Visualization using VeloViz

In this example, we will compare the velocity-informed 2D embedding created by VeloViz to other commonly used embeddings. We will go through the standard workflow needed to generate the VeloViz visualization using the pancreas endocrinogenesis dataset as an example.

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

#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

#choose colors based on clusters for plotting later
cell.cols = rainbow(8)[as.numeric(clusters)]
names(cell.cols) = names(clusters)
```

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)
proj.norm = normalizeDepth(proj)

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

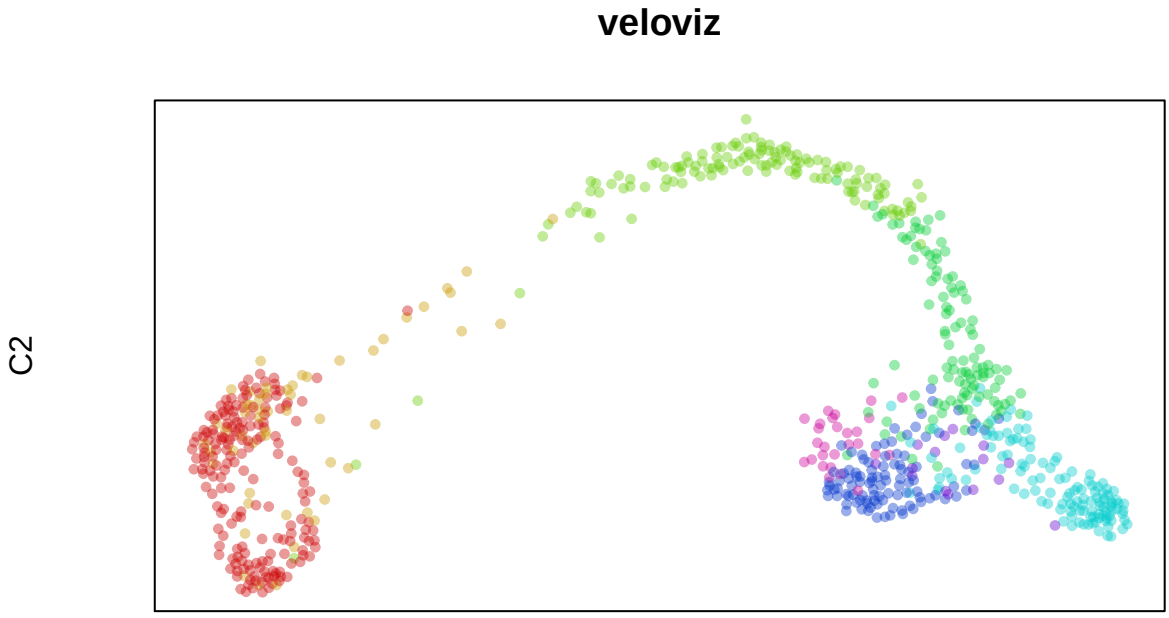
```
#log normalize
curr.pca = log10(curr.varnorm + 1)
proj.pca = log10(proj.varnorm + 1)
```

```r
#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]
```

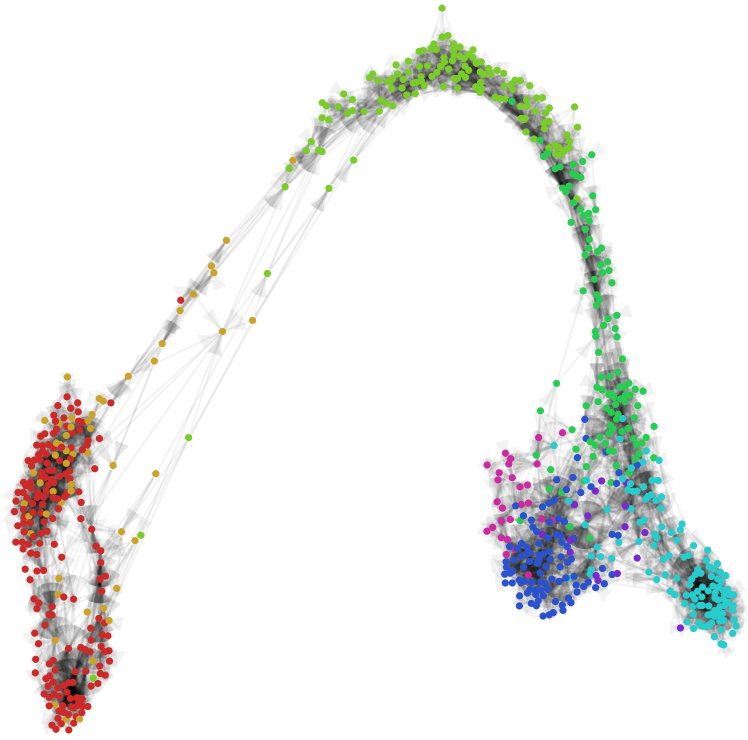
VeloViz

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 component of composite distance - 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 most similar
   distance_threshold: quantile threshold specifying minimum distance in PC space between projected state and neighbor for out-edge to be included - e.g. a distance threshold of 0.2 means that any edges where the distance component is not in the smallest 20% of distances in PC space will be removed from the graph
   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')
```



