Pancreas example

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Vignette Template

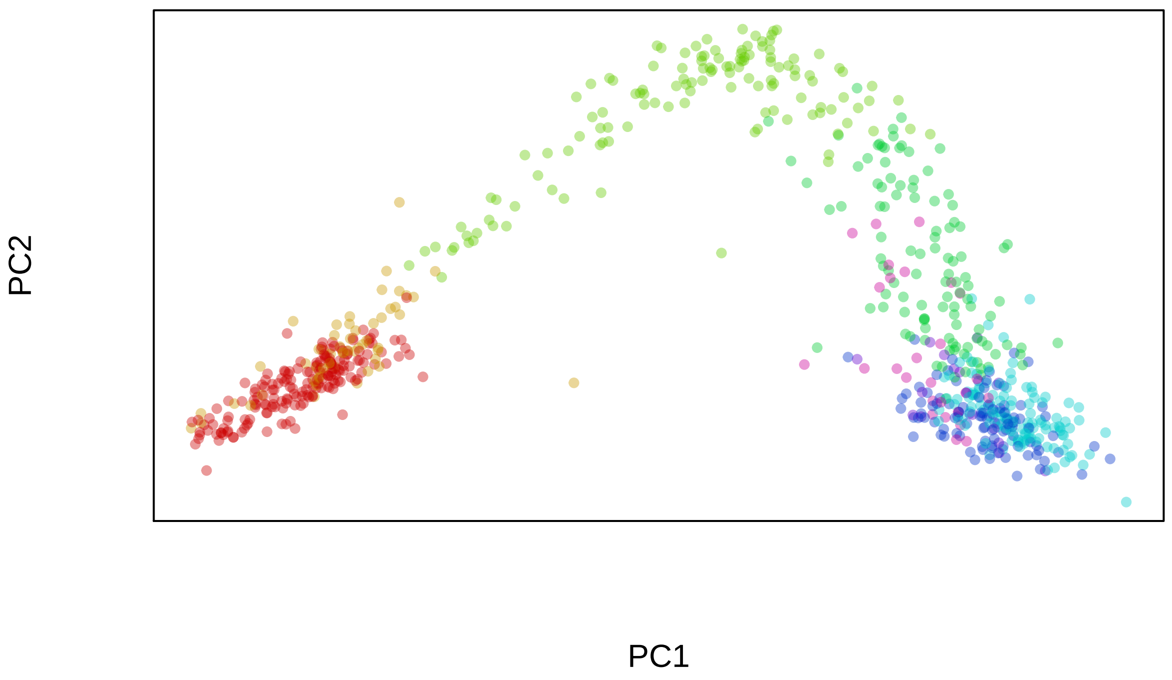
```r
library(reticulate)
use_condaenv("r-velocity", required = TRUE)
scv = import("scvelo")
adata = scv$datasets$pancreas()

## spliced and unspliced expression matrices
spliced = as.matrix(t(adata$layers['spliced']))
unspliced = as.matrix(t(adata$layers['unspliced']))
cells = adata$obs_names$values
genes = adata$var_names$values
colnames(spliced) = colnames(unspliced) = cells
rownames(spliced) = rownames(unspliced) = genes
## extract clusters
clusters = adata$obs$clusters
names(clusters) = adata$obs_names$values
## old embedding
emb.original = adata$obsm['X_umap'] #extract umap embedding
rownames(emb.original) <- names(clusters)
## plot
par(mfrow = c(1,1))
plotEmbedding(emb.original, groups=clusters,
              xlab = "UMAP X", ylab = "UMAP Y",
              mark.clusters=TRUE)

## subsample to create smaller dataset
## that can be included with package
set.seed(0)
good.cells = sample(rownames(emb.original), nrow(emb.original)/5)
spliced = spliced[,good.cells]
unspliced = unspliced[,good.cells]
clusters = clusters[good.cells]
emb.original = emb.original[good.cells,]
## plot
par(mfrow = c(1,1))
plotEmbedding(emb.original, groups=clusters,
              xlab = "UMAP X", ylab = "UMAP Y",
              mark.clusters=TRUE)

## filter to well detected genes
vi = rowSums(spliced) > 10 & rowSums(unspliced) > 10
```

```r
spliced = spliced[vi,]
unspliced = unspliced[vi,]

## analyze
all.counts = spliced + unspliced # use combined spliced and unspliced counts
all.cpm = normalizeCounts(all.counts) # cpm normalize
ods.genes = normalizeVariance(all.cpm) # identify overdispersed genes
all.logODS = log10(all.cpm[ods.genes,]+1) # log transform
pca = RSpectra::svds(A = t(all.logODS),
                     k=50, # 50 PCs
                     opts = list(center = TRUE, scale = TRUE))
pcs = pca$u
rownames(pcs) <- colnames(all.counts)
colnames(pcs) <- paste0('PC', 1:ncol(pcs))
cell.dist = as.dist(1-cor(t(pcs))) # cell distance in PC space

## velocity model
library(velocyto.R)
vel = gene.relative.velocity.estimates(spliced,
                                       unspliced,
                                       kCells=30,
                                       cell.dist=cell.dist,
                                       fit.quantile=0.1)

pancreas <- list(
  spliced = spliced,
  unspliced = unspliced,
  clusters = clusters,
  pcs = pcs,
  cell.dist = cell.dist,
  vel = vel
)
usethis::use_data(pancreas, overwrite=TRUE)
```

