A quick guide to JSNMF algorithm with mouse brain (RNA+H3K4me3, Paired-Tag) data

Load data, including single-cell transcriptome and epigenomic proiles.

Data preprocessing using R package "Seurat 4.0", preprocessed data was used in the analysis below. We provide script "preprocessing.R" to conduct data preprocess, normalization and high variable gene selection steps in R environment

```
addpath('../data')
load('kidney_5k_10k.mat')
load('housekeeping_genes.mat')
load('Kidney_sciCAR_data.mat','RNA')
```

Preparing for computing RAGI

```
[~,ix1,ix2]=intersect(share_bd,RNA.Cells,'stable');
genes = RNA.Features; data = RNA.data;
data = data(:,ix2);
sM = sum(data,2); zero_row = find(sM==0);
data(zero_row,:) = []; genes(zero_row) = [];
data = data./repmat(sum(data),size(data,1),1)*10000;
data = log(data+1);


label_name = label;
num_clu = length(unique(label_name));
tag = unique(label_name); tag = cellstr(tag);
[~, label] = ismember(label_name,tag);
```

marker genes are given by the original publication; house-keeping genes are provided in the website "http://www.housekeeping.unicamp.br/"

```
marker_genes =
["Slc12a3";'Trpm6';'Abca13';'Klhl3';'Wnk1';'Tsc22d1';'Cadps2';'Egfem1';'Calb1';'Kl';'Temem72';'Dach1';'Ptprm';'Sgms2';'Tox3';'Frmpd4';'Rbms3';'Fxyd4';'Dnm3';'Cacnb2';'Pde1c';'Pde8b'];
[~, ~, ind1] = intersect(marker_genes, genes,'stable');
[~, ~, ind2] = intersect(hp_genes, genes,'stable');
data = sparse(data);
clear ix1 ix2 sM zero_row tag share_bd RNA peaks marker_genes hp_genes genes
```

Parameter selection

```
addpath('../codes')
addpath('../external')
[alpha, gamma, Inits] = parameter_selection(X1, X2, label);


disp(['value of alpha:',num2str(alpha)]);
disp(['value of gamma:',num2str(gamma)]);
```

```
value of alpha:0.31629
value of gamma:3287.3936
```

Start time

```
tic
[W1,W2,H1,H2,S,iter,objs] = jsnmf(X1,X2,alpha,gamma,Inits);
disp('JSNMF runtime:');
toc
```

```
iteration starts!
number of iteration:10  obj:3730831.8015
number of iteration:20  obj:3720740.5389
number of iteration:30  obj:3717563.2642
number of iteration:40  obj:3715983.1244
number of iteration:50  obj:3715001.5325
number of iteration:60  obj:3714346.0563
number of iteration:70  obj:3713900.8529
converged!

JSNMF runtime:
Elapsed time is 157.382742 seconds.
```

Evaluating performance

```
% KNN on S, k is insensitive to the performance, here we suggested k=50
A = Wtrim(S,50);
% implement louvain clustering
[clust,~,~] = getNCluster(A,num_clu,0,3,20);
if length(unique(clust))== num_clu
   [ac, nmi_value, ~] = CalcMetrics(label, clust);
   [ave_mk_gini, ave_hk_gini, difgini] = RAGI(data,ind1,ind2,clust);
else
% using spectral clustering instead if number of clusering doesn't equals to the pointed number
   [~, clust, ~] = SpectralClustering(A, num_clu);
   [ac, nmi_value, ~] = CalcMetrics(label, clust);
   [ave_mk_gini, ave_hk_gini, difgini] = RAGI(data,ind1,ind2,clust);
```

```
end
```

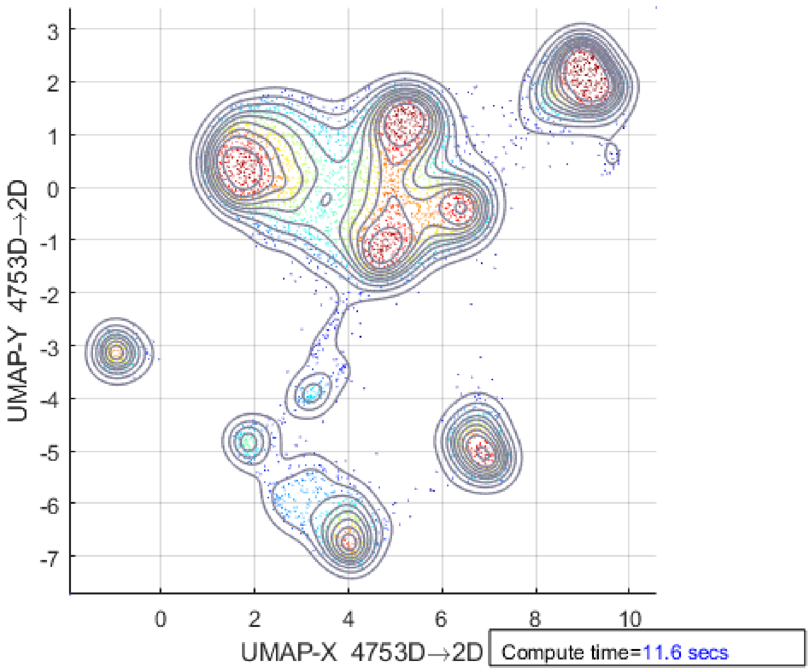
```
ac: 0.6093     1857/4753      nmi:0.5430
ave_marker_gene: 0.5968       ave_hk_gini:0.1555     diff_gini:0.4413
```