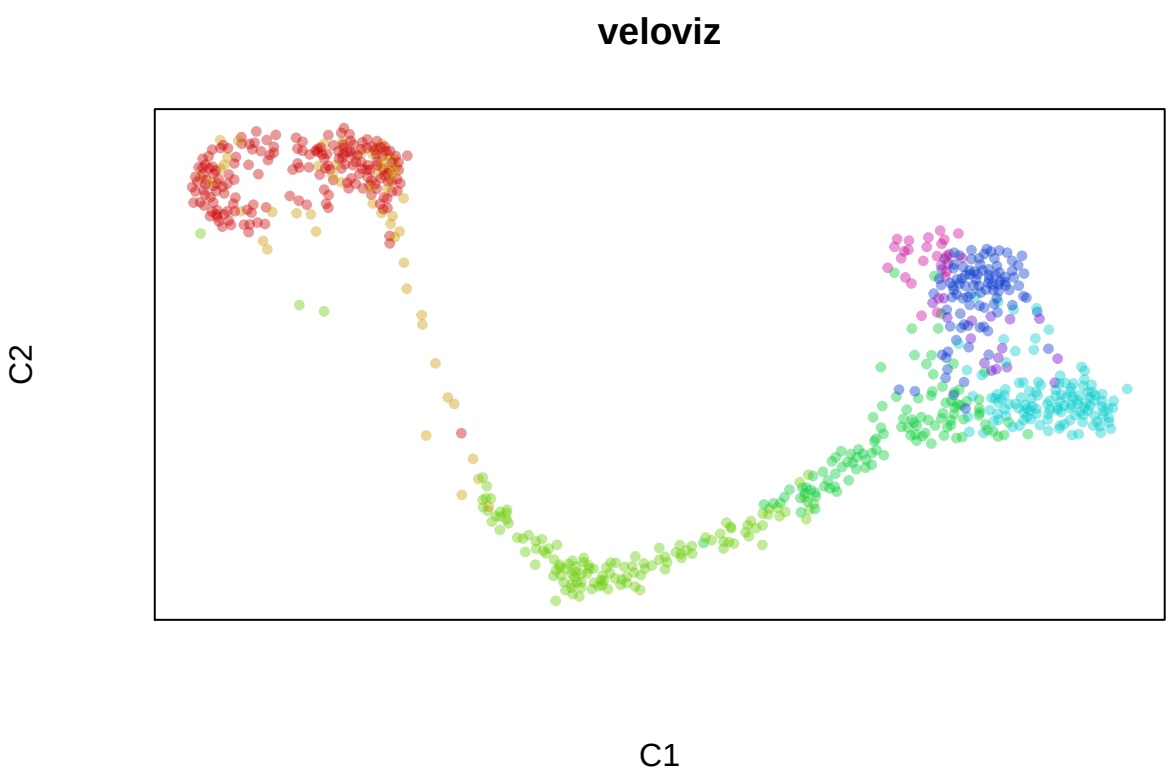

```
par(mfrow=c(1,1), mar=rep(1,4))
g = plotVeloviz(veloviz, clusters=clusters[rownames(emb.veloviz)], seed=0, verbose=TRUE)
```