Compare visualizations

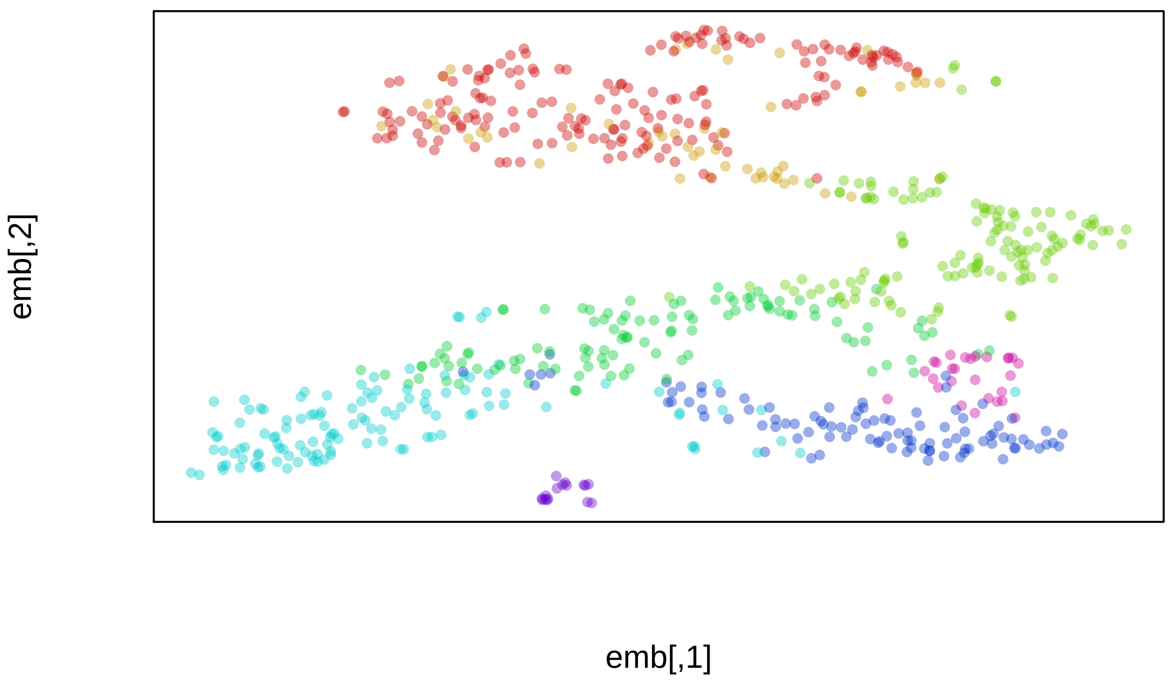
```r
## load data
library(veloviz)
data("pancreas")

## 2D embedding by PCA
emb.pcs = pancreas$pcs[,1:2]
plotEmbedding(emb.pcs, groups=pancreas$clusters)
```

```
## using provided groups as a factor
```



```
## 2D embedding by tSNE
set.seed(0)
emb.tsne = Rtsne::Rtsne(pancreas$pcs[,1:10], perplexity=30)$Y
rownames(emb.tsne) <- rownames(pancreas$pcs)
plotEmbedding(emb.tsne, groups=pancreas$clusters)
```

