Dimensionality Reduction

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Dimensionality Reduction & Visualization

In this tutorial we will look at different ways to visualize single cell RNA-seq datasets using dimensionality reduction. We will apply the Principal Component Analysis (PCA), and t-distributed Stochastic Neighbor Embedding (t-SNE) algorithms. Alternatively, Uniform Manifold Approximation and Projection (UMAP) algorithm is also mentioned, however, it is optional for the current exercise. Further, we will look at different ways to plot the dimensionality reduced data and augment them with additional information, such as gene expression or meta-information.

Datasets

For this tutorial we will continue with the dataset you have preprocessed in the previous practicals. We will also give you some cell type labels for visualization purposes. During a later practical, you will learn how to annotate the cells yourself.

Data preprocessing

Load required packages. Here, we will use the Seurat functions for the dimensionality reduction. They are also available as pure R functions but Seurat nicely packages them for our data. We will use ggplot for creating the plots.

```
library(Seurat)
library(ggplot2)
```

Data loading and preprocessing

First, we load the Seurat object from the previous practical and attach the celltype labels.

```
pbmc = readRDS('pbmc3k_QC.rds')
labels = read.delim('celltype_labels.tsv', row.names = 1)
pbmc <- AddMetaData(
    object = pbmc,
    metadata = labels)
```

Since the data is already normalized in the previous practical, we can skip these steps here. We will only need to scale the data.

```
# Run the standard workflow for visualization and clustering
pbmc <- ScaleData(pbmc, verbose = FALSE)
```

Dimensionality Reduction

From here on, we will have a look at the different dimensionality reduction methods and their parameterizations.

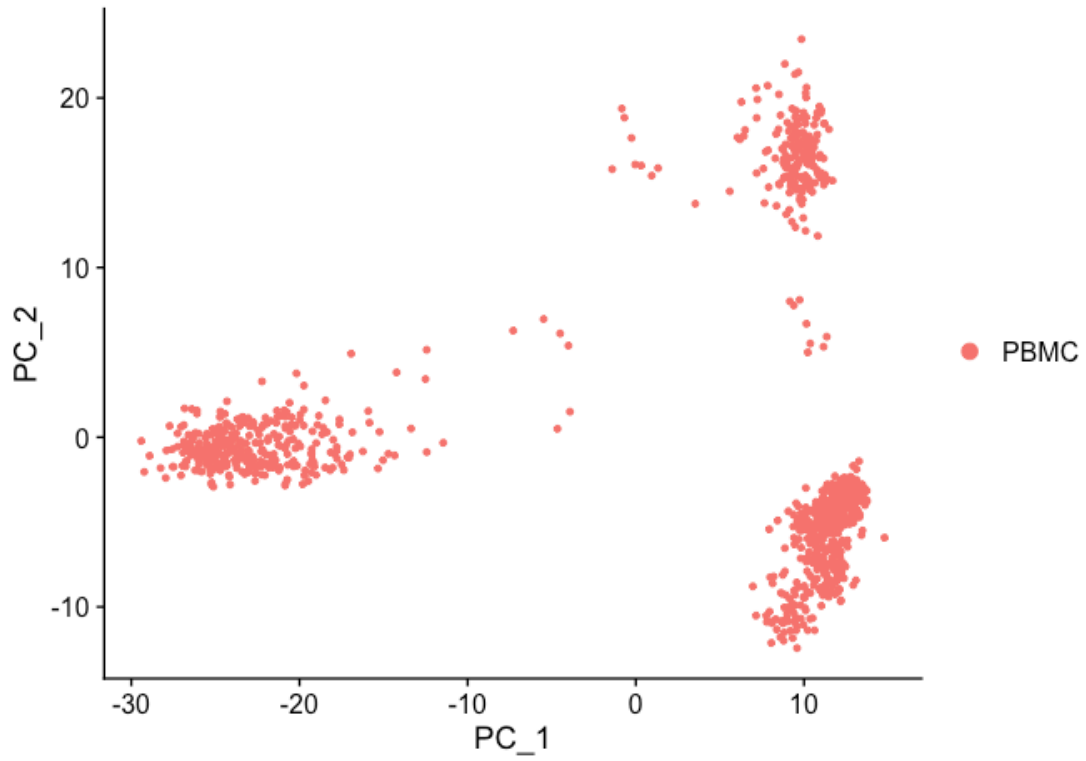
PCA

We start with PCA. Seurat provides the `RunPCA` function, we use `pbmc` as input data. `npcs` refers to the number of principal components to compute. We set it to `100`. This will take a bit longer to compute but will allow use to explore the differences using different numbers of PCs below. By assigning the result to our `pbmc` data object it will be available in the object with the default name `pca`.

```
pbmc <- RunPCA(pbmc, npcs = 100, verbose = FALSE)
```

