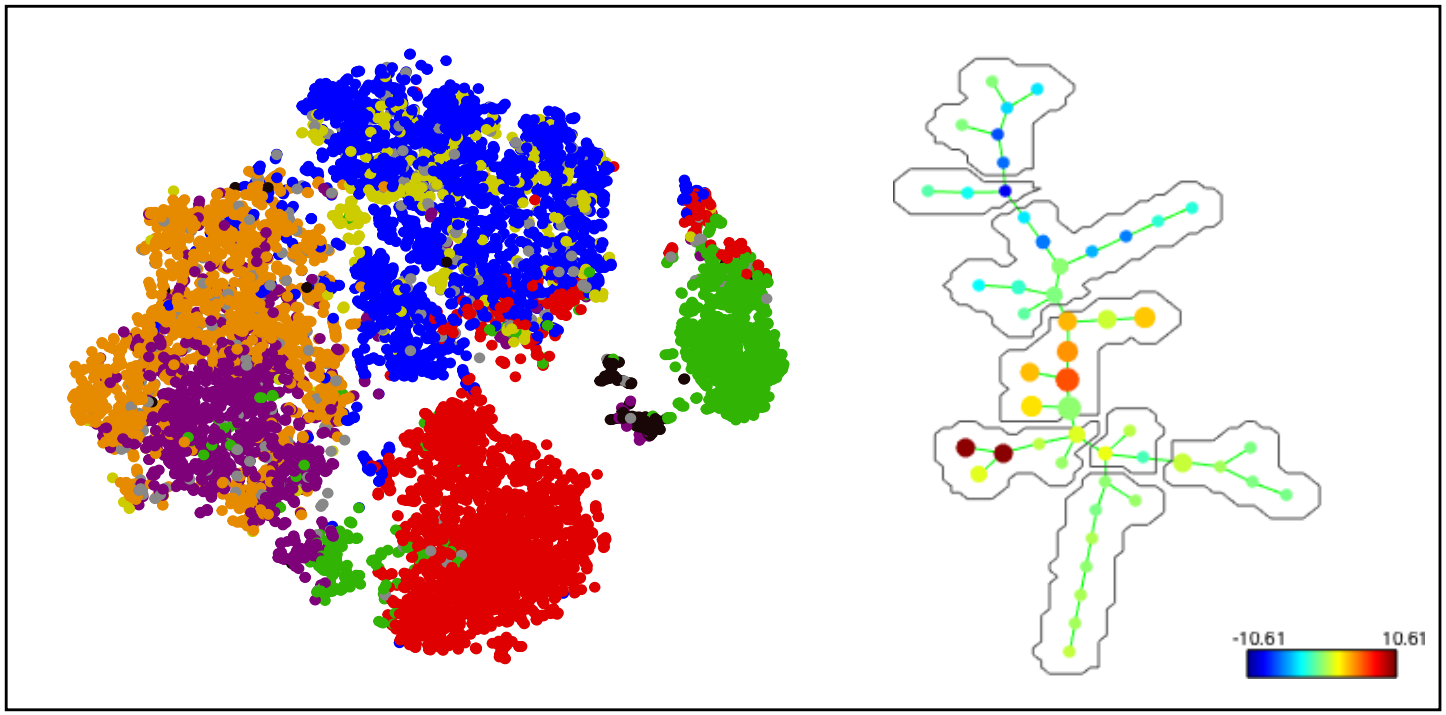
How to Prepare your FCS files for Algorithmic Analysis



