Pancreas example

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11/13/2020

Visualization using VeloViz

Preprocessing

Inputs to VeloViz are the scores in PCA space of the current and projected transcriptional states, which we get here by calculating RNA velocity using velocyto.
To get current and projected PC scores from raw counts, we first follow standard filtering, normalization, and dimensional reduction steps and then calculate velocity. (Steps 1-4 can be skipped by loading the example dataset in the VeloViz package - see 4*).

0) Get Data:

```
#getting pancreas data from scVelo
use_condaenv("cellrank", required = TRUE)
scv = import("scvelo")
adata = scv$datasets$pancreas()

#extract spliced, unspliced counts
spliced <- as.matrix(Matrix::t(adata$layers['spliced']))
unspliced <- as.matrix(Matrix::t(adata$layers['unspliced']))
cells <- adata$obs_names$values
genes <- adata$var_names$values
colnames(spliced) <- colnames(unspliced) <- cells
rownames(spliced) <- rownames(unspliced) <- genes

#clusters
clusters <- adata$obs$clusters
names(clusters) <- adata$obs_names$values

#subsample to make things faster
set.seed(0)
good.cells <- sample(cells, length(cells)/5)
spliced <- spliced[,good.cells]
unspliced <- unspliced[,good.cells]
clusters <- clusters[good.cells]

dim(spliced)
dim(unspliced)
```

1) Filter good genes

```
#keep genes with >10 total counts
good.genes = genes[rowSums(spliced) > 10 & rowSums(unspliced) > 10]
spliced = spliced[good.genes,]
unspliced = unspliced[good.genes,]

dim(spliced)
dim(unspliced)
```

2) Normalize

```
counts = spliced + unspliced # use combined spliced and unspliced counts
cpm = normalizeDepth(counts) # normalize to counts per million
```

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

```
## Converting to sparse matrix ...
```

```
## Normalizing matrix with 739 cells and 7192 genes
```

```
varnorm = normalizeVariance(cpm) # variance stabilize, find overdispersed genes
```

```
## Using general additive modeling with k = 5...
```

```
## Identifed 1730 overdispersed genes using
##     adjusted p-value threshold alpha = 0.05
```

```
lognorm = log10(varnorm + 1) # log normalize
```

3) Reduce Dimensions
After filtering and normalizing, we reduce dimensions, and calculate cell-cell distance in PC space. This distance will be used to compute velocity.

```
#PCA on centered and scaled expression of overdispersed genes
pcs = reduceDimensions(lognorm, center = TRUE, scale = TRUE, nPCs = 50)
```

```
## Centering...
```

```
## Using unit variance...
```

```
#cell distance in PC space
cell.dist = as.dist(1-cor(t(pcs))) # cell distance in PC space
```

Velocity

4) Calculate velocity
Next, we compute velocity from spliced and unspliced counts and cell-cell distances using velocyto. This will give us the current and projected transcriptional states.

```
vel = gene.relative.velocity.estimates(spliced,
                                       unspliced,
                                       kCells = 30,
                                       cell.dist = cell.dist,
                                       fit.quantile = 0.1)
```

4*) Load example from VeloViz
This example dataset is available with the veloviz package.

```
clusters = pancreas$clusters # cell type annotations
pcs = pancreas$pcs # PCs used to make other embeddings (UMAP,tSNE..)
vel = pancreas$vel # velocity
```

5) Normalize current and projected
Now that we have the current and projected expression, we want to go through a similar normalization process as we did with the raw counts and then reduce dimensions in PCA. Steps 5-7 can be done together using the `buildVeloviz` function (see 7*).

```
curr = vel$current
proj = vel$projected

#normalize depth
curr.norm = normalizeDepth(curr)
```

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

```
## Converting to sparse matrix ...
```

```
## Normalizing matrix with 739 cells and 4933 genes
```

```
proj.norm = normalizeDepth(proj)
```

