A quick guide to JSNMF algorithm with mouse kidney (RNA+ATAC, sci-CAR) 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';'Pde
1c';'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
```

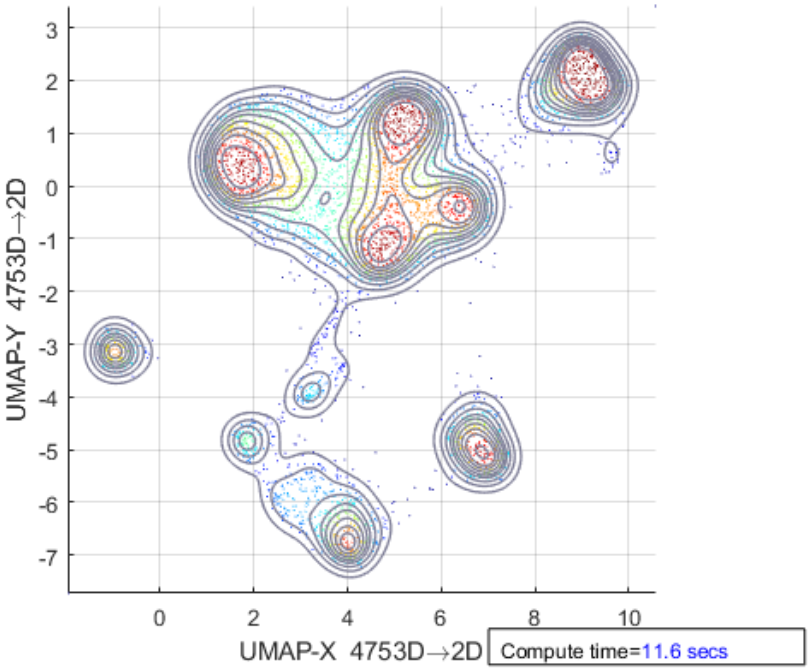
```
ac: 0.6093      1857/4753       nmi:0.5430
ave_marker_gene: 0.5968         ave_hk_gini:0.1555      diff_gini:0.4413
```

Downstream analysis including visualization

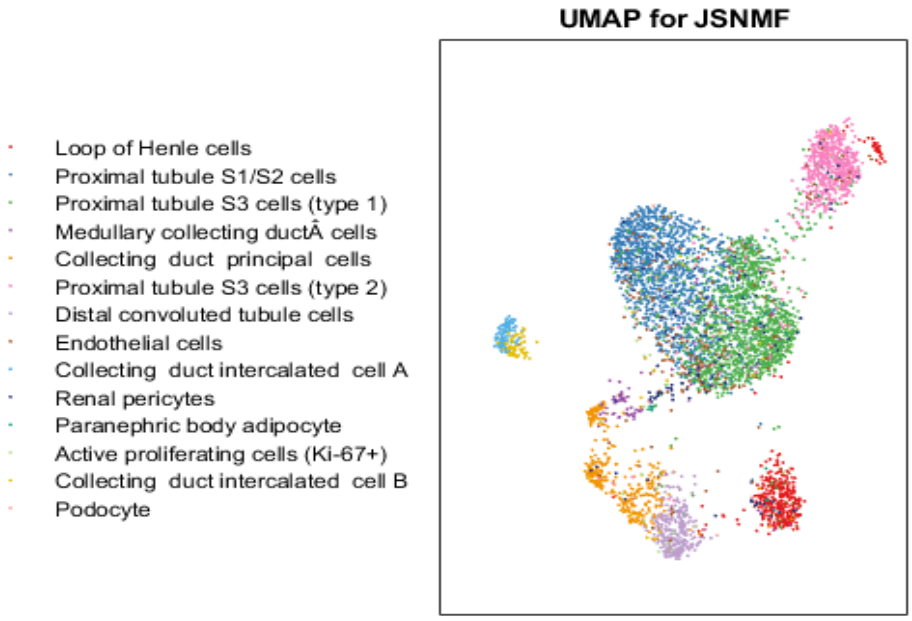
visualization on S obtained from JSNMF