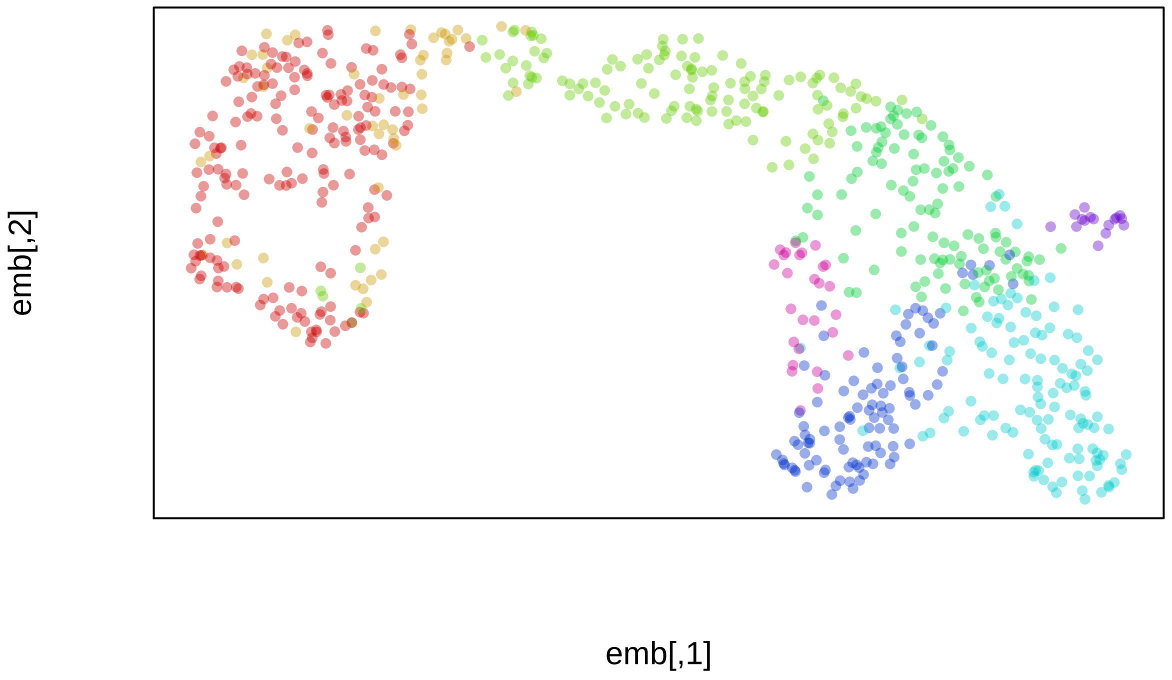
```
## using provided groups as a factor
```



```
## 2D embedding by UMAP
set.seed(0)
emb.umap = uwot::umap(pancreas$pcs[,1:10], min_dist = 0.5)
rownames(emb.umap) <- rownames(pancreas$pcs)
```

```r
plotEmbedding(emb.umap, groups=pancreas$clusters)
```

```
## using provided groups as a factor
```