Lisa Borghesi
Professor of Immunology
Director, Unified Flow Core

Basic Workflow

export gated fcs files

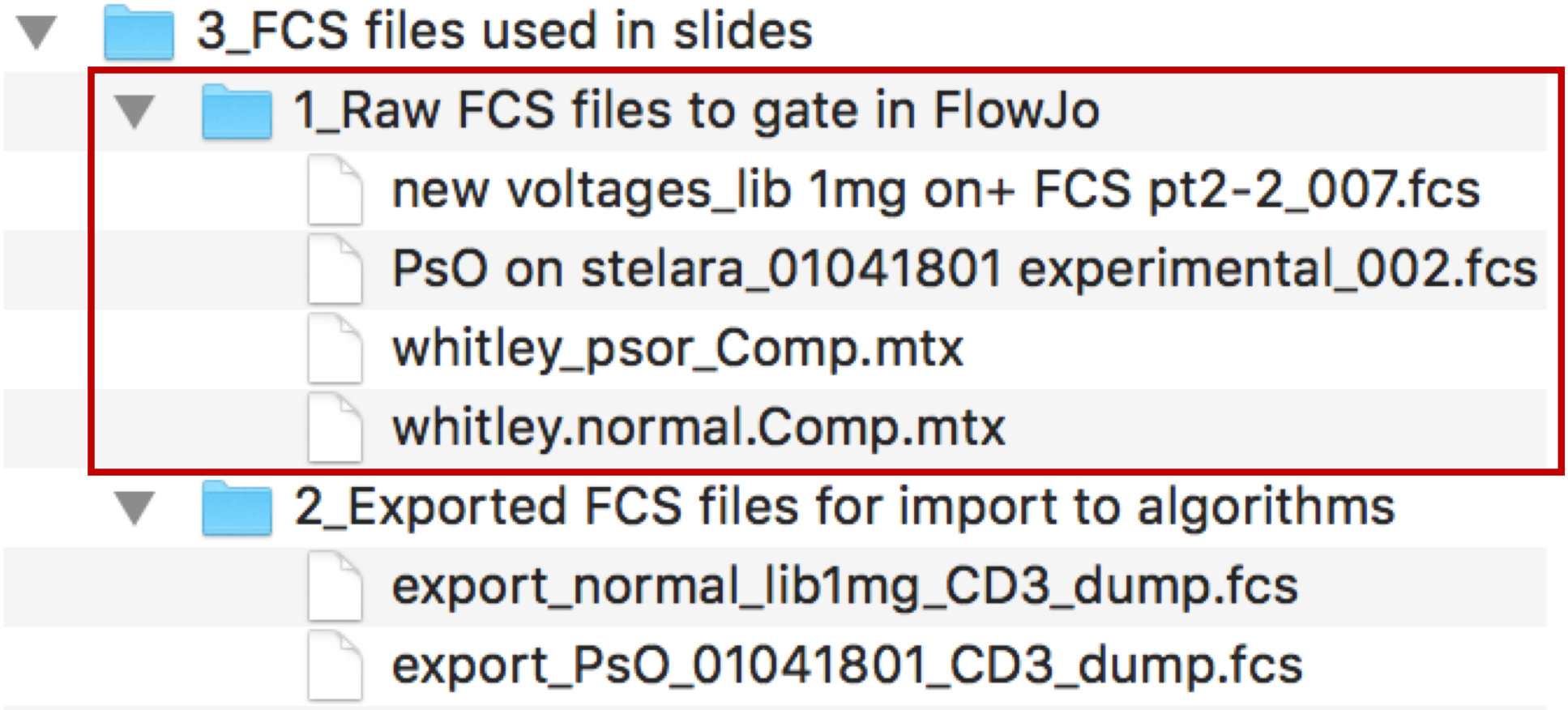
viSNE, PhenoGraph

SPADE

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



