Flow cytometry data-Analysis and Visualization

ChUG
Cytometry

Challenges in analyzing high-dimensional data

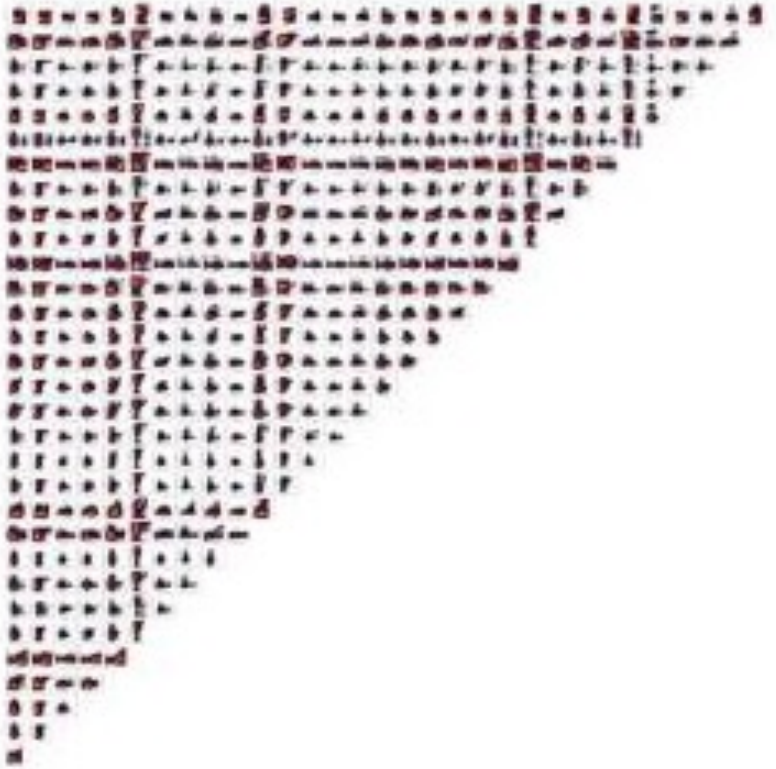
- Historically
  - manual gating
  - histograms and biaxial plots
- Manual gating pitfalls-subjective, time consuming, will not work for high-dimensional data)
- Histograms and biaxial plots are almost impossible for high parameter flow cytometry.
- Supervised vs semi-supervised vs unsupervised

9 parameters

36 plots

32 parameters

496 plots



Bendall et al., Trends Immunol. 2012 Jul; 33(7): 323–332.

Supervised vs unsupervised vs semisupervised

Supervised, useful for hypothesis testing

- manual gating
- visual QC with 2 and 3D plots
- Abundance, MFI, statistics etc.

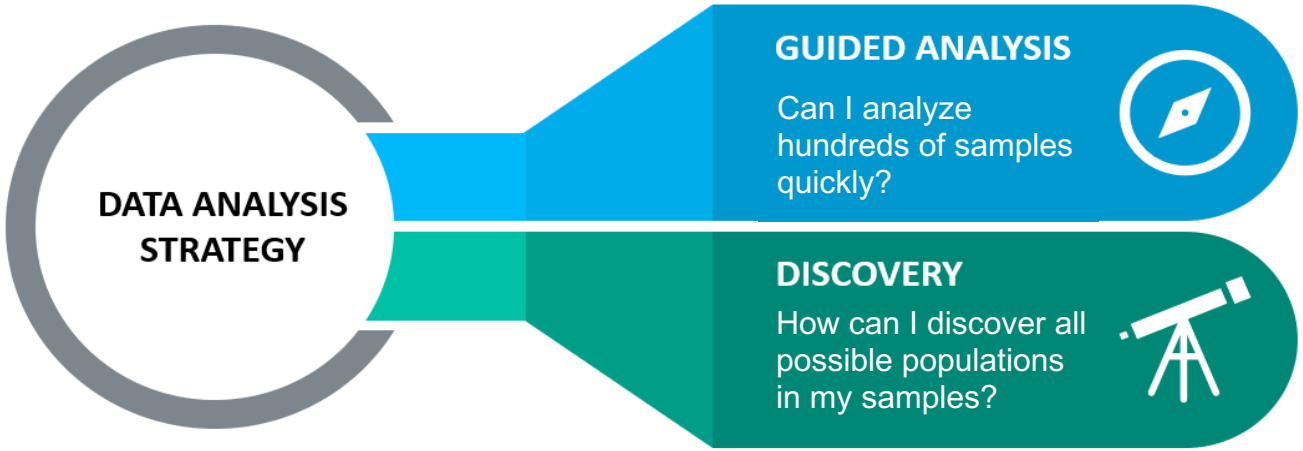
Unsupervised, approach is better suited for exploratory analysis and hypothesis-generating experiments

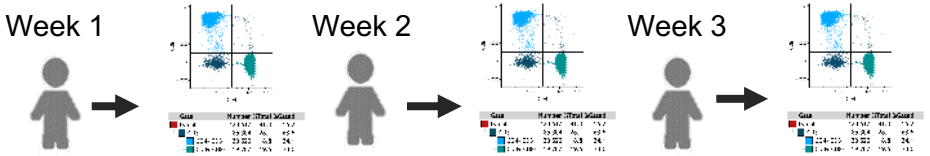
- Dimensionality reduction
- Clustering
- HeatMaps

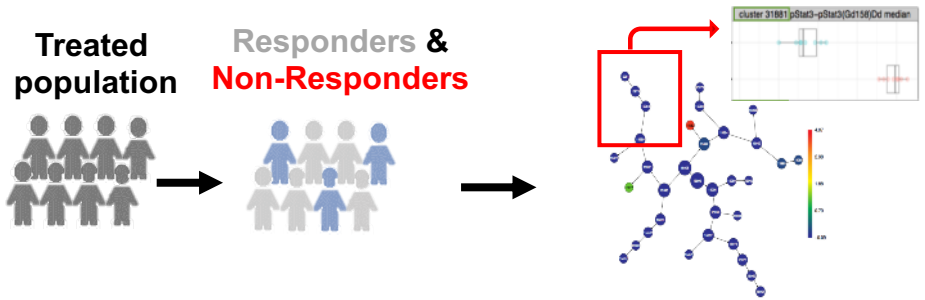
With well designed multi-parameter panel of antibodies this two approaches can be combined in one experiment. We postulate that most experiments would benefit from dual approach and will be demonstrated today.

The Best Data Analysis Approach Depends on Your Experiment

- Report defined population abundance
- Report marker intensity endpoints for user specified populations







- What previously unkown cellular subset exist in the sample?
- Which specific biomarker(s) differentiate the two groups?

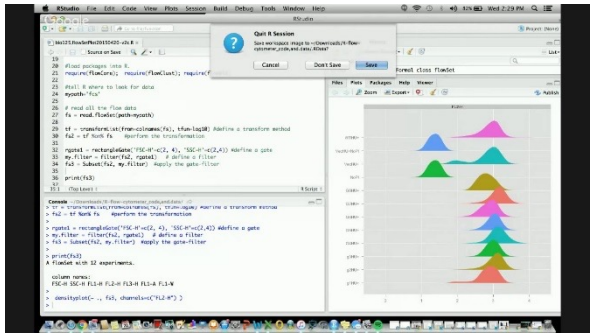
Slide courtesy of Cytobank, Beckman Coulter

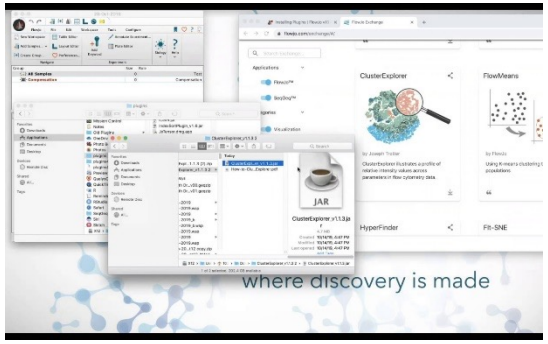
Some of the common platforms

R

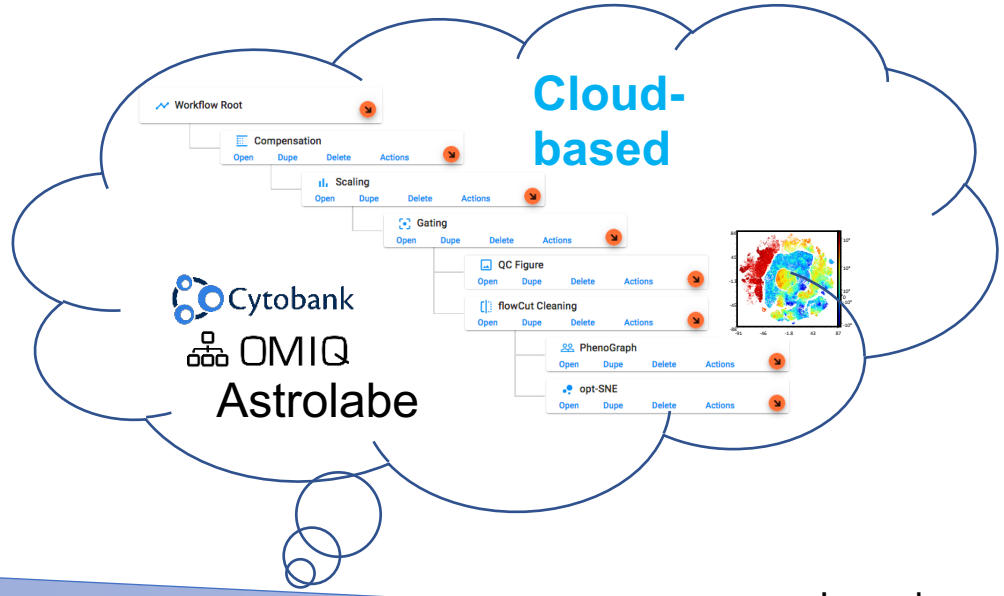
Desktop

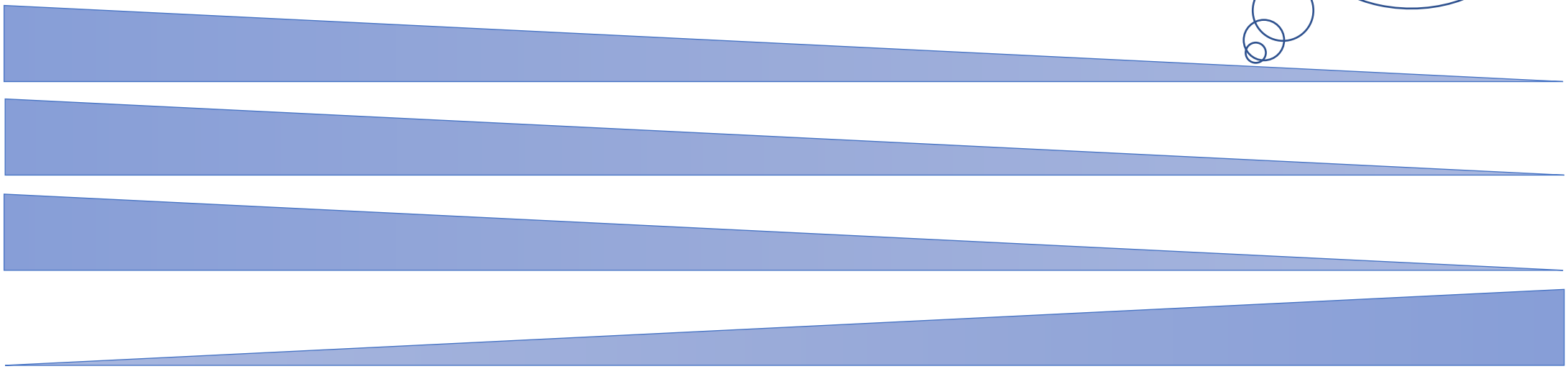
FlowJo
FCS Express
Kaluza





Cloud-based





Learning curve

Computational resources

Flexibility

$$$ Cost

Cloud based analysis vs Desktop based

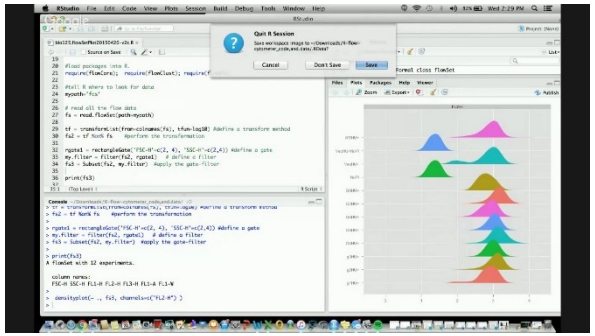
- Easier management of large data
- Easier sharing of data and analysis
- Data is secure
- Cloud computing
- Desktop- Comfort Zone
- Need high-efficiency computer
- Most likely resort to the cloud for data sharing

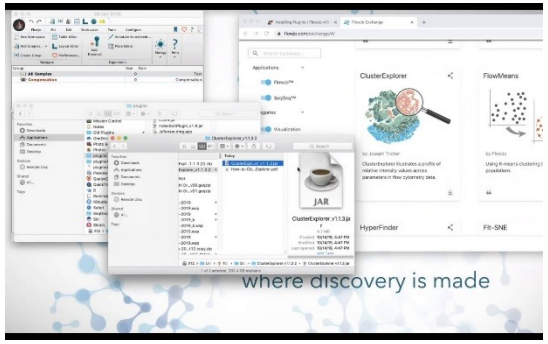
Some of the common platforms

R

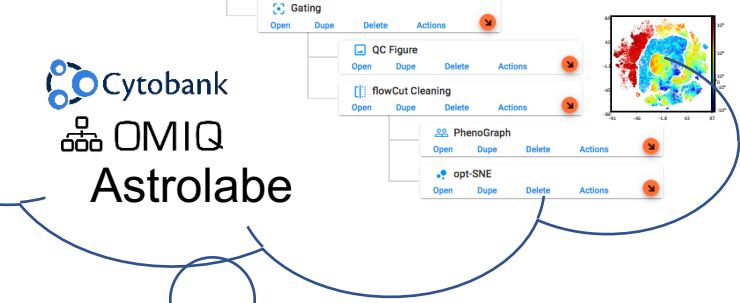
Desktop

FlowJo
FCS Express
Kaluza





Cloud-based



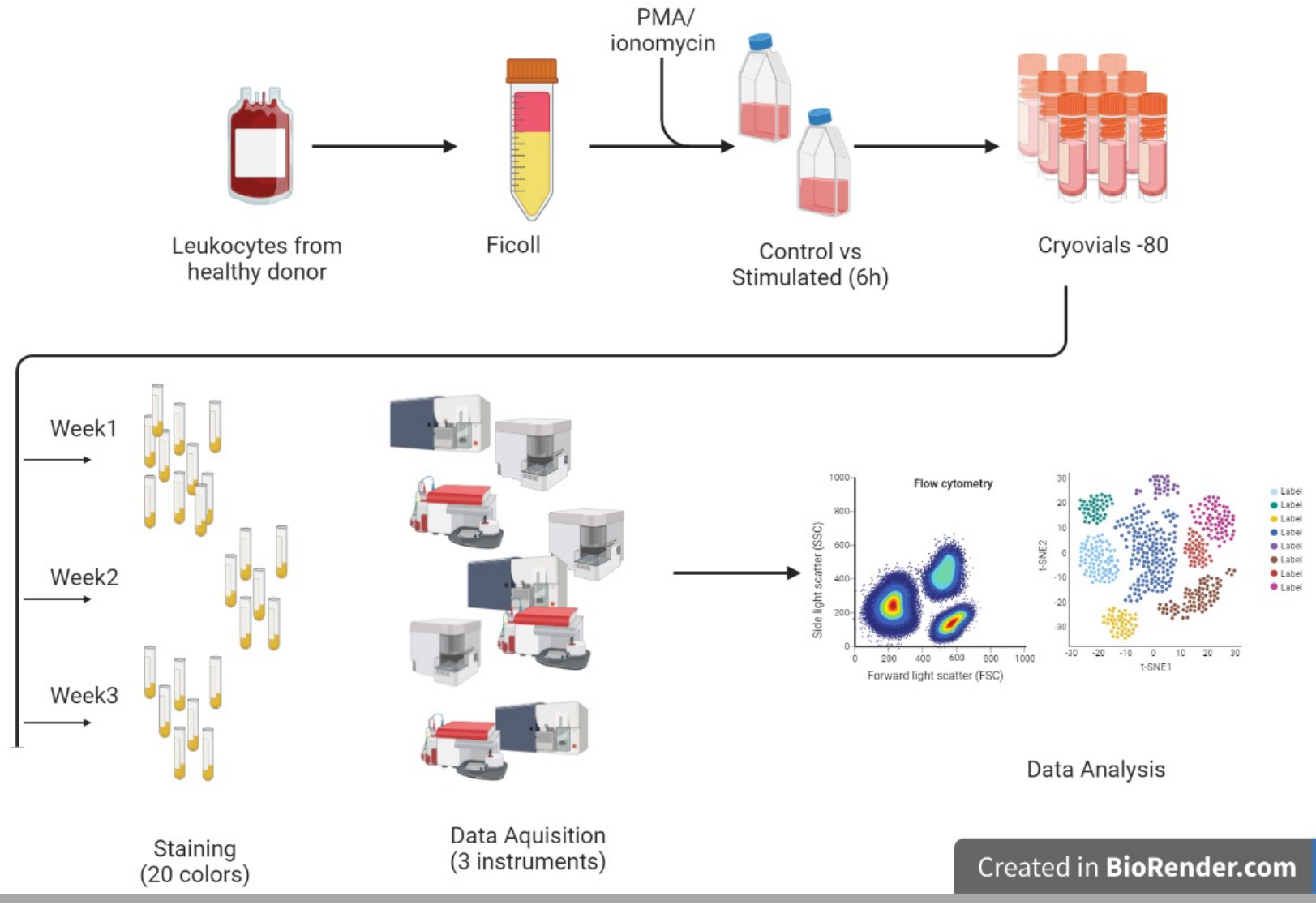
Learning curve

Computational resources

Flexibility

$$$ Cost

Experiment outline



Antibodies donated by:

BD Biosciences

Antibodies donated by:

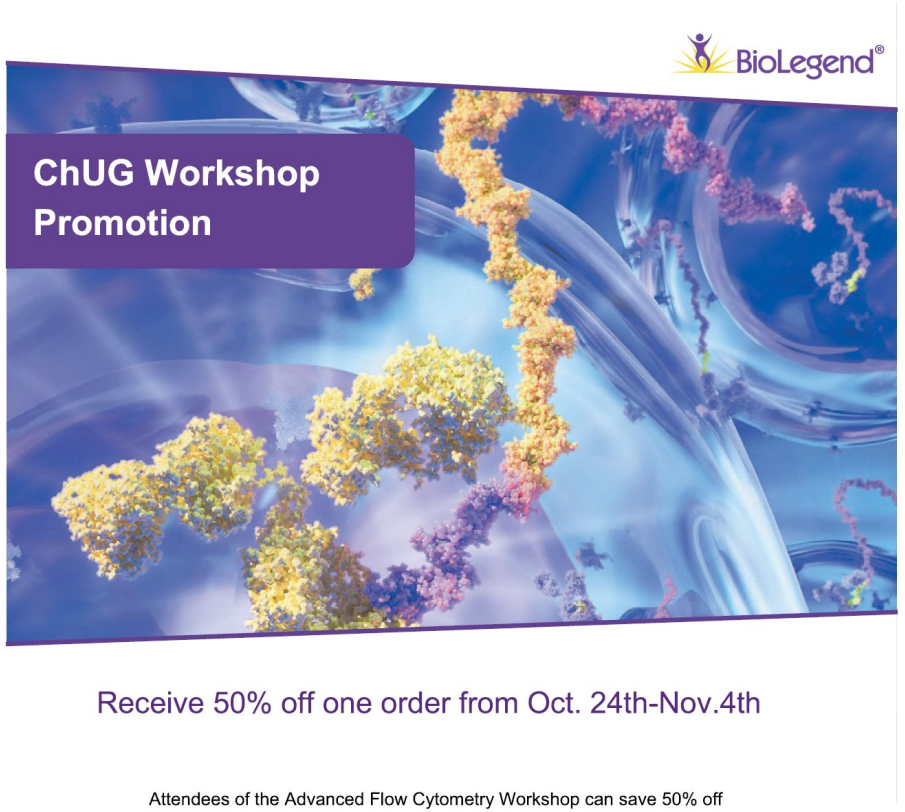
I would be happy to offer a one-time 50% promo valid for 2 weeks (excluding OptiBuild) to help academic researchers They would just need to reach out to me directly for a quote.

BD
Shari Sanders
Research Reagent Specialist
Single Cell Multiomics (scM)
BD Biosciences
2350 Qume Drive
San Jose, California, 95131-1807
mobile: 331-302-6716

Antibodies donated by:

BioLegend®

Enabling Legendary Discovery™



Antibodies donated by:

ThermoFisher SCIENTIFIC

I am happy to let you know I was able to create a promotion that will give all ChUG attendees **50% off all Flow Antibodies and Reagents** (comp beads, Live/Dead Dyes, Molecular Probe Dyes, etc…) and this promotion will extend until December 15th, 2022

Emily Hennessy
Technical Sales Specialist
Flow Cytometry Reagents
MN, WI, IA, IL, MI, IN
Thermo Fisher Scientific
Mobile +1 978-766-9552
Emily.Hennessy@Thermofisher.com

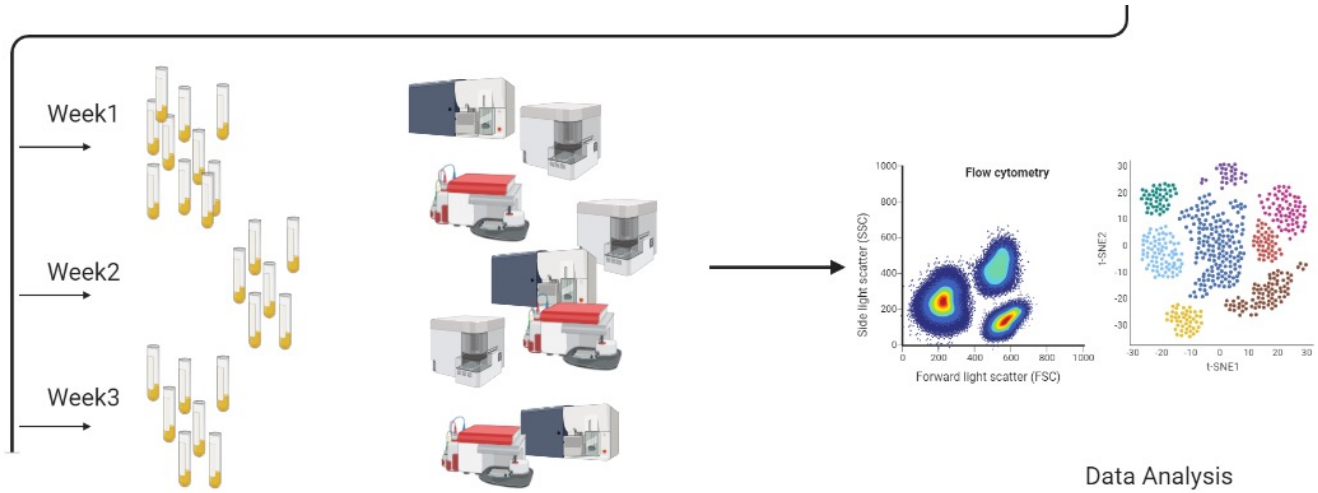
Antibodies donated by:

Yes! 50% off the first three StarBright antibodies per customer until the end of this year, and I will do 50% off any other flow antibody for the rest of the year (up to three per customer). They are welcome to reach out to me directly and you are welcome to provide them with my contact information.

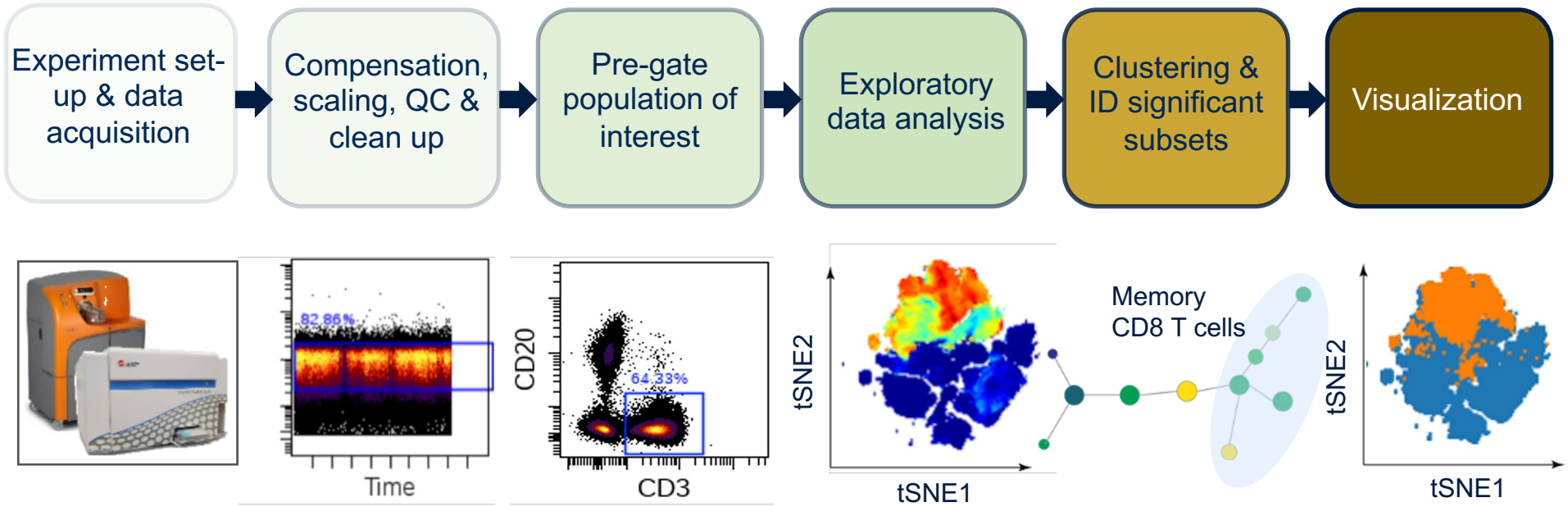
Don Boyer
Senior Account Executive | Cell Biology
Southeast and Midwest Regions
Bio-Rad, Inc.
Email: Donald_Boyer@Bio-Rad.com
Cell: (510) 806-5779

Using this data set we may be able to:

- **Evaluate different analysis methods**
- Observe, quantify and correct batch effect
- Take a closer look at spectral unmixing methods
- Compare data from different instruments

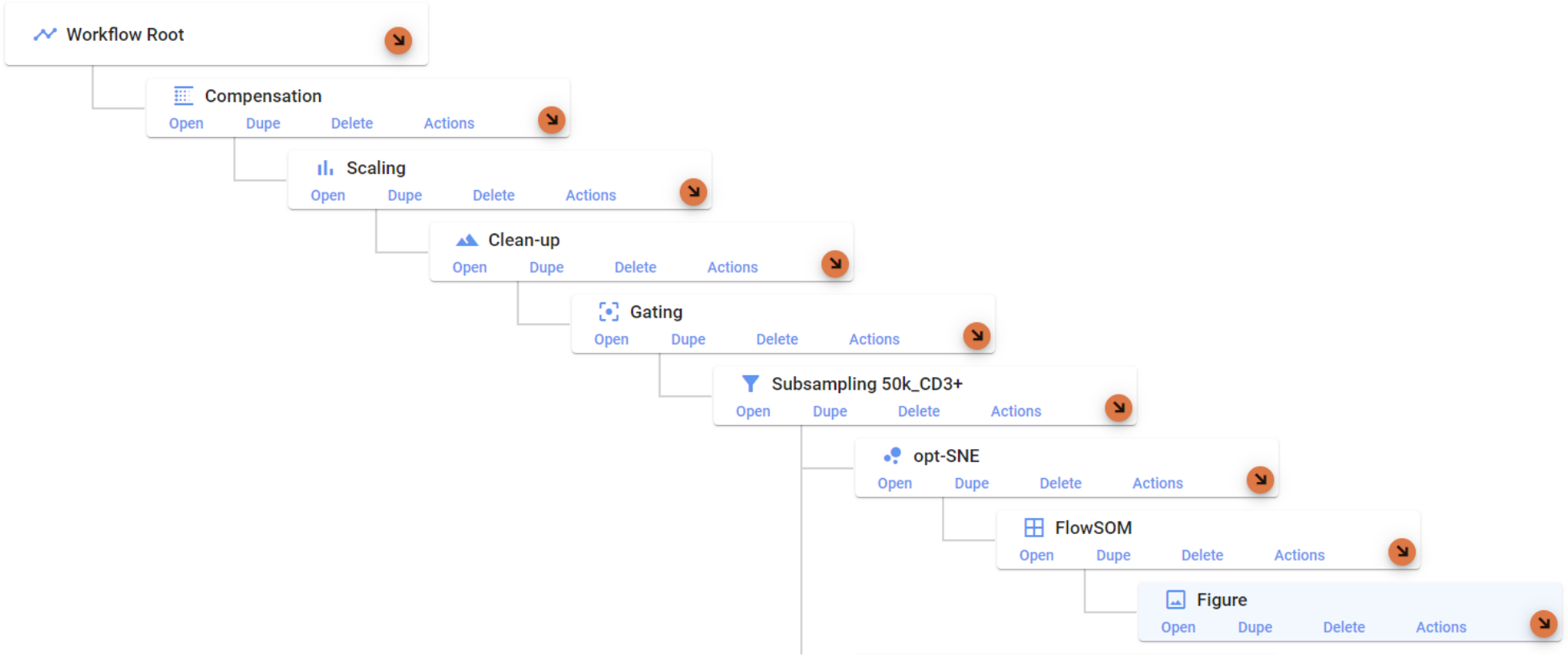


Computational Cytometry Data Analysis Workflow for Cytobank



Data Acquisition	QC and clean up	Pre-gate (optional)	viSNE, tSNE-CUDA, opt-SNE, UMAP	SPADE, FlowSOM, CITRUS	viSNE Overlay