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

```
## Converting to sparse matrix ...
## Normalizing matrix with 739 cells and 4933 genes
```

```
#variance stabilize current
curr.varnorm.info = normalizeVariance(curr.norm, details = TRUE)
```

```
## Using general additive modeling with k = 5...
```

```
## Identifed 1122 overdispersed genes using
##     adjusted p-value threshold alpha = 0.05
```

```
curr.varnorm = curr.varnorm.info$matnorm

#use same model for projected
scale.factor = curr.varnorm.info$df$scale_factor #gene scale factors
names(scale.factor) = rownames(curr.varnorm.info$df)

m = proj.norm
rmean = Matrix::rowMeans(m) #row mean
```

```
sumx = Matrix::rowSums(m)
sumxx = Matrix::rowSums(m^2)
rsd = sqrt((sumxx - 2 * sumx * rmean + ncol(m) * rmean ^ 2) / (ncol(m)-1)) #row sd

proj.varnorm = proj.norm / rsd * scale.factor[names(rsd)]
proj.varnorm = proj.norm[rownames(curr.varnorm),]
```

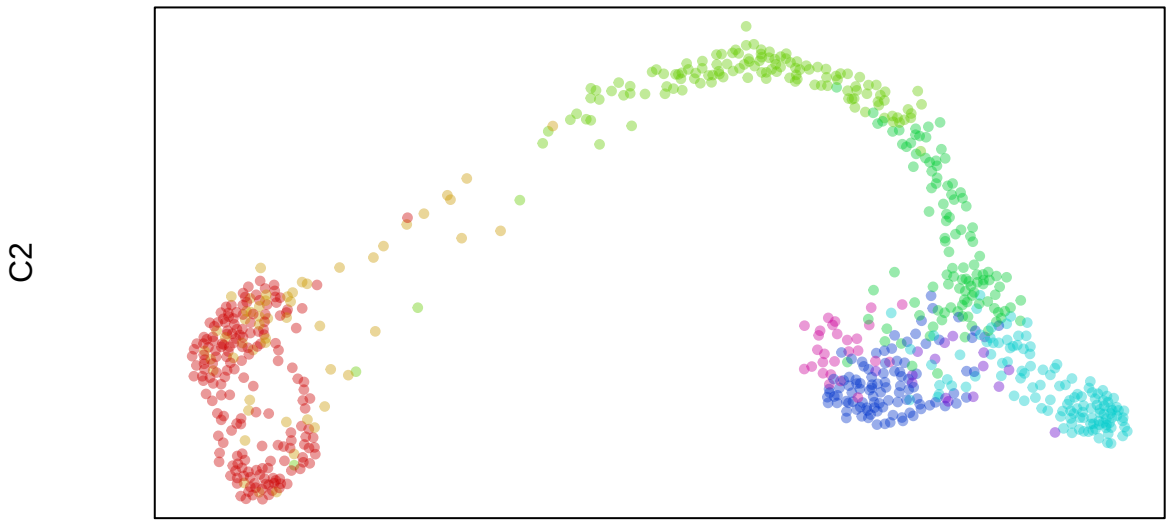
6) Project current and projected into PC space

```
#log normalize
curr.pca = log10(curr.varnorm + 1)
proj.pca = log10(proj.varnorm + 1)

#mean center
c.rmean = Matrix::rowMeans(curr.pca)
curr.pca = curr.pca - c.rmean
p.rmean = Matrix::rowMeans(proj.pca)
proj.pca = proj.pca - p.rmean

#scale variance
c.sumx = Matrix::rowSums(curr.pca)
c.sumxx = Matrix::rowSums(curr.pca^2)
c.rsd = sqrt((c.sumxx - 2*c.sumx*c.rmean + ncol(curr.pca)*c.rmean^2)/(ncol(curr.pca)-1))
curr.pca = curr.pca/c.rsd

p.sumx = Matrix::rowSums(proj.pca)
p.sumxx = Matrix::rowSums(proj.pca^2)
p.rsd = sqrt((p.sumxx - 2*p.sumx*p.rmean + ncol(proj.pca)*p.rmean^2)/(ncol(proj.pca)-1))
proj.pca = proj.pca/p.rsd

#PCA
pca = RSpectra::svds(A = Matrix::t(curr.pca), k=20,
                     opts = list(
                       center = FALSE, ## already done
                       scale = FALSE, ## already done
                       maxitr = 2000,
                       tol = 1e-10))

#scores of current and projected
curr.scores = Matrix::t(curr.pca) %*% pca$v[,1:10]
proj.scores = Matrix::t(proj.pca) %*% pca$v[,1:10]
```

