
DigitalCellSorter Documentation

Release 1.3.7

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Single cell RNA-seq data analysis toolkit.

Digital Cell Sorter (DCS): a cell type identification, anomaly detection, and Hopfield landscapes toolkit for single-cell transcriptomics. Sergii Domanskyi, Alex Hakansson, Thomas Bertus, Giovanni Paternostro & Carlo Piermarocchi, *PeerJ*, 9:e10670 (2021). <https://doi.org/10.7717/peerj.10670>

Polled Digital Cell Sorter (p-DCS): Automatic identification of hematological cell types from single cell RNA-sequencing clusters. Sergii Domanskyi, Anthony Szedlak, Nathaniel T Hawkins, Jiayin Wang, Giovanni Paternostro & Carlo Piermarocchi, *BMC Bioinformatics* volume 20, Article number: 369 (2019). <https://doi.org/10.1186/s12859-019-2951-x>

OVERVIEW

1.1 Publications

Identification of hematological cell types from heterogeneous single cell RNA-seq data.

Polled Digital Cell Sorter (p-DCS): Automatic identification of hematological cell types from single cell RNA-sequencing clusters. Sergii Domanskyi, Anthony Szedlak, Nathaniel T Hawkins, Jiayin Wang, Giovanni Paternostro & Carlo Piermarocchi, *BMC Bioinformatics* volume 20, Article number: 369 (2019) <https://doi.org/10.1186/s12859-019-2951-x>

1.2 Description of the package functionality

The main class of DigitalCellSorter. The class includes tools for:

1. **Pre-preprocessing** of single cell RNA sequencing data
2. **Quality control**
3. **Batch effects correction**
4. **Cells anomaly score evaluation**
5. **Dimensionality reduction**
6. **Clustering**
7. **Annotation of cell types**
8. **Vizualization**
9. **Post-processing**

1.3 Versions change log

- **1.3.7.6**
 - Minor bug fix of fail-safe fitsne package import
- **1.3.7.5**
 - Upgraded function makeViolinPlot to enable combinations of columns
- **1.3.7.3-4**
 - Added option of the fixed random seed (option *random_state*) for the whole prcessing pipeline

- **1.3.7.2**
 - Added documentation of the function makeViolinPlot
- **1.3.7.1**
 - Added makeViolinPlot visualization function enabling exploratory analysis of the numeric values distributions for comparison between categorical variables
 - Updated visualization functions returning
- **1.3.7**
 - Added a function to import data from kallisto-bustools and cellranger
 - Updated documentation
- **1.3.6**
 - Added quick-demo materials
- **1.3.5**
 - Miscellaneous code improvements and bug fixes
- **1.3.4.0-1.3.4.11**
 - Integrated plotly offline figure saving (when orca is unavailable)
 - Added Quality Control pre-cut
- **1.3.2**
 - Added Hopfield landscape visualization capability
 - Added network of underlying biological gene-gene interaction to the Hopfield annotation scheme
- **1.3.1**
 - Minor API modifications
- **1.3.0**
 - Modified pDCS algorithm for cell type identification to account for markers that should not be expressed in a given cell type (negative markers)
 - Modified pDCS celltype/marker matrix normalization
 - Modified pDCS algorithm account for low quality scores
 - Added Hopfield classifier for cell type annotation
 - Added ratio method for cell type annotation
 - Added options for consensus cell type annotation
 - Added cell markers pie summary function and plot
 - Added t-test for individual gene plot
 - Added several new user functions, for efficient and flexible extraction of cells, genes, clusters, etc.
 - Added anomaly score calculation and visualization
 - Refactored function for extraction of new markers based on cell type annotations to separate it from function process() of class DigitalCellSorter
 - Optimized implementation (for higher performance) of various function of this package
 - Detailed visualization functions API

- Incorporated different clustering methods in addition to the widely-utilized hierarchical clustering
 - Incorporated several types of high-dimensional data projection methods, such as efficient t-SNE, UMAP and simple PCA components.
 - Extended options for input data format
 - Included a set of functions to load data from Human Cell Atlas (HCA) and prepare it for processing
- **1.2.3**
 - API updates, documentation updates
- **1.2.1**
 - Minor updates, reshaped DigitalCellSorter into a stand-alone package
- **1.2.0**
 - More features, better runtime efficiency
- **1.1**
 - Updated method, signature matrices
- **1.0**
 - First Release

GETTING STARTED

These instructions will get you a copy of the project up and running on your machine for data analysis, development or testing purposes.

2.1 Installation

Install of the latest release of `DigitalCellSorter`:

```
$ pip install DigitalCellSorter
```

For detailed instructions and other ways to install `DigitalCellSorter` as well as list of optional packages and instructions on how to install them see **Prerequisites** section at <https://github.com/sdomanskyi/DigitalCellSorter>

2.2 Loading the package

In your script import the package:

```
import DigitalCellSorter
```

Create an instance of class `DigitalCellSorter`. Here, for simplicity, we use Default parameter values:

```
DCS = DigitalCellSorter.DigitalCellSorter()
```


USER FUNCTIONS

User functions from **DigitalCellSorter.core.DigitalCellSorter** class.

Note: All of the tools listed below in this section are intended to use from an instance of a `DigitalCellSorter` class. For example:

```
DCS = DigitalCellSorter.DigitalCellSorter()

DCS.dataName = 'my_data_name'
DCS.saveDir = os.path.join(os.path.dirname(__file__), 'output', DCS.dataName, '')

data = DCS.prepare(raw_data)

DCS.process(DCS.prepare(data))

DCS.makeIndividualGeneExpressionPlot('CCL5')

DCS.makeIndividualGeneTtestPlot('CCL5', analyzeBy='celltype')

cells = DCS.getCells(celltype='T cell')
DCS.makeAnomalyScoresPlot(cells)

# ...
```

Direct use of function from where they are stored may result in undefined behavior.

Description of the package functionality

The main class of `DigitalCellSorter`. The class includes tools for:

1. **Pre-preprocessing** of single cell RNA sequencing data
2. **Quality control**
3. **Batch effects correction**
4. **Cells anomaly score evaluation**
5. **Dimensionality reduction**
6. **Clustering**
7. **Annotation of cell types**
8. **Vizualization**
9. **Post-processing**

3.1 Primary tools

Primary tools are used for pre-processing of the input data, quality control, batch correction, dimensionality reduction, clustering and cell type annotation.

Note: We recommend to use only functions `prepare()`, `process()`, and `visualize()` of the Primary tools. All processing workflow is contained within `process()`. If you wish to modify the workflow use the other components of the Primary tools, such as `cluster()`, `project()` etc.

References to DigitalCellSorter class:

<code>prepare(obj)</code>	Prepare pandas.DataFrame for input to function <code>process()</code> If input is <code>pd.DataFrame</code> validate the input whether it has correct structure.
<code>convert([nameFrom, nameTo])</code>	Convert index to hugo names, if any names in the index are duplicated, remove duplicates
<code>clean()</code>	Clean pandas.DataFrame: validate index, remove index duplicates, replace missing with zeros, remove all-zero rows and columns
<code>project([PCAonly, do_fast_tsne])</code>	Project pandas.DataFrame to lower dimensions
<code>cluster()</code>	Cluster PCA-reduced data into a desired number of clusters
<code>annotate([mapNonexpressedCelltypes])</code>	Produce cluster voting results, annotate cell types, and update marker expression with cell type labels
<code>process([dataIsNormalized, cleanData])</code>	Process data before using any annotation of visualization functions
<code>visualize()</code>	Aggregate of visualization tools of this class.

Function `prepare()`: prepare input data for function `process()`

DigitalCellSorter.**prepare** (*obj*)

Prepare pandas.DataFrame for input to function `process()` If input is `pd.DataFrame` validate the input whether it has correct structure.

Parameters:

obj: **str, pandas.DataFrame, pandas.Series** Expression data in a form of pandas.DataFrame, pandas.Series, or name and path to a csv file with data

Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`dDCS.preapre('data.csv')`

Function `convert()`: convert gene index of a DataFrame prepared by function `prepare()` from one naming

convention to another

`DigitalCellSorter.convert` (*nameFrom=None, nameTo=None, **kwargs*)

Convert index to hugo names, if any names in the index are duplicated, remove duplicates

Parameters:

nameFrom: str, Default 'alias' Gene name type to convert from

nameTo: str, Default 'hugo' Gene name type to convert to

Any parameters that function 'mergeIndexDuplicates' can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.convertIndex()

Function `clean()`: validate index, replace missing with zeros, remove all-zero rows and columns of a DataFrame

`DigitalCellSorter.clean()`

Clean pandas.DataFrame: validate index, remove index duplicates, replace missing with zeros, remove all-zero rows and columns

Parameters: None

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.clean()

Function `normalize()`: rescale all cells, log-transform data, remove constant genes, and sort index of a DataFrame

`DigitalCellSorter.normalize` (*median=None*)

Normalize pandas.DataFrame: rescale all cells, log-transform data, remove constant genes, sort index

Parameters:

median: float, Default None Scale factor, if not provided will be computed as median across all cells in data

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.normalize()

Function `project()`: project data to lower dimensions

`DigitalCellSorter.project (PCAonly=False, do_fast_tsne=True)`

Project pandas.DataFrame to lower dimensions

Parameters:

PCAonly: boolean, Default False Perform Principal component analysis only

do_fast_tsne: boolean, Default True Do FI-tSNE instead of “exact” tSNE This option is ignored if layout is not ‘TSNE’

Returns:

tuple Processed data

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`xPCA, PCs, tSNE = DCS.project()`

Function `cluster()`: cluster PCA-reduced data into a desired number of clusters

`DigitalCellSorter.cluster()`

Cluster PCA-reduced data into a desired number of clusters

Parameters: None

Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.cluster()`

Function `annotate()`: produce cluster voting results

`DigitalCellSorter.annotate (mapNonexpressedCelltypes=True)`

Produce cluster voting results, annotate cell types, and update marker expression with cell type labels

Parameters:

mapNonexpressedCelltypes: boolean, Default True If True then cell types coloring will be consistent across all datasets, regardless what cell types are annotated in all datasets for a given input marker list file.

Returns:

dictionary Voting results, a dictionary in form of: {cluster label: assigned cell type}

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`results = DCS.annotate(df_markers_expr, df_marker_cell_type)`

Function `process()`: main function

`DigitalCellSorter.process` (*dataIsNormalized=False, cleanData=True*)

Process data before using any annotation of visualization functions

Parameters:

dataIsNormalized: boolean, Default False Whether DCS.df_expr is normalized or not

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

Function `visualize()`: make all default plots of to visualize results of function `process()`

`DigitalCellSorter.visualize()`

Aggregate of visualization tools of this class.

Parameters: None

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.visualize()

3.2 Extraction tools

Warning: Use these functions only after `process()`

References to DigitalCellSorter class:

<code>getExprOfGene</code> (gene[, analyzeBy])	Get expression of a gene.
<code>getExprOfCells</code> (cells)	Get expression of a set of cells.
<code>getCells</code> ([celltype, clusterIndex, clusterName])	Get cell annotations in a form of pandas.Series
<code>getAnomalyScores</code> (trainingSet, testingSet[, ...])	Function to get anomaly score of cells based on some reference set
<code>getNewMarkerGenes</code> ([cluster, top, ...])	Extract new marker genes based on the cluster annotations
<code>getIndexOfGoodQualityCells</code> ([QCplotsSubDir])	Get index of sells that satisfy the QC criteria
<code>getCountsDataFrame</code> (se1, se2[, tagForMissing])	Get a pandas.DataFrame with cross-counts (overlaps) between two pandas.Series

Function `getExprOfGene()`: Get expression of a gene

`DigitalCellSorter.getExprOfGene` (*gene, analyzeBy='cluster'*)

Get expression of a gene. Run this function only after function process()

Parameters:

cells: **pandas.MultiIndex** Index of cells of interest

analyzeBy: **str, Default 'cluster'** What level of labels to include. Other possible options are 'label' and 'celltype'

Returns:

pandas.DataFrame With expression of the cells of interest

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.getExprOfGene('SDC1')

Function `getExprOfCells()`: Get expression of a set of cells

`DigitalCellSorter.getExprOfCells(cells)`

Get expression of a set of cells. Run this function only after function process()

Parameters:

cells: **pandas.MultiIndex** 2-level Index of cells of interest, must include levels 'batch' and 'cell'

Returns:

pandas.DataFrame With expression of the cells of interest

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.getExprOfCells(cells)

Function `getCells()`: get cells index by celltype, clusterIndex or clusterName

`DigitalCellSorter.getCells(celltype=None, clusterIndex=None, clusterName=None)`

Get cell annotations in a form of pandas.Series

Parameters:

celltype: **str, Default None** Cell type to extract

clusterIndex: **int, Default None** Cell type to extract

clusterName: **str, Default None** Cell type to extract

Returns:

pandas.MultiIndex Index of labelled cells

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

labels = DCS.getCells()

