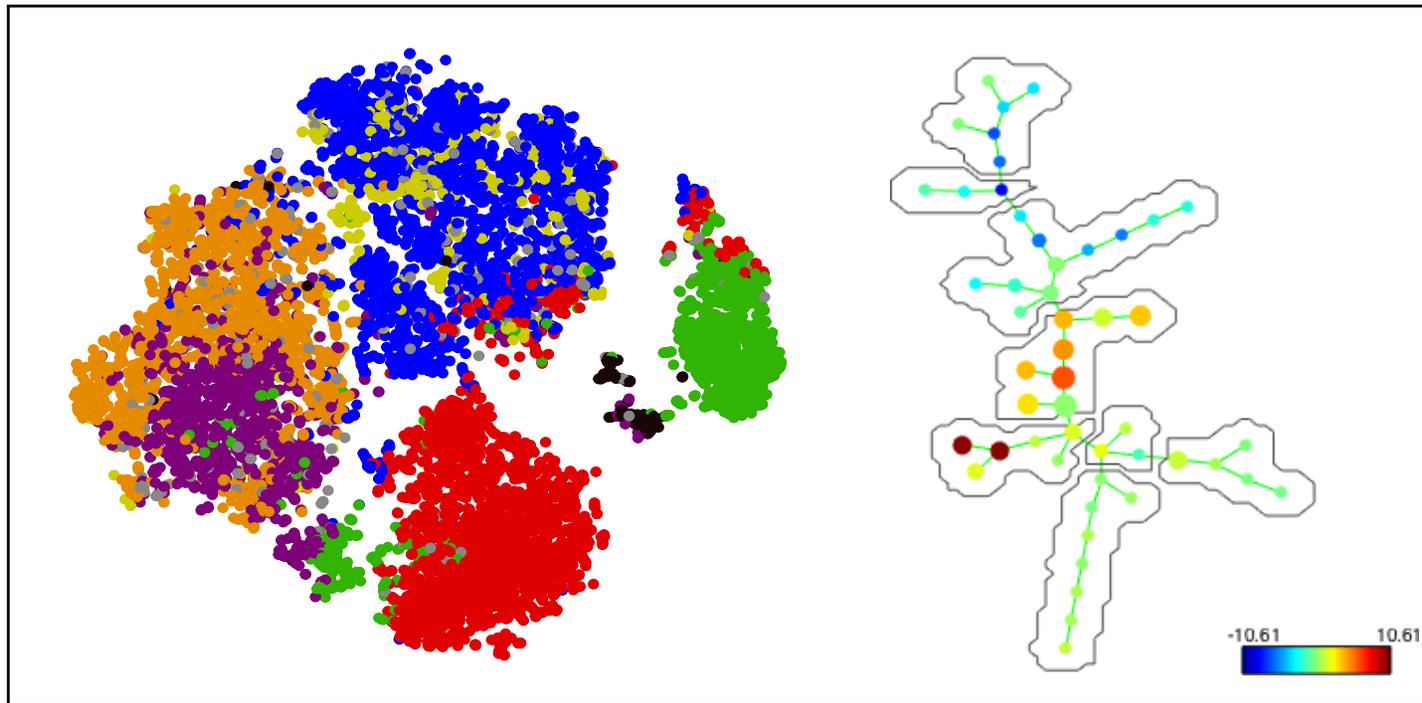
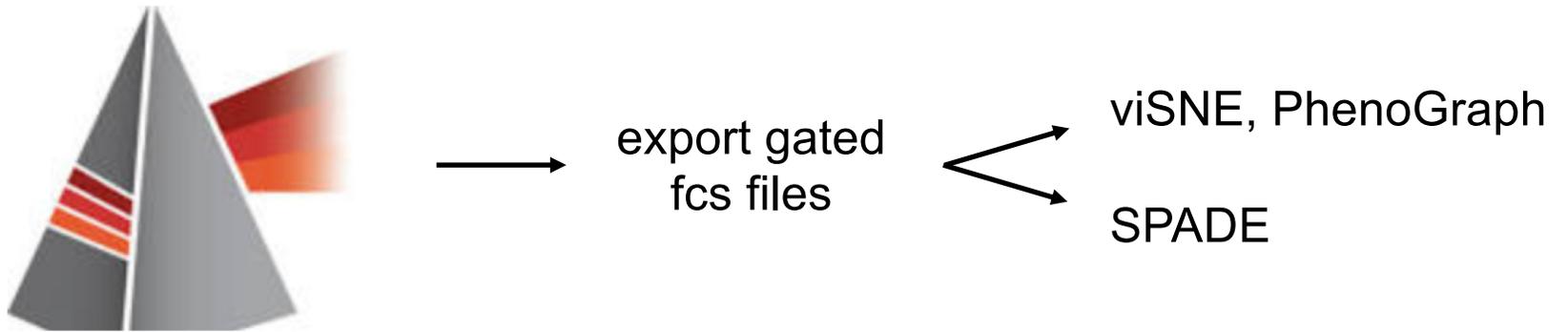


