Getting Started with Harmony

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Introduction

`harmony` enables scalable integration of single-cell RNA-seq data for batch correction and meta analysis. In this tutorial, we will demonstrate the utility of `harmony` to jointly analyze single-cell RNA-seq PBMC datasets from two healthy individuals.

Installation

First, install `harmony` if you have not already done so.

```
library(devtools)
install_github("immunogenomics/harmony")
```

Now we can load `harmony`

```
library(harmony)
```

```
## Loading required package: Rcpp
```

For this tutorial, we will use single-cell RNA-seq PBMC datasets that are readily available as part of the `MUDAN` package.

```
install_github("JEFworks/MUDAN")
```

Now we can load the data.

```
library(MUDAN)
```

```
## Loading required package: Matrix
```

```
data("pbmcA")
data("pbmcB")
```

For the purposes of a quick demonstration, we will downsize the number of cells in each PBMC dataset. To create a more challenging scenario, we will also make one dataset much smaller than the other.

```
# downsample
print(dim(pbmcA))
print(dim(pbmcB))
pbmcA <- pbmcA[, 1:500] # take 500 cells
pbmcB <- pbmcB[, 1:2000] # take 2000 cells
```

```
## [1] 13939  2896
## [1] 15325  7765
```

We can now combine the two datasets into one cell by gene counts matrix and use a `meta` vector to keep track of which cell belongs to which sample.

```
# combine into one counts matrix
genes.int <- intersect(rownames(pbmcA), rownames(pbmcB))
cd <- cbind(pbmcA[genes.int,], pbmcB[genes.int,])
```

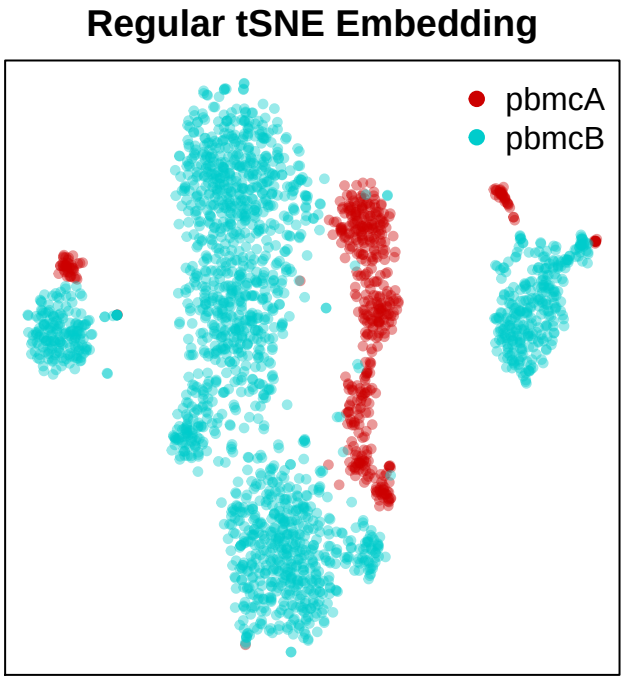
```
# meta data
meta <- c(rep('pbmcA', ncol(pbmcA)), rep('pbmcB', ncol(pbmcB)))
names(meta) <- c(colnames(pbmcA), colnames(pbmcB))
meta <- factor(meta)

print(cd[1:5,1:2])
print(meta[1:5])
```

```
## 5 x 2 sparse Matrix of class "dgCMatrix"
##               frozen_pbmc_donor_a_AAACATTGCACTAG
## AL627309.1                                     .
## RP11-206L10.2                                  .
## RP11-206L10.9                                  .
## LINC00115                                      .
## NOC2L                                          .
##               frozen_pbmc_donor_a_AAACATTGGCTAAC
## AL627309.1                                     .
## RP11-206L10.2                                  .
## RP11-206L10.9                                  .
## LINC00115                                      .
## NOC2L                                          .
## frozen_pbmc_donor_a_AAACATTGCACTAG frozen_pbmc_donor_a_AAACATTGGCTAAC
##                              pbmcA                              pbmcA
## frozen_pbmc_donor_a_AAACATTGTAACCG frozen_pbmc_donor_a_AAACCGTGTGGTCA
##                              pbmcA                              pbmcA
## frozen_pbmc_donor_a_AAACCGTGTTACCT
##                              pbmcA
## Levels: pbmcA pbmcB
```

Given this counts matrix, we can normalize our data, derive principal components, and perform dimensionality reduction using tSNE. However, we see prominent separation by sample due to batch effects.