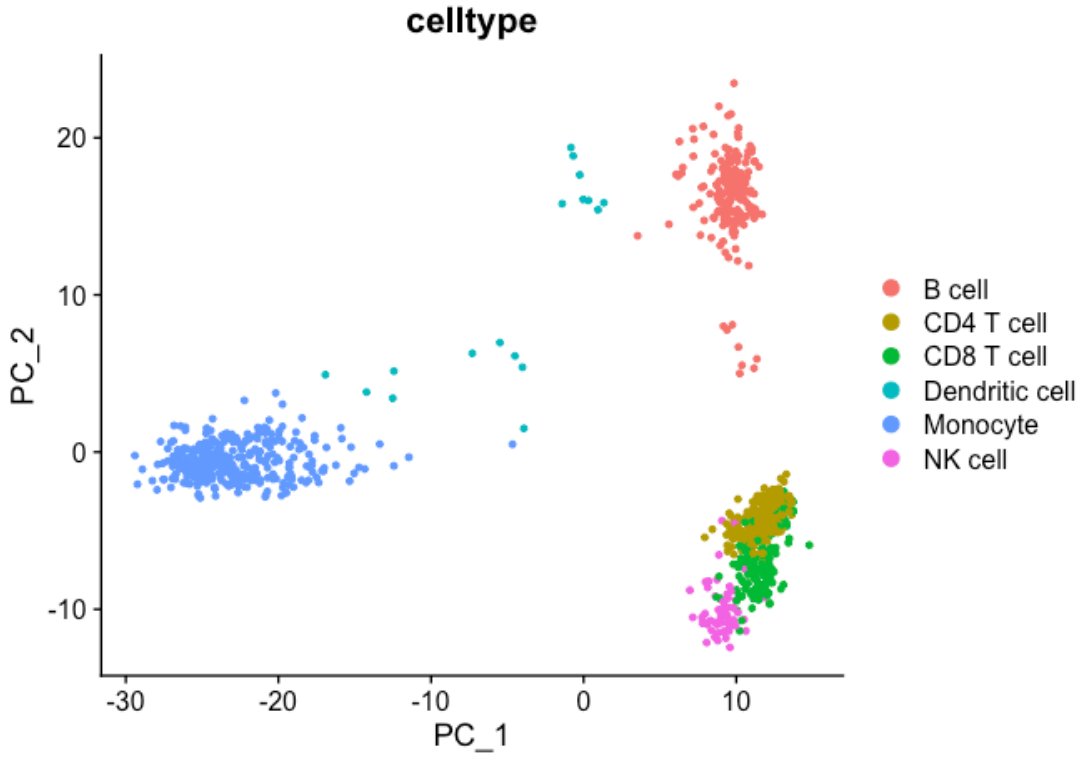
We can now plot the first two components using the `DimPlot` function. The first argument here is the Seurat data object `pbmc`. By providing the `Rreduction = "pca"` argument, `DimPlot` looks in the object for the PCA we created and assigned above.

```
DimPlot(pbmc, reduction = "pca")
```



This plot shows some structure of the data. Every dot is a cell, but we do not know which cells belong to which dot, etc. We can add meta information by the `group.by` parameter. We use the `celltype` that we have read from the metadata object.

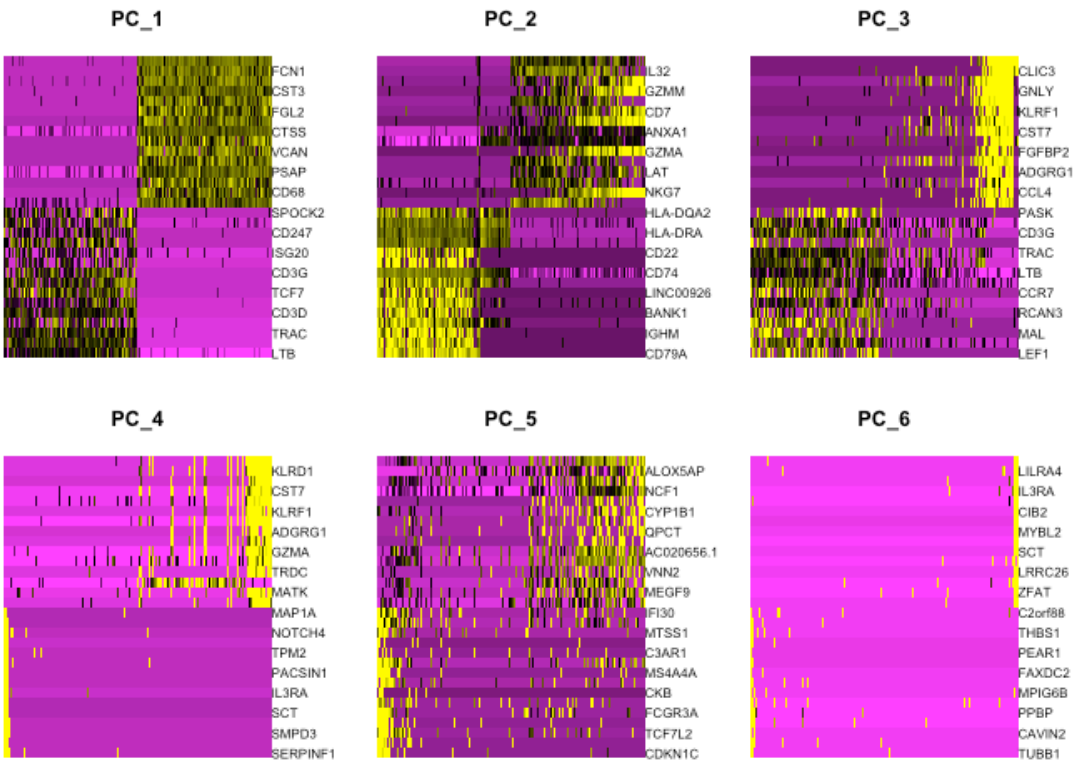
```
DimPlot(pbmc, reduction = "pca", group.by = "celltype")
```



We can see that only a few of the labeled cell-types separate well but many are clumped together on the bottom of the plot.