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

Courtesy: fcs data Sarah Whitely

Start in FlowJo

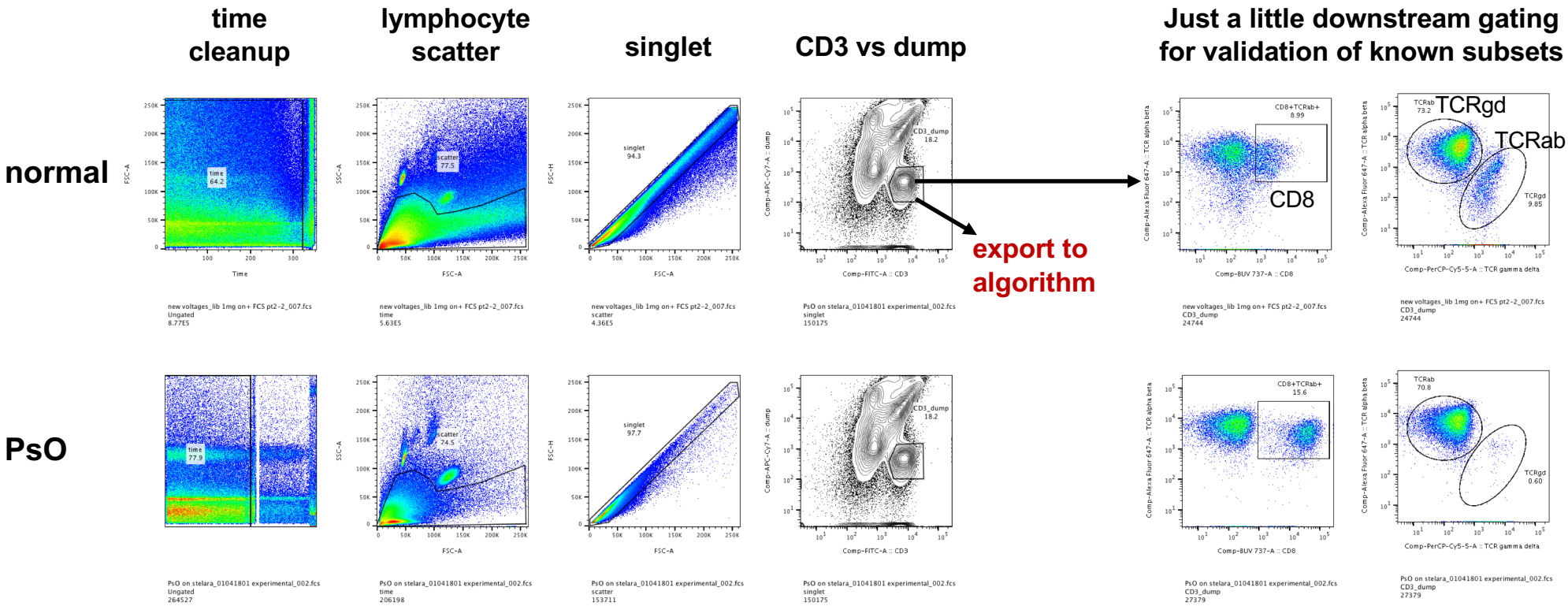
1. Import two FCS files:

- **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)