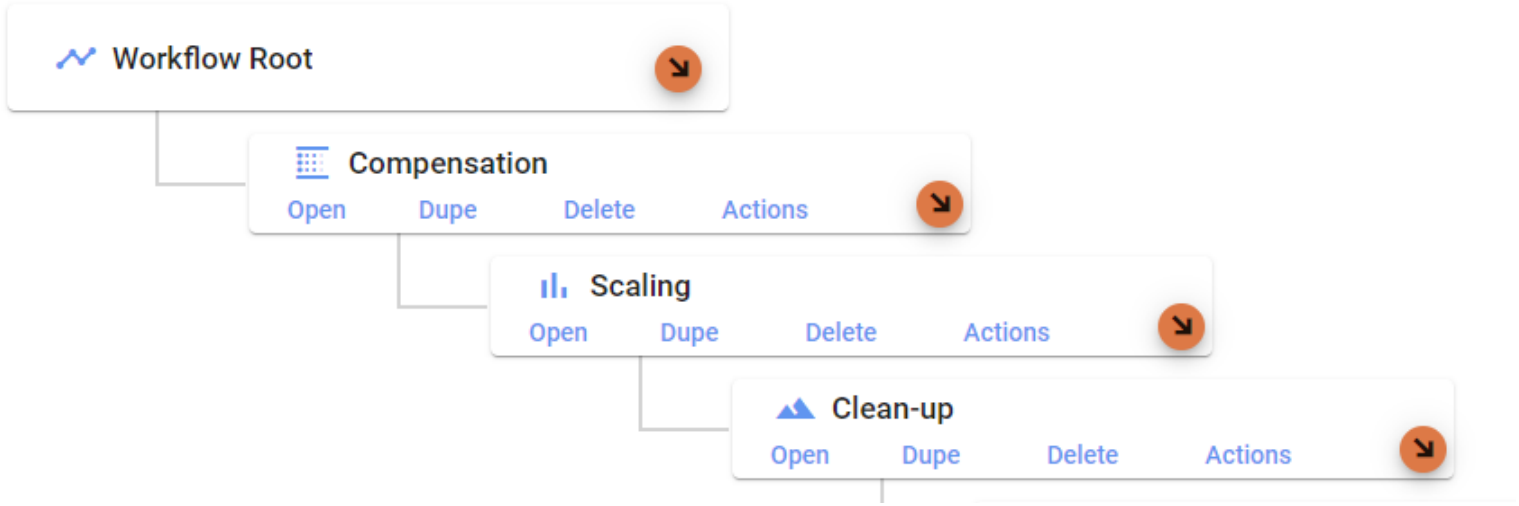
Preprocessing → supervised → unsupervised



Dimensionality reduction

Clustering

Preprocessing



Compensation

Select a Compensation Group

Comp Group 1

Create New Comp | Find File | More

Files in Group (10)

File Name
PBMC Unstim_exp3_3.fcs
PBMC Unstim EXP 2.fcs
PBMC Unstim.fcs
PBMC Unstim_exp3_2.fcs
PBMC Stim_exp3_1.fcs
PBMC Stim.fcs
PBMC Unstim_exp3_1.fcs
PBMC Stim_exp3_3.fcs
PBMC Stim EXP 2.fcs
PBMC Stim_exp3_2.fcs

Comp Matrix Actions

- Copy
- Paste
- Highlight Feature Pair
- Add Files to Group
- Remove Files from Group
- Copy Features Only
- Revert to Original
- Delete Group

Features	V805-A	BV421-A	LIVE DEAD Aqua-A	StarBright570-A	BV605-A	StarBright670-A	BV711-A	BV785-A	BB515-A	NovaBlue 610-A	NovaBlue 660-A	PE-A	PE-CF594-A	PE-Cy7-A	APC-A	APC-R700-A	APC-Fire 750-A	AF-A
BUV395-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BUV496-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BUV737-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BUV805-A	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BV421-A	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIVE DEAD Aqua-A	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
StarBright570-A	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BV605-A	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0
StarBright670-A	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
BV711-A	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
BV785-A	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
BB515-A	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
NovaBlue 610-A	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0
NovaBlue 660-A	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
PE-A	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
PE-CF594-A	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
PE-Cy7-A	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0
APC-A	0	0	0	0	0	0	0	0	0	0	0	0	-2.7	0	100	0	0	0
APC-R700-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0
APC-Fire 750-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
AF-A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100

Scaling - aka transformation

Filter Features

Feature Name	Scaling Type	Cofactor	Min	Max	
Time	None (linear)		0	6023025	
SSC-H	None (linear)		0	4194304	
SSC-A	None (linear)		0	4194304	
FSC-H	None (linear)		0	4194304	
FSC-A	None (linear)		0	4194304	
SSC-B-H	None (linear)		0	4194304	
SSC-B-A	None (linear)		0	4194304	
BUV395-A | CD11b	Arcsinh	600	-13000	4194304	
BUV496-A | CD16	Arcsinh	2000	-13000	4194304	
BUV737-A | IgD	Arcsinh	6000	-13000	4194304	
BUV805-A | CD14	Arcsinh	6000	-13000	4194304	
BV421-A | CD66b	Arcsinh	6000	-13000	4194304	
LIVE DEAD Aqua-A | Live/Dead	Arcsinh	3000	-20000	4194304	
StarBright570-A | CD27	Arcsinh	6000	-13000	4194304	
BV605-A | IgM	Arcsinh	6000	-13000	4194304	
StarBright670-A | CD19	Arcsinh	1000	-20000	4194304	
BV711-A | CD20	Arcsinh	6000	-13000	4194304	
BV785-A | CD8a	Arcsinh	1000	-13000	4194304	Y
BB515-A | CD35	Arcsinh	6000	-13000	4194304	
NovaBlue 610-A | CD45	Arcsinh	400	-13000	4194304	
NovaBlue 660-A | CD3	Arcsinh	1500	-13000	4194304	
PE-A | TCRab	Arcsinh	1000	-13000	4194304	X

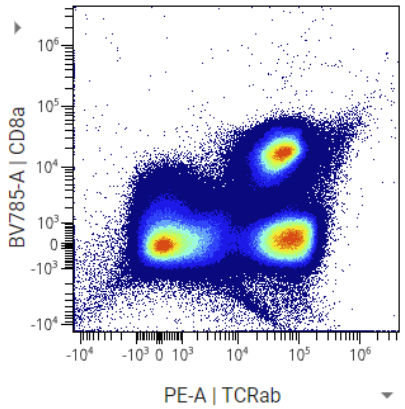
Select File: PBMC Unstim.fcs

Select Plot Type: Scatterplot (Density)

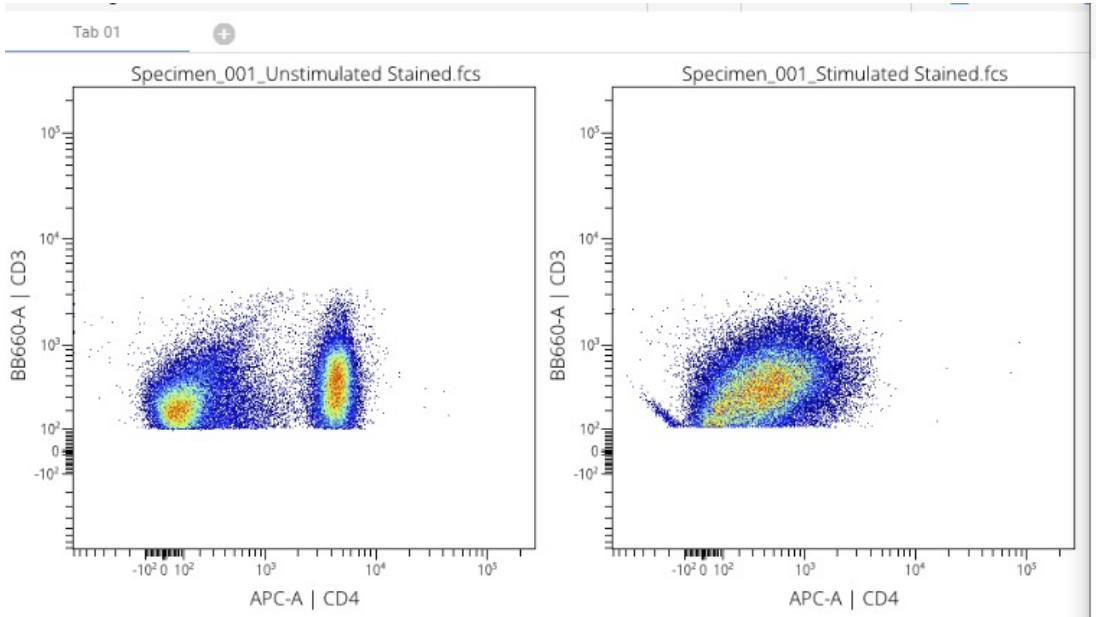
Select Filter

Y Min: -13000 Y Max: 4194304

X Min: -13000 X Max: 4194304



Scaling - aka transformation



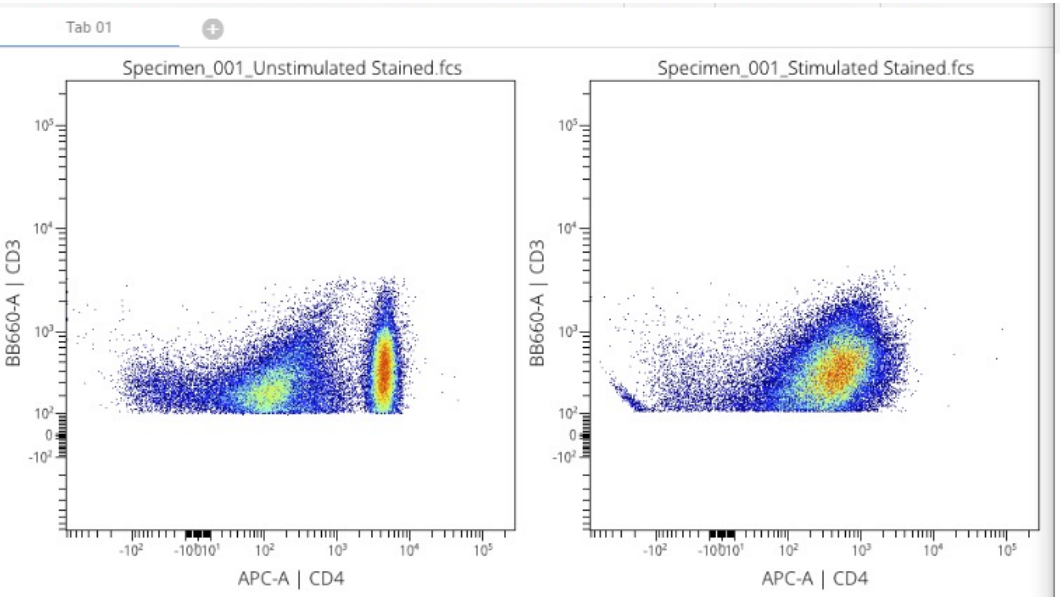
Scaling copy

Save Task | Copy Settings

Filter Features

Feature Name	Scaling Type	Cofactor	Min	Max
FSC-A	None (linear)		0	262144
SSC-A	None (linear)		0	262144
BUV395-A | CD11b	Arcsinh	400	-800	262144
BUV496-A | CD16	Arcsinh	700	-800	262144
BUV737-A | IgD	Arcsinh	400	-800	262144
BUV805-A | CD14	Arcsinh	400	-800	262144
BV421-A | CD66b	Arcsinh	400	-800	262144
BV570-A | CD27	Arcsinh	400	-800	262144
BV605-A | IgM	Arcsinh	400	-800	262144
BV650-A | CD19	Arcsinh	400	-800	262144
BV711-A | CD20	Arcsinh	400	-800	262144
BV786-A | CD8a	Arcsinh	400	-800	262144
BB515-A | CD35	Arcsinh	400	-800	262144
BB630-A | CD45	Arcsinh	400	-800	262144
BB660-A | CD3	Arcsinh	200	-800	262144
PE-A | TCRab	Arcsinh	200	-800	262144
PE-CF594-A | CD56	Arcsinh	200	-800	262144
PE-Cy7-A | CD38	Arcsinh	400	-800	262144
APC-A | CD4	Arcsinh	250	-800	262144

Scaling - aka transformation



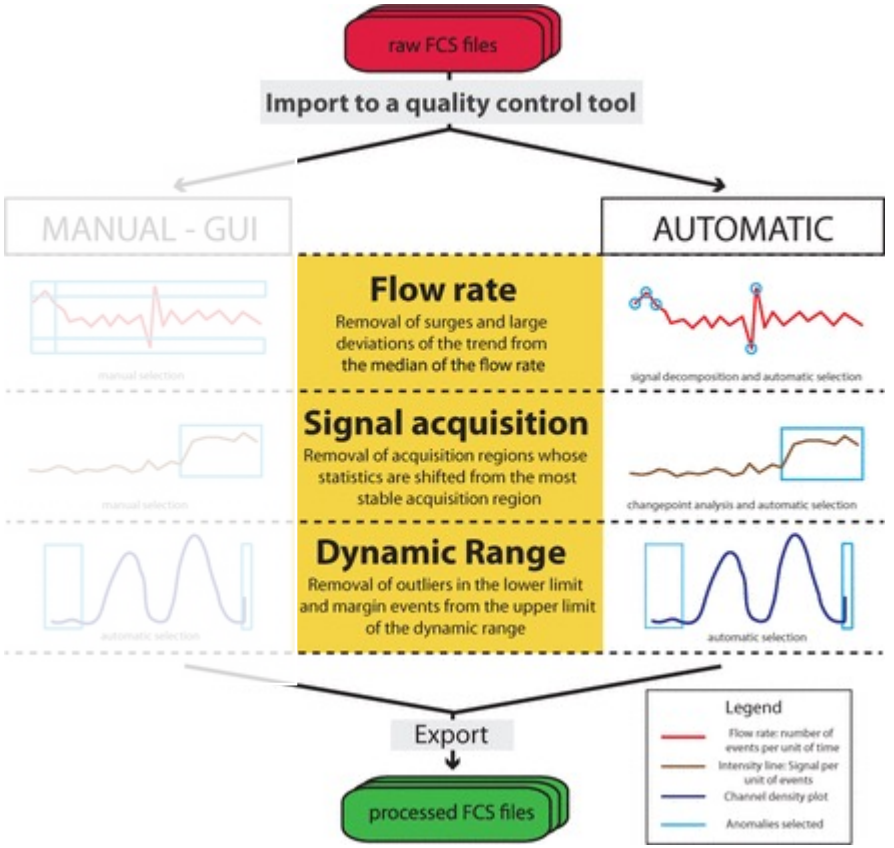
Scaling copy ..._exp1 Chug S6 Cez S6... Save Task Copy Settings

Filter Features

Feature Name	Scaling Type	Cofactor	Min	Max	
FSC-A	None (linear)		0	262144	
SSC-A	None (linear)		0	262144	Y
BUV395-A | CD11b	Arcsinh	400	-800	262144	
BUV496-A | CD16	Arcsinh	700	-800	262144	
BUV737-A | IgD	Arcsinh	400	-800	262144	
BUV805-A | CD14	Arcsinh	400	-800	262144	
BV421-A | CD66b	Arcsinh	400	-800	262144	
BV570-A | CD27	Arcsinh	400	-800	262144	
BV605-A | IgM	Arcsinh	400	-800	262144	
BV650-A | CD19	Arcsinh	400	-800	262144	
BV711-A | CD20	Arcsinh	400	-800	262144	
BV786-A | CD8a	Arcsinh	400	-800	262144	
BB515-A | CD35	Arcsinh	400	-800	262144	
BB630-A | CD45	Arcsinh	400	-800	262144	
BB660-A | CD3	Arcsinh	200	-800	262144	
PE-A | TCRab	Arcsinh	200	-800	262144	
PE-CF594-A | CD56	Arcsinh	200	-800	262144	
PE-Cy7-A | CD38	Arcsinh	400	-800	262144	
APC-A | CD4	Arcsinh	25	-800	262144	X
APC_R700-A | CD25	Arcsinh	300	-800	262144	

Data cleanup

Automated methods that can identify and remove artefacts generated during data acquisition (clogs, drastic flow rate changes, out of range signal)



Data cleanup

3 cleanup algorithms were run (all default settings) on our data set (20 files from Aurora) for comparison purpose.

Data Cleaning

https://doi.org/10.1093/bioinformatics/btw191

https://doi.org/10.1002/cyto.a.24670

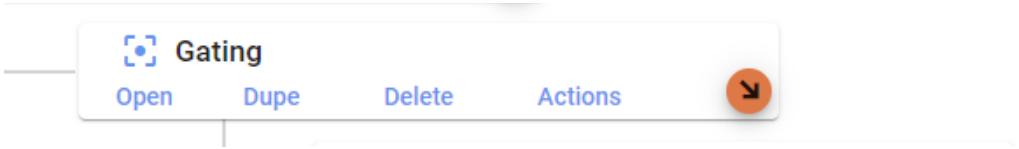
https://doi.org/10.1002/cyto.a.24501

% of rejected events

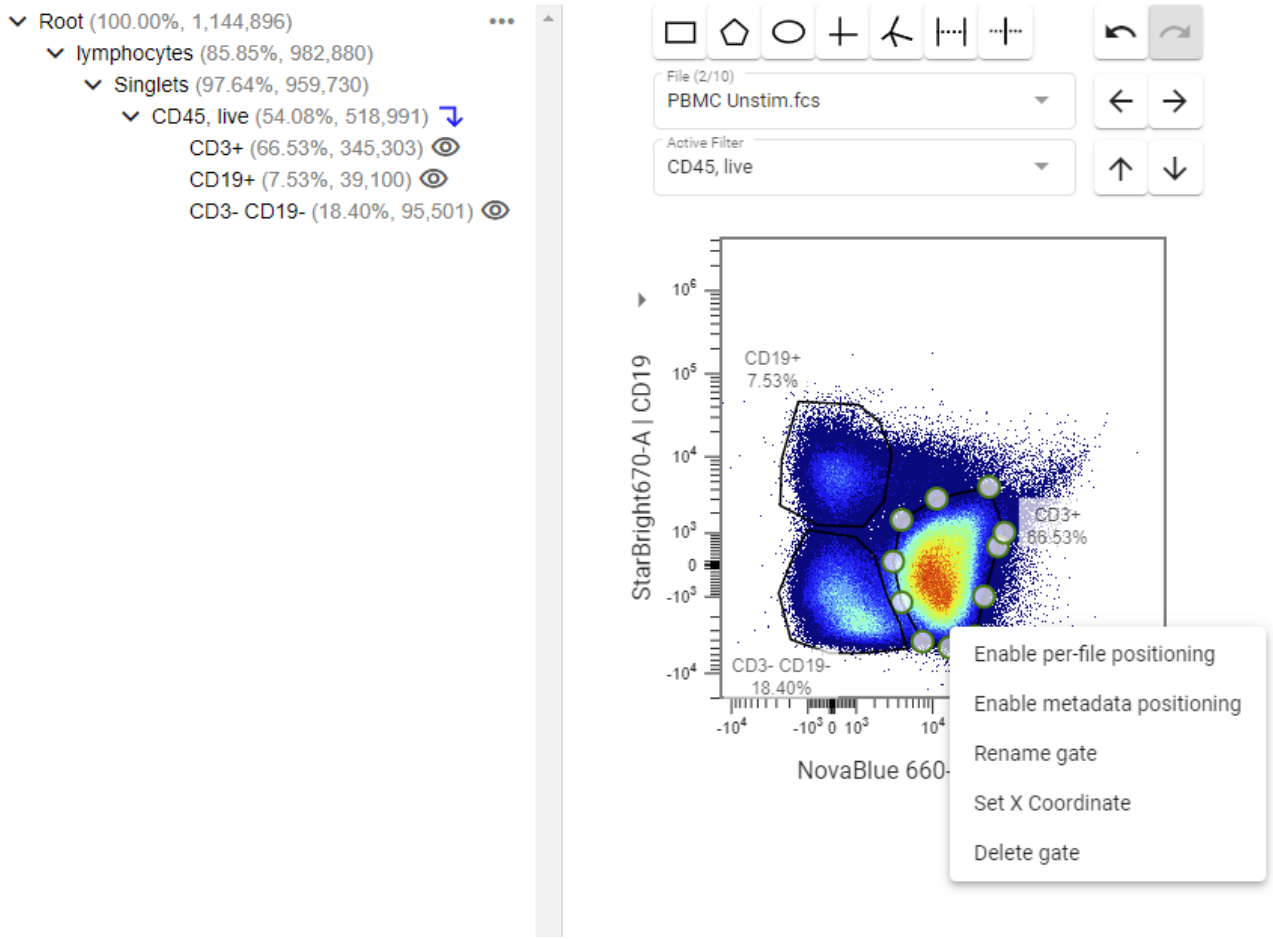
	FlowAI (12min)	flowCut (36min)	PeacoQC (11min)
PBMC Stim	7.39	0	3.30
PBMC Unstim	14.6	2.48	13.26
PBMC Stim exp2	10.27	0.05	4.60
PBMC Unstim exp2	9	0	5.64
PBMC Stim_exp3_1	6.28	0	3.28
PBMC Stim_exp3_2	6.47	3.8	5.27
PBMC Stim_exp3_3	4.89	0.05	4.48
PBMC Unstim_exp3_1	8.59	0	7.56
PBMC Unstim_exp3_2	8.16	0	8.30
PBMC Unstim_exp3_3	8.38	0	6.64

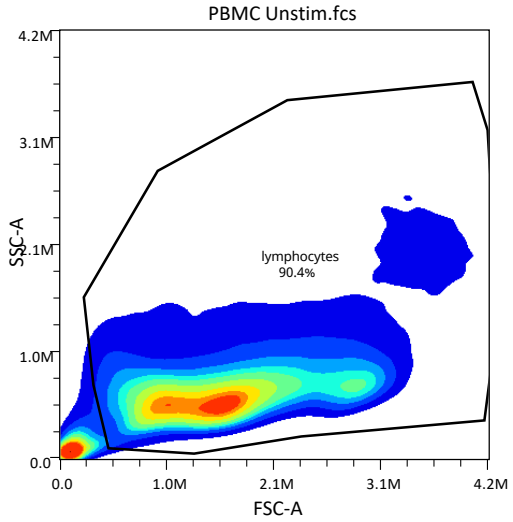
Gating = cleanup too

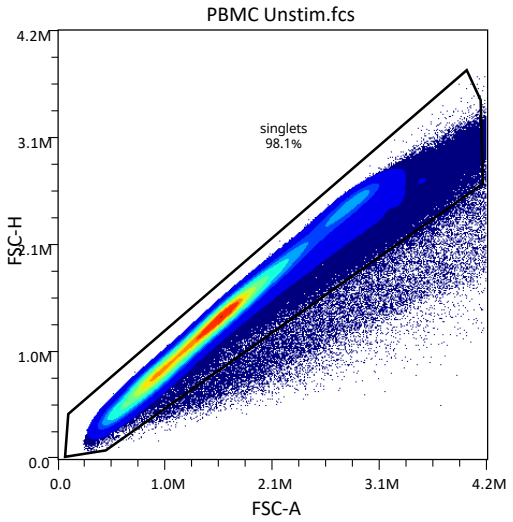
supervised

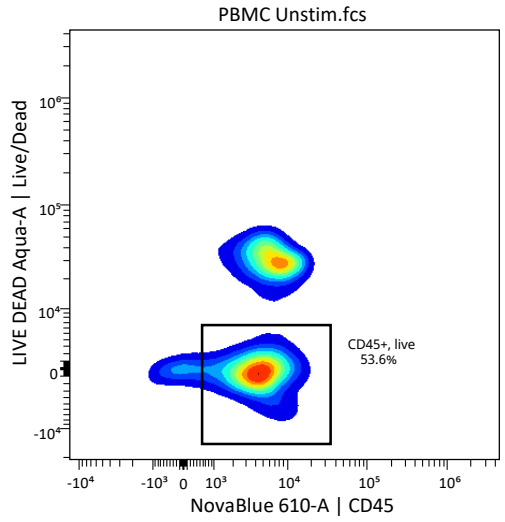


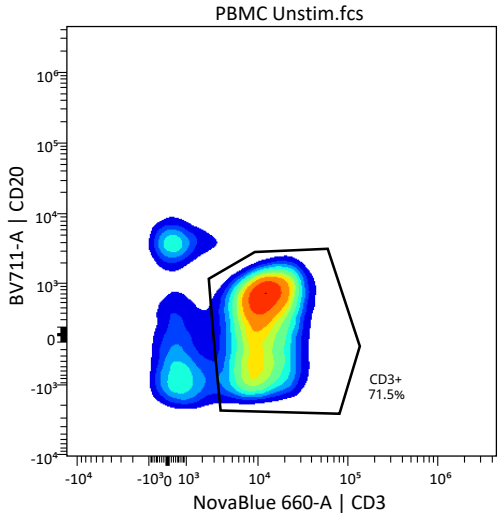
Supervised, gating

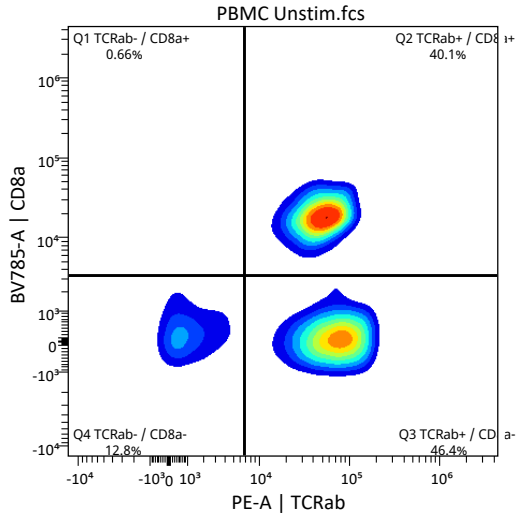



PBMC Unstim.fcs
lymphocytes
90.4%
SSC-A
FSC-A
4.2M
3.1M
2.1M
1.0M
0.0


PBMC Unstim.fcs
singlets
98.1%
FSC-H
FSC-A
4.2M
3.1M
2.1M
1.0M
0.0

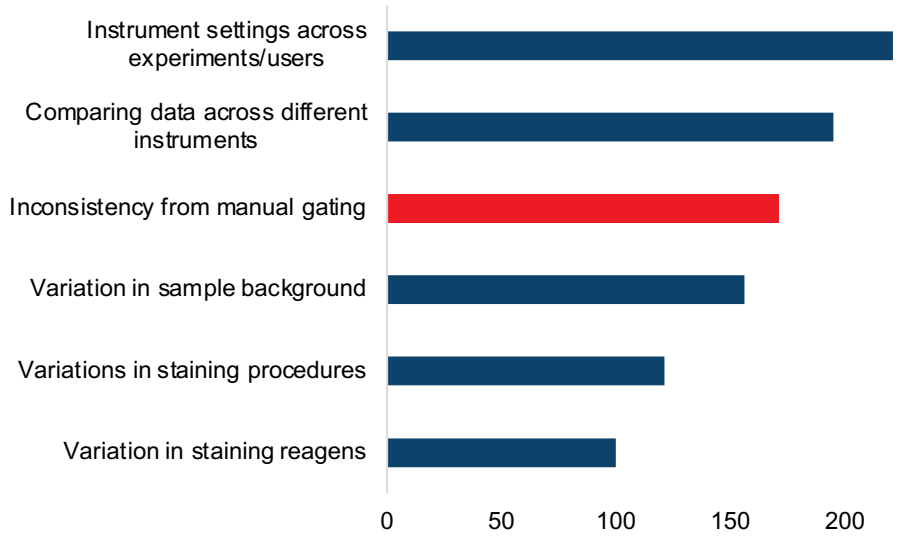

PBMC Unstim.fcs
CD45+, live
53.6%
LIVE DEAD Aqua-A | Live/Dead
NovaBlue 610-A | CD45


PBMC Unstim.fcs
CD3+
71.5%
BV711-A | CD20
NovaBlue 660-A | CD3


PBMC Unstim.fcs
Q1 TCRab- / CD8a+
0.66%
Q2 TCRab+ / CD8a+
40.1%
Q4 TCRab- / CD8a-
12.8%
Q3 TCRab+ / CD8a-
46.4%
BV785-A | CD8a
PE-A | TCRab

Manual gating is a concern!

What aspects of reproducibility are cytometrists most concerned about? [multi-select]



Source:
- 2019 Flow and Mass Cytometry User Survey, >300 participants

Cytobank v10 has recently added a new Automatic gating feature

- Automate the gating process to reduce intra- and inter-operator variability.
- Eliminate time spent on repetitive, manual tasks, to focus result interpretation and the generation of novel insights.

Cytobank Automatic gating is trained on your panel and gating strategy

Share your Cytobank Automatic gating model easily with other users

Complete gating in less than one minute per sample*

Slide courtesy of Cytobank, Beckman Coulter

* For files with 300 000 events, 19 parameters and 42 populations. Performance dependent on gating strategy and file size.

Subsampling/Downsampling

Select Filter: CD3+

Target Count *: 50000

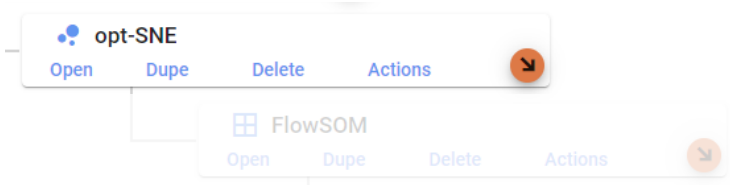
Counting Method: (•) Per File ( ) Total

Sampling Method: ( ) Equal (•) Max Equal

[ ] Group Files by Metadata

File Name (10)	Available	Available in Filter	Sampling Count
PBMC Stim.fcs	618,808	63,910	50,000
PBMC Unstim.fcs	1,144,896	245,692	50,000
PBMC Stim EXP 2.fcs	1,036,896	93,528	50,000
PBMC Unstim EXP 2.fcs	2,060,040	453,656	50,000
PBMC Stim_exp3_3.fcs	806,640	78,542	50,000
PBMC Stim_exp3_1.fcs	776,728	76,020	50,000
PBMC Stim_exp3_2.fcs	987,632	118,508	50,000
PBMC Unstim_exp3_3.fcs	1,193,664	242,162	50,000
PBMC Unstim_exp3_1.fcs	1,286,360	223,917	50,000
PBMC Unstim_exp3_2.fcs	1,316,456	236,166	50,000
Total	**11,228,120**	**1,832,101**	**500,000**

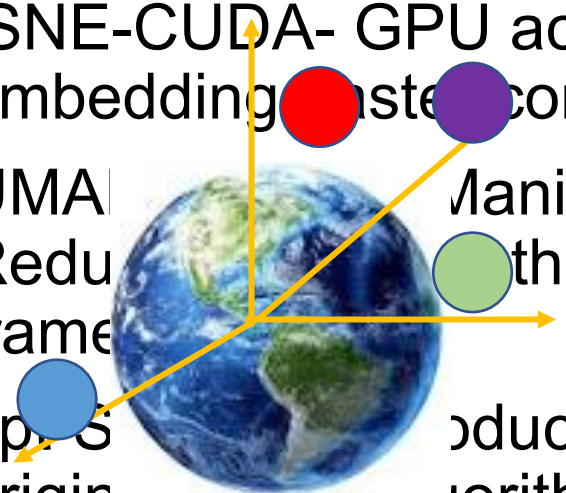
unsupervised



Dimensionality reduction

Common approaches to dimensionality reduction-

- viSNE/tSNE- non-linear dimensionality reduction algorithm developed based on Stochastic Neighbor Embedding-similar objects are modeled by nearby points and dissimilar objects are modeled by distant points with high probability
- tSNE-CUDA- GPU accelerated t-distributed stochastic neighbor embedding[illegible] computational time
- UMA[illegible] Manifold Appr[illegible] ection for Dimension Redu[illegible] the underly[illegible] aset using a topological frame[illegible]
- op[illegible] oduce superior dimensionality reduction results than the original t-SNE algorithms



Fast Dimensionality Reduction: How To Choose?

	viSNE	Opt-SNE	tSNE-CUDA	UMAP
Set the Seed	Yes	Yes	-	-
Max # total events	1.3M	3M	10M	10M
Global structure	-	-	-	Yes
Speed	Slower	Medium	Fast	Fast
Low events/sample	More sparse map			More condense map/ islands
Visualization	Bigger, less compact islands with points more spread			More dense islands with overlapping dots

- UMAP can generate islands that are distance from each other and dense, therefore, it might be more clear than a tSNE map for samples with few events
- tSNE-CUDA islands are bigger, less compact, the points are more spread out and don't overlap each other as much as UMAP. It will be easier to discriminate subpopulations within the tSNE-CUDA islands than the dense UMAP islands
- Distance between islands in UMAP is meaningful while it’s not for the tSNEs maps
- tSNE-CUDA might be a fast option to explore large datasets working well with default settings BUT if you want to be able to include the seed for your run in your paper, then you need to use opt-SNE

The good and the bad...