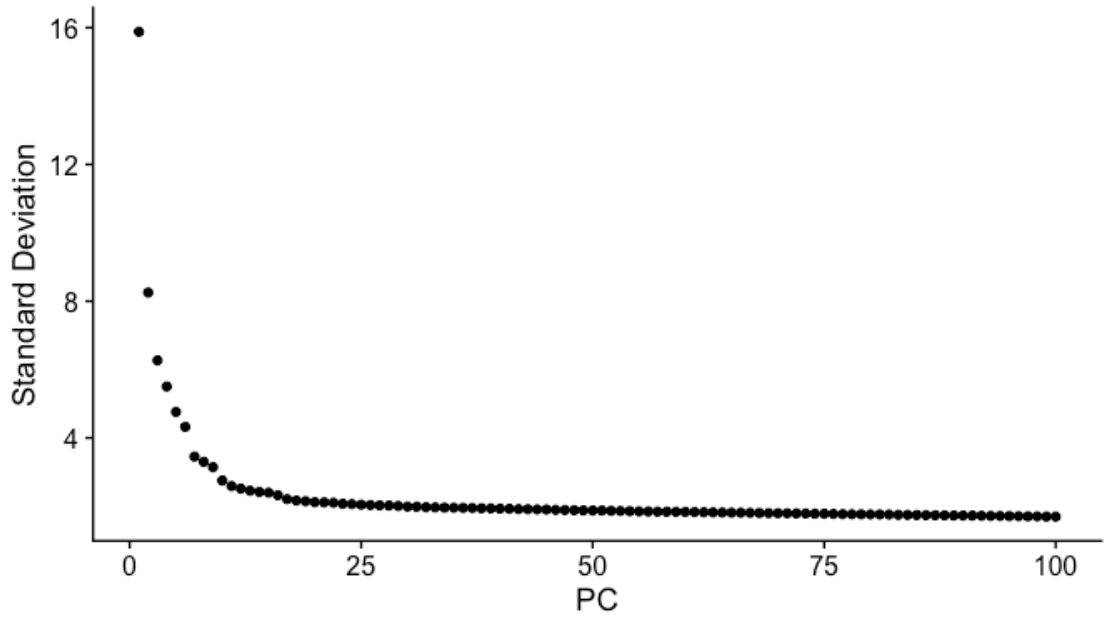
Let's have a look at the PCs to understand a bit better how PCA separates the data. Using the `DimHeatmap` function, we can plot the expression of the top genes for each PC for a number of cells. We will plot the first six components `dims = 1:6` for 500 random cells `cells = 500`. Each component will produce one heatmap, the cells will be the columns in the heatmap and the top genes for each component the rows.

```
DimHeatmap(pbmc, dims = 1:6, cells = 500, balanced = TRUE)
```



As discussed in the lecture, and indicated above, PCA is not optimal for visualization, but can be very helpful in reducing the complexity before applying non-linear dimensionality reduction methods. For that, let's have a look how many PCs actually cover the main variation. A very simple, fast-to-compute way is simply looking at the standard deviation per PC. We use the `ElbowPlot`.

```
ElbowPlot(pbmc, ndims = 100)
```



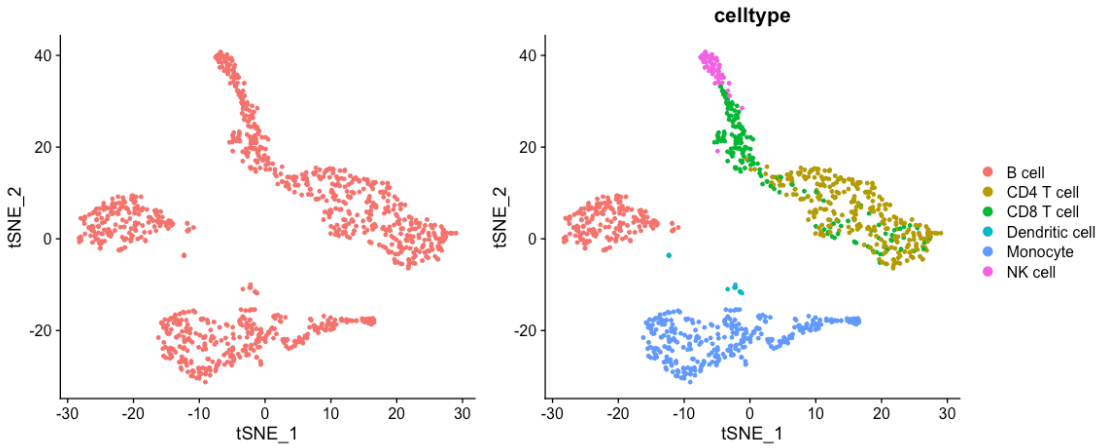
As we can see there is a very steep drop in standard deviation within the first 20 or so PCs indicating that we will likely be able to use roughly that number of PCs as input to following computations with little impact on the results.

t-SNE

Let's try out t-SNE. Seurat by default uses the Barnes Hut (BH) SNE implementation.

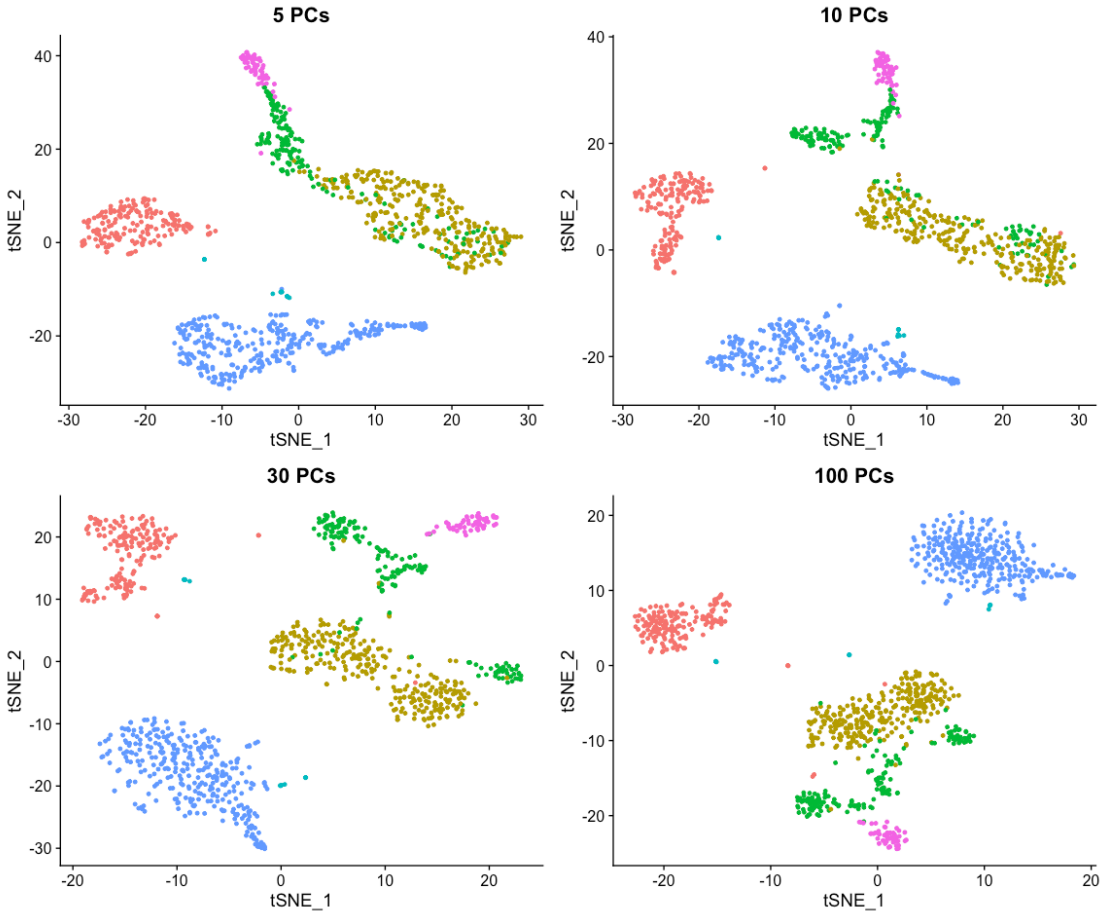
Similar to the PCA, Seurat provides a convenient function to run t-SNE called `RunTSNE`. We provide the `pbmc` data object as parameter. By default `RunTSNE` will look for and use the PCA we created above as input, we can also force it with `reduction = "pca"`. Again we use `DimPlot` to plot the result, this time using `reduction = "tsne"` to indicate that we want to plot the t-SNE computation. We create two plots, the first without and the second with the cell-types used for grouping. Already without the coloring, we can see much more structure in the plot than in the PCA plot. With the color overlay we see that most cell-types are nicely separated in the plot.

```
pbmc <- RunTSNE(pbmc, reduction = "pca")
p1 <- DimPlot(pbmc, reduction = "tsne") + NoLegend()
p2 <- DimPlot(pbmc, reduction = "tsne", group.by = "celltype")
p1 + p2
```