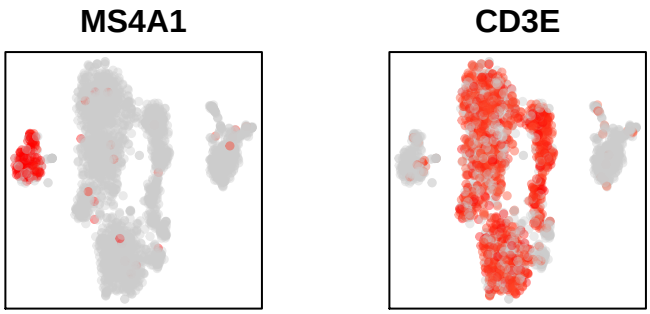
```
## CPM normalization
mat <- MUDAN::normalizeCounts(cd,
                      verbose=FALSE)
## variance normalize, identify overdispersed genes
matnorm.info <- MUDAN::normalizeVariance(mat,
                                details=TRUE,
                                verbose=FALSE)
## log transform
matnorm <- log10(matnorm.info$mat+1)
## 30 PCs on overdispersed genes
pcs <- MUDAN::getPcs(matnorm[matnorm.info$ods,],
              nGenes=length(matnorm.info$ods),
              nPcs=30,
              verbose=FALSE)

# TSNE embedding with regular PCs
emb <- Rtsne::Rtsne(pcs,
                    is_distance=FALSE,
                    perplexity=30,
                    num_threads=1,
                    verbose=FALSE)$Y
rownames(emb) <- rownames(pcs)
```

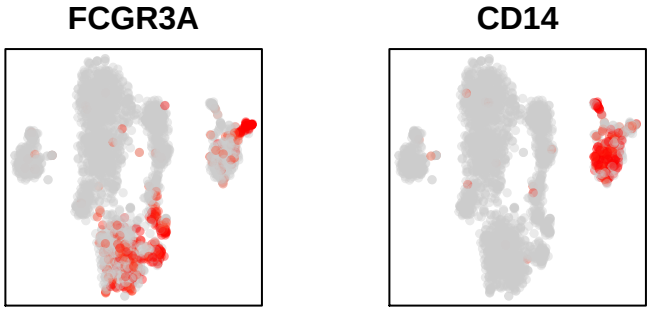
```
# Plot
par(mfrow=c(1,1), mar=rep(2,4))
MUDAN::plotEmbedding(emb, groups=meta,
                     show.legend=TRUE, xlab=NA, ylab=NA,
                     main='Regular tSNE Embedding',
                     verbose=FALSE)
```



Indeed, when we inspect certain cell-type specific marker genes (MS4A1/CD20 for B-cells, CD3E for T-cells, FCGR3A/CD16 for NK cells, macrophages, and monocytes, CD14 for dendritic cells, macrophages, and monocytes), we see that cells are separating by batch rather than by their expected cell-types.

