scvelo documentation

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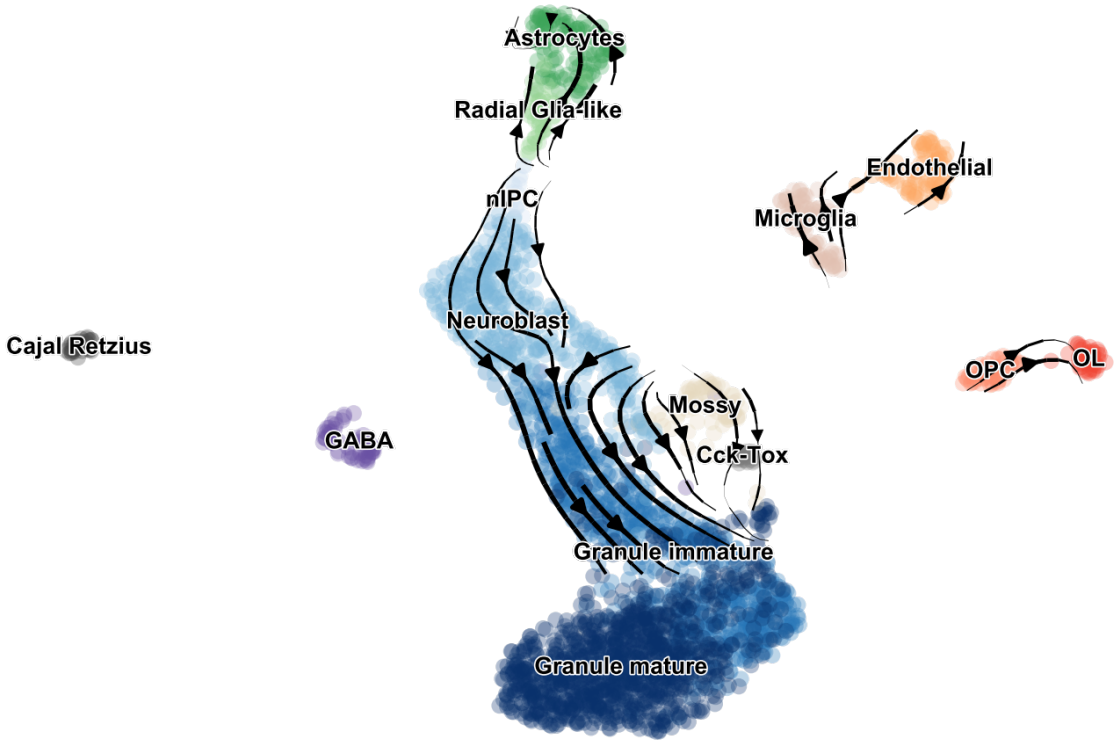

scVelo is a scalable toolkit for estimating and analyzing RNA velocities in single cells.

RNA velocity, the time derivative of mRNA abundance, enables you to infer directionality in your data by superimposing splicing information. The main principles have been presented in La Manno et al. (2018), and are based on a deterministic steady-state model of transcriptional dynamics. scVelo provides two extensions: A stochastic model that incorporates second-order moments, and a dynamical model that captures the full splicing kinetics. It thereby adapts RNA velocity to widely varying specifications such as non-stationary populations.

It is compatible with scanpy (Wolf et al., 2018). Making use of sparse implementation, iterative neighbors search and other techniques, it is remarkably efficient in terms of memory and runtime without loss in accuracy and runs easily on your local machine (30k cells in a few minutes).

Report issues and see the code on GitHub.

CHAPTER 1

About RNA velocity

RNA velocity enables you to infer directionality in your data by superimposing splicing information.

With the astounding finding that unspliced and spliced mRNA abundances can be distinguished in standard single cell protocols, La Manno et al., (2018) introduced the concept of RNA velocity. Inference of directional trajectories is explored by connecting measurements to the underlying mRNA splicing kinetics: Transcriptional induction for a particular gene results in an increase of (newly transcribed) precursor unspliced mRNAs while, conversely, repression or absence of transcription results in a decrease of unspliced mRNAs. Hence, by distinguishing unspliced from mature spliced mRNA, the change of mRNA abundance, i.e. its time derivative, denoted as RNA velocity, can be approximated. The combination of velocities across mRNAs can then be used to estimate the future state of an individual cell. The movement of the cells is visualized by projecting the velocities into a lower-dimensional embedding.

1.1 How scVelo estimates RNA velocities

RNA velocity estimation can currently be tackled with three existing approaches:

- steady-state / deterministic model (as being used in velocyto)
- stochastic model (using second-order moments),
- dynamical model (using a likelihood-based framework).

The **steady-state / deterministic model**, as being used in velocyto, estimates velocities as follows: Under the assumption that transcriptional phases (induction and repression) endure long enough to reach a steady-state equilibrium (active and inactive), velocities are quantified as how the actual observations deviate from the steady-state equilibrium. The equilibrium mRNA levels are approximated with a linear regression on the presumed steady states in the lower and upper quantiles. This simplification is obtained by assuming of a common splicing rate across genes and steady-state mRNA levels to be reflected in the data. It can lead to errors in velocity estimates and cellular states as the assumptions are often violated, in particular when a population comprises multiple heterogeneous subpopulation dynamics.

The **stochastic model** aims to better capture the steady states, yet relying on the same assumptions as the steady-state model. It is obtained by treating transcription, splicing and degradation as probabilistic events, thereby incorporating second-order moments. That is, steady state levels are approximated not only from mRNA levels, but also from intrinsic expression variability.

The **dynamical model** (most powerful while computationally most expensive) solves the full dynamics of splicing kinetics for each gene. It thereby adapts RNA velocity to widely varying specifications such as non-stationary populations, as it does not rely on the restrictions of a common splicing rate or steady states to be sampled. The splicing dynamics is solved in a likelihood-based expectation-maximization framework, by iteratively estimating the identifiable parameters of reaction rates and latent cell-specific variables, i.e. transcriptional state and cell-internal latent time. More precisely, we explicitly model four transcriptional states to account for all possible configurations of gene activity: two dynamic transient states (induction and repression) and two steady states (active and inactive) potentially reached after each dynamic transition. For each observation per state a model-optimal latent time is computed to obtain a mapping onto the learned curve of unspliced/spliced dynamics. The cell-to-curve mapping then yields likelihoods of respective state membership, and individual reaction rate parameters via maximizing the overall likelihood.

This yields more consistent velocity estimates and better identification of transcriptional states. The model further enables to systemically identifies dynamics-driving genes in a likelihood-based way, thereby finding the key drivers that govern cell fate transitions. Moreover, the dynamical model infers a universal cell-internal latent time shared across genes that enables relating genes and identifying regimes of transcriptional changes (e.g. branching points).

For best results we obviously recommend using the superior dynamical model. If runtime matters, we recommend using the stochastic model, which is designed to approximate the dynamical model. The stochastic model takes less than a minute on 30k cells. The dynamical yet can take up to one hour, however, enhancing efficiency is in progress.

1.2 Beyond RNA velocity

There are multiple extensions that can be easily explored, including terminal states (root and end points), pseudotemporal ordering based on velocities, infer directionality in abstracted graph and many more. A more detailed description will follow very soon.

CHAPTER 2

Getting Started

Welcome to scVelo!

scVelo is a scalable toolkit for estimating and analyzing RNA velocities in single cells.

2.1 Installation

scVelo requires Python 3.6 or later. We recommend to use Miniconda.

Install scVelo from PyPI using:

```
pip install -U scvelo
```

To work with the latest development version, install from source using:

```
pip install git+https://github.com/theislab/scvelo
```

or:

```
git clone git+https://github.com/theislab/scvelo
pip install -e scvelo
```

Parts of scVelo require (optional):

```
conda install -c conda-forge numba pytables louvain
```

The splicing data can be obtained using the velocyto pipeline or the kallisto pipeline.

2.2 scVelo in action

Import scvelo as:

```
import scvelo as scv
```

For beautified visualization you can change the matplotlib settings to our defaults with:

```
scv.settings.set_figure_params('scvelo')
```

2.2.1 Read your data

Read your data file (loom, h5ad, csv, . . .) using:

```
adata = scv.read(filename, cache=True)
```

which stores the data matrix (`adata.X`), annotation of cells / observations (`adata.obs`) and genes / variables (`adata.var`), unstructured annotation such as graphs (`adata.uns`) and additional data layers where spliced and unspliced counts are stored (`adata.layers`) .

If you already have an existing preprocessed adata object you can simply merge the spliced/unspliced counts via:

```
ldata = scv.read(filename.loom, cache=True)
adata = scv.utils.merge(adata, ldata)
```

If you do not have a datasets yet, you can still play around using one of the in-built datasets, e.g.:

```
adata = scv.datasets.dentategyrus()
```

The typical workflow consists of subsequent calls of preprocessing (`scv.pp.*`), analysis tools (`scv.tl.*`) and plotting (`scv.pl.*`).

2.2.2 Basic preprocessing

After basic preprocessing (gene selection and normalization is sufficient), we compute the first- and second-order moments (basically means and variances) for velocity estimation:

```
scv.pp.filter_and_normalize(adata, **params)
scv.pp.moments(adata, **params)
```

2.2.3 Velocity Tools

The core of the software is the efficient and robust estimation of velocities, obtained with:

```
scv.tl.velocity(adata, mode='stochastic', **params)
```

The velocities are vectors in gene expression space obtained by solving a stochastic model of transcriptional dynamics. The solution to the deterministic model is obtained by setting `mode='deterministic'`.