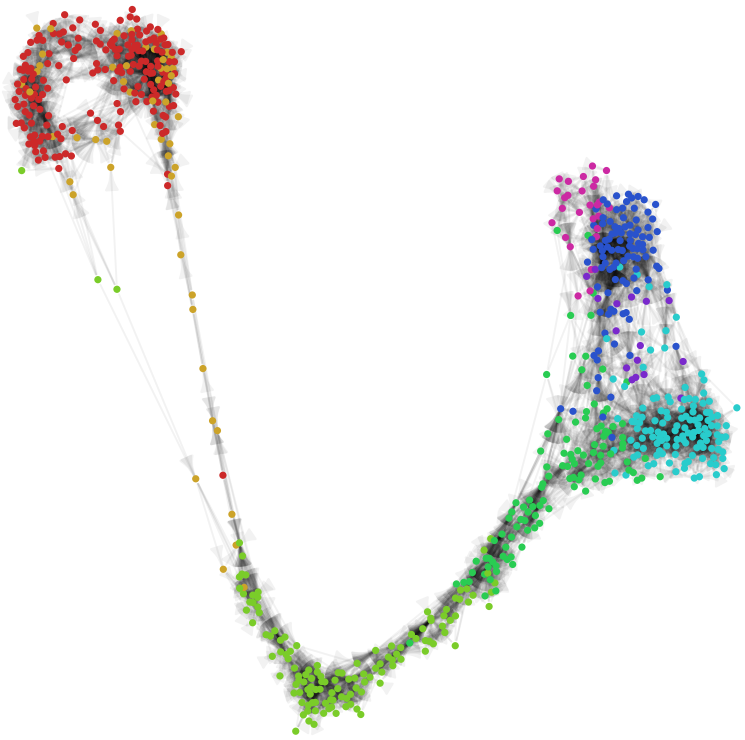
7*) Build VeloViz graph from current and projected using `buildVeloviz`

```
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
)

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

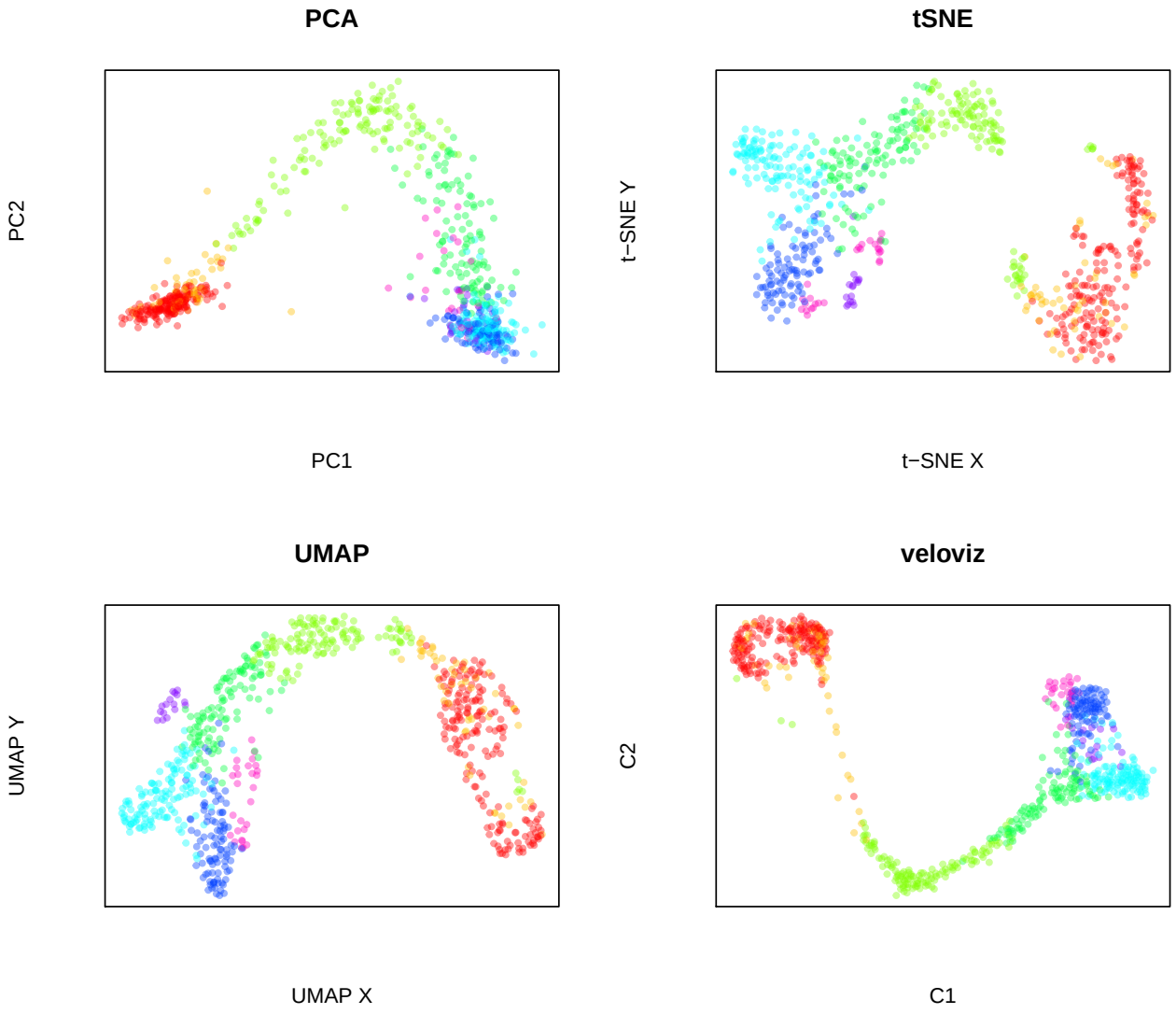