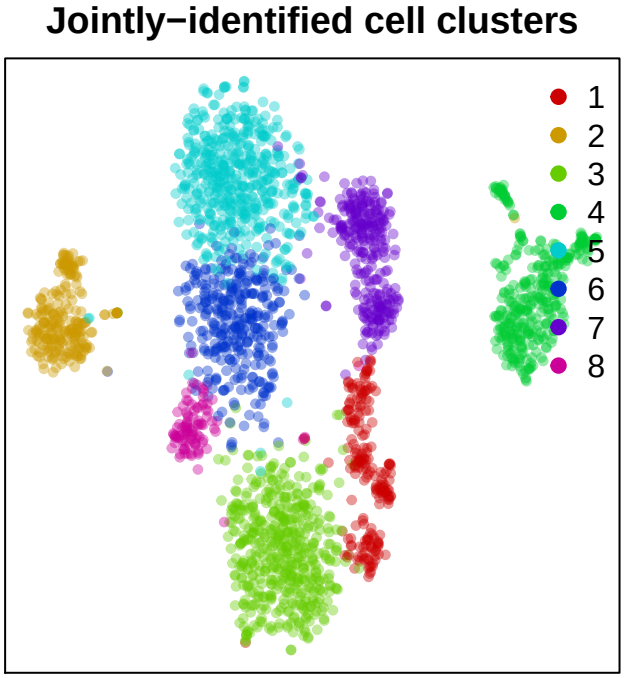
```
par(mfrow=c(2,2), mar=rep(2,4))
invisible(lapply(c('MS4A1', 'CD3E', 'FCGR3A', 'CD14'), function(g) {
  gexp <- log10(mat[g,]+1)
  plotEmbedding(emb, col=gexp,
                xlab=NA, ylab=NA, main=g,
                verbose=FALSE)
}))
```





If we were attempt to identify cell-types using clustering analysis at this step, we would identify a number of sample-specific clusters driven by batch effects.

```
# Joint clustering
annot.bad <- getComMembership(pcs, k=30, method=igraph::cluster_louvain)
par(mfrow=c(1,1), mar=rep(2,4))
plotEmbedding(emb, groups=annot.bad,
              show.legend=TRUE, xlab=NA, ylab=NA,
              main='Jointly-identified cell clusters',
              verbose=FALSE)
```



```
# Look at cell-type proportions per sample
print(t(table(annot.bad, meta))/as.numeric(table(meta)))
```

```
## [1] "finding approximate nearest neighbors ..."
## [1] "calculating clustering ..."
## [1] "graph modularity: 0.740534386510747"
## [1] "identifying cluster membership ..."
## com
##   1   2   3   4   5   6   7   8
## 204 229 499 352 492 338 286 100
##        annot.bad
## meta         1      2      3      4      5      6      7      8
##   pbmcA 0.2860 0.0860 0.0040 0.0880 0.0000 0.0000 0.5360 0.0000
##   pbmcB 0.0305 0.0930 0.2485 0.1540 0.2460 0.1690 0.0090 0.0500
```

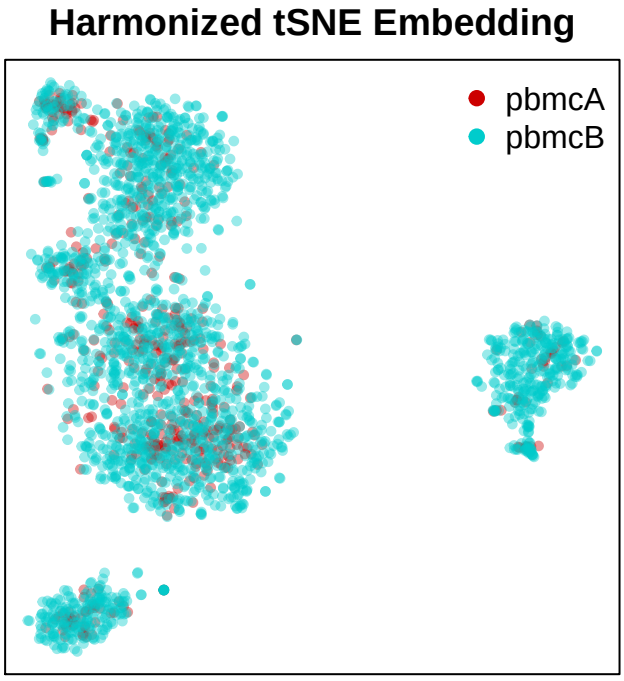
In order to better identify cell-types that may be common to both samples, we will use **harmony** to integrate the cells into a unified embedding.

```
# Now harmonize PCs
harmonized <- HarmonyMatrix(pcs, meta)
```

```
## Harmony 1/10
## Harmony 2/10
## Harmony 3/10
## Harmony 4/10
## Harmony 5/10
## Harmony 6/10
## Clustered for 65 iterations
## Clustered for 29 iterations
## Clustered for 56 iterations
## Clustered for 21 iterations
## Clustered for 24 iterations
## Clustered for 19 iterations
## Harmony converged after 6 iterations
```