How to Prepare your FCS files for Algorithmic Analysis



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Basic Workflow



Gate on live singlets, population to cluster

Ex. Live single CD45+ cells

Ex. Live single CD19+ B cells

Ex. Live single CD4+Foxp3+ Tregs

Follow Along Using the "Raw" FCS files located in same PittBox as this ppt

- ▼  3_FCS files used in slides
 - ▼  1_Raw FCS files to gate in FlowJo
 -  new voltages_lib 1mg on+ FCS pt2-2_007.fcs
 -  PsO on stelara_01041801 experimental_002.fcs
 -  whitley_psor_Comp.mtx
 -  whitley.normal.Comp.mtx
 - ▼  2_Exported FCS files for import to algorithms
 -  export_normal_lib1mg_CD3_dump.fcs
 -  export_PsO_01041801_CD3_dump.fcs

Goal: Multidimensional profiling of human skin T cells

→gate on dump^{neg}CD3^{pos}

Two specimens

1. Normal skin “lib 1mg”
2. Psoriasis (PsO_stelara)

Two compensation matrices

1. Normal
2. Psoriasis



Antibody Panel:

CD4 BUV 395

CD8 BUV 737

CD3 FITC = population to cluster

TCRab APC (A647)

TCRgd PerCP/Cy5.5

CD45RO BV510

CTLA4/CD152 PE-TxRed

CD69 AF700

CD103 PE-Cy7

Foxp3 PE

Tbet BV605

RORgt BV421

Dump: live/dead, CD11c, CD19, CD14 APCCy7

Start in FlowJo

1. Import two FCS files:

- **Normal skin “lib 1mg”** (full file name new voltages_lib 1mg on+ FCS pt2-2_007)
- **Psoriasis** (full file name PsO on stelara_01041801 experimental_002)

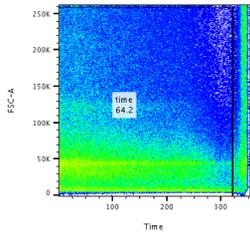
2. Apply appropriate compensation matrix to each fcs file

- **Normal** (full file name whitley.normal.Comp.mtx)
- **Psoriasis** (full file name whitley_psor_Comp.mtx)

Gate on live single CD3+ T cells

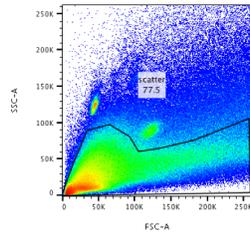
normal

time cleanup



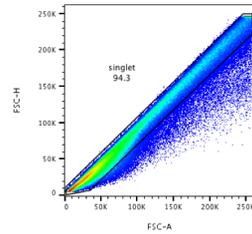
new voltages_lib 1mg on+ FCS pt2-2_007.fcs
Ungated
8.77E5

lymphocyte scatter



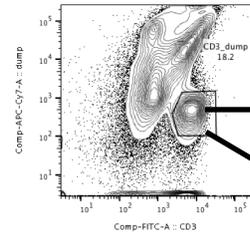
new voltages_lib 1mg on+ FCS pt2-2_007.fcs
time
5.63E5

singlet



new voltages_lib 1mg on+ FCS pt2-2_007.fcs
scatter
4.36E5

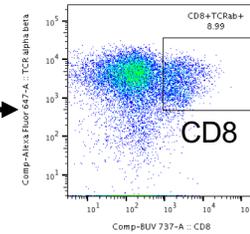
CD3 vs dump



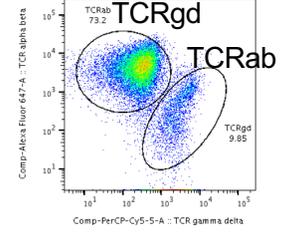
PsO on stelara_01041801 experimental_002.fcs
singlet
150175

export to algorithm

Just a little downstream gating for validation of known subsets

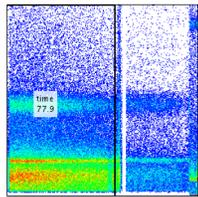


new voltages_lib 1mg on+ FCS pt2-2_007.fcs
CD3_dump
24744

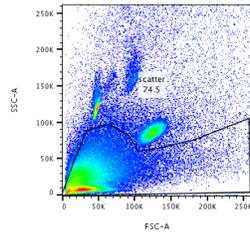


new voltages_lib 1mg on+ FCS pt2-2_007.fcs
CD3_dump
24744

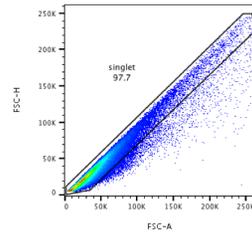
PsO



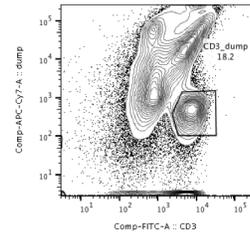
PsO on stelara_01041801 experimental_002.fcs
Ungated
264527