Function `getAnomalyScores()`: get anomaly score of cells based on some reference set

`DigitalCellSorter.getAnomalyScores(trainingSet, testingSet, printResults=False)`

Function to get anomaly score of cells based on some reference set

Parameters:

trainingSet: `pandas.DataFrame` With cells to train isolation forest on

testingSet: `pandas.DataFrame` With cells to score

printResults: `boolean`, **Default False** Whether to print results

Returns:

1d numpy.array Anomaly score(s) of tested cell(s)

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

cutoff = DCS.getAnomalyScores(df_expr.iloc[:, 5:], df_expr.iloc[:, :5])

Function `getNewMarkerGenes()`: extract new markers from the annotated clusters and produce plot of the new markers

`DigitalCellSorter.getNewMarkerGenes(cluster=None, top=100, zScoreCutoff=None, removeUnknown=False, **kwargs)`

Extract new marker genes based on the cluster annotations

Parameters:

cluster: `int`, **Default None** Cluster #, if provided genes of only this cluster will be returned

top: `int`, **Default 100** Upper bound for number of new markers per cell type

zScoreCutoff: `float`, **Default 0.3** Lower bound for a marker z-score to be significant

removeUnknown: `boolean`, **Default False** Whether to remove type "Unknown"

Any parameters that function 'makePlotOfNewMarkers' can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.extractNewMarkerGenes()

Function `getIndexofGoodQualityCells()`: Get index of cells that satisfy the QC criteria

`DigitalCellSorter.getIndexOfGoodQualityCells(QCplotsSubDir='QC_plots', **kwargs)`

Get index of sells that satisfy the QC criteria

Parameters:

count_depth_cutoff: float, Default 0.5 Fraction of median to take as count depth cutoff

number_of_genes_cutoff: float, Default 0.5 Fraction of median to take as number of genes cutoff

mitochondrial_genes_cutoff: float, Default 3.0 The cutoff is median + standard_deviation * this_parameter

Any parameters that function 'makeQualityControlHistogramPlot' can accept

Returns:

pandas.Index Index of cells

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

index = DCS.getIndexOfGoodQualityCells()

Function `getCountsDataframe()`: Get a pandas.DataFrame with cross-counts (overlaps) between two pandas.Series

`DigitalCellSorter.getCountsDataframe(se1, se2, tagForMissing='N/A')`

Get a pandas.DataFrame with cross-counts (overlaps) between two pandas.Series

Parameters:

se1: pandas.Series Series with the first set of items

se2: pandas.Series Series with the second set of items

tagForMissing: str, Default 'N/A' Label to assign to non-overlapping items

Returns:

pandas.DataFrame Contains counts

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

df = DCS.getCountsDataframe(se1, se2)

3.3 Visualization tools

Warning: Use these functions only after <code>process()</code>

References to DigitalCellSorter class:

<code>makeProjectionPlotAnnotated(**kwargs)</code>	Produce projection plot colored by cell types
--	---

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Table 3 – continued from previous page

<code>makeProjectionPlotByBatches(**kwargs)</code>	Produce projection plot colored by batches
<code>makeProjectionPlotByClusters(**kwargs)</code>	Produce projection plot colored by clusters
<code>makeProjectionPlotsQualityControl(**kwargs)</code>	Produce Quality Control projection plots
<code>makeMarkerSubplots(**kwargs)</code>	Produce subplots on each marker and its expression on all clusters
<code>makeAnomalyScoresPlot([cells, suffix, noPlot])</code>	Make anomaly scores plot
<code>makeIndividualGeneTtestPlot(gene[, analyzeBy])</code>	Produce individual gene t-test plot of the two-tailed p-value.
<code>makeIndividualGeneExpressionPlot(genes, **kwargs)</code>	Produce individual gene expression plot on a 2D layout

References to VisualizationFunctions class:

<code>makeQualityControlHistogramPlot(*args, **kwargs)</code>	Function to calculate QC quality cutoff and visualize it on a histogram
<code>makeHistogramNullDistributionPlot(*args, ...)</code>	Produce histogram plot of the voting null distributions
<code>makeAnnotationResultsMatrixPlot(*args, **kwargs)</code>	Produce voting results voting matrix plot
<code>makeMarkerExpressionPlot(*args, **kwargs)</code>	Produce image on marker genes and their expression on all clusters.
<code>makeStackedBarplot(*args, **kwargs)</code>	Produce stacked barplot with cell fractions
<code>makeSankeyDiagram(*args, **kwargs)</code>	Make a Sankey diagram, also known as ‘river plot’ with two groups of nodes

Function `makeProjectionPlotAnnotated()`: Produce t-SNE plot colored by cell types

DigitalCellSorter.**makeProjectionPlotAnnotated**(**kwargs)

Produce projection plot colored by cell types

Parameters: Any parameters that function ‘makeProjectionPlot’ can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.makeProjectionPlotAnnotated()

Example output:

Function `makeProjectionPlotByBatches()`: Produce t-SNE plot colored by batches

DigitalCellSorter.**makeProjectionPlotByBatches**(**kwargs)

Produce projection plot colored by batches

Parameters: Any parameters that function ‘makeProjectionPlot’ can accept

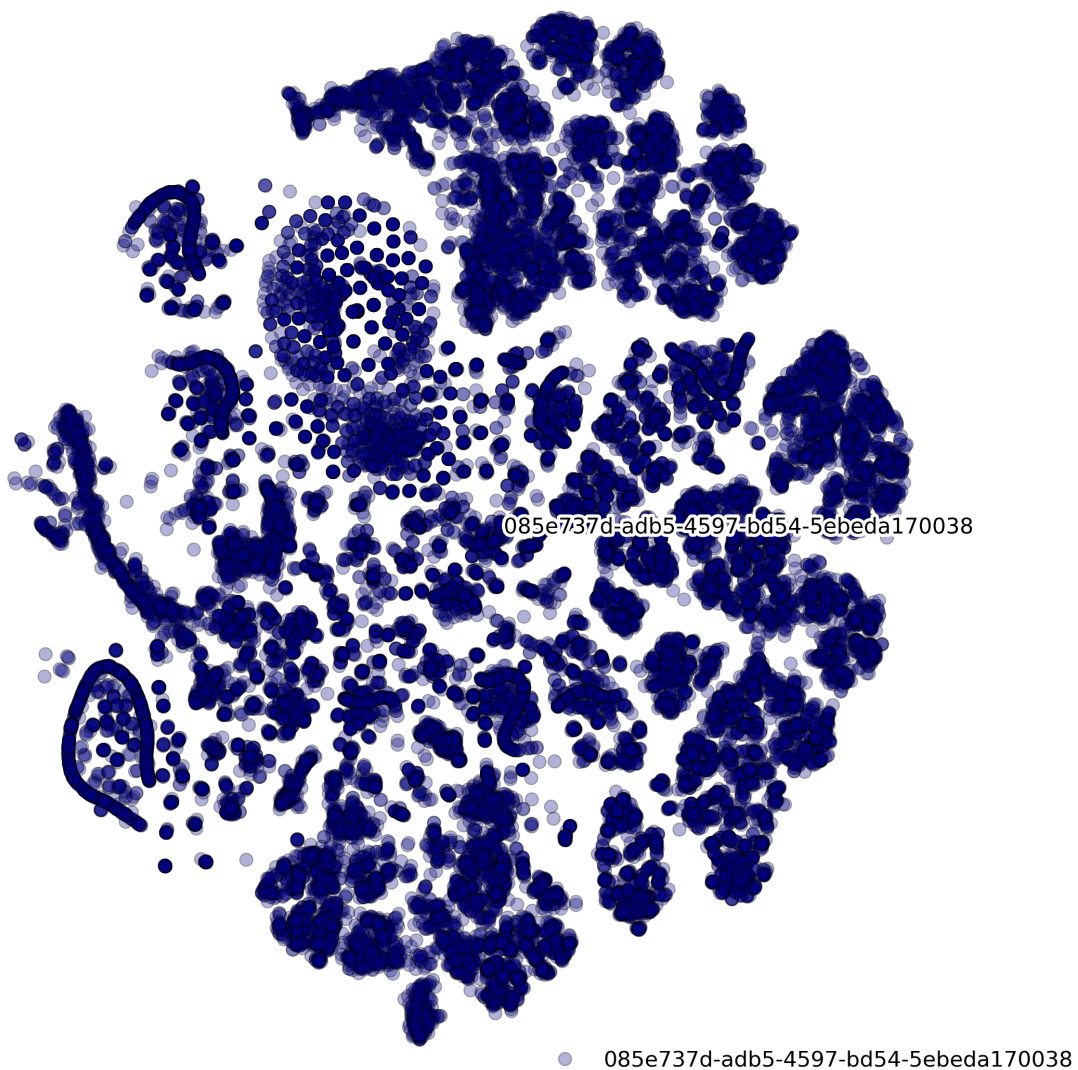
Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.makeProjectionPlotByBatches()

Example output:



Function `makeProjectionPlotByClusters()`: Produce t-SNE plot colored by clusters

DigitalCellSorter.**makeProjectionPlotByClusters** (**kwargs)

Produce projection plot colored by clusters

Parameters: Any parameters that function ‘makeProjectionPlot’ can accept

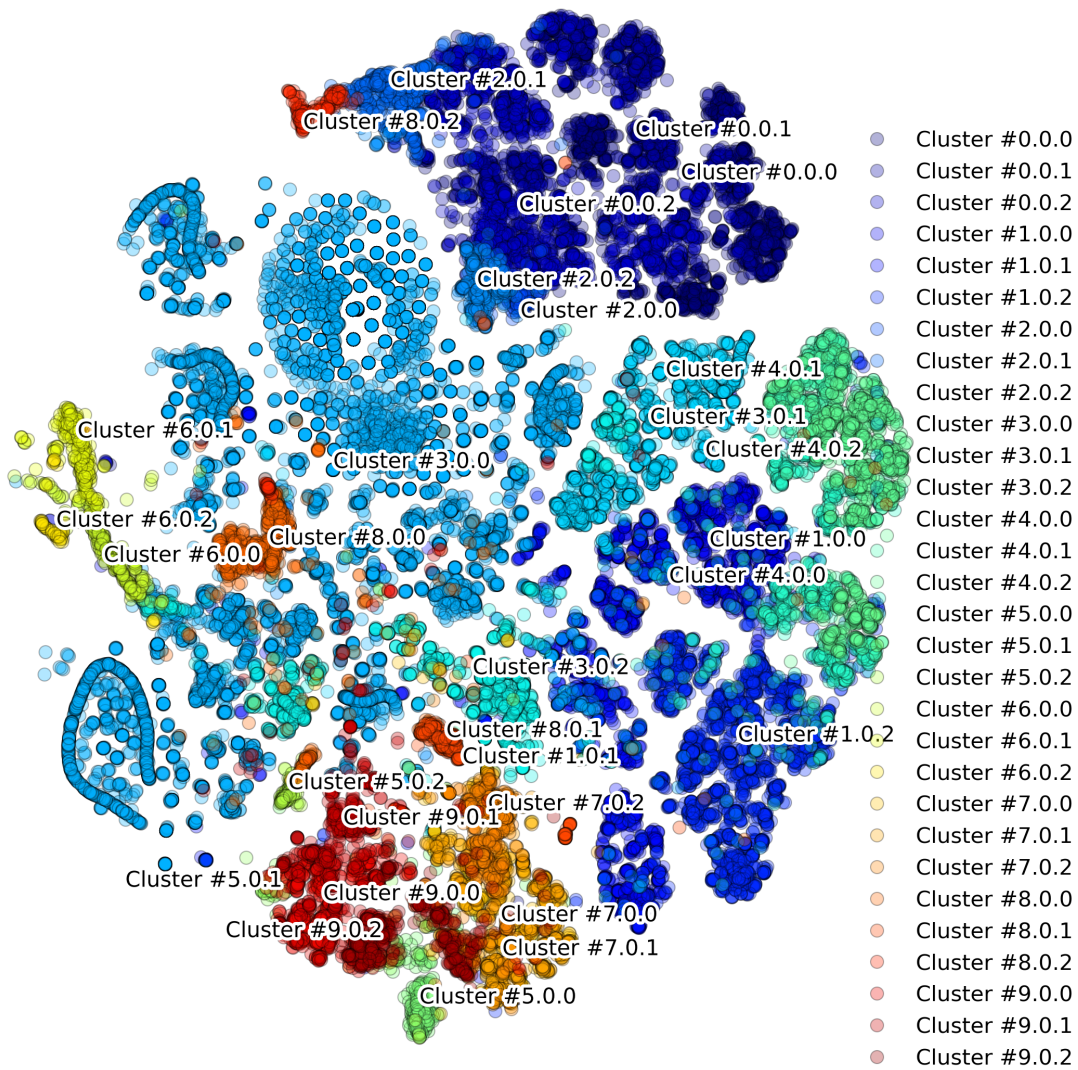
Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.makeProjectionPlotByClusters()

