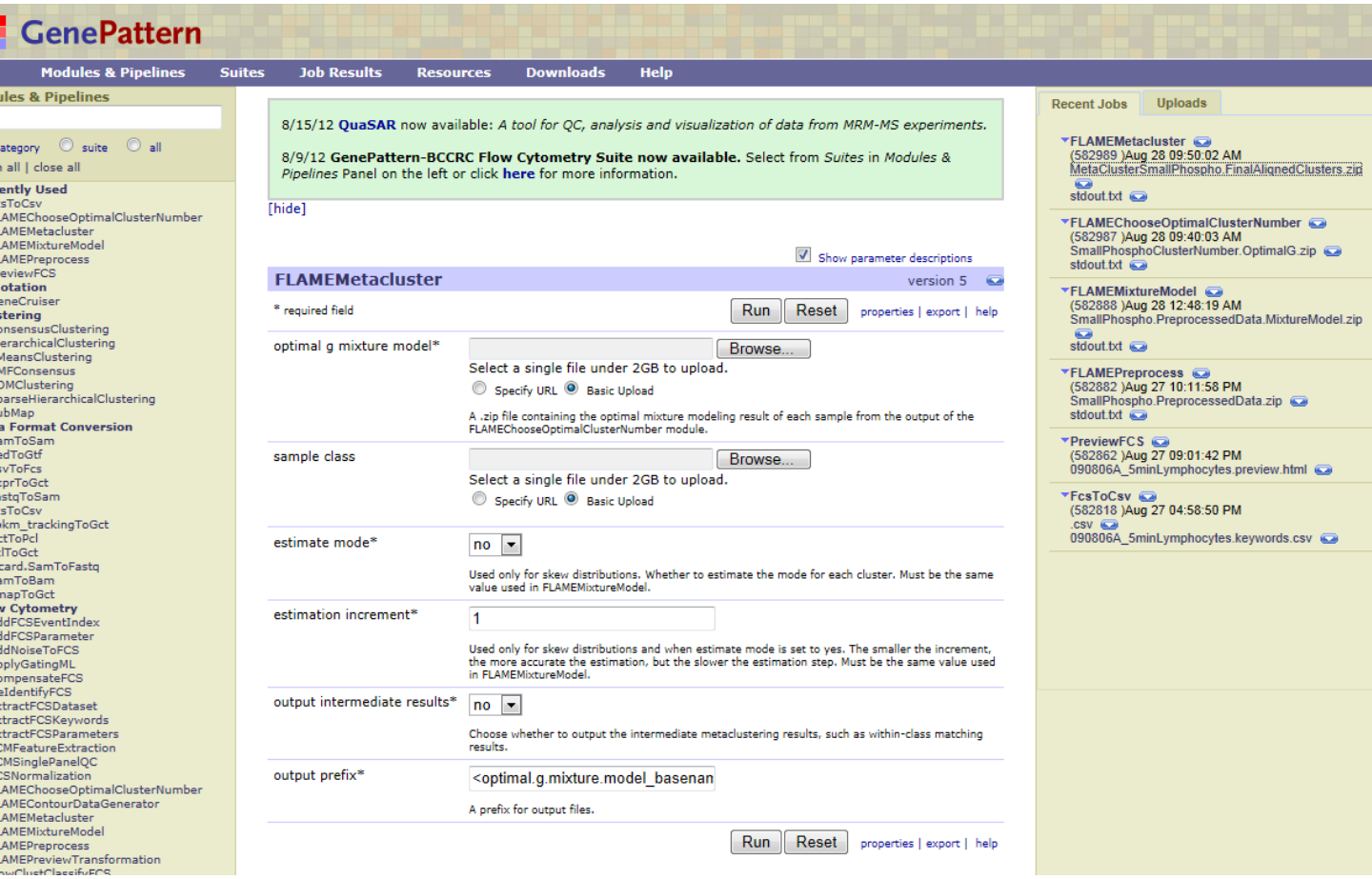
2	412	164	222	288	462	0	562	0
3	388	164	124	74	144	0	726	0
4	361	140	109	98	428	0	212	0
5	424	237	139	306	489	0	278	1
6	414	109	210	204	479	0	679	1
7	443	181	191	162	290	0	118	1
8	388	176	212	322	246	0	381	1
9	418	128	108	95	168	0	753	1
10	459	221	181	249	320	0	804	1
11	360	110	117	233	484	0	671	1
12	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