Advantages of Dimensionality Reduction

- Data compression and better visualization
- Reduces time of analysis
- Allows automation, identification of unique populations, biomarker discovery and predictive modelling to correlate with clinical outcome.

Disadvantages of Dimensionality Reduction

- Potential data loss
- Requires either a good code writer or potentially expensive software

Multiparameter data analysis

Tools/algorithms

Dimensionality Reduction

t-SNE

opt-SNE

t-SNE-CUDA

FIt-SNE

UMAP

PCA

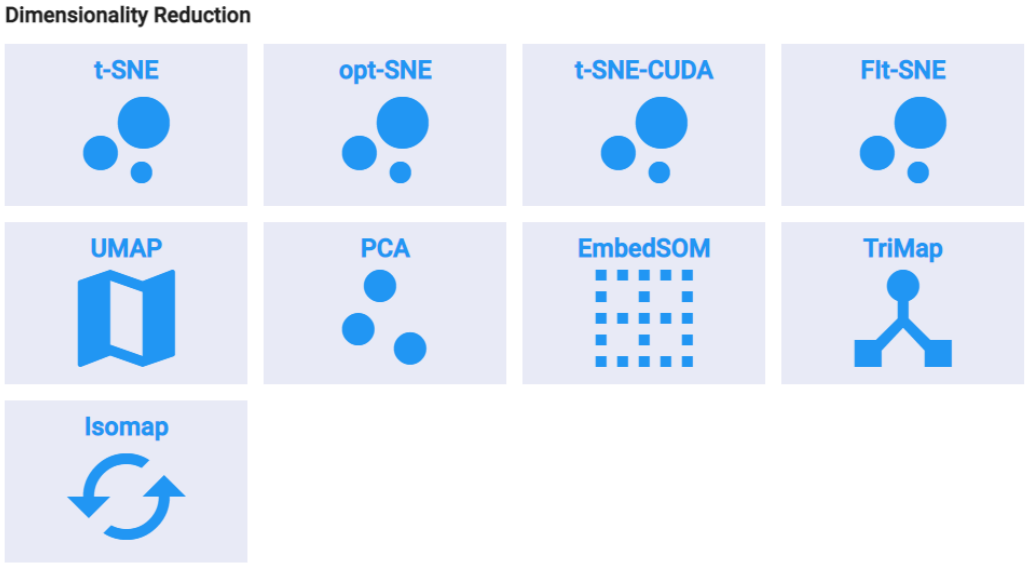
EmbedSOM

TriMap

Isomap

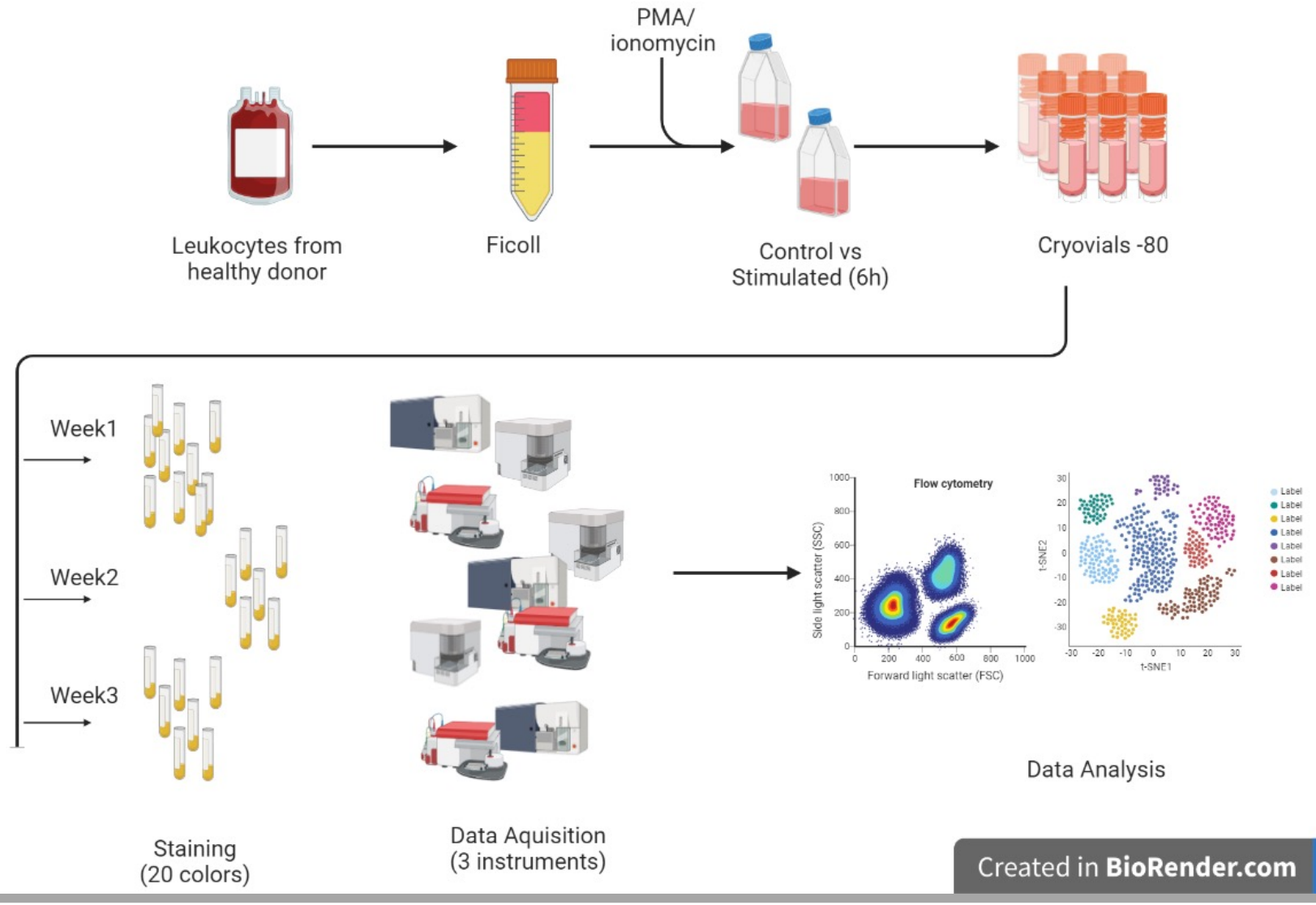
Multiparameter data analysis

Tools/algorithms

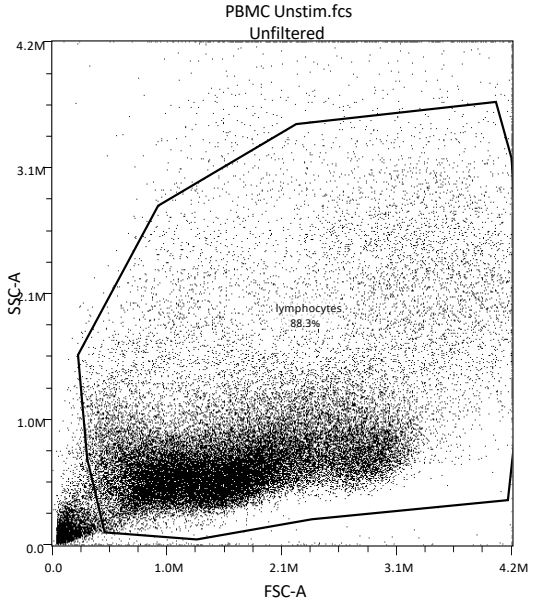


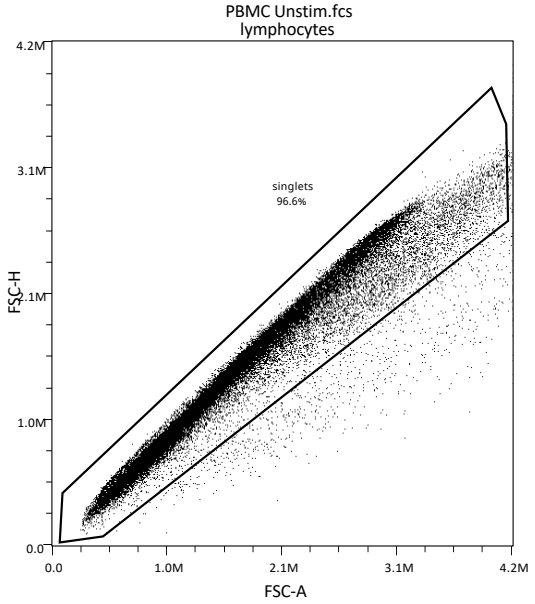
Opt-SNE: https://doi.org/10.1101/451690
UMAP: https://arxiv.org/abs/1802.03426
EmbedSOM: https://doi.org/10.12688%2Ff1000research.21642.2
TriMap: https://arxiv.org/abs/1910.00204
Isomap: https://doi.org/10.1126/science.290.5500.2319

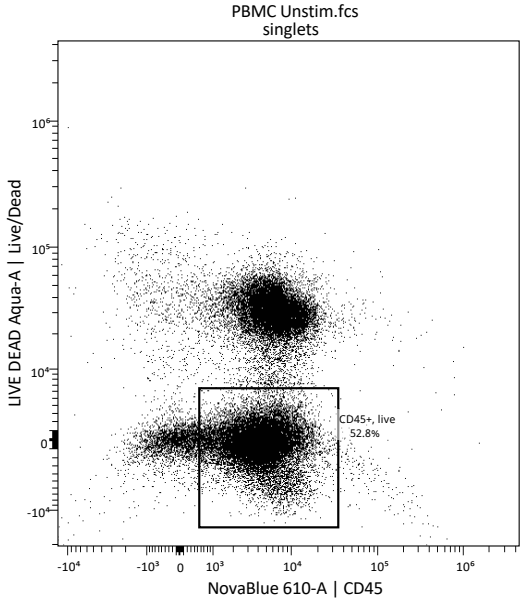
Experiment outline

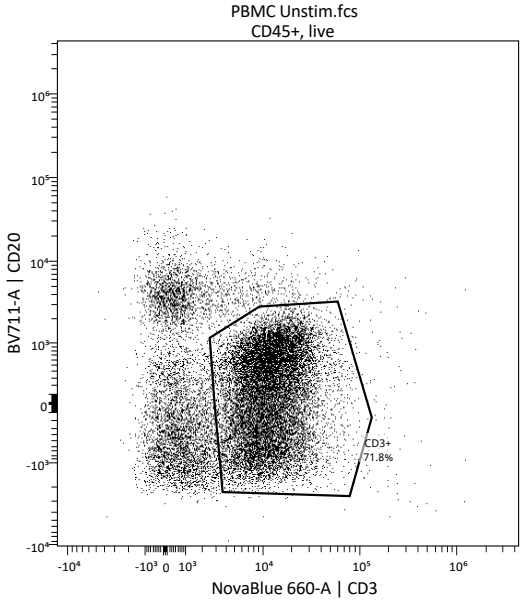


Supervised, gating





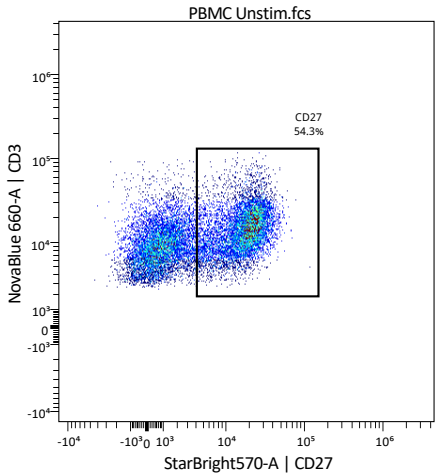


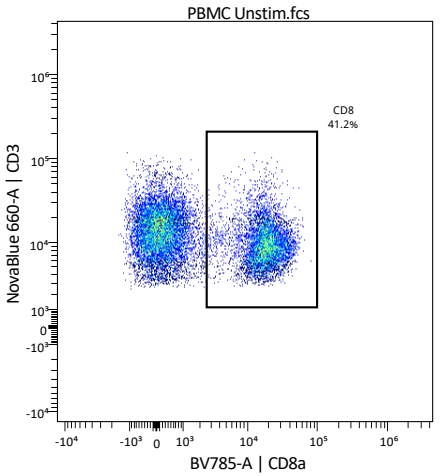


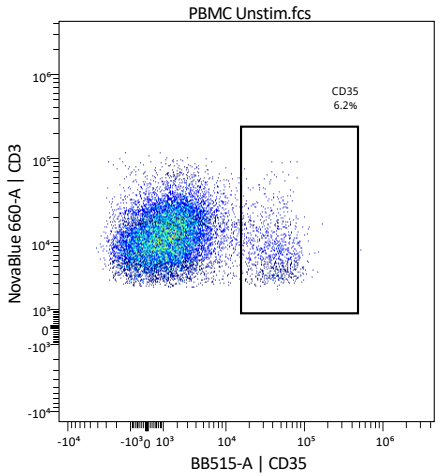
BUV395	CD11b	**BB515**	CD35
BUV496	CD16	**NB610**	CD45
Live/Dead Aqua	Viability	**NB660**	CD3
BUV737	IgD	**PE**	TCRa/b
BUV805	CD14	**PE-CF594**	CD56
BV421	CD66b	**PE-Cy7**	CD38
SBV570	CD27	**APC**	CD4
BV605	IgM	**APC-R700**	CD25
SBV670	CD19	**APC-Fire750**	CD62L
BV711	CD20		
BV785	CD8a		

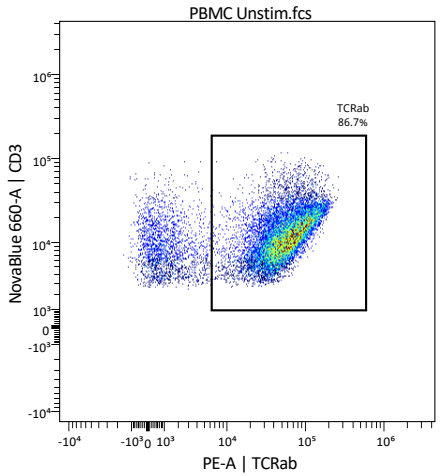
SBV570	CD27
BV785	CD8a
BB515	CD35
PE	TCRa/b
PE-CF594	CD56
PE-Cy7	CD38
APC	CD4
APC-R700	CD25
APC-Fire750	CD62L

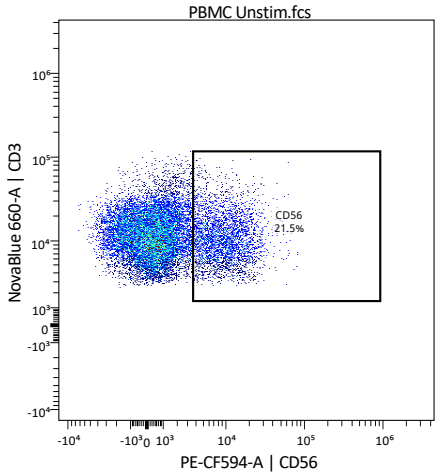
Supervised, gating

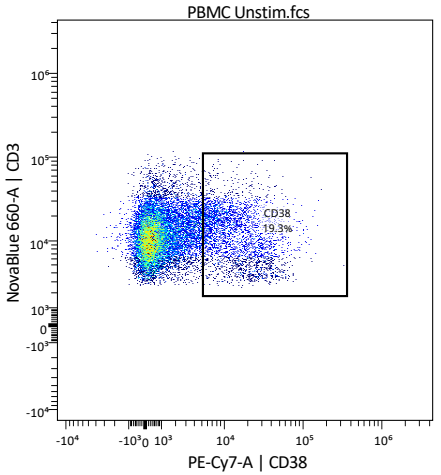


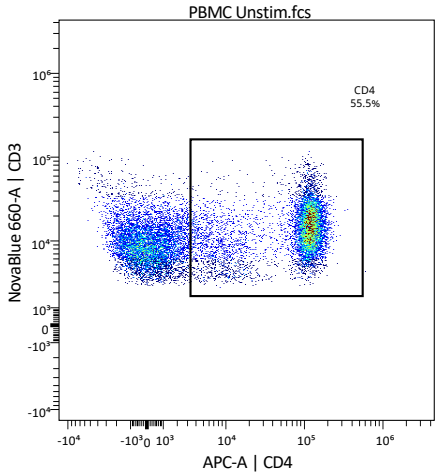


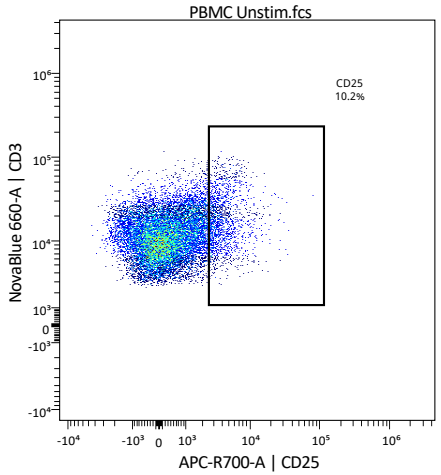


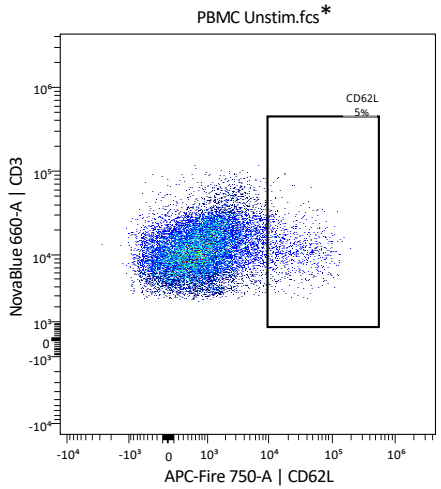








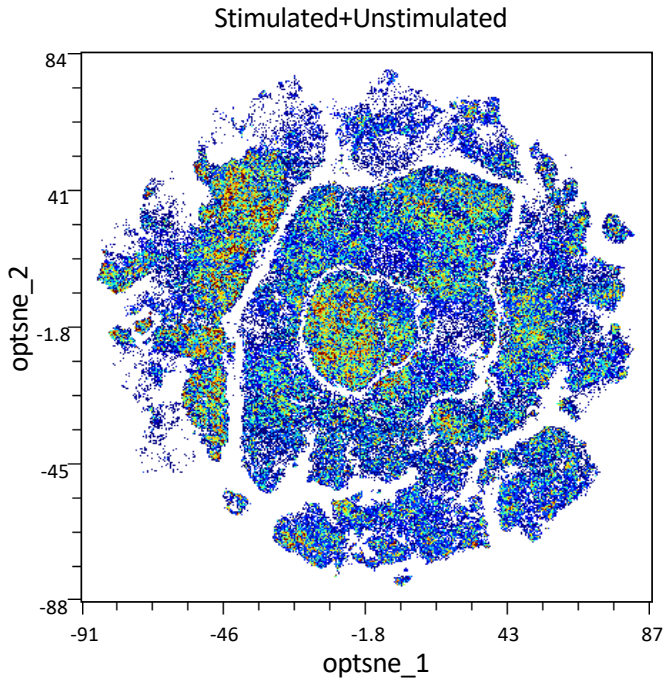




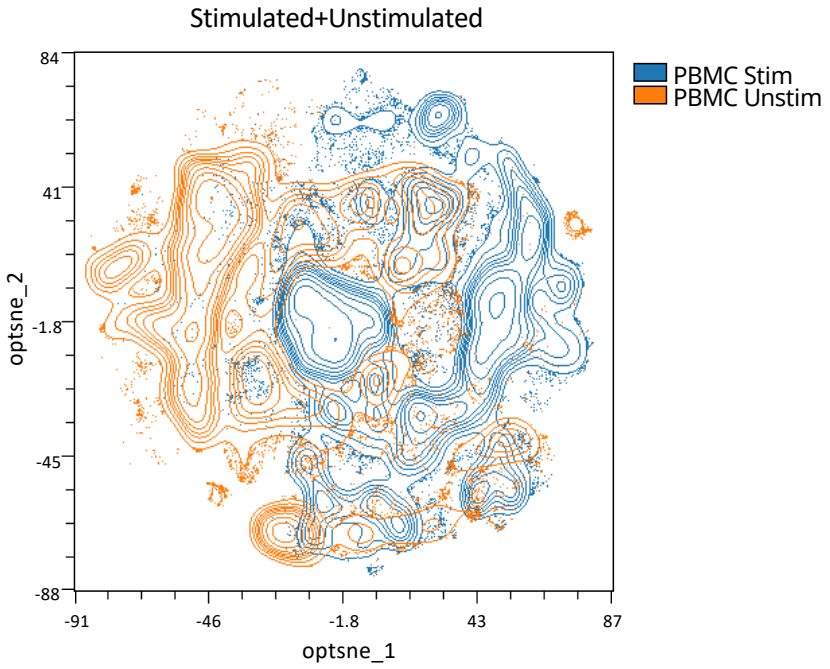
*CD62L expression pattern seems to be incorrect, new antibody the same pattern but titration on fresh PBMC ok, Possible that freeze/thaw cycle affect CD62L expression https://doi.org/10.1016/s0022-1759(03)00202-3

opt-SNE

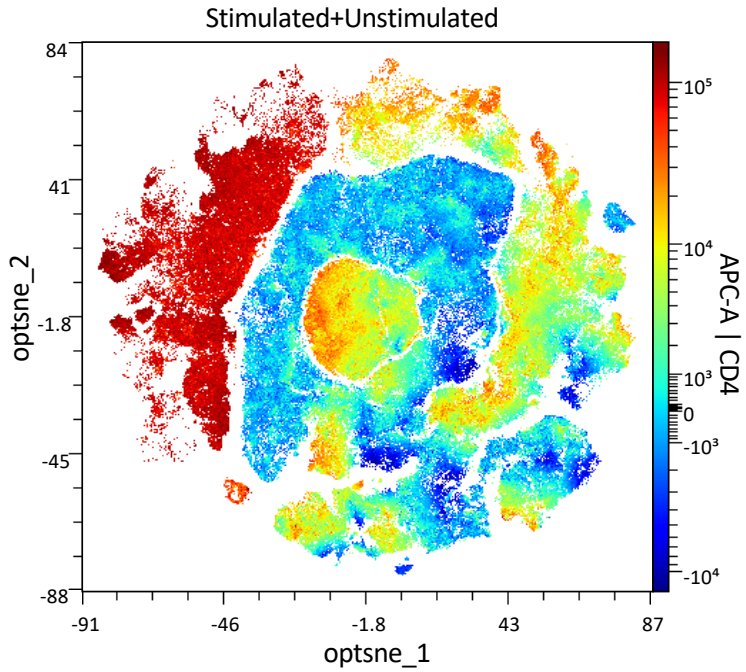
Modified version of t-SNE that enables high-quality embeddings in the optimal amount of compute time without having to tune algorithm parameters.



tSNE with Scatterplot (density)

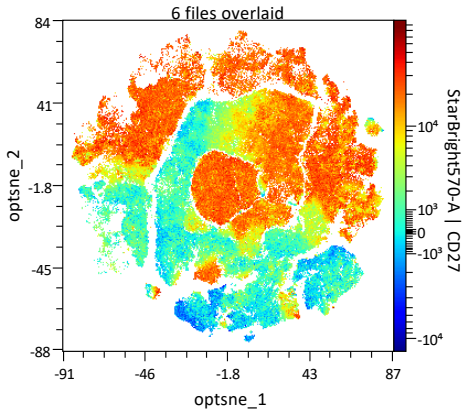


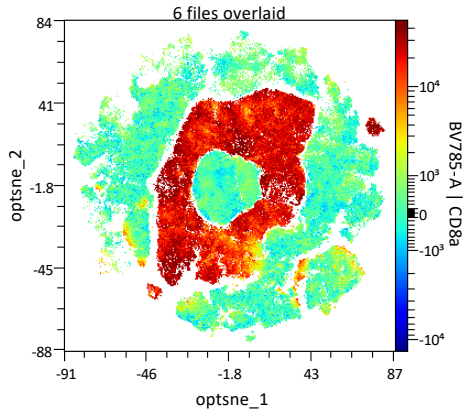
tSNE with Contour Plot

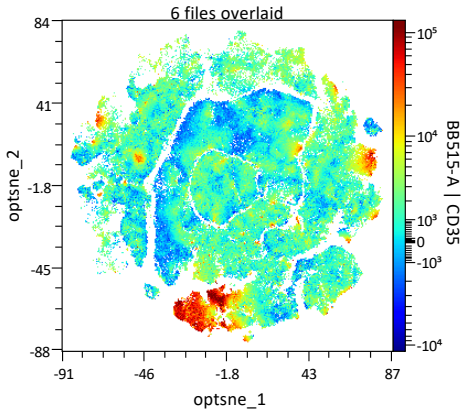


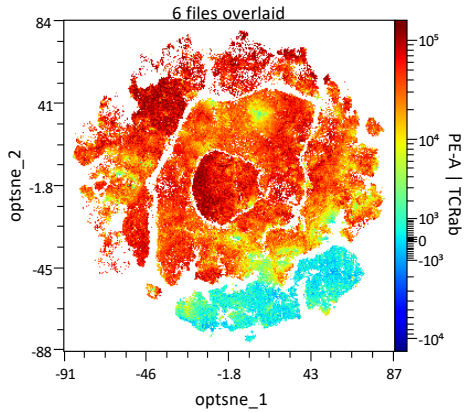
Scatterplot (colored-continuous)

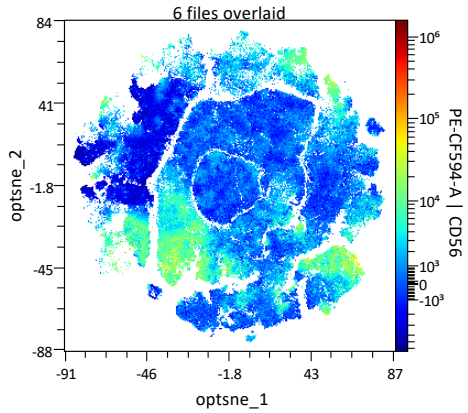
Visualization of data in higher number of dimension