Downstream analysis including visualization

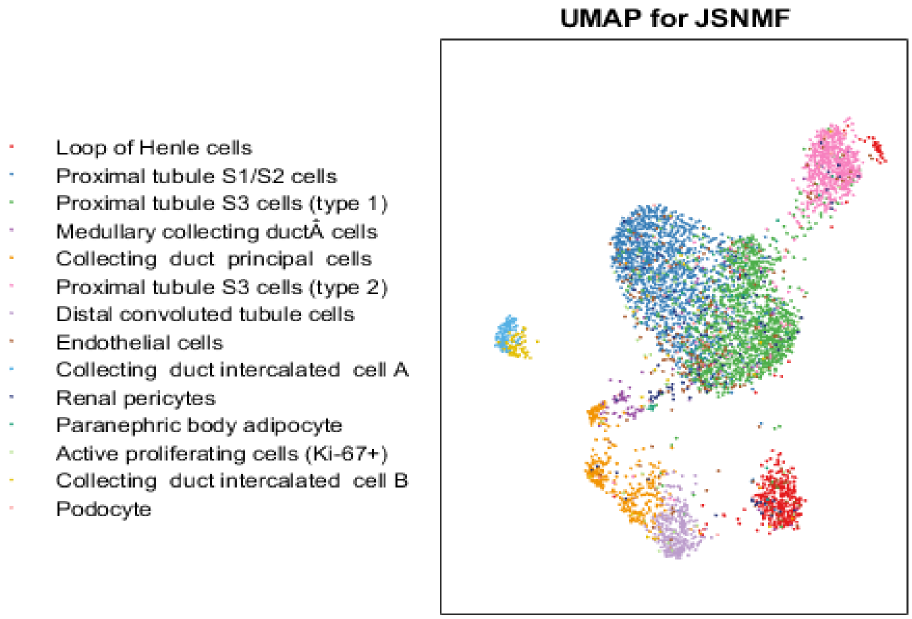
visualization on S obtained from JSNMF

```
% load UMAP package
addpath('../umapFileExchange/umap')
addpath('../umapFileExchange/util')


term = '.';
colors = generateColors(max(length(unique(label_name))),length(unique(term)));


D_jsnfm = 1 - S; D_jsnfm = D_jsnfm - diag(diag(D_jsnfm));
[reduction_jsnmf, ~, ~, ~] = run_umap(D_jsnfm, 'metric', 'precomputed');
gscatter(reduction_jsnmf(:,1), reduction_jsnmf(:,2), label_name, colors, [], 4);
set(gca,'xtick', [], 'ytick', []);
title('UMAP for JSNMF')


legend('Location', 'westoutside', 'Box', 'off', 'FontSize', 9.5);
legendmarkeradjust(16)
legend('boxoff')
```

```
Parallelizing UMAP with MATLAB's 8 assigned logical cores for
nn_descent_tasks, sgd_tasks

Running basic (ub) reduction, v2.1.3
(ub=basic, us=supervised, ubt=template, ust=supervised template)
UMAP(method=MEX, n_neighbors=15, n_components=2, metric='precomputed',
n_epochs=[], learning_rate=1, init=spectral, min_dist=0.3, spread=1,
set_op_mix_ratio=1, local_connectivity=1, repulsion_strength=1,
negative_sample_rate=5, transform_queue_size=4, a=0.992174408960354,
b=1.11225576844318, randomize=true, target_n_neighbors=-1,
target_metric='categorical', target_metric_kwds=[], target_weight=0.5,
verbose=true, initial_alpha=1, sparse_data=false, small_data=true,
distance_func='precomputed', dist_args=[]
```



```
UMAP reduction finished (cost 11.6 secs)
 Finished basic (ub) reduction
```


UMAP for JSNMF
Loop of Henle cells
Proximal tubule S1/S2 cells
Proximal tubule S3 cells (type 1)
Medullary collecting ductÂ cells
Collecting duct principal cells
Proximal tubule S3 cells (type 2)
Distal convoluted tubule cells
Endothelial cells
Collecting duct intercalated cell A
Renal pericytes
Paranephric body adipocyte
Active proliferating cells (Ki-67+)
Collecting duct intercalated cell B
Podocyte