FLAME Workflow

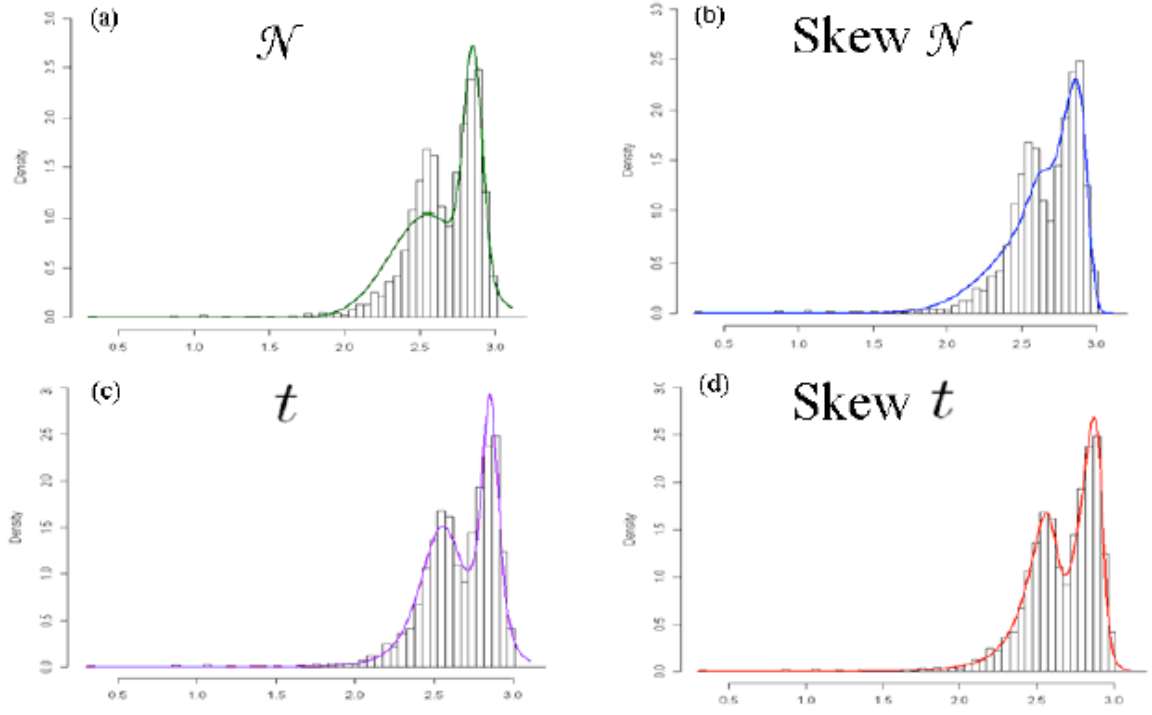


Supplementary Figure S1. Schematic representation of FLAME dataflow. In this flowchart, we outline the dataflow for FLAME's computational pipeline beginning with the raw flow cytometric data files (in .fcs format) for all the samples and ending with the assignment of their components to metaclusters. 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.