VeloViz

7) Build graph using VeloViz
   Now we can use the PC projections of the current and projected transcriptional states to build the VeloViz graph. To build the graph, we have to specify multiple parameters that control the features of the graph:
   k: how many out-edges each cell can have
   similarity_threshold: cosine similarity threshold specifying how similar the velocity and cell transition vectors have to be for an out-edge to be included
   distance_weight: weight for distance in PC space between projected state and neighbor - with large weights, graph will prioritize linking cells where projected states and neighbors are close in PC space; with small weights, graph will prioritize linking cells where velocity and cell transition vectors are similar
   distance_threshold: quantile threshold specifying minimum distance in PC space between projected state and neighbor for out-edge to be included
   weighted: whether to use composite distance to determine graph edge weights (TRUE) or to assign all edges equal weights (FALSE).

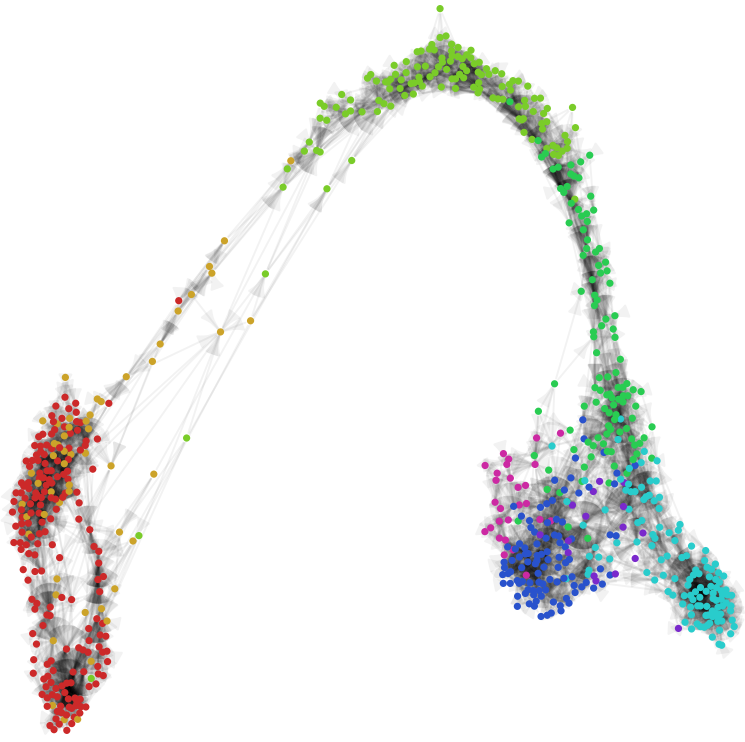
```
#VeloViz graph parameters
k = 5
similarity.threshold = 0.25
distance.weight = 1
distance.threshold = 0.5
weighted = TRUE

#build graph
set.seed(0)
veloviz = graphViz(t(curr.scores), t(proj.scores), k,
                   cell.colors=NA,
                   similarity_threshold=similarity.threshold,
                   distance_weight = distance.weight,
                   distance_threshold = distance.threshold,
                   weighted = weighted,
                   plot = FALSE,
                   return_graph = TRUE)


emb.veloviz = veloviz$fdg_coords
plotEmbedding(emb.veloviz, groups=clusters[rownames(emb.veloviz)], main='veloviz')
```




```
par(mfrow=c(1,1), mar=rep(1,4))
g = plotVeloviz(veloviz, clusters=clusters[rownames(emb.veloviz)], seed=0, verbose=TRUE)
```

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

```
## Using provided clusters...
```



