

# Package ‘veloviz’

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**Title** VeloViz: RNA-velocity informed 2D embeddings for visualizing cell state trajectories

**Version** 1.17.0

**Description** VeloViz uses each cell’s current observed and predicted future transcriptional states inferred from RNA velocity analysis to build a nearest neighbor graph between cells in the population. Edges are then pruned based on a cosine correlation threshold and/or a distance threshold and the resulting graph is visualized using a force-directed graph layout algorithm. VeloViz can help ensure that relationships between cell states are reflected in the 2D embedding, allowing for more reliable representation of underlying cellular trajectories.

**biocViews** Transcriptomics, Visualization, GeneExpression, Sequencing, RNASeq, DimensionReduction

**License** GPL-3

**Encoding** UTF-8

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**Imports** Rcpp, Matrix, igraph, mgcv, RSpectra, grDevices, graphics, stats

**LinkingTo** Rcpp

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**Suggests** knitr, rmarkdown, testthat

**VignetteBuilder** knitr

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|           |                                                                           |
|-----------|---------------------------------------------------------------------------|
| asNNGraph | <i>Function to produce idx and dist representation of a VeloViz graph</i> |
|-----------|---------------------------------------------------------------------------|

---

**Description**

Function to produce idx and dist representation of a VeloViz graph

**Usage**

asNNGraph(vig)

**Arguments**

vig                      output of buildVeloviz

**Value**

idx numVertices x numNeighbors matrix, where each row i contains indices of vertex i’s neighbors  
dist numVertices x numNeighbors matrix, where each row i contains distances from vertex i to its neighbors

## Examples

```
data(vel)
curr <- vel$current
proj <- vel$projected

vv <- buildVeloviz(curr = curr, proj = proj, normalize.depth = TRUE,
  use.ods.genes = FALSE, alpha = 0.05, pca = TRUE, nPCs = 3, center = TRUE,
  scale = TRUE, k = 10, similarity.threshold = -1, distance.weight = 1,
  distance.threshold = 1, weighted = TRUE, verbose = FALSE)

asNNGraph(vv)
```

---

|              |                                                                                                             |
|--------------|-------------------------------------------------------------------------------------------------------------|
| buildVeloviz | <i>Creates VeloViz graph and FDG layout from PC scores of current and projected transcriptional states.</i> |
|--------------|-------------------------------------------------------------------------------------------------------------|

---

## Description

Creates VeloViz graph and FDG layout from PC scores of current and projected transcriptional states.

## Usage

```
buildVeloviz(
  curr,
  proj,
  normalize.depth = TRUE,
  depth = 1e+06,
  use.ods.genes = TRUE,
  max.ods.genes = 2000,
  alpha = 0.05,
  pca = TRUE,
  center = TRUE,
  scale = TRUE,
  nPCs = 10,
  k = 10,
  similarity.threshold = 0,
  distance.weight = 1,
  distance.threshold = 1,
  weighted = TRUE,
  remove.unconnected = TRUE,
  verbose = FALSE,
  details = FALSE
)
```