Example output:



Function `makeQualityControlHistogramPlot()`: Produce Quality Control histogram plots

`DigitalCellSorter.makeQualityControlHistogramPlot(*args, **kwargs)`

Function to calculate QC quality cutoff and visualize it on a histogram

Parameters:

subset: `pandas.Series` Data to analyze

cutoff: `float` Cutoff to display

plotPathAndName: `str`, **Default** `None` Text to include in the figure title and file name

N_bins: `int`, **Default** `100` Number of bins of the histogram

mito: `boolean`, **Default** `False` Whether the analysis of mitochondrial genes fraction

displayMeasures: `boolean`, **Default** `True` Print vertical dashed lines along with mean, median, and standard deviation

precision: `int`, **Default** `4` Number of digits after decimal

quantilePlotCutoff: `float`, **Default** `0.99` Distributions are cut to display the range from 0 to quantilePlot-Cutoff

dpi: `int`, **Default** `600` Resolution of the figure image

extension: `str`, **Default** `'png'` Format of the figure file

fontScale: `float`, **Default** `1.5` Scale most of the figure fonts

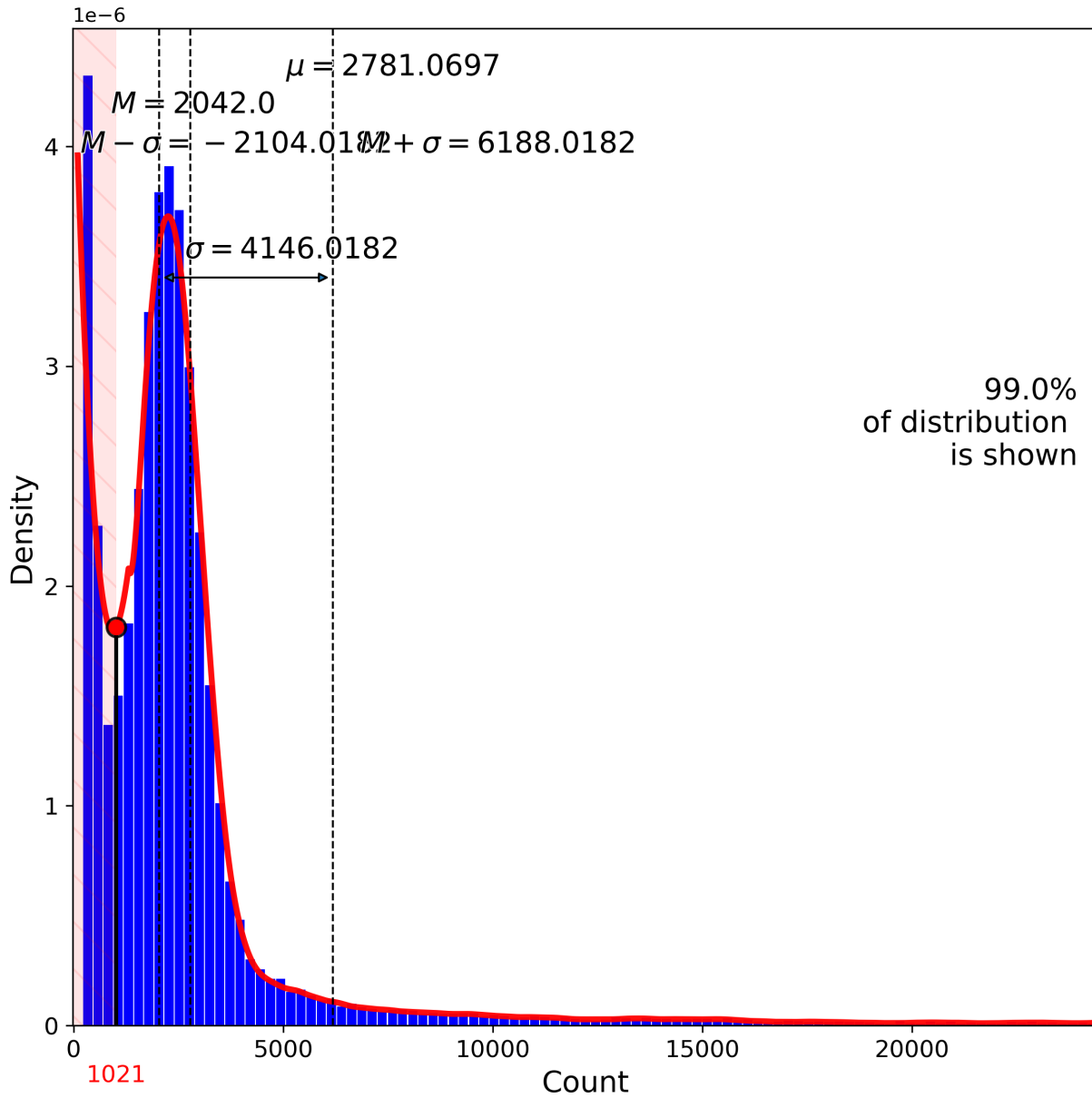
includeTitle: `boolean`, **Default** `False` Whether to include title on the figure

Returns: `None`

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`cutoff = DCS.makeQualityControlHistogramPlot(subset, cutoff)`

Example output:



Function `makeProjectionPlotsQualityControl()`: Produce Quality Control t-SNE plots

`DigitalCellSorter.makeProjectionPlotsQualityControl(**kwargs)`

Produce Quality Control projection plots

Parameters: Any parameters that function ‘makeProjectionPlot’ can accept

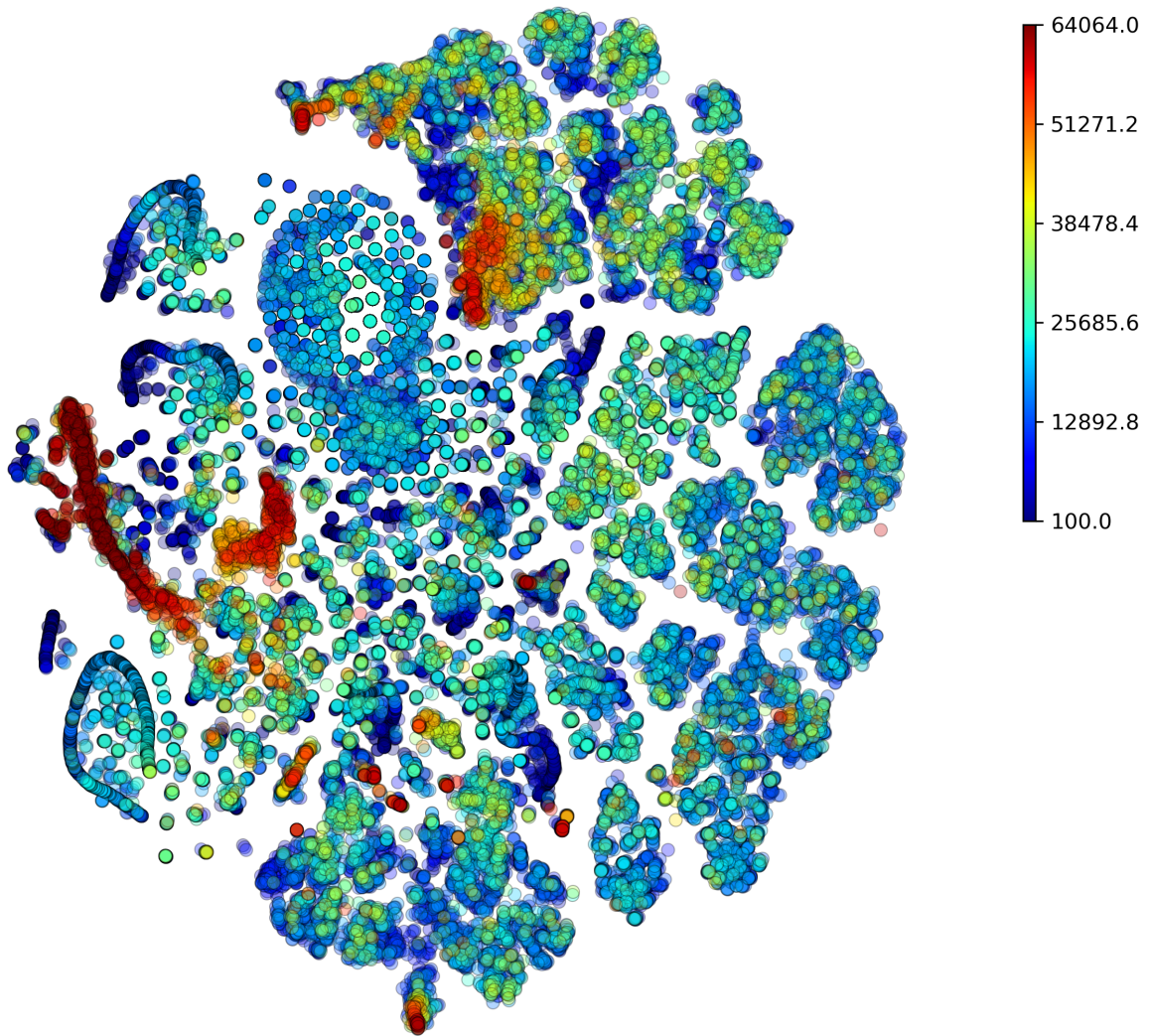
Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`


```
DCS.process()
```

```
DCS.makeProjectionPlotsQualityControl()
```

Example output:



Function `makeMarkerSubplots()`: Produce subplots on each marker and its expression on all clusters

`DigitalCellSorter.makeMarkerSubplots (**kwargs)`

Produce subplots on each marker and its expression on all clusters

Parameters: Any parameters that function ‘internalMakeMarkerSubplots’ can accept

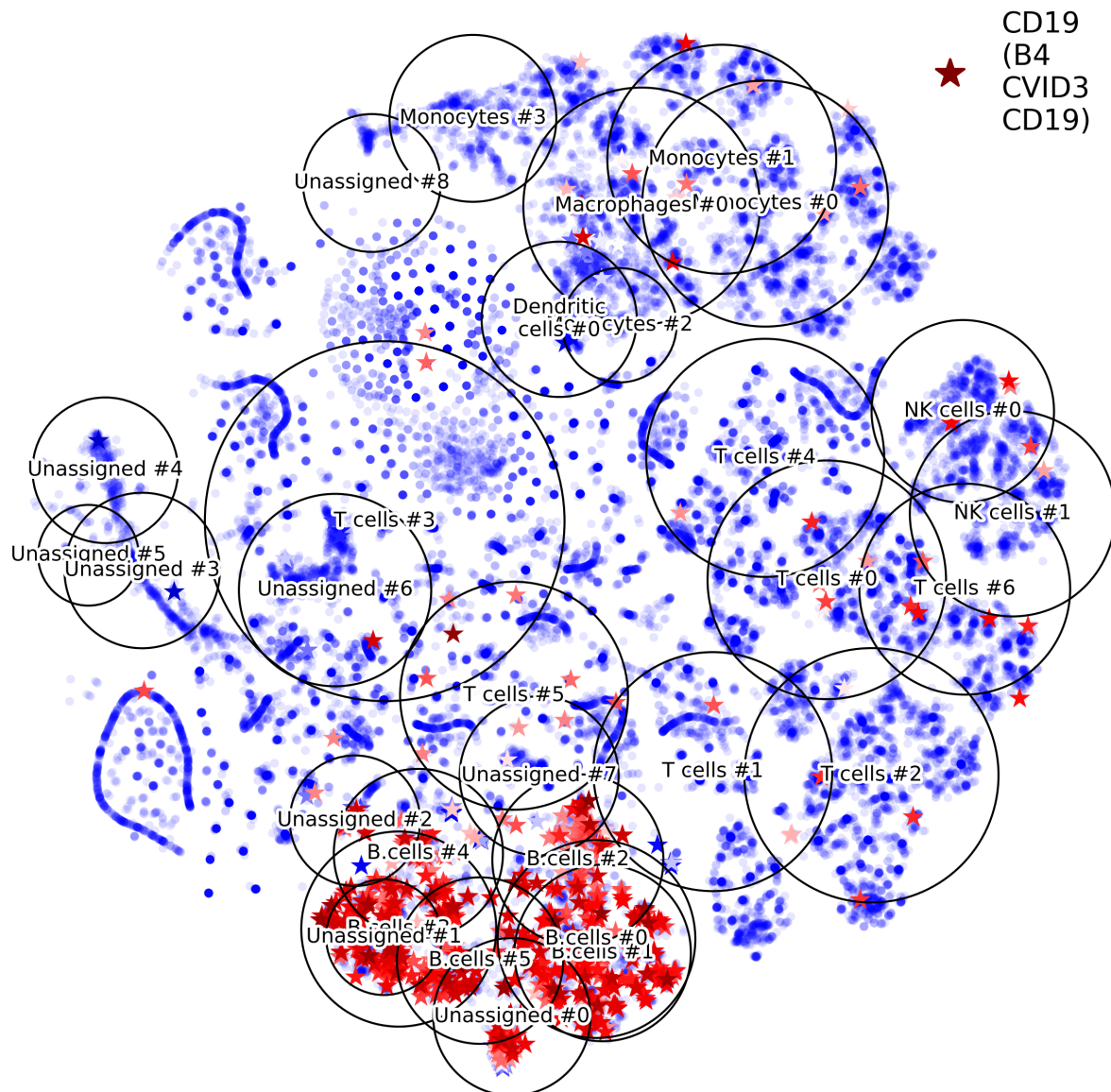
Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.process()`

`DCS.makeMarkerSubplots()`

Example output:



Function `makeAnomalyScoresPlot()`: Make anomaly scores plot

`DigitalCellSorter.makeAnomalyScoresPlot(cells='All', suffix='', noPlot=False, **kwargs)`
Make anomaly scores plot

Parameters:

cells: `pandas.MultiIndex`, Default 'All' Index of cells of interest

Any parameters that function 'makeProjectionPlot' can accept

Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.process()`

`cells = DCS.getCells(celltype='T cell')`

`DCS.makeAnomalyScoresPlot(cells)`

Example output:

Function `makeIndividualGeneTtestPlot()`: Produce individual gene t-test plot of the two-tailed p-value

`DigitalCellSorter.makeIndividualGeneTtestPlot(gene, analyzeBy='label', **kwargs)`
Produce individual gene t-test plot of the two-tailed p-value.