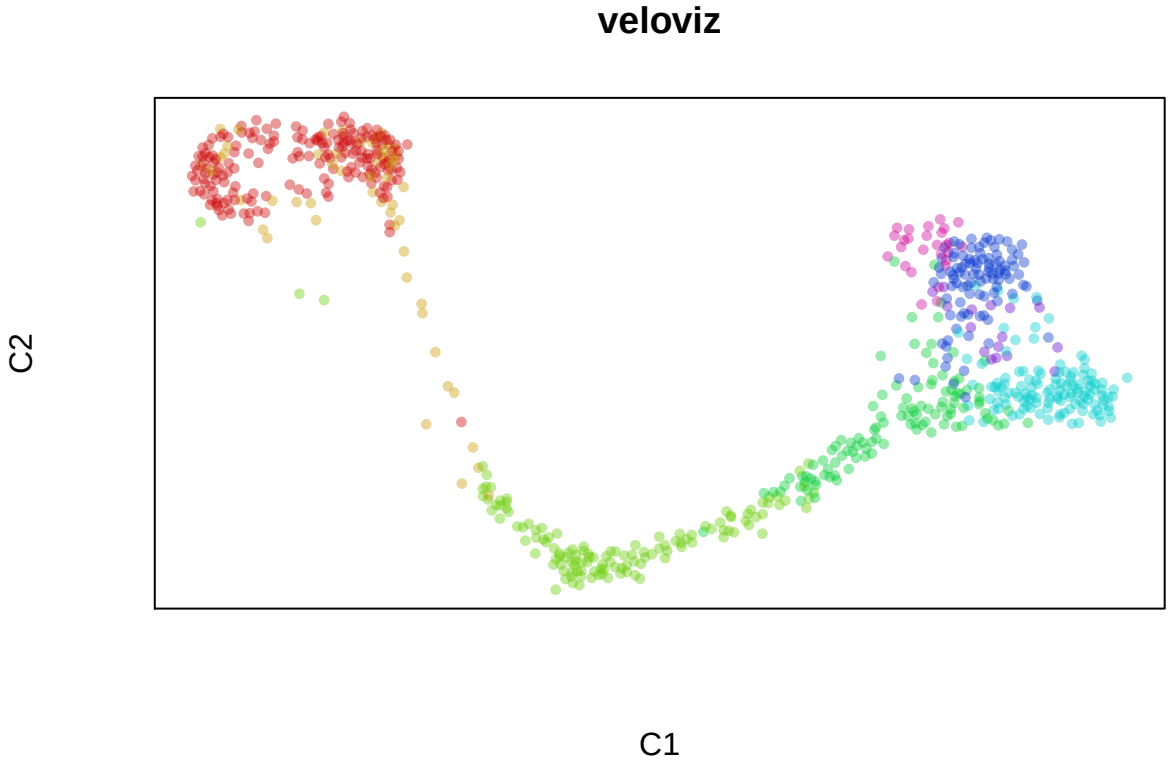
7*) Build VeloViz graph from current and projected using `buildVeloviz`

```
curr = vel$current
proj = vel$projected

veloviz = buildVeloviz(
  curr = curr, proj = proj,
  normalize.depth = TRUE,
  use.ods.genes = TRUE,
  alpha = 0.05,
  pca = TRUE,
  nPCs = 20,
  center = TRUE,
  scale = TRUE,
  k = 5,
  similarity.threshold = 0.25,
  distance.weight = 1,
  distance.threshold = 0.5,
  weighted = TRUE,
  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
```

