

Single-cell Interpretation via Multi-kernel LeaRning (SIMLR)

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Overview. Single-cell RNA-seq technologies enable high throughput gene expression measurement of individual cells, and allow the discovery of heterogeneity within cell populations. Measurement of cell-to-cell gene expression similarity is critical for the identification, visualization and analysis of cell populations. However, single-cell data introduce challenges to conventional measures of gene expression similarity because of the high level of noise, outliers and dropouts. We develop a novel similarity-learning framework, SIMLR (Single-cell Interpretation via Multi-kernel LeaRning), which learns an appropriate distance metric from the data for dimension reduction, clustering and visualization.

In this vignette, we give an overview of the package by presenting some of its main functions.

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1 Changelog

- 1.0.0 implements SIMLR and SIMLR feature ranking algorithms.
- 1.0.2 implements SIMLR large scale algorithms.
- 1.5.1 implements CIMLR clustering method.
- 1.7.3 removes CIMLR clustering method; please refer to <https://github.com/danro9685/CIMLR>.

2 Algorithms and useful links

Acronym	Extended name	Reference
SIMLR	Single-cell Interpretation via Multi-kernel LeaRning	Publication
Package	A Tool for Large-Scale Genomic Analyses	Publication

3 Using the SIMLR R package

We first load the data provided as examples in the package. The dataset BuettnerFlorian is used for an example of the standard SIMLR, while the dataset ZeiselAmit is used for an example of SIMLR large scale.

```
library(SIMLR)
data(BuettnerFlorian)
data(ZeiselAmit)
```

The external R package igraph is required for the computation of the normalized mutual information to assess the results of the clustering.

```
library(igraph)
```

We now run SIMLR as an example on an input dataset from Buettner, Florian, et al. "Computational analysis of cell-to-cell heterogeneity in single-cell RNA-sequencing data reveals hidden subpopulations of cells." Nature biotechnology 33.2 (2015): 155-160. For this dataset we have a ground true of 3 cell populations, i.e., clusters.

Single-cell Interpretation via Multi-kernel LeaRning (*SIMLR*)

```
set.seed(11111)
example = SIMLR(X = BuettnerFlorian$in_X, c = BuettnerFlorian$n_clust, cores.ratio = 0)

## Computing the multiple Kernels.
##Performing network diffusion.
##Iteration: 1
##Iteration: 2
##Iteration: 3
##Iteration: 4
##Iteration: 5
##Iteration: 6
##Iteration: 7
##Iteration: 8
##Iteration: 9
##Iteration: 10
##Iteration: 11
##Performing t-SNE.
##Epoch: Iteration # 100 error is: 0.1140084
##Epoch: Iteration # 200 error is: 0.06181848
##Epoch: Iteration # 300 error is: 0.05932105
##Epoch: Iteration # 400 error is: 0.05923113
##Epoch: Iteration # 500 error is: 0.05914954
##Epoch: Iteration # 600 error is: 0.05907505
##Epoch: Iteration # 700 error is: 0.05900841
##Epoch: Iteration # 800 error is: 0.05894696
##Epoch: Iteration # 900 error is: 0.05889082
##Epoch: Iteration # 1000 error is: 0.0588387
##Performing Kmeans.
##Performing t-SNE.
##Epoch: Iteration # 100 error is: 11.05456
##Epoch: Iteration # 200 error is: 0.7603752
##Epoch: Iteration # 300 error is: 0.4287243
##Epoch: Iteration # 400 error is: 0.4185942
##Epoch: Iteration # 500 error is: 0.5603325
##Epoch: Iteration # 600 error is: 0.5102124
##Epoch: Iteration # 700 error is: 0.5367624
##Epoch: Iteration # 800 error is: 0.5724108
##Epoch: Iteration # 900 error is: 0.461722
##Epoch: Iteration # 1000 error is: 0.4045293
```

We now compute the normalized mutual information between the inferred clusters by SIMLR and the true ones. This measure with values in $[0,1]$, allows us to assess the performance of the clustering with higher values reflecting better performance.

```
nmi_1 = compare(BuettnerFlorian$true_labs[,1], example$y$cluster, method="nmi")
nmi_1
## [1] 0.888298
```

As a further understanding of the results, we now visualize the cell populations in a plot.