Above we did not specify the number of PCs to use as input. Let's have a look what happens with different numbers of PCs as input. We simply run `RunTSNE` multiple times with `dims` defining a range of PCs. *Note* every run overwrites the `tsne` object nested in the `pbmc` object. Therefore we plot the tsne directly after each run and store all plots in a object. We add `+ NoLegend() + ggtitle("n PCs")` to remove the list of cell types for compactness and add a title.

```
# PC_1 to PC_5
pbmc <- RunTSNE(pbmc, reduction = "pca", dims = 1:5)
p1 <- DimPlot(pbmc, reduction = "tsne", group.by = "celltype") + NoLegend() +
ggtitle("5 PCs")
# PC_1 to PC_10
pbmc <- RunTSNE(pbmc, reduction = "pca", dims = 1:10)
p2 <- DimPlot(pbmc, reduction = "tsne", group.by = "celltype") + NoLegend() +
ggtitle("10 PCs")
# PC_1 to PC_30
pbmc <- RunTSNE(pbmc, reduction = "pca", dims = 1:30)
p3 <- DimPlot(pbmc, reduction = "tsne", group.by = "celltype") + NoLegend() +
ggtitle("30 PCs")
# PC_1 to PC_100
pbmc <- RunTSNE(pbmc, reduction = "pca", dims = 1:100)
p4 <- DimPlot(pbmc, reduction = "tsne", group.by = "celltype") + NoLegend() +
ggtitle("100 PCs")
p1 + p2 + p3 + p4
```



Looking at these plots, it seems `RunTSNE` by default only uses 5 PCs (the plot is identical to the plot with default parameters above), but we get much clearer separation of clusters using 10 or even 30 PCs. This is not surprising considering the plot above of the standard deviation within the PCs above. Therefore we will use 30 PCs in the following, by explicitly setting `dims = 1:30`. *Note* that t-SNE is slower with more input dimensions (here the PCs), so it is good to find a middleground between capturing as much variation as possible with as few PCs as possible. However, using the default 5 does clearly not produce optimal results for this dataset. When using t-SNE with PCA preprocessing with your own data, always check how many PCs you need to cover the variance, as done above.

t-SNE has a few hyper-parameters that can be tuned for better visualization. There is an excellent tutorial. The main parameter is the perplexity, basically indicating how many neighbors to look at. We will run different perplexities to see the effect. As we will see, a perplexity of 30 is the default. This value often works well, again it might be advisable to test different values with other data. *Note*, higher perplexity values make t-SNE slower to compute.

```
# Perplexity 3
pbmc <- RunTSNE(pbmc, reduction = "pca", dims = 1:30, perplexity = 3)
p1 <- DimPlot(pbmc, reduction = "tsne", group.by = "celltype") + NoLegend() +
ggtitle("30PCs, Perplexity 3")
```

Question 3 *Visualize tSNE plots at different perplexity: Run the tSNE algorithm at perplexity 10, 30 and 200. Save the plots in p2, p3 and p4 plots and thereafter plot all four plots together.*

Another important parameter is the number of iterations. t-SNE gradually optimizes the low-dimensional space. The more iterations the more there is to optimize. We will run different numbers of iterations to see the effect. As we will see, 1000 iterations is the default. This value often works well, again it might be advisable to test different values with other data. Especially for larger datasets you will need more iterations. *Note*, more iterations make t-SNE slower to compute.

```
# 100 iterations
pbmc <- RunTSNE(pbmc, reduction = "pca", dims = 1:30, max_iter = 100)
p1 <- DimPlot(pbmc, reduction = "tsne", group.by = "celltype") + NoLegend() +
ggtitle("100 iterations")
```

Question 4: *Visualize tSNE plots at different iterations: Run the tSNE algorithm at iterations 500, 1000 and 2000. Save the plots in p2, p3 and p4 plots and thereafter plot all four plots together.*

Question 5: *What are the differences between the four tSNE plots from different iterations. At what iteration do you observe a good structure? Where did you observed good convergence of optimization? Upto which iteration can provide you good results?*

