Walkthrough with SCC dataset

Load the Data

```
addpath('./data')
load('SCC_dataset.mat')
```

Process data and select features

Remove low-quality cells and select features:

- filter cells don't express min_exp percentage of genes
- select top #gene_selected informative genes

```
min_exp = 0.95; gene_selected = 3000;
[prodata,progene_name,procell_label] = preprocessing(data,gene_name,true_label,min_exp, ...
gene_selected);
```

Consensus clustering

If choosing SC3 package to do the consensus clustering on the processed data, we can get the following cell-cell similarity matrix M:

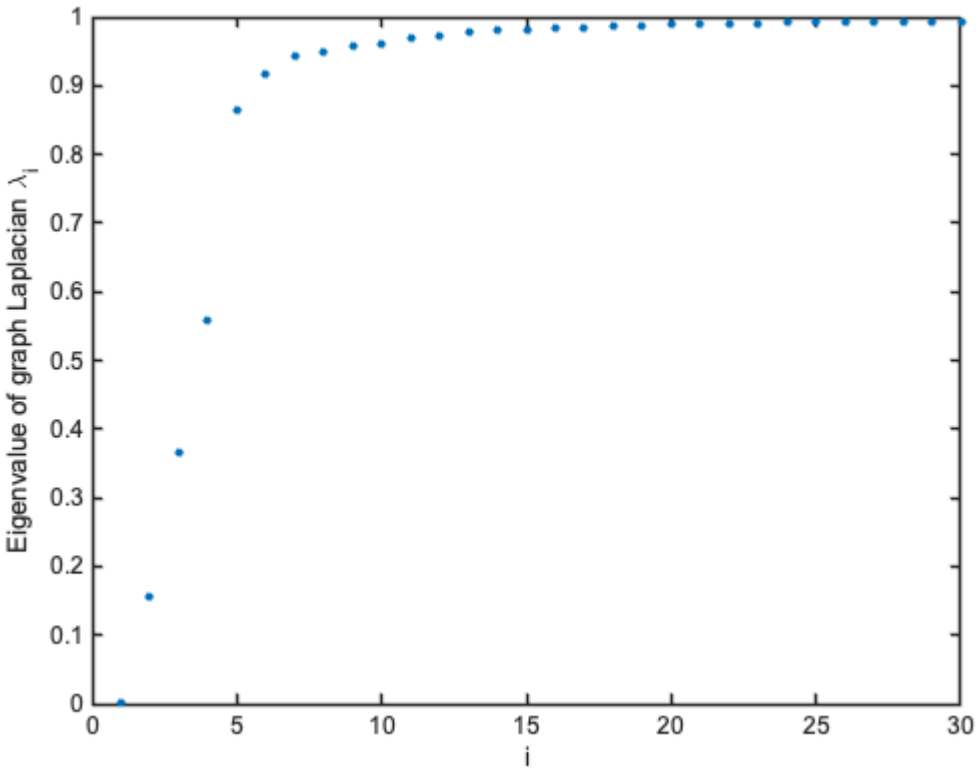
```
M = csvread('SCC_cell-cell.csv');
```

Number of clusters

The number of clusters is estimated by analyzing the largest gap of sorted eigenvalues of symmetric normalized graph Laplacian:

```
[eigenvalues] = plot_eigen_gap(M);
```

```
Number of cluster based on zero eigenvalues & Largest gap
     1     4
```




```
No_cluster = 4;
```