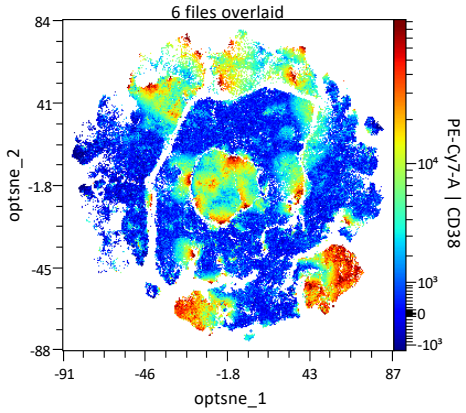


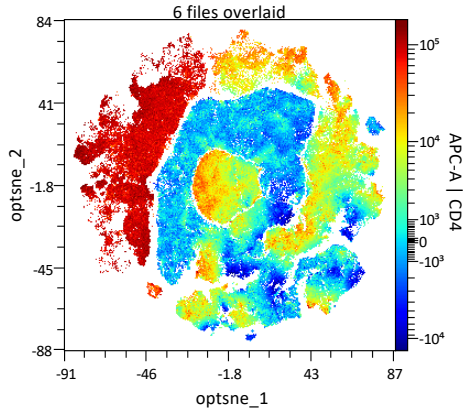


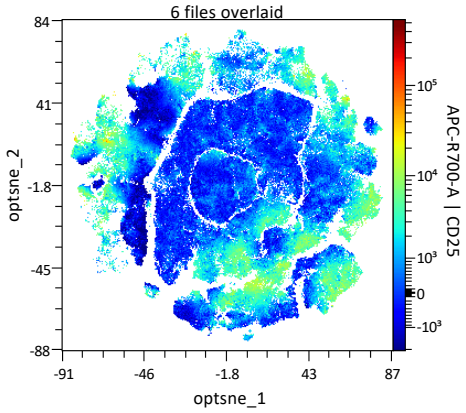


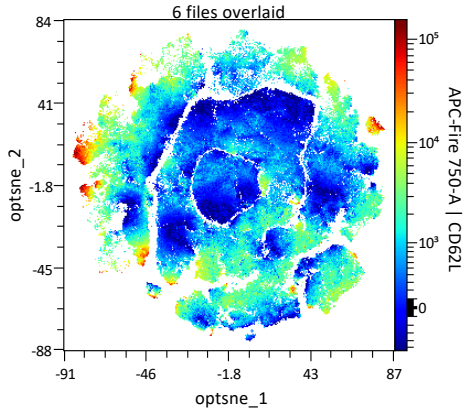




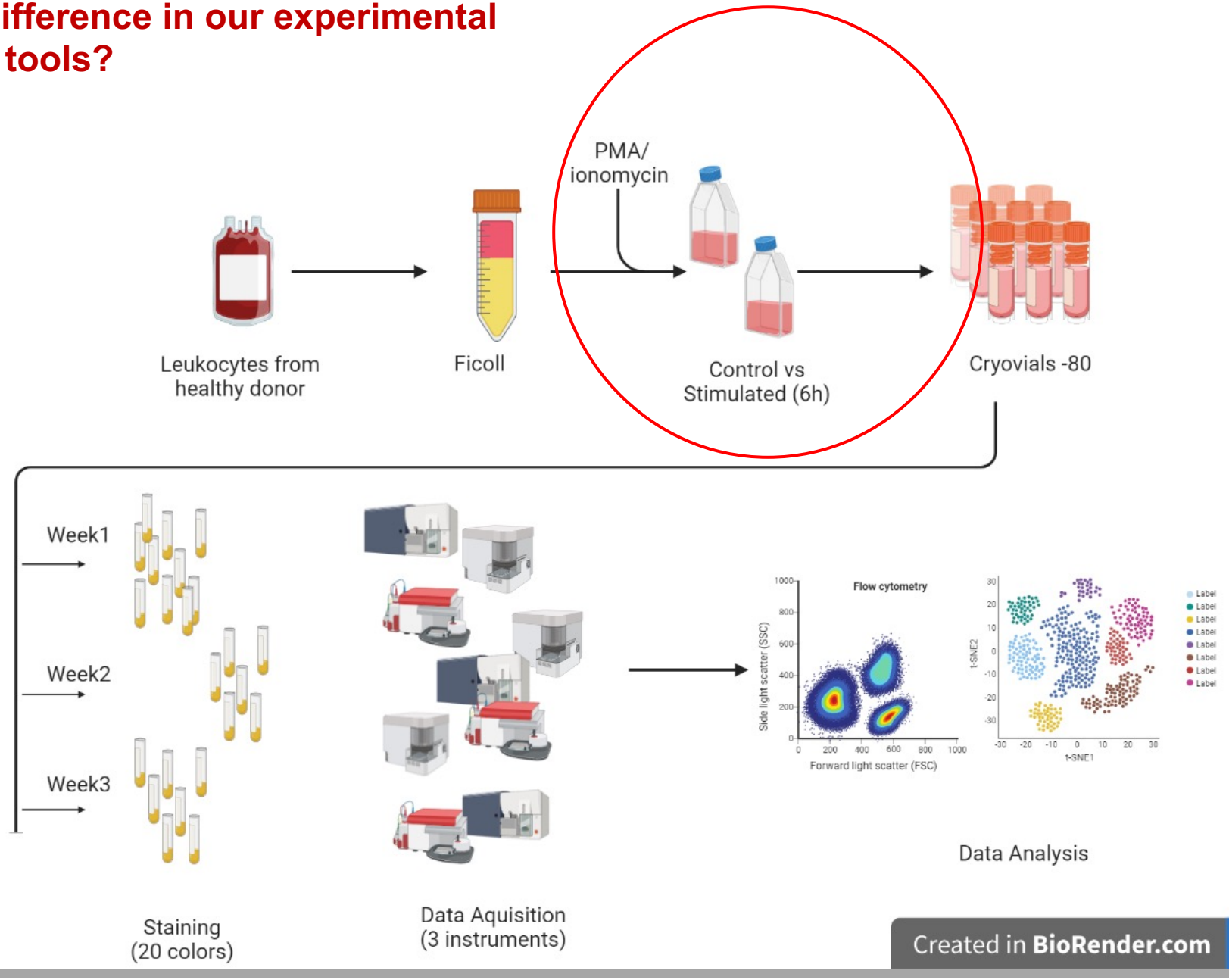




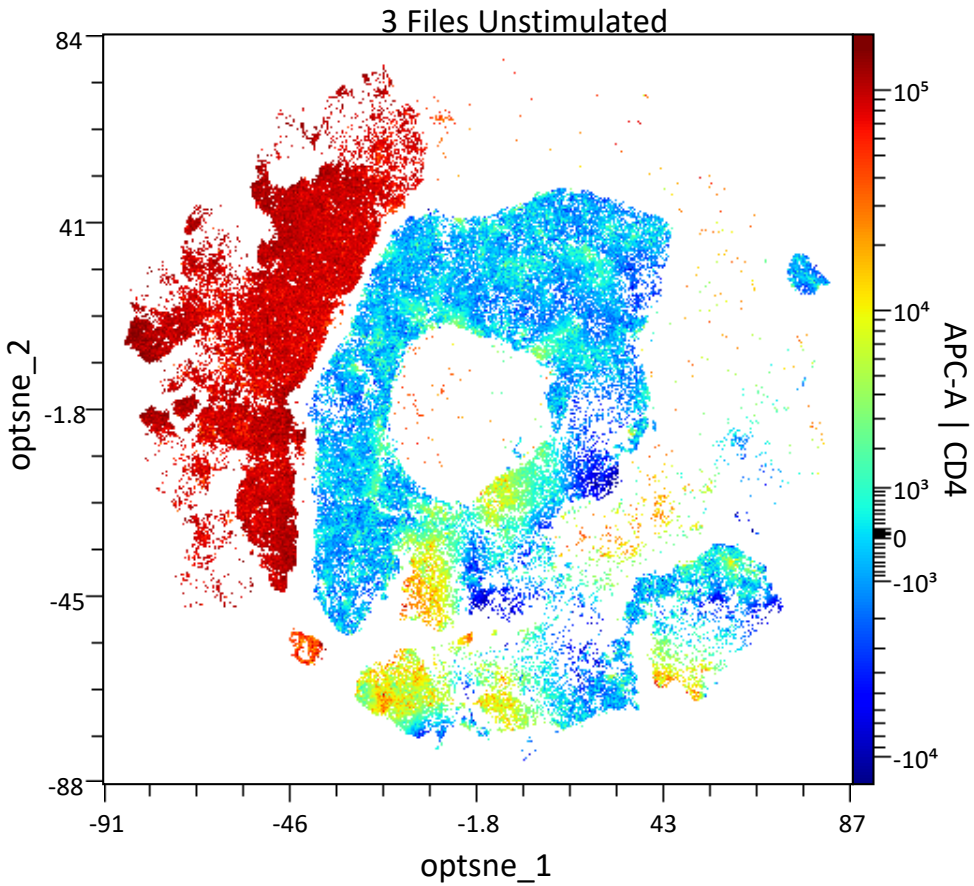


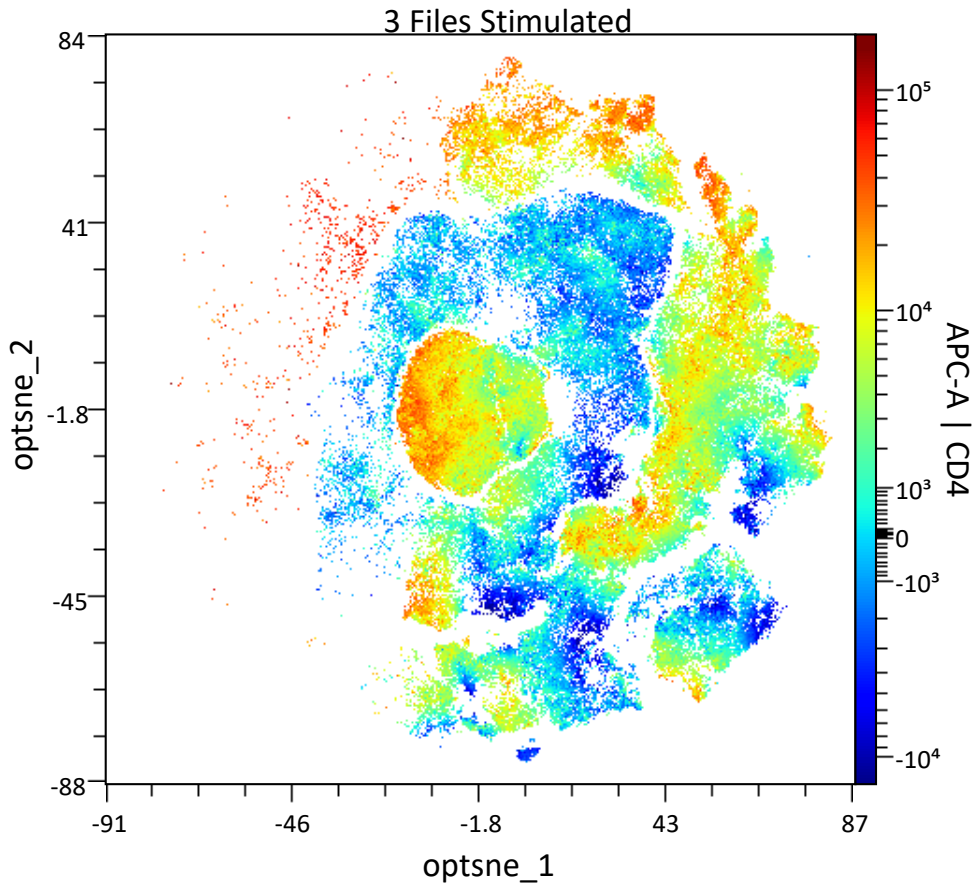


Can we see the difference in our experimental model using this tools?



Yes, we can!


3 Files Unstimulated
84
41
-1.8
-45
-88
optsne_2
-91
-46
-1.8
43
87
optsne_1
10^5
10^4
10^3
0
-10^3
-10^4
APC-A | CD4


3 Files Stimulated
84
41
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optsne_2
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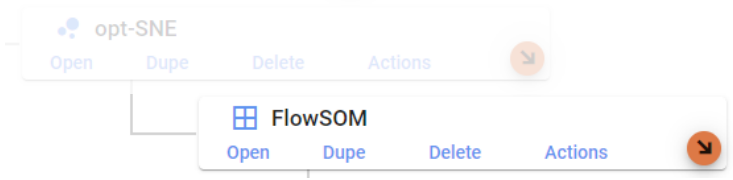
-10^4
APC-A | CD4

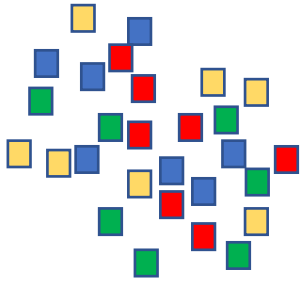
unsupervised

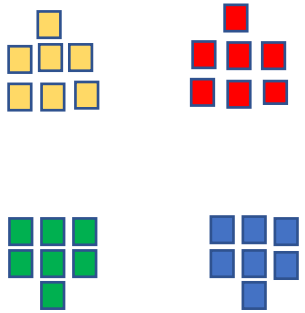


Clustering

Clustering

- Dividing the population or data points into groups where data points in the same groups are more similar to other data points and dissimilar to the data points in other groups.





Clustering

- *SPADE*=Spanning-tree Progression Analysis of Density-normalized Events

  -clusters phenotypically-similar cells into a hierarchy that allows high-throughput, multidimensional analysis of heterogeneous samples.

- *FlowSOM*= analysis and quality of clustering with Self-Organizing Maps (SOMs)

  -Quicker analysis, clusters cells based on chosen markers

CITRUS=algorithm developed to automatically find stratifying signatures from within a data set to explain differences between multiple groups of samples (responders vs non-responders). Very useful for human studies with a high number of experimental samples.

Some references-

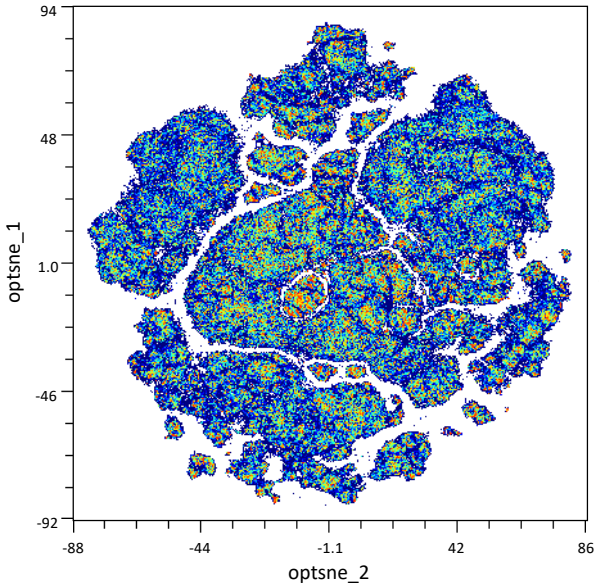
- Toward deterministic and semi-automated SPADE analysis-Cytometry A. 2017 Mar; 91(3): 281–289.
- viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia-Nat Biotechnol. 2013 Jun; 31(6): 545–552.
- Analyzing high-dimensional cytometry data using FlowSOM-*Nature Protocols* **volume 16**, pages3775–3801 (2021)
- A Beginner's Guide to Analyzing and Visualizing Mass Cytometry Data 2018 Jan 1;200(1):3-22. doi: 10.4049/jimmunol.1701494

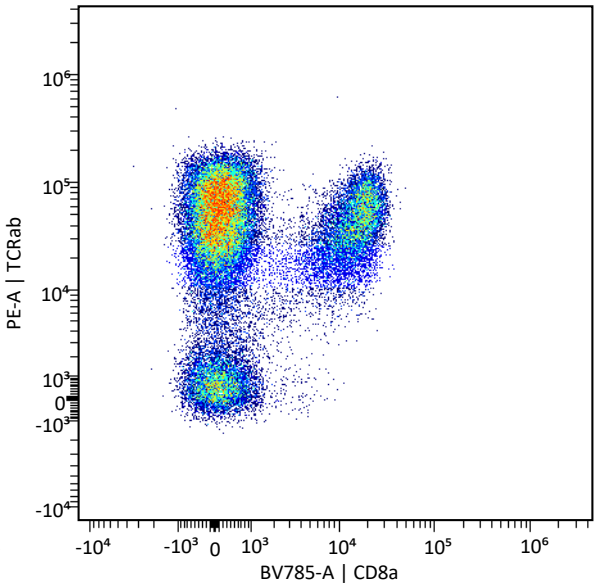
Advantages vs limitations for clustering

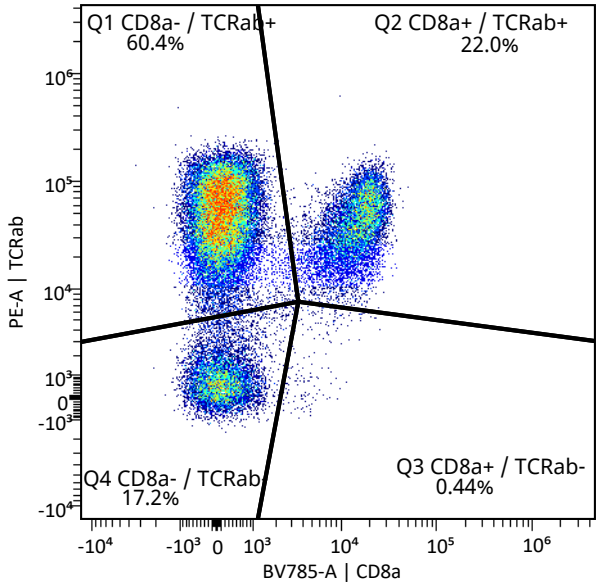
Individual cells are not displayed, only clusters are.

No automatic methods to determine the number of clusters and meta-clusters based on the individual panel. It is user-defined and often needs to be optimized by a couple of runs

Clustering

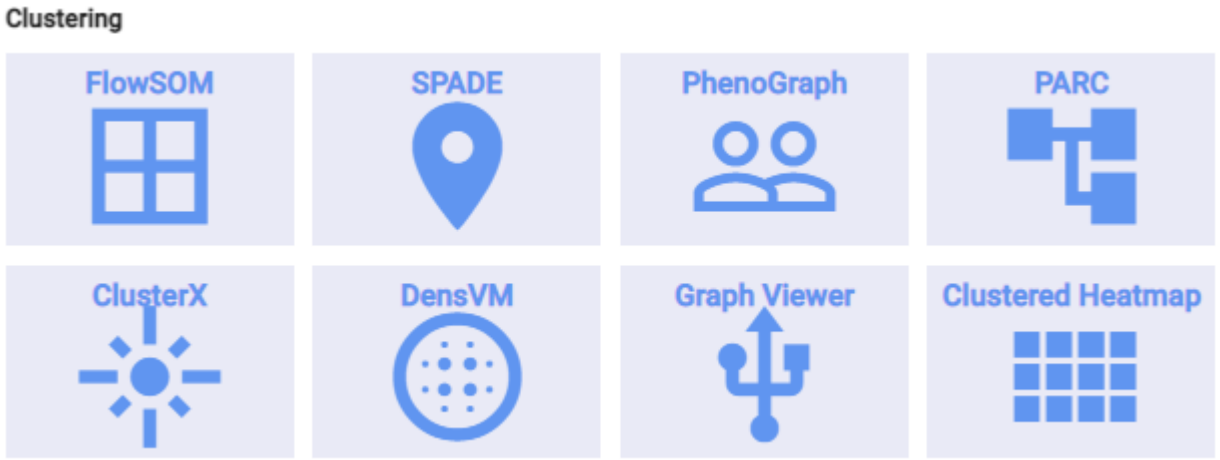




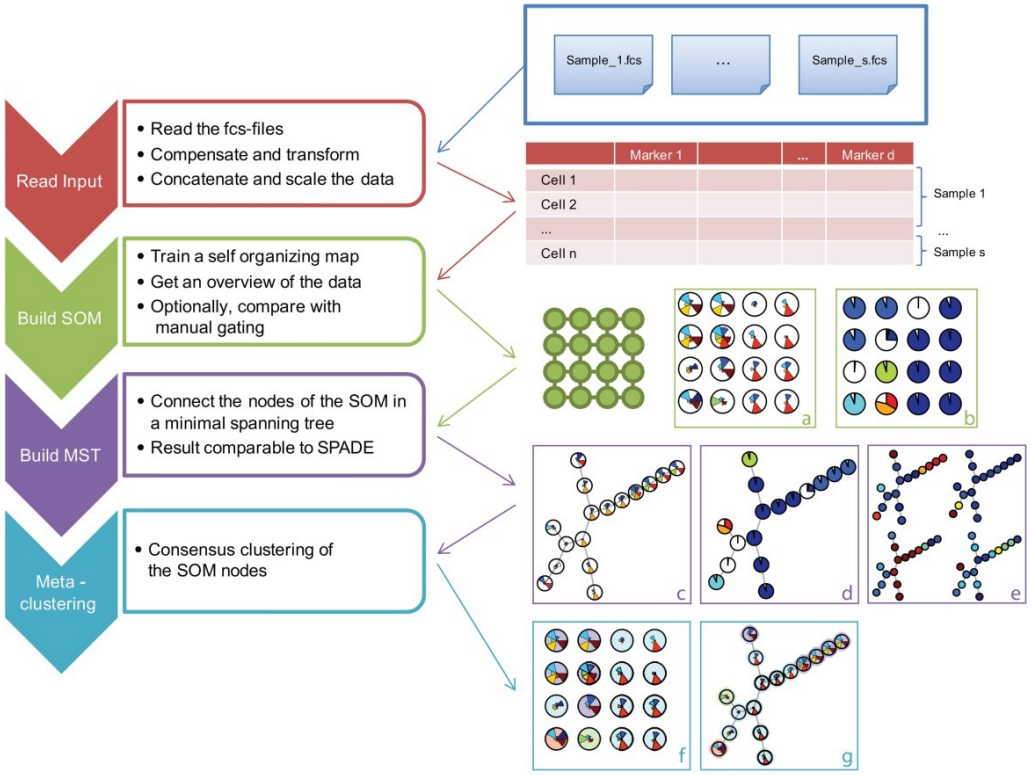


Clustering is like gating but for multidimensional data set

Clustering


Clustering
FlowSOM
SPADE
PhenoGraph
PARC
ClusterX
DensVM
Graph Viewer
Clustered Heatmap

Clustering with FlowSOM

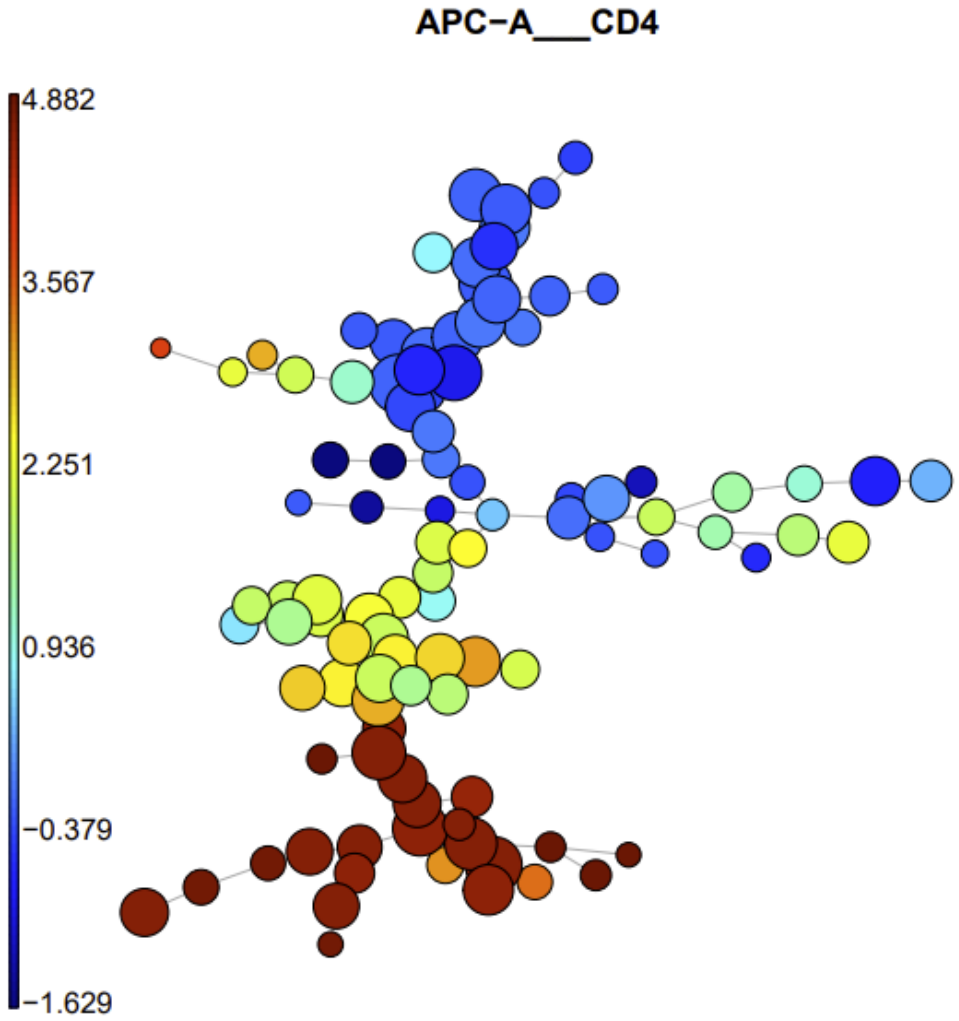


Uses Self-Organizing Map
Allows to set seed
Reproducible
Fast
Works with large number of events and samples
Outputs clusters (~overclustering) and metaclusters

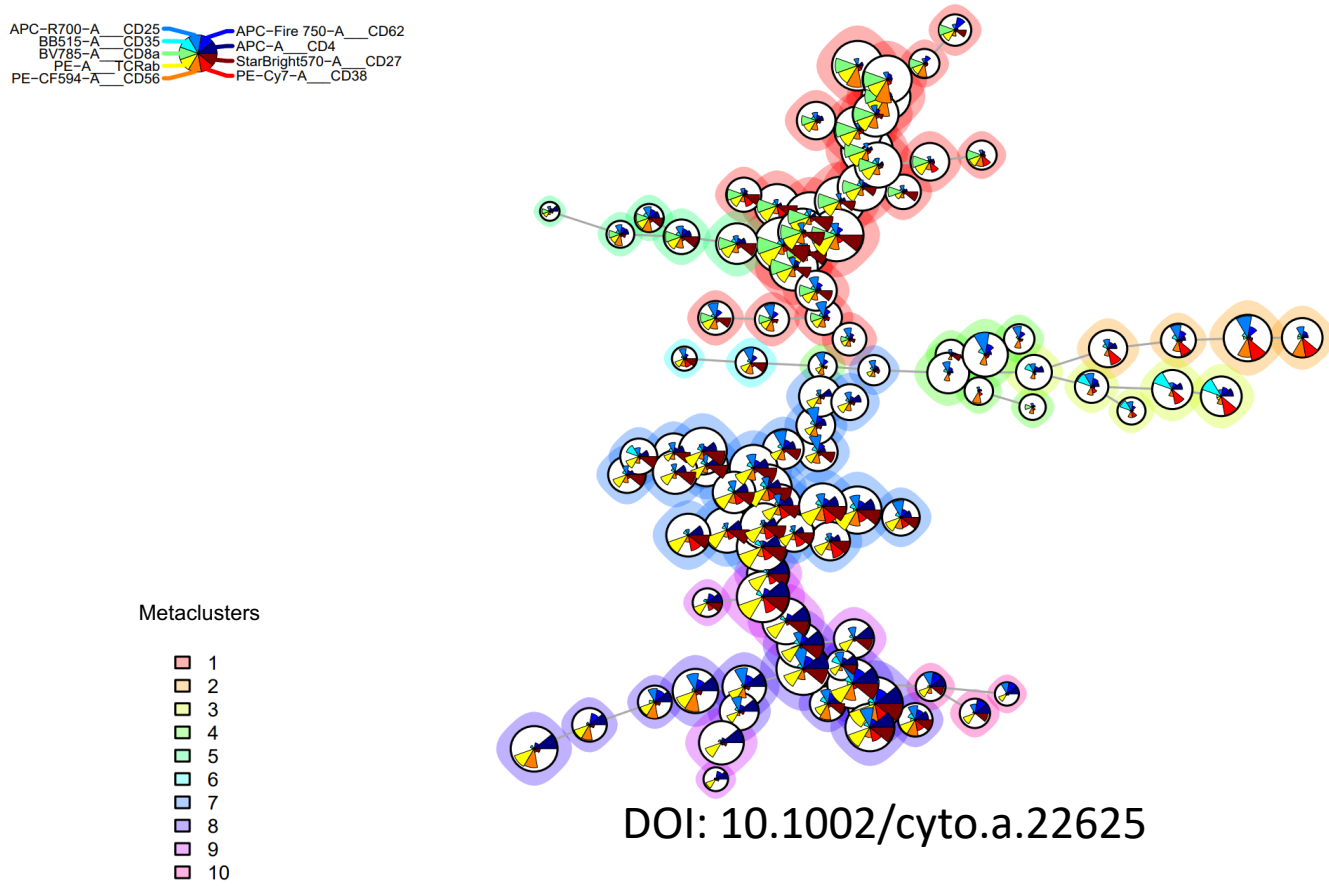
DOI: 10.1002/cyto.a.22625

Clusters and metaclusters

Minimal spanning tree

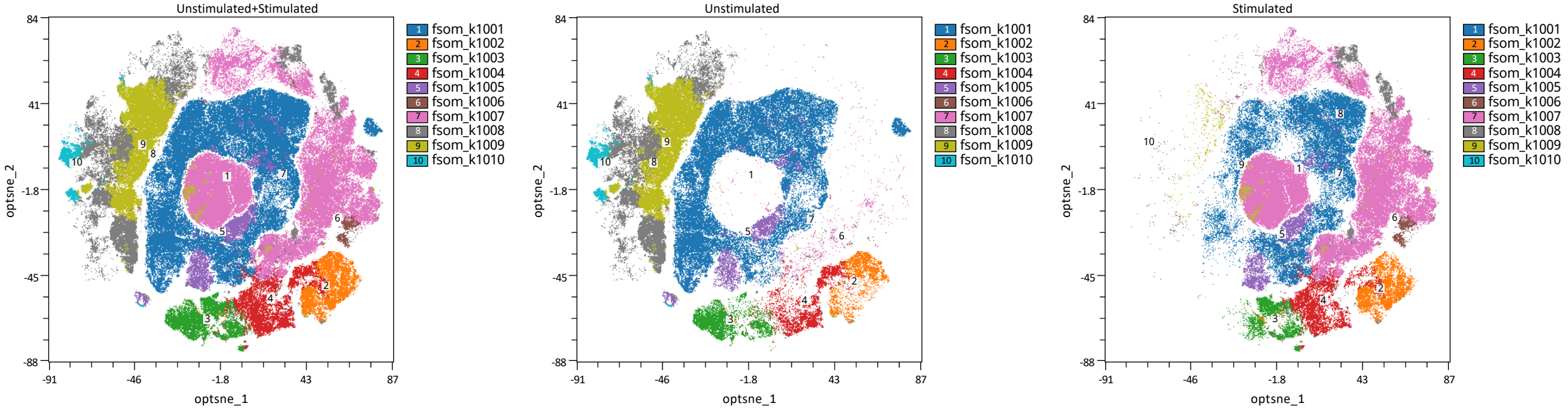


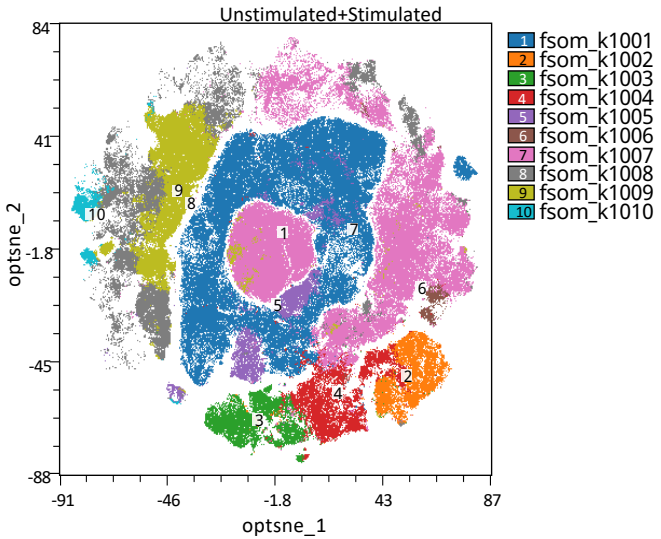
Metaclusters from consensus method (k=10) visualized on tree



DOI: 10.1002/cyto.a.22625

Clustering, allows us to derive multiple statistics for different populations




Unstimulated+Stimulated
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optsne_1
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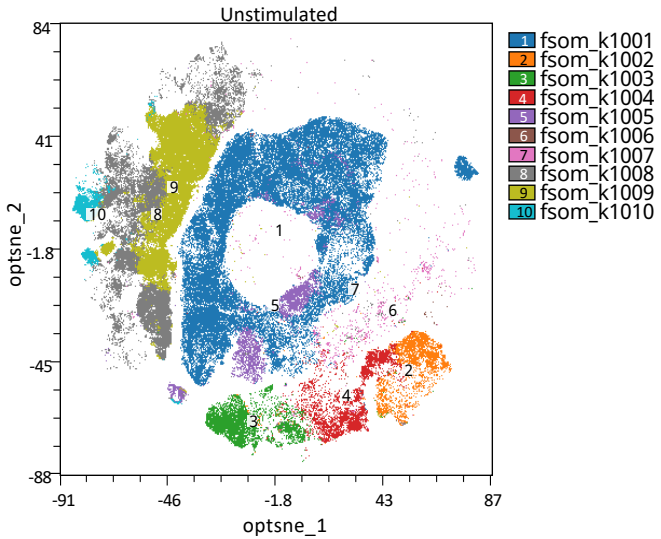
41
-1.8
-45
-88
-91
-46
-1.8
43
87
fsom_k1001
fsom_k1002
fsom_k1003
fsom_k1004
fsom_k1005
fsom_k1006
fsom_k1007
fsom_k1008
fsom_k1009
fsom_k1010