The solution to the dynamical model is obtained by setting `mode='dynamical'`, which requires to run `scv.tl.recover_dynamics(adata, **params)` beforehand.

The velocities are stored in `adata.layers` just like the count matrices.

The velocities are projected into a lower-dimensional embedding by translating them into likely cell transitions. That is, for each velocity vector we find the likely cell transitions that are accordance with that direction. The probabilities of one cell transitioning into another cell are computed using cosine correlation (btw. the potential cell transition and the velocity vector) and are stored in a matrix denoted as velocity graph:

```
scv.tl.velocity_graph(adata, **params)
```

2.2.4 Visualization

Finally the velocities can be projected and visualized in any embedding (e.g. UMAP) on single cell level, grid level, or as streamplot:

```
scv.pl.velocity_embedding(adata, basis='umap', **params)
scv.pl.velocity_embedding_grid(adata, basis='umap', **params)
scv.pl.velocity_embedding_stream(adata, basis='umap', **params)
```

For every tool module there is a plotting counterpart, which allows you to examine your results in detail, e.g.:

```
scv.pl.velocity(adata, var_names=['gene_A', 'gene_B'], **params)
scv.pl.velocity_graph(adata, **params)
```

scvelo - RNA velocity using dynamical modeling

CHAPTER 3

API

Import scVelo as:

```
import scvelo as scv
```

3.1 Read / Load

read(filename[, backed, sheet, ext, ...])	Read file and return AnnData object.
read_loom(filename[, sparse, cleanup, ...])	Read *.loom*-formatted hdf5 file.

3.1.1 scvelo.read

scvelo.**read**(*filename*, *backed=None*, *sheet=None*, *ext=None*, *delimiter=None*, *first_column_names=False*, *backup_url=None*, *cache=False*, ***kwargs*)

Read file and return AnnData object.

To speed up reading, consider passing cache=True, which creates an hdf5 cache file.

Parameters

filename : Path, str If the filename has no file extension, it is interpreted as a key for generating a filename via sc.settings.writedir / (filename + sc.settings.file_format_data). This is the same behavior as in sc.read(filename, ...).

backed : {None, 'r', 'r+'} If 'r', load AnnData in backed mode instead of fully loading it into memory (*memory* mode). If you want to modify backed attributes of the AnnData object, you need to choose 'r+'.

sheet : str, None Name of sheet/table in hdf5 or Excel file.

ext : str, None Extension that indicates the file type. If None, uses extension of filename.

delimiter : str, None Delimiter that separates data within text file. If `None`, will split at arbitrary number of white spaces, which is different from enforcing splitting at any single white space `' '`.

first_column_names : bool Assume the first column stores row names. This is only necessary if these are not strings: strings in the first column are automatically assumed to be row names.

backup_url : str, None Retrieve the file from an URL if not present on disk.

cache : bool If `False`, read from source, if `True`, read from fast 'h5ad' cache.

kwargs Parameters passed to `read_loom()`.

Return type `AnnData`

Returns An `AnnData` object

3.1.2 scvelo.read_loom

scvelo.**read_loom**(*filename*, *sparse=True*, *cleanup=False*, *X_name='spliced'*, *obs_names='CellID'*, *var_names='Gene'*, *dtype='float32'*, ***kwargs*)

Read *.loom*-formatted hdf5 file.

This reads the whole file into memory.

Beware that you have to explicitly state when you want to read the file as sparse data.

Parameters

filename : PathLike The filename.

sparse : bool Whether to read the data matrix as sparse.

cleanup : bool Whether to collapse all obs/var fields that only store one unique value into *.uns['loom-']*.

X_name : str Loompy key with which the data matrix *.X* is initialized.

obs_names : str Loompy key where the observation/cell names are stored.

var_names : str Loompy key where the variable/gene names are stored.

****kwargs** Arguments to loompy.connect

Return type `AnnData`

3.2 Preprocessing (pp)

pp.filter_genes(data[, min_counts, ...])	Filter genes based on number of cells or counts.
pp.filter_genes_dispersion(data[, flavor, ...])	Extract highly variable genes.
pp.normalize_per_cell(data[, ...])	Normalize each cell by total counts over all genes.
pp.filter_and_normalize(data[, min_counts, ...])	Filtering, normalization and log transform
pp.moments(data[, n_neighbors, n_pcs, mode, ...])	Computes moments for velocity estimation.

3.2.1 scvelo.pp.filter_genes

scvelo.pp.**filter_genes**(*data*, *min_counts=None*, *min_cells=None*, *max_counts=None*, *max_cells=None*, *min_counts_u=None*, *min_cells_u=None*, *max_counts_u=None*, *max_cells_u=None*, *min_shared_counts=None*, *min_shared_cells=None*, *copy=False*)

Filter genes based on number of cells or counts. Keep genes that have at least *min_counts* counts or are expressed in at least *min_cells* cells or have at most *max_counts* counts or are expressed in at most *max_cells* cells. Only provide one of the optional parameters *min_counts*, *min_cells*, *max_counts*, *max_cells* per call.

Parameters

data : AnnData, *np.ndarray*, *sp.spmatrix* The (annotated) data matrix of shape *n_obs* × *n_vars*. Rows correspond to cells and columns to genes.

min_counts : *int*, optional (default: *None*) Minimum number of counts required for a gene to pass filtering.

min_cells : *int*, optional (default: *None*) Minimum number of cells expressed required for a gene to pass filtering.

max_counts : *int*, optional (default: *None*) Maximum number of counts required for a gene to pass filtering.

max_cells : *int*, optional (default: *None*) Maximum number of cells expressed required for a gene to pass filtering.

min_counts_u : *int*, optional (default: *None*) Minimum number of unspliced counts required for a gene to pass filtering.

min_cells_u : *int*, optional (default: *None*) Minimum number of unspliced cells expressed required for a gene to pass filtering.

max_counts_u : *int*, optional (default: *None*) Maximum number of unspliced counts required for a gene to pass filtering.

max_cells_u : *int*, optional (default: *None*) Maximum number of unspliced cells expressed required for a gene to pass filtering.

min_shared_counts : *int*, optional (default: *None*) Minimum number of counts (in cells expressed simultaneously in unspliced and spliced) required for a gene.

min_shared_cells : *int*, optional (default: *None*) Minimum number of cells required for a gene to be expressed simultaneously in unspliced and spliced.

copy : *bool*, optional (default: *False*) Determines whether a copy is returned.

Returns Filters the object and adds *n_counts* to *adata.var*.

3.2.2 scvelo.pp.filter_genes_dispersion

scvelo.pp.**filter_genes_dispersion**(*data*, *flavor='seurat'*, *min_disp=None*, *max_disp=None*, *min_mean=None*, *max_mean=None*, *n_bins=20*, *n_top_genes=None*, *log=True*, *copy=False*)

Extract highly variable genes. The normalized dispersion is obtained by scaling with the mean and standard deviation of the dispersions for genes falling into a given bin for mean expression of genes. This means that for each bin of mean expression, highly variable genes are selected.

Parameters

data : AnnData, *np.ndarray*, *sp.sparse* The (annotated) data matrix of shape *n_obs* × *n_vars*. Rows correspond to cells and columns to genes.

flavor : *{'seurat', 'cell_ranger', 'svr'}, optional (default: 'seurat')* Choose the flavor for computing normalized dispersion. If choosing 'seurat', this expects non-logarithmized data - the logarithm of mean and dispersion is taken internally when *log* is at its default value *True*. For 'cell_ranger', this is usually called for logarithmized data - in this case you should set *log* to *False*. In their default workflows, Seurat passes the cutoffs whereas Cell Ranger passes *n_top_genes*.

max_mean=3, min_disp=0.5, max_disp=`None` : *min_mean=0.0125,* If *n_top_genes* unequals *None*, these cutoffs for the means and the normalized dispersions are ignored.

n_bins : *int* (default: 20) Number of bins for binning the mean gene expression. Normalization is done with respect to each bin. If just a single gene falls into a bin, the normalized dispersion is artificially set to 1. You'll be informed about this if you set *settings.verbosity = 4*.

n_top_genes : *int* or *None* (default: *None*) Number of highly-variable genes to keep.

log : *bool*, optional (default: *True*) Use the logarithm of the mean to variance ratio.

copy : *bool*, optional (default: *False*) If an AnnData is passed, determines whether a copy is returned.

Returns If an AnnData *adata* is passed, returns or updates *adata* depending on *copy*. It filters the *adata* and adds the annotations

3.2.3 scvelo.pp.normalize_per_cell

scvelo.pp.**normalize_per_cell**(*data, counts_per_cell_after=None, counts_per_cell=None, key_n_counts=None, max_proportion_per_cell=None, use_initial_size=True, layers=['spliced', 'unspliced'], enforce=False, copy=False*)

Normalize each cell by total counts over all genes.

Parameters

data : AnnData, *np.ndarray*, *sp.sparse* The (annotated) data matrix of shape *n_obs* × *n_vars*. Rows correspond to cells and columns to genes.

counts_per_cell_after : *float* or *None*, optional (default: *None*) If *None*, after normalization, each cell has a total count equal to the median of the *counts_per_cell* before normalization.

counts_per_cell : *np.array*, optional (default: *None*) Precomputed counts per cell.

key_n_counts : *str*, optional (default: *'n_counts'*) Name of the field in *adata.obs* where the total counts per cell are stored.

max_proportion_per_cell : *int* (default: *None*) Exclude genes counts that account for more than a specific proportion of cell size, e.g. 0.05.

use_initial_size : *bool* (default: *True*) Whether to use initial cell sizes oder actual cell sizes.

layers : *str* or *list* (default: *{'spliced', 'unspliced'}*) Keys for layers to be also considered for normalization.

copy : *bool*, optional (default: *False*) If an AnnData is passed, determines whether a copy is returned.

