

scAlign Tutorial

Nelson Johansen, Gerald Quon

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Introduction

This tutorial provides a guided alignment for two groups of cells from cellbench RNA mixture experiments. In this tutorial we demonstrate the unsupervised alignment strategy of `scAlign` described in Johansen et al, 2018 along with typical analysis utilizing the aligned dataset, and show how `scAlign` can identify and match cell types across platforms without using the labels as input.

Alignment goals

The following is a walkthrough of a typical alignment problem for `scAlign` and has been designed to provide an overview of data preprocessing, alignment and finally analysis in our joint embedding space. Here, our primary goals include:

1. Learning a low-dimensional cell state space in which cells group by function and type, regardless of condition (platform).
2. Accurately labeling old cells with cell cycle and cell type information using only the young cell annotations.

Installation

```
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install("scAlign")
```

Guide to installing python and tensorflow on different operating systems.

On Windows:

Download Python 3.6.8. Note, newer versions of Python (e.g. 3.7) cannot use TensorFlow at this time.

Make sure pip is included in the installation.

Open Windows Command Prompt, or PowerShell.

Navigate to your Python installation directory (for Windows 10, the default seems to be C:\Users\userid

Run `.\pip install --upgrade tensorflow`

On Ubuntu:

```
sudo apt update
```

```
sudo apt install python3-dev python3-pip
```

```
pip3 install --user --upgrade tensorflow # install in $HOME
```

```
python3 -c "import tensorflow as tf; tf.enable_eager_execution(); print(tf.reduce_sum(tf.random_normal(
```

On MacOS (homebrew):

```
/usr/bin/ruby -e "$(curl -fsSL https://raw.githubusercontent.com/Homebrew/install/master/install)"
```

```
export PATH="/usr/local/bin:/usr/local/sbin:$PATH"
```

```
brew update
```

```
brew install python # Python 3
```

```
pip3 install --user --upgrade tensorflow # install in $HOME
```

```
python3 -c "import tensorflow as tf; tf.enable_eager_execution(); print(tf.reduce_sum(tf.random_normal(
```

Further details at: <https://www.tensorflow.org/install>

Data setup

The gene count matrices used for this tutorial are hosted on the cellbench github: [here](#).

First, we load in the normalized cellbench data. The data was normalized following the procedures defined on the cellbench github.

```
library(scAlign)
library(SingleCellExperiment)
library(ggplot2)

## Load in cellbench data
data("cellbench", package = "scAlign", envir = environment())

## Extract RNA mixture cell types
mix.types = unlist(lapply(strsplit(colnames(cellbench), "-"), "[", 2))

## Extract Platform
batch = c(rep("CEL", length(which(!grepl("sortseq", colnames(cellbench)) == TRUE))),
          rep("SORT", length(which(grepl("sortseq", colnames(cellbench)) == TRUE))))
```

scAlign setup

The general design of scAlign's makes it agnostic to the input RNA-seq data representation. Thus, the input data can either be gene-level counts, transformations of those gene level counts or a preliminary step of dimensionality reduction such as canonical correlates or principal component scores. Here we create the scAlign object from the previously normalized cellbench data and perform CCA on the unaligned data.

```
## Create SCE objects to pass into scAlignCreateObject
youngMouseSCE <- SingleCellExperiment(
  assays = list(scale.data = cellbench[,batch=='CEL'])
)

oldMouseSCE <- SingleCellExperiment(
  assays = list(scale.data = cellbench[,batch=='SORT'])
)

## Build the scAlign class object and compute PCs
scAlignCB = scAlignCreateObject(sce.objects = list("CEL"=youngMouseSCE,
                                                  "SORT"=oldMouseSCE),
                               labels = list(mix.types[batch=='CEL'],
                                              mix.types[batch=='SORT']),
                               data.use="scale.data",
                               pca.reduce = FALSE,
                               cca.reduce = TRUE,
                               ccs.compute = 5,
                               project.name = "scAlign_cellbench")

## [1] "Computing CCA using Seurat."
```

```

## Centering and scaling data matrix
## Centering and scaling data matrix

## Running CCA

## Merging objects

```

Alignment of cellbench RNAmixture

Now we align the cell populations from both protocols. `scAlign` returns a low-dimensional joint embedding space where the effect of platform is removed allowing us to use the complete dataset for downstream analyses such as clustering or differential expression.

```

## Run scAlign with all_genes
scAlignCB = scAlign(scAlignCB,
                    options=scAlignOptions(steps=1000,
                                           log.every=1000,
                                           norm=TRUE,
                                           early.stop=TRUE),
                    encoder.data="scale.data",
                    supervised='none',
                    run.encoder=TRUE,
                    run.decoder=FALSE,
                    log.dir=file.path('~/models_temp', 'gene_input'),
                    device="CPU")