Parameters:

gene: `str` Name of gene of interest

analyzeBy: `str`, Default 'label' What level of labels to include. Other possible options are 'label' and 'celltype'

Any parameters that function 'makeTtestPlot' can accept

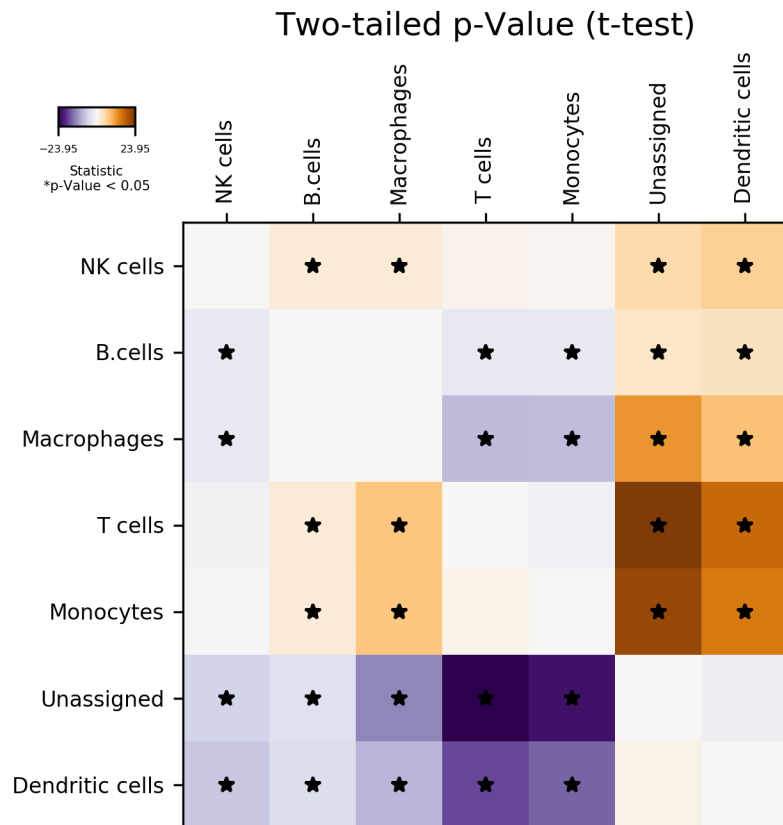
Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.makeIndividualGeneTtestPlot('SDC1')`

Example output:

CD4
(CD4
CD4mut)



Function `makeIndividualGeneExpressionPlot()`: Produce individual gene expression plot on a 2D layout

`DigitalCellSorter.makeIndividualGeneExpressionPlot` (*genes*, ***kwargs*)

Produce individual gene expression plot on a 2D layout

Parameters:

gene: str, or list-like Name of gene of interest. E.g. 'CD4, CD33', 'PECAM1', ['CD4', 'CD33']

hideClusterLabels: boolean, Default False Whether to hide the clusters labels

outlineClusters: boolean, Default True Whether to outline the clusters with circles

Any parameters that function 'internalMakeMarkerSubplots' can accept

Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.makeIndividualGeneExpressionPlot('CD4')`

Example output:

Function `makeHistogramNullDistributionPlot()` : Produce histogram plot of the voting null distributions

`DigitalCellSorter.makeHistogramNullDistributionPlot(*args, **kwargs)`

Produce histogram plot of the voting null distributions

Parameters:

dpi: int, Default 600 Resolution of the figure image

extension: str, Default 'png' Format of the figure file

Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.makeHistogramNullDistributionPlot()`

Example output:



Function `makeAnnotationResultsMatrixPlot()`: Produce voting results voting matrix plot

`DigitalCellSorter.makeAnnotationResultsMatrixPlot(*args, **kwargs)`

Produce voting results voting matrix plot

Parameters:

dpi: int, Default 600 Resolution of the figure image

extension: str, Default 'png' Format of the figure file

Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.makeAnnotationResultsMatrixPlot()`

Example output:

Function `makeMarkerExpressionPlot()`: Produce image on marker genes and their expression on all clusters

`DigitalCellSorter.makeMarkerExpressionPlot(*args, **kwargs)`

Produce image on marker genes and their expression on all clusters. Uses files generated by function `DCS.Vote`

Parameters:

dpi: int, Default 600 Resolution of the figure image

extension: str, Default 'png' Format of the figure file

Returns: None

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.makeMarkerExpressionPlot()`

Example output:

Function `makeStackedBarplot()`: Produce stacked barplot with cell fractions

`DigitalCellSorter.makeStackedBarplot(*args, **kwargs)`

Produce stacked barplot with cell fractions

Parameters:

clusterName: str, Default None Label to include at the bar bottom. If None the `self.dataName` value will be used

legendStyle: boolean, Default False Use one out of two styles of this figure

includeLowQC: boolean, Default True Whether to include low quality cells

dpi: int, Default 600 Resolution of the figure image

extension: str, Default 'png' Format of the figure file

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.makeStackedBarplot(clusterName)

Example output:

Function `makeSankeyDiagram()`: Make a Sankey diagram, also known as 'river plot' with two groups of nodes

`DigitalCellSorter.makeSankeyDiagram(*args, **kwargs)`

Make a Sankey diagram, also known as 'river plot' with two groups of nodes

Parameters:

df: pandas.DataFrame With counts (overlaps)

colormapForIndex: dictionary, Default None Colors to use for nodes specified in the DataFrame index

colormapForColumns: dictionary, Default None Colors to use for nodes specified in the DataFrame columns

linksColor: str, Default 'rgba(100,100,100,0.6)' Color of the non-overlapping links

title: str, Default '' Title to print on the diagram

interactive: boolean, Default False Whether to launch interactive JavaScript-based graph

quality: int, Default 4 Proportional to the resolution of the figure to save

nodeLabelsFontSize: int, Default 15 Font size for node labels

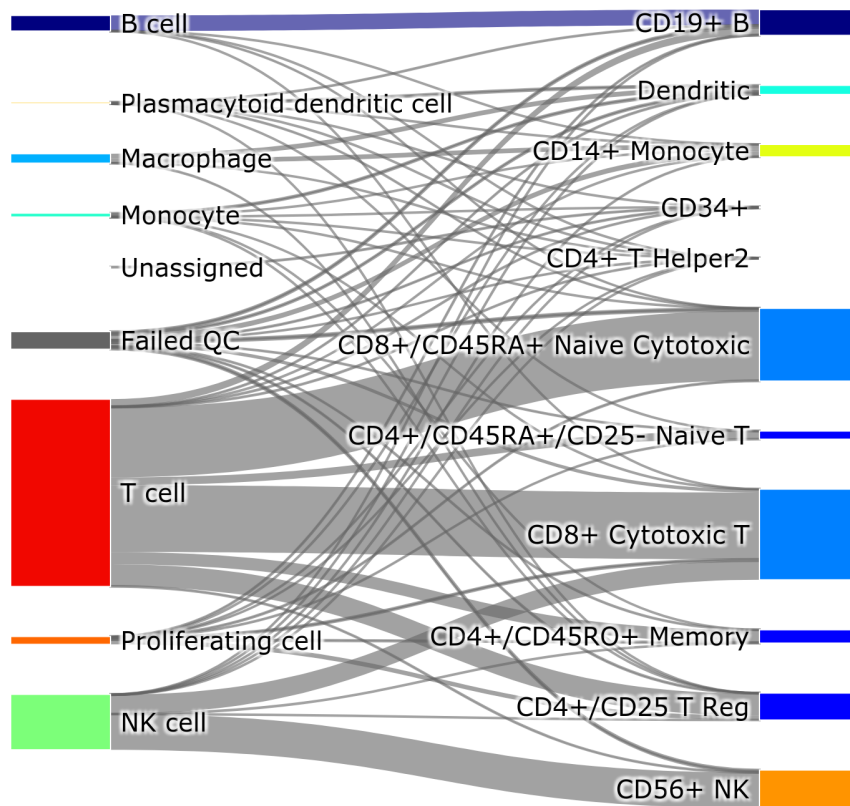
nameAppend: str, Default '_Sankey_diagram' Name to append to the figure file

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.makeSankeyDiagram(df)

Example output:



CORE CLASS

Submodule core

Description of the package functionality

The main class of DigitalCellSorter. The class includes tools for:

1. **Pre-preprocessing** of single cell RNA sequencing data
2. **Quality control**
3. **Batch effects correction**
4. **Cells anomaly score evaluation**
5. **Dimensionality reduction**
6. **Clustering**
7. **Annotation of cell types**
8. **Vizualization**
9. **Post-processing**

```
class DigitalCellSorter(df_expr=None, dataName='dataName', species='Human', geneNamesType='alias', geneListFileName=None, mitochondrialGenes=None, sigmaOverMeanSigma=0.01, nClusters=10, nFineClusters=3, doFineClustering=True, splitFineClusters=False, subSplitSize=100, medianScaleFactor=10000, minSizeForFineClustering=50, clusteringFunction=<class 'sklearn.cluster._agglomerative.AgglomerativeClustering'>, nComponentsPCA=200, nSamples_pDCS=3000, nSamples_Hopfield=200, saveDir="", makeMarkerSubplots=False, availableCPUsCount=2, zScoreCutoff=0.3, subclusteringName=None, doQualityControl=True, doBatchCorrection=False, makePlots=True, useUnderlyingNetwork=True, minimumNumberOfMarkersPerCelltype=10, nameForUnknown='Unassigned', nameForLowQC='Failed QC', matplotlibMode='Agg', countDepthCutoffQC=0.5, numberOfGenesCutoffQC=0.5, mitochondrialGenesCutoffQC=1.5, excludedFromQC=None, countDepthPrecutQC=500, numberOfGenesPrecutQC=250, precutQC=False, minSubclusterSize=25, thresholdForUnknown_pDCS=0.0, thresholdForUnknown_ratio=0.0, thresholdForUnknown_Hopfield=0.0, thresholdForUnknown=0.2, layout='TSNE', safePlotting=True, HopfieldTemperature=0.1, annotationMethod='ratio-pDCS-Hopfield', useNegativeMarkers=True, removeLowQualityScores=True, updateConversionDictFile=True, verbose=1, random_state=None)
```

Bases: DigitalCellSorter.VisualizationFunctions.VisualizationFunctions

Class of Digital Cell Sorter with methods for processing single cell RNA-seq data. Includes analyses and visualization tools.