Returns Returns or updates *adata* with normalized version of the original *adata.X*, depending on *copy*.

3.2.4 scvelo.pp.filter_and_normalize

scvelo.pp.**filter_and_normalize**(*data*, *min_counts=None*, *min_counts_u=None*, *min_cells=None*, *min_cells_u=None*, *min_shared_counts=None*, *min_shared_cells=None*, *n_top_genes=None*, *flavor='seurat'*, *log=True*, *copy=False*)

Filtering, normalization and log transform

Expects non-logarithmized data. If using logarithmized data, pass *log=False*.

Runs the following steps

```
scv.pp.filter_genes(adata)
scv.pp.normalize_per_cell(adata)
if n_top_genes is not None:
    scv.pp.filter_genes_dispersion(adata)
if log:
    scv.pp.log1p(adata)
```

Parameters

data : AnnData Annotated data matrix.

min_counts : *int* (default: *None*) Minimum number of counts required for a gene to pass filtering (spliced).

min_counts_u : *int* (default: *None*) Minimum number of counts required for a gene to pass filtering (unspliced).

min_cells : *int* (default: *None*) Minimum number of cells expressed required for a gene to pass filtering (spliced).

min_cells_u : *int* (default: *None*) Minimum number of cells expressed required for a gene to pass filtering (unspliced).

min_shared_counts : *int*, optional (default: *None*) Minimum number of counts (in cells expressed simultaneously in unspliced and spliced) required for a gene.

min_shared_cells : *int*, optional (default: *None*) Minimum number of cells required for a gene to be expressed simultaneously in unspliced and spliced.

n_top_genes : *int* (default: *None*) Number of genes to keep.

flavor : *{'seurat', 'cell_ranger', 'svr'}, optional (default: 'seurat')* Choose the flavor for computing normalized dispersion. If choosing 'seurat', this expects non-logarithmized data.

log : *bool* (default: *True*) Take logarithm.

copy : *bool* (default: *False*) Return a copy of *adata* instead of updating it.

Returns Returns or updates *adata* depending on *copy*.

3.2.5 scvelo.pp.moments

scvelo.pp.**moments**(*data*, *n_neighbors=30*, *n_pcs=None*, *mode='connectivities'*, *method='umap'*, *use_rep=None*, *copy=False*)

Computes moments for velocity estimation.

Parameters

data : AnnData Annotated data matrix.

n_neighbors : *int* (default: 30) Number of neighbors to use.

n_pcs : *int* (default: None) Number of principal components to use. If not specified, the full space is used of a pre-computed PCA, or 30 components are used when PCA is computed internally.

mode : *'connectivities'* or *'distances'* (default: *'connectivities'*) Distance metric to use for moment computation.

method : {{'umap', 'gauss', 'hnsw', 'sklearn', *None*}} (default: *'umap'*) Use 'umap' [McInnes18] or 'gauss' (Gauss kernel following [Coifman05] with adaptive width [Haghverdi16]) for computing connectivities.

use_rep : *None*, *'X'* or any key for *.obsm* (default: None) Use the indicated representation. If *None*, the representation is chosen automatically: for .n_vars < 50, .X is used, otherwise 'X_pca' is used.

copy : *bool* (default: *False*) Return a copy instead of writing to adata.

Returns

- Returns or updates *adata* with the attributes
- **Ms** (*.layers*) – dense matrix with first order moments of spliced counts.
- **Mu** (*.layers*) – dense matrix with first order moments of unspliced counts.

3.3 Tools (tl)

tl.velocity(data[, vkey, mode, fit_offset, ...])	Estimates velocities in a gene-specific manner
tl.velocity_graph(data[, vkey, xkey, tkey, ...])	Computes velocity graph based on cosine similarities.
tl.velocity_embedding(data[, basis, vkey, ...])	Computes the single cell velocities in the embedding
tl.recover_dynamics(data[, var_names, ...])	Recovers the full splicing kinetics of specified genes
tl.transition_matrix(adata[, vkey, basis, ...])	Computes transition probabilities from velocity graph
tl.terminal_states(data[, vkey, groupby, ...])	Computes terminal states (root and end points).
tl.rank_velocity_genes(data[, vkey, ...])	Rank genes for velocity characterizing groups.
tl.velocity_confidence(data[, vkey, copy])	Computes confidences of velocities.

3.3.1 scvelo.tl.velocity

scvelo.tl.**velocity**(*data*, *vkey='velocity'*, *mode='stochastic'*, *fit_offset=False*, *fit_offset2=False*, *filter_genes=False*, *groups=None*, *groupby=None*, *groups_for_fit=None*, *constrain_ratio=None*, *use_raw=False*, *use_latent_time=None*, *perc=[5, 95]*, *min_r2=0.01*, *min_likelihood=0.001*, *r2_adjusted=None*, *copy=False*, ***kwargs*)

Estimates velocities in a gene-specific manner

Parameters

data : AnnData Annotated data matrix.

vkey : *str* **(default:** *'velocity'***)** Name under which to refer to the computed velocities for *velocity_graph* and *velocity_embedding*.

mode : *'steady_state'*, *'deterministic'*, *'stochastic'* **or** *'dynamical'* **(default:** *'stochastic'***)** Whether to run the estimation using the deterministic or stochastic model of transcriptional dynamics. The *'steady_state'* model is default and refers to the deterministic model. The dynamical model requires to run *tl.recover_dynamics* first; it is yet under development.

fit_offset : *bool* **(default:** *False***)** Whether to fit with offset for first order moment dynamics.

fit_offset2 : *bool*, **(default:** *False***)** Whether to fit with offset for second order moment dynamics.

filter_genes : *bool* **(default:** *True***)** Whether to remove genes that are not used for further velocity analysis.

groups : *str*, *list* **(default:** *None***)** Subset of groups, e.g. ['g1', 'g2', 'g3'], to which velocity analysis shall be restricted.

groupby : *str*, *list* **or** *np.ndarray* **(default:** *None***)** Key of observations grouping to consider.

groups_for_fit : *str*, *list* **or** *np.ndarray* **(default:** *None***)** Subset of groups, e.g. ['g1', 'g2', 'g3'], to which steady-state fitting shall be restricted.

constrain_ratio : *float* **or tuple of type** *float* **or None: (default:** *None***)** Bounds for the steady-state ratio gamma'.

use_raw : *bool* **(default:** *False***)** Whether to use raw data for estimation.

use_latent_time : *bool'or 'None* **(default: 'None)** Whether to use latent time as a regularization for velocity when using dynamical mode.

perc : *float* **(default:** *None***)** Percentile, e.g. 98, upon for extreme quantile fit (to better capture steady states for velocity estimation).

min_r2 : *float* **(default: 0.01)** Minimum threshold for coefficient of determination

min_likelihood : *float* **(default:** *None***)** Minimal likelihood for velocity genes to fit the model on.

r2_adjusted : *bool* **(default:** *None***)** Whether to compute coefficient of determination on full data fit (adjusted) or extreme quantile fit (None)

copy : *bool* **(default:** *False***)** Return a copy instead of writing to *adata*.

Returns

- Returns or updates *adata* with the attributes
- **velocity** (*.layers*) – velocity vectors for each individual cell
- **variance_velocity** (*.layers*) – velocity vectors for the cell variances
- **velocity_offset, velocity_beta, velocity_gamma, velocity_r2** (*.var*) – parameters

3.3.2 scvelo.tl.velocity_graph

scvelo.tl.**velocity_graph**(*data*, *vkey='velocity'*, *xkey='Ms'*, *tkey=None*, *basis=None*, *n_neighbors=None*, *n_recurse_neighbors=None*, *random_neighbors_at_max=None*, *sqrt_transform=None*, *variance_stabilization=None*, *gene_subset=None*, *approx=None*, *copy=False*)

Computes velocity graph based on cosine similarities.

The cosine similarities are computed between velocities and potential cell state transitions.

Parameters

data : AnnData Annotated data matrix.

vkey : *str* (default: *'velocity'*) Name of velocity estimates to be used.

xkey : *str* (default: *'Ms'*) Layer key to extract count data from.

tkey : *str* (default: *None*) Observation key to extract time data from.

basis : *str* (default: *None*) Basis / Embedding to use.

n_neighbors : *int* or *None* (default: None) Use fixed number of neighbors or do recursive neighbor search (if *None*).

n_recurse_neighbors : *int* (default: 2) Number of recursions to be done for neighbors search.

random_neighbors_at_max : *int* or *None* (default: *None*) If number of iterative neighbors for an individual cell is higher than this threshold, a random selection of such are chosen as reference neighbors.

sqrt_transform : *bool* (default: *False*) Whether to variance-transform the cell states changes and velocities before computing cosine similarities.

gene_subset : *list* of *str*, subset of adata.var_names or *None'(default: 'None*) Subset of genes to compute velocity graph on exclusively.

approx : *bool* or *None* (default: *None*) If True, first 30 pc's are used instead of the full count matrix

copy : *bool* (default: *False*) Return a copy instead of writing to adata.