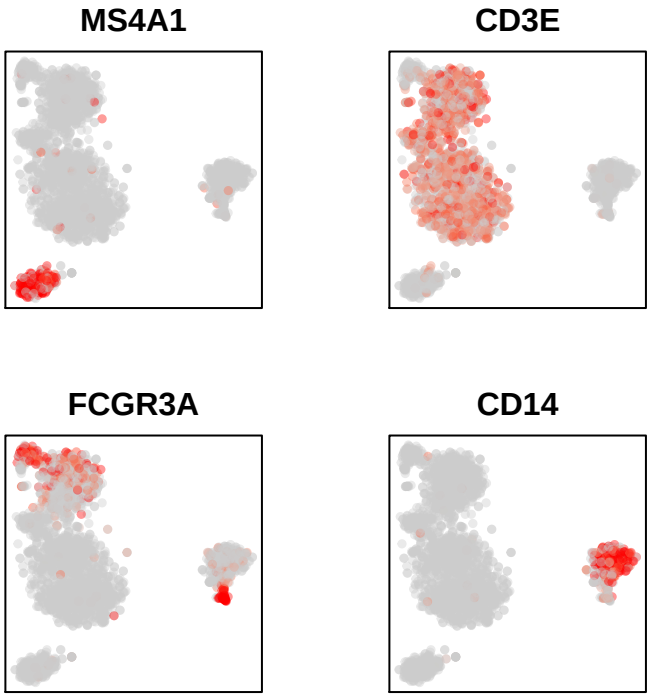
Now, the two samples are well mixed.

```
# TSNE embedding with harmonized PCs
emb.harmony <- Rtsne::Rtsne(harmonized,
                    is_distance=FALSE,
                    perplexity=30,
                    num_threads=1,
                    verbose=FALSE)$Y
rownames(emb.harmony) <- rownames(harmonized)

# Plot
par(mfrow=c(1,1), mar=rep(2,4))
MUDAN::plotEmbedding(emb.harmony, groups=meta,
               show.legend=TRUE, xlab=NA, ylab=NA,
               main='Harmonized tSNE Embedding',
               verbose=FALSE)
```



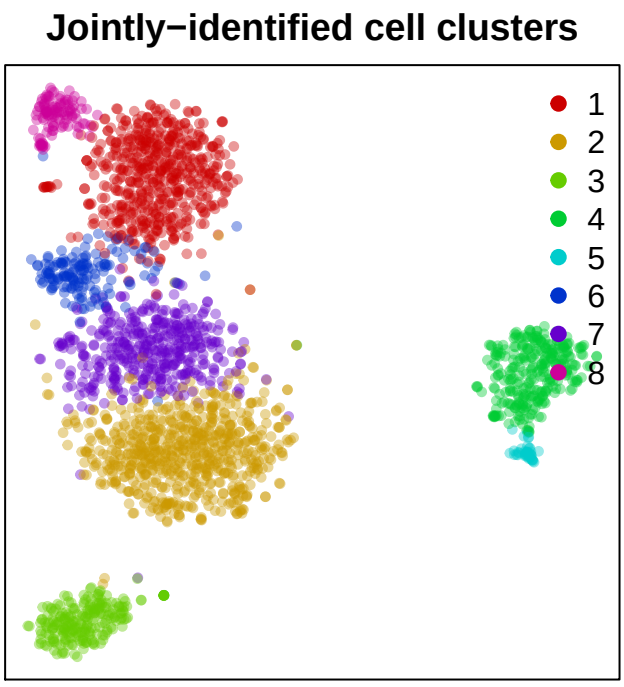
Indeed, when we inspect the same cell-type specific markers as we did previously, we now see that cells are clustered by putative cell-type rather than separating by batch.

```
par(mfrow=c(2,2), mar=rep(2,4))
invisible(lapply(c('MS4A1', 'CD3E', 'FCGR3A', 'CD14'), function(g) {
  gexp <- mat[g,]
  plotEmbedding(emb.harmony, col=gexp,
                xlab=NA, ylab=NA, main=g,
                verbose=FALSE)
}))
```