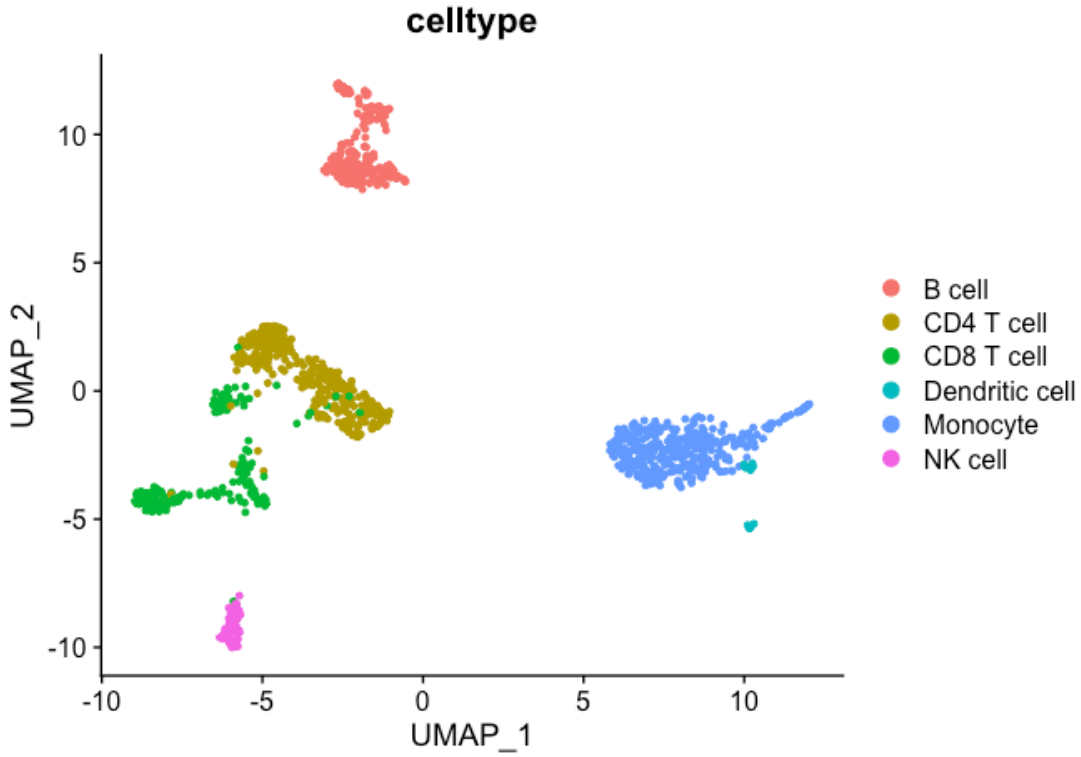
UMAP (OPTIONAL)

We have seen t-SNE and it's main parameters. Let's have a look at UMAP. Its main function call is very similar to t-SNE and PCA and called `RunUMAP`. Again, by default it looks for the PCA in the `pbmc` data object, but we have to provide it with the number of PCs (or `dims`) to use. Here, we use `30`. As expected, the plot looks rather similar to the t-SNE plot, with more compact clusters.

```
pbmc <- RunUMAP(pbmc, dims = 1:30, verbose = FALSE)

## Warning: The default method for RunUMAP has changed from calling Python UM
AP via reticulate to the R-native UWOT using the cosine metric
## To use Python UMAP via reticulate, set umap.method to 'umap-learn' and met
ric to 'correlation'
## This message will be shown once per session

DimPlot(pbmc, reduction = "umap", group.by = "celltype")
```



Just like t-SNE, UMAP has a bunch of parameters. In fact, Seurat exposes quite a few more than for t-SNE. We will look at the most important in the following. An interactive tutorial can be found here, and a comparison with the same datasets between UMAP and t-SNE.

Again, we start with a different number of PCs. Similar to t-SNE, 5 is clearly not enough, 30 provides decent separation and detail. Just like for t-SNE, test this parameter to match your own data in real-world experiments.

```
# PC_1 to PC_5
pbmc <- RunUMAP(pbmc, dims = 1:5, verbose = FALSE)
p1 <- DimPlot(pbmc, reduction = "umap", group.by = "celltype") + NoLegend() +
ggtitle("5 PCs")
```

Question UMAP1 (Optional) *Run UMAP based on different dimensions i.e. 1:10, 1:30 and 1:100. Save the plots in p2, p3 and p4 plots and thereafter plot all four plots together.*

The `n.neighbors` parameter sets the number of neighbors to consider for UMAP. This parameter is similar to the perplexity in t-SNE. We try a similar range of values for comparison. The results are quite similar to t-SNE. With low values, clearly the structures are too spread out, but quickly the embeddings become quite stable. The default vaule for RunUMAP is 30. Similar to t-SNE this value is quite general. Again, it's always a good idea to run some test with new data to find a good value.

```
# 3 Neighbors
pbmc <- RunUMAP(pbmc, dims = 1:30, n.neighbors = 3, verbose = FALSE)
```

```
p1 <- DimPlot(pbmc, reduction = "umap", group.by = "celltype") + NoLegend() +
ggtitle("3 Neighbors")
```

Question UMAP2 (Optional) *Run UMAP based on different neighbors i.e. 10, 30 and 200. Save the plots in p2, p3 and p4 plots and thereafter plot all four plots together.*

Another parameter is `min.dist`. There is no directly comparable parameter in t-SNE. Generally, `min.dist` defines the compactness of the final embedding. The default value is 0.3.

```
# Min Distance 0.01
pbmc <- RunUMAP(pbmc, dims = 1:30, min.dist = 0.01, verbose = FALSE)
p1 <- DimPlot(pbmc, reduction = "umap", group.by = "celltype") + NoLegend() +
ggtitle("Min Dist 0.01")
```

Question UMAP3 (Optional) *Run UMAP based on different distances i.e. 0.1, 0.3 and 1.0. Save the plots in p2, p3 and p4 plots and thereafter plot all four plots together.*

`n.epochs` is comparable to the number of iterations in t-SNE. Typically, UMAP needs fewer of these to converge, but also changes more when it is run longer. The default is 500. Again, this should be adjusted to your data.

```
# 10 epochs
pbmc <- RunUMAP(pbmc, dims = 1:30, n.epochs = 10, verbose = FALSE)
p1 <- DimPlot(pbmc, reduction = "umap", group.by = "celltype") + NoLegend() +
ggtitle("10 Epochs")
```

Question UMAP4 (Optional) *Run UMAP based on different epochs i.e. 100, 500 and 1000. Save the plots in p2, p3 and p4 plots and thereafter plot all four plots together.*

Finally `RunUMAP` allows to set the distance metric for the high-dimensional space. While in principle this is also possible with t-SNE, `RunTSNE`, and most other implementations, do not provide this option. The four possibilities, `Euclidean`, `cosine`, `manhattan`, and `hamming` distance are shown below. `Cosine` distance is the default (RunTSNE uses Euclidean distances).