Returns

- Returns or updates *adata* with the attributes
- **velocity_graph** (*.uns*) – sparse matrix with transition probabilities

3.3.3 scvelo.tl.velocity_embedding

scvelo.tl.**velocity_embedding**(*data*, *basis=None*, *vkey='velocity'*, *scale=10*, *self_transitions=True*, *use_negative_cosines=True*, *direct_pca_projection=None*, *retain_scale=False*, *autoscale=True*, *all_comps=True*, *T=None*, *copy=False*)

Computes the single cell velocities in the embedding

Parameters

data : AnnData Annotated data matrix.

basis : *str* (default: *'tsne'*) Which embedding to use.

vkey : *str* (default: *'velocity'*) Name of velocity estimates to be used.

scale : *int* (default: 10) Scale parameter of gaussian kernel for transition matrix.

self_transitions : *bool* (default: *True*) Whether to allow self transitions, based on the confidences of transitioning to neighboring cell.

use_negative_cosines : *bool* (default: *True*) Whether to use not only positive, but also negative cosines and use those transitions to the opposite way.

direct_pca_projection : *bool* (default: *None*) Whether to directly project the velocities into PCA space, thus skipping velocity graph.

retain_scale : *bool* (default: *False*) Whether to retain scale from high dimensional space in embedding.

autoscale : *bool* (default: *True*) Whether to scale the embedded velocities by a scalar multiplier, which simply ensures that the arrows in the embedding are properly scaled.

all_comps : *bool* (default: *True*) Whether to compute the velocities on all embedding components or just the first two.

T : *csr_matrix* (default: *None*) Allows the user to directly pass a transition matrix.

copy : *bool* (default: *False*) Return a copy instead of writing to *adata*.

Returns

- Returns or updates *adata* with the attributes
- **velocity_basis** (*.obsm*) – coordinates of velocity projection on embedding

3.3.4 scvelo.tl.recover_dynamics

scvelo.tl.**recover_dynamics**(*data*, *var_names='velocity_genes'*, *n_top_genes=None*, *max_iter=10*, *assignment_mode='projection'*, *t_max=None*, *fit_time=True*, *fit_scaling=True*, *fit_steady_states=True*, *fit_connected_states=None*, *fit_basal_transcription=None*, *use_raw=False*, *load_pars=None*, *return_model=None*, *plot_results=False*, *steady_state_prior=None*, *add_key='fit'*, *copy=False*, ***kwargs*)

Recovers the full splicing kinetics of specified genes

The model infers transcription rates, splicing rates, degradation rates, as well as cell-specific latent time and transcriptional states.

Parameters

data : AnnData Annotated data matrix.

var_names : *str*, list of *str* (default: *'velocity_genes'*) Names of variables/genes to use for the fitting.

n_top_genes : *int* or *None* (default: *None*) Number of top velocity genes to use for the dynamical model.

max_iter : *int* (default: *10*) Maximal iterations in the EM-Algorithm.

assignment_mode : *str* (default: *projection*) Determined how times are assigned to observations. If *projection*, observations are projected onto the model trajectory. Else uses an inverse approximating formula.

t_max : *float* or *None* (default: *None*) Total range for time assignments.

fit_scaling : *bool* or *float* or *None* (default: *True*) Whether to fit scaling between unspliced and spliced or keep initially given scaling fixed.

fit_time : *bool* or *float* or *None* (default: *True*) Whether to fit time or keep initially given time fixed.

fit_steady_states : *bool* or *None* (default: *True*) Allows fitting of observations to steady states next to repression and induction.

fit_connected_states : *bool* or *None* (default: *None*) Restricts fitting to neighbors given by connectivities.

fit_basal_transcription : *bool* or *None* (default: *None*) Enables model to incorporate basal transcriptions.

use_raw : *bool* or *None* (default: *None*) if True, use .layers['sliced'], else use moments from .layers['Ms']

load_pars : *bool* or *None* (default: *None*) Load parameters from past fits.

return_model : *bool* or *None* (default: *True*) Whether to return the model as :DynamicsRecovery: object.

plot_results : *bool* or *None* (default: *False*) Plot results after parameter inference.

steady_state_prior : list of *bool* or *None* (default: *None*) Mask for indices used for steady state regression.

add_key : *str* (default: *'fit'*) Key to add to parameter names, e.g. 'fit_t' for fitted time.

copy : *bool* (default: *False*) Return a copy instead of writing to *adata*.

Returns Returns or updates *adata*

3.3.5 scvelo.tl.transition_matrix

scvelo.tl.**transition_matrix**(*adata*, *vkey='velocity'*, *basis=None*, *backward=False*, *self_transitions=True*, *scale=10*, *perc=None*, *use_negative_cosines=False*, *weight_diffusion=0*, *scale_diffusion=1*, *weight_indirect_neighbors=None*, *n_neighbors=None*, *vgraph=None*)

Computes transition probabilities from velocity graph

Parameters

adata : AnnData Annotated data matrix.

vkey : *str* (default: *'velocity'*) Name of velocity estimates to be used.

basis : *str* or *None* (default: *None*) Restrict transition to embedding if specified

backward : *bool* (default: *False*) Whether to use the transition matrix to push forward (*False*) or to pull backward (*True*)

self_transitions : *bool* (default: *False*) Allow transitions from one node to itself.

scale : *float* (default: 10) Scale parameter of gaussian kernel.

perc : *float* between *0* and *100* or *None* (default: *None*) Determines threshold of transitions to include.

use_negative_cosines : *bool* (default: *False*) If True, negatively similar transitions are taken into account.

weight_diffusion : *float* (default: 0) Relative weight to be given to diffusion kernel (Brownian motion)

scale_diffusion : *float* (default: 1) Scale of diffusion kernel.

weight_indirect_neighbors : *float* between *0* and *1* or *None* (default: *None*) Weight to be assigned to indirect neighbors (i.e. neighbors of higher degrees).

n_neighbors : *int* (default: None) Number of nearest neighbors to consider around each cell.

vgraph : csr matrix or *None* (default: *None*) Sparse velocity graph representation to use instead of adata.uns[vkey + '_graph'].

Returns *Returns sparse matrix with transition probabilities.*

3.3.6 scvelo.tl.terminal_states

scvelo.tl.**terminal_states**(*data*, *vkey='velocity'*, *groupby=None*, *groups=None*, *self_transitions=False*, *basis=None*, *weight_diffusion=0*, *scale_diffusion=1*, *eps=0.001*, *copy=False*)

Computes terminal states (root and end points).

Parameters

data : AnnData Annotated data matrix.

vkey : *str* (default: *'velocity'*) Name of velocity estimates to be used.

groupby : *str*, *list* or *np.ndarray* (default: *None*) Key of observations grouping to consider.

groups : *str*, *list* or *np.ndarray* (default: *None*) Groups selected to find terminal states on. Must be an element of adata.obs[groupby].

self_transitions : *bool* (default: *False*) Allow transitions from one node to itself.

basis : *str* (default: *None*) Basis to use.

weight_diffusion : *float* (default: 0) Relative weight to be given to diffusion kernel (Brownian motion)

scale_diffusion : *float* (default: 1) Scale of diffusion kernel.

eps : *float* (default: 1e-3) Tolerance for eigenvalue selection.

copy : *bool* (default: *False*) Return a copy instead of writing to data.

Returns

- Returns or updates *data* with the attributes
- **root** (*.obs*) – sparse matrix with transition probabilities.
- **end** (*.obs*) – sparse matrix with transition probabilities.

3.3.7 scvelo.tl.rank_velocity_genes

scvelo.tl.**rank_velocity_genes**(*data*, *vkey='velocity'*, *n_genes=10*, *groupby=None*, *match_with=None*, *resolution=None*, *min_counts=None*, *min_r2=None*, *min_dispersion=None*, *min_likelihood=None*, *copy=False*)

Rank genes for velocity characterizing groups.

Parameters

data : AnnData Annotated data matrix.

vkey : *str* (default: *'velocity'*) Key of velocities computed in *tl.velocity*

n_genes : *int*, optional (default: 100) The number of genes that appear in the returned tables.

groupby : *str*, *list* or *np.ndarray* (default: *None*) Key of observations grouping to consider.

match_with : *str* or *None* (default: *None*) adata.obs key to separatively rank velocities on.

resolution : *str* or *None* (default: *None*) Resolution for louvain modularity.

min_counts : *float* (default: None) Minimum count of genes for consideration.

min_r2 : *float* (default: None) Minimum r2 value of genes for consideration.

min_dispersion : *float* (default: None) Minimum dispersion norm value of genes for consideration.

min_likelihood : *float* between *0* and *1* or *None* (default: *None*) Only rank velocity of genes with a likelihood higher than min_likelihood.

copy : *bool* (default: *False*) Return a copy instead of writing to data.

Returns

- Returns or updates *data* with the attributes
- **rank_velocity_genes** (*.uns*) – Structured array to be indexed by group id storing the gene names. Ordered according to scores.
- **velocity_score** (*.var*) – Storing the score for each gene for each group. Ordered according to scores.

3.3.8 scvelo.tl.velocity_confidence

scvelo.tl.**velocity_confidence**(*data*, *vkey='velocity'*, *copy=False*)

Computes confidences of velocities.

Parameters

data : AnnData Annotated data matrix.

vkey : *str* (default: *'velocity'*) Name of velocity estimates to be used.

copy : *bool* (default: *False*) Return a copy instead of writing to adata.

Returns

- Returns or updates *adata* with the attributes
- **velocity_length** (*.obs*) – Length of the velocity vectors for each individual cell
- **velocity_confidence** (*.obs*) – Confidence for each cell

3.4 Plotting (pl)

pl.scatter([adata, x, y, basis, vkey, ...])	Scatter plot along observations or variables axes.

Continued on next page

Table 4 – continued from previous page

pl.velocity(adata[, var_names, basis, vkey, ...])	Phase and velocity plot for set of genes.
pl.velocity_graph(adata[, basis, vkey, ...])	Plot of the velocity graph.
pl.velocity_embedding(adata[, basis, vkey, ...])	Scatter plot of velocities on the embedding.
pl.velocity_embedding_grid(adata[, basis, ...])	Scatter plot of velocities on a grid.
pl.velocity_embedding_stream(adata[, basis, ...])	Stream plot of velocities on the embedding.