Now, we can jointly identify cell-type clusters. In this case, the cell-types are comparably represented in proportion across the two samples.

```
# Joint clustering
com <- getComMembership(harmonized, k=30, method=igraph::cluster_louvain)
par(mfrow=c(1,1), mar=rep(2,4))
plotEmbedding(emb.harmony, groups=com,
              show.legend=TRUE, xlab=NA, ylab=NA,
              main='Jointly-identified cell clusters',
              verbose=FALSE)
```




```
# Look at cell-type proportions per sample
print(t(table(com, meta))/as.numeric(table(meta)))
```

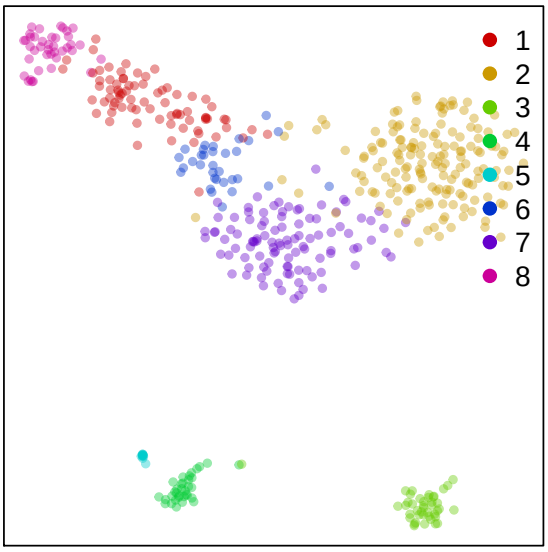
```
## [1] "finding approximate nearest neighbors ..."
## [1] "calculating clustering ..."
## [1] "graph modularity: 0.701384788890046"
## [1] "identifying cluster membership ..."
## com
##   1   2   3   4   5   6   7   8
## 555 672 227 308  45 156 427 110
##        com
## meta         1      2      3      4      5      6      7      8
##   pbmcA 0.1540 0.3240 0.0840 0.0680 0.0220 0.0640 0.2040 0.0800
##   pbmcB 0.2390 0.2550 0.0925 0.1370 0.0170 0.0620 0.1625 0.0350
```

We can also analyze each sample separately and see how our jointly-dervied cell-type clusters map onto each individual sample's embeddings.

```
# Assess validity of joint-derived clusters in individual samples
emb1 <- Rtsne::Rtsne(pcs[meta=='pbmcA',],
                     is_distance=FALSE,
                     perplexity=30,
                     num_threads=1,
                     verbose=FALSE)$Y
```

```
rownames(emb1) <- rownames(pcs)[meta=='pbmcA']
emb2 <- Rtsne::Rtsne(pcs[meta=='pbmcB',],
                     is_distance=FALSE,
                     perplexity=30,
                     num_threads=1,
                     verbose=FALSE)$Y
rownames(emb2) <- rownames(pcs)[meta=='pbmcB']

# Plot
par(mfrow=c(1,2), mar=rep(2,4))
MUDAN::plotEmbedding(emb1, groups=com,
                     show.legend=TRUE, xlab=NA, ylab=NA,
                     main='pbmcA with joint cluster annotations',
                     verbose=FALSE)
MUDAN::plotEmbedding(emb2, groups=com,
                     show.legend=TRUE, xlab=NA, ylab=NA,
                     main='pbmcB with joint cluster annotations',
                     verbose=FALSE)
```

pbmcA with joint cluster annotations



pbmcB with joint cluster annotations