Package ‘velociraptor’

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Description This package provides Bioconductor-friendly wrappers for RNA velocity calculations in single-cell RNA-seq data. We use the basilisk package to manage Conda environments, and the zellkonverter package to convert data structures between SingleCellExperiment (R) and AnnData (Python). The information produced by the velocity methods is stored in the various components of the SingleCellExperiment class.
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velociraptor: Toolkit for Single-Cell Velocity

Description

This package provides Bioconductor-friendly wrappers for RNA velocity calculations in single-cell RNA-seq data. We use the basilisk package to manage Conda environments, and the zellkonverter package to convert data structures between SingleCellExperiment (R) and AnnData (Python). The information produced by the velocity methods is stored in the various components of the SingleCellExperiment class.

Author(s)

Maintainer: Kevin Rue-Albrecht <kevinrue67@gmail.com> (ORCID)

Authors:

- Aaron Lun <infinite.monkeys.with.keyboards@gmail.com> (ORCID)
- Charlotte Soneson <charlottesoneson@gmail.com> (ORCID)
- Michael Stadler <michael.stadler@fmi.ch> (ORCID)

See Also

Useful links:

- https://github.com/kevinrue/velociraptor
- Report bugs at https://github.com/kevinrue/velociraptor/issues
**Description**

Project the velocity vector for each cell onto an existing low-dimensional embedding.

**Usage**

```r
embedVelocity(x, vobj, ...)
## S4 method for signature 'ANY'
embedVelocity(x, vobj, ...)
## S4 method for signature 'SingleCellExperiment'
embedVelocity(x, vobj, ..., use.dimred = 1)
```

**Arguments**

- `x` A numeric matrix of low-dimensional coordinates, e.g., after t-SNE. Alternatively, a `SingleCellExperiment` containing such coordinates in its `reducedDims`.
- `vobj` A `SingleCellExperiment` containing the output of the velocity calculations, typically after running `scvelo`.
- `...` For the generic, further arguments to pass to specific methods.
- `use.dimred` String or integer scalar specifying the reduced dimensions to retrieve from `x`.

**Details**

This is a simple wrapper around the `scvelo.tools.velocity_embedding` function. Briefly, we construct a cell-cell transition matrix where a cell is more likely to transition to one of its neighbors if its velocity vector is pointing in the same direction as that neighbor. The resulting matrix is then used to compute a weighted average of the positions in `x`, allowing us to compute a velocity in the low-dimensional embedding.

**Value**

A numeric matrix of the same dimensions as `x`, containing the projected velocity vectors in that embedding.

**Author(s)**

Aaron Lun
Examples

```r
example(scvelo, echo=FALSE) # recycling that example.

# Making up a new embedding.
tsne.results <- matrix(rnorm(2*ncol(out)), ncol=2)

# Projecting the future state of each cell:
projected <- embedVelocity(tsne.results, out)
```

### Description

Summarize the velocity vectors into a grid, usually for easy plotting.

#### Usage

```r
gridVectors(x, embedded, ...
```

#### Arguments

- **x**: A numeric matrix of low-dimensional coordinates, e.g., after t-SNE. Alternatively, a `SingleCellExperiment` containing such coordinates in its `reducedDims`.
- **embedded**: A low-dimensional projection of the velocity vectors into the embedding of `x`. This should be of the same dimensions as `x` and is typically produced by `embedVelocity`.
- **...**: For the generic, further arguments to pass to specific methods.
- **resolution**: Integer scalar specifying the resolution of the grid, in terms of the number of grid intervals along each axis.
gridVectors

scale Logical scalar indicating whether the averaged vectors should be scaled by the grid resolution.
as.data.frame Logical scalar indicating whether the output should be a data.frame. If FALSE, a list of two matrices is returned.
return.intermediates Logical scalar indicating whether intermediate objects should also be returned. This enforces as.data.frame=FALSE and throws a warning if it is TRUE.
use.dimred String or integer scalar specifying the reduced dimensions to retrieve from x.