3.4.1 scvelo.pl.scatter

scvelo.pl.**scatter**(*adata=None*, *x=None*, *y=None*, *basis=None*, *vkey=None*, *color=None*, *use_raw=None*, *layer=None*, *color_map=None*, *colorbar=None*, *palette=None*, *size=None*, *alpha=None*, *linewidth=None*, *perc=None*, *sort_order=True*, *groups=None*, *components=None*, *projection='2d'*, *legend_loc=None*, *legend_fontsize=None*, *legend_fontweight=None*, *right_margin=None*, *left_margin=None*, *xlabel=None*, *ylabel=None*, *title=None*, *fontsize=None*, *figsize=None*, *xlim=None*, *ylim=None*, *show_density=None*, *show_assignments=None*, *show_linear_fit=None*, *show_polyfit=None*, *rug=None*, *n_convolve=None*, *smooth=None*, *rescale_color=None*, *dpi=None*, *frameon=None*, *show=True*, *save=None*, *ax=None*, *zorder=None*, *ncols=None*, ***kwargs*)

Scatter plot along observations or variables axes.

Parameters

adata : AnnData Annotated data matrix.

x : *str*, *np.ndarray* or *None* (default: *None*) x coordinate

y : *str*, *np.ndarray* or *None* (default: *None*) y coordinate

vkey : *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes.

basis : *str* (default='umap') Key for embedding.

color : *str*, list of *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes

use_raw : *bool* (default: *None*) Use *raw* attribute of *adata* if present.

layer : *str*, list of *str* or *None* (default: *None*) Specify the layer for *color*.

color_map : *str* (default: *matplotlib.rcParams['image.cmap']*) String denoting matplotlib color map.

colorbar : *bool* (default: *False*) Whether to show colorbar.

palette : list of *str* (default: *None*) Colors to use for plotting groups (categorical annotation).

size : *float* (default: 5) Point size.

alpha : *float* (default: 1) Set blending - 0 transparent to 1 opaque.

linewidth : *float* (default: 1) Scaling factor for the width of occurring lines.

perc : tuple, e.g. [2,98] (default: *None*) Specify percentile for continuous coloring.

sort_order : *bool* (default: *True*) For continuous annotations used as color parameter, plot data points with higher values on top of others.

groups : *str* (default: *all groups*) Restrict to a few categories in categorical observation annotation.

components : *str* or list of *str* (default: '1,2') For instance, ['1,2', '2,3'].

projection : *{'2d', '3d'} (default: '2d')* Projection of plot.

legend_loc : *str (default: 'none')* Location of legend, either 'on data', 'right margin' or valid keywords for matplotlib.legend.

legend_fontsize : *int* (default: *None*) Legend font size.

legend_fontweight : {'normal', 'bold', ...} (default: *None*) Legend font weight. Defaults to 'bold' if *legend_loc = 'on data'*, otherwise to 'normal'. Available are *['light', 'normal', 'medium', 'semibold', 'bold', 'heavy', 'black']*.

right_margin : *float* or list of *float* (default: *None*) Adjust the width of the space right of each plotting panel.

left_margin : *float* or list of *float* (default: *None*) Adjust the width of the space left of each plotting panel.

xlabel : *str* (default: *None*) Label of x-axis.

ylabel : *str* (default: *None*) Label of y-axis.

title : *str* (default: *None*) Provide title for panels either as, e.g. *["title1", "title2", ...]*.

fontsize : *float* (default: *None*) Label font size.

figsize : tuple (default: *(7,5)*) Figure size.

xlim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict x-limits of the axis.

ylim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict y-limits of the axis.

show_density : *bool* or *str* or *None* (default: *None*) Whether to show density of counts attached to the x and y axes. Color of the plot can also be given as *str*.

show_assignments : *bool* or *str* or *None* (default: *None*) Whether to show assignments to the model curve. Color of the assignments can also be given as *str*.

show_linear_fit : *bool* or *str* or *None* (default: *None*) Whether to show linear fit to the data. Color of the plot can also be given as *str*.

show_polyfit : *bool* or *str* or *int* or *None* (default: *None*) Whether to show polynomial fit to ???. Color of the polyfit plot can also be given as *str*. If *int* is given, determines the degree of the polynomial fit (else the degree defaults to 2).

rug : *str* or *None* (default: *None*) If categorical observation annotation (e.g. 'clusters') is given, a rugplot is attached to the x-axis showing the distribution of data membership to each of the categories.

n_convolve : *int* or *None* (default: *None*) If *int* is given, data is smoothed by convolution along the x-axis with kernel size n_convolve.

smooth : *bool* or *int* (default: *None*) Whether to convolve/average the color values over the nearest neighbors. If *int*, it specifies number of neighbors.

dpi : *int* (default: 80) Figure dpi.

frameon : *bool* (default: *True*) Draw a frame around the scatter plot.

ncols : *int* (default: *None*) Number of panels per row.

show : *bool*, optional (default: *None*) Show the plot, do not return axis.

save : *bool* or *str*, optional (default: *None*) If *True* or a *str*, save the figure. A string is appended to the default filename. Infer the filetype if ending on {'.pdf', '.png', '.svg'}.

ax : *matplotlib.Axes*, optional (default: *None*) A matplotlib axes object. Only works if plotting a single component.

Returns If *show==False* a *matplotlib.Axis*

3.4.2 scvelo.pl.velocity

scvelo.pl.**velocity**(*adata, var_names=None, basis=None, vkey='velocity', mode=None, fits='all', layers='all', color=None, color_map='RdBu_r', colorbar=False, perc=[2, 98], alpha=0.5, size=None, groupby=None, groups=None, use_raw=False, fontsize=None, figsize=None, dpi=None, show=True, save=None, ax=None, ncols=None, **kwargs*)

Phase and velocity plot for set of genes.

The phase plot shows spliced against unspliced expressions with steady-state fit. Further the embedding is shown colored by velocity and expression.

Parameters

adata : AnnData Annotated data matrix.

var_names : *str* or list of *str* (default: *None*) Which variables to show.

basis : *str* (default: *'umap'*) Key for embedding coordinates.

mode : *'stochastic'* or *None* (default: *None*) Whether to show show covariability phase portrait.

fits : *str* or list of *str* (default: *'all'*) Which steady-state estimates to show.

layers : *str* or list of *str* (default: *'all'*) Which layers to show.

color : *str*, list of *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes

color_map : *str* (default: *matplotlib.rcParams['image.cmap']*) String denoting matplotlib color map.

perc : tuple, e.g. [2,98] (default: *None*) Specify percentile for continuous coloring.

groups : *str*, *list* (default: *None*) Subset of groups, e.g. ['g1', 'g2', 'g3'], to which the plot shall be restricted.

groupby : *str*, *list* or *np.ndarray* (default: *None*) Key of observations grouping to consider.

size : *float* (default: 5) Point size.

alpha : *float* (default: 1) Set blending - 0 transparent to 1 opaque.

fontsize : *float* (default: *None*) Label font size.

figsize : tuple (default: *(7,5)*) Figure size.

dpi : *int* (default: 80) Figure dpi.

show : *bool*, optional (default: *None*) Show the plot, do not return axis.

save : *bool* or *str*, optional (default: *None*) If *True* or a *str*, save the figure. A string is appended to the default filename. Infer the filetype if ending on {'.pdf', '.png', '.svg'}.

ax : *matplotlib.Axes*, optional (default: *None*) A matplotlib axes object. Only works if plotting a single component.

ncols : *int* or *None* (default: *None*) Number of columns to arange multiplots into.

3.4.3 scvelo.pl.velocity_graph

scvelo.pl.**velocity_graph**(*adata*, *basis=None*, *vkey='velocity'*, *which_graph='velocity'*, *n_neighbors=10*, *alpha=0.8*, *perc=90*, *edge_width=0.2*, *edge_color='grey'*, *color=None*, *use_raw=None*, *layer=None*, *color_map=None*, *colorbar=True*, *palette=None*, *size=None*, *sort_order=True*, *groups=None*, *components=None*, *projection='2d'*, *legend_loc='on data'*, *legend_fontsize=None*, *legend_fontweight=None*, *right_margin=None*, *left_margin=None*, *xlabel=None*, *ylabel=None*, *title=None*, *fontsize=None*, *figsize=None*, *dpi=None*, *frameon=None*, *show=True*, *save=None*, *ax=None*)

Plot of the velocity graph.