```

```

## [1] "==== Step 1/3: Encoder training ====="
## [1] "Graph construction"
## [1] "Adding source walker loss"
## [1] "Adding target walker loss"
## [1] "Done random initialization"
## [1] "Step: 1      Loss: 68.2143   Alignment: 0.8687"
## [1] "Step: 100   Loss: 49.1458   Alignment: 0.7286"
## [1] "Step: 200   Loss: 39.5291   Alignment: 0.6807"
## [1] "Step: 300   Loss: 32.5206   Alignment: 0.7588"
## [1] "Step: 400   Loss: 27.3723   Alignment: 0.8568"
## [1] "Step: 500   Loss: 26.1896   Alignment: 0.867"
## [1] "Step: 600   Loss: 24.1721   Alignment: 0.8655"
## [1] "Step: 700   Loss: 24.0994   Alignment: 0.8614"
## [1] "Step: 800   Loss: 24.0894   Alignment: 0.8478"
## [1] "Step: 900   Loss: 24.1644   Alignment: 0.8362"
## [1] "Step: 1000  Loss: 24.7685   Alignment: 0.8355"

```

```

# ## Additional run of scAlign with CCA
# scAlignCB = scAlign(scAlignCB,
#                     options=scAlignOptions(steps=1000,
#                                             log.every=1000,
#                                             norm=TRUE,
#                                             early.stop=TRUE),
#                     encoder.data="CCA",
#                     supervised='none',
#                     run.encoder=TRUE,
#                     run.decoder=FALSE,
#                     log.dir=file.path('~/models', 'cca_input'),
#                     device="CPU")

```

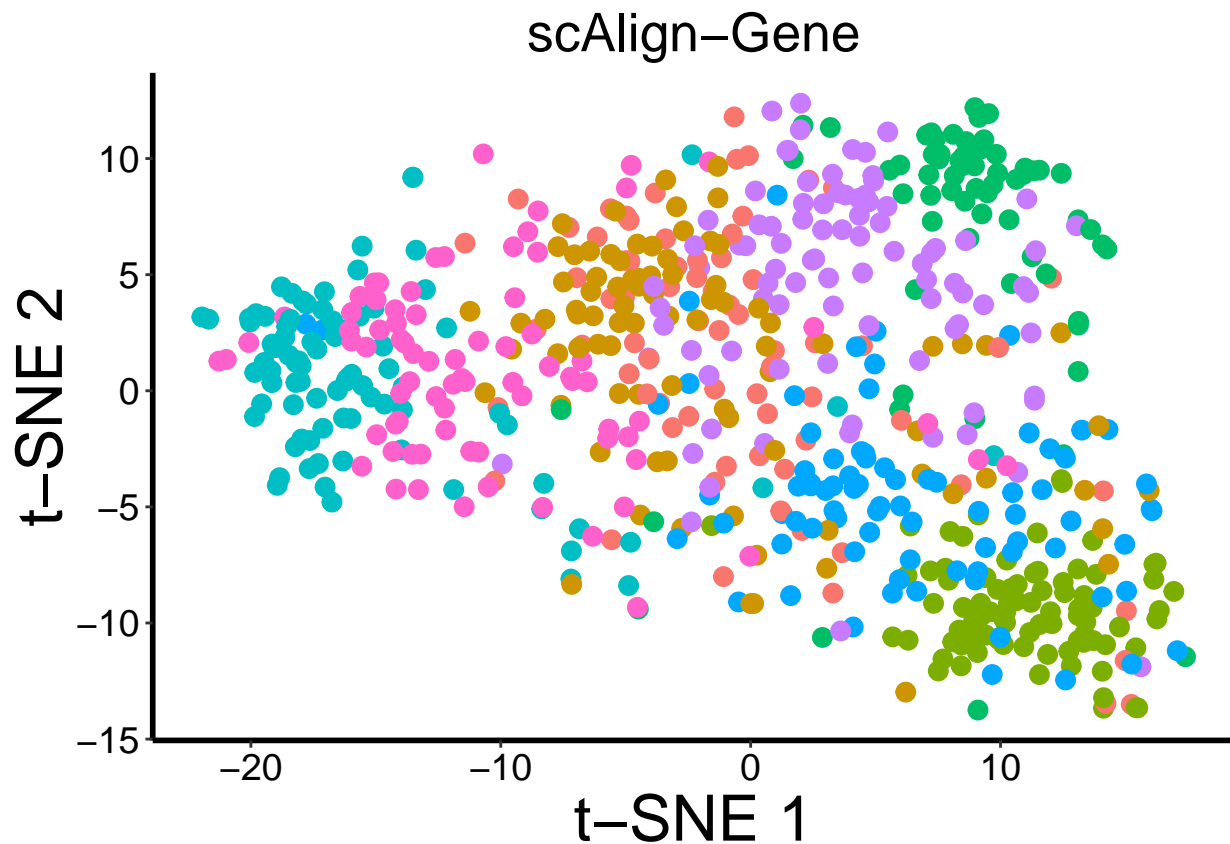
```

## Plot aligned data in tSNE space, when the data was processed in three different ways:
## 1) either using the original gene inputs,
## 2) after CCA dimensionality reduction for preprocessing.
## Cells here are colored by input labels

set.seed(5678)
gene_plot = PlotTSNE(scAlignCB,
                     "ALIGNED-GENE",
                     title="scAlign-Gene",
                     perplexity=30)

## Show plot
gene_plot