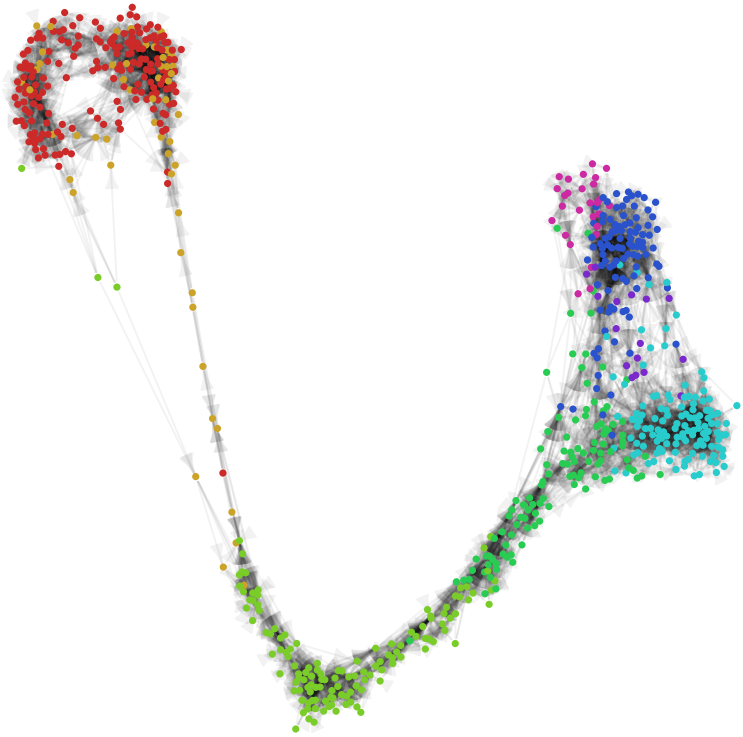
```
emb.veloviz = veloviz$fdg_coords
plotEmbedding(emb.veloviz, groups=clusters[rownames(emb.veloviz)], main='veloviz')
```



```
par(mfrow=c(1,1), mar=rep(1,4))
g = plotVeloviz(veloviz, clusters=clusters[rownames(emb.veloviz)], seed=0, verbose=TRUE)
```

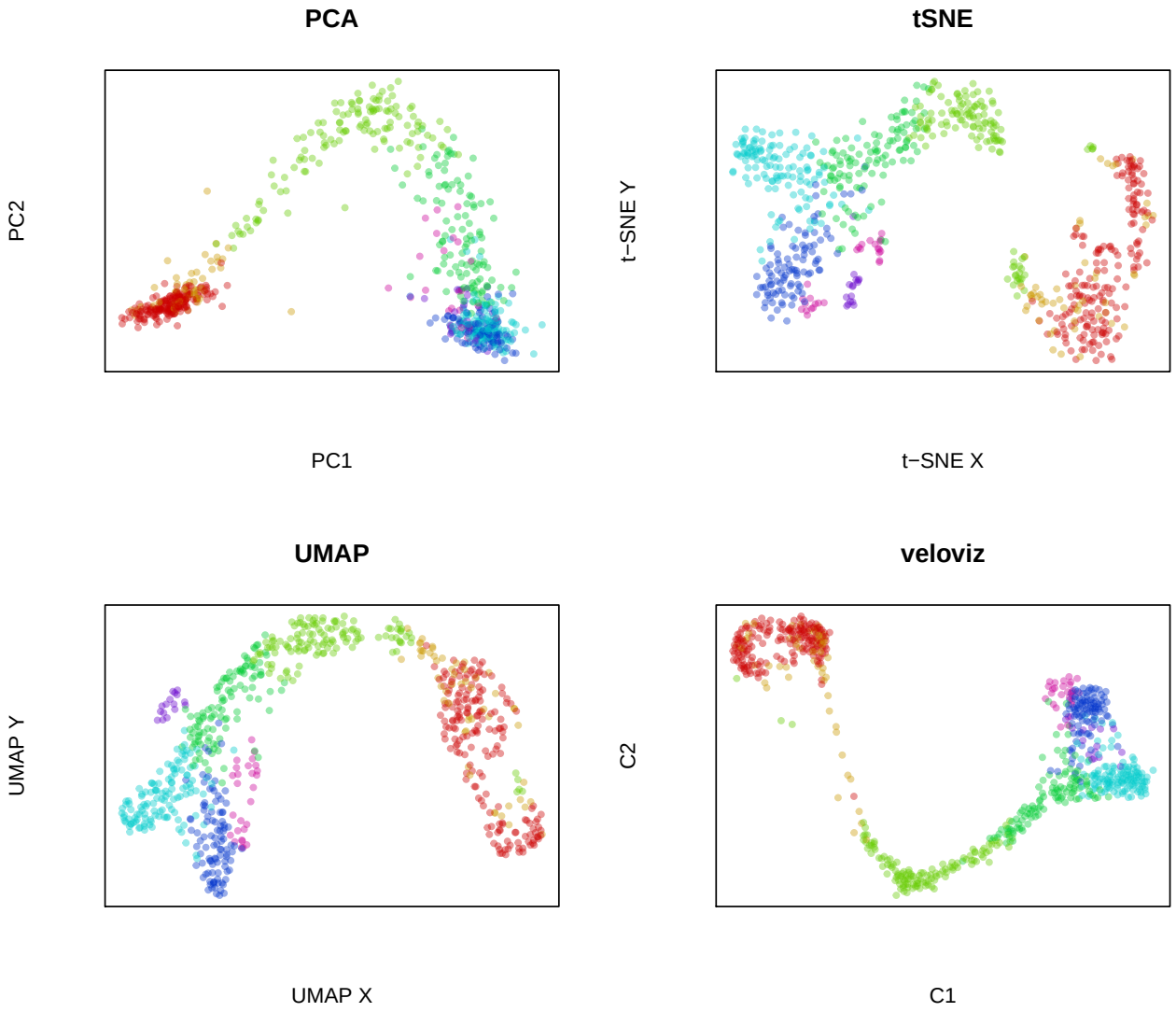
```
## Warning in if (!is.na(clusters) & is.na(col)) {: the condition has length > 1
## and only the first element will be used
```

```
## Using provided clusters...
```