Unstimulated
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84
41
-1.8
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-1.8
43
87
fsom_k1001
fsom_k1002
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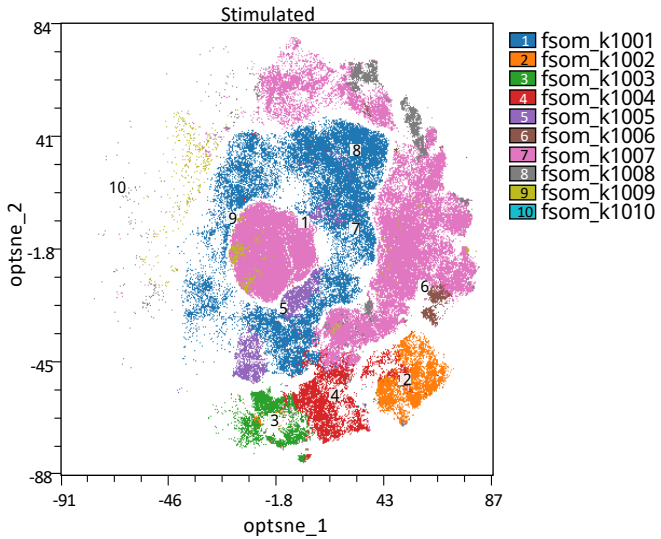
Stimulated
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optsne_1
84
41
-1.8
-45
-88
-91
-46
-1.8
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87
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fsom_k1002
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fsom_k1008
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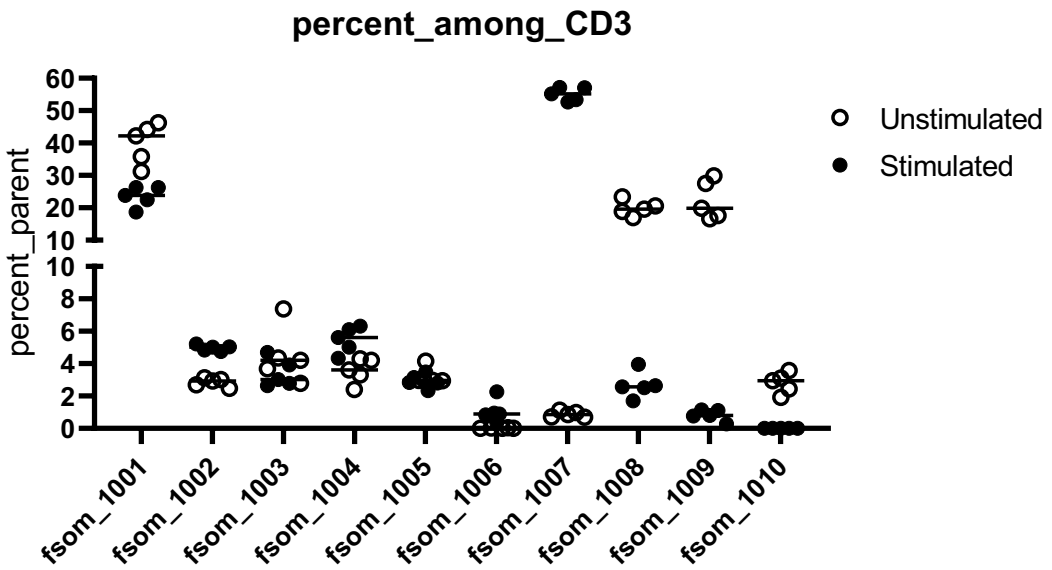
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percent_parent
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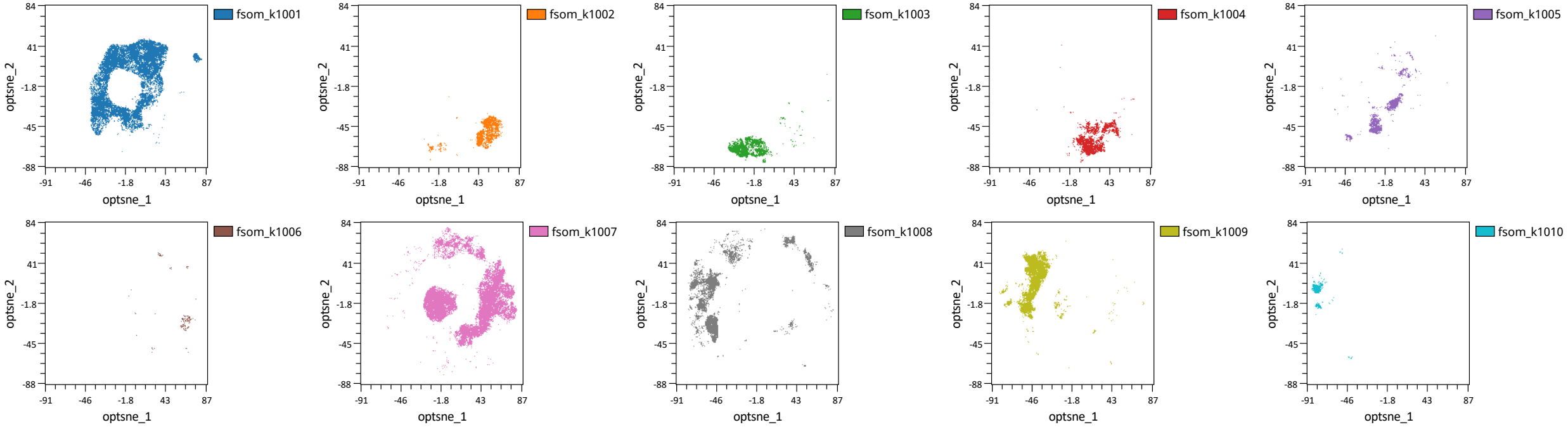
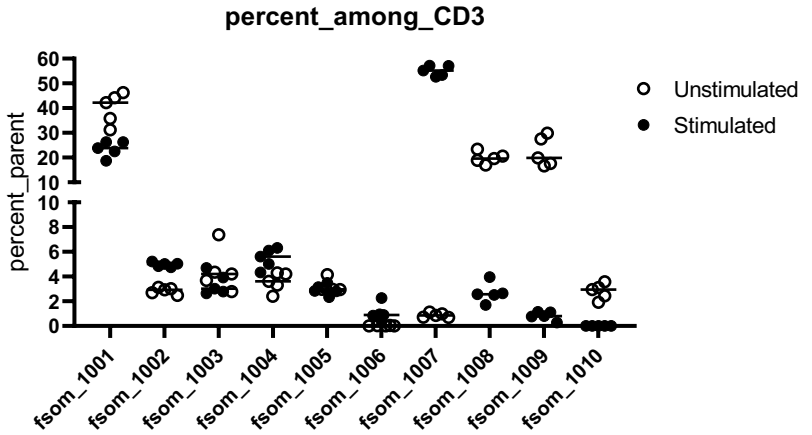
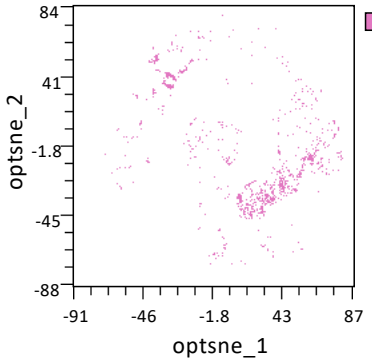
50
40
30
20
10
10
8
6
4
2
0
Unstimulated
Stimulated
fsom_1001
fsom_1002
fsom_1003
fsom_1004
fsom_1005
fsom_1006
fsom_1007
fsom_1008
fsom_1009
fsom_1010

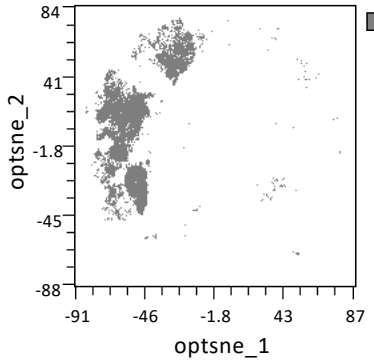
Clustering, individual clusters visualization (couple clicks in OMIQ)

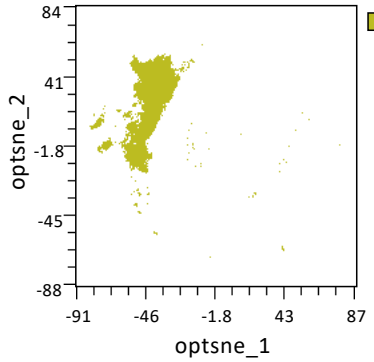


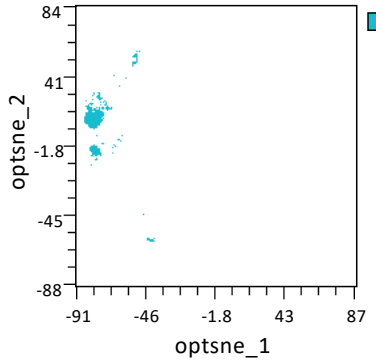


Clustering, focus on cluster 8 and 9

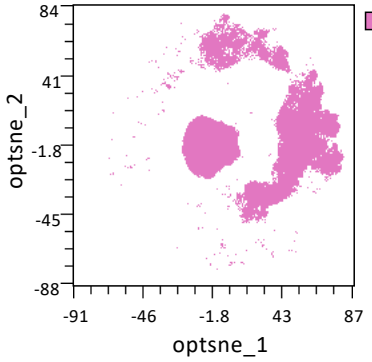


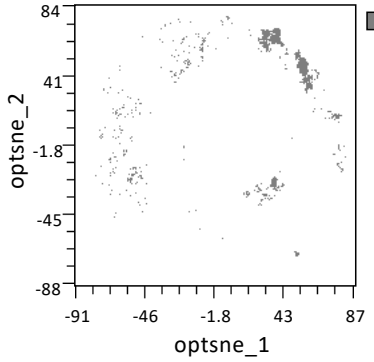


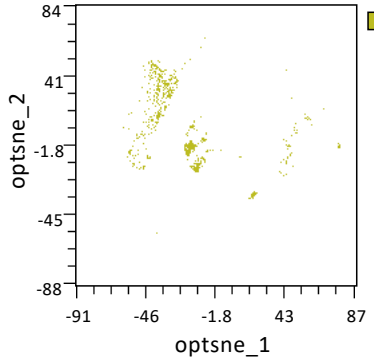


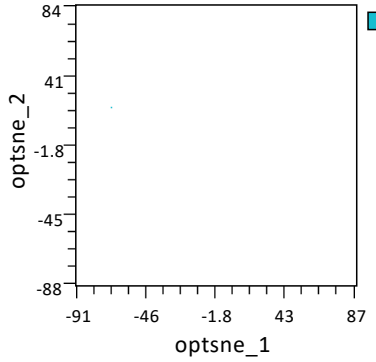


Unstimulated

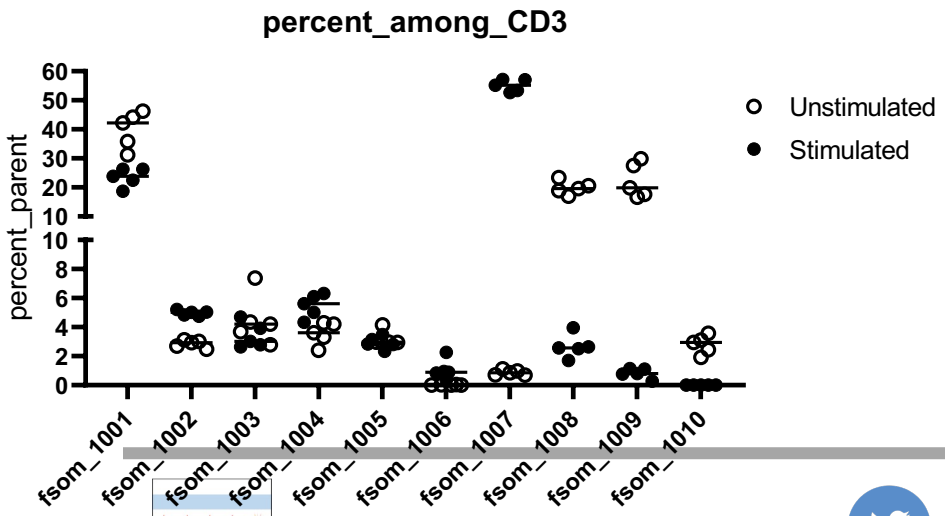


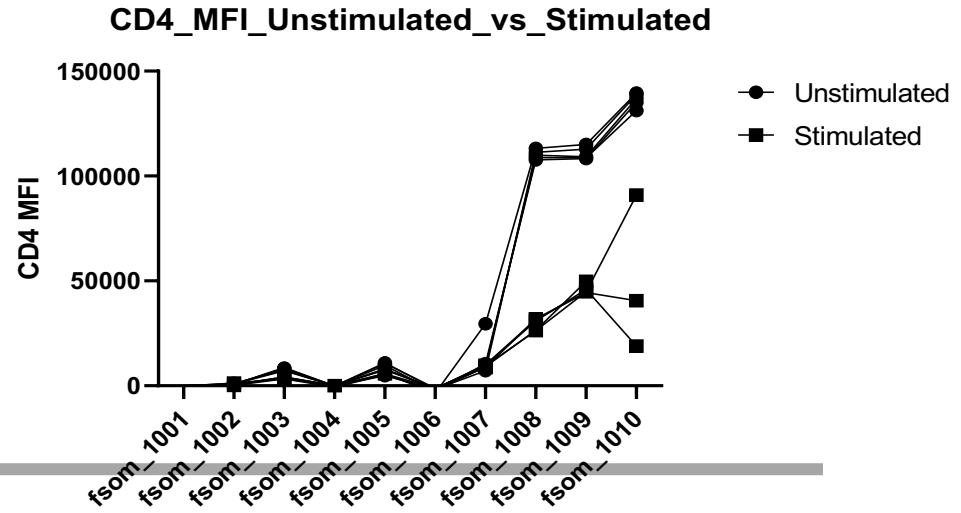




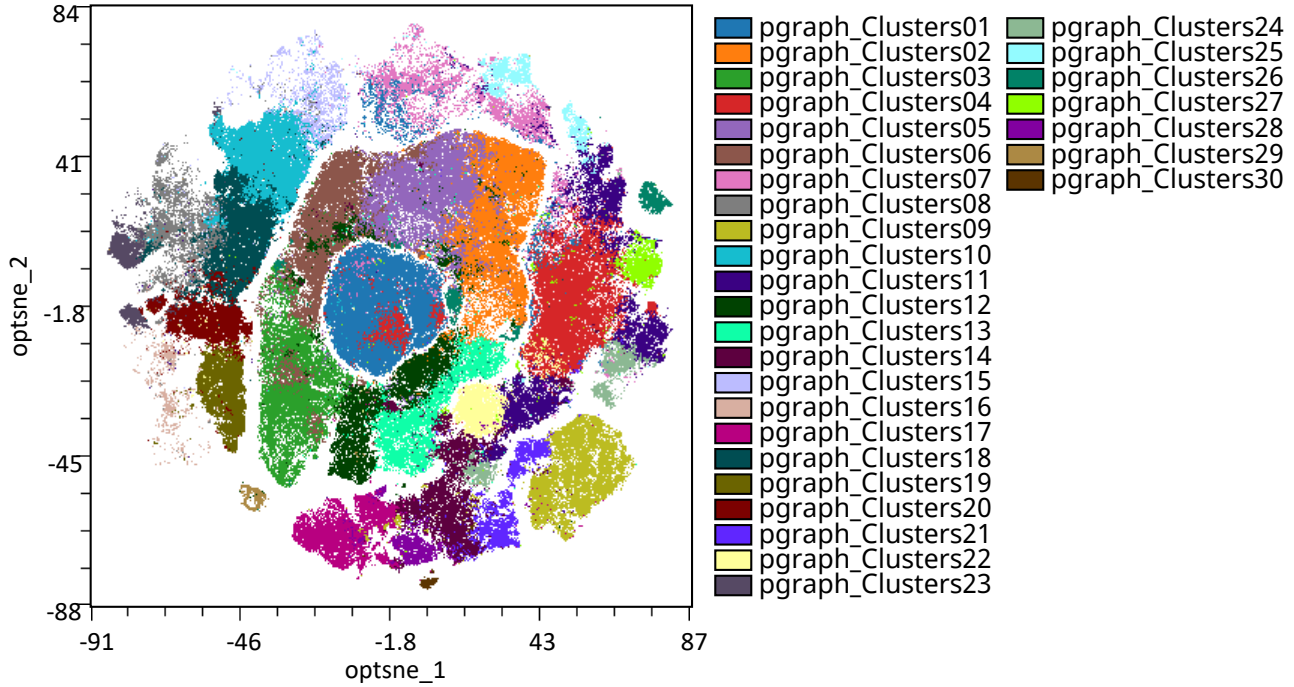


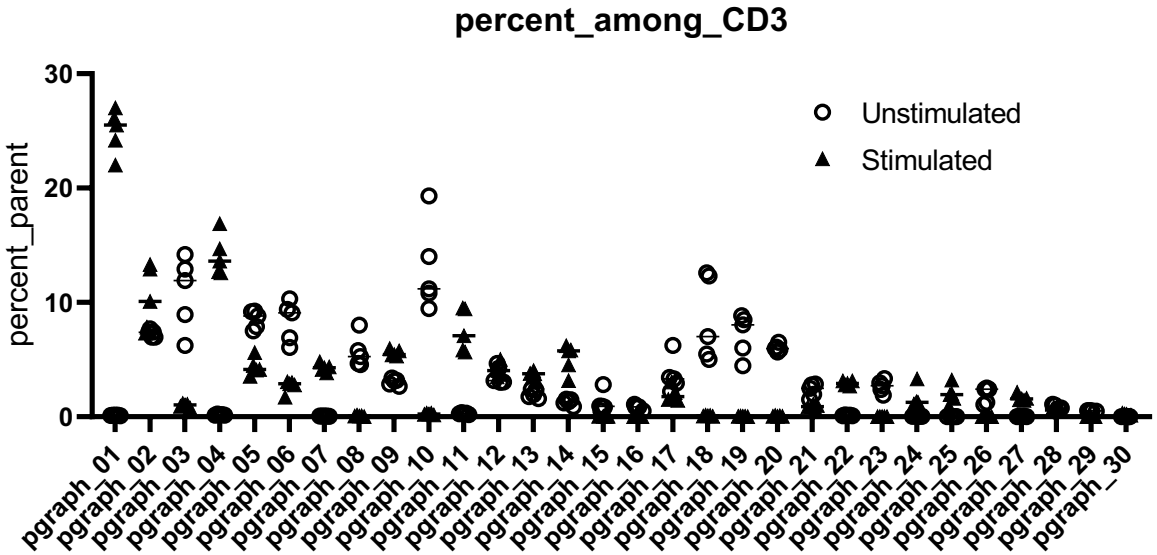
Stimulated



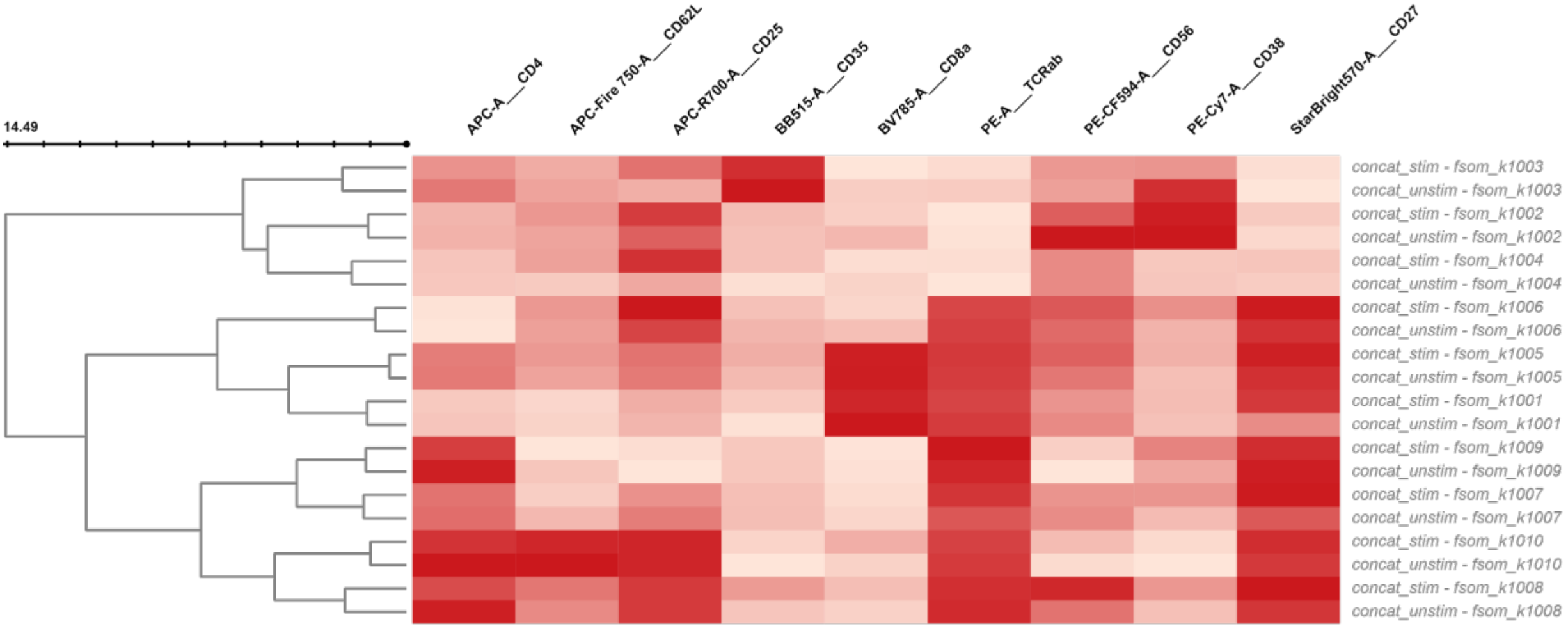


How many clusters to pick? 30 Clusters with Phenograph

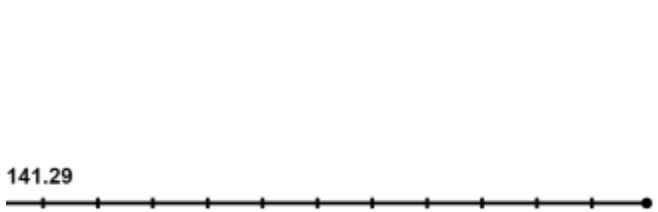




Clusters heatmap, results from OMIQ for advanced statistics (MFI)

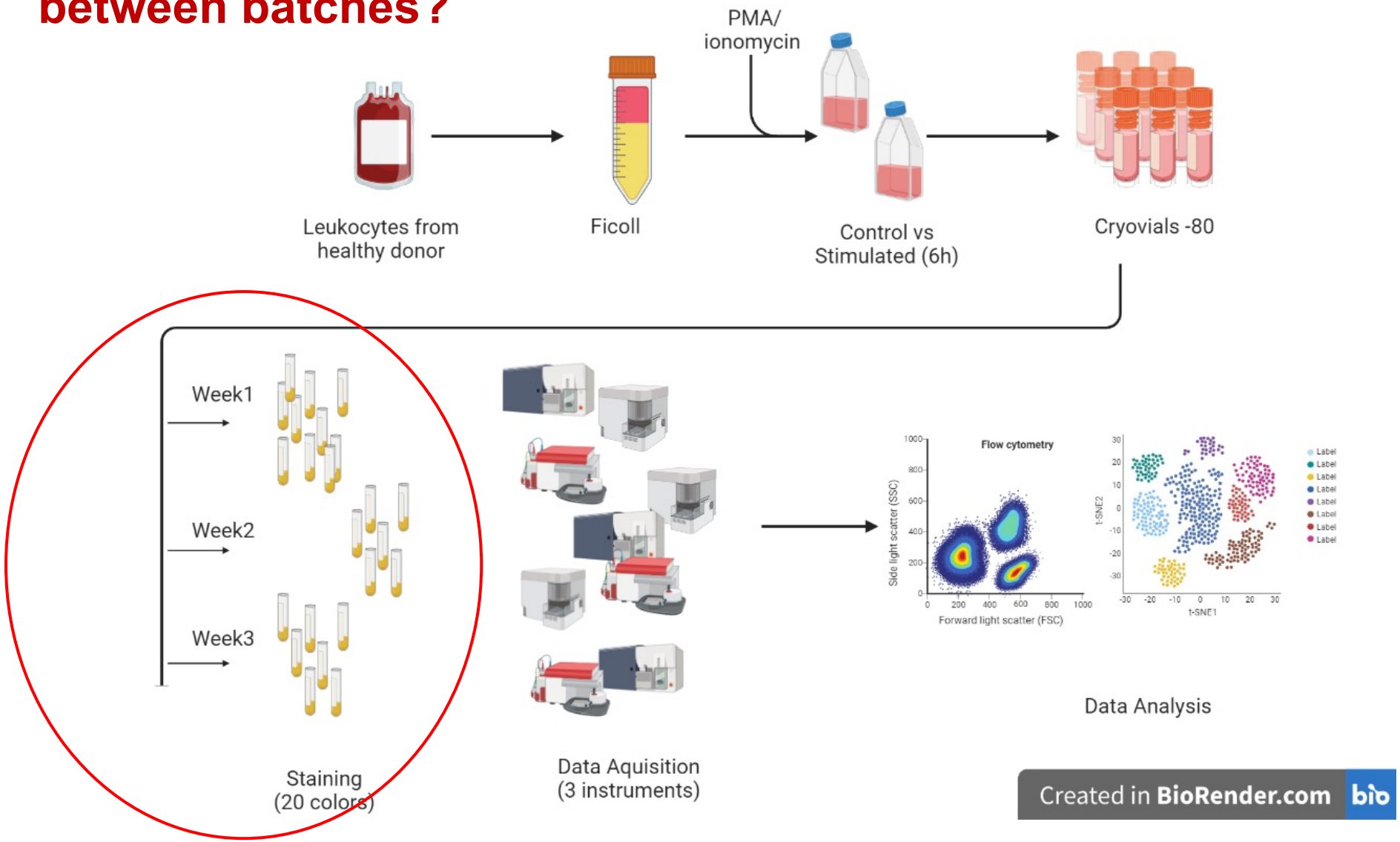


Clusters heatmap results from OMIQ for advanced statistics (Abundance)



% of file - fsom_k1007	% of file - fsom_k1001	% of file - fsom_k1003	% of file - fsom_k1005	% of file - fsom_k1004	% of file - fsom_k1002	% of file - fsom_k1006	% of file - fsom_k1010	% of file - fsom_k1009	% of file - fsom_k1008	Sample	
53.4	26.3	2.64	3.14	5.02	4.83	0.84	0.01	1.14	2.64	stim	*PBMC Stim_exp3_3-1.fcs*
52.7	26.3	2.78	2.84	6.1	4.74	0.89	0	1.1	2.52	stim	*PBMC Stim_exp3_1-1.fcs*
57.2	18.7	4.69	3.49	5.61	5.22	0.38	0	0.8	3.96	stim	*PBMC Stim-1.fcs*
57.1	22.5	3.91	2.81	4.34	5.04	2.26	0	0.28	1.71	stim	*PBMC Stim EXP 2-1.fcs*
55.2	23.8	3.02	2.33	6.31	5.02	0.94	0	0.77	2.57	stim	*PBMC Stim_exp3_2-1.fcs*
0.72	42.2	3.68	2.97	3.62	2.7	0.01	3.58	19.9	20.6	unstim	*PBMC Unstim_exp3_3-1.fcs*
0.7	46.3	4.21	3.07	4.21	3.02	0.01	2.94	16.6	18.9	unstim	*PBMC Unstim_exp3_1-1.fcs*
0.85	44.2	4.35	2.96	4.3	2.93	0.02	3.11	17.6	19.6	unstim	*PBMC Unstim_exp3_2-1.fcs*
0.97	35.8	7.37	2.95	3.3	3.12	0.02	1.92	27.5	17	unstim	*PBMC Unstim-1.fcs*
1.13	31.2	2.78	4.15	2.41	2.48	0.04	2.44	29.9	23.4	unstim	*PBMC Unstim EXP 2-1.fcs*

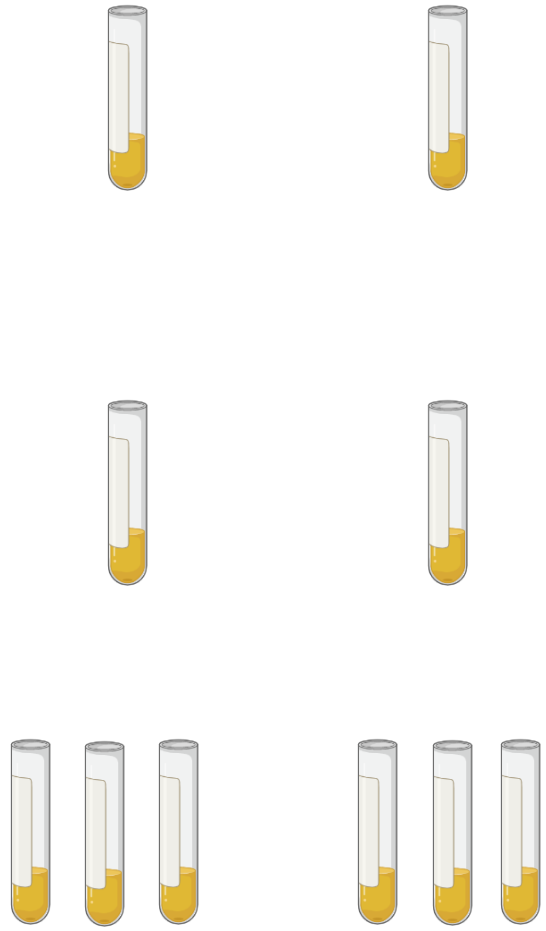
Can we identify differences between batches?