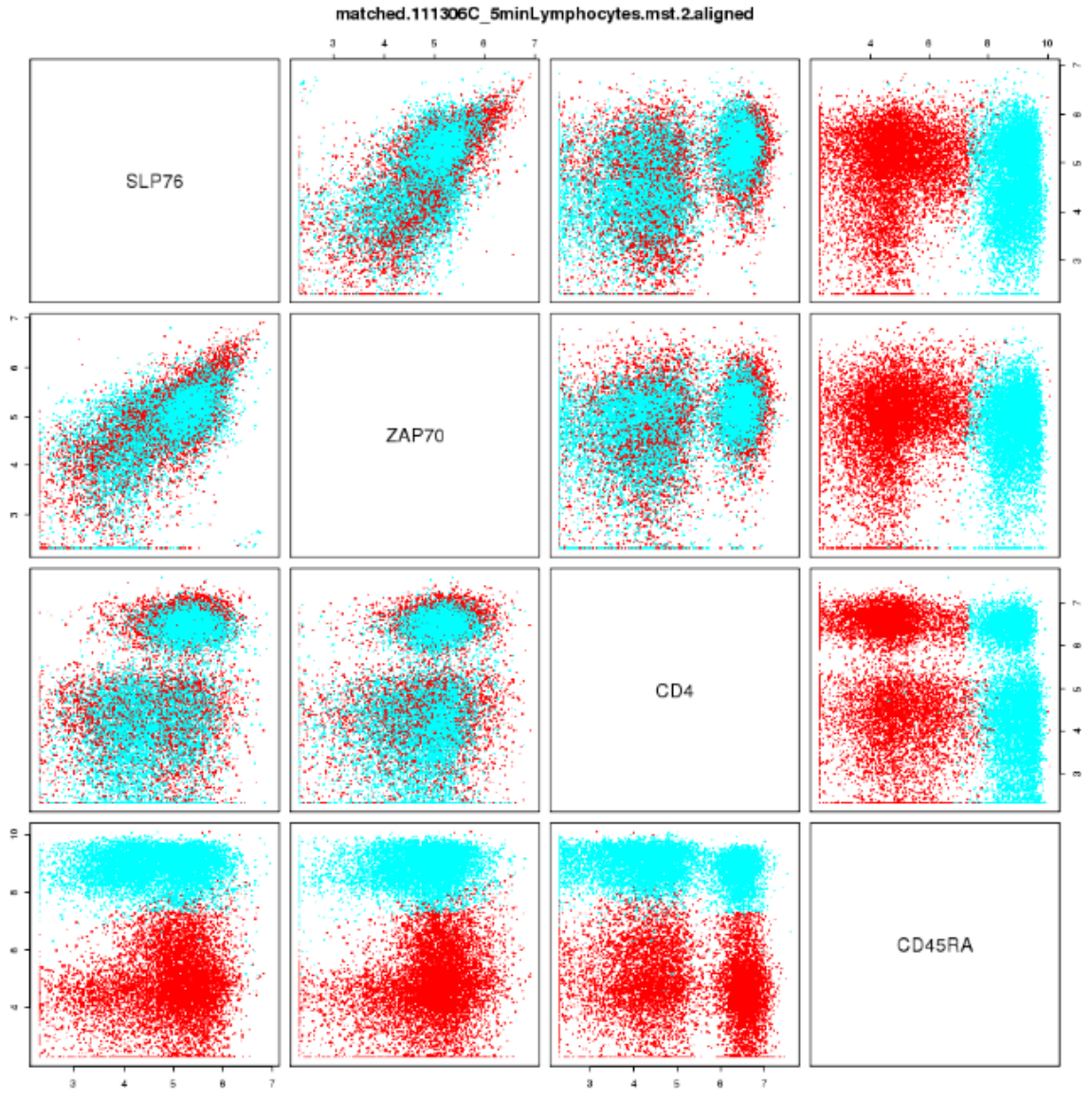
Pyne etal. Automated high-dimensional flow cytometric data analysis. Proc Natl Acad Sci U S A. 2009 May 26; 106(21): 8519–8524.



http://genepattern.broadinstitute.org/gp/pages/index.jsf



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.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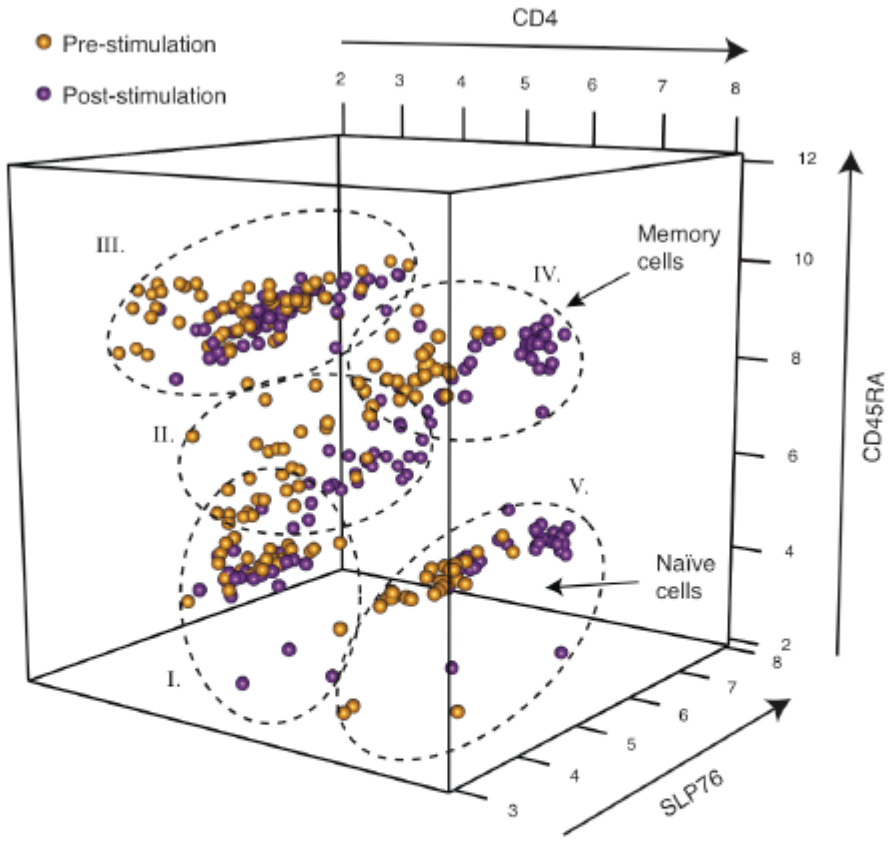
CD45RA



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.