There is not necessarily a clear winner. Hamming distances perform worse but they are usually used for different data, such as text as they ignore the numerical difference for a given comparison. Going with the default cosine is definitely not a bad choice in most applications.

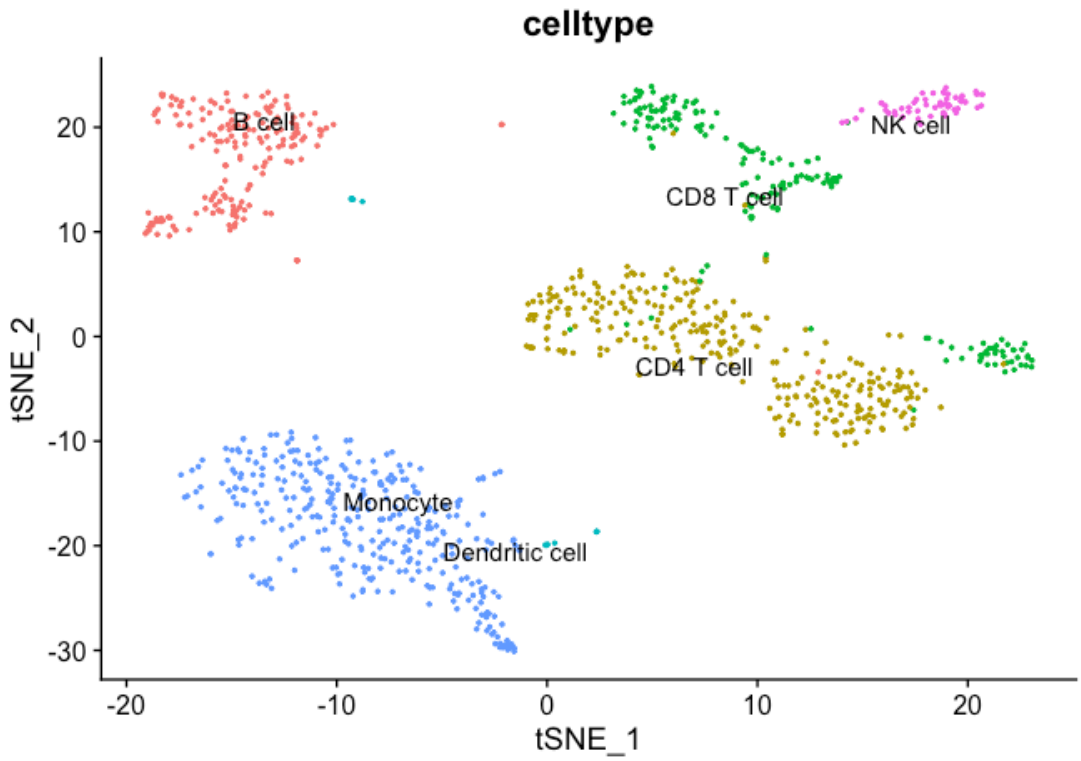
```
# Euclidean distance
pbmc <- RunUMAP(pbmc, dims = 1:30, metric = "euclidean", verbose = FALSE)
p1 <- DimPlot(pbmc, reduction = "umap", group.by = "celltype") + NoLegend() +
ggtitle("Euclidean")
```

Question UMAP5 (Optional) *Run UMAP based on different distance metrics i.e. cosine, manhattan and hamming. Save the plots in p2, p3 and p4 plots and thereafter plot all four plots together.*

Visualization

Finally, let's have a brief look at some visualization options. We have already use color for grouping. With `label = TRUE` we can add a text-label to each group and with `repel = TRUE` we can make sure those labels don't clump together. Finally, `pt.size = 0.5` changes the size of the dots used in the plot.

```
# Re-run a t-SNE so we do not rely on changes above
pbmc <- RunTSNE(pbmc, dims = 1:30)
DimPlot(pbmc, reduction = "tsne", group.by = "celltype", label = TRUE, repel
= TRUE, pt.size = 0.5) + NoLegend()
```



Another property we might want to look at in our dimensionality reduction plot is the expression of individual genes. We can overlay gene expression as color using the `FeaturePlot`. Here, we first find the *top two* features correlated to the *first and second PCs* and combine them into a single vector which will be the parameter for the `FeaturePlot`.

Finally, we call `FeaturePlot` with the `pbmc` data object, `features = topFeaturesPC` uses the extracted feature vector to create one plot for each feature in the list and lastly, `reduction = "pca"` will create PCA plots.