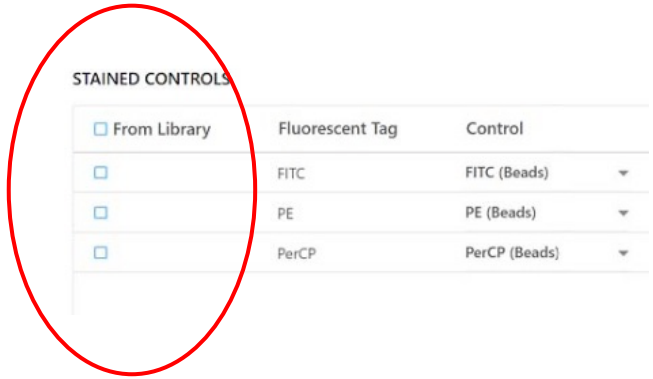


Week1

Week2

Week3





Unstimulated Stimulated

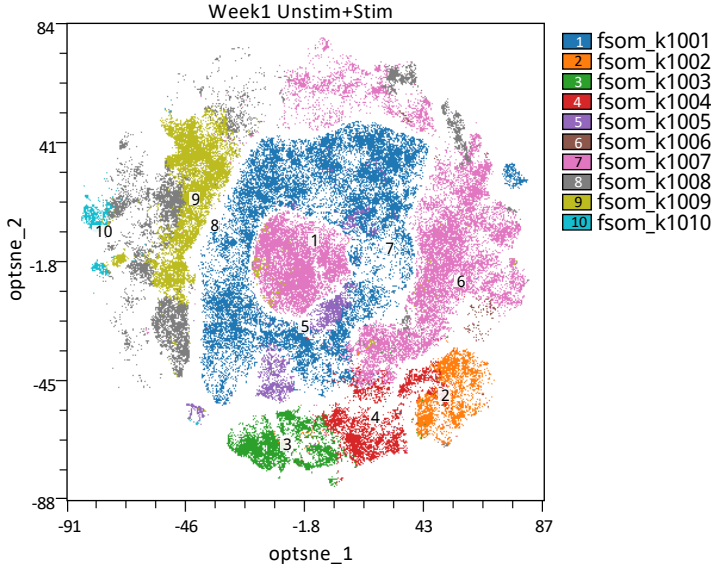
10 Samples

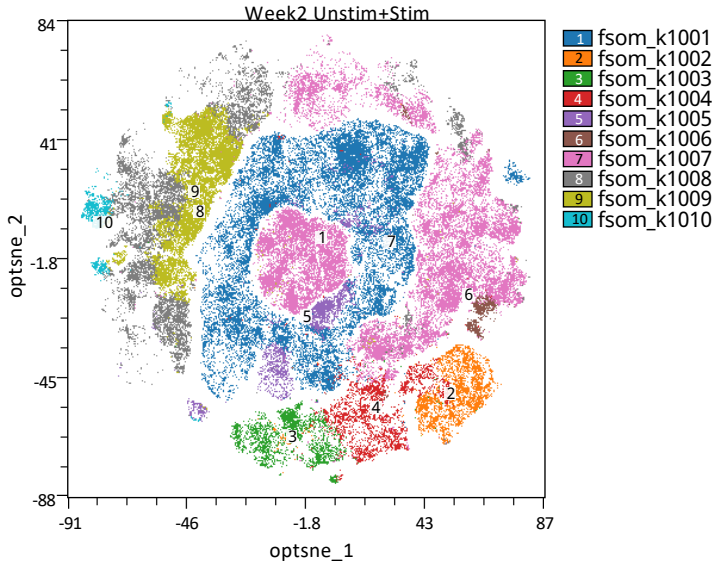
Aurora - Unmixing 2x

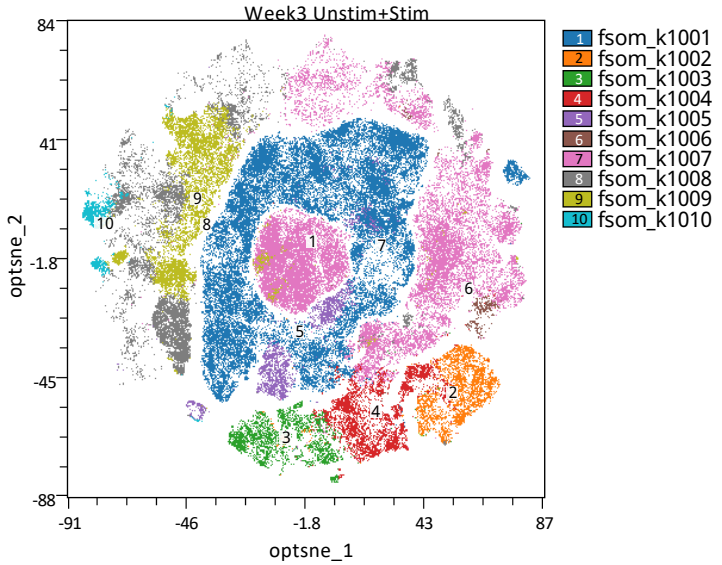
20 data files
10- unmixed with new set of reference controls for each “week”

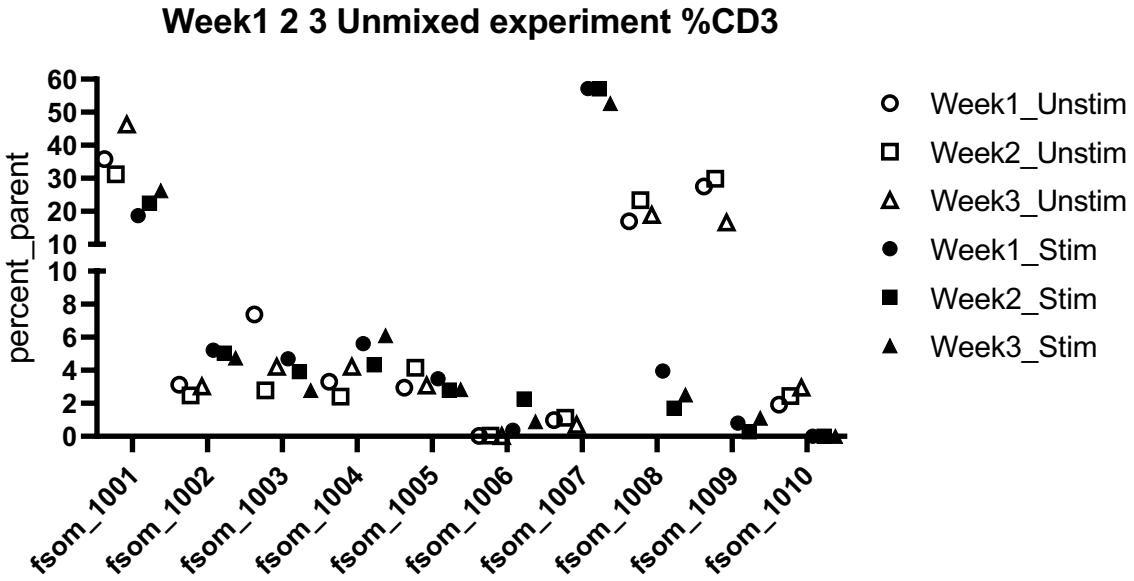
10- unmixed using reference controls recorded in library on week1

tSNE and Clustering overtime

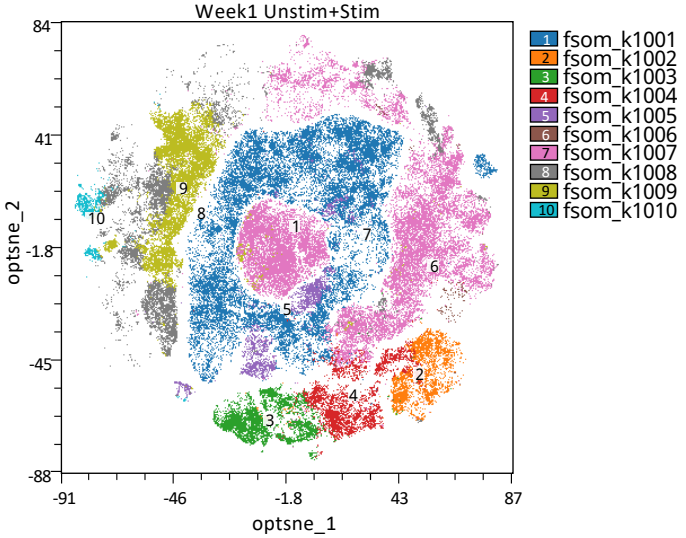


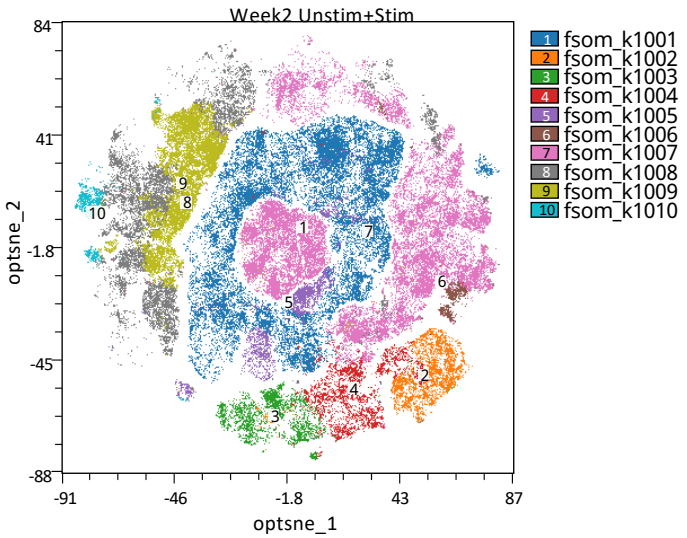


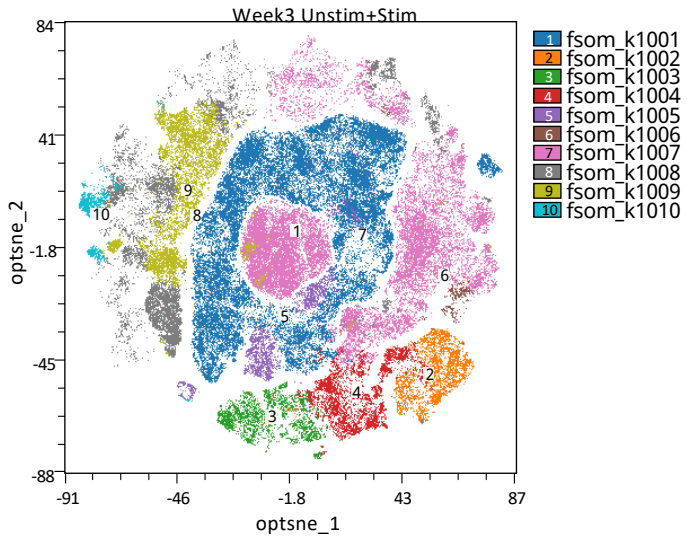




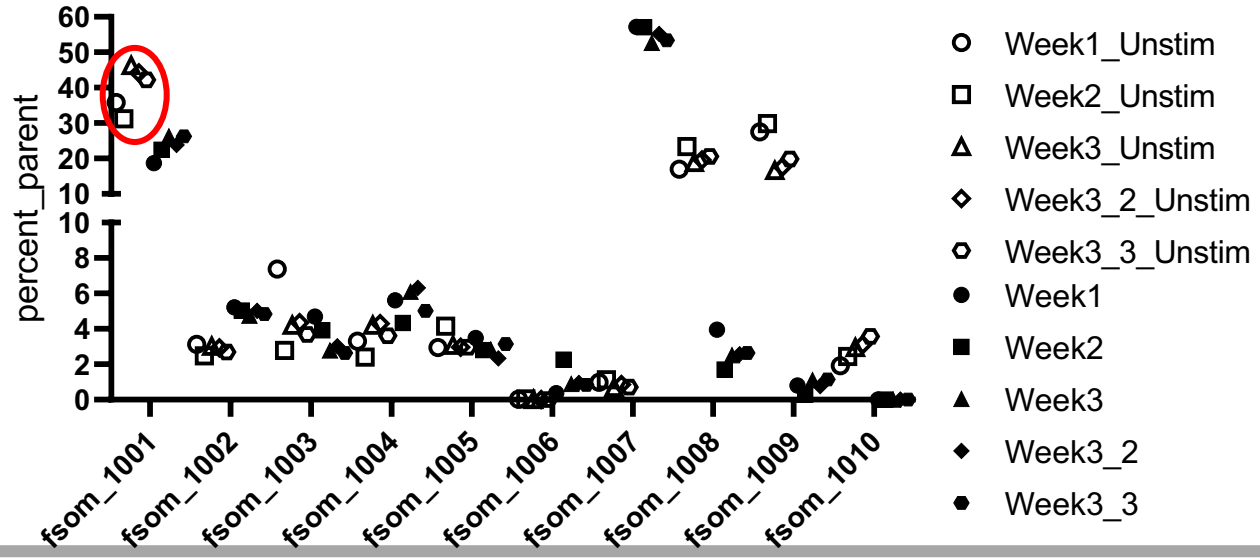
tSNE and Clustering overtime



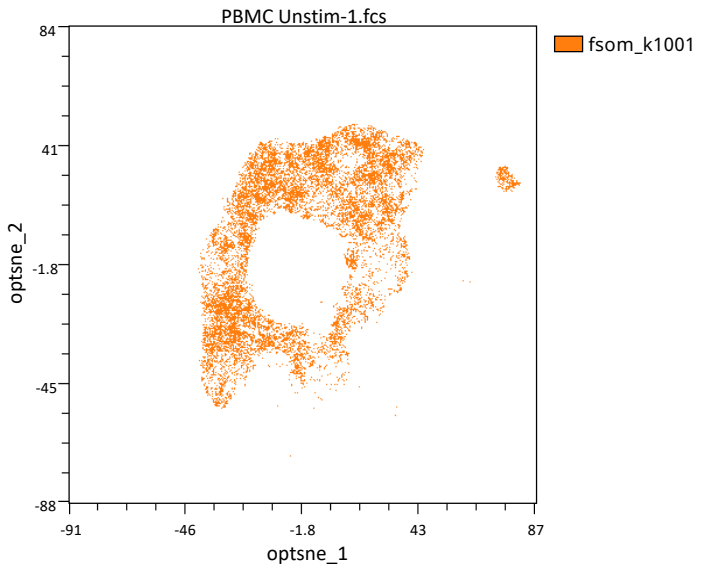


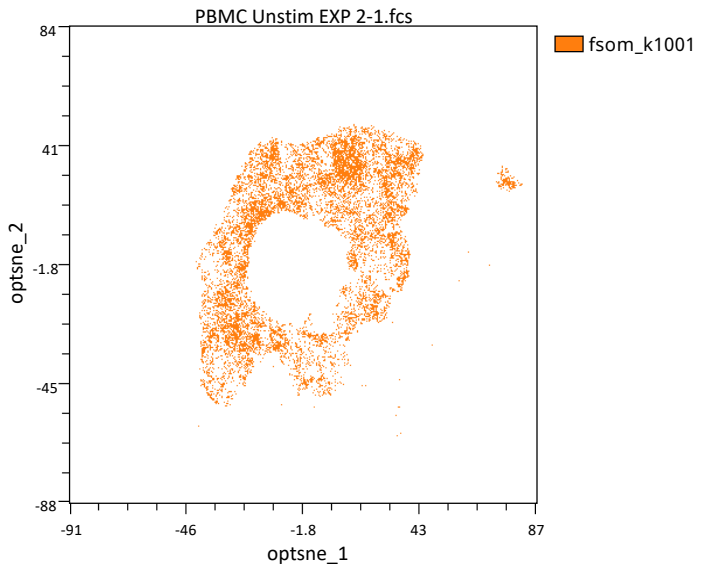


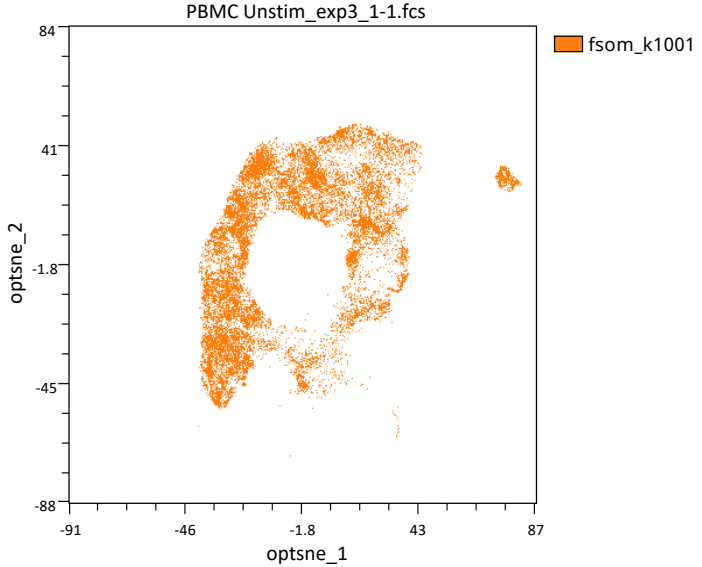
Week1 2 3 Unmixed experiment %CD3_n=5

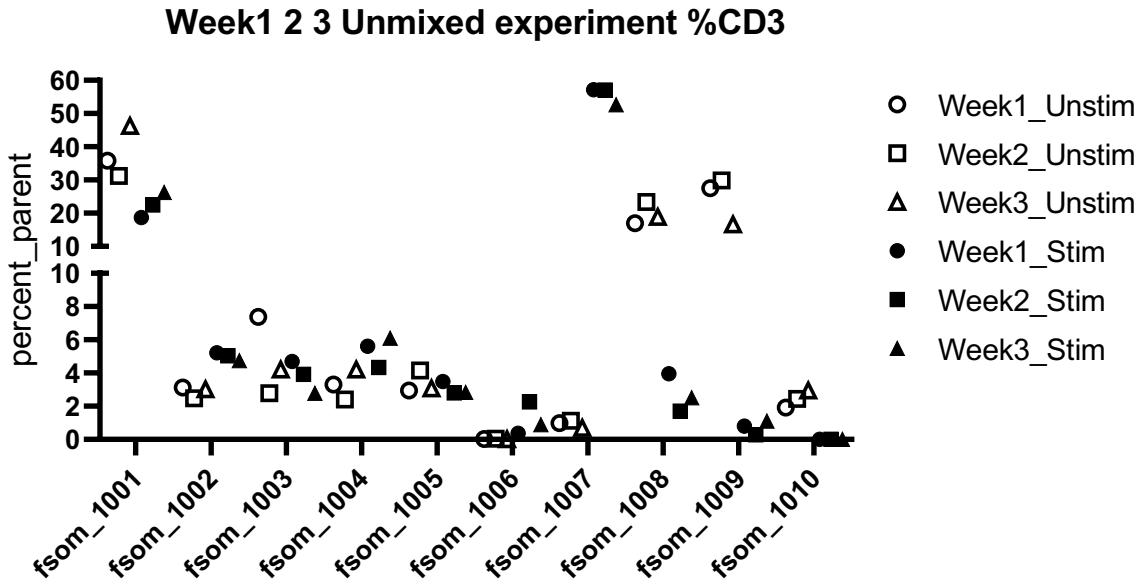


tSNE and Clustering overtime



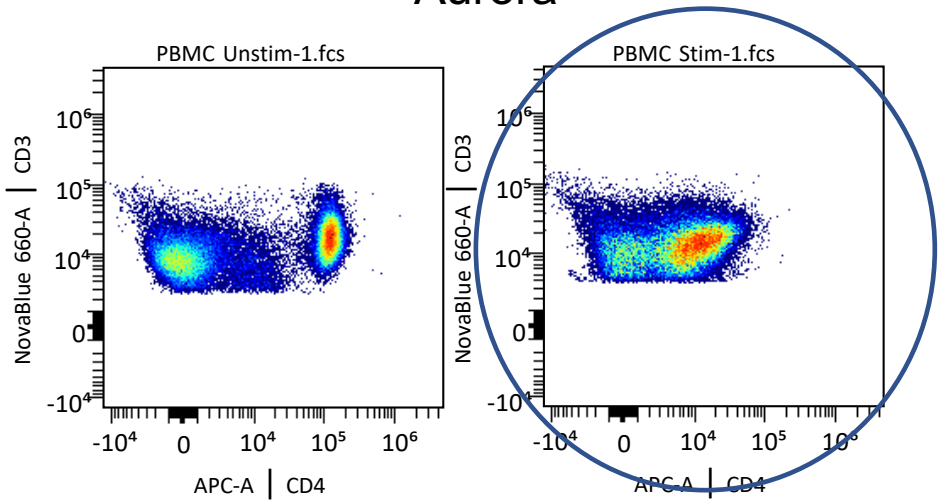




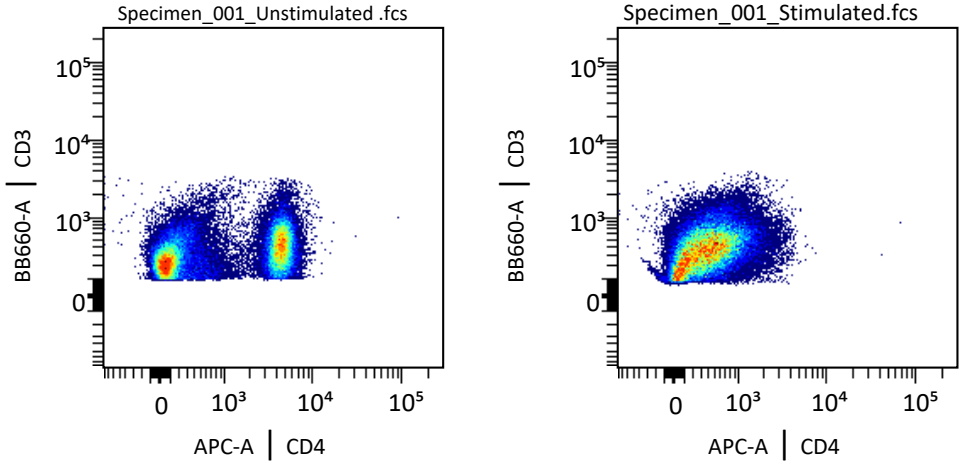


Instruments comparison, sensitivity

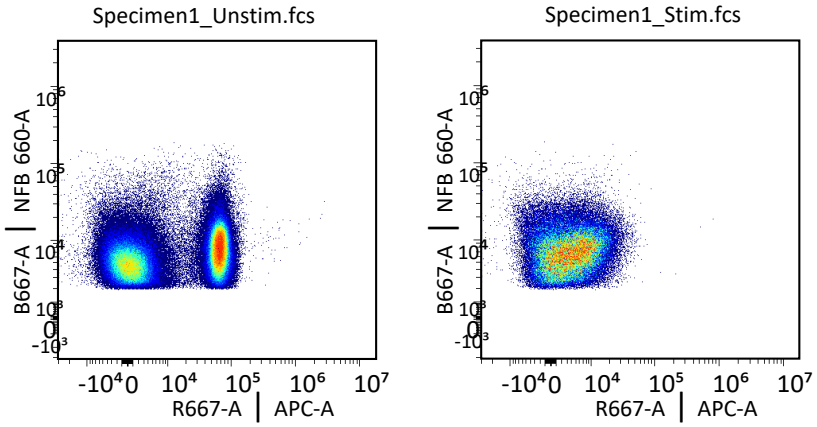
Aurora



Symphony S6



Penteon



BD Cell Sorter Symphony S6 Data (compensation matrix generated in OMIQ)

Week1

Channels	BUV395-A	BUV496-A	BUV737-A	BUV805-A	BV421-A	BV570-A	BV605-A	BV650-A	BV711-A	BV786-A	BB515-A	BB630-A	BB660-A	PE-A	PE-CF594-A	PE-Cy7-A	APC-A	APC_R700-A	APC-Cy7-A	BV510-A
BUV395-A	100	5.28	0.117	0	0.158	0.318	0.311	0.181	0.147	0	0	0.296	0.15	0	0	0	0	0	-0.124	0.655
BUV496-A	20.5	100	2.43	1	0.405	8.28	4.73	1.48	0.966	0.35	2.81	1.83	0.82	0	0.215	0	0.238	0.151	0.238	41
BUV737-A	2.69	0.507	100	46.6	0	0.167	0.117	0.13	2.39	1.79	0	0.163	0.161	0	0	1.69	0.246	15.6	33.9	0.384
BUV805-A	4.88	0.833	3.29	100	0	0.23	0.182	0.109	0.125	1.33	0	0.198	0	0	0	1.17	0	0.147	15.6	0.396
BV421-A	1.97	1.81	0.598	0.199	100	1.63	1.11	0.585	0.376	0	0.172	0.892	0.495	0	0.142	0.144	0	0	0.224	7.8
BV570-A	0.613	3.2	3.71	1.49	1.18	100	61.6	17.3	10.7	2.36	0.325	6.88	1.87	5.37	3.01	0.287	0	0	0	38.8
BV605-A	1.48	0.573	10.8	4.17	9.86	34.6	100	50.3	32.3	8.17	0	8.58	3.77	17.5	27	5.02	0.29	0.311	0.305	1.78
BV650-A	0.492	0.956	39.2	20.6	5.8	16.6	51.3	100	82.7	29	0	3.54	8.26	1.18	3.11	5.72	5	3.48	2.67	5.71
BV711-A	2.76	1.01	34.7	16.2	9.21	1.24	1.05	6.6	100	31.9	0.121	1	1.16	0.187	0.17	4.22	4.16	63.3	54.4	2.82
BV786-A	2.48	0.61	49.1	39.5	8.44	1.21	1.01	0.564	31.2	100	0	1.08	0.517	0.131	0.111	3.23	0.12	4.84	48.8	2.47
BB515-A	1.36	7.56	0.279	0.164	0	0.947	0.795	0.304	0.258	0	100	16.1	2.8	0	0	0.101	0	0	0.167	4.5
BB630-A	3.03	0.841	1.6	0.65	0.346	1.94	9.8	3.75	2.49	0.552	2.2	100	29.8	5.88	34.5	3.82	0.945	1.01	0.826	2.51
BB660-A	3.19	0.961	2.72	1.12	0	1.49	1.22	15.8	8.16	1.71	2.66	2.84	100	0	0.139	2.54	172	111	65	2.94
PE-A	0.35	0.141	0.84	0.279	0	11.4	5.26	1.38	0.852	0.151	0	43.4	8.9	100	45.3	2.61	0	0	0	0.414
PE-CF594-A	0.559	0.158	2.26	0.871	0	1.25	7.93	3.08	2.13	0.48	0	85	27.4	22.6	100	12.5	0.256	0.295	0.226	0.442
PE-Cy7-A	0.336	0.121	1.58	11.3	0	0.185	0.153	0	0.146	4.01	0	0.431	0.189	0.489	0.276	100	0	0.107	14.2	0.262
APC-A	1.7	0.453	12	3.92	0.239	0.681	0.979	11.4	5.7	1.29	0.155	0.864	2.75	0	1.15	9.11	100	71.9	46.6	1.29
APC_R700-A	0.498	0.141	11.7	4.15	0	0.204	0.16	0.624	5.66	1.46	0	0.18	0.313	0	0	9.79	8.71	100	67	0.37
APC-Cy7-A	0.2	0	1.34	13	0	0	0	0.192	0.17	3.22	0	0	0	0	0	17	1.29	1.5	100	0.157
BV510-A	-4.73	56.8	2.52	0.545	-1	33.3	21.1	5.44	2.61	0.391	-0.106	-0.469	-0.379	0	0	0	0	-0.165	-0.243	100

Week2

Channels	BUV395-A	BUV496-A	BUV737-A	BUV805-A	BV421-A	BV570-A	BV605-A	BV650-A	BV711-A	BV786-A	BB515-A	BB630-A	BB660-A	PE-A	PE-CF594-A	PE-Cy7-A	APC-A	APC_R700-A	APC-Cy7-A	BV510-A
BUV395-A	100	5.59	0.302	0.148	0.369	0.515	0.444	0.26	0.155	0	0.108	0.411	0.242	0	0	0.101	0	0	0	0.832
BUV496-A	17.1	100	2.72	1.18	0.758	7.94	5.03	1.4	0.985	0.208	3.11	1.76	0.779	0.111	0.218	0.215	0	0.163	0.277	38.9
BUV737-A	2.25	0.45	100	44.2	0	0.147	0.152	0	1.84	1.35	0	0.162	0.149	0	0	1.66	0.216	12.4	26.5	0.355
BUV805-A	4.86	0.92	3.64	100	0.133	0.173	0.165	0.107	0	1.05	0	0.196	0	0	0	1.14	0	0.151	11.5	0.314
BV421-A	2.05	1.78	0.394	0.275	100	1.58	1.2	0.577	0.439	0.145	0.121	0.626	0.474	0.128	0.186	0.226	0.102	0.108	0.226	7.93
BV570-A	0.533	3.03	3.74	1.53	1.08	100	62	16.8	9.97	2.23	0.366	7.47	2.01	5.71	3.2	0.352	0	0	0.316	37.9
BV605-A	1.47	0.625	10.4	4.14	8.97	33.8	100	46.7	28.9	7.06	0.101	9.09	3.94	19.2	29.4	5.19	0.195	0.207	0	1.65
BV650-A	0.385	0.89	40.6	20.2	4.98	16.5	52.5	100	77.8	26.1	0	4.05	9.49	1.31	3.4	5.79	4.39	2.94	2.23	5.35
BV711-A	2.82	1.34	37.7	17.3	10.7	1.36	1.32	6.83	100	31.4	0.173	1.05	1.24	0.102	0.279	4.4	4.09	57.7	47.5	3
BV786-A	2.81	1.56	54.9	42.7	9.43	1.31	1.38	0.692	32.6	100	0.151	0.913	0.439	0.134	0.234	4.15	0.162	5.13	46.5	2.94
BB515-A	0.714	5.8	0.133	0	0	0.599	0.412	0.172	0.144	0	100	15.5	2.62	0	0	0	0	0	0	3.46
BB630-A	2.39	1	1.27	0.442	0.657	1.71	7.96	2.87	1.82	0.363	2.46	100	29.9	5.88	33.5	3.42	0.875	0.786	0.389	2.73
BB660-A	2.32	0.609	2.28	0.81	0.204	1.2	1.19	11.5	5.7	1.28	3.04	2.96	100	0	0.276	2.37	124	76.9	44	2.66
PE-A	0.26	0	0.599	0.21	0	8.35	3.82	0.937	0.566	0	0	44	8.78	100	44.3	2.37	0	0	0	0.356
PE-CF594-A	0.355	0.109	1.7	0.623	0	0.954	6.14	2.26	1.47	0.272	0	86.5	27.6	22.8	100	11.5	0.198	0.215	0.138	0.308
PE-Cy7-A	0.246	0	1.29	8.33	0	0.148	0.106	0	0	2.73	0	0.447	0.182	0.522	0.284	100	0	0	9.48	0.224
APC-A	1.47	0.61	11.9	4.02	0.454	0.756	0.909	10.6	5.04	1.2	0	0.61	3.12	0	1.39	10.3	100	68.6	43.2	1.5
APC_R700-A	0.436	0.152	12.1	4.16	0	0.207	0.207	0.641	5.37	1.29	0	0.187	0.406	0	0	12.2	9.02	100	62.9	0.455
APC-Cy7-A	0.391	0.217	1.33	13.1	0.229	0.128	0.142	0.182	0.178	3.13	0	0.112	0	0	0	14.4	1.1	1.33	100	0.276
BV510-A	-0.701	41.7	3.35	1.71	1.32	33.3	21.8	6.58	4.36	1.28	0.16	0.862	0.733	0.529	0.743	0.965	1.13	1.49	2.09	100