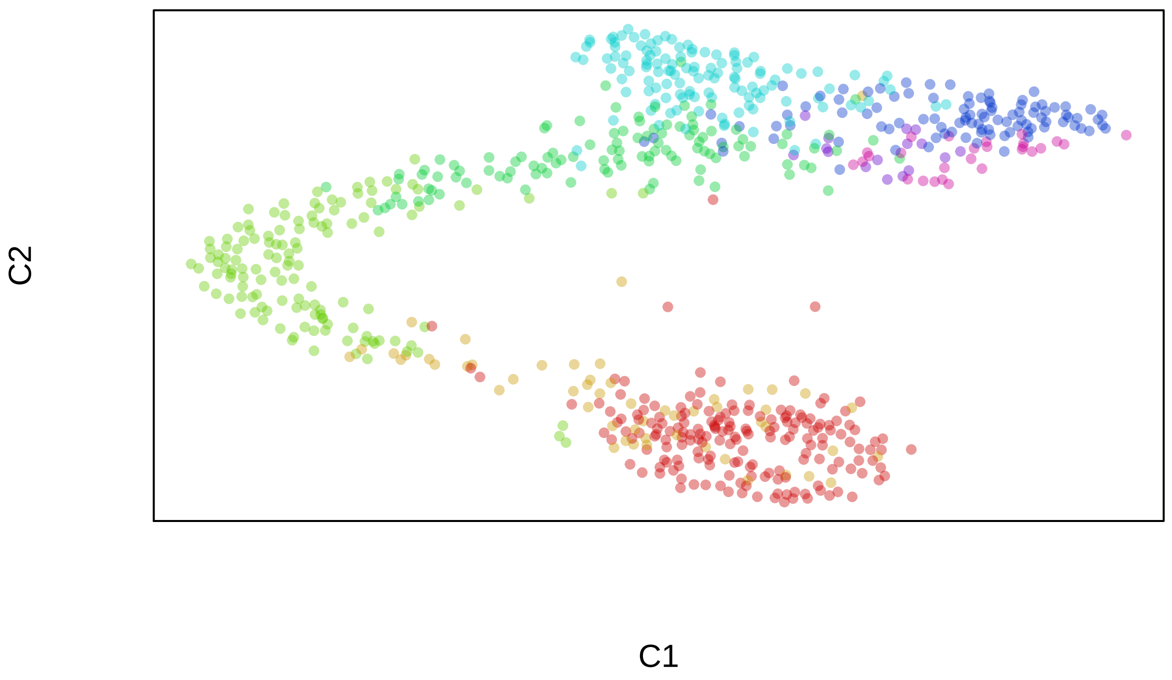


```r
## 2D embedding by veloviz
vig = buildVeloviz(
  curr = pancreas$vel$curr,
  proj = pancreas$vel$proj,
  normalize.depth = TRUE,
  use.ods.genes = TRUE,
  alpha = 0.05,
  pca = TRUE,
  nPCs = 10,
  center = TRUE,
  scale = TRUE,
  k = 10,
  seed = 0,
  verbose = FALSE
)
```

```
## Warning in if (!class(curr) %in% c("dgCMatrix", "dgTMatrix")) {: the condition
## has length > 1 and only the first element will be used

## Warning in if (!class(proj) %in% c("dgCMatrix", "dgTMatrix")) {: the condition
## has length > 1 and only the first element will be used

## [1] "Done finding neighbors"
## [1] "Done making graph"
```

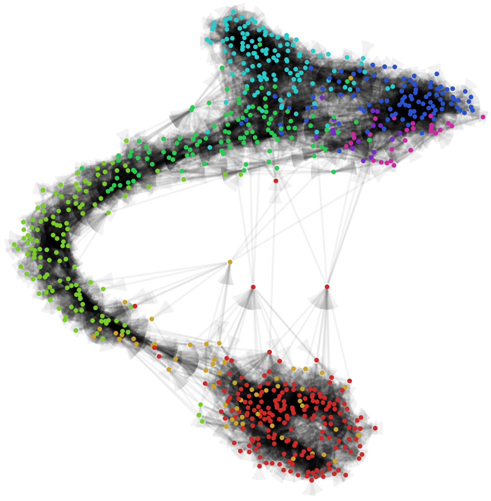
```r
emb.veloviz = vig$fdg_coords
plotEmbedding(emb.veloviz, groups=pancreas$clusters)
```

```
## using provided groups as a factor
```



```
g = plotVeloviz(vig, clusters=pancreas$clusters, seed=0)
```

```
## Warning in if (is.na(clusters)) {: the condition has length > 1 and only the
## first element will be used
```