Compare to other embeddings

```
par(mfrow = c(2,2))

#PCA
emb.pca = pcs[,1:2]
plotEmbedding(emb.pca, groups=pancreas$clusters, main='PCA')

#tSNE
set.seed(0)
emb.tsne = Rtsne::Rtsne(pcs, perplexity=30)$Y
rownames(emb.tsne) = rownames(pcs)
plotEmbedding(emb.tsne, groups=pancreas$clusters, main='tSNE',
              xlab = "t-SNE X", ylab = "t-SNE Y")

##UMAP
set.seed(0)
emb.umap = uwot::umap(pcs, min_dist = 0.5)
rownames(emb.umap) <- rownames(pcs)
plotEmbedding(emb.umap, groups=pancreas$clusters, main='UMAP',
              xlab = "UMAP X", ylab = "UMAP Y")

#veloviz
plotEmbedding(emb.veloviz, groups=clusters[rownames(emb.veloviz)], main='veloviz')
```


PCA
PC2
PC1
tSNE
t−SNE Y
t−SNE X
UMAP
UMAP Y
UMAP X
veloviz
C2
C1

Visualization with missing intermediates using VeloViz

Load data: this is the same dataset as above but missing a proportion of Ngn3 high EP cells

```
clusters = pancreasWithGap$clusters # cell type annotations
pcs = pancreasWithGap$pcs # PCs used to make other embeddings (UMAP,tSNE..)
vel = pancreasWithGap$vel # velocity
```

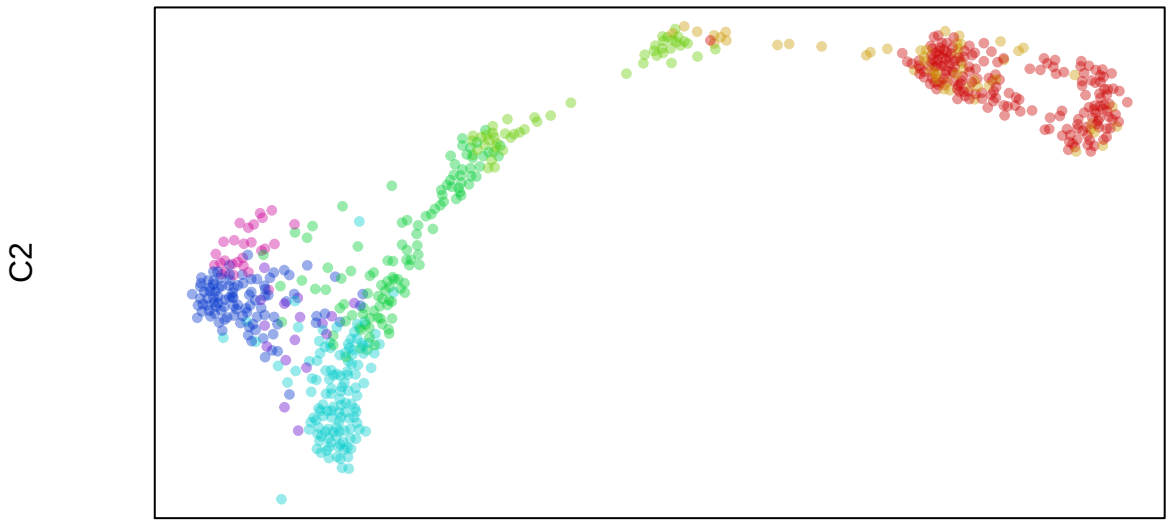
Create VeloViz embedding

```
curr = vel$current
proj = vel$projected

veloviz = buildVeloviz(
  curr = curr, proj = proj,
  normalize.depth = TRUE,
  use.ods.genes = TRUE,
  alpha = 0.05,
  pca = TRUE,
  nPCs = 20,
  center = TRUE,
  scale = TRUE,
  k = 5,
  similarity.threshold = 0.25,
  distance.weight = 1,
  distance.threshold = 0.5,
  weighted = TRUE,
  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
```