Parameters

adata : AnnData Annotated data matrix.

vkey : *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes.

which_graph : *'velocity'* or *'neighbors'* (default: *'velocity'*) Whether to show transitions from velocity graph or connectivities from neighbors graph.

basis : *str* (default='umap') Key for embedding.

color : *str*, list of *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes

use_raw : *bool* (default: *None*) Use *raw* attribute of *adata* if present.

layer : *str*, list of *str* or *None* (default: *None*) Specify the layer for *color*.

color_map : *str* (default: *matplotlib.rcParams['image.cmap']*) String denoting matplotlib color map.

colorbar : *bool* (default: *False*) Whether to show colorbar.

palette : list of *str* (default: *None*) Colors to use for plotting groups (categorical annotation).

size : *float* (default: 5) Point size.

alpha : *float* (default: 1) Set blending - 0 transparent to 1 opaque.

linewidth : *float* (default: 1) Scaling factor for the width of occurring lines.

perc : tuple, e.g. [2,98] (default: *None*) Specify percentile for continuous coloring.

sort_order : *bool* (default: *True*) For continuous annotations used as color parameter, plot data points with higher values on top of others.

groups : *str* (default: *all groups*) Restrict to a few categories in categorical observation annotation.

components : *str* or list of *str* (default: '1,2') For instance, ['1,2', '2,3'].

projection : *{'2d', '3d'} (default: '2d')* Projection of plot.

legend_loc : *str (default: 'none')* Location of legend, either 'on data', 'right margin' or valid keywords for matplotlib.legend.

legend_fontsize : *int* (default: *None*) Legend font size.

legend_fontweight : {'normal', 'bold', ...} (default: *None*) Legend font weight. Defaults to 'bold' if *legend_loc = 'on data'*, otherwise to 'normal'. Available are *['light', 'normal', 'medium', 'semibold', 'bold', 'heavy', 'black']*.

right_margin : *float* or list of *float* (default: *None*) Adjust the width of the space right of each plotting panel.

left_margin : *float* or list of *float* (default: *None*) Adjust the width of the space left of each plotting panel.

xlabel : *str* (default: *None*) Label of x-axis.

ylabel : *str* (default: *None*) Label of y-axis.

title : *str* (default: *None*) Provide title for panels either as, e.g. *["title1", "title2", ...]*.

fontsize : *float* (default: *None*) Label font size.

figsize : tuple (default: *(7,5)*) Figure size.

xlim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict x-limits of the axis.

ylim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict y-limits of the axis.

show_density : *bool* or *str* or *None* (default: *None*) Whether to show density of counts attached to the x and y axes. Color of the plot can also be given as *str*.

show_assignments : *bool* or *str* or *None* (default: *None*) Whether to show assignments to the model curve. Color of the assignments can also be given as *str*.

show_linear_fit : *bool* or *str* or *None* (default: *None*) Whether to show linear fit to the data. Color of the plot can also be given as *str*.

show_polyfit : *bool* or *str* or *int* or *None* (default: *None*) Whether to show polynomial fit to ???. Color of the polyfit plot can also be given as *str*. If *int* is given, determines the degree of the polynomial fit (else the degree defaults to 2).

rug : *str* or *None* (default: *None*) If categorical observation annotation (e.g. 'clusters') is given, a rugplot is attached to the x-axis showing the distribution of data membership to each of the categories.

n_convolve : *int* or *None* (default: *None*) If *int* is given, data is smoothed by convolution along the x-axis with kernel size n_convolve.

smooth : *bool* or *int* (default: *None*) Whether to convolve/average the color values over the nearest neighbors. If *int*, it specifies number of neighbors.

dpi : *int* (default: 80) Figure dpi.

frameon : *bool* (default: *True*) Draw a frame around the scatter plot.

ncols : *int* (default: *None*) Number of panels per row.

show : *bool*, optional (default: *None*) Show the plot, do not return axis.

save : *bool* or *str*, optional (default: *None*) If *True* or a *str*, save the figure. A string is appended to the default filename. Infer the filetype if ending on {'.pdf', '.png', '.svg'}.

ax : *matplotlib.Axes*, optional (default: *None*) A matplotlib axes object. Only works if plotting a single component.

Returns *matplotlib.Axis* if *show==False*

3.4.4 scvelo.pl.velocity_embedding

scvelo.pl.**velocity_embedding**(*adata*, *basis=None*, *vkey='velocity'*, *density=None*, *arrow_size=None*, *arrow_length=None*, *scale=None*, *X=None*, *V=None*, *recompute=None*, *color=None*, *use_raw=None*, *layer=None*, *color_map=None*, *colorbar=True*, *palette=None*, *size=None*, *alpha=0.2*, *perc=None*, *sort_order=True*, *groups=None*, *components=None*, *projection='2d'*, *legend_loc='none'*, *legend_fontsize=None*, *legend_fontweight=None*, *right_margin=None*, *left_margin=None*, *xlabel=None*, *ylabel=None*, *title=None*, *fontsize=None*, *figsize=None*, *dpi=None*, *frameon=None*, *show=True*, *save=None*, *ax=None*, *ncols=None*, ***kwargs*)

Scatter plot of velocities on the embedding.

Parameters

adata : AnnData Annotated data matrix.

vkey : *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes.

density : *float* (default: 1) Amount of velocities to show - 0 none to 1 all

arrow_size : *float* or 3-tuple for headlength, headwidth and headaxislength (default: 1) Size of arrows.

arrow_length : *float* (default: 1) Length of arrows.

scale : *float* (default: 1) Length of velocities in the embedding.

basis : *str* (default='umap') Key for embedding.

color : *str*, list of *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes

use_raw : *bool* (default: *None*) Use *raw* attribute of *adata* if present.

layer : *str*, list of *str* or *None* (default: *None*) Specify the layer for *color*.

color_map : *str* (default: *matplotlib.rcParams['image.cmap']*) String denoting matplotlib color map.

colorbar : *bool* (default: *False*) Whether to show colorbar.

palette : list of *str* (default: *None*) Colors to use for plotting groups (categorical annotation).

size : *float* (default: 5) Point size.

alpha : *float* (default: 1) Set blending - 0 transparent to 1 opaque.

linewidth : *float* (default: 1) Scaling factor for the width of occurring lines.

perc : tuple, e.g. [2,98] (default: *None*) Specify percentile for continuous coloring.

sort_order : *bool* (default: *True*) For continuous annotations used as color parameter, plot data points with higher values on top of others.

groups : *str* (default: *all groups*) Restrict to a few categories in categorical observation annotation.

components : *str* or list of *str* (default: '1,2') For instance, ['1,2', '2,3'].

projection : *{'2d', '3d'} (default: '2d')* Projection of plot.

legend_loc : *str (default: 'none')* Location of legend, either 'on data', 'right margin' or valid keywords for matplotlib.legend.

legend_fontsize : *int* (default: *None*) Legend font size.

legend_fontweight : {'normal', 'bold', ...} (default: *None*) Legend font weight. Defaults to 'bold' if *legend_loc = 'on data'*, otherwise to 'normal'. Available are *['light', 'normal', 'medium', 'semibold', 'bold', 'heavy', 'black']*.

right_margin : *float* or list of *float* (default: *None*) Adjust the width of the space right of each plotting panel.

left_margin : *float* or list of *float* (default: *None*) Adjust the width of the space left of each plotting panel.

xlabel : *str* (default: *None*) Label of x-axis.

ylabel : *str* (default: *None*) Label of y-axis.

title : *str* (default: *None*) Provide title for panels either as, e.g. *["title1", "title2", ...]*.

fontsize : *float* (default: *None*) Label font size.

figsize : tuple (default: *(7,5)*) Figure size.

xlim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict x-limits of the axis.

ylim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict y-limits of the axis.

show_density : *bool* or *str* or *None* (default: *None*) Whether to show density of counts attached to the x and y axes. Color of the plot can also be given as *str*.

show_assignments : *bool* or *str* or *None* (default: *None*) Whether to show assignments to the model curve. Color of the assignments can also be given as *str*.

show_linear_fit : *bool* or *str* or *None* (default: *None*) Whether to show linear fit to the data. Color of the plot can also be given as *str*.

show_polyfit : *bool* or *str* or *int* or *None* (default: *None*) Whether to show polynomial fit to ???. Color of the polyfit plot can also be given as *str*. If *int* is given, determines the degree of the polynomial fit (else the degree defaults to 2).

rug : *str* or *None* (default: *None*) If categorical observation annotation (e.g. 'clusters') is given, a rugplot is attached to the x-axis showing the distribution of data membership to each of the categories.

n_convolve : *int* or *None* (default: *None*) If *int* is given, data is smoothed by convolution along the x-axis with kernel size n_convolve.

smooth : *bool* or *int* (default: *None*) Whether to convolve/average the color values over the nearest neighbors. If *int*, it specifies number of neighbors.

dpi : *int* (default: 80) Figure dpi.

frameon : *bool* (default: *True*) Draw a frame around the scatter plot.

ncols : *int* (default: *None*) Number of panels per row.

show : *bool*, optional (default: *None*) Show the plot, do not return axis.

save : *bool* or *str*, optional (default: *None*) If *True* or a *str*, save the figure. A string is appended to the default filename. Infer the filetype if ending on {'.pdf', '.png', '.svg'}.

ax : *matplotlib.Axes*, optional (default: *None*) A matplotlib axes object. Only works if plotting a single component.

Returns *matplotlib.Axis* if *show==False*

3.4.5 scvelo.pl.velocity_embedding_grid

scvelo.pl.**velocity_embedding_grid**(*adata*, *basis=None*, *vkey='velocity'*, *density=None*, *smooth=None*, *min_mass=None*, *arrow_size=None*, *arrow_length=None*, *arrow_color=None*, *scale=None*, *autoscale=True*, *n_neighbors=None*, *recompute=None*, *X=None*, *V=None*, *X_grid=None*, *V_grid=None*, *principal_curve=False*, *color=None*, *use_raw=None*, *layer=None*, *color_map=None*, *colorbar=True*, *palette=None*, *size=None*, *alpha=0.2*, *perc=None*, *sort_order=True*, *groups=None*, *components=None*, *projection='2d'*, *legend_loc='none'*, *legend_fontsize=None*, *legend_fontweight=None*, *right_margin=None*, *left_margin=None*, *xlabel=None*, *ylabel=None*, *title=None*, *fontsize=None*, *figsize=None*, *dpi=None*, *frameon=None*, *show=True*, *save=None*, *ax=None*, *ncols=None*, ***kwargs*)

Scatter plot of velocities on a grid.