Now we remove cells

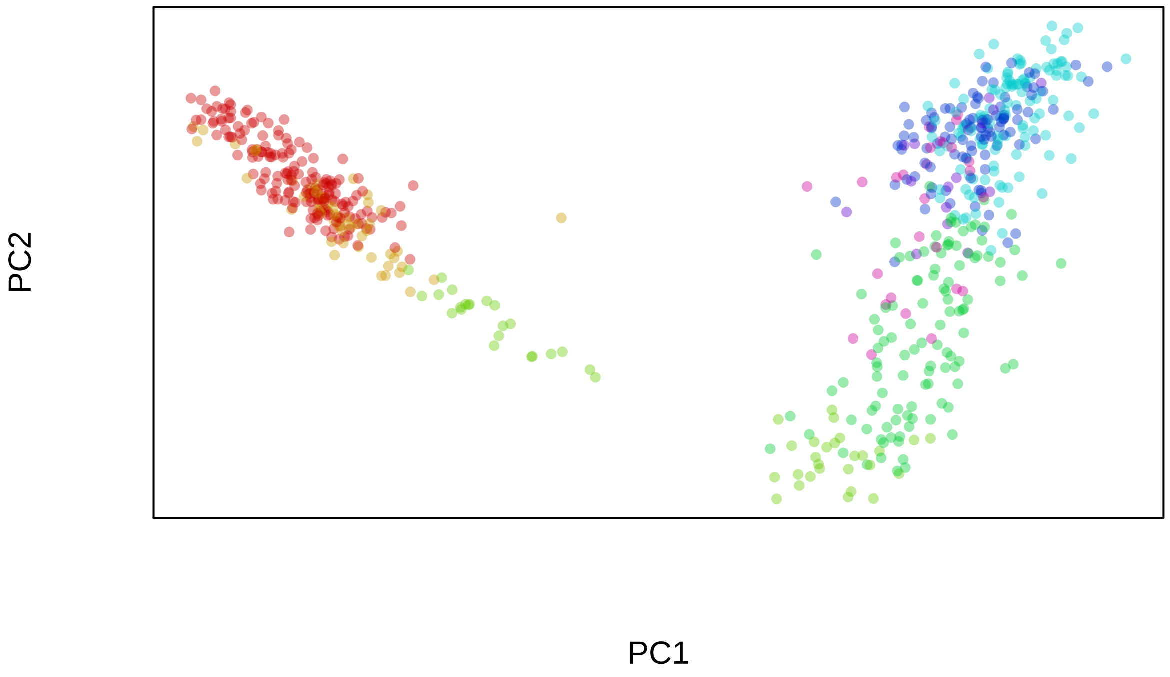
```
## remove EP cells along original trajectory
x = emb.original[,1]
vi = x > -5 & x < 0
good.cells = rownames(emb.original)[!vi]
plotEmbedding(emb.original[good.cells,], groups=clusters,
              xlab = "UMAP X", ylab = "UMAP Y", mark.clusters=TRUE)
spliced = spliced[,good.cells]
unspliced = unspliced[,good.cells]
clusters = clusters[good.cells]

## analyze
```

```r
all.counts = spliced + unspliced # use combined spliced and unspliced counts
all.cpm = normalizeCounts(all.counts) # cpm normalize
ods.genes = normalizeVariance(all.cpm) # identify overdispersed genes
all.logODS = log10(all.cpm[ods.genes,]+1) # log transform
pca = RSpectra::svds(A = t(all.logODS),
                     k=50, # 50 PCs
                     opts = list(center = TRUE, scale = TRUE))
pcs = pca$u
rownames(pcs) <- colnames(all.counts)
colnames(pcs) <- paste0('PC', 1:ncol(pcs))
cell.dist = as.dist(1-cor(t(pcs))) # cell distance in PC space

## velocity model
library(velocyto.R)
vel = gene.relative.velocity.estimates(spliced,
                                       unspliced,
                                       kCells=30,
                                       cell.dist=cell.dist,
                                       fit.quantile=0.1)

pancreasWithGap <- list(
  spliced = spliced,
  unspliced = unspliced,
  clusters = clusters,
  pcs = pcs,
  cell.dist = cell.dist,
  vel = vel
)
usethis::use_data(pancreasWithGap, overwrite=TRUE)
```