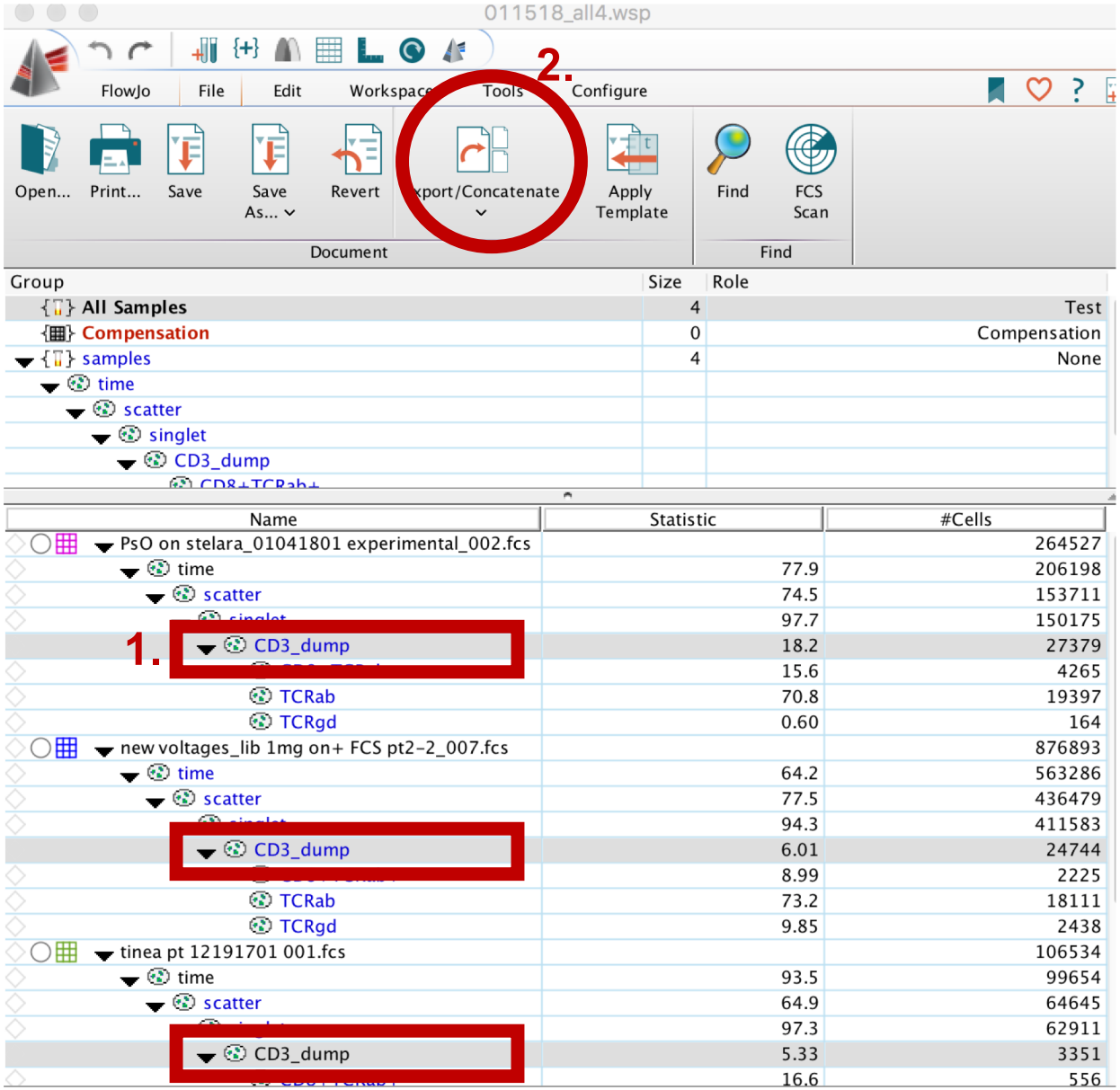
2. Apply appropriate compensation matrix to each fcs file