```
par(mfrow=c(1,1), mar=rep(1,4))
g = plotVeloviz(veloviz, clusters=clusters[rownames(emb.veloviz)], seed=0, verbose=TRUE)
```



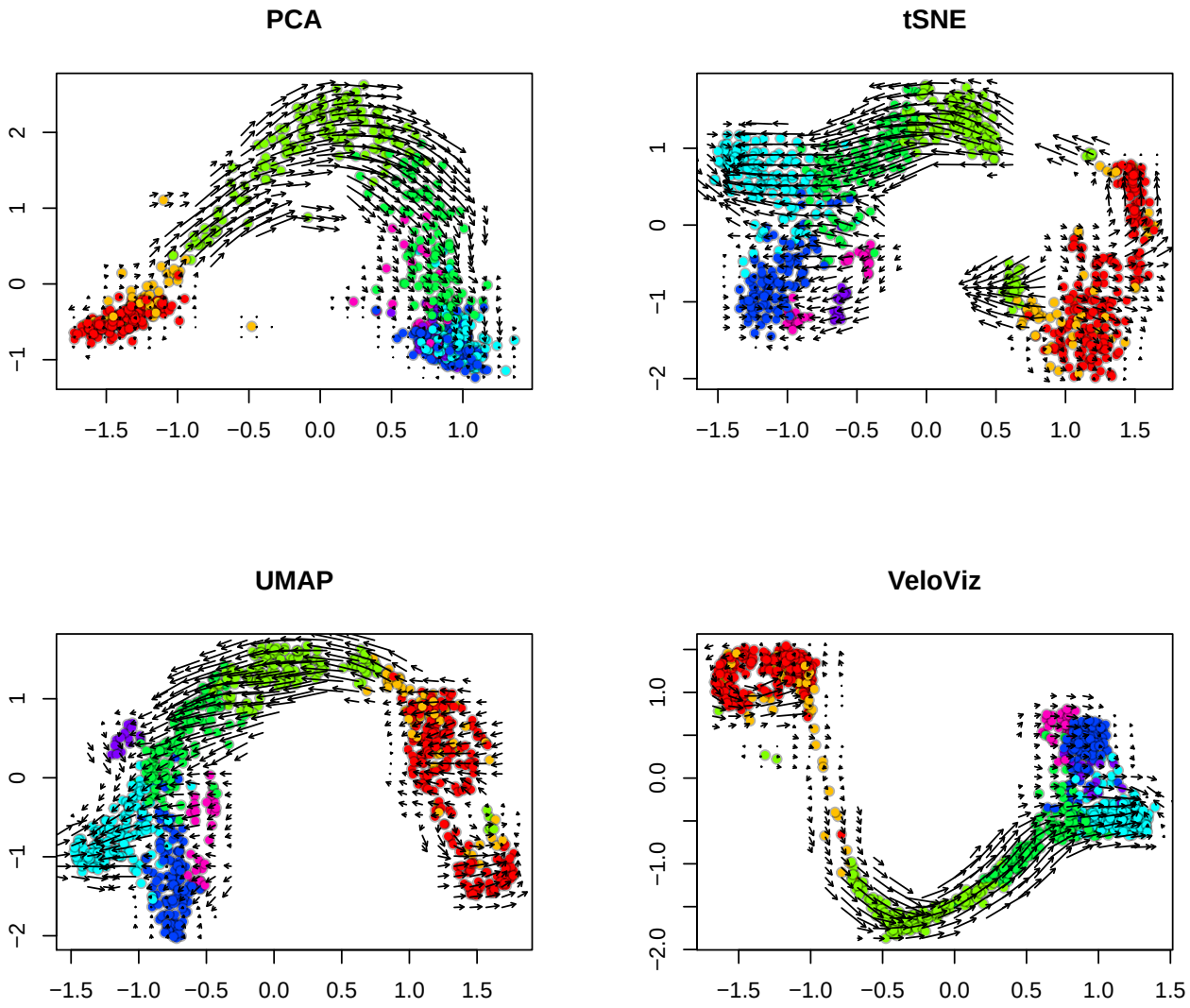
Compare to other embeddings

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

#PCA
emb.pca = pcs[,1:2]
plotEmbedding(emb.pca, colors = cell.cols, main='PCA',
              xlab = "PC1", ylab = "PC2")

#tSNE
set.seed(0)
emb.tsne = Rtsne::Rtsne(pcs, perplexity=30)$Y
rownames(emb.tsne) = rownames(pcs)
plotEmbedding(emb.tsne, colors = cell.cols, 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, colors = cell.cols, main='UMAP',
              xlab = "UMAP X", ylab = "UMAP Y")

#veloviz
plotEmbedding(emb.veloviz, colors = cell.cols[rownames(emb.veloviz)], main='veloviz')
```