Single-cell Interpretation via Multi-kernel LeaRning (*SIMLR*)

```
plot(example$ydata,
      col = c(topo.colors(BuettnerFlorian$n_clust))[BuettnerFlorian$true_labs[,1]],
      xlab = "SIMLR component 1",
      ylab = "SIMLR component 2",
      pch = 20,
      main="SIMLR 2D visualization for BuettnerFlorian")
```

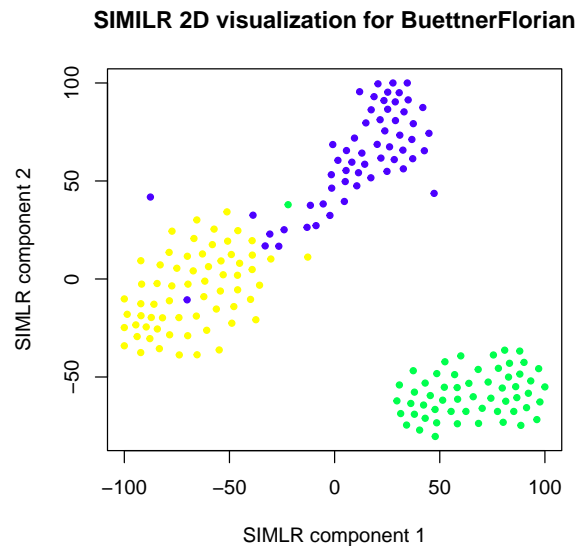


Figure 1: Visualization of the 3 cell populations retrieved by SIMLR on the dataset by Florian, et al

We also run SIMLR feature ranking on the same inputs to get a rank of the key genes with the related pvalues.

```
set.seed(11111)
ranks = SIMLR_Feature_Ranking(A=BuettnerFlorian$results$S,X=BuettnerFlorian$in_X)
```

```
head(ranks$pval)
## [1] 1.086748e-128 1.189327e-90 5.504924e-80 4.652359e-75 5.593957e-73
## [6] 3.373056e-69
head(ranks$aggR)
## [1] 5701 1689 7549 57 2653 8081
```

Similarly, we show an example for SIMLR large scale on an input dataset being a reduced version of the dataset provided in Buettner, Zeisel, Amit, et al. "Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq." Science 347.6226 (2015): 1138-1142. For this dataset we have a ground true of 9 cell populations, i.e., clusters. But we should notice that here we use for computational reasons a reduced version of the data, so please refer to the original publication for the full data.

```
set.seed(11111)
example_large_scale = SIMLR_Large_Scale(X = ZeiselAmit$in_X, c = ZeiselAmit$n_clust, kk = 10)
## Performing fast PCA.
```

Single-cell Interpretation via Multi-kernel LeaRning (*SIMLR*)

```
## Performing k-nearest neighbour search.
## Computing the multiple Kernels.
## Performing the iterative procedure 5 times.
## Iteration: 1
## Iteration: 2
## Iteration: 3
## Iteration: 4
## Iteration: 5
## Performing Kmeans.
## Performing t-SNE.
## The main loop will be now performed with a maximum of 300 iterations.
## Performing iteration 1.
## Performing iteration 2.
## Performing iteration 3.
## Performing iteration 4.
## Performing iteration 5.
## Performing iteration 6.
## Performing iteration 7.
## Performing iteration 8.
## Performing iteration 9.
## Performing iteration 10.
## Performing iteration 11.
## Performing iteration 12.
## Performing iteration 13.
## Performing iteration 14.
## Performing iteration 15.
## Performing iteration 16.
## Performing iteration 17.
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## Performing iteration 19.
## Performing iteration 20.
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## Performing iteration 30.
## Performing iteration 31.
## Performing iteration 32.
## Performing iteration 33.
## Performing iteration 34.
## Performing iteration 35.
## Performing iteration 36.
## Performing iteration 37.
## Performing iteration 38.
## Performing iteration 39.
## Performing iteration 40.
```

Single-cell Interpretation via Multi-kernel LeaRning (*SIMLR*)

```
## Performing iteration 41.  
## Performing iteration 42.  
## Performing iteration 43.  
## Performing iteration 44.  
## Performing iteration 45.  
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## Performing iteration 90.  
## Performing iteration 91.
```

Single-cell Interpretation via Multi-kernel LeaRning (*SIMLR*)

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## Performing iteration 92.  
## Performing iteration 93.  
## Performing iteration 94.  
## Performing iteration 95.  
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## Performing iteration 97.  
## Performing iteration 98.  
## Performing iteration 99.  
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## Performing iteration 139.  
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## Performing iteration 141.  
## Performing iteration 142.
```

Single-cell Interpretation via Multi-kernel LeaRning (*SIMLR*)

```
## Performing iteration 143.
## Performing iteration 144.
## Performing iteration 145.
## Performing iteration 146.
## Performing iteration 147.
## Performing iteration 148.
## Performing iteration 149.
## Performing iteration 150.
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## Performing iteration 192.
## Performing iteration 193.
```


Single-cell Interpretation via Multi-kernel LeaRning (*SIMLR*)

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## Performing iteration 194.  
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## Performing iteration 244.
```