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

Gate on live single CD3+ T cells



Export gated population that you want to cluster



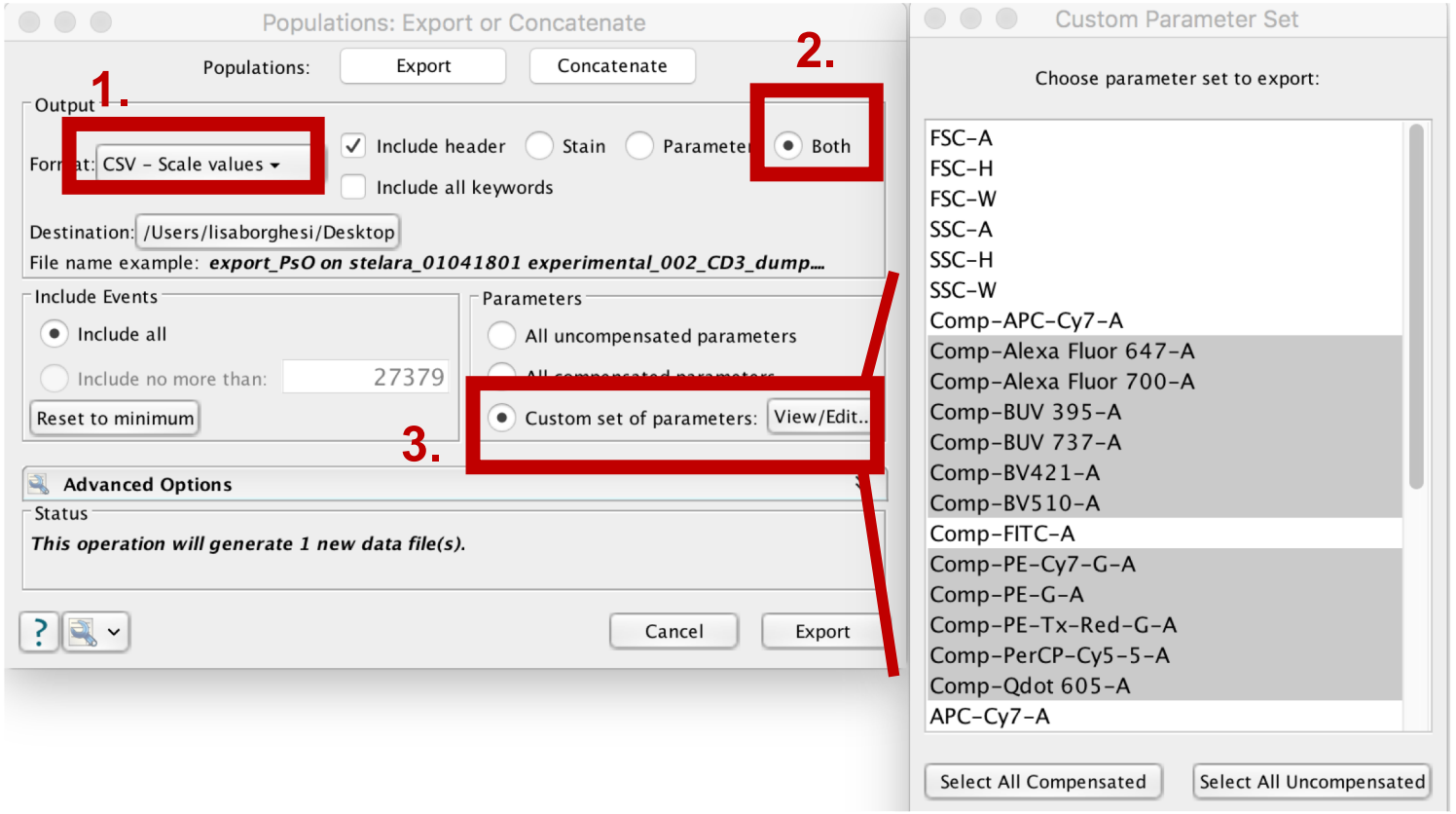
1. Highlight gates to export

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

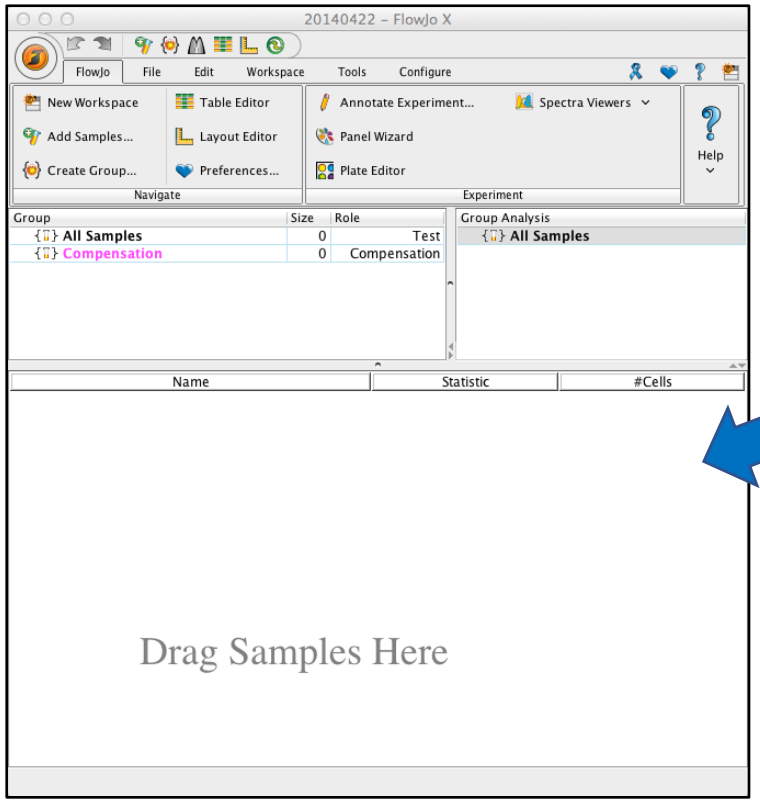


Convert CSV → FCS

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