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

Now let's project velocity inferred from `velocyto.R` onto these embeddings.

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

show.velocity.on.embedding.cor(scale(emb.pca), vel,
                               n = 50,
                               scale='sqrt',
                               cex=1, arrow.scale=1, show.grid.flow=TRUE,
                               min.grid.cell.mass=0.5, grid.n=30, arrow.lwd=1, do.par = F,
                               cell.colors=cell.cols, main='PCA')
show.velocity.on.embedding.cor(scale(emb.tsne), vel,
                               n = 50,
                               scale='sqrt',
                               cex=1, arrow.scale=1, show.grid.flow=TRUE,
                               min.grid.cell.mass=0.5, grid.n=30, arrow.lwd=1,do.par = F,
                               cell.colors=cell.cols, main='tSNE')
show.velocity.on.embedding.cor(scale(emb.umap), vel,
                               n = 50,
                               scale='sqrt',
                               cex=1, arrow.scale=1, show.grid.flow=TRUE,
                               min.grid.cell.mass=0.5, grid.n=30, arrow.lwd=1,do.par = F,
                               cell.colors=cell.cols, main='UMAP')
show.velocity.on.embedding.cor(scale(emb.veloviz), vel,
                               n = 50,
                               scale='sqrt',
                               cex=1, arrow.scale=1, show.grid.flow=TRUE,
                               min.grid.cell.mass=0.5, grid.n=30, arrow.lwd=1,do.par = F,
                               cell.colors=cell.cols, main='VeloViz')
```


PCA
tSNE
UMAP
VeloViz

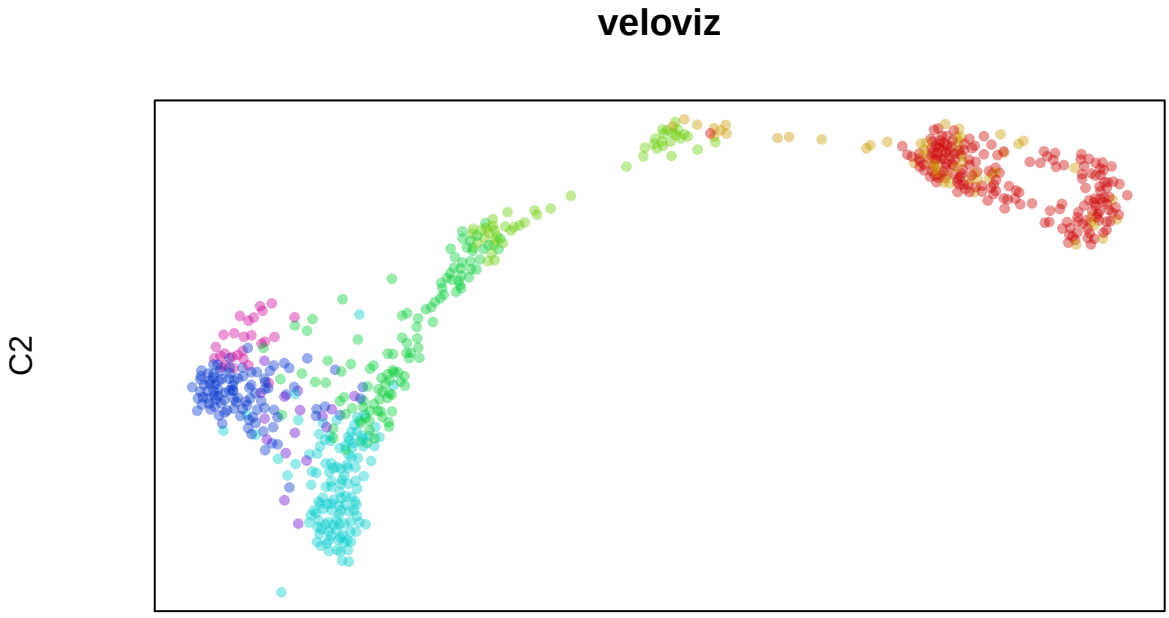
Visualization with missing intermediates using VeloViz

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

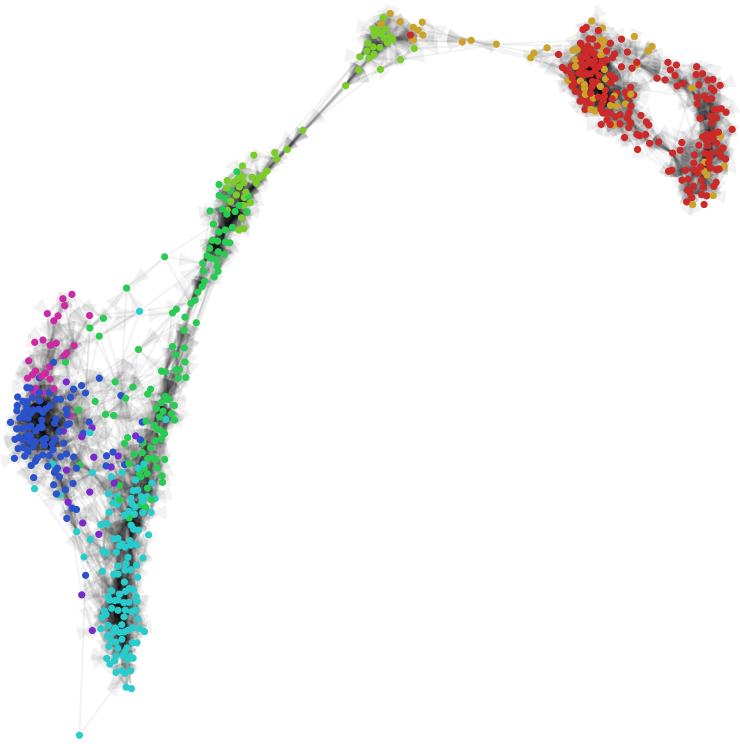
```
clusters = pancreasWithGap$clusters # cell type annotations
pcs = pancreasWithGap$pcs # PCs used to make other embeddings (UMAP,tSNE..)
vel = pancreasWithGap$vel # velocity