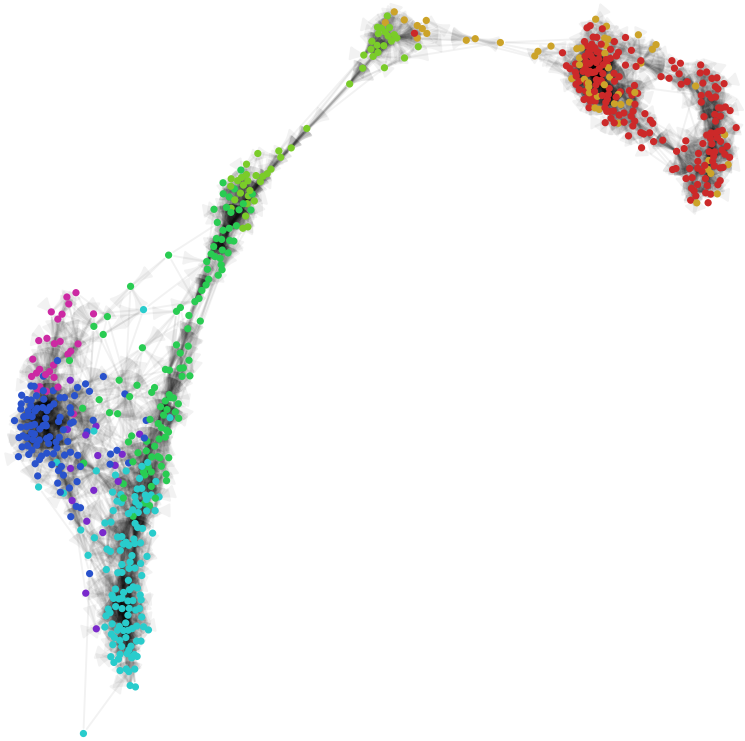
```
emb.veloviz = veloviz$fdg_coords
plotEmbedding(emb.veloviz, groups=clusters[rownames(emb.veloviz)], main='veloviz')
```




```
par(mfrow=c(1,1), mar=rep(1,4))
g = plotVeloviz(veloviz, clusters=clusters[rownames(emb.veloviz)], seed=0, verbose=TRUE)
```