Parameters:

df_expr: pandas.DataFrame, Default None Gene expression in a form of a table, where genes are rows, and cells/batches are columns

dataName: str, Default 'dataName' Name used in output files

geneNamesType: str, Default 'alias' Input gene name convention

geneListFileName: str, Default None Name of the marker genes file

mitochondrialGenes: list, Default None List of mitochondrial genes to use in quality control

sigmaOverMeanSigma: float, Default 0.1 Threshold to consider a gene constant

nClusters: int, Default 10 Number of clusters

nFineClusters: int, Default 3 Number of fine clusters to determine with Spectral Co-clustering routine. This option is ignored is doFineClustering is False.

doFineClustering: boolean, Default True Whether to do fine clustering or not

minSizeForFineClustering: int, Default 50 Minimum number of cells required to do fine clustering of a cluster. This option is ignored is doFineClustering is False.

clusteringFunction: function, Default AgglomerativeClustering Clustering function to use. Other options: KMeans, {k_neighbors:40}, etc. Note: the function should have .fit method and same input and output. For Network-based clustering pass a dictionary {'k_neighbors':40, 'metric':'euclidean', 'clusterExpression':True}, this way the best number of clusters will be determined automatically

nComponentsPCA: int, Default 200 Number of pca components

nSamples_pDCS: int, Default 3000 Number of random samples in distribution for pDCS annotation method

nSamples_Hopfield: int, Default 500 Number of repetitions for Hopfield annotation method

saveDir: str, Default `os.path.join('')` Directory for output files

makeMarkerSubplots: boolean, Default `False` Whether to make subplots on markers

makePlots: boolean, Default `True` Whether to make all major plots

availableCPUsCount: int, Default `min(12, os.cpu_count())` Number of CPUs used in pDCS method

zScoreCutoff: float, Default `0.3` Z-Score cutoff when setting expression of a cluster as significant

thresholdForUnknown: float, Default `0.3` Threshold when assigning label “Unknown”. This option is used only with a combination of 2 or more annotation methods

thresholdForUnknown_pDCS: float, Default `0.1` Threshold when assigning label “Unknown” in pDCS method

thresholdForUnknown_ratio: float, Default `0.1` Threshold when assigning label “Unknown” in ratio method

thresholdForUnknown_Hopfield: float, Default `0.1` Threshold when assigning label “Unknown” in Hopfield method

annotationMethod: str, Default `‘ratio-pDCS-Hopfield’`

Method to use for annotation of cell types to clusters. Options are: ‘pDCS’: main DCS voting scheme with null testing

‘ratio’: simple voting score

‘Hopfield’: Hopfield Network classifier

‘pDCS-ratio’: ‘pDCS’ adjusted with ‘ratio’

‘pDCS-Hopfield’: ‘pDCS’ adjusted with ‘Hopfield’

‘ratio-Hopfield’: ‘ratio’ adjusted with ‘Hopfield’

‘pDCS-ratio-Hopfield’: ‘pDCS’ adjusted with ‘ratio’ and ‘Hopfield’

subclusteringName: str, Default `None` Parameter used in for certain labels on plots

doQualityControl: boolean, Default `True` Whether to remove low quality cells

doBatchCorrection: boolean, Default `False` Whether to correct data for batches

minimumNumberOfMarkersPerCelltype: int, Default `10` Minimum number of markers per cell type to keep that cell type in annotation options

nameForUnknown: str, Default `‘Unassigned’` Name to use for clusters where label assignment yielded uncertain results

nameForLowQC: str, Default `‘Failed QC’` Name to use for cell that do not pass quality control

layout: str, Default `‘TSNE’`

Projection layout used in visualization. Options are: ‘TSNE’: t-SNE layout L.J.P. van der Maaten. Accelerating t-SNE using Tree-Based Algorithms. Journal of Machine Learning Research 15(Oct):3221-3245, 2014.

‘PCA’: use two largest principal components

‘UMAP’: use uniform manifold approximation, McInnes, L., Healy, J., UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction, ArXiv e-prints 1802.03426, 2018

‘PHATE’: use potential of heat diffusion for affinity-based transition embedding, Moon, K.R., van Dijk, D., Wang, Z. et al. Visualizing structure and transitions in high-dimensional biological data. Nat Biotechnol 37, 1482–1492 (2019).

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

df_data = DCS.Clean(df_data)

Methods:

<i>KeyInFile</i> (key, file)	Check if a key exists in a HDF file.
<i>alignSeries</i> (se1, se2, tagForMissing)	Align two pandas.Series
<i>annotate</i> ([mapNonexpressedCelltypes])	Produce cluster voting results, annotate cell types, and update marker expression with cell type labels
<i>annotateWith_Hopfield_Scheme</i> (...)	Produce cluster annotation results
<i>annotateWith_pDCS_Scheme</i> (df_markers_expr, ...)	Produce cluster annotation results
<i>annotateWith_ratio_Scheme</i> (df_markers_expr, ...)	Produce cluster annotation results
<i>batchEffectCorrection</i> ([method])	Batch effect correction.
<i>calculateQCmeasures</i> ()	Calculate Quality Control (QC) measures
<i>calculateV</i> (args)	Calculate the voting scores (celltypes by clusters)
<i>clean</i> ()	Clean pandas.DataFrame: validate index, remove index duplicates, replace missing with zeros, remove all-zero rows and columns
<i>cluster</i> ()	Cluster PCA-reduced data into a desired number of clusters
<i>convert</i> ([nameFrom, nameTo])	Convert index to hugo names, if any names in the index are duplicated, remove duplicates
<i>convertColormap</i> (colormap)	Convert colormap from the form (1.,1.,1.,1.) to 'rgba(255,255,255,1.)'
<i>createReverseDictionary</i> (inputDictionary)	Efficient way to create a reverse dictionary from a dictionary.
<i>getAnomalyScores</i> (trainingSet, testingSet[, ...])	Function to get anomaly score of cells based on some reference set
<i>getCells</i> ([celltype, clusterIndex, clusterName])	Get cell annotations in a form of pandas.Series
<i>getCountsDataframe</i> (se1, se2[, tagForMissing])	Get a pandas.DataFrame with cross-counts (overlaps) between two pandas.Series
<i>getExprOfCells</i> (cells)	Get expression of a set of cells.
<i>getExprOfGene</i> (gene[, analyzeBy])	Get expression of a gene.
<i>getHugoName</i> (gene[, printAliases])	Get gene hugo name(s).
<i>getIndexOfGoodQualityCells</i> ([QCplotsSubDict])	Get index of cells that satisfy the QC criteria
<i>getNewMarkerGenes</i> ([cluster, top, ...])	Extract new marker genes based on the cluster annotations
<i>getQualityControlCutoff</i> (se, cutoff[, ...])	Function to calculate QC quality cutoff
<i>getSubnetworkOfPCN</i> (subnetworkGenes[, ...])	Extract subnetwork of PCN network
<i>loadAnnotatedLabels</i> ([detailed, ...])	Load cell annotations resulted from function 'annotate'
<i>loadExpressionData</i> ()	Load processed expression data from the internal HDF storage.
<i>makeAnomalyScoresPlot</i> ([cells, suffix, no-Plot])	Make anomaly scores plot
<i>makeHopfieldLandscapePlot</i> ([...])	Make and plot Hopfield landscape
<i>makeIndividualGeneExpressionPlot</i> (genes, **kwargs)	Produce individual gene expression plot on a 2D layout

Continued on next page

Table 1 – continued from previous page

<code>makeIndividualGeneTtestPlot(gene[, analyzeBy])</code>	Produce individual gene t-test plot of the two-tailed p-value.
<code>makeMarkerSubplots(**kwargs)</code>	Produce subplots on each marker and its expression on all clusters
<code>makeProjectionPlotAnnotated(**kwargs)</code>	Produce projection plot colored by cell types
<code>makeProjectionPlotByBatches(**kwargs)</code>	Produce projection plot colored by batches
<code>makeProjectionPlotByClusters(**kwargs)</code>	Produce projection plot colored by clusters
<code>makeProjectionPlotsQualityControl(**kwargs)</code>	Produce Quality Control projection plots
<code>mergeIndexDuplicates(df_expr[, method, ...])</code>	Merge index duplicates
<code>normalize([median])</code>	Normalize pandas.DataFrame: rescale all cells, log-transform data, remove constant genes, sort index
<code>prepare(obj)</code>	Prepare pandas.DataFrame for input to function process() If input is pd.DataFrame validate the input whether it has correct structure.
<code>prepareMarkers([expressedGenes, ...])</code>	Get dictionary of markers for each cell types.
<code>process([dataIsNormalized, cleanData])</code>	Process data before using any annotation of visualization functions
<code>project([PCAonly, do_fast_tsne])</code>	Project pandas.DataFrame to lower dimensions
<code>propagateHopfield([sigma, xi, T, tmax, ...])</code>	Function is used internally to propagate Hopfield network over a set number of time steps
<code>qualityControl(**kwargs)</code>	Remove low quality cells
<code>readMarkerFile([mergeFunction, mergeCut-off])</code>	Read markers file, prepare markers
<code>recordAnnotationResults(df_marker_cell_type, ...)</code>	Record cell type annotation results to spreadsheets.
<code>recordExpressionData()</code>	Record expression data from the internal HDF storage.
<code>visualize()</code>	Aggregate of visualization tools of this class.
<code>zScoreOfSeries(se)</code>	Calculate z-score of pandas.Series and modify the Series in place

Attributes:

<code>df_expr</code>
<code>fileHDFpath</code>
<code>geneListFileName</code>
<code>saveDir</code>

property `saveDir`**property** `fileHDFpath`**property** `df_expr`**property** `geneListFileName`**prepare** (*obj*)

Prepare pandas.DataFrame for input to function process() If input is pd.DataFrame validate the input whether it has correct structure.

Parameters:

obj: str, pandas.DataFrame, pandas.Series Expression data in a form of pandas.DataFrame, pan-

das.Series, or name and path to a csv file with data

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

dDCS.preapre('data.csv')

convert (*nameFrom=None, nameTo=None, **kwargs*)

Convert index to hugo names, if any names in the index are duplicated, remove duplicates

Parameters:

nameFrom: str, Default 'alias' Gene name type to convert from

nameTo: str, Default 'hugo' Gene name type to convert to

Any parameters that function 'mergeIndexDuplicates' can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.convertIndex()

clean ()

Clean pandas.DataFrame: validate index, remove index duplicates, replace missing with zeros, remove all-zero rows and columns

Parameters: None

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.clean()

normalize (*median=None*)

Normalize pandas.DataFrame: rescale all cells, log-transform data, remove constant genes, sort index

Parameters:

median: float, Default None Scale factor, if not provided will be computed as median across all cells in data

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.normalize()

project (*PCAonly=False, do_fast_tsne=True*)

Project pandas.DataFrame to lower dimensions

Parameters:

PCAonly: boolean, Default False Perform Principal component analysis only

do_fast_tsne: boolean, Default True Do FI-tSNE instead of "exact" tSNE This option is ignored if layout is not 'TSNE'

Returns:

tuple Processed data

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

xPCA, PCs, tSNE = DCS.project()

cluster()

Cluster PCA-reduced data into a desired number of clusters

Parameters: None

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.cluster()

annotate (*mapNonexpressedCelltypes=True*)

Produce cluster voting results, annotate cell types, and update marker expression with cell type labels

Parameters:

mapNonexpressedCelltypes: boolean, Default True If True then cell types coloring will be consistent across all datasets, regardless what cell types are annotated in all datasets for a given input marker list file.

Returns:

dictionary Voting results, a dictionary in form of: {cluster label: assigned cell type}

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

results = DCS.annotate(df_markers_expr, df_marker_cell_type)

process (*dataIsNormalized=False, cleanData=True*)

Process data before using any annotation of visualization functions

Parameters:

dataIsNormalized: boolean, Default False Whether DCS.df_expr is normalized or not

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

visualize()

Aggregate of visualization tools of this class.

Parameters: None

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.visualize()

makeProjectionPlotAnnotated (***kwargs*)

Produce projection plot colored by cell types

Parameters: Any parameters that function 'makeProjectionPlot' can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.makeProjectionPlotAnnotated()

makeProjectionPlotByBatches (***kwargs*)

Produce projection plot colored by batches

Parameters: Any parameters that function ‘makeProjectionPlot’ can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.makeProjectionPlotByBatches()

makeProjectionPlotByClusters (***kwargs*)

Produce projection plot colored by clusters

Parameters: Any parameters that function ‘makeProjectionPlot’ can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.makeProjectionPlotByClusters()

makeProjectionPlotsQualityControl (***kwargs*)

Produce Quality Control projection plots

Parameters: Any parameters that function ‘makeProjectionPlot’ can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.makeProjectionPlotsQualityControl()

makeMarkerSubplots (***kwargs*)

Produce subplots on each marker and its expression on all clusters

Parameters: Any parameters that function ‘internalMakeMarkerSubplots’ can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.makeMarkerSubplots()

makeAnomalyScoresPlot (*cells='All', suffix='', noPlot=False, **kwargs*)

Make anomaly scores plot

Parameters:

cells: pandas.MultiIndex, Default ‘All’ Index of cells of interest

Any parameters that function ‘makeProjectionPlot’ can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

cells = DCS.getCells(celltype=’T cell’)

DCS.makeAnomalyScoresPlot(cells)

makeIndividualGeneTtestPlot (*gene, analyzeBy='label', **kwargs*)

Produce individual gene t-test plot of the two-tailed p-value.