Details
This partitions the bounding box of x into a grid with resolution units in each dimension. The locations and vectors of all cells in each block are averaged to obtain a representative of that block. This is most obviously useful for visualization to avoid overplotting of velocity vectors.
If scale=TRUE, per-block vectors are scaled so that the median vector length is comparable to the spacing between blocks. This improves visualization when the scales of x and embedded are not immediately comparable.

Value
If as.data.frame=FALSE, a list is returned containing start and end, two numeric matrices with one row per non-empty block in the grid and one column per column in x. start contains the mean location of all cells inside that block, and end contains the endpoint after adding the (scaled) average of the block’s cell’s velocity vectors.
If as.data.frame=TRUE, a data.frame is returned with numeric columns of the same contents as the list above. Column names are prefixed by start.* and end.*.
If return.intermediates=TRUE, a list is returned (irrespective of the value of as.data.frame) that in addition to start and end also contains intermediate objects limits (the ranges in x and y), delta (the grid intervals in x and y), categories (a DataFrame with integer row and column indices for each cell that specify the grid field that it is contained in), grp (numerical index of grid fields for each cell) and vec (velocity vectors for non-empty grid fields).

Author(s)
Aaron Lun

See Also
embedVelocity, to generate embedded.

Examples

tsne.results <- matrix(rnorm(10000), ncol=2)
tsne.vectors <- matrix(rnorm(10000), ncol=2)

out <- gridVectors(tsne.results, tsne.vectors)

# Demonstration for plotting.
plot(tsne.results[,1], tsne.results[,2], col='grey')
plotVelocity

Phase and velocity graphs for a set of genes

Description

For a each gene in a set of genes, show the phase graph (spliced versus unspliced counts and fitted model) and reduced dimension graphs with cell colored by velocity and (spliced) expression.

Usage

plotVelocity(
  x,
  genes,
  use.dimred = 1,
  assay.splicedM = "Ms",
  assay.unsplicedM = "Mu",
  which.plots = c("phase", "velocity", "expression"),
  genes.per.row = 1,
  color_by = "#222222",
  color.alpha = 0.4,
  colors.velocity = c("#A50026", "#D73027", "#F46D43", "#F4AE61", "#FEE08B", "#FFFFBF",
  "#D9EF8B", "#A696A", "#66BD63", "#1A9850", "#006837"),
  colors.expression = c("#440154", "#482576", "#414487", "#35608D", "#2A788E", "#21908C",
  "#22A884", "#43BF71", "#7AD151", "#BBDF27", "#FDE725"),
  max.abs.velo = 0.001
)

Arguments

x A SingleCellExperiment object with RNA velocity results as returned by scvelo, and low-dimensional coordinates, e.g., after t-SNE, in its reducedDims.

genes A character vector with one or several genes for which to plot phase and velocity graphs. genes have to be in rownames(x).

use.dimred String or integer scalar specifying the reduced dimensions to retrieve from x.

assay.splicedM An integer scalar or string specifying the assay of x containing the moments of spliced abundances.

assay.unsplicedM An integer scalar or string specifying the assay of x containing the moments unspliced abundances.

which.plots A character vector specifying which plots to create for each gene. Possible values are "phase", "velocity", "expression" and correspond to the phase graph or reduced dimension graphs with cells colored by velocity or (spliced) expression.
genes.per.row  An integer scalar with the numbers of genes to visualize per row of plots. For example, if `which.plots = c("phase","expression")` and `genes.per.row = 2`, the resulting figure will have four plot panels per row.

color_by  A character scalar specifying a column in `colData(x)` to color cells in the phase graph. Alternatively, `color_by` can be set to vector of valid R colors, either of length one (recycled for all cells) or of length `ncol(x)`, which will then be used to color cells in the phase graph.

color.alpha  An integer scalar giving the transparency of colored cells. Possible values are between 0 (fully transparent) and 1.0 (opaque).

colors.velocity, colors.expression  Character vectors specifying the color ranges used for mapping velocities and expression values. The defaults are `RColorBrewer::brewer.pal(11, "RdYlGn")` for the velocities and `viridisLite::viridis(11)` for the expression values.

max.abs.velo  A numeric scalar greater than zero giving the maximum absolute velocity to limit the color scale for the "velocity" graph.