**Arguments**

|                                   |                                                                                                                                        |
|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| <code>curr</code>                 | Genes (rows) x cells (columns) matrix of observed current transcriptional state                                                        |
| <code>proj</code>                 | Genes (rows) x cells (columns) matrix of predicted future transcriptional state                                                        |
| <code>normalize.depth</code>      | logical to normalize raw counts to counts per million, default = TRUE                                                                  |
| <code>depth</code>                | Depth scaling, default = 1e6 for counts per million (CPM)                                                                              |
| <code>use.ods.genes</code>        | Use only overdispersed genes to create VeloViz graph, default = TRUE                                                                   |
| <code>max.ods.genes</code>        | number of most highly expressed overdispersed genes to use to create VeloViz graph, default = 2000                                     |
| <code>alpha</code>                | Significance threshold for overdispersed genes, default = 0.05                                                                         |
| <code>pca</code>                  | logical to use PC scores to create VeloViz graph, default = TRUE. FALSE = use gene expression to create VeloViz graph                  |
| <code>center</code>               | logical to mean center gene expression before PCA, default = TRUE                                                                      |
| <code>scale</code>                | logical to scale gene expression variance before PCA, default = TRUE                                                                   |
| <code>nPCs</code>                 | number of principal components to use to create VeloViz graph, default = 10                                                            |
| <code>k</code>                    | Number of nearest neighbors to assign each cell                                                                                        |
| <code>similarity.threshold</code> | similarity threshold below which to remove edges, default = -1 i.e. no edges removed                                                   |
| <code>distance.weight</code>      | Weight of distance component of composite distance, default = 1                                                                        |
| <code>distance.threshold</code>   | quantile threshold for distance component above which to remove edges, default = 1 i.e. no edges removed                               |
| <code>weighted</code>             | logical indicating whether to compute VeloViz edges based on composite distance, default = TRUE. FALSE = all edges are of equal weight |
| <code>remove.unconnected</code>   | logical indicating whether to remove cells with no edges in the VeloViz graph from the output embedding, default = TRUE (removed)      |
| <code>verbose</code>              | logical for verbosity setting, default = FALSE                                                                                         |
| <code>details</code>              | logical to return detailed data frame or names of genes, default = FALSE                                                               |

**Value**

`graph` igraph object of VeloViz graph  
`fdg_coords` cells (rows) x 2 coordinates of force-directed layout of VeloViz graph  
`projectedNeighbors` output of `projectedNeighbors`

**See Also**

[projectedNeighbors](#)

**Examples**

```
data(vel)
curr <- vel$current
proj <- vel$projected

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, verbose = FALSE)
```

graphViz

*Visualize as velocity informed force directed graph***Description**

Visualize as velocity informed force directed graph

**Usage**

```
graphViz(
  observed,
  projected,
  k,
  distance_metric = "L2",
  similarity_metric = "cosine",
  distance_weight = 1,
  distance_threshold = 1,
  similarity_threshold = -1,
  weighted = TRUE,
  remove_unconnected = TRUE,
  return_graph = FALSE,
  plot = TRUE,
  cell.colors = NA,
  title = NA
)
```

**Arguments**

|                 |                                                                                                                      |
|-----------------|----------------------------------------------------------------------------------------------------------------------|
| observed        | PCs (rows) x cells (columns) matrix of observed transcriptional state projected into PC space                        |
| projected       | PCs (rows) x cells (columns) matrix of projected transcriptional states. Cell should be in same order as in observed |
| k               | Number of nearest neighbors to assign each cell                                                                      |
| distance_metric | Method to compute distance component of composite distance. "L1" or "L2", default = "L2"                             |

|                      |                                                                                                                                                                                        |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| similarity_metric    | Method to compute similarity between velocity and cell transition matrices. "cosine" or "pearson", default = "cosine"                                                                  |
| distance_weight      | Weight of distance component of composite distance, default = 1                                                                                                                        |
| distance_threshold   | quantile threshold for distance component above which to remove edges, default = 1 i.e. no edges removed                                                                               |
| similarity_threshold | similarity threshold below which to remove edges, default = -1 i.e. no edges removed                                                                                                   |
| weighted             | if TRUE, assigns edge weights based on composite distance, if FALSE assigns equal weights to all edges, default = TRUE                                                                 |
| remove_unconnected   | if TRUE, does not plot cells with no edges, default = TRUE                                                                                                                             |
| return_graph         | if TRUE, returns igraph object graph, force-directed layout coordinates fdg_coords, and projected_neighbors object detailing composite distance values and components, default = FALSE |
| plot                 | if TRUE, plots graph and force-directed layout                                                                                                                                         |
| cell.colors          | cell.colors to use for plotting                                                                                                                                                        |
| title                | title to use for plot                                                                                                                                                                  |

### Value

graph igraph object of VeloViz graph  
 fdg\_coords cells (rows) x 2 coordinates of force-directed layout of VeloViz graph  
 projectedNeighbors output of projectedNeighbors

### See Also

[projectedNeighbors](#)