Parameters:

gene: **str** Name of gene of interest

analyzeBy: **str, Default 'label'** What level of labels to include. Other possible options are 'label' and 'celltype'

Any parameters that function 'makeTtestPlot' can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.makeIndividualGeneTtestPlot('SDC1')

makeIndividualGeneExpressionPlot (*genes, **kwargs*)

Produce individual gene expression plot on a 2D layout

Parameters:

gene: **str, or list-like** Name of gene of interest. E.g. 'CD4, CD33', 'PECAM1', ['CD4', 'CD33']

hideClusterLabels: **boolean, Default False** Whether to hide the clusters labels

outlineClusters: **boolean, Default True** Whether to outline the clusters with circles

Any parameters that function 'internalMakeMarkerSubplots' can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.makeIndividualGeneExpressionPlot('CD4')

makeHopfieldLandscapePlot (*meshSamplingRate=1000, plot3D=True, reuseData=False, **kwargs*)

Make and plot Hopfield landscape

Parameters:

meshSamplingRate: **int, Default 1000** Defines quality of sampling around attractor states

plot3D: **boolean, Default False** Whether to plot 2D or 3D figure

reuseData: **boolean, Default False** Whether to attempt using precalculated data.

Any parameters that function 'HopfieldLandscapePlot' or 'HopfieldLandscapePlot3D' can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter() DCS.makeHopfieldLandscapePlot()

getAnomalyScores (*trainingSet, testingSet, printResults=False*)

Function to get anomaly score of cells based on some reference set

Parameters:

trainingSet: **pandas.DataFrame** With cells to train isolation forest on

testingSet: **pandas.DataFrame** With cells to score

printResults: **boolean, Default False** Whether to print results

Returns:

1d numpy.array Anomaly score(s) of tested cell(s)

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

cutoff = DCS.getAnomalyScores(df_expr.iloc[:, 5:], df_expr.iloc[:, :5])

getHugoName (*gene*, *printAliases=False*)

Get gene hugo name(s).

Parameters:

gene: **str** 'hugo' or 'alias' name of a gene

Returns:

str Hugo name if found, otherwise input name

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.getHugoName('CD138')

getExprOfGene (*gene*, *analyzeBy='cluster'*)

Get expression of a gene. Run this function only after function process()

Parameters:

cells: **pandas.MultiIndex** Index of cells of interest

analyzeBy: **str**, **Default 'cluster'** What level of labels to include. Other possible options are 'label' and 'celltype'

Returns:

pandas.DataFrame With expression of the cells of interest

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.getExprOfGene('SDC1')

getExprOfCells (*cells*)

Get expression of a set of cells. Run this function only after function process()

Parameters:

cells: **pandas.MultiIndex** 2-level Index of cells of interest, must include levels 'batch' and 'cell'

Returns:

pandas.DataFrame With expression of the cells of interest

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

DCS.getExprOfCells(cells)

getCells (*celltype=None*, *clusterIndex=None*, *clusterName=None*)

Get cell annotations in a form of pandas.Series

Parameters:

celltype: **str**, **Default None** Cell type to extract

clusterIndex: **int**, **Default None** Cell type to extract

clusterName: **str**, **Default None** Cell type to extract

Returns:

pandas.MultiIndex Index of labelled cells

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.process()

labels = DCS.getCells()

getIndexOfGoodQualityCells (*QCplotsSubDir='QC_plots', **kwargs*)

Get index of sells that satisfy the QC criteria

Parameters:

count_depth_cutoff: float, Default 0.5 Fraction of median to take as count depth cutoff

number_of_genes_cutoff: float, Default 0.5 Fraction of median to take as number of genes cutoff

mitochondrial_genes_cutoff: float, Default 3.0 The cutoff is median + standard_deviation * this_parameter

Any parameters that function 'makeQualityControlHistogramPlot' can accept

Returns:

pandas.Index Index of cells

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

index = DCS.getIndexOfGoodQualityCells()

getQualityControlCutoff (*se, cutoff, precut=1.0, mito=False, MakeHistogramPlot=True, **kwargs*)

Function to calculate QC quality cutoff

Parameters:

se: pandas.Series With data to analyze

cutoff: float Parameter for calculating the quality control cutoff

mito: boolean, Default False Whether the analysis of mitochondrial genes fraction

plotPathAndName: str, Default None Text to include in the figure title and file name

MakeHistogramPlot: boolean, Default True Whether to make a histogram plot

Any parameters that function 'makeQualityControlHistogramPlot' can accept

Returns:

float Cutoff value

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

cutoff = DCS.getQualityControlCutoff(se)

getCountsDataFrame (*se1, se2, tagForMissing='N/A'*)

Get a pandas.DataFrame with cross-counts (overlaps) between two pandas.Series

Parameters:

se1: pandas.Series Series with the first set of items

se2: pandas.Series Series with the second set of items

tagForMissing: str, Default 'N/A' Label to assign to non-overlapping items

Returns:

pandas.DataFrame Contains counts

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

df = DCS.getCountsDataframe(se1, se2)

getNewMarkerGenes (*cluster=None, top=100, zScoreCutoff=None, removeUnknown=False, **kwargs*)

Extract new marker genes based on the cluster annotations

Parameters:

cluster: int, Default None Cluster #, if provided genes of only this cluster will be returned

top: int, Default 100 Upper bound for number of new markers per cell type

zScoreCutoff: float, Default 0.3 Lower bound for a marker z-score to be significant

removeUnknown: boolean, Default False Whether to remove type “Unknown”

Any parameters that function ‘makePlotOfNewMarkers’ can accept

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.extractNewMarkerGenes()

classmethod calculateV (*args*)

Calculate the voting scores (celltypes by clusters)

Parameters:

args: tuple Tuple of sub-arguments

df_M: pandas.DataFrame Marker cell type DataFrame

df_X: pandas.DataFrame Markers expression DataFrame

cluster_index: 1d numpy.array Clustering index

cutoff: float Significance cutoff, i.e. a threshold for a given marker to be significant

giveSignificant: boolean Whether to return the significance matrix along with the scores

removeLowQCScores: boolean Whether to remove low quality scores, i.e. those with less than 10% of markers that are supporting

Returns:

pandas.DataFrame Contains voting scores per celltype per cluster

Usage: Function is used internally.

df = calculateV((df_M, df_X, cluster_index, 0.3, False, True))

annotateWith_pDCS_Scheme (*df_markers_expr, df_marker_cell_type*)

Produce cluster annotation results

Parameters:

df_markers_expr: pandas.DataFrame Data with marker genes by cells expression

df_marker_cell_type: pandas.DataFrame Data with marker genes by cell types

Returns: tuple

Usage: Function should be called internally only

annotateWith_ratio_Scheme (*df_markers_expr, df_marker_cell_type*)

Produce cluster annotation results

Parameters:

df_markers_expr: `pandas.DataFrame` Data with marker genes by cells expression

df_marker_cell_type: `pandas.DataFrame` Data with marker genes by cell types

Returns: tuple

Usage: Function should be called internally only

annotateWith_Hopfield_Scheme (*df_markers_expr, df_marker_cell_type*)

Produce cluster annotation results

Parameters:

df_markers_expr: `pandas.DataFrame` Markers expression DataFrame

df_marker_cell_type: `pandas.DataFrame` Marker cell type DataFrame

Returns: tuple

Usage: Function should be called internally only

recordAnnotationResults (*df_marker_cell_type, df_markers_expr, df_L, df_V, dict_expressed_markers, df_null_distributions=None*)

Record cell type annotation results to spreadsheets.

Parameters:

df_marker_cell_type: `pandas.DataFrame` Markers to cell types table

df_markers_expr: `pandas.DataFrame` Markers expression in each cluster

df_L: `pandas.DataFrame` Annotation scores along with other information

df_V: `pandas.DataFrame` Annotation scores along with other information

dict_expressed_markers: `dictionary` Dictionary of markers significantly expressed in each cluster

df_null_distributions: `pandas.DataFrame`, **Default None** Table with null distributions

Returns: None

Usage: This function is intended to be used internally only

propagateHopfield (*sigma=None, xi=None, T=0.2, tmax=200, fractionToUpdate=0.5, mode=4, meshSamplingRate=200, underlyingNetwork=None, typesNames=None, clustersNames=None, printInfo=False, recordTrajectories=True, id=None, printSwitchingFraction=False, path=None, verbose=0*)

Function is used internally to propagate Hopfield network over a set number of time steps

Parameters:

sigma: `pandas.DataFrame`, **Default None** Markers expression

xi: `pandas.DataFrame`, **Default None** Marker cell type DataFrame

T: `float`, **Default 0.2** Noise (Temperature) parameter

tmax: `int`, **Default 200** Number of step to iterate through

fractionToUpdate: `float`, **Default 0.5** Fraction of nodes to randomly update at each iteration

mode: `int`, **Default 4**

Options are: 1: non-orthogonalized, non-weighted attractors 2: orthogonalized, non-weighted attractors 3: orthogonalized, weighted attractors 4: orthogonalized, weighted attractors, asymmetric and diluted dynamics

meshSamplingRate: int, Default 100 Visualization parameter to control the quality of the color mesh near the attractors

underlyingNetwork: 2d numpy.array, Default None Network of underlying connections between genes

typesNames: list-like, Default None Names of cell types

clustersNames: list-like, Default None Names or identifiers of the clusters

printInfo: boolean, Default False Whether to print details

recordTrajectories: boolean, Default True Whether to record trajectories data to files

id: int, Default None Identifier of this function call

printSwitchingFraction: boolean, Default False Whether to print fraction of clusters that switch their maximum overlapping attractor

path: str, Default None Path for saving trajectories data

Returns:

2d numpy.array Overlaps

Usage: `result = propagateHopfield(sigma=sigma, xi=df_attr)`

classmethod `convertColormap` (*colormap*)

Convert colormap from the form (1.,1.,1.,1.) to 'rgba(255,255,255,1.)'

Parameters:

colormap: dictionary Colormap to convert

Returns:

dictionary Converted colormap

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`colormap = DCS.convertColormap(colormap)`

classmethod `zScoreOfSeries` (*se*)

Calculate z-score of pandas.Series and modify the Series in place

Parameters:

se: pandas.Series Series to process

Returns:

pandas.Series Processed series

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`se = DCS.zScoreOfSeries(se)`

classmethod `KeyInFile` (*key, file*)

Check if a key exists in a HDF file.

Parameters:

key: str Key name to check

file: str HDF file name to check

Returns:

boolean True if the key is found False otherwise

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.KeyInFile('df_expr', 'data/file.h5')

getSubnetworkOfPCN (*subnetworkGenes, min_shared_first_targets=30*)

Extract subnetwork of PCN network

Parameters:

subnetworkGenes: list-like Set of genes that the subnetwork should contain

min_shared_first_targets: int, Default 30 Number of minimum first shared targets to connect two nodes

Returns:

pandas.DataFrame Adjacency matrix

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

df_subnetwork = DCS.getSubnetworkOfPCN(genes)

alignSeries (*se1, se2, tagForMissing*)

Align two pandas.Series

Parameters:

se1: pandas.Series Series with the first set of items

se2: pandas.Series Series with the second set of items

tagForMissing: str, Default 'Missing' Label to assign to non-overlapping items

Returns:

pandas.DataFrame Contains two aligned pandas.Series

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

df = DCS.alignSeries(pd.Index(['A', 'B', 'C', 'D']).to_series(), pd.Index(['B', 'C', 'D', 'E', 'F']).to_series())

createReverseDictionary (*inputDictionary*)

Efficient way to create a reverse dictionary from a dictionary. Utilizes Pandas.DataFrame.groupby and Numpy arrays indexing.

Parameters:

inputDictionary: dictionary Dictionary to reverse

Returns:

dictionary Reversed dictionary

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

revDict = DCS.createReverseDictionary(Dict)

readMarkerFile (*mergeFunction='mean', mergeCutoff=0.25*)

Read markers file, prepare markers

Parameters:

mergeCutoff: str, Default 'mean'

Function used for grouping of the cell sub-types. Options are: 'mean': average of the values
'max': maximum of the values, effectively a logical OR function

mergeCutoff: float, Default 0.25 Values below cutoff are set to zero. This option is used if merge-Cutoff is 'mean'

Returns:

pandas.DataFrame Celltype/markers matrix

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

df_marker_cell_type = DCS.readMarkerFile()

mergeIndexDuplicates (*df_expr, method='average', printDuplicates=False, verbose=1*)

Merge index duplicates

Parameters:

df_expr: pandas.DataFrame Gene expression table

method: str, Default None

How to deal with index duplicates. Option are: 'average': average values of duplicates

'first': keep only first of duplicates, discard rest

Returns:

pandas.DataFrame Gene expression table

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

df_expr = DCS.mergeIndexDuplicates(df_expr)

recordExpressionData ()

Record expression data from the internal HDF storage.

Parameters: None

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.recordExpressionData()

loadAnnotatedLabels (*detailed=False, includeLowQC=True, infoType='label'*)

Load cell annotations resulted from function 'annotate'

Parameters:

detailed: boolean, Default False Whether to give cluster- or celltype- resolution data

includeLowQC: boolean, Default False Whether to include low quality cells in the output

Returns: pandas.Series

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.loadAnnotatedLabels()

loadExpressionData ()

Load processed expression data from the internal HDF storage.