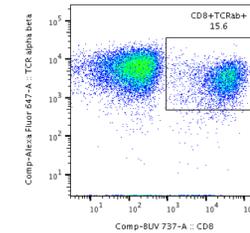
PsO on stelara_01041801 experimental_002.fcs
time
206198



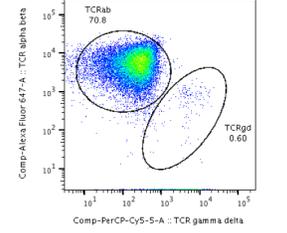
PsO on stelara_01041801 experimental_002.fcs
scatter
153711



PsO on stelara_01041801 experimental_002.fcs
singlet
150175



PsO on stelara_01041801 experimental_002.fcs
CD3_dump
27379



PsO on stelara_01041801 experimental_002.fcs
CD3_dump
27379

Export gated **population** that you want to cluster

The screenshot shows the FlowJo software interface. The 'Tools' menu is open, and the 'Export/Concatenate' option is circled in red with a '2.' annotation. Below the menu, a table displays gated populations. Three 'CD3_dump' entries are highlighted in red with a '1.' annotation.

Name	Statistic	#Cells
PsO on stelara_01041801 experimental_002.fcs		264527
time	77.9	206198
scatter	74.5	153711
singlet	97.7	150175
CD3_dump	18.2	27379
TCRab	15.6	4265
TCRgd	70.8	19397
TCRgd	0.60	164
new voltages_lib 1mg on+ FCS pt2-2_007.fcs		876893
time	64.2	563286
scatter	77.5	436479
singlet	94.3	411583
CD3_dump	6.01	24744
TCRab	8.99	2225
TCRgd	73.2	18111
TCRgd	9.85	2438
tinea pt 12191701 001.fcs		106534
time	93.5	99654
scatter	64.9	64645
singlet	97.3	62911
CD3_dump	5.33	3351
TCRab	16.6	556