Compare

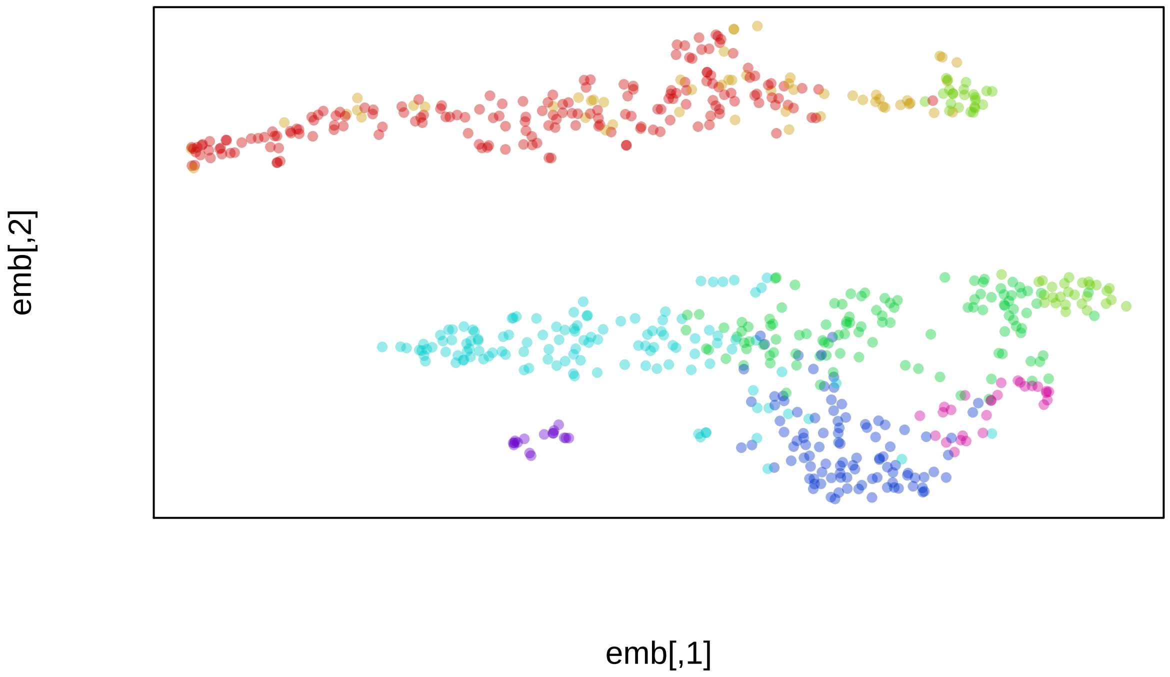
```r
## load data
library(veloviz)
data("pancreasWithGap")

## 2D embedding by PCA
emb.pcs = pancreasWithGap$pcs[,1:2]
plotEmbedding(emb.pcs, groups=pancreasWithGap$clusters)
```

```
## using provided groups as a factor
```



```
## 2D embedding by tSNE
set.seed(0)
emb.tsne = Rtsne::Rtsne(pancreasWithGap$pcs[,1:10], perplexity=30)$Y
rownames(emb.tsne) <- rownames(pancreasWithGap$pcs)
plotEmbedding(emb.tsne, groups=pancreasWithGap$clusters)
```