### Examples

```
data(vel)
curr = vel$current
proj = vel$projected

m <- log10(curr+1)
pca <- RSpectra::svds(A = Matrix::t(m), k=3,
  opts = list(center = FALSE, scale = FALSE, maxitr = 2000, tol = 1e-10))
pca.curr <- Matrix::t(m) %*% pca$v[,1:3]

m <- log10(proj+1)
pca.proj <- Matrix::t(m) %*% pca$v[,1:3]

graphViz(t(pca.curr), t(pca.proj), k=10,
```

```
cell.colors=NA, similarity_threshold=-1, distance_weight = 1,  
distance_threshold = 1, weighted = TRUE, remove_unconnected = TRUE,  
plot = FALSE, return_graph = TRUE)
```

---

|                |                                 |
|----------------|---------------------------------|
| normalizeDepth | <i>Normalizes counts to CPM</i> |
|----------------|---------------------------------|

---

### Description

Normalizes raw counts to counts per million

### Usage

```
normalizeDepth(counts, depthScale = 1e+06, verbose = TRUE)
```

### Arguments

|            |                                                                                         |
|------------|-----------------------------------------------------------------------------------------|
| counts     | Read count matrix. The rows correspond to genes, columns correspond to individual cells |
| depthScale | Depth scaling. Using a million for CPM (default: 1e6)                                   |
| verbose    | Boolean for verbosity setting (default: TRUE)                                           |

### Value

a normalized matrix

### Examples

```
data(vel)  
curr <- vel$current  
  
normalizeDepth(curr)
```

---

|                   |                                                                                                                                                                                    |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| normalizeVariance | <i>Identify overdispersed genes by normalizing counts per million (CPM) gene expression variance relative to transcriptome-wide expectations (Modified from SCDE/PAGODA2 code)</i> |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

---

### Description

Normalizes gene expression magnitudes to with respect to its ratio to the transcriptome-wide expectation as determined by local regression on expression magnitude

**Usage**

```
normalizeVariance(  
  cpm,  
  gam.k = 5,  
  alpha = 0.05,  
  max.adjusted.variance = 1000,  
  min.adjusted.variance = 0.001,  
  verbose = TRUE,  
  plot = FALSE,  
  details = FALSE  
)
```

**Arguments**

|                       |                                                                                                                 |
|-----------------------|-----------------------------------------------------------------------------------------------------------------|
| cpm                   | Counts per million (CPM) matrix. Rows are genes, columns are cells.                                             |
| gam.k                 | Generalized additive model parameter; the dimension of the basis used to represent the smooth term (default: 5) |
| alpha                 | Significance threshold for overdispersed genes (default: 0.05)                                                  |
| max.adjusted.variance | Ceiling on maximum variance after normalization to prevent infinities (default: 1e3)                            |
| min.adjusted.variance | Floor on minimum variance after normalization (default: 1e-3)                                                   |
| verbose               | Boolean for verbosity setting (default: TRUE)                                                                   |
| plot                  | Boolean to plot mean variance plots before and after correction                                                 |
| details               | Boolean to return detailed data frame or names of genes (default: FALSE)                                        |

**Value**

A list with two items: (1) an adjusted CPM matrix with the same dimensions as the input and (2) a dataframe with the summary statistics for each gene.

**Examples**

```
data(vel)  
curr <- vel$current  
  
normalizeDepth(curr)
```



---

|          |                                |
|----------|--------------------------------|
| pancreas | <i>Pancreas scRNA-seq data</i> |
|----------|--------------------------------|

---

**Description**

Pancreatic endocrinogenesis scRNA-seq from Bastidas-Ponce et. al., Development 2019 accessed via scVelo package and subsampled to 739 cells.