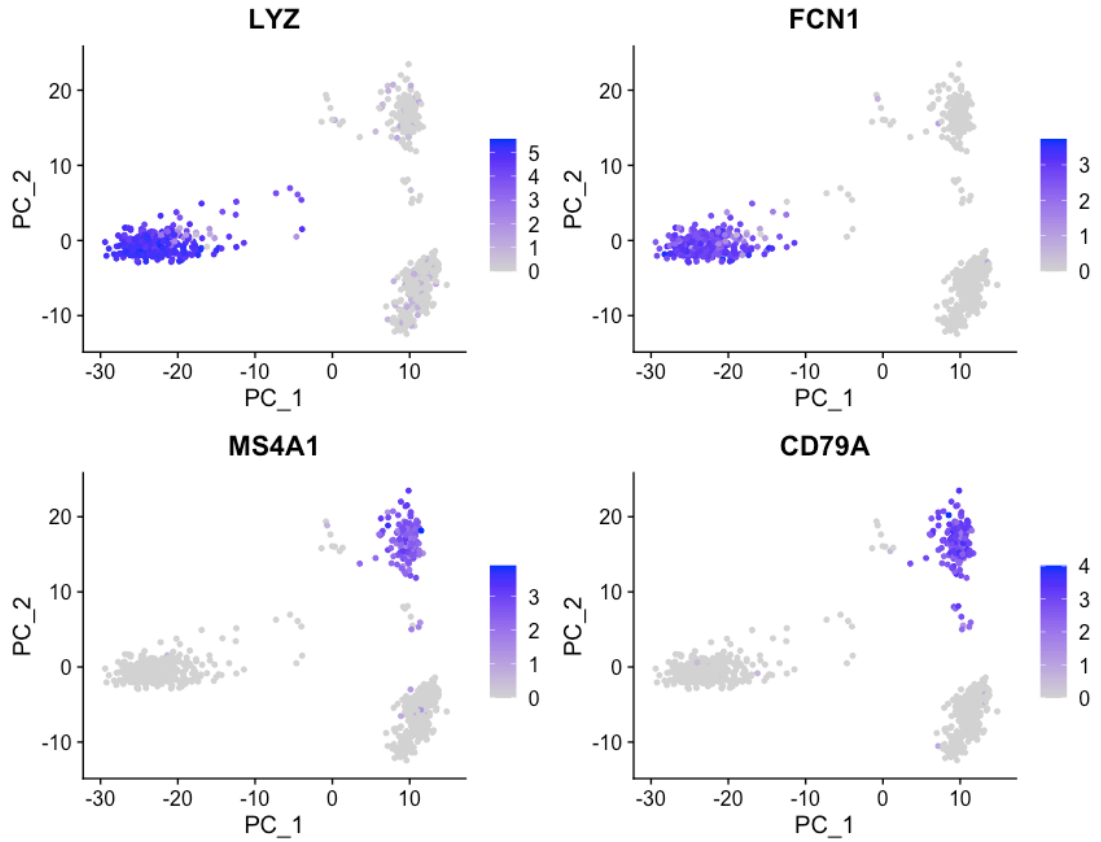
Not surprisingly, the top two features of the first PC form a smooth gradient on the PC_1 axis and the top two features of the second PC a smooth gradient on the PC_2 axis.

```
# find top genes for PCs 1 and 2
topFeaturesPC1 <- TopFeatures(object = pbmc[["pca"]], nfeatures = 2, dim = 1)
```

```
topFeaturesPC2 <- TopFeatures(object = pbmc[["pca"]], nfeatures = 2, dim = 2)
# combine the genes into a single vector
topFeaturesPC <- c(topFeaturesPC1, topFeaturesPC2)
print(topFeaturesPC)

## [1] "LYZ"   "FCN1"  "MS4A1" "CD79A"

# feature plot with the defined genes
FeaturePlot(pbmc, features = topFeaturesPC, reduction = "pca")
```



Question 6 *Make a featurePlot of the data using a t-SNE plot and observe the differences between PCA and tSNE based feature plot.*

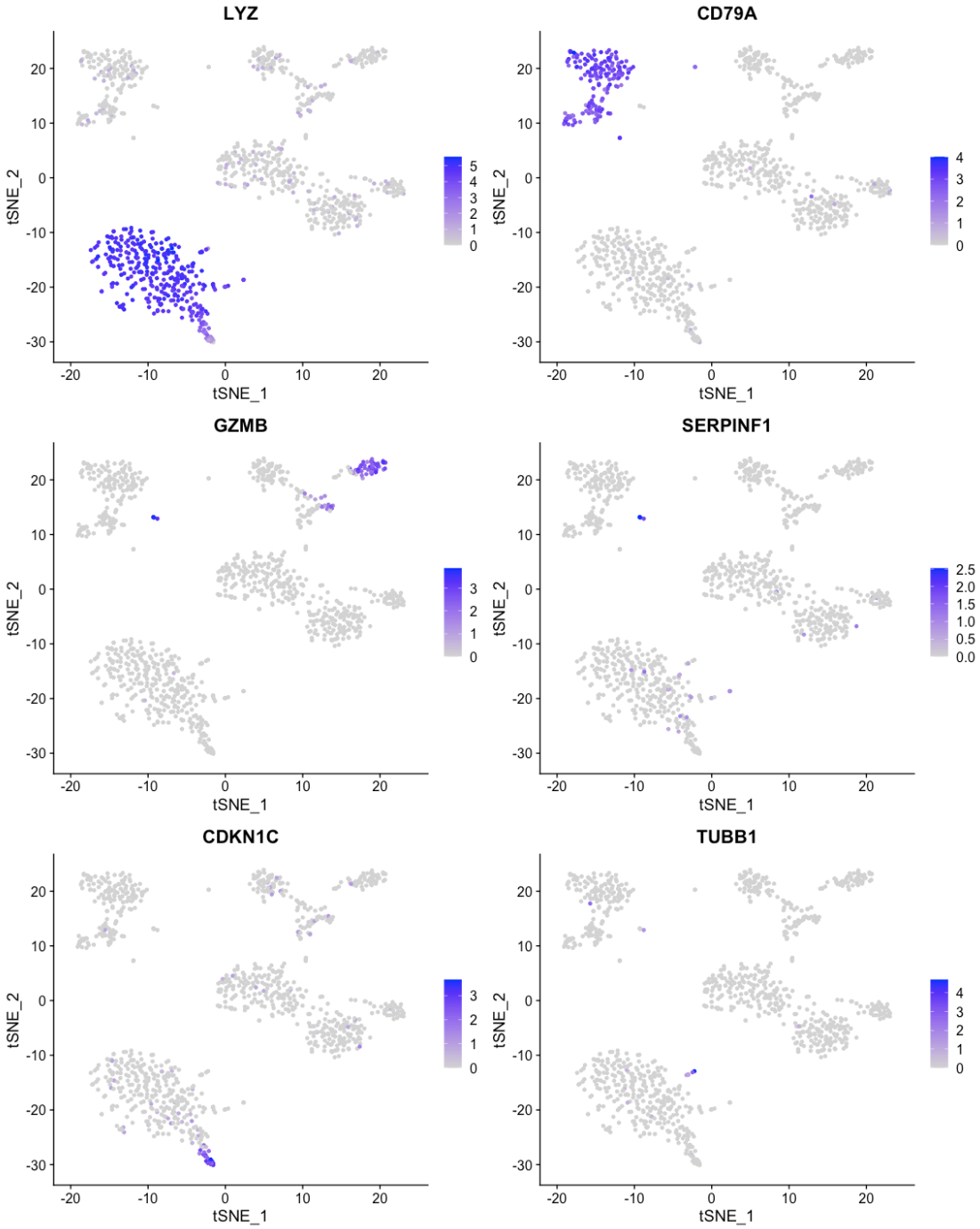
The behavior here is quite different, with the high expression being very localized to specific clusters in the maps. Again, not surprising as t-SNE uses these