Week3

Channels	BUV395-A	BUV496-A	BUV737-A	BUV805-A	BV421-A	BV570-A	BV605-A	BV650-A	BV711-A	BV786-A	BB515-A	BB630-A	BB660-A	PE-A	PE-CF594-A	PE-Cy7-A	APC-A	APC_R700-A	APC-Cy7-A	BV510-A
BUV395-A	100	5.63	0.288	0.148	0.462	0.52	0.429	0.254	0.183	0	0	0.362	0.19	0	0	0	0	0	0.108	0.991
BUV496-A	17.8	100	2.73	1.22	0.835	8.12	5.08	1.55	1.13	0.225	2.99	1.75	0.776	0.134	0.319	0.257	0.159	0.199	0.279	39.5
BUV737-A	2.31	0.54	100	43.4	0.12	0.183	0.188	0.112	1.85	1.32	0	0.133	0.134	0	0	1.63	0.213	12.9	27.6	0.452
BUV805-A	4.64	0.945	3.54	100	0.165	0.241	0.206	0.131	0.102	1.09	0	0.184	0	0	0	1.21	0	0.189	12.9	0.529
BV421-A	2.32	2.29	0.404	0.279	100	1.74	1.41	0.521	0.427	0.134	0	0.726	0.38	0.116	0.254	0.158	0.145	0.196	0.122	8.39
BV570-A	0.53	2.98	3.58	1.4	1.09	100	61.5	16.3	9.64	2.1	0.342	7.09	1.91	5.73	3.16	0.284	0	0	0	37.3
BV605-A	1.55	0.761	10.4	4.05	9.11	33.5	100	46.4	29.2	6.99	0.107	9.04	3.98	19.4	29.1	5.11	0.306	0.283	0.291	1.85
BV650-A	0.435	0.945	40.2	19.4	5.17	16.4	52.5	100	78.4	26.1	0	3.8	9.08	1.32	3.37	5.8	4.68	3.18	2.45	5.51
BV711-A	3.32	1.61	36.9	16.4	9.77	1.19	1.31	6.63	100	30.7	0	0.937	1.06	0.167	0.221	4.48	4.18	62.4	52.6	3.26
BV786-A	2.59	1.39	54.8	42	9.57	1.26	1.18	0.763	32.2	100	0	0.783	0.518	0.107	0.244	3.96	0.177	5.08	48.6	3.04
BB515-A	0.685	6.29	0.187	0	0	0.718	0.482	0.195	0.106	0	100	15.3	2.62	0	0	0	0	0	0	3.71
BB630-A	2.43	1.12	1.41	0.676	0.545	2.05	8.55	3.04	1.95	0.451	2.51	100	30.1	6.15	34.2	3.72	0.877	0.907	0.388	3.16
BB660-A	2.76	1.21	2.64	0.811	0.612	1.41	1.15	12.6	6.26	1.31	2.92	2.74	100	0.184	0.385	2.45	143	87.6	51.4	3.08
PE-A	0.247	0	0.677	0.225	0	9.16	4.18	1.04	0.622	0.105	0	43.3	8.69	100	44	2.34	0	0	0	0.339
PE-CF594-A	0.367	0.174	1.93	0.715	0	1.03	6.65	2.47	1.64	0.351	0	85.7	27.2	23	100	11.7	0.256	0.259	0.159	0.371
PE-Cy7-A	0.275	0.11	1.39	8.94	0	0.156	0.14	0	0.111	2.94	0	0.397	0.175	0.558	0.302	100	0	0.103	10.9	0.258
APC-A	1.2	0.483	11.6	3.94	0.174	0.556	0.771	10.2	4.92	1.14	0	0.498	3.01	0.121	1.38	9.98	100	70.4	43.5	1.14
APC_R700-A	0.329	0.125	11.6	3.78	0	0.131	0.135	0.571	4.98	1.24	0	0.111	0.309	0	0	10.7	8.97	100	64.2	0.282
APC-Cy7-A	0.37	0.262	1.4	12.8	0.225	0.169	0.15	0.208	0.21	2.97	0	0.104	0.107	0	0	14.7	1.14	1.36	100	0.374
BV510-A	-1.13	46.4	2.81	1.12	0.382	32.7	21.5	6.31	3.96	1.26	-0.103	0	0.216	0.278	0.465	0.561	0.903	1.14	1.3	100

Panteon Data (compensation matrix generated in OMIQ)

Week1

Channels	B525-A	B615-A	B667-A	R667-A	R695-A	R725-A	UV445-A	UV525-A	UV725-A	UV780-A	V445-A	V525-A	V586-A	V615-A	V667-A	V725-A	V780-A	Y586-A	Y615-A	Y780-A
B525-A	100	2.69	0.542	0	0	0	0.235	2	0	0	0.13	1.43	0.186	0	0	0	0	0	0	0
B615-A	19.6	100	33.1	1.13	0.746	1.06	3.03	1.63	0.442	0	1.26	4.98	2.61	4.94	2.37	0.654	0	7.13	57.5	0.658
B667-A	21.2	2.95	100	123	41.9	64.7	3.17	1.66	0.538	0.336	1.23	5.59	1.69	0.541	9.39	2.17	0.482	-0.183	0.417	0.653
R667-A	1.1	0.861	2.88	100	25.6	62.3	1.82	0.68	2.54	0.753	0.856	2.45	0.745	0.374	7.74	2	0.371	0.188	2.42	2.89
R695-A	1.38	0.671	1.08	15.2	100	353	2.03	1.01	12.1	3.67	1.37	2.62	0.803	0.313	1.29	8.83	1.57	0	0.448	12.9
R725-A	6.34	4.14	4.07	51.4	18.1	100	7.43	3.27	3.33	66.9	1.59	10.7	3.38	1.06	4.18	3.9	40.5	1.6	7.98	236
UV445-A	0.478	0.282	0.153	0	0	0	100	6.55	0	0	0.705	1.09	0.289	0.108	0	0	0	0	0	0
UV525-A	9.82	0.903	0.477	0	0	0	23.1	100	0.523	0	3.46	35.2	3.98	0.947	0.378	0.202	0	0	0.256	0
UV725-A	1.37	0.655	0.547	0.384	5.64	84.3	20.3	2.48	100	50	1.1	2.62	0.856	0.243	0.167	6.12	2.03	0.177	0.422	1.89
UV780-A	2.29	1.37	0.941	-0.239	0.331	1.43	63.7	6.9	2.98	100	2.56	5.18	1.64	0.628	0.378	0	2.85	0.389	0.647	2.8
V445-A	1.1	0.61	0.361	0	0	0.129	11.9	1.5	0	0	100	8.74	1.05	0.27	0.191	0	0	0.122	0.242	0
V525-A	-0.234	0	0	0.304	0.186	0.365	-1.35	30.2	0.249	0.121	9.74	100	20.2	4.69	1.66	0.374	0	0	0.223	0
V586-A	6.13	7.04	2.14	0	0	0	0.894	13	1.47	0.483	6.23	73.2	100	23.3	8.9	2.3	0.483	9.89	4.71	0
V615-A	2.4	15.4	7.92	0.6	0.237	0.601	6.29	1.82	7.72	2.55	44.9	7.93	103	100	67.6	19.6	4.17	49.7	99.4	2.9
V667-A	0.897	7.04	17.6	7.89	2.42	5.07	5.63	3.2	31.1	13	73.5	29.2	42.8	45.4	100	42.4	12.7	3.4	10.8	3.4
V725-A	6.73	3.54	3.37	2.33	26.3	94.9	13	2.94	34.4	12.3	58	18.2	5	1.23	7.37	100	24.4	1.16	2.34	2.38
V780-A	8.56	4.35	4.44	1.31	1.26	33.4	16.6	5.66	31.1	48.4	81.7	25.5	7.08	2.37	2.57	103	100	2.53	1.73	3.65
Y586-A	2.71	35.6	8.82	0	0	0	0.288	0.203	0.118	0	0	0.893	23	3.91	1.28	0.307	0	100	35	0.375
Y615-A	0.725	75.5	27	0.333	0.158	0.223	0.499	0.239	0.355	0	0.135	0.637	2.54	6.28	2.89	0.843	0.189	21.2	100	2.33
Y780-A	1.3	1.39	0.629	0.15	0	1.23	0.99	0.56	0.192	5.01	0.404	1.65	0.836	0.17	0.15	0.45	4.97	3.91	1.63	100

Week2

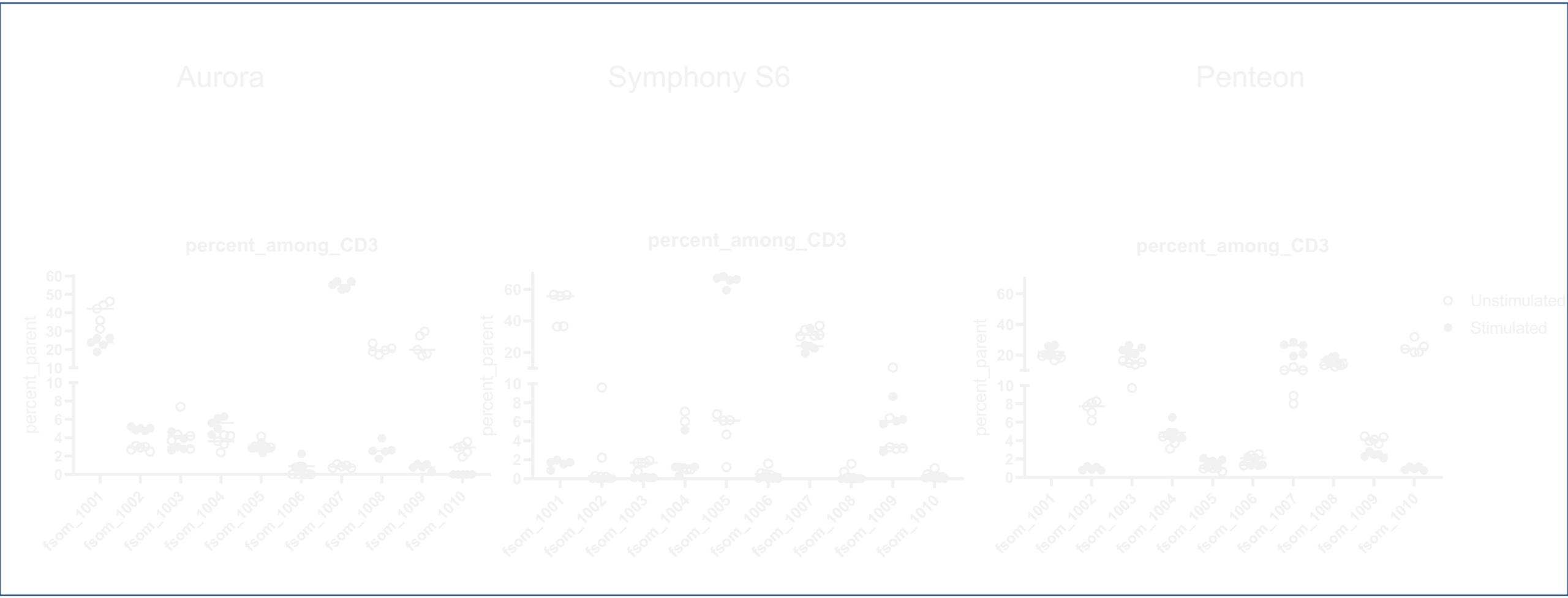
Channels	B525-A	B615-A	B667-A	R667-A	R695-A	R725-A	UV445-A	UV525-A	UV725-A	UV780-A	V445-A	V525-A	V586-A	V615-A	V667-A	V725-A	V780-A	Y586-A	Y615-A	Y780-A
B525-A	100	2.73	0.542	0	0	0	0.174	2.11	0	0	0	1.37	0.154	0	0	0	0	0	0	0
B615-A	18.8	100	33.5	0.953	0.707	0.376	2.41	1.03	0.541	0.348	1.22	4.37	2.35	5.03	2.27	0.639	0.172	7.46	59.6	0.89
B667-A	20.9	2.62	100	124	42.5	65.1	2.05	0.875	0.752	0.123	0.773	4.67	1.24	0.419	9.31	2.22	0.564	0	0.524	0.407
R667-A	0.811	0.271	2.81	100	25.8	62.4	1.25	0.504	2.47	0.581	0.769	1.41	0.626	0.316	7.63	1.98	0.378	0.148	2.19	2.9
R695-A	0.966	0.62	0.922	15.8	100	357	1.75	0.629	12.5	3.63	0.884	1.83	0.483	0.236	1.16	8.5	1.67	0.247	0.243	13.1
R725-A	6.92	3.48	4.78	42.8	16.7	100	7.5	3.91	4.79	67.4	5.6	13.2	5.02	1.42	4.03	4.55	41.2	2.92	7.86	234
UV445-A	0.36	0.241	0.138	0.105	0	0	100	6.57	0	0	1.18	1.09	0.27	0.188	0.111	0	0	0.104	0.189	0
UV525-A	9.66	0.851	0.423	0	0	0	23.7	100	0.424	0	3.61	35.2	3.97	0.939	0.323	0	0.111	0.144	0.344	0
UV725-A	1.11	0.771	0.312	0.355	5.91	83.5	19.8	2.29	100	49.1	0.236	1.64	0.621	0.235	0.266	5.95	2.02	0	0.176	2
UV780-A	1.44	1.07	0.461	0.282	0.223	1.71	70.2	8.15	3.39	100	2.64	3.87	1.2	0.319	0.195	0.26	2.72	0.185	0.878	3.13
V445-A	0.604	0.485	0.342	0	0	0.112	10.9	1.35	0	0	100	7.8	0.853	0.303	0.147	0.147	0	0.205	0	0
V525-A	0.75	0.505	0.36	0.359	0.279	0.474	-5.16	27.5	0.403	0.12	10	100	20.3	4.81	1.83	0.473	0.14	0.285	0.452	0.127
V586-A	5.94	6.93	2.17	0	0	0	1.14	13.1	1.56	0.486	6.21	74.4	100	23.3	8.97	2.29	0.477	9.72	4.64	0.182
V615-A	1.44	14.3	7.66	0.553	0.542	0.371	6.45	1.66	7.85	2.57	43.7	7.23	102	100	67.6	19.3	4.06	50	96.9	3.03
V667-A	0.779	6.98	17.4	8.16	2.5	5.13	5.69	3.24	31.2	13.2	73.9	29	42.9	45	100	42.5	12.7	3.6	11.3	3.43
V725-A	4.32	2.03	3.84	2.77	25.5	90.9	11.7	4.26	33.7	13.2	64.6	16.1	4.28	1.32	6.72	100	23.6	1.61	1.35	2.61
V780-A	4.23	4.45	1.6	0.293	-0.776	33.5	14.8	5.15	31	45.6	74.9	14	4.22	1.91	0.484	99.7	100	1.66	1.83	2.76
Y586-A	2.68	34.2	8.64	0	0	0	0.288	0.236	0.114	0	0.136	0.885	22	3.79	1.22	0.265	0	100	35.3	0.452
Y615-A	0.494	74.9	26.7	0.286	0.159	0.312	0.267	0.203	0.231	0	0.182	0.418	2.42	6.1	2.8	0.784	0.163	21.2	100	2.26
Y780-A	0.98	1.22	0.557	0	0.214	1.22	1.05	0.488	0.269	5.1	0.61	1.07	0.55	0	0.138	0.373	5.1	3.95	1.76	100

Week3

Channels	B525-A	B615-A	B667-A	R667-A	R695-A	R725-A	UV445-A	UV525-A	UV725-A	UV780-A	V445-A	V525-A	V586-A	V615-A	V667-A	V725-A	V780-A	Y586-A	Y615-A	Y780-A
B525-A	100	2.69	0.522	0	0	0	0.145	1.92	0	0	0	1.26	0.131	0	0	0	0	0	0	0
B615-A	17.7	100	33.6	1.01	0.686	0.536	2.18	0.834	0.113	0.194	1.06	3.99	2.48	4.54	2.09	0.705	0.114	7.07	58.6	0.926
B667-A	20.2	2.35	100	121	42.1	64.2	2.64	1.05	0.647	0.274	0	3.71	1.22	0.463	9.06	2.07	0.491	0.467	0.434	0.728
R667-A	0.342	0.332	2.69	100	26.3	63	0.97	0.28	2.47	0.813	0	1.08	0.337	0.247	7.77	2.04	0.397	0.243	2.69	2.99
R695-A	0.495	0.187	0.896	15.7	100	353	0.903	0.329	12.3	3.55	0.597	1.42	0.484	0.105	1.15	8.56	1.56	0.158	0.387	13
R725-A	3.07	2.53	3.74	42.4	16.9	100	9.11	4.58	4.02	65.9	0.886	13.9	3.24	1.52	4.23	4.61	40.5	2.12	8.2	230
UV445-A	0.247	0.163	0.146	0	0	0	100	6.55	0	0	0.827	0.89	0.248	0	0	0	0	0.145	0.211	0
UV525-A	9.4	0.725	0.399	0	0	0	23.4	100	0.467	0.146	2.88	35.1	3.98	0.934	0.337	0.104	0	0.128	0.209	0
UV725-A	0.411	0.401	0.26	0.298	5.7	83.6	20.2	2.57	100	49.6	1.05	1.75	0.516	0.245	0.19	6.07	1.93	0.181	0.341	1.9
UV780-A	0.834	0.855	0.548	0	0.12	1.55	66.5	7.22	3.02	100	1.92	3.67	1.14	0.45	0	0	2.7	0.513	0.677	2.66
V445-A	0.415	0.186	0.203	0	0	0	11.3	1.25	0	0	100	7.49	0.848	0.206	0.114	0.105	0	0	0.268	0
V525-A	0.142	0.13	0.143	0.232	0.155	0.273	-3.91	28.7	0.226	0	9.34	100	19.9	4.69	1.75	0.402	0.108	0	0.265	0
V586-A	5.78	6.94	2.03	0	0	0	0.837	13	1.53	0.463	6.19	74.5	100	23.6	8.99	2.29	0.484	10.1	4.58	0
V615-A	1.32	13.9	7.42	0.44	0.501	0.285	5.48	1.22	7.55	2.3	45.6	6.33	103	100	68.4	19.1	3.97	50.4	99.3	2.88
V667-A	0.499	6.69	17.4	7.76	2.39	5.12	5.48	2.99	30.6	13.1	72.9	28.9	42.3	44.6	100	41.9	12.6	3.44	11	3.3
V725-A	2.43	2.99	1.33	2.63	26.8	92.3	11.7	4.19	34.4	12.1	60.2	15.3	4.1	1.11	7.7	100	23.6	0.918	1.2	2.84
V780-A	3.9	2.85	1.52	-0.386	0.963	33.1	13.1	5.33	31.7	46.7	78.1	17.8	5.59	1.47	0.972	102	100	0	1.71	2.81
Y586-A	2.65	35.6	8.85	0	0	0	0.219	0.186	0.126	0	0	0.817	23.2	3.98	1.26	0.286	0	100	35.3	0.38
Y615-A	0.442	75.5	27	0.316	0.195	0.254	0.301	0.105	0.363	0	0	0.37	2.43	6.23	2.88	0.784	0.186	21.2	100	2.21
Y780-A	0.84	1.02	0.539	0	0	1.13	0.735	0.433	0.299	4.99	0.477	1.13	0.53	0.127	0.132	0.445	4.97	4.06	1.76	100

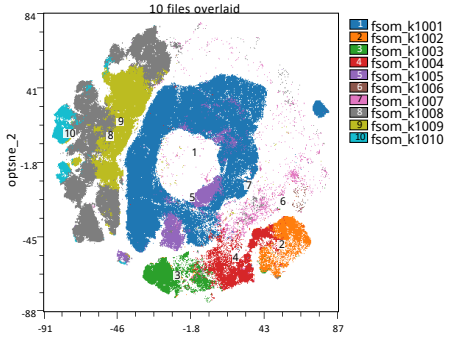
Symphony S6 – is comparing cell sorters to analyzers fair?

Instruments comparison



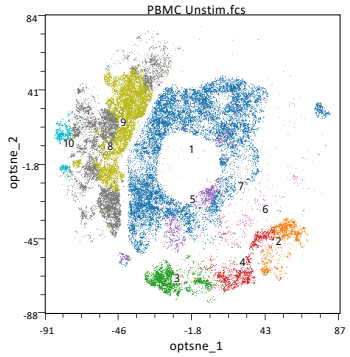
Symphony S6 – is comparing cell sorters to analyzers fair?

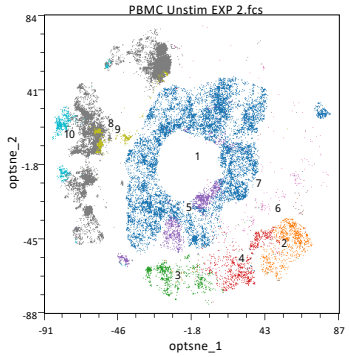
Library vs experiment reference controls unmixing analysis, Unstimulated samples:

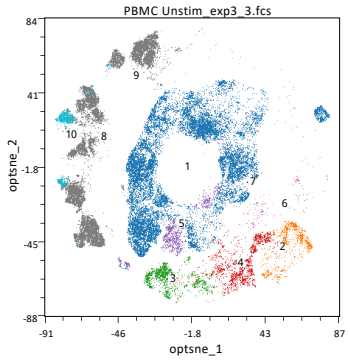


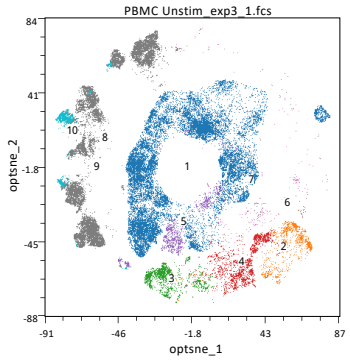
STAINED CONTROLS

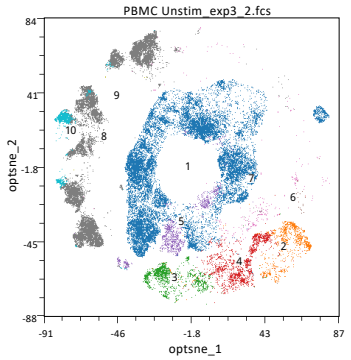
From Library	Fluorescent Tag	Control
☐	FITC	FITC (Beads)
☐	PE	PE (Beads)
☐	PerCP	PerCP (Beads)



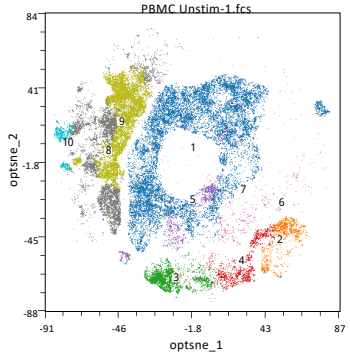


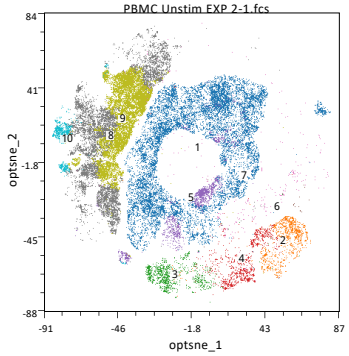


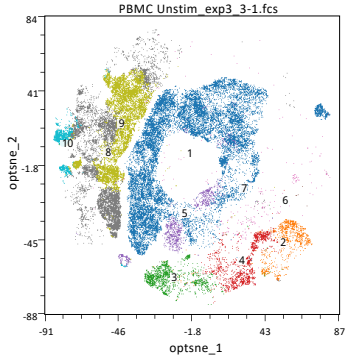


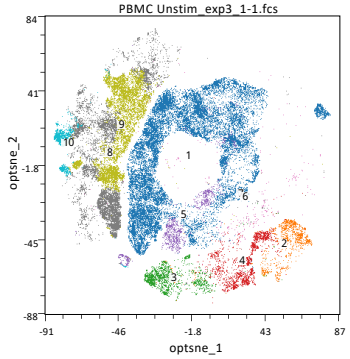


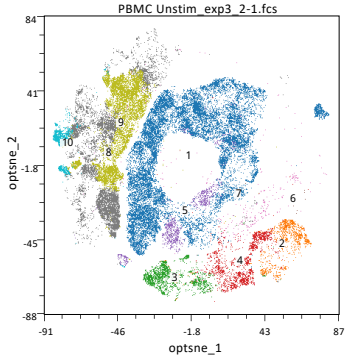
Library







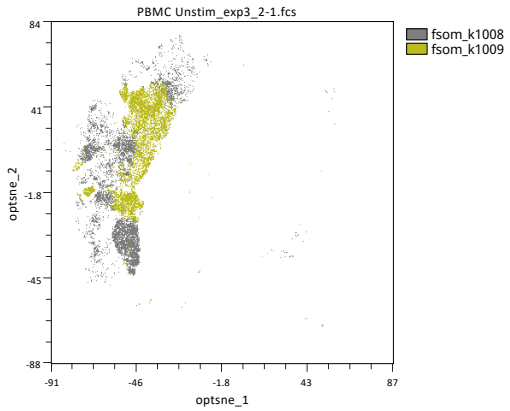




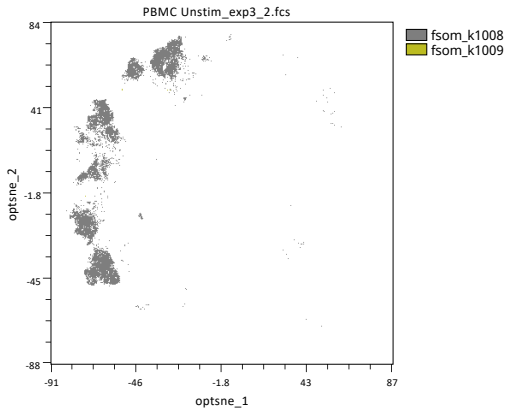
Experiment

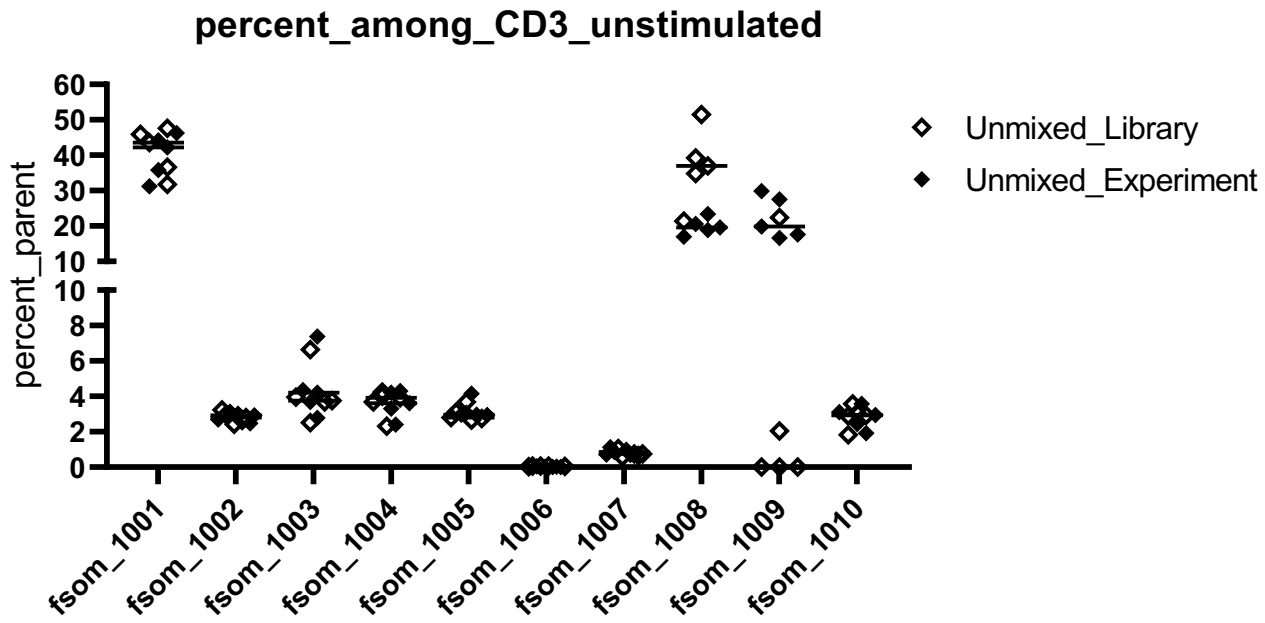
Library vs experiment reference controls unmixing analysis Unstimulated samples:

Experiment



Library





Normalization

Data normalization refers to the process of transforming variables to fit a limited range of values and create consistency between samples.