Single-cell Interpretation via Multi-kernel LeaRning (*SIMLR*)

```
## Performing iteration 245.  
## Performing iteration 246.  
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## Performing iteration 290.  
## Performing iteration 291.  
## Performing iteration 292.  
## Performing iteration 293.  
## Performing iteration 294.  
## Performing iteration 295.
```

Single-cell Interpretation via Multi-kernel LeaRning (*SIMLR*)

```
## Performing iteration 296.  
## Performing iteration 297.  
## Performing iteration 298.  
## Performing iteration 299.  
## Performing iteration 300.
```

We compute the normalized mutual information between the inferred clusters by SIMLR large scale and the true ones.

```
nmi_2 = compare(ZeiselAmit$true_labs[,1], example_large_scale$y$cluster, method="nmi")  
nmi_2  
## [1] 0.04158302
```

As a further understanding of the results, also in this case we visualize the cell populations in a plot.

```
plot(example_large_scale$data,  
      col = c(topo.colors(ZeiselAmit$n_clust))[ZeiselAmit$true_labs[,1]],  
      xlab = "SIMLR component 1",  
      ylab = "SIMLR component 2",  
      pch = 20,  
      main="SIMLR 2D visualization for ZeiselAmit")
```

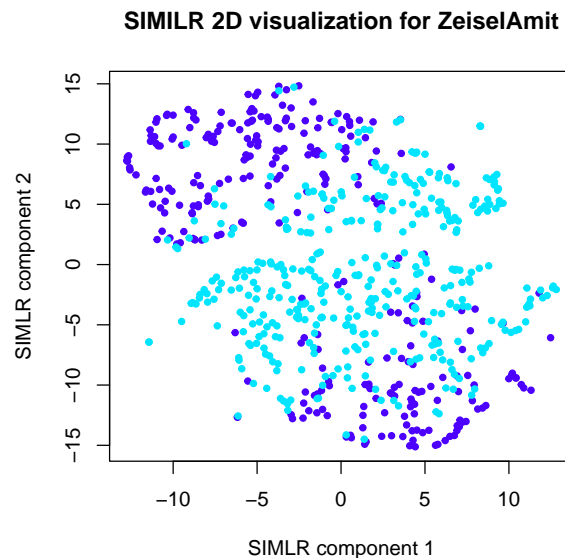


Figure 2: Visualization of the 9 cell populations retrieved by SIMLR large scale on the dataset by Zeisel, Amit, et al

Now, as a final example, we also provide the results of two heuristics (see the original SIMLR paper) to estimate the number of clusters from data.

```
set.seed(53900)  
NUMC = 2:5  
res_example = SIMLR_Estimate_Number_of_Clusters(BuettnerFlorian$in_X,  
          NUMC = NUMC,  
          cores.ratio = 0)
```

Single-cell Interpretation via Multi-kernel LeaRning (SIMLR)

Best number of clusters, K1 heuristic:

```
NUMC[which.min(res_example$K1)]  
## [1] 2
```

K2 heuristic:

```
NUMC[which.min(res_example$K2)]  
## [1] 2
```

Results of the two heuristics:

```
res_example  
## $K1  
## [1] -63.04223 -19.49278 -23.77182 13.30109  
##  
## $K2  
## [1] -94.56335 -25.99037 -29.71477 15.96130
```

4 sessionInfo()

- R version 4.1.1 Patched (2021-08-22 r80813), x86_64-apple-darwin17.0
- Locale: C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
- Running under: macOS Mojave 10.14.6
- Matrix products: default
- BLAS:
/Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.0.dylib
- LAPACK:
/Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: SIMLR 1.19.1, igraph 1.2.6, knitr 1.34
- Loaded via a namespace (and not attached): BiocManager 1.30.16, BiocStyle 2.21.3, Matrix 1.3-4, RSpectra 0.16-0, Rcpp 1.0.7, RcppAnnoy 0.0.19, codetools 0.2-18, compiler 4.1.1, digest 0.6.27, evaluate 0.14, fastmap 1.1.0, grid 4.1.1, highr 0.9, htmltools 0.5.2, lattice 0.20-44, magrittr 2.0.1, parallel 4.1.1, pkgconfig 2.0.3, pracma 2.3.3, rlang 0.4.11, rmarkdown 2.11, stringi 1.7.4, stringr 1.4.0, tools 4.1.1, xfun 0.26, yaml 2.2.1