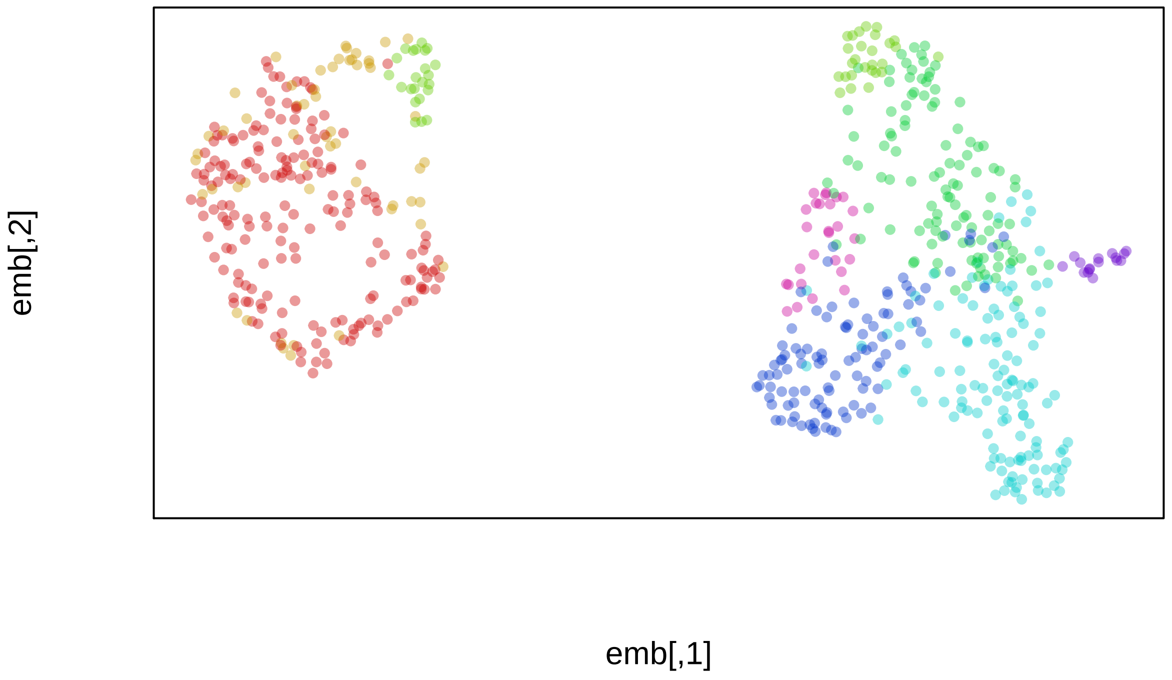
```
## using provided groups as a factor
```



```
## 2D embedding by UMAP
set.seed(0)
emb.umap = uwot::umap(pancreasWithGap$pcs[,1:10], min_dist = 0.5)
rownames(emb.umap) <- rownames(pancreasWithGap$pcs)
```

```r
plotEmbedding(emb.umap, groups=pancreasWithGap$clusters)
```

```
## using provided groups as a factor
```



```r
## 2D embedding by veloviz
vig = buildVeloviz(
  curr = pancreasWithGap$vel$curr,
  proj = pancreasWithGap$vel$proj,
  normalize.depth = TRUE,
  use.ods.genes = TRUE,
  alpha = 0.05,
  pca = TRUE,
  nPCs = 10,
  center = TRUE,
  scale = TRUE,
  k = 10,
  seed = 0,
  verbose = FALSE
)
```

```
## Warning in if (!class(curr) %in% c("dgCMatrix", "dgTMatrix")) {: the condition
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## [1] "Done finding neighbors"
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```r
emb.veloviz = vig$fdg_coords
plotEmbedding(emb.veloviz, groups=pancreasWithGap$clusters)
```

```
## using provided groups as a factor
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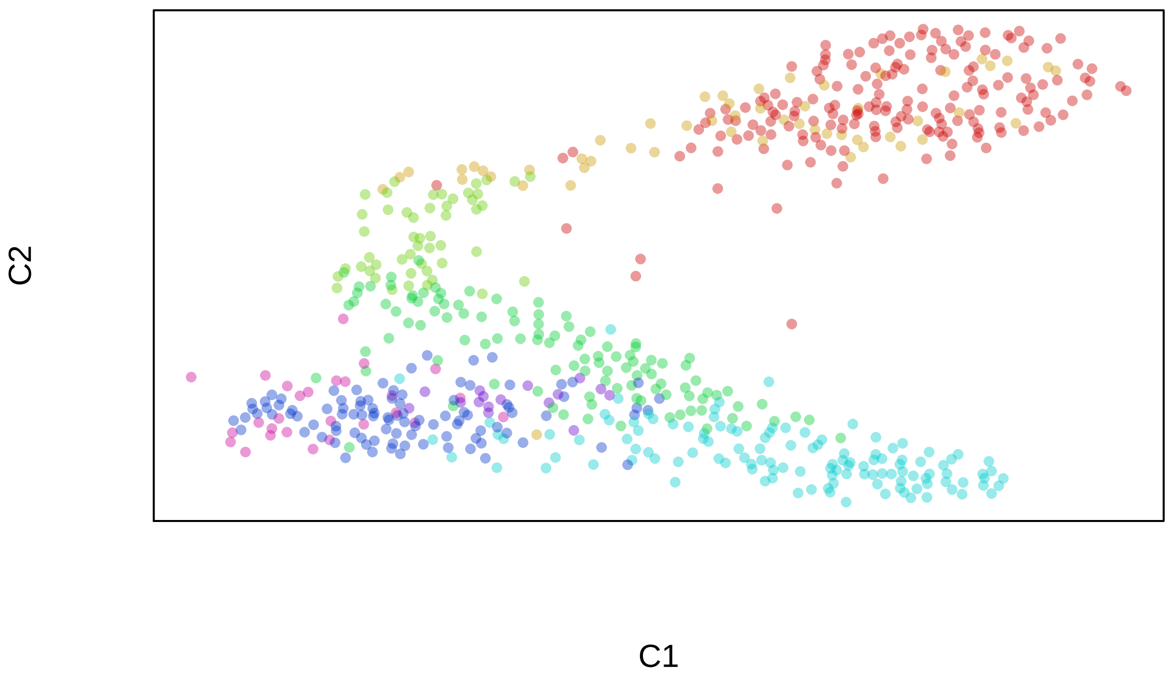

```
g = plotVeloviz(vig, clusters=pancreasWithGap$clusters, seed=0)
```

```
## Warning in if (is.na(clusters)) {: the condition has length > 1 and only the
## first element will be used
```