Parameters

adata : AnnData Annotated data matrix.

vkey : *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes.

density : *float* (default: 1) Amount of velocities to show - 0 none to 1 all

arrow_size : *float* or 3-tuple for headlength, headwidth and headaxislength (default: 1) Size of arrows.

arrow_length : *float* (default: 1) Length of arrows.

scale : *float* (default: 1) Length of velocities in the embedding.

min_mass : *float* or *None* (default: *None*) Minimum threshold for mass to be shown. It can range between 0 (all velocities) and 100 (large velocities).

smooth : *bool* or *int* (default: *None*) Multiplication factor for scale in Gaussian kernel around grid point.

n_neighbors : *int* (default: None) Number of neighbors to consider around grid point.

X : *np.ndarray* (default: None) embedding grid point coordinates

V : *np.ndarray* (default: None) embedding grid velocity coordinates

basis : *str* (default='umap') Key for embedding.

color : *str*, list of *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes

use_raw : *bool* (default: *None*) Use *raw* attribute of *adata* if present.

layer : *str*, list of *str* or *None* (default: *None*) Specify the layer for *color*.

color_map : *str* (default: *matplotlib.rcParams['image.cmap']*) String denoting matplotlib color map.

colorbar : *bool* (default: *False*) Whether to show colorbar.

palette : list of *str* (default: *None*) Colors to use for plotting groups (categorical annotation).

size : *float* (default: 5) Point size.

alpha : *float* (default: 1) Set blending - 0 transparent to 1 opaque.

linewidth : *float* (default: 1) Scaling factor for the width of occurring lines.

perc : tuple, e.g. [2,98] (default: *None*) Specify percentile for continuous coloring.

sort_order : *bool* (default: *True*) For continuous annotations used as color parameter, plot data points with higher values on top of others.

groups : *str* (default: *all groups*) Restrict to a few categories in categorical observation annotation.

components : *str* or list of *str* (default: '1,2') For instance, ['1,2', '2,3'].

projection : *{'2d', '3d'} (default: '2d')* Projection of plot.

legend_loc : *str (default: 'none')* Location of legend, either 'on data', 'right margin' or valid keywords for matplotlib.legend.

legend_fontsize : *int* (default: *None*) Legend font size.

legend_fontweight : {'normal', 'bold', ...} (default: *None*) Legend font weight. Defaults to 'bold' if *legend_loc = 'on data'*, otherwise to 'normal'. Available are *['light', 'normal', 'medium', 'semibold', 'bold', 'heavy', 'black']*.

right_margin : *float* or list of *float* (default: *None*) Adjust the width of the space right of each plotting panel.

left_margin : *float* or list of *float* (default: *None*) Adjust the width of the space left of each plotting panel.

xlabel : *str* (default: *None*) Label of x-axis.

ylabel : *str* (default: *None*) Label of y-axis.

title : *str* (default: *None*) Provide title for panels either as, e.g. *["title1", "title2", ...]*.

fontsize : *float* (default: *None*) Label font size.

figsize : tuple (default: *(7,5)*) Figure size.

xlim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict x-limits of the axis.

ylim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict y-limits of the axis.

show_density : *bool* or *str* or *None* (default: *None*) Whether to show density of counts attached to the x and y axes. Color of the plot can also be given as *str*.

show_assignments : *bool* or *str* or *None* (default: *None*) Whether to show assignments to the model curve. Color of the assignments can also be given as *str*.

show_linear_fit : *bool* or *str* or *None* (default: *None*) Whether to show linear fit to the data. Color of the plot can also be given as *str*.

show_polyfit : *bool* or *str* or *int* or *None* (default: *None*) Whether to show polynomial fit to ???. Color of the polyfit plot can also be given as *str*. If *int* is given, determines the degree of the polynomial fit (else the degree defaults to 2).

rug : *str* or *None* (default: *None*) If categorical observation annotation (e.g. 'clusters') is given, a rugplot is attached to the x-axis showing the distribution of data membership to each of the categories.

n_convolve : *int* or *None* (default: *None*) If *int* is given, data is smoothed by convolution along the x-axis with kernel size n_convolve.

smooth Whether to convolve/average the color values over the nearest neighbors. If *int*, it specifies number of neighbors.

dpi : *int* (default: 80) Figure dpi.

frameon : *bool* (default: *True*) Draw a frame around the scatter plot.

ncols : *int* (default: *None*) Number of panels per row.

show : *bool*, optional (default: *None*) Show the plot, do not return axis.

save : *bool* or *str*, optional (default: *None*) If *True* or a *str*, save the figure. A string is appended to the default filename. Infer the filetype if ending on {'.pdf', '.png', '.svg'}.

ax : *matplotlib.Axes*, optional (default: *None*) A matplotlib axes object. Only works if plotting a single component.

Returns *matplotlib.Axis* if *show==False*

3.4.6 scvelo.pl.velocity_embedding_stream

scvelo.pl.**velocity_embedding_stream**(*adata*, *basis=None*, *vkey='velocity'*, *density=None*, *smooth=None*, *min_mass=None*, *cutoff_perc=None*, *arrow_color=None*, *linewidth=None*, *n_neighbors=None*, *recompute=None*, *color=None*, *use_raw=None*, *layer=None*, *color_map=None*, *colorbar=True*, *palette=None*, *size=None*, *alpha=0.3*, *perc=None*, *X=None*, *V=None*, *X_grid=None*, *V_grid=None*, *sort_order=True*, *groups=None*, *components=None*, *legend_loc='on data'*, *legend_fontsize=None*, *legend_fontweight=None*, *right_margin=None*, *left_margin=None*, *xlabel=None*, *ylabel=None*, *title=None*, *fontsize=None*, *figsize=None*, *dpi=None*, *frameon=None*, *show=True*, *save=None*, *ax=None*, *ncols=None*, ***kwargs*)

Stream plot of velocities on the embedding.

Parameters

adata : AnnData Annotated data matrix.

vkey : *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes.

density : *float* (default: 1) Amount of velocities to show - 0 none to 1 all

smooth : *bool* or *int* (default: *None*) Multiplication factor for scale in Gaussian kernel around grid point.

min_mass : *float* (default: 1) Minimum threshold for mass to be shown. It can range between 0 (all velocities) and 5 (large velocities only).

cutoff_perc : *float* (default: *None*) If set, mask small velocities below a percentile threshold (range between 0 and 100).

linewidth : *float* (default: 1) Line width for streamplot.

n_neighbors : *int* (default: None) Number of neighbors to consider around grid point.

X : *np.ndarray* (default: None) Embedding grid point coordinates

V : *np.ndarray* (default: None) Embedding grid velocity coordinates

basis : *str* (default='umap') Key for embedding.

color : *str*, list of *str* or *None* (default: *None*) Key for annotations of observations/cells or variables/genes

use_raw : *bool* (default: *None*) Use *raw* attribute of *adata* if present.

layer : *str*, list of *str* or *None* (default: *None*) Specify the layer for *color*.

color_map : *str* (default: *matplotlib.rcParams['image.cmap']*) String denoting matplotlib color map.

colorbar : *bool* (default: *False*) Whether to show colorbar.

palette : list of *str* (default: *None*) Colors to use for plotting groups (categorical annotation).

size : *float* (default: 5) Point size.

alpha : *float* (default: 1) Set blending - 0 transparent to 1 opaque.

linewidth Scaling factor for the width of occurring lines.

perc : tuple, e.g. [2,98] (default: *None*) Specify percentile for continuous coloring.

sort_order : *bool* (default: *True*) For continuous annotations used as color parameter, plot data points with higher values on top of others.

groups : *str* (default: *all groups*) Restrict to a few categories in categorical observation annotation.

components : *str* or list of *str* (default: '1,2') For instance, ['1,2', '2,3'].

projection : *{'2d', '3d'} (default: '2d')* Projection of plot.

legend_loc : *str (default: 'none')* Location of legend, either 'on data', 'right margin' or valid keywords for matplotlib.legend.

legend_fontsize : *int* (default: *None*) Legend font size.

legend_fontweight : {'normal', 'bold', ...} (default: *None*) Legend font weight. Defaults to 'bold' if *legend_loc = 'on data'*, otherwise to 'normal'. Available are *['light', 'normal', 'medium', 'semibold', 'bold', 'heavy', 'black']*.

right_margin : *float* or list of *float* (default: *None*) Adjust the width of the space right of each plotting panel.

left_margin : *float* or list of *float* (default: *None*) Adjust the width of the space left of each plotting panel.

xlabel : *str* (default: *None*) Label of x-axis.

ylabel : *str* (default: *None*) Label of y-axis.

title : *str* (default: *None*) Provide title for panels either as, e.g. *["title1", "title2", ...]*.

fontsize : *float* (default: *None*) Label font size.

figsize : tuple (default: *(7,5)*) Figure size.

xlim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict x-limits of the axis.

ylim : tuple, e.g. [0,1] or *None* (default: *None*) Restrict y-limits of the axis.

show_density : *bool* or *str* or *None* (default: *None*) Whether to show density of counts attached to the x and y axes. Color of the plot can also be given as *str*.

show_assignments : *bool* or *str* or *None* (default: *None*) Whether to show assignments to the model curve. Color of the assignments can also be given as *str*.

show_linear_fit : *bool* or *str* or *None* (default: *None*) Whether to show linear fit to the data. Color of the plot can also be given as *str*.

show_polyfit : *bool* or *str* or *int* or *None* (default: *None*) Whether to show polynomial fit to ???. Color of the polyfit plot can also be given as *str*. If *int* is given, determines the degree of the polynomial fit (else the degree defaults to 2).

rug : *str* or *None* (default: *None*) If categorical observation annotation (e.g. 'clusters') is given, a rugplot is attached to the x-axis showing the distribution of data membership to each of the categories.

n_convolve : *int* or *None* (default: *None*) If *int* is given, data is smoothed by convolution along the x-axis with kernel size n_convolve.

smooth Whether to convolve/average the color values over the nearest neighbors. If *int*, it specifies number of neighbors.

dpi : *int* (default: 80) Figure dpi.

frameon : *bool* (default: *True*) Draw a frame around the scatter plot.

ncols : *int* (default: *None*) Number of panels per row.

show : *bool*, optional (default: *None*) Show the plot, do not return axis.

save : *bool* or *str*, optional (default: *None*) If *True* or a *str*, save the figure. A string is appended to the default filename. Infer the filetype if ending on {'.pdf', '.png', '.svg'}.

ax : *matplotlib.Axes*, optional (default: *None*) A matplotlib axes object. Only works if plotting a single component.