#choose colors based on clusters for plotting later
cell.cols = rainbow(8)[as.numeric(clusters)]
names(cell.cols) = names(clusters)
```

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
)

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



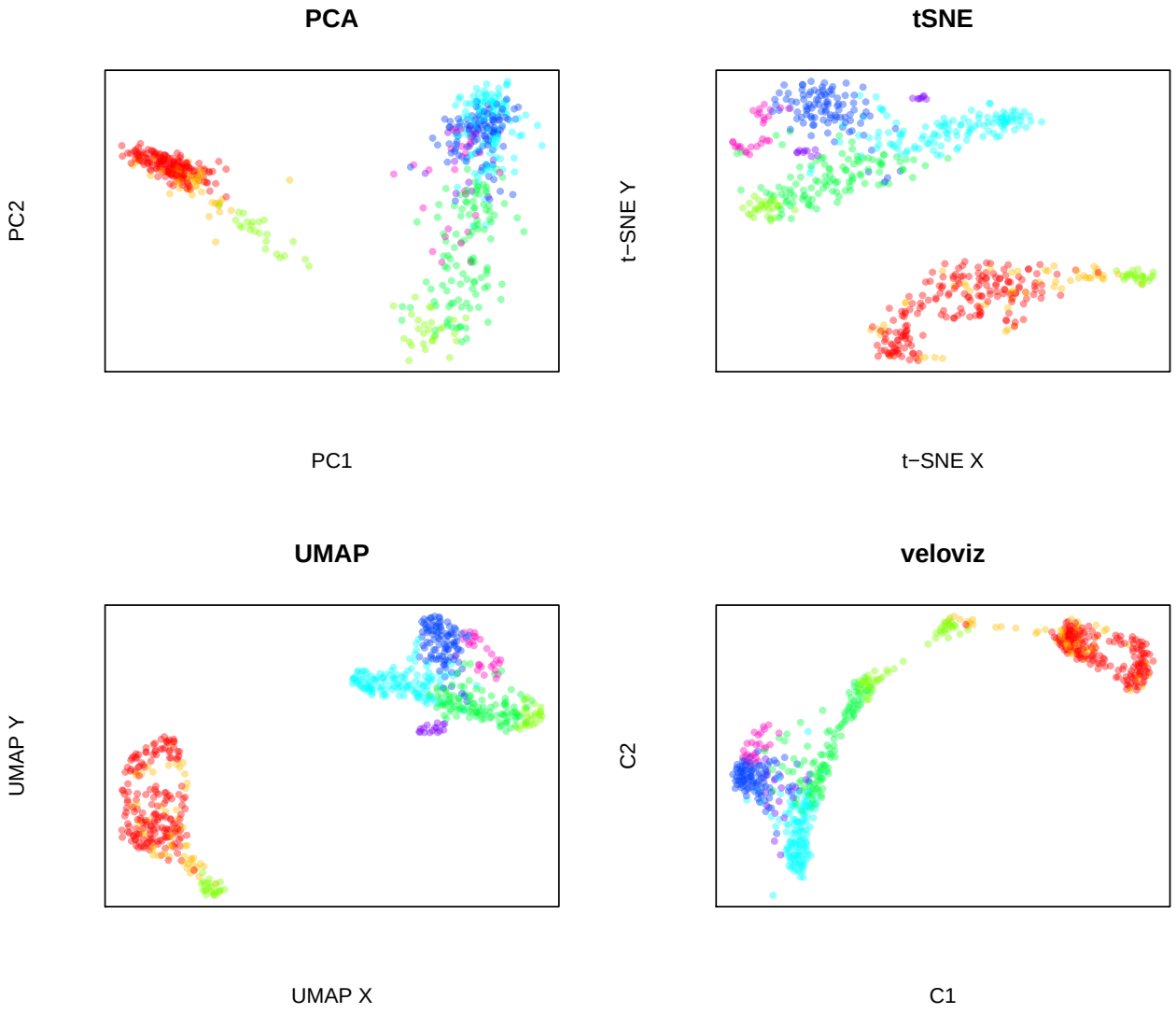

