viSNE & PhenoGraph Step-By-Step

Lisa Borghesi
Associate Professor of Immunology
Director, Unified Flow Core
Steps for viSNE analysis in Matlab/Cyt3

1. **Import** fcs data gated on the population you wish to cluster

2. **Transform**, arcsinh 150

3. **Downsample** (i.e., subsample a portion of total events)
   - to reduce computational burden
   - to select a small subset of events for a quick first-pass analysis
   - to normalize events across comparative analyses

4. Invoke **bh-SNE**
Launch Matlab/Cyt3

1. Launch Matlab
2. At the >> prompt type “cyt”
3. A new window should appear name "SightOf" at the top
Import pre-gated fcs files (live, singlet, gated on population to cluster) into Cyt3 “SightOf”

Use your fcs files or choose the “Exported FCS files” below. Files are located in the same PittBox as this ppt.
Import pre-gated fcs files (live, singlet, gated on population to cluster) into Cyt3 “SightOf”

Cyt3

1. Click the “+” sign to import fcs files
2. Navigate folder and select files
3. Select “Add”
4. Click “Done”
Pre-processing steps for viSNE analysis: Transform

1. Import fcs files
2. Select “navigator wheel” icon, and scroll to Transform
3. In popup window Select Channels to transform
4. Enter Cofactor 150 for flow cytometry (or 5 for mass cytometry)
5. Click “OK”
Why transformation value of 150 for fcs files?

• fcs data can have negative numbers due to compensation correction and instrument baseline correction. **Algorithms can’t handle negative numbers.**

• hyperbolic arcsine (arcsinh) transformation is similar to biexponential transformation in FlowJo. See [http://docs.flowjo.com/d2/graphs-and-gating/gw-transform-overview/](http://docs.flowjo.com/d2/graphs-and-gating/gw-transform-overview/)
Pre-processing steps for viSNE analysis: Downsampling

1. Select fcs files to downsample

2. Select “navigator wheel” icon, and scroll to “Subsample” (or “Subsample Each” if you have multiple samples to simultaneously downsample)

3. In popup window specify the number of events

4. Click “OK”. Then type a short prefix to be appended to sample name.

5. The new subsampled files you created should appear in the list
Invoke bh-SNE

1. Select the (transformed, downsampled) fcs files to cluster

2. Select fluors to include in clustering

3. Under “lower navigator wheel” select bh-SNE

4. Check original Matlab window (remember, you are currently in Cyt window) for algorithm progress

→Once algorithm has finished, the two new derived parameters will appear in lower pane: bh-SNE1 and bh-SNE2
5. Make sure the files you clustered are still selected.

6. Select the two new derived parameters bh-SNE1 and bh-SNE2

7. Select “Plot”
viSNE: normal vs PsO

- downsample Normal, PsO_stel to 24K/ea
- cluster all markers except for pre-gated channels (CD3, dump)
Marker heatmaps
L1 statistic – difference between marker distributions

- RORgt: diff: 2.03712
- CD69: diff: 1.98166
- CD4: diff: 1.72107
- CD103: diff: 1.51811
- TCR gamma delta: diff: -1.13759
- TCR alpha beta: diff: 0.945807
- CD45RO: diff: -0.855818
- Tbet: diff: 0.830009
- CD8: diff: 0.749534
- Foxp3: diff: 0.539077
Manual gating overlays

Gate on tSNE clusters

Gate on fluors

TCRgd

TCRab

CD8hi

CD8lo

CD4

foxp3

CD4+

TCRgd

CD8lo

foxp3+CD4+
Summary: Multidimensional profiling of human skin T cells
PhenoGraph

1. Make sure the files you clustered are still selected.

2. Select the two new derived parameters bh-SNE1 and bh-SNE2

3. Under navigation wheel select “PhenoGraph”
PhenoGraph – automated clustering
References

Reviews

Algorithms
UMAP
for MatLab savvy users who don’t need a GUI; available in MathWorks as Add On

PCA – preserves largest pairwise difference, loss of local structure

tSNE – dimensionality reduction algorithm that preserves local and global structure but NOT cluster distance