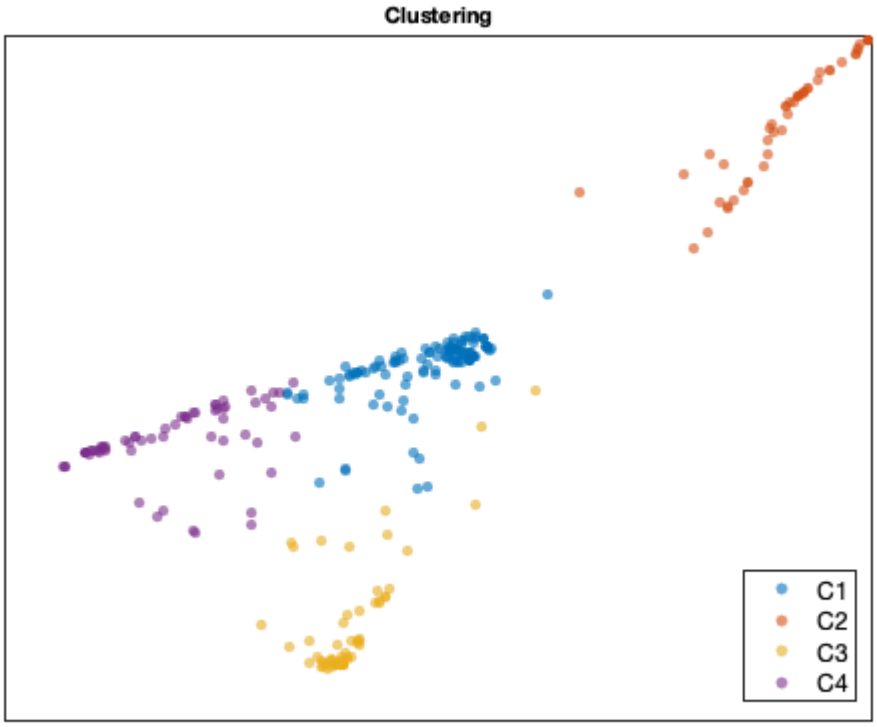
Run QuanTC

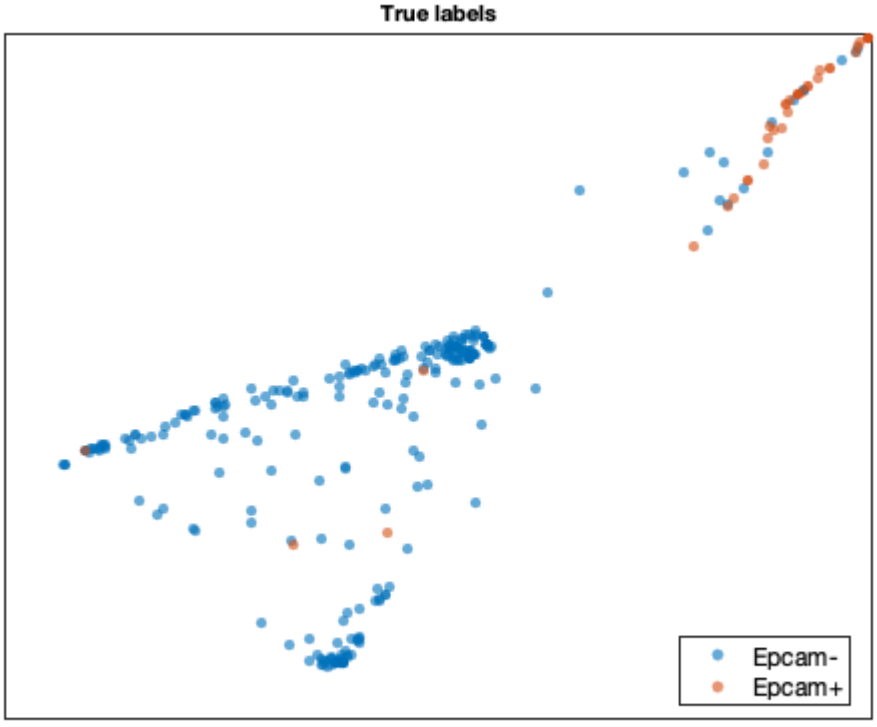
- **Soft clustering**: get the likelihoods of cells belonging to each cluster based on symmetric non-negative matrix factorization of M
- **CPI**: compute CPI value of each cell, use TC_cut to select cell with higher CPI values to be TC

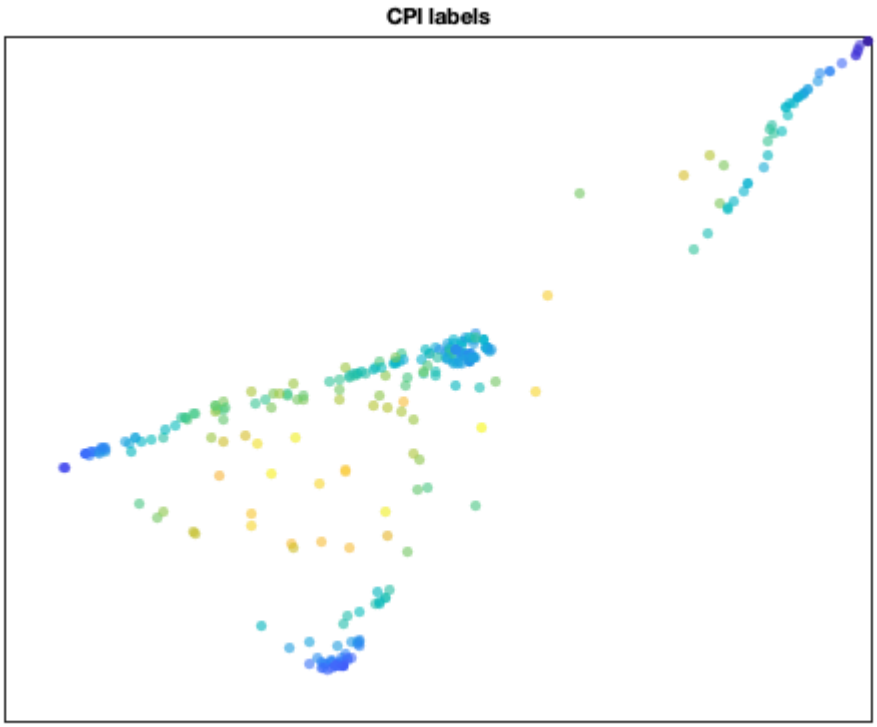
```
TC_cut = 0.34;
[result] = run_QuanTC(prodata,M,No_cluster,TC_cut);
```