Details
Please note that `plotVelocity` will modify parameters of the current graphics device using `layout` and `par`, in order to create the layout for the generated graph panels.

Value
A patchwork object with the plots selected by `which.plot` for the genes in `genes`, arranged in a grid according to `genes.per.row`.

Author(s)
Michael Stadler

See Also
`svelo`, `brewer.pal` and `viridis` for creation of color palettes, packages `ggplot2` and `patchwork` used to generate and arrange the plots.

Examples
```r
library(scuttle)
set.seed(42)
sce1 <- mockSCE(ncells = 100, ngenes = 500)
sce2 <- mockSCE(ncells = 100, ngenes = 500)
datlist <- list(X=counts(sce1), spliced=counts(sce1), unspliced=counts(sce2))
out1 <- svelo(datlist, mode = "steady_state")
out2 <- svelo(datlist, mode = "dynamical")
plotVelocity(out1, c("Gene_0031","Gene_0268"))
plotVelocity(out2, c("Gene_0031","Gene_0268"))
```
plotVelocityStream

Velocity stream plot in low-dimensional space

Description

Plot velocities embedded into low-dimensional space as a stream plot. Stream lines are lines that follow the gradient in the velocity field and illustrate paths that cells could follow based on observed RNA velocities.

Usage

```r
plotVelocityStream(
  sce,
  embedded,
  use.dimred = 1,
  color_by = "#444444",
  color.alpha = 0.2,
  grid.resolution = 60,
  scale = TRUE,
  stream.L = 10,
  stream.min.L = 0,
  stream.res = 4,
  stream.width = 8,
  color.streamlines = FALSE,
  color.streamlines.map = c("#440154", "#482576", "#414487", "#35608D", "#2A788E",
                           "#21908C", "#22A884", "#43BF71", "#7AD151", "#BBDF27", "#FDE725"),
  arrow.angle = 8,
  arrow.length = 0.8
)
```

Arguments

- **sce** A `SingleCellExperiment` object containing low-dimensional coordinates, e.g., after t-SNE, in its `reducedDims`.
- **embedded** A low-dimensional projection of the velocity vectors into the embedding of `sce`. This should be of the same dimensions as `sce` and is typically produced by `embedVelocity`.
- **use.dimred** String or integer scalar specifying the reduced dimensions to retrieve from `sce`.
- **color_by** A character scalar specifying a column in `colData(sce)` to color cells in the phase graph. Alternatively, `color_by` can be set to a valid R color to be used to color cells.
- **color.alpha** An integer scalar giving the transparency of colored cells. Possible values are between 0 (fully transparent) and 1.0 (opaque).
- **grid.resolution** Integer scalar specifying the resolution of the grid, in terms of the number of grid intervals along each axis.
scale Logical scalar indicating whether the averaged vectors should be scaled by the grid resolution.

stream.L Integer scalar giving the typical length of a streamline low-dimensional space units.

stream.min.L A numeric scalar with the minimum length of segments to be shown.

stream.res Numeric scalar specifying the resolution of estimated streamlines (higher numbers increase smoothness of lines but also the time for computation).

stream.width A numeric scalar controlling the width of streamlines.

color.streamlines Logical scalar. If TRUE streamlines will be colored by local velocity. Arrows cannot be shown in that case.

color.streamlines.map A character vector specifying the color range used for mapping local velocities to streamline colors. The default is viridisLite::viridis(11).

arrow.angle, arrow.length Numeric scalars giving the angle and length of arrowheads.

Details

grid.resolution and scale are passed to gridVectors, which is used to summarized the velocity vectors into an initial grid. A full regular grid is computed from that and used in geom_streamline to calculate streamlines. The following arguments are passed to the arguments given in parenthesis of geom_streamline: stream.L (L), stream.res (res), stream.min.L (min.L), arrow.angle (arrow.angle) and arrow.length (arrow.length). Streamlines are computed by simple integration with a forward Euler method, and stream.L and stream.res are used to compute the number of steps and the time interval between steps for the integration. stream.width is multiplied with ..step.. estimated by geom_streamline to control the width of streamlines.