```
par(mfrow=c(1,1), mar=rep(1,4))
g = plotVeloviz(veloviz, clusters=clusters[rownames(emb.veloviz)], seed=0, verbose=TRUE)
```



Compare to other embeddings

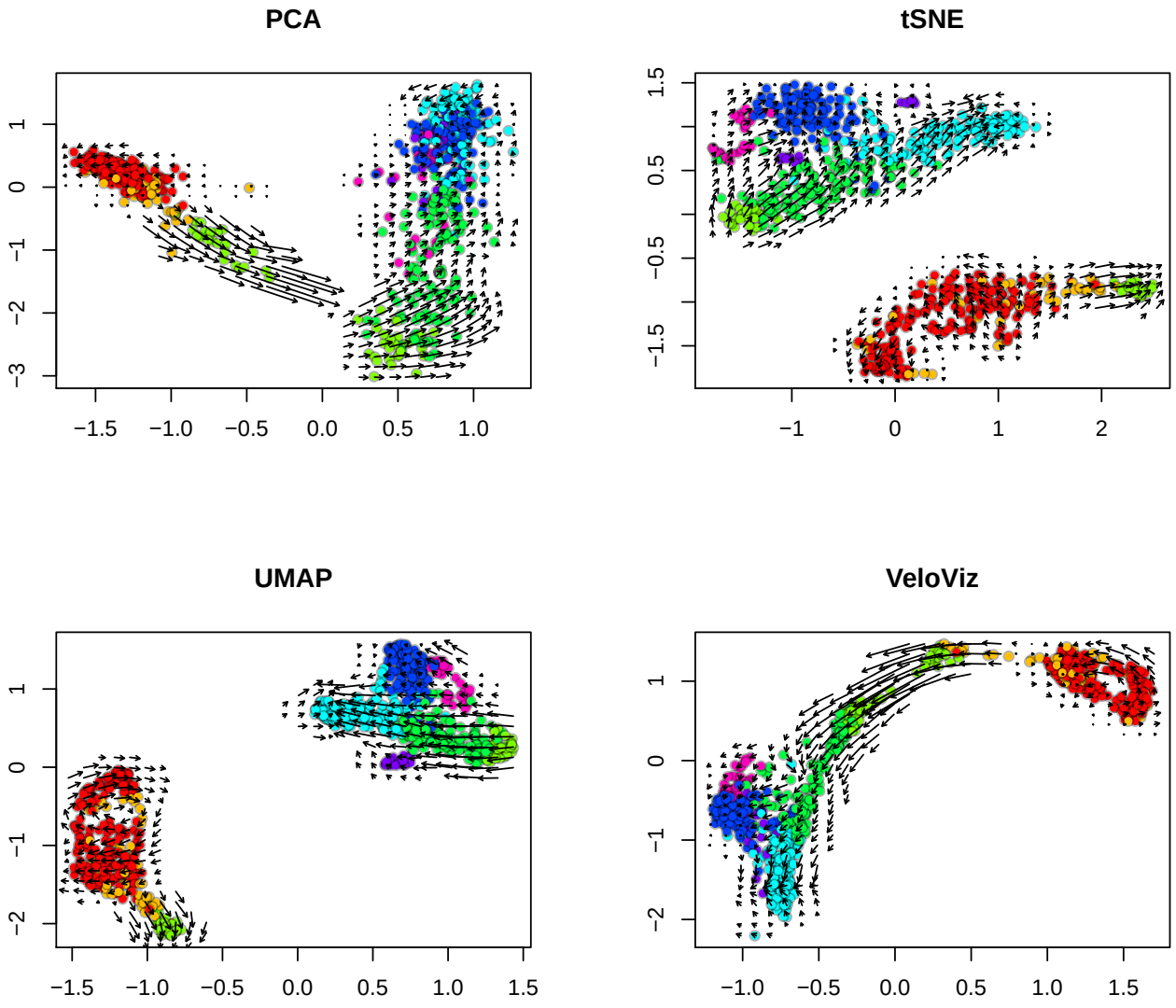
```
par(mfrow = c(2,2))
```