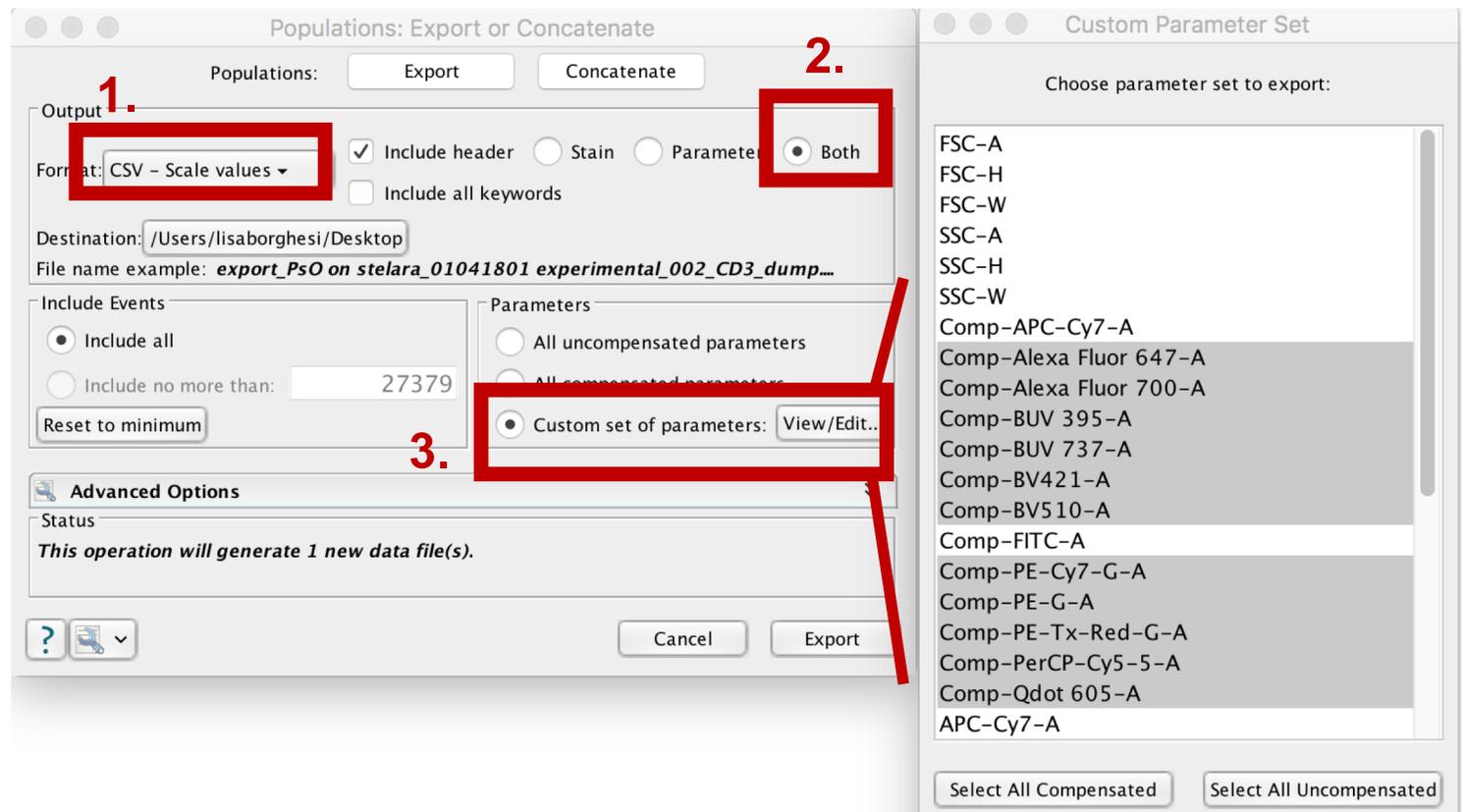
1. Highlight gates to export

2. Select Export → Export/Concatenate Populations

3. You'll get a pop-up window (next slide)

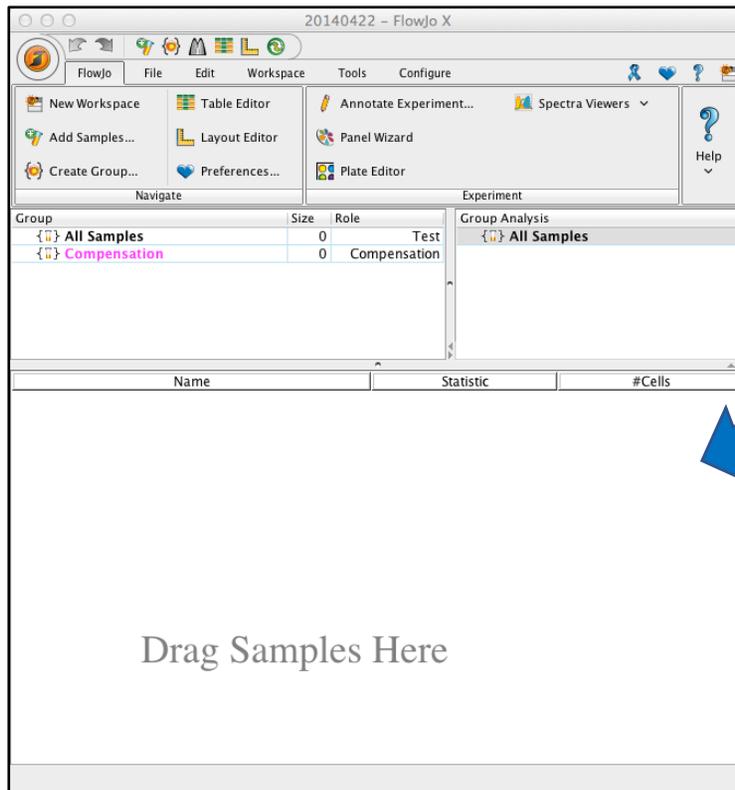
Export **custom parameters** that you want to cluster

1. Under Format choose “CSV – Scale values”
2. Select “both” stain & parameter
3. Select the compensated fluors you wish to export for clustering
 - leave behind: viability dye APC-Cy7 (you’ve already excluded dead cells)
 - leave behind: CD3 FITC (you’ve already gated on CD3)
 - leave behind: uncompensated parameters



Convert CSV → FCS

1. Open a new FlowJo workspace
2. Drop the CSV file onto the workspace
3. **A new FSC file will appear** in the same location where you saved the CSV file
 - conversion may take a minute or two so be patient



File.csv



File.fcs –
will appear in same
folder as your .csv

That's it!

Launch your favorite algorithm and import the cleaned-up FCS files