Value

A ggplot2 object with the streamline plot.

Author(s)

Michael Stadler

See Also

gridVectors used to summarize velocity vectors into a grid (velocity field), the ggplot2 package used for plotting, geom_streamline in package metR used to calculate and add streamlines from the RNA velocity field to the plot, viridis for creation of color palettes.

Examples

library(scuttle)
set.seed(42)
sce1 <- mockSCE(ncells = 100, ngenes = 500)
sce2 <- mockSCE(ncells = 100, ngenes = 500)
scvelo <- list(X=counts(sce1), spliced=counts(sce1), unspliced=counts(sce2))

out <- scvelo(datlist, mode = "dynamical")

em <- embedVelocity(reducedDim(out, 1), out)[,1:2]

plotVelocityStream(out, em)
plotVelocityStream(out, em, color.streamlines = TRUE)

---

RNA velocity with scVelo

Description

Perform RNA velocity calculations with the scVelo package.

Usage

scvelo(x, ...)

# S4 method for signature 'ANY'
scvelo(
  x,
  subset.row = NULL,
  sf.X = NULL,
  sf.spliced = NULL,
  sf.unspliced = NULL,
  use.theirs = FALSE,
  mode = c("steady_state", "deterministic", "stochastic", "dynamical"),
  scvelo.params = list(),
  dimred = NULL,
  ncomponents = 30,
  BPPARAM = SerialParam(),
  BSPARAM = bsparam()
)

# S4 method for signature 'SummarizedExperiment'
scvelo(
  x,
  ...,
  assay.X = "counts",
  assay.spliced = "spliced",
  assay.unspliced = "unspliced"
)

# S4 method for signature 'SingleCellExperiment'
scvelo(x, ..., sf.X = sizeFactors(x), dimred = NULL, use.dimred = NULL)
Arguments

A named list of three matrices of the same dimensions where genes are in rows and cells are in columns. The list should contain "spliced" and "unspliced" entries containing spliced and unspliced counts, respectively. It should also contain an "X" entry containing the "usual" count matrix, see details below. Alternatively, a SummarizedExperiment object containing three such matrices among its assays.

For the generic, further arguments to pass to specific methods. For the SummarizedExperiment and SingleCellExperiment methods, further arguments to pass to the ANY method.

subset.row A character, integer or logical vector specifying the genes to use for the velocity calculations. Defaults to all genes.

sf.X A numeric vector containing size factors for usual count matrix. Defaults to librarySizeFactors on the "X" matrix in x.

sf.spliced A numeric vector containing size factors for the spliced counts for each cell. Defaults to librarySizeFactors on the "spliced" matrix in x.

sf.unspliced A numeric vector containing size factors for the unspliced counts for each cell. Defaults to librarySizeFactors on the "unspliced" matrix in x.

use.theirs Logical scalar indicating whether scVelo's gene filtering and normalization should be used.

mode String specifying the method to use to estimate the transcriptional dynamics.

scvelo.params List of lists containing arguments for individual scVelo functions, see details below.

dimred A low-dimensional representation of the cells with number of rows equal to the number of cells in x, used to find the nearest neighbors.

ncomponents Numeric scalar indicating the number of principal components to obtain. Only used if use.theirs=FALSE and dimred=NULL.

BPPARAM A BiocParallelParam object specifying whether the PCA calculations should be parallelized. Only used if use.theirs=FALSE and dimred=NULL.

BSPARAM A BiocSingularParam object specifying which algorithm should be used to perform the PCA. Only used if use.theirs=FALSE and dimred=NULL.

assay.X An integer scalar or string specifying the assay of x containing the usual count matrix.

assay.spliced An integer scalar or string specifying the assay of x containing the spliced counts.

assay.unspliced An integer scalar or string specifying the assay of x containing the unspliced counts.

use.dimred String naming the entry of reducedDims(x) to use for nearest neighbor calculations. Ignored if dimred is supplied.
Details

This function uses the scVelo Python package (https://pypi.org/project/scvelo/) for RNA velocity calculations. The main difference from the original velocyto approach is that the dynamical model of scVelo does not rely on the presence of observed steady-state populations, which should improve the reliability of the velocity calculations in general applications.