- **2d-visualization**: cells are visualized through the probabilistic regularized embedding (PRE) approach

```
label_legend =  {'Epcam-','Epcam+'};
cell_visu_PRE(result,procell_label, label_legend)
```


Clustering
C1
C2
C3
C4


True labels
Epcam-
Epcam+



Feature plot

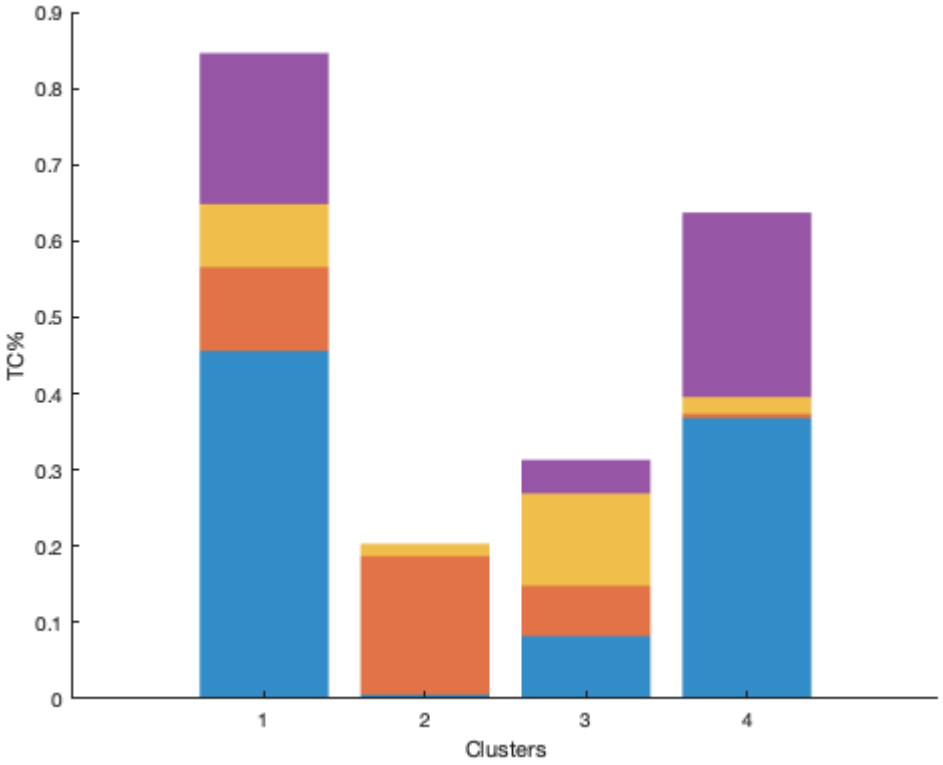
visualizes feature expressions on PRE

```
gene_plot = {'Epcam'};
featureplot(result,prodata,progene_name,gene_plot)
```

Transition trajectory

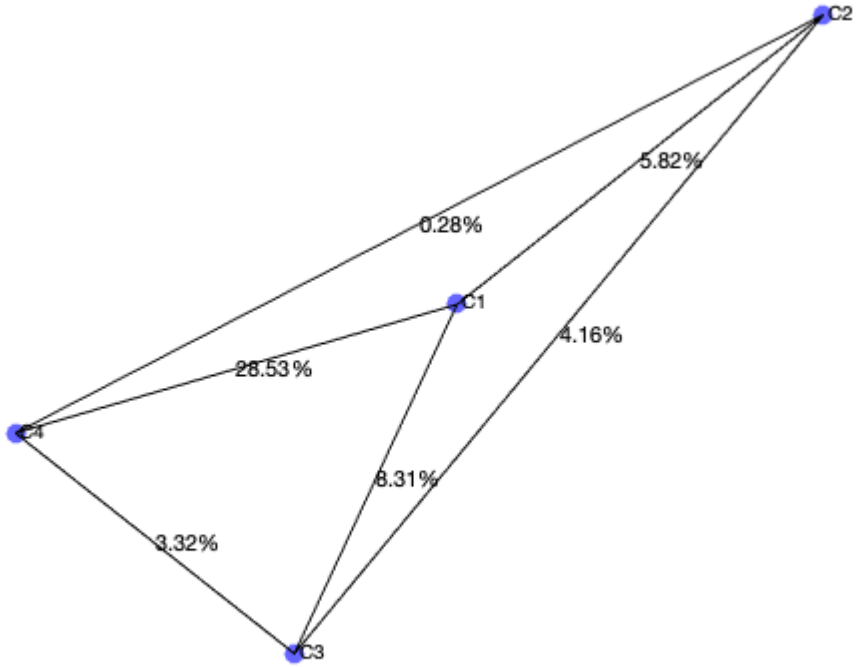
- **Start/ end cluster**: choose non-ICS based on the percentage of TC associated with each cluster relative to the total number of TC

```
pTC(result,No_cluster)
```