```



```

# cca_plot = PlotTSNE(scAlignCB,
#                     "ALIGNED-CCA",
#                     title="scAlign-CCA",
#                     perplexity=30)
#
# multi_plot_labels = grid.arrange(gene_plot, cca_plot, nrow = 1)

```

Session Info

```
sessionInfo()
```

```
## R version 3.6.0 (2019-04-26)
```

```

## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 18.04.2 LTS
##
## Matrix products: default
## BLAS: /home/biocbuild/bbs-3.9-bioc/R/lib/libRblas.so
## LAPACK: /home/biocbuild/bbs-3.9-bioc/R/lib/libRlapack.so
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8 LC_NUMERIC=C
## [3] LC_TIME=en_US.UTF-8 LC_COLLATE=C
## [5] LC_MONETARY=en_US.UTF-8 LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=en_US.UTF-8 LC_NAME=C
## [9] LC_ADDRESS=C LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel stats4 stats graphics grDevices utils datasets
## [8] methods base
##
## other attached packages:
## [1] ggplot2_3.1.1 SingleCellExperiment_1.6.0
## [3] SummarizedExperiment_1.14.0 DelayedArray_0.10.0
## [5] BiocParallel_1.18.0 matrixStats_0.54.0
## [7] Biobase_2.44.0 GenomicRanges_1.36.0
## [9] GenomeInfoDb_1.20.0 IRanges_2.18.0
## [11] S4Vectors_0.22.0 BiocGenerics_0.30.0
## [13] scAlign_1.0.0
##
## loaded via a namespace (and not attached):
## [1] Seurat_3.0.0 Rtsne_0.15 colorspace_1.4-1
## [4] ggirdges_0.5.1 XVector_0.24.0 base64enc_0.1-3
## [7] listenv_0.7.0 npsurv_0.4-0 ggrepel_0.8.0
## [10] codetools_0.2-16 splines_3.6.0 R.methodsS3_1.7.1
## [13] lsei_1.2-0 knitr_1.22 config_0.3
## [16] jsonlite_1.6 ica_1.0-2 cluster_2.0.9
## [19] png_0.1-7 R.oo_1.22.0 tfruns_1.4
## [22] sctransform_0.2.0 compiler_3.6.0 httr_1.4.0
## [25] assertthat_0.2.1 Matrix_1.2-17 lazyeval_0.2.2
## [28] htmltools_0.3.6 tools_3.6.0 rsvd_1.0.0
## [31] igraph_1.2.4.1 gtable_0.3.0 glue_1.3.1
## [34] GenomeInfoDbData_1.2.1 RANN_2.6.1 reshape2_1.4.3
## [37] dplyr_0.8.0.1 Rcpp_1.0.1 gdata_2.18.0
## [40] ape_5.3 nlme_3.1-139 gbRd_0.4-11
## [43] lmtest_0.9-37 xfun_0.6 stringr_1.4.0
## [46] globals_0.12.4 irlba_2.3.3 gtools_3.8.1
## [49] future_1.12.0 MASS_7.3-51.4 zlibbioc_1.30.0
## [52] zoo_1.8-5 scales_1.0.0 RColorBrewer_1.1-2
## [55] yaml_2.2.0 reticulate_1.12 pbapply_1.4-0
## [58] gridExtra_2.3 stringi_1.4.3 tensorflow_1.13.1
## [61] caTools_1.17.1.2 bibtext_0.4.2 Rdpack_0.11-0
## [64] SDMTools_1.1-221.1 rlang_0.3.4 pkgconfig_2.0.2
## [67] bitops_1.0-6 evaluate_0.13 lattice_0.20-38
## [70] ROCR_1.0-7 purrr_0.3.2 labeling_0.3
## [73] htmlwidgets_1.3 cowplot_0.9.4 tidysselect_0.2.5

```

```
## [76] plyr_1.8.4          magrittr_1.5          R6_2.4.0
## [79] gplots_3.0.1.1      pillar_1.3.1          whisker_0.3-2
## [82] withr_2.1.2         fitdistrplus_1.0-14  survival_2.44-1.1
## [85] RCurl_1.95-4.12     tibble_2.1.1          future.apply_1.2.0
## [88] tsne_0.1-3          crayon_1.3.4          KernSmooth_2.23-15
## [91] plotly_4.9.0        rmarkdown_1.12       grid_3.6.0
## [94] data.table_1.12.2   FNN_1.1.3            metap_1.1
## [97] digest_0.6.18       tidyr_0.8.3          R.utils_2.8.0
## [100] munsell_0.5.0       viridisLite_0.3.0
```