```
% 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
```



Preparing for gene ontology (GO) enrichment analysis

```
% analysis on W1
W1 = full(W1);
% normalization is not necessary
% W1_norm = W1 * diag(1./sqrt(sum(W1.^2)));


% sort each column of W1 to find key genes
[B, I] = sort(W1, 1, 'descend');
nfactor = size(W1, 2);
```

loading genes and peaks

```
load('kidney_5k_10k.mat','genes','peaks')


% selecting top 200 genes with large values
topk = 200;
IX = cell(topk+1, 1);
IX{1} = 'gene symbol';


% save top genes for each factor, and these genes can be used for pathway
% enrichment analysis.


% need to construct Directory 'kidney\W1' in advance
for i = 1:nfactor
   filenm = ['kidney\W1\factor_' num2str(i) '.csv'];
   IX(2:end) = genes(I(1:topk, i));
   dlmcell(filenm, IX);
   disp(['Successfully write factor_',num2str(i),' for gene enrichment analysis']);
end
```

```
Successfully write factor_1 for gene enrichment analysis
Successfully write factor_2 for gene enrichment analysis
Successfully write factor_3 for gene enrichment analysis
Successfully write factor_4 for gene enrichment analysis
Successfully write factor_5 for gene enrichment analysis
Successfully write factor_6 for gene enrichment analysis
Successfully write factor_7 for gene enrichment analysis
Successfully write factor_8 for gene enrichment analysis
Successfully write factor_9 for gene enrichment analysis
Successfully write factor_10 for gene enrichment analysis
Successfully write factor_11 for gene enrichment analysis
Successfully write factor_12 for gene enrichment analysis
Successfully write factor_13 for gene enrichment analysis
Successfully write factor_14 for gene enrichment analysis
```

```
% analysis on W2(region loading)
% check peaks with abnormal name, such as {'chrUn-GL456239-39648-39894'},
% these peaks should be removed from peak list
% peaks(1101,:) = [];
[B,I] = sort(W2, 1,'descend');
nfactor = size(W2,2);
topk = 1000;
IX = cell(topk,nfactor);
loci = cell(topk,3);


% check peak names with special symbols
peaks = strrep(peaks,':','-');


% need to construct Directory 'kidney/W2' in advance
for i = 1:nfactor
   IX(:,i) = peaks(I(1:topk,i),:);
   IX(:,i) = regexp(IX(:,i), '-', 'split');
   filenm = ['kidney/W2/factor_' num2str(i) '.bed' ];
   for j = 1:topk
       loci{j, 1} = char(IX{j,i}{1,1});
       loci{j, 2} = char(IX{j,i}{1,2});
       loci{j, 3} = char(IX{j,i}{1,3});
       dlmcell(filenm, loci);
   end
   disp(['Successfully write factor_',num2str(i),' for region enrichment analysis']);

end


% after obtaining genes from W1 and peaks from W2, gene ontology (GO) enrichment
analyis
% can be implemented using some online tools, such as GREAT, Metascape and
% so on
```

```
Successfully write factor_1 for region enrichment analysis
Successfully write factor_2 for region enrichment analysis
Successfully write factor_3 for region enrichment analysis
Successfully write factor_4 for region enrichment analysis
Successfully write factor_5 for region enrichment analysis
Successfully write factor_6 for region enrichment analysis
Successfully write factor_7 for region enrichment analysis
Successfully write factor_8 for region enrichment analysis
Successfully write factor_9 for region enrichment analysis
Successfully write factor_10 for region enrichment analysis
Successfully write factor_11 for region enrichment analysis
Successfully write factor_12 for region enrichment analysis
```

```
Successfully write factor_13 for region enrichment analysis
Successfully write factor_14 for region enrichment analysis
```