Parameters: None

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.loadExpressionData()

prepareMarkers (*expressedGenes=None, createColormapForCelltypes=True*)

Get dictionary of markers for each cell types.

Parameters:

expressedGenes: pandas.Index, Default None If not None then the marker DataFrame will be intersected with this index, i.e. all non-expressed genes will be filtered from the marker file

createColormapForCelltypes: boolean, Default True Create (or update) a colormap for cell types based on a marker-celltype matrix. This will make coloring of cell clusters consistent across all plots.

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.prepareMarkers()

calculateQCmeasures ()

Calculate Quality Control (QC) measures

Parameters: None

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.calculateQCmeasures()

qualityControl (***kwargs*)

Remove low quality cells

Parameters: None

Returns: Any parameters that function 'getIndexOfGoodQualityCells' can accept

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.qualityControl()

batchEffectCorrection (*method='COMBAT'*)

Batch effect correction.

Parameters:

method: str, Default 'COMBAT' Stein, C.K., Qu, P., Epstein, J. et al. Removing batch effects from purified plasma cell gene expression microarrays with modified ComBat. BMC Bioinformatics 16, 63 (2015)

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.batchEffectCorrection()

VISUALIZATION FUNCTIONS API

The package contains a set of visualization functions that are used at the stage of post-processing.

Note: These functions are not intended to be accessed directly by a user. See **User Functions** for the list of visualization tools that utilize visualization functions detailed here.

Submodule VisualizationFunctions

5.1 Cell type markers pie plot

This visualization function can be launched from *class DigitalCellSorter* at the stage of pre- and post-processing.

From submodule *VisualizationFunctions*:

```
class VisualizationFunctions (dataName='dataName', saveDir="", matplotlibMode='Agg', safe-  
                                Plotting=True, verbose=1)
```

Class of visualization functions for DigitalCellSorter

```
makeCellMarkersPiePlot (*args, **kwargs)
```

Make summary of markers comparison between two cell types.

Parameters:

type1: str Name of the first cell type to compare

type2: str Name of the second cell type to compare

df_marker_cell_type: pandas.DataFrame or str, Default 'all' Celltypes/Markers matrix. If 'expressed', then only expressed markers will be used. If 'all' then all markers of the input marker list will be used. If an instance of a pandas.DataFrame is passed, then its all markers will be used.

nameToAppend: str, Default None String to append to the figure file name.

listUnexpressedMarkers: boolean, Default True List (highlight) markers that are not expressed. This option is ignored unless df_marker_cell_type=='all'

orthogonalSectorsShift: float, Default 0.1 Sectors marked as '+/-' and '-/+' are shifted off-center. Set this parameter to zero to have round continuous pie chart.

rotationAngle: int or float, Default 0 Angle in degrees that will rotate the whole pie chart counter-clockwise.

dpi: int, Default 600 Resolution of the figure image

extension: str, Default 'png' Format of the figure file

rightShift: float, Default 0.3 Fraction of space to leave on the right-hand side of the plot. This parameter is useful for adjusting legend overlap with data points.

dpi: int, Default 600 Resolution of the figure image

extension: str, Default 'png' Format of the figure file

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.makeProjectionPlot(projection, cellClusterIndexLabel, suffix)

Example output:

5.3 Marker subplots

This visualization function can be launched from *class DigitalCellSorter* at the stage of post-processing.

From submodule *VisualizationFunctions*:

```
class VisualizationFunctions (dataName='dataName', saveDir="", matplotlibMode='Agg', safe-  
                               Plotting=True, verbose=1)
```

Class of visualization functions for DigitalCellSorter

```
internalMakeMarkerSubplots (*args, **kwargs)
```

Produce subplots on each marker and its expression on all clusters

Parameters:

df: pandas.DataFrame Data with marker genes expression

X_projection: 2d numpy.array 2D coordinates for each cell

hugo_cd_dict: dictionary With aliases for hugo names of genes

NoFrameOnFigures: boolean, Default False Whether to include frame on the figure

HideClusterLabels: boolean, Default False Whether to print cluster labels on the figure

outlineClusters: boolean, Default True Whether to outline the clusters with circles

analyzeBy: str, Default 'cluster' What level of labels to include. Other possible option is 'label'

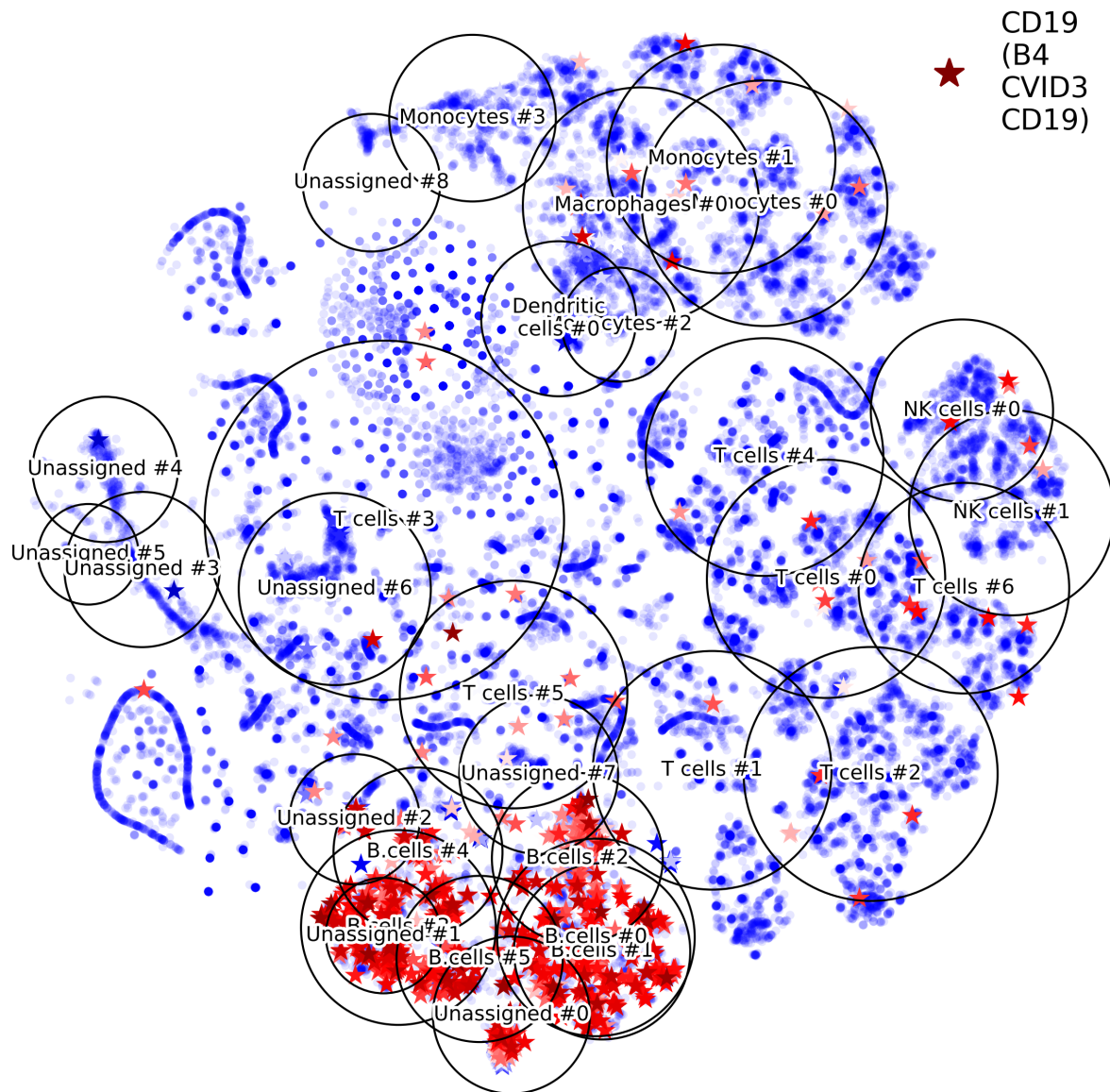
Returns: None

Usage: Function used internally

DCS = DigitalCellSorter.DigitalCellSorter()

DCS.internalMakeMarkerSubplots(df_markers_expr, projection, hugo_cd_dict)

Example output:



5.4 Quality control histogram plot

This visualization function can be launched from *class DigitalCellSorter* at the stage of post-processing.

From submodule *VisualizationFunctions*:

```
class VisualizationFunctions (dataName='dataName', saveDir='', matplotlibMode='Agg', safe-
                               Plotting=True, verbose=1)
```

Class of visualization functions for DigitalCellSorter

```
makeQualityControlHistogramPlot (*args, **kwargs)
```

Function to calculate QC quality cutoff and visualize it on a histogram

Parameters:

subset: `pandas.Series` Data to analyze

cutoff: `float` Cutoff to display

plotPathAndName: `str`, **Default** `None` Text to include in the figure title and file name

N_bins: `int`, **Default** `100` Number of bins of the histogram

mito: `boolean`, **Default** `False` Whether the analysis of mitochondrial genes fraction

displayMeasures: `boolean`, **Default** `True` Print vertical dashed lines along with mean, median, and standard deviation

precision: `int`, **Default** `4` Number of digits after decimal

quantilePlotCutoff: `float`, **Default** `0.99` Distributions are cut to display the range from 0 to quantile-PlotCutoff

dpi: `int`, **Default** `600` Resolution of the figure image

extension: `str`, **Default** `'png'` Format of the figure file

fontScale: `float`, **Default** `1.5` Scale most of the figure fonts

includeTitle: `boolean`, **Default** `False` Whether to include title on the figure

Returns: `None`

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`cutoff = DCS.makeQualityControlHistogramPlot(subset, cutoff)`

Example output:

5.5 Histogram null distribution plot

This visualization function can be launched from *class* `DigitalCellSorter` at the stage of post-processing.

From submodule `VisualizationFunctions`:

```
class VisualizationFunctions (dataName='dataName', saveDir="", matplotlibMode='Agg', safe-Plotting=True, verbose=1)
```

Class of visualization functions for `DigitalCellSorter`

```
makeHistogramNullDistributionPlot (*args, **kwargs)
```

Produce histogram plot of the voting null distributions

Parameters:

dpi: `int`, **Default** `600` Resolution of the figure image

extension: `str`, **Default** `'png'` Format of the figure file

Returns: `None`

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.makeHistogramNullDistributionPlot()`

Example output:

5.6 Sankey diagram

This visualization function can be launched from *class DigitalCellSorter* at the stage of post-processing.

From submodule *VisualizationFunctions*:

```
class VisualizationFunctions (dataName='dataName', saveDir="", matplotlibMode='Agg', safe-  
                               Plotting=True, verbose=1)
```

Class of visualization functions for DigitalCellSorter

```
makeSankeyDiagram (*args, **kwargs)
```

Make a Sankey diagram, also known as ‘river plot’ with two groups of nodes

Parameters:

df: pandas.DataFrame With counts (overlaps)

colormapForIndex: dictionary, Default None Colors to use for nodes specified in the DataFrame index

colormapForColumns: dictionary, Default None Colors to use for nodes specified in the DataFrame columns

linksColor: str, Default ‘rgba(100,100,100,0.6)’ Color of the non-overlapping links

title: str, Default ‘’ Title to print on the diagram

interactive: boolean , Default False Whether to launch interactive JavaScript-based graph

quality: int, Default 4 Proportional to the resolution of the figure to save

nodeLabelsFontSize: int, Default 15 Font size for node labels

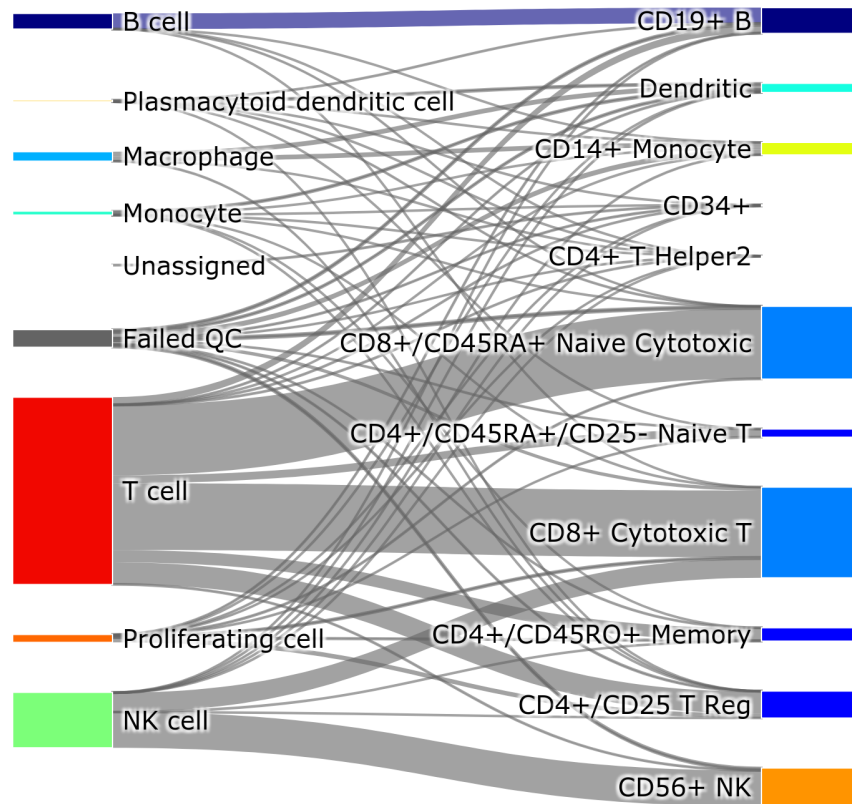
nameAppend: str, Default ‘_Sankey_diagram’ Name to append to the figure file

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.makeSankeyDiagram(df)

Example output:



5.7 Stacked bar plot

This visualization function can be launched from *class DigitalCellSorter* at the stage of post-processing.