**Usage**

pancreas

**Format**

list of 4 objects:

**spliced** matrix, 7192 genes x 739 cells of spliced counts

**unspliced** matrix, 7192 genes x 739 cells of unspliced counts

**pcs** matrix, 739 x 50 cell scores in 50 PCs

**clusters** factor of cell type annotations from scVelo

**Source**

<https://dev.biologists.org/content/146/12/dev173849.long>

---

|               |                                                  |
|---------------|--------------------------------------------------|
| plotEmbedding | <i>Plot 2D embedding From scde/pagoda2/MUDAN</i> |
|---------------|--------------------------------------------------|

---

**Description**

Plot 2D embedding From scde/pagoda2/MUDAN

**Usage**

```
plotEmbedding(  
  emb,  
  groups = NULL,  
  colors = NULL,  
  cex = 0.6,  
  alpha = 0.4,  
  gradientPalette = NULL,  
  zlim = NULL,  
  s = 1,  
  v = 0.8,  
  min.group.size = 1,
```

```

    show.legend = FALSE,
    mark.clusters = FALSE,
    mark.cluster.cex = 2,
    shuffle.colors = FALSE,
    legend.x = "topright",
    gradient.range.quantile = 0.95,
    verbose = TRUE,
    unclassified.cell.color = "gray70",
    group.level.colors = NULL,
    ...
)

```

### Arguments

|                                      |                                                                                      |
|--------------------------------------|--------------------------------------------------------------------------------------|
| <code>emb</code>                     | dataframe with x and y coordinates                                                   |
| <code>groups</code>                  | factor annotations for rows on <code>emb</code> for visualizing cluster annotations  |
| <code>colors</code>                  | color or numeric values for rows on <code>emb</code> for visualizing gene expression |
| <code>cex</code>                     | point size                                                                           |
| <code>alpha</code>                   | point opacity                                                                        |
| <code>gradientPalette</code>         | palette for colors if numeric values provided                                        |
| <code>zlim</code>                    | range for colors                                                                     |
| <code>s</code>                       | saturation of rainbow for group colors                                               |
| <code>v</code>                       | value of rainbow for group colors                                                    |
| <code>min.group.size</code>          | minimum size of group in order for group to be colored                               |
| <code>show.legend</code>             | whether to show legend                                                               |
| <code>mark.clusters</code>           | whether to mark clusters with name of cluster                                        |
| <code>mark.cluster.cex</code>        | cluster marker point size                                                            |
| <code>shuffle.colors</code>          | whether to shuffle group colors                                                      |
| <code>legend.x</code>                | legend position ie. 'topright', 'topleft', 'bottomleft', 'bottomright'               |
| <code>gradient.range.quantile</code> | quantile for mapping colors to gradient palette                                      |
| <code>verbose</code>                 | verbosity                                                                            |
| <code>unclassified.cell.color</code> | cells not included in groups will be labeled in this color                           |
| <code>group.level.colors</code>      | set group level colors. Default uses rainbow.                                        |
| <code>...</code>                     | Additional parameters to pass to <code>BASE::plot</code>                             |

### Value

embedding plot

**Examples**

```

data(vel)
curr <- vel$current
proj <- vel$projected

vv <- 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, verbose = FALSE)

plotEmbedding(vv$fdg_coords)

```

---

|             |                      |
|-------------|----------------------|
| plotVeloviz | <i>Plot function</i> |
|-------------|----------------------|

---

**Description**

Plot function

**Usage**

```

plotVeloviz(
  vig,
  layout.method = igraph::layout_with_fr,
  clusters = NA,
  cluster.method = igraph::cluster_louvain,
  col = NA,
  alpha = 0.05,
  verbose = TRUE
)

```

**Arguments**

|                |                                                                                                     |
|----------------|-----------------------------------------------------------------------------------------------------|
| vig            | output of buildVeloviz                                                                              |
| layout.method  | igraph method to use for generating 2D graph representation, default = igraph::layout_with_fr       |
| clusters       | cluster annotations for cells in data                                                               |
| cluster.method | igraph method to use for clustering if clusters are not provided, default = igraph::cluster_louvain |
| col            | colors to use for plotting                                                                          |
| alpha          | transparency for plotting graph nodes                                                               |
| verbose        | logical for verbosity setting, default = FALSE                                                      |

**Value**

cells (rows) x 2 coordinates of force-directed layout of VeloViz graph

**Examples**

```

data(vel)
curr <- vel$current
proj <- vel$projected

vv <- 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, verbose = FALSE)

plotVeloviz(vv)

```

---

|                    |                                                                                                                                            |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| projectedNeighbors | <i>Computes composite distances between all cell pairs and returns k-nearest neighbors and edge weights needed to build VeloViz graph.</i> |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------|

---

**Description**

Computes composite distances between all cell pairs and returns k-nearest neighbors and edge weights needed to build VeloViz graph.

