Package 'veloviz'

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

Version 1.16.0

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

LazyData false

Roxygen list(markdown = TRUE)

RoxygenNote 7.1.1

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

LinkingTo Rcpp

Depends R (>= 4.1)

Suggests knitr, rmarkdown, testthat

VignetteBuilder knitr

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`asNNGraph` *Function to produce idx and dist representation of a VeloViz graph*

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

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

`curr`	Genes (rows) x cells (columns) matrix of observed current transcriptional state
`proj`	Genes (rows) x cells (columns) matrix of predicted future transcriptional state
`normalize.depth`	logical to normalize raw counts to counts per million, default = TRUE
`depth`	Depth scaling, default = 1e6 for counts per million (CPM)
`use.ods.genes`	Use only overdispersed genes to create VeloViz graph, default = TRUE
`max.ods.genes`	number of most highly expressed overdispersed genes to use to create VeloViz graph, default = 2000
`alpha`	Significance threshold for overdispersed genes, default = 0.05
`pca`	logical to use PC scores to create VeloViz graph, default = TRUE. FALSE = use gene expression to create VeloViz graph
`center`	logical to mean center gene expression before PCA, default = TRUE
`scale`	logical to scale gene expression variance before PCA, default = TRUE

nPCs	number of principal components to use to create VeloViz graph, default = 10
k	Number of nearest neighbors to assign each cell
similarity.threshold	similarity threshold below which to remove edges, default = -1 i.e. no edges removed
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
weighted	logical indicating whether to compute VeloViz edges based on composite distance, default = TRUE. FALSE = all edges are of equal weight
remove.unconnected	logical indicating whether to remove cells with no edges in the VeloViz graph from the output embedding, default = TRUE (removed)
verbose	logical for verbosity setting, default = FALSE
details	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 *Normalizes counts to CPM*

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` *Identify overdispersed genes by normalizing counts per million (CPM) gene expression variance relative to transcriptome-wide expectations (Modified from SCDE/PAGODA2 code)*

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 infinites (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` *Pancreas scRNA-seq data*

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` *Plot 2D embedding From scde/pagoda2/MUDAN*

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

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

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 *Plot function*

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

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)
```

---

reduceDimensions *Reduce dimension using Principal Components Analysis via* svds *from* RSpectra

---

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 *veloviz*

Description

Package for creating RNA velocity informed embeddings for single cell transcriptomics

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