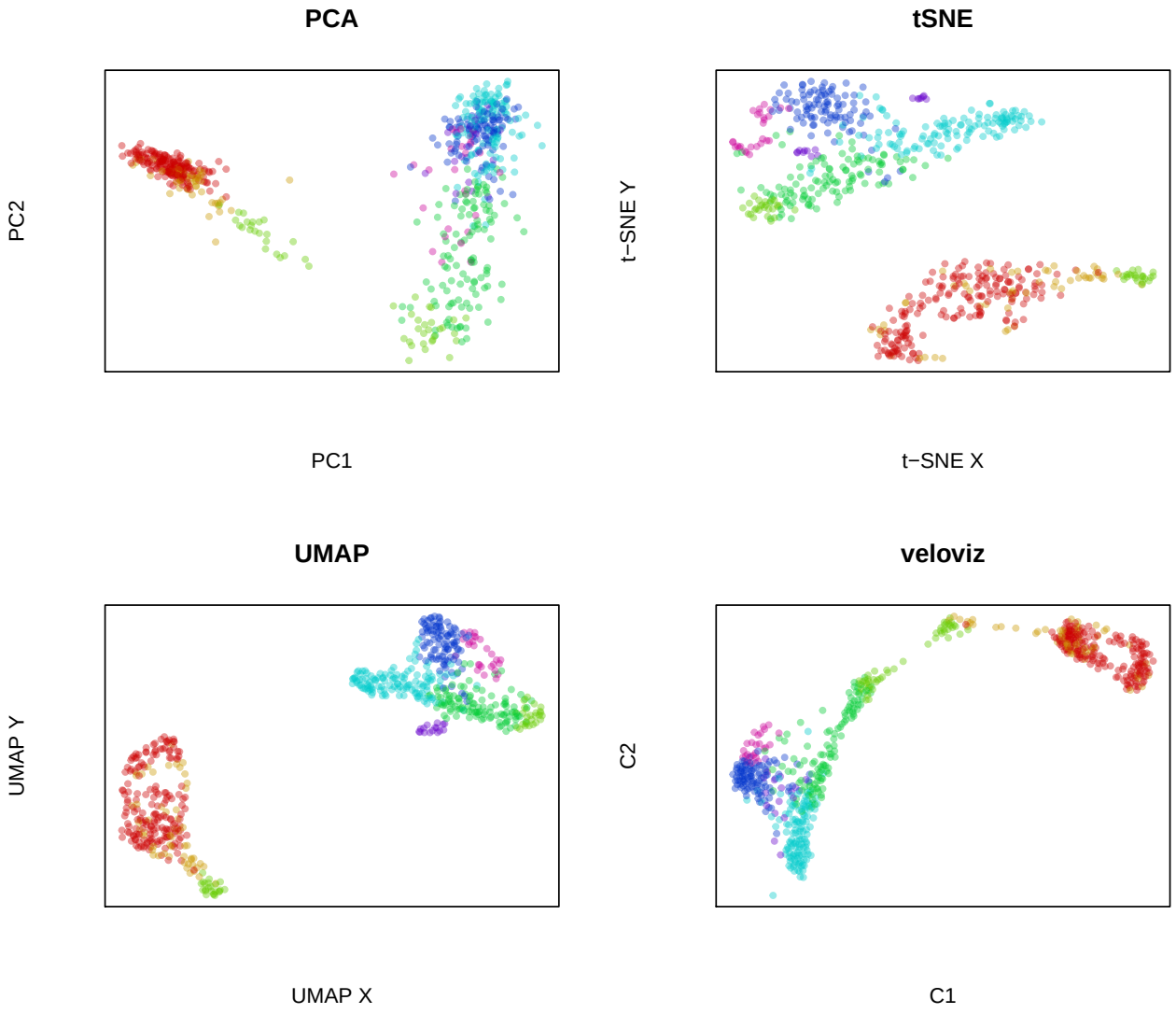
```
## Warning in if (!is.na(clusters) & is.na(col)) {: the condition has length > 1
## and only the first element will be used
```

```
## Using provided clusters...
```



Compare to other embeddings

```
par(mfrow = c(2,2))

#PCA
emb.pca = pcs[,1:2]
plotEmbedding(emb.pca, groups=pancreas$clusters, main='PCA')

#tSNE
set.seed(0)
emb.tsne = Rtsne::Rtsne(pcs, perplexity=30)$Y
rownames(emb.tsne) = rownames(pcs)
plotEmbedding(emb.tsne, groups=pancreas$clusters, main='tSNE',
              xlab = "t-SNE X", ylab = "t-SNE Y")

##UMAP
set.seed(0)
emb.umap = uwot::umap(pcs, min_dist = 0.5)
rownames(emb.umap) <- rownames(pcs)
plotEmbedding(emb.umap, groups=pancreas$clusters, main='UMAP',
              xlab = "UMAP X", ylab = "UMAP Y")

#veloviz
plotEmbedding(emb.veloviz, groups=clusters[rownames(emb.veloviz)], main='veloviz')
```


PCA
PC2
PC1
tSNE
t−SNE Y
t−SNE X
UMAP
UMAP Y
UMAP X
veloviz
C2
C1