```r
#PCA
emb.pca = pcs[,1:2]
plotEmbedding(emb.pca, colors = cell.cols, main='PCA')

#tSNE
set.seed(0)
emb.tsne = Rtsne::Rtsne(pcs, perplexity=30)$Y
rownames(emb.tsne) = rownames(pcs)
plotEmbedding(emb.tsne, colors = cell.cols, 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, colors = cell.cols, main='UMAP',
              xlab = "UMAP X", ylab = "UMAP Y")

#veloviz
plotEmbedding(emb.veloviz, colors = cell.cols[rownames(emb.veloviz)], main='veloviz')
```


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

Now let's project velocity inferred from `velocyto.R` onto these embeddings.

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

show.velocity.on.embedding.cor(scale(emb.pca), vel,
                               n = 50,
                               scale='sqrt',
                               cex=1, arrow.scale=1, show.grid.flow=TRUE,
                               min.grid.cell.mass=0.5, grid.n=30, arrow.lwd=1, do.par = F,
                               cell.colors=cell.cols, main='PCA')
show.velocity.on.embedding.cor(scale(emb.tsne), vel,
                               n = 50,
                               scale='sqrt',
                               cex=1, arrow.scale=1, show.grid.flow=TRUE,
                               min.grid.cell.mass=0.5, grid.n=30, arrow.lwd=1,do.par = F,
                               cell.colors=cell.cols, main='tSNE')
show.velocity.on.embedding.cor(scale(emb.umap), vel,
                               n = 50,
                               scale='sqrt',
                               cex=1, arrow.scale=1, show.grid.flow=TRUE,
                               min.grid.cell.mass=0.5, grid.n=30, arrow.lwd=1,do.par = F,
                               cell.colors=cell.cols, main='UMAP')
show.velocity.on.embedding.cor(scale(emb.veloviz), vel,
                               n = 50,
                               scale='sqrt',
                               cex=1, arrow.scale=1, show.grid.flow=TRUE,
                               min.grid.cell.mass=0.5, grid.n=30, arrow.lwd=1,do.par = F,
                               cell.colors=cell.cols, main='VeloViz')
```


PCA
tSNE
UMAP
VeloViz