It is clear that the top PCs are fundamental to forming the clusters, so let's have a look at more PCs and pick the top gene per PC for a few more PCs.

```
for(i in 1:6) {
  topFeaturesPC[[i]] <- TopFeatures(object = pbmc[["pca"]], nfeatures = 1, di
m = i)
}
print(topFeaturesPC)
```

```
## [1] "LYZ"      "CD79A"    "GZMB"     "SERPINF1" "CDKN1C"   "TUBB1"
```

```
FeaturePlot(pbmc, features = topFeaturesPC, reduction = "tsne")
```



Finally, let's create a more interactive plot. First we create a regular `FeaturePlot`, here with just one gene `features = "CD3D"`, a marker of T cells. Instead of plotting it directly we save the plot in the `interactivePlot` variable.

With `HoverLocator` we can then embed this plot into an interactive version. The `information = FetchData(pbmc, vars = c("celltype", topFeaturesPC))` creates a set of properties that will be shown on hover over each point. Im this case, we show the cell-type from the meta information.

The result is a plot that allows us to inspect single cells in detail.

```
interactivePlot <- FeaturePlot(pbmc, reduction = "tsne", features = "CD3D")
HoverLocator(plot = interactivePlot, information = FetchData(pbmc, vars = c("
celltype", topFeaturesPC)))
```

Saving the data

Save the Seurat object with the new embeddings for future use downstream.

```
saveRDS(pbmc, file = "pbmc3k_DR.rds")
```

If you're interested in more ways to visualize your data, this vignette might be useful.

Session info

```
sessionInfo()

## R version 4.1.0 (2021-05-18)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Mojave 10.14.6
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRbla
s.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlap
ack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] ggplot2_3.3.5      SeuratObject_4.0.2 Seurat_4.0.4
##
## loaded via a namespace (and not attached):
##   [1] nlme_3.1-153          spatstat.sparse_2.0-0 matrixStats_0.60.1
##   [4] RcppAnnoy_0.0.19      RColorBrewer_1.1-2    httr_1.4.2
##   [7] sctransform_0.3.2     tools_4.1.0           utf8_1.2.2
##  [10] R6_2.5.1              irlba_2.3.3           rpart_4.1-15
##  [13] KernSmooth_2.23-20    uwot_0.1.10           mgcv_1.8-36
##  [16] DBI_1.1.1             lazyeval_0.2.2        colorspace_2.0-2
##  [19] withr_2.4.2           tidyselect_1.1.1      gridExtra_2.3
##  [22] compiler_4.1.0        plotly_4.9.4.1        labeling_0.4.2
##  [25] scales_1.1.1          spatstat.data_2.1-0   lmtest_0.9-38
```

```
##  [28] ggridges_0.5.3        pbapply_1.5-0         goftest_1.2-2
##  [31] stringr_1.4.0         digest_0.6.27         spatstat.utils_2.2-0
##  [34] rmarkdown_2.11        pkgconfig_2.0.3       htmltools_0.5.2
##  [37] parallelly_1.28.1     highr_0.9             fastmap_1.1.0
##  [40] htmlwidgets_1.5.4     rlang_0.4.11          FNN_1.1.3
##  [43] shiny_1.6.0           farver_2.1.0          generics_0.1.0
##  [46] zoo_1.8-9             jsonlite_1.7.2        ica_1.0-2
##  [49] dplyr_1.0.7           magrittr_2.0.1        patchwork_1.1.1
##  [52] Matrix_1.3-4          Rcpp_1.0.7            munsell_0.5.0
##  [55] fansi_0.5.0           abind_1.4-5           reticulate_1.22
##  [58] lifecycle_1.0.0       stringi_1.7.4         yaml_2.2.1
##  [61] MASS_7.3-54           Rtsne_0.15            plyr_1.8.6
##  [64] grid_4.1.0            parallel_4.1.0        listenv_0.8.0
##  [67] promises_1.2.0.1      ggrepel_0.9.1         crayon_1.4.1
##  [70] deldir_0.2-10         miniUI_0.1.1.1        lattice_0.20-44
##  [73] cowplot_1.1.1         splines_4.1.0         tensor_1.5
##  [76] knitr_1.34            pillar_1.6.2          igraph_1.2.6
##  [79] spatstat.geom_2.2-2   future.apply_1.8.1    reshape2_1.4.4
##  [82] codetools_0.2-18      leiden_0.3.9          glue_1.4.2
##  [85] evaluate_0.14         data.table_1.14.0     png_0.1-7
##  [88] vctrs_0.3.8           httpuv_1.6.3          polyclip_1.10-0
##  [91] gtable_0.3.0          RANN_2.6.1            purrr_0.3.4
##  [94] spatstat.core_2.3-0   tidyr_1.1.3           scattermore_0.7
##  [97] future_1.22.1         assertthat_0.2.1      xfun_0.26
## [100] mime_0.11             xtable_1.8-4          RSpectra_0.16-0
## [103] later_1.3.0           survival_3.2-13       viridisLite_0.4.0
## [106] tibble_3.1.4          cluster_2.1.2         globals_0.14.0
## [109] fitdistrplus_1.1-5    ellipsis_0.3.2        ROCR_1.0-11
```