**Usage**

```

projectedNeighbors(
  observed,
  projected,
  k,
  distance_metric = "L2",
  similarity_metric = "cosine",
  distance_weight = 1,
  distance_threshold = 1,
  similarity_threshold = -1
)

```

**Arguments**

|                 |                                                                                                                       |
|-----------------|-----------------------------------------------------------------------------------------------------------------------|
| observed        | PCs (rows) x cells (columns) matrix of observed transcriptional state projected into PC space                         |
| projected       | PCs (rows) x cells (columns) matrix of projected transcriptional states. Cells should be in same order as in observed |
| k               | Number of nearest neighbors to assign each cell                                                                       |
| distance_metric | Method to compute distance component of composite distance. "L1" or "L2", default = "L2"                              |

`similarity_metric`  
 Method to compute similarity between velocity and cell transition matrices. "cosine" or "pearson", default = "cosine"

`distance_weight`  
 Weight of distance component of composite distance, default = 1

`distance_threshold`  
 quantile threshold for distance component above which to remove edges, default = 1 i.e. no edges removed

`similarity_threshold`  
 similarity threshold below which to remove edges, default = -1 i.e. no edges removed

### Value

`kNNs` cells (rows) x `k` (columns) matrix of indices of each cell's nearest neighbors computed based on composite distance. Edges removed based on distance or similarity threshold will be NA.

`edge_weights` cells (rows) x `k` (columns) matrix of edge weights computed based on composite distance. Edges removed based on distance or similarity threshold will be NA.

`all_dists` cells x cells matrix of all pairwise composite distances

`dist_comp` components of composite distance: `invDist` distance component, `negSim` similarity component

### See Also

[graphViz](#)

### Examples

```
data(vel)
curr <- vel$current
proj <- vel$projected

projectedNeighbors(curr, proj, 10)
```

---

|                               |                                                                                    |
|-------------------------------|------------------------------------------------------------------------------------|
| <code>reduceDimensions</code> | <i>Reduce dimension using Principal Components Analysis via svds from RSpectra</i> |
|-------------------------------|------------------------------------------------------------------------------------|

---

### Description

Reduce dimension using Principal Components Analysis via svds from RSpectra

**Usage**

```
reduceDimensions(
  matnorm,
  center = TRUE,
  scale = TRUE,
  max.ods.genes = 2000,
  nPCs = 50,
  verbose = TRUE,
  plot = FALSE,
  details = FALSE
)
```

**Arguments**

|               |                                                                                |
|---------------|--------------------------------------------------------------------------------|
| matnorm       | matrix on which to perform PCA                                                 |
| center        | logical to mean center gene expression before PCA, default = TRUE              |
| scale         | logical to scale gene expression variance before PCA, default = TRUE           |
| max.ods.genes | number of most highly expressed overdispersed genes to include, default = 2000 |
| nPCs          | number of principal components to reduce to return, default = 50               |
| verbose       | logical for verbosity setting, default = TRUE                                  |
| plot          | plot singular values vs number of components                                   |
| details       | logical to return pca object, default = FALSE                                  |

**Value**

matrix of cell scores in nPCs components

**Examples**

```
data(vel)
curr <- vel$current

curr.norm <- normalizeDepth(curr)
curr.norm <- log10(curr.norm+1)
reduceDimensions(curr.norm, nPCs=3)
```

---

vel

---

*MERFISH velocity subset*

---

**Description**

output of `velocyto.R::gene.relative.velocity.estimates` for 40 cell subset of MERFISH data. Used to run examples

**Usage**

vel

**Format**

list of 1:

**vel** velocity output containing current observed ("current") and predicted future ("projected") estimates

**Source**

<https://www.pnas.org/content/116/39/19490>

---

|         |                |
|---------|----------------|
| veloviz | <i>veloviz</i> |
|---------|----------------|

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**Description**

Package for creating RNA velocity informed embeddings for single cell transcriptomics

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