Normalization in OMIQ

Normalization

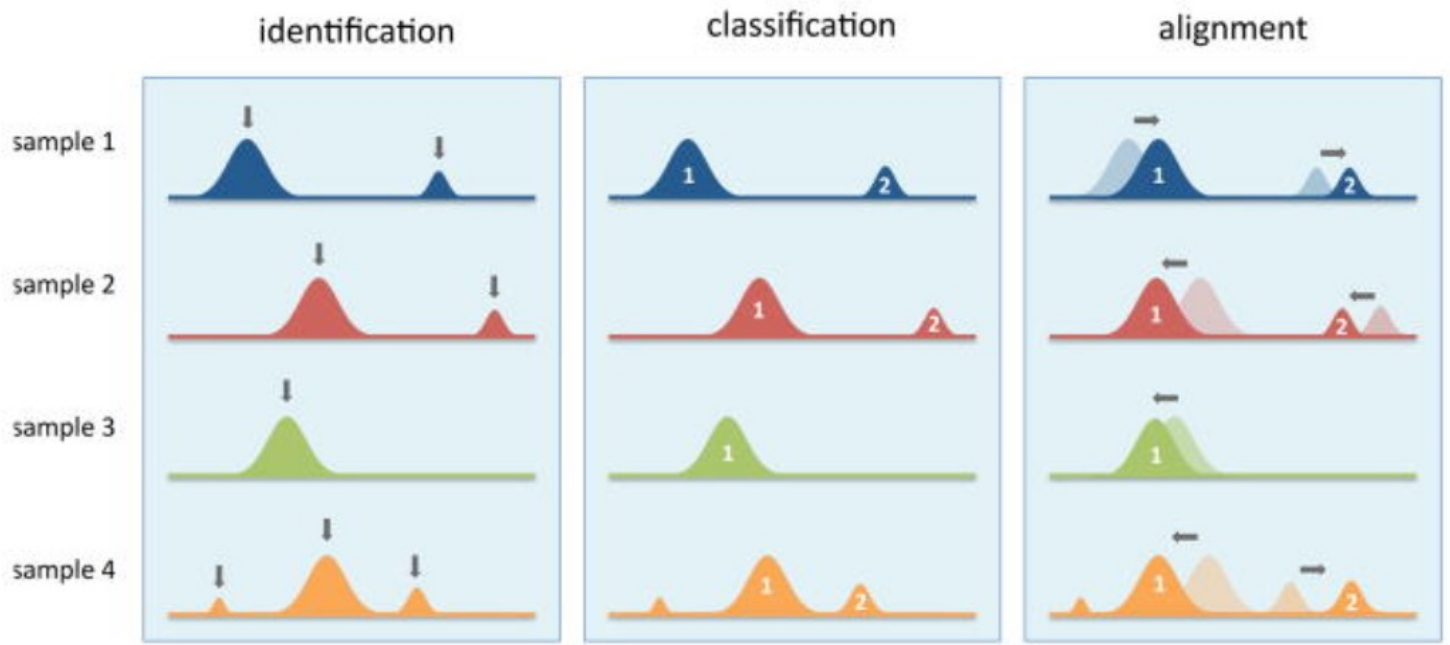
fdaNorm

gaussNorm

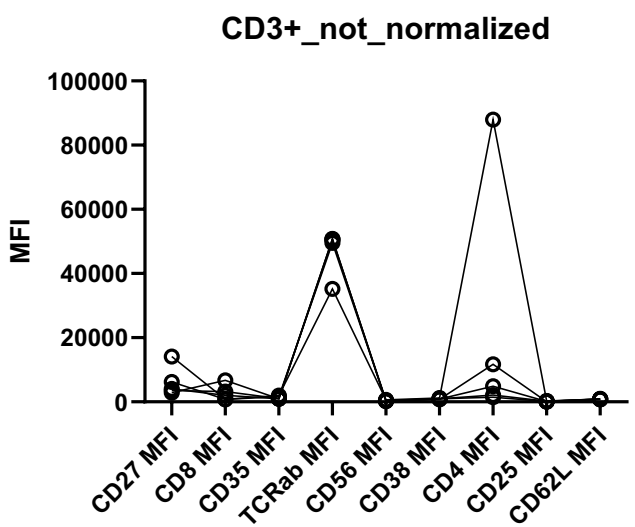
CytoNorm

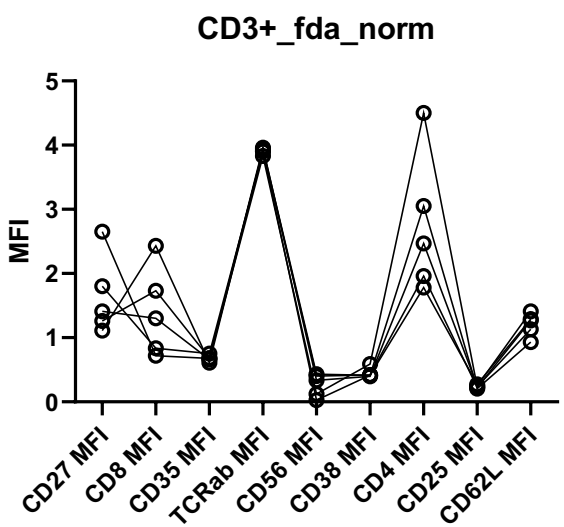
https://doi.org/10.1002%2Fcyto.a.20823

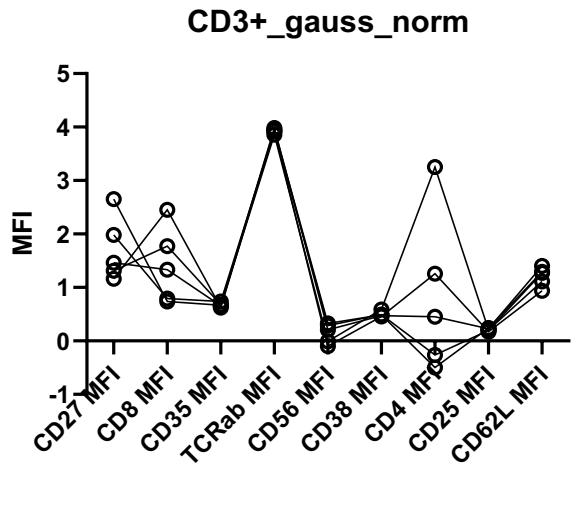
https://doi.org/10.1002%2Fcyto.a.23904

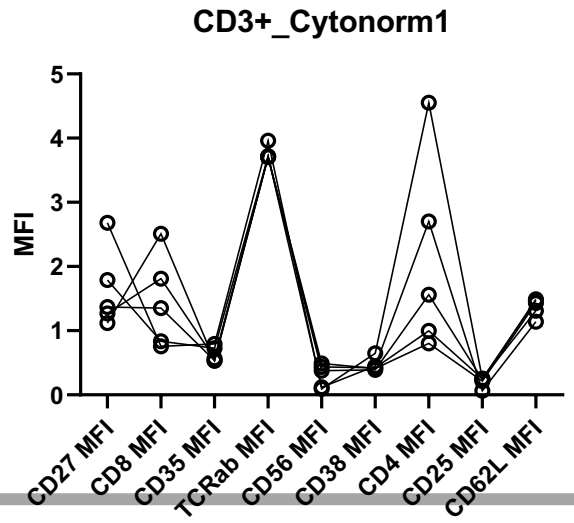


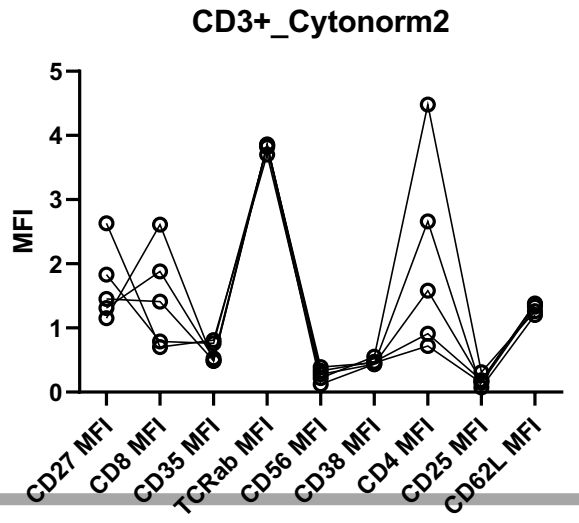
normalization



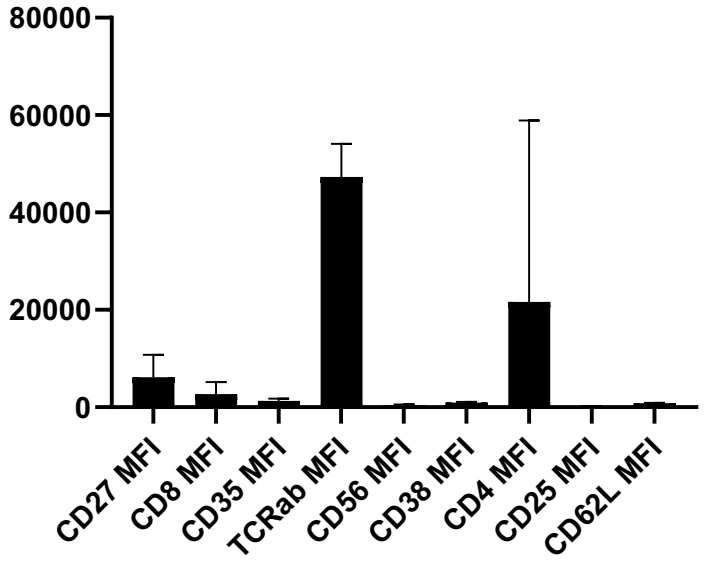


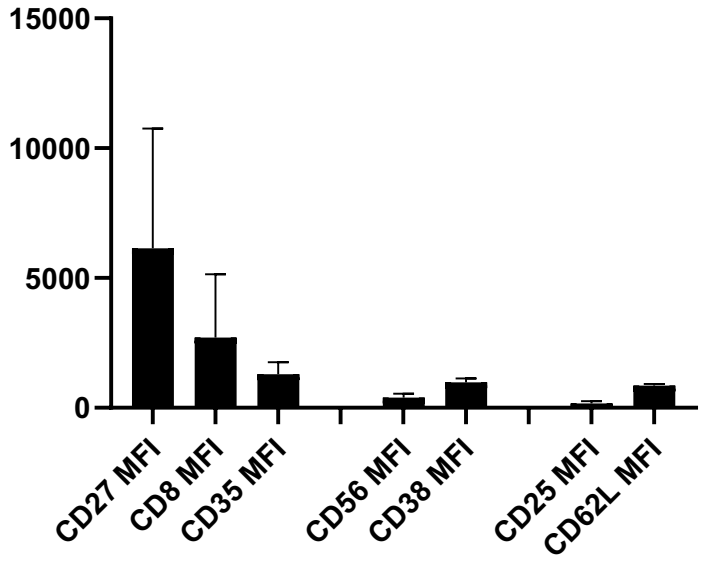






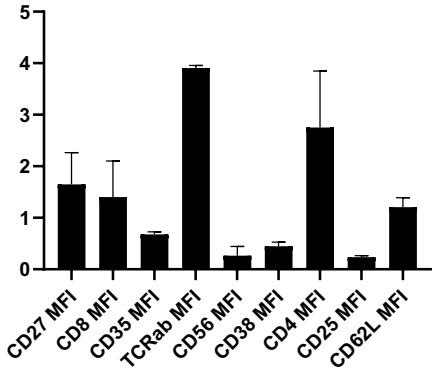
MFI_Unstimulated_not_normalize





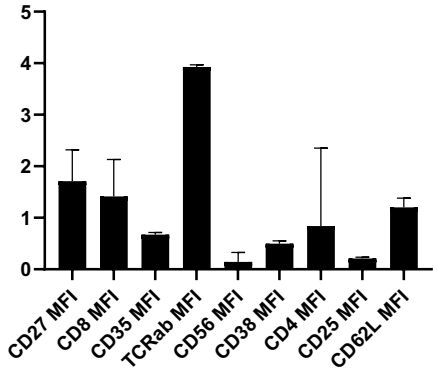
	Mean	%CV
CD27 MFI	6147.8	**74.9**
CD8 MFI	2705	**90.3**
CD35 MFI	1295	**35.8**
TCRab MFI	47323.6	**14.3**
CD56 MFI	390.6	**40.1**
CD38 MFI	980.4	**15.1**
CD4 MFI	21625.6	**172.4**
CD25 MFI	169.56	**52.0**
CD62L MFI	853.4	**7.8**

MFI_Unstimulated_fda_norm



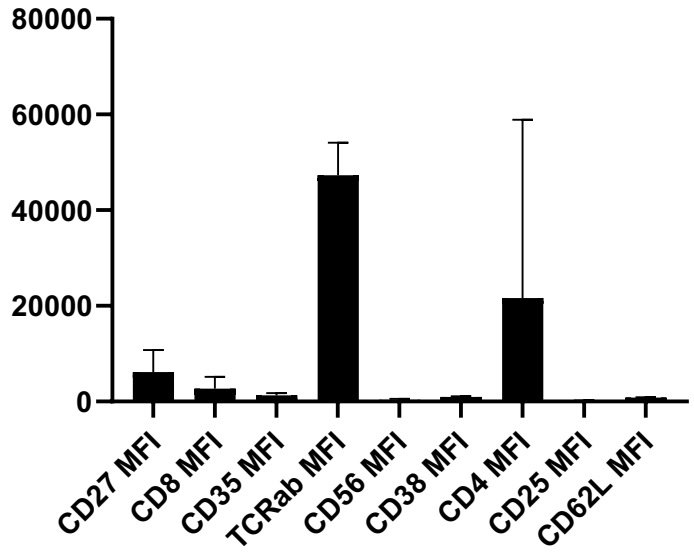
	Mean	%CV
CD27 MFI	1.646	**37.4**
CD8 MFI	1.4028	**49.9**
CD35 MFI	0.6758	**7.5**
TCRab MFI	3.908	**1.2**
CD56 MFI	0.26422	**67.7**
CD38 MFI	0.447	**17.8**
CD4 MFI	2.752	**39.7**
CD25 MFI	0.2368	**10.9**
CD62L MFI	1.2066	**15.1**

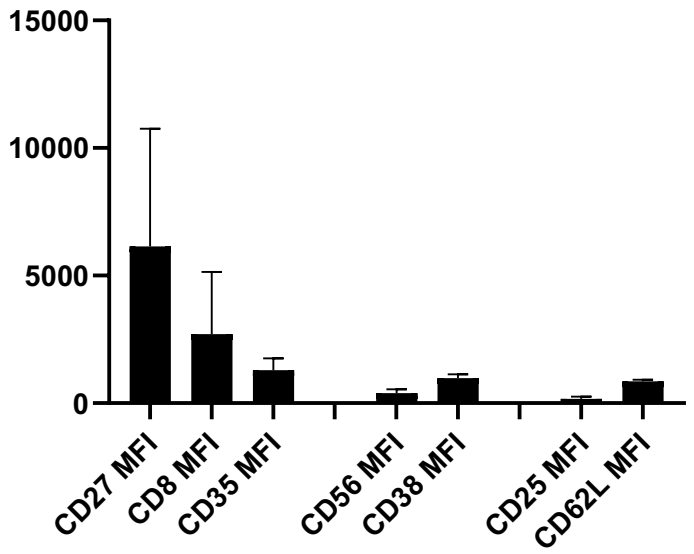
MFI_Unstimulated_gauss_norm



	Mean	%CV
CD27 MFI	1.712	**35.5**
CD8 MFI	1.416	**50.5**
CD35 MFI	0.6728	**6.4**
TCRab MFI	3.926	**1.1**
CD56 MFI	0.14238	**130.8**
CD38 MFI	0.4998	**10.4**
CD4 MFI	0.8396	**180.1**
CD25 MFI	0.2086	**12.7**
CD62L MFI	1.2014	**15.0**

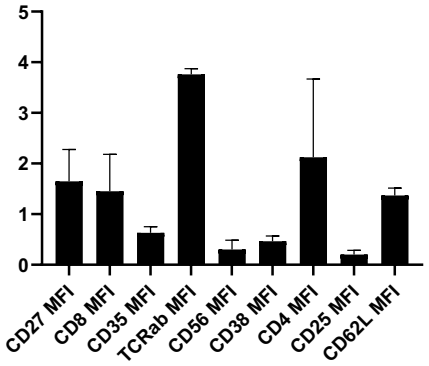
MFI_Unstimulated_not_normalize





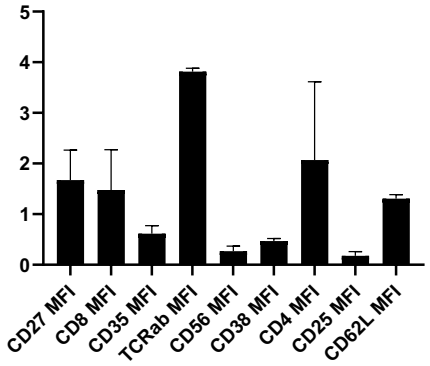
	Mean	%CV
CD27 MFI	6147.8	**74.9**
CD8 MFI	2705	**90.3**
CD35 MFI	1295	**35.8**
TCRab MFI	47323.6	**14.3**
CD56 MFI	390.6	**40.1**
CD38 MFI	980.4	**15.1**
CD4 MFI	21625.6	**172.4**
CD25 MFI	169.56	**52.0**
CD62L MFI	853.4	**7.8**

MFI_Unstimulated_Cytonorm1



	Mean	%CV
CD27 MFI	1.646	**38.2**
CD8 MFI	1.453	**50.0**
CD35 MFI	0.630	**19.3**
TCRab MFI	3.76	**2.9**
CD56 MFI	0.303	**60.7**
CD38 MFI	0.462	**22.7**
CD4 MFI	2.122	**72.8**
CD25 MFI	0.203	**39.1**
CD62L MFI	1.368	**10.6**

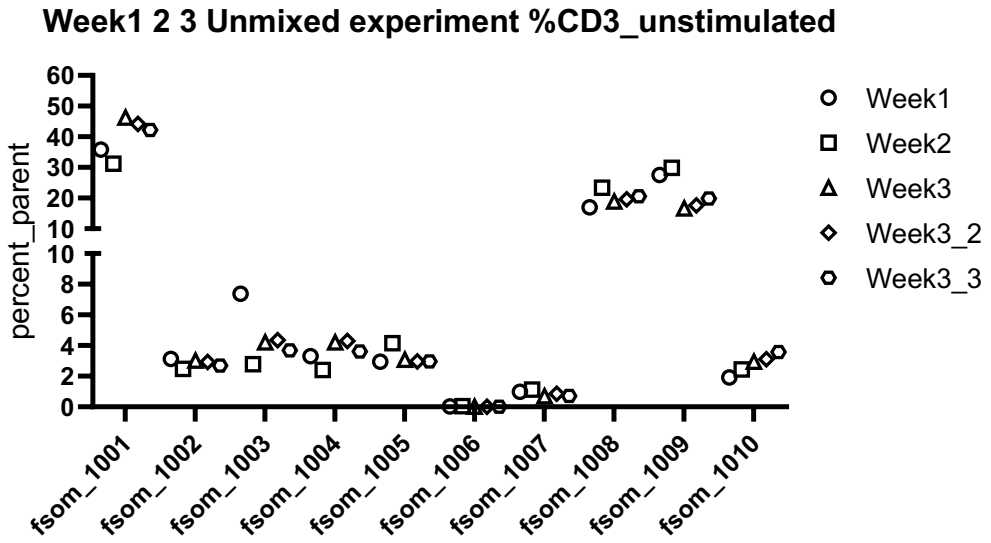
MFI_Unstimulated_Cytonorm2

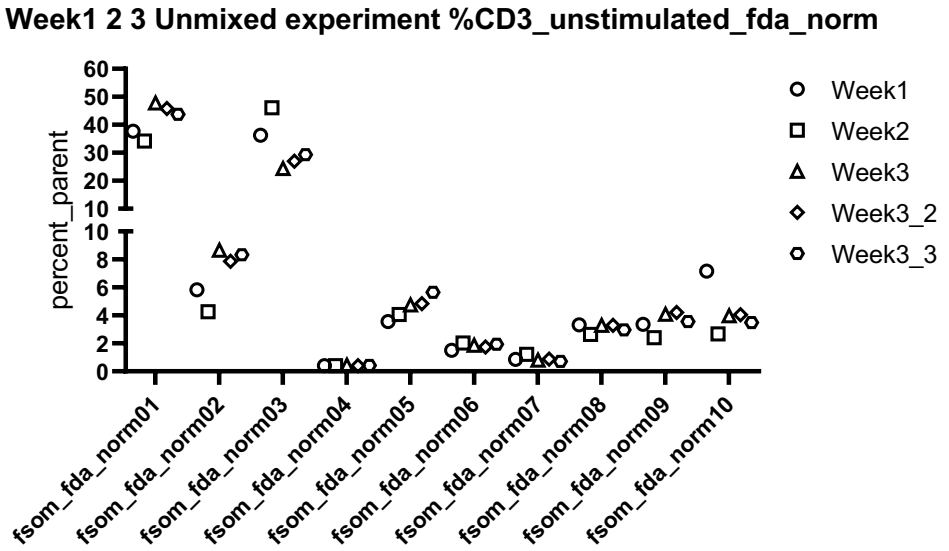


	Mean	%CV
CD27 MFI	1.674	**35.2**
CD8 MFI	1.4784	**53.7**
CD35 MFI	0.615	**25.7**
TCRab MFI	3.814	**1.7**
CD56 MFI	0.2668	**38.3**
CD38 MFI	0.4704	**10.2**
CD4 MFI	2.0702	**74.6**
CD25 MFI	0.1771	**48.1**
CD62L MFI	1.308	**5.8**

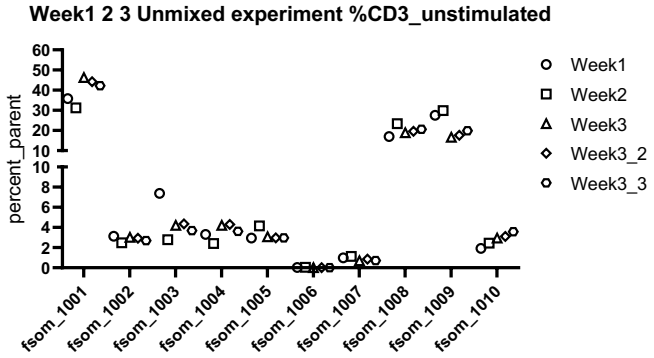
	not_normalized		fda_norm		gauss_norm		cytonorm1		cytonorm2	
	Mean	**%CV**	Mean	**%CV**	Mean	**%CV**	Mean	**%CV**	Mean	**%CV**
CD27 MFI	6147.8	**74.9**	1.646	**37.4**	1.712	**35.5**	1.646	**38.2**	1.674	**35.2**
CD8 MFI	2705	**90.3**	1.4028	**49.9**	1.416	**50.5**	1.453	**50**	1.4784	**53.7**
CD35 MFI	1295	**35.8**	0.6758	**7.5**	0.6728	**6.4**	0.63	**19.3**	0.615	**25.7**
TCRab MFI	47324	**14.3**	3.908	**1.2**	3.926	**1.1**	3.76	**2.9**	3.814	**1.7**
CD56 MFI	390.6	**40.1**	0.2642	**67.7**	0.1424	**130.8**	0.303	**60.7**	0.2668	**38.3**
CD38 MFI	980.4	**15.1**	0.447	**17.8**	0.4998	**10.4**	0.462	**22.7**	0.4704	**10.2**
CD4 MFI	21626	**172.4**	2.752	**39.7**	0.8396	**180.1**	2.122	**72.8**	2.0702	**74.6**
CD25 MFI	169.56	**52**	0.2368	**10.9**	0.2086	**12.7**	0.203	**39.1**	0.1771	**48.1**
CD62L MFI	853.4	**7.8**	1.2066	**15.1**	1.2014	**15**	1.368	**10.6**	1.308	**5.8**

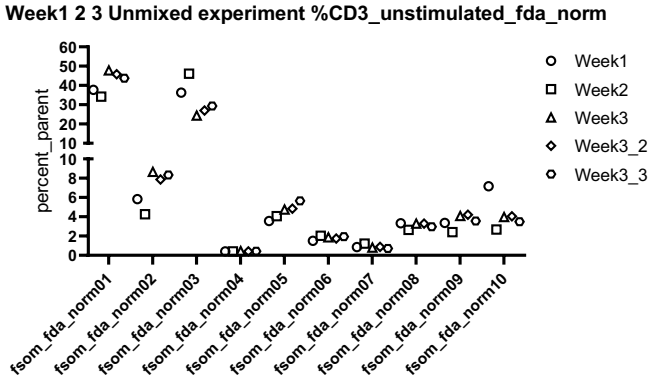
Normalization

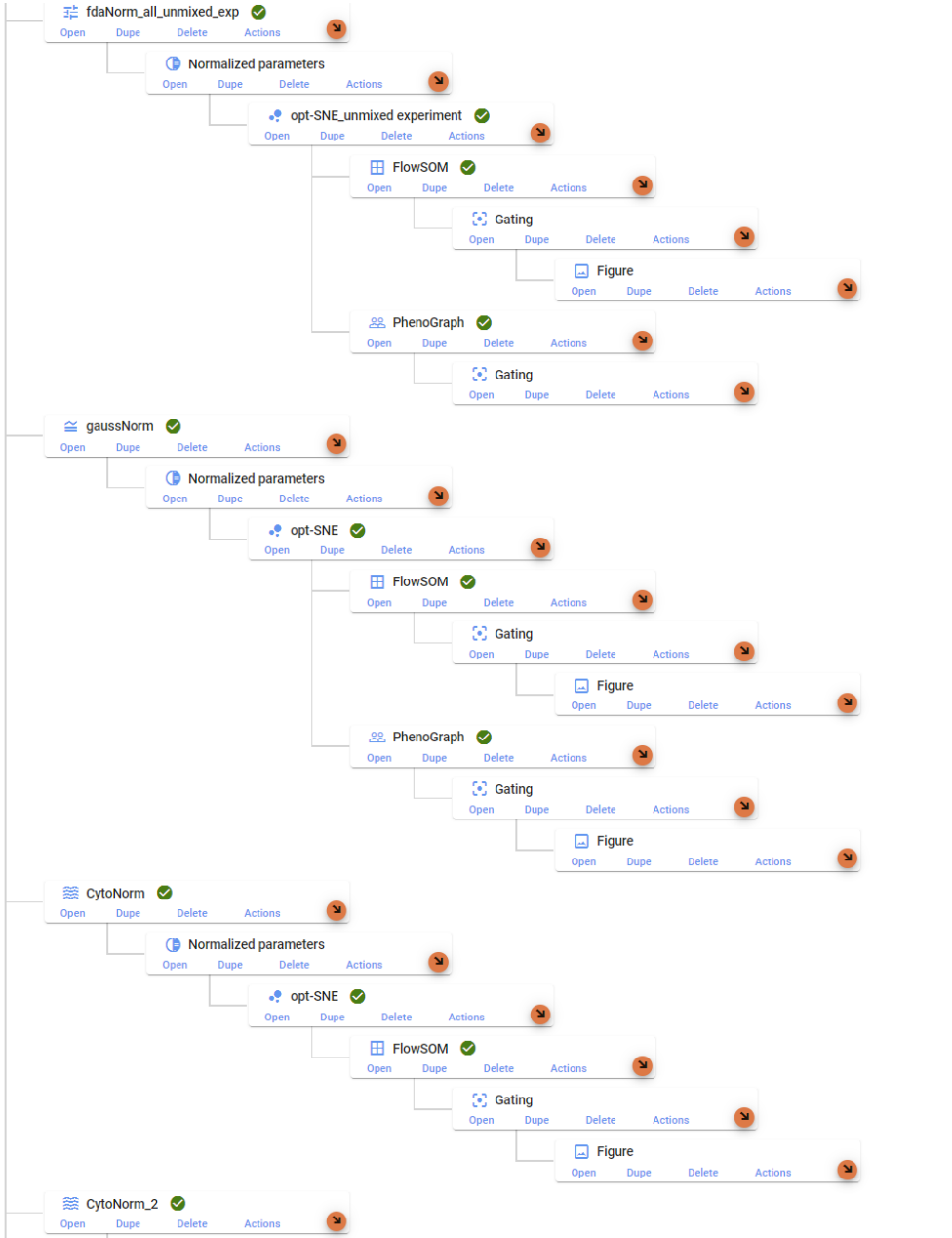




Normalization

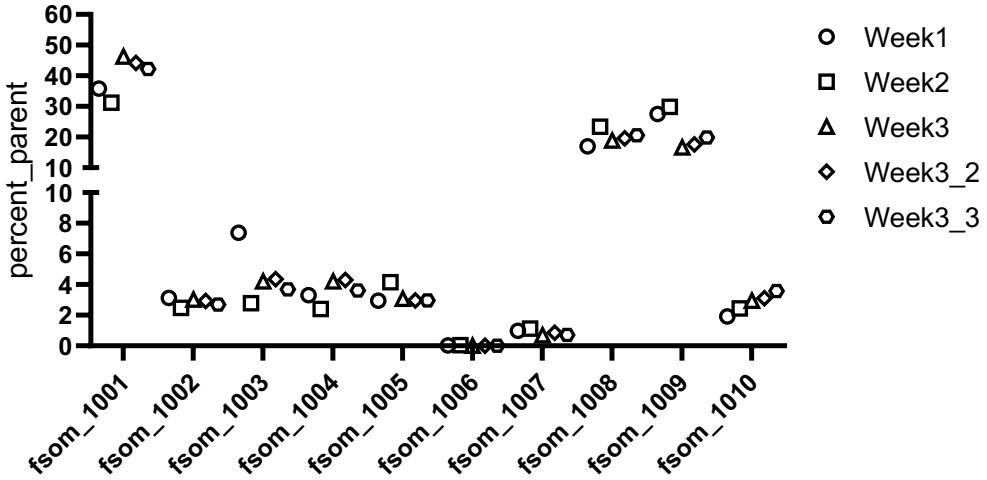




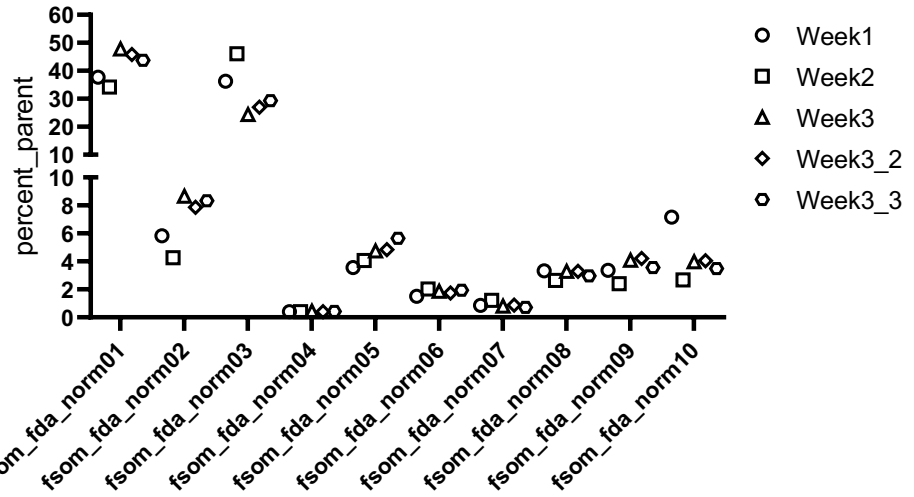


Normalization

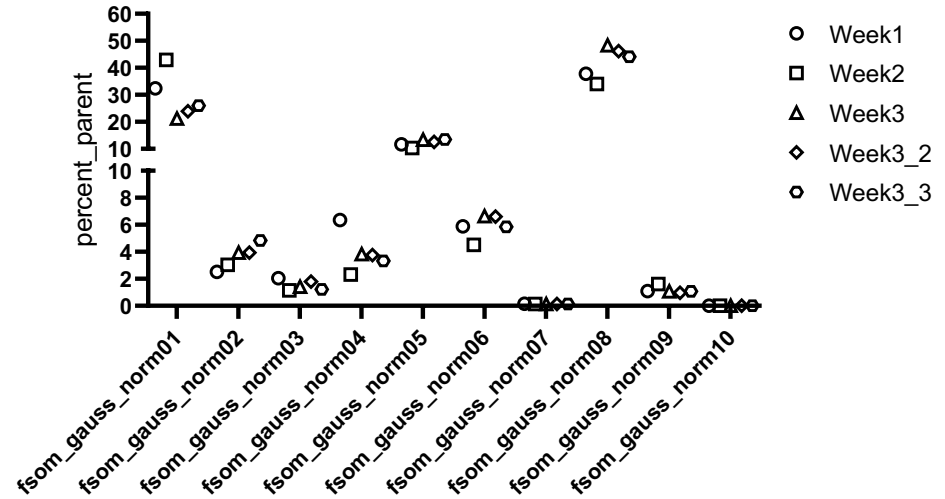
Week1 2 3 Unmixed experiment %CD3_unstimulated



Week1 2 3 Unmixed experiment %CD3_unstimulated_fda_norm



Week1 2 3 Unmixed experiment %CD3_unstimulated_guauss_norm



Week1 2 3 Unmixed experiment %CD3_unstimulated_CytoNorm2