Returns *matplotlib.Axis* if *show==False*

3.5 Datasets

datasets.toy_data(n_obs)	Randomly samples from the Dentate Gyrus dataset.
datasets.dentategyrus([adjusted])	Dentate Gyrus dataset from Hochgerner et al.
datasets.forebrain()	Developing human forebrain.

3.5.1 scvelo.datasets.toy_data

scvelo.datasets.**toy_data**(*n_obs*)

Randomly samples from the Dentate Gyrus dataset.

Parameters

n_obs : *int* Size of the sampled dataset

Returns Returns *adata* object

3.5.2 scvelo.datasets.dentategyrus

scvelo.datasets.**dentategyrus**(*adjusted=True*)
Dentate Gyrus dataset from Hochgerner et al. (2018).

Dentate gyrus is part of the hippocampus involved in learning, episodic memory formation and spatial coding. It is measured using 10X Genomics Chromium and described in Hochgerner et al. (2018). The data consists of 25,919 genes across 3,396 cells and provides several interesting characteristics.

Returns Returns *adata* object

3.5.3 scvelo.datasets.forebrain

scvelo.datasets.**forebrain**()
Developing human forebrain. Forebrain tissue of a week 10 embryo, focusing on the glutamatergic neuronal lineage.

Returns Returns *adata* object

3.6 Utils

utils.show_proportions(adata)	Fraction of spliced/unspliced/ambiguous abundances
utils.cleanup(data[, clean, keep, copy])	Deletes attributes not needed.
utils.clean_obs_names(data[, base, ...])	Cleans up the obs_names and identifies sample names.
utils.merge(adata, ldata[, copy])	Merges two annotated data matrices.

3.6.1 scvelo.utils.show_proportions

scvelo.utils.**show_proportions**(*adata*)
Fraction of spliced/unspliced/ambiguous abundances

Parameters

adata : AnnData Annotated data matrix.

Returns *Prints the fractions of abundances.*

3.6.2 scvelo.utils.cleanup

scvelo.utils.**cleanup**(*data*, *clean='layers'*, *keep=None*, *copy=False*)
Deletes attributes not needed.

Parameters

data : AnnData Annotated data matrix.

clean : *str* or list of *str* (default: *layers*) Which attributes to consider for freeing memory.

keep : *str* or list of *str* (default: None) Which attributes to keep.

copy : *bool* (default: *False*) Return a copy instead of writing to adata.

Returns Returns or updates *adata* with selection of attributes kept.

3.6.3 scvelo.utils.clean_obs_names

scvelo.utils.**clean_obs_names**(*data*, *base='[AGTCBDHKMNRSVWY]'*, *ID_length=12*, *copy=False*)

Cleans up the obs_names and identifies sample names. For example an obs_name 'samlple1_AGTCdate' is changed to 'AGTC' of the sample 'sample1_date'. The sample name is then saved in obs['sample_batch']. The genetic codes are identified according to according to https://www.neb.com/tools-and-resources/usage-guidelines/the-genetic-code.

Parameters

adata : AnnData Annotated data matrix.

base : *str* (default: *[AGTCBDHKMNRSVWY]*) Genetic code letters to be identified.

ID_length : *int* (default: 12) Length of the Genetic Codes in the samples.

copy : *bool* (default: *False*) Return a copy instead of writing to adata.

Returns

- Returns or updates *adata* with the attributes
- **obs_names** (*list*) – updated names of the observations
- **sample_batch** (*.obs*) – names of the identified sample batches

3.6.4 scvelo.utils.merge

scvelo.utils.**merge**(*adata*, *ldata*, *copy=True*)

Merges two annotated data matrices.

Parameters

adata : AnnData Annotated data matrix (reference data set).

ldata : AnnData Annotated data matrix (to be merged into adata).

Returns Returns a AnnData object

3.7 Settings

settings.set_figure_params([style, figsize, ...])	Set resolution/size, styling and format of figures.

3.7.1 scvelo.settings.set_figure_params

scvelo.settings.**set_figure_params**(*style='scvelo'*, *figsize=None*, *dpi=None*, *dpi_save=None*, *frameon=None*, *vector_friendly=True*, *color_map=None*, *format='pdf'*, *transparent=False*, *ipython_format='png2x'*)

Set resolution/size, styling and format of figures.

Parameters

style : *str* (default: *None*) Init default values for matplotlib.rcParams suited for *scvelo* or *scanpy*. Use *None* for the default matplotlib values.

figsize : *[float, float]* (default: *None*) Width and height for default figure size.

dpi : *int* (default: *None*) Resolution of rendered figures - this influences the size of figures in notebooks.

dpi_save : *int* (default: *None*) Resolution of saved figures. This should typically be higher to achieve publication quality.

frameon : *bool* (default: *None*) Add frames and axes labels to scatter plots.

vector_friendly : *bool* (default: *True*) Plot scatter plots using *png* backend even when exporting as *pdf* or *svg*.

color_map : *str* (default: *None*) Convenience method for setting the default color map.

format : *{'png', 'pdf', 'svg', etc.} (default: 'pdf')* This sets the default format for saving figures: *file_format_figs*.

transparent : *bool* (default: *True*) Save figures with transparent back ground. Sets *rcParams['savefig.transparent']*.

ipython_format : list of *str* (default: 'png2x') Only concerns the notebook/IPython environment; see *IPython.core.display.set_matplotlib_formats* for more details.

CHAPTER 4

Release Notes

4.1 Version 0.1.20 Sep 5, 2019

Tools:

- *tl.recover_dynamics*: introduced a dynamical model inferring the full splicing kinetics, thereby identifying all kinetic rates of transcription, splicing and degradation.
- *tl.recover_latent_time*: infers a shared latent time across all genes based on the learned splicing dynamics.

Plotting:

- enhancements in *pl.scatter*: multiplots, rugplot, linear and polynomical fits, densityplots, etc.
- *pl.heatmap*: heatmap / clsutermap of genes along time coordinate sorted by expression along dynamics.

Preprocessing:

- New attributes in pp.filter_genes: *min_shared_counts* and *min_shared_genes*.
- Added fast neighbor search method: Hierarchical Navigable Small World graphs (HNSW)

4.2 Version 0.1.14 Dec 7, 2018

Plotting:

- New attriutes *arrow_length* and *arrow_size* for flexible adjustment of embedded velocities.
- *pl.velocity_graph*: Scatter plot of embedding with cell-to-cell transition connectivities.
- *pl.velocity_embedding_stream*: Streamplot visualization of velocities.
- Improve visualization of embedded single cell velocities (autosize, colors etc.)

Tools:

- *tl.cell_fate*: compute cell-specific terminal state likelihood

- New attribute *approx=True* in *tl.velocity_graph* to enable approximate graph computation by performing cosine correlations on PCA space.

Preprocessing:

- Automatically detect whether data is already preprocessed.

4.3 Version 0.1.11 Oct 27, 2018

Plotting:

- *settings.set_figure_params()*: adjust matplotlib defaults for beautified plots
- improved default point and arrow sizes; improved quiver autoscale
- enable direct plotting of

Tools:

- *tl.velocity_confidence*: Added two confidence measures 'velocity_confidence' and 'velocity_confidence_transition'.
- *tl.rank_velocity_genes*: Added functionality to rank genes for velocity characterizing groups using a t-test.
- New attribute *perc* in *tl.velocity* enables extreme quantile fit, e.g. set *perc=95*.
- New attribute *groups* in *tl.velocity* enables velocity estimation only on a subset of the data.
- Improved *tl.transition_matrix* by incorporating self-loops via *self_transitions=True* and state changes that have negative correlation with velocity (opposite direction) via *use_negative_cosines=True*

Utils:

- *utils.merge* to merge to AnnData objects such as already existing AnnData and newly generated Loom File.

4.4 Version 0.1.8 Sep 12, 2018

Plotting:

- support saving plots as pdf, png etc.
- support multiple colors and layers
- quiver autoscaling for velocity plots
- attributes added: figsize and dpi

Preprocessing:

- filter_and_normalize() instead of recipe_velocity()
- normalization of layers is done automatically when computing moments

Tools:

- terminal_states: computes root and end points via eigenvalue decomposition thanks to M Lange

4.5 Version 0.1.5 Sep 4, 2018

- Support writing loom files
- Support both dense and sparse layers
- Plotting bugfixes
- Added pp.recipe_velocity()

4.6 Version 0.1.2 Aug 21, 2018

First alpha release of scvelo.

CHAPTER 5

References

Bibliography

[Wolf18] Wolf *et al.* (2018), *Scanpy: large-scale single-cell gene expression data analysis*, Genome Biology.

[Manno18] La Manno *et al.* (2018), *RNA velocity of single cells*, Nature.

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