For consistency with other Bioconductor workflows, we perform as many standard steps in R as we can before starting the velocity calculations with scVelo. This involves:

1. Size factor-based normalization with sf.* values and normalizeCounts. For "X", log-transformation is performed as well, while for the others, only scaling normalization is performed.
2. Subsetting all matrices to subset.row, most typically to a subset of interest, e.g., highly variable genes. Note that, if set, any subsetting is done after normalization so that library sizes are correctly computed.
3. If dimred=NULL, the PCA step on the log-expression values derived from the "X" matrix, using the specified BSPARAM to obtain the first ncomponents PCs.

This allows us to guarantee that, for example, the log-expression matrix of HVGs or the PCA coordinates are the same as that used in other applications like clustering or trajectory reconstruction.

Nonetheless, one can set use.theirs=TRUE to directly use the entire scVelo normalization and filtering pipeline. This ignores all of the size factors arguments (sf.*), all of the PCA-related arguments (ncomponents, BSPARAM) and subset.row. However, if a low-dimensionality result is supplied via dimred or use.dimred, the scVelo PCA will always be omitted.

Upon first use, this function will instantiate a conda environment containing the scVelo package. This is done via the basilisk package - see the documentation for that package for trouble-shooting.

Value

A SingleCellExperiment is returned containing the output of the velocity calculations. Of particular interest are:

- the velocity_pseudotime field in the colData, containing the velocity pseudotime for each cell.
- the velocity entry of the assays, containing the velocity vectors for each cell.

The output will always have number of columns equal to the number of cells supplied in x, though the number of rows will depend on whether any subsetting (if subset.row is supplied) or feature selection (if use.theirs=TRUE) was performed.

Comments on the three matrices

Strictly speaking, only the spliced and unspliced matrices are necessary for the velocity calculations. However, it is often the case that the spliced matrix is not actually the same as a “usual” count matrix (e.g., generated by summing counts across all exons for all mapped genes). This is due to differences in the handling of ambiguous reads that map across exon-intron boundaries, or to genomic regions that can be either exonic or intronic depending on the isoform; the spliced count matrix is more likely to exclude such reads.

We request the usual count matrix as the "X" entry of x to ensure that the PCA and nearest neighbor detection in scVelo are done on the same data as that used in other steps of the large analysis (e.g.,
clustering, visualization, trajectory reconstruction). In practice, if the usual count matrix is not available, one can often achieve satisfactory results by simply re-using the spliced count matrix as both the "X" and "spliced" entries of x.

Note that if reduced dimensions are supplied in dimred, any "X" entry is only used to create the AnnData object and is not used in any actual calculations.

Additional arguments to Python

Additional arguments to scVelo functions are provided via scvelo.params. This is a named list where each entry is named after a function and is itself a named list of arguments for that function. The following function names are currently recognized:

- "filter_and_normalize", for gene selection and normalization. This is not used unless use.theirs=TRUE.
- "moments", for PCA and nearest neighbor detection. The PCA is not performed if dimred or use.dimred is already supplied.
- "recover_dynamics"
- "velocity"
- "velocity_graph"
- "velocity_pseudotime"
- "latent_time"
- "velocity_confidence"

See the scVelo documentation for more details about the available arguments and the examples below for a syntax example.

Author(s)

Aaron Lun, Charlotte Soneson

References


Examples

# Using mock data to demonstrate the process:
library(scuttle)
sce1 <- mockSCE()
sce2 <- mockSCE()

spliced <- counts(sce1)
unspliced <- counts(sce2)

out <- scvelo(list(X=spliced, spliced=spliced, unspliced=unspliced))

# make scvelo use 10 rather than the default 30 neighbors to compute moments for velocity estimation:
out <- scvelo(list(X=spliced, spliced=spliced, unspliced=unspliced),
scvelo.params=list(moments=list(n_neighbors=10L)))
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