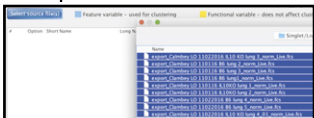


## A Dataset Importation:

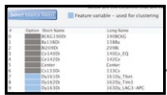
### 1. Sample selection:



Select all relevant FCS files pre-gated for live singlet events

Determine how many events from each FCS file will be imported

### 2. Importation settings:



### 3. Parameters selection:

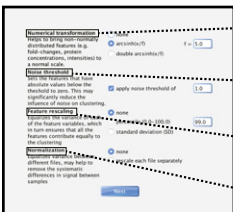
Select all relevant phenotypic markers for clustering

Before clustering the user should confirm that the size and dimensions of the dataset are appropriate

### 4. Successful dataset importation:



## B Clustering settings:



### 1. Transformation method:

Select "arcsinh (x/f)" with f= 5.0

### 2. Noise threshold:

Apply a noise threshold of 1.0

### 3. Feature rescaling:

Select "none"

### 4. Normalization:

Select "none"



### 5. Distance Measure:

Select "Angular Distance"

### 6. Clustering Algorithm

Select the "X-shift algorithm"

### 7. Density Estimate

Select the density estimation method "N nearest neighbors"

### 8. Define K values

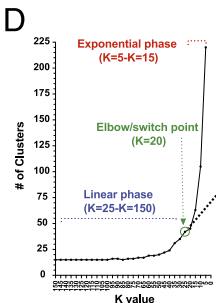
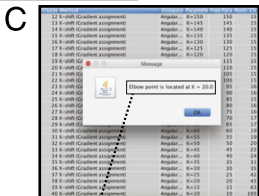
Determine what K values will be calculated, (e.g. K values from 5 to 150 at 30 step intervals)

### 9. Number of neighbors:

Select "determine automatically"

### 10. Begin clustering

Press "Go" to begin clustering calculations (progress of calculations appears in the box)



The elbow/switch point identifies the optimal number of cell clusters in the dataset, to minimize overfragmentation (exponential phase) or underclustering (linear phase).

### Elbow Point Location:

The results of the clustering calculations for all given K values can be used to determine an optimal K value, known as an "elbow point".

## E Force directed layout settings:

### 1. Events sampled:

The number of events from each cluster can be selected by the user

### 2. Proportional sampling:

The events can also be sampled proportionally to the cluster size

### 3. Distance measurement:

Choose "angular distance"

### 4. Num. nearest neighbors:

Use the automatic value here of 10



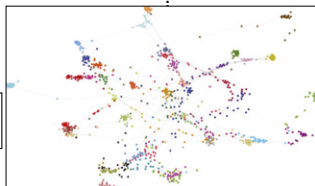
### 5. Edge settings:

The connections between nodes can be determined based on node density or by a specific parameter

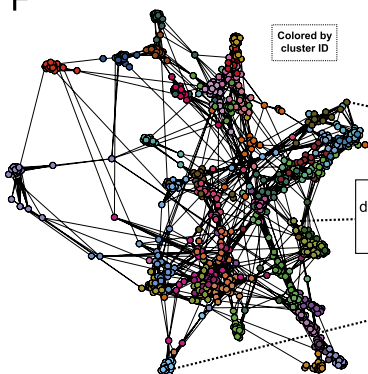
### 6. Sample selection:

A force directed layout can be created from one or more samples

The force directed layout generated in VorteX will look like this and can be colored by cluster ID or expression and can be visualized across individuals or experimental conditions.



## F



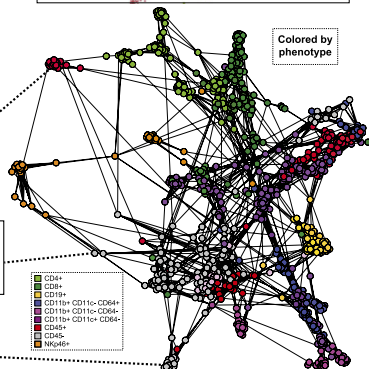
Colored by cluster ID

The force directed layout can be opened in the Gephi app to customize figure.

Force directed layout shows different cell clusters (phenotypes) spatially distributed based on phenotypic similarity.

Clusters can be identified by cluster ID or phenotype.

## G



Colored by phenotype

- CD4+
- CD8+
- CD19+
- CD11b+ CD11c+ CD68+
- CD11b+ CD11c+ CD68+
- CD11b+ CD11c+ CD68+
- CD45+
- CD45+
- MS6d6+