- **Potential transition trajectories**: list the percetage of cells over whole cell population to choose the potential transition trajectories

```
start_cluster = 2; %choose starting cluster based on the above graph
%plot TC% between clusters among all the cells
[path,ordered_cell] = traj(result,start_cluster,No_cluster);
```




```
trajectory: 2  1  3  4, percentage of cells involved: 0.50693
trajectory: 2  1  4, percentage of cells involved: 0.65651
trajectory: 2  3  1  4, percentage of cells involved: 0.84211
trajectory: 2  3  4, percentage of cells involved: 0.26039
trajectory: 2  4, percentage of cells involved: 0.1385
```

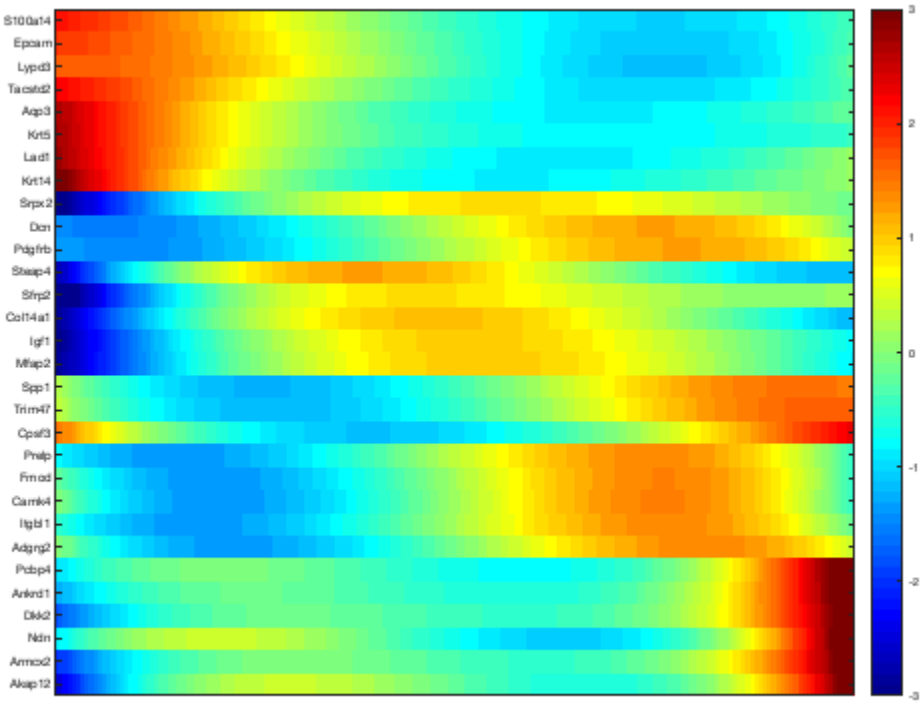
Finding cluster marker genes and the transition genes that mark transition from one trajectory

```
lam2 = 10;
[marker_gene,transition_gene] = markers(result,prodata,M,path{1},ordered_cell{1},lam2)
```

```
ans = 0.9783
Consistency of clustering: 0.97835
marker_gene = 4×1 cell array
    {  75×1 double}
    { 260×1 double}
    { 936×1 double}
    {1046×1 double}
transition_gene = 3×2 cell array
    {7×1 double}    {79×1 double}
    {4×1 double}    { 0×1 double}
    {[    2263]}    { 0×1 double}
```

Heatmap

```
a = 5; b = 3; %plot top a marker genes and top 2b transition genes
[gene_plot,gene_cluster] = heatmap(prodata,marker_gene,transition_gene,path{1}, ...
ordered_cell{1},progene_name,a,b);
```



Plot transition genes along the transiton trajectory

```
trans_gene_plot = transition_gene{1,1}(1);
tran_gene_plot(result,prodata,trans_gene_plot,path{1},ordered_cell{1},progene_name)
```