From submodule *VisualizationFunctions*:

```
class VisualizationFunctions (dataName='dataName', saveDir="", matplotlibMode='Agg', safe-
                               Plotting=True, verbose=1)
```

Class of visualization functions for DigitalCellSorter

```
makeStackedBarplot (*args, **kwargs)
```

Produce stacked barplot with cell fractions

Parameters:

clusterName: str, Default None Label to include at the bar bottom. If None the self.dataName value will be used

legendStyle: boolean, Default False Use one out of two styles of this figure

includeLowQC: boolean, Default True Whether to include low quality cells

dpi: int, Default 600 Resolution of the figure image

extension: str, Default 'png' Format of the figure file

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.makeStackedBarplot(clusterName)

Example output:

5.8 Annotation Results Matrix plot

This visualization function can be launched from *class DigitalCellSorter* at the stage of post-processing.

From submodule *VisualizationFunctions*:

class VisualizationFunctions (*dataName='dataName', saveDir='', matplotlibMode='Agg', safe-Plotting=True, verbose=1*)

Class of visualization functions for DigitalCellSorter

makeAnnotationResultsMatrixPlot (**args, **kwargs*)

Produce voting results voting matrix plot

Parameters:

dpi: int, Default 600 Resolution of the figure image

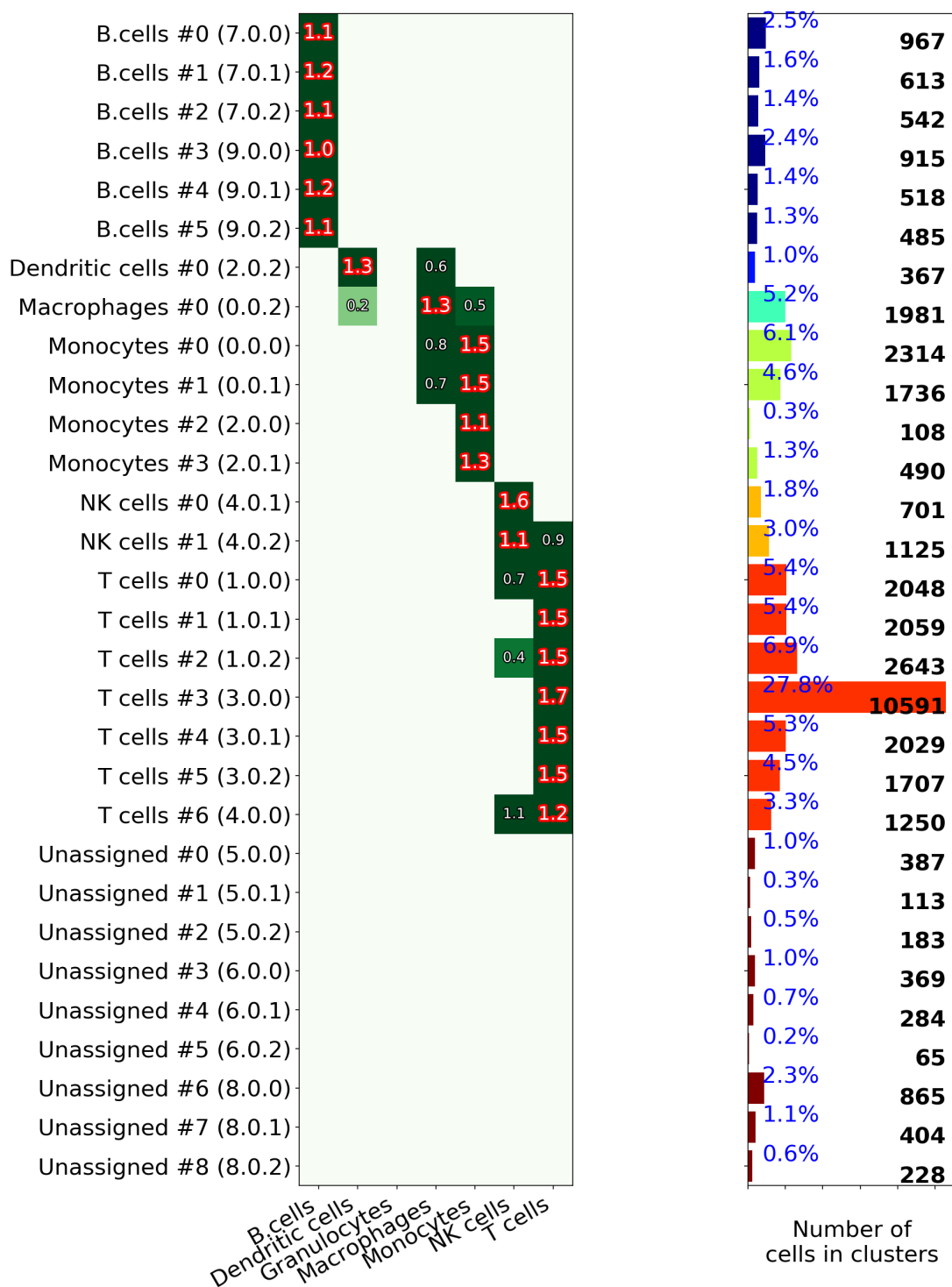
extension: str, Default 'png' Format of the figure file

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()

DCS.makeAnnotationResultsMatrixPlot()

Example output:



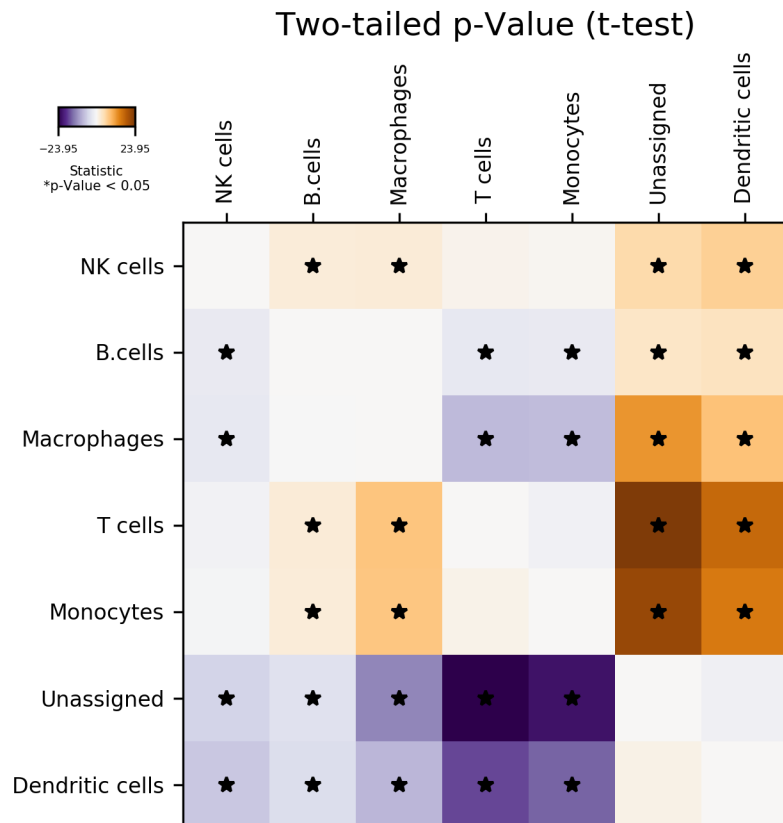
p_value_cutoff: float, Default 0.05 p-Value cutoff
dpi: int, Default 600 Resolution of the figure image
extension: str, Default 'png' Format of the figure file

Returns: None

Usage: DCS = DigitalCellSorter.DigitalCellSorter()
 DCS.makeTtestPlot(df)

Example output:

CD4
 (CD4
 CD4mut)



5.11 Plot of new markers

This visualization function can be launched from *class DigitalCellSorter* at the stage of post-processing.

From submodule *VisualizationFunctions*:

class VisualizationFunctions (*dataName='dataName', saveDir='', matplotlibMode='Agg', safe-Plotting=True, verbose=1*)

Class of visualization functions for DigitalCellSorter

makePlotOfNewMarkers (**args, **kwargs*)

Produce plot of the new markers extracted from the annotated clusters

Parameters:

df_marker_cell_type: `pandas.DataFrame` Known markers per cell types

df_new_marker_cell_type: `pandas.DataFrame` New markers per cell types

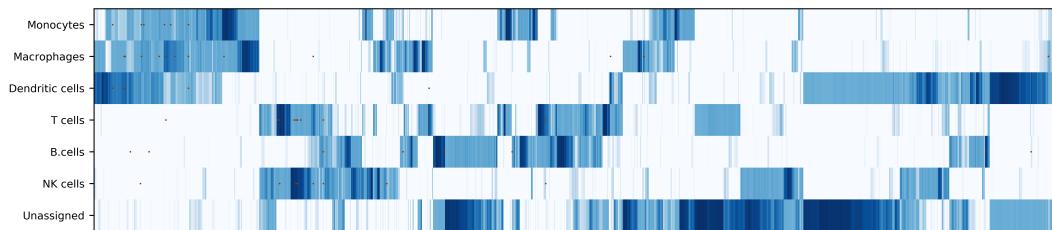
dpi: `int`, **Default 600** Resolution of the figure image

extension: `str`, **Default 'png'** Format of the figure file

Returns: `None`

Usage: `DCS = DigitalCellSorter.DigitalCellSorter()`

`DCS.makePlotOfNewMarkers(df_marker_cell_type, df_new_marker_cell_type)`

Example output:

5.12 Customized violin plot

This visualization function can be launched from *class DigitalCellSorter*. By default the function also exports a summary of the data.

From submodule *VisualizationFunctions*:

class VisualizationFunctions (*dataName='dataName', saveDir='', matplotlibMode='Agg', safe-Plotting=True, verbose=1*)

Class of visualization functions for DigitalCellSorter

makeViolinPlot (**args, **kwargs*)

Exploratory analysis of the numeric values distributions using matplotlib violinplot.

Parameters:

df_sel: `pandas.DataFrame`

Table where rows are unique object identifiers, columns are [`dimPanels`, `dimCategories`, `gene1`, `gene2`, ...]
Numeric columns should be without any missing values

genes: `list` List of genes names to plot, these should be a (sub)set of the `df_sel` columns

dimPanels: `str` Name of the categorical variable is for separation into panels. Option 'All' can be used too

dimCategories: `str` Name of the categorical variable is for separation into categories within a panel. Option 'All' can be used too

panelWidth: `float`, **Default 5** Width of a panel, including the tick labels

panelHeight: `float`, **Default 5** Height of a panel, including the tick labels

title: `str`, **Default '{name} {gene}'** Template for panel names

exportData: float, Default **True** Whether to export data summary into an excel file

xlabel: str, Default **“\$log(count+1)\$”** x-axis label

ylabel: str, Default **“ ”** y-axis label

addPoints: boolean, Default **True** Whether to include scattered points on violins

linesColor: str, Default **‘black’** Line color

linesWidth: float, Default **1.0** Line width

cmap: matplotlib.colormap or callable, Default **cm.jet** Colormap or its string name

fontSize: float, Default **10** Size of labels font

showMedians: boolean, Default **True** Whether to display median

showExtrema: boolean, Default **True** Whether to display max and min

excludeZeroValues: boolean, Default **False** If True then zeros and missing values are not used in calculation of the probability densities

violinWidths: float, Default **0.85** Relative violin widths

violinAlpha: float, Default **0.7** Transparency of the violins

pointsColor: str, Default **‘black’** Color of the points

pointsSize: float, Default **1.0** Size of the points

pointsAlpha: float, Default **0.7** Transparency of the points

pointsPushBack: boolean, Default **True** If False then points will be drawn in front of all other objects

sharex: boolean, Default **True** Whether to share x-axis

sharey: boolean, Default **True** Whether to share y-axis

dpi: float, Default **300** Resolution of the figure

extension: str, Default **‘png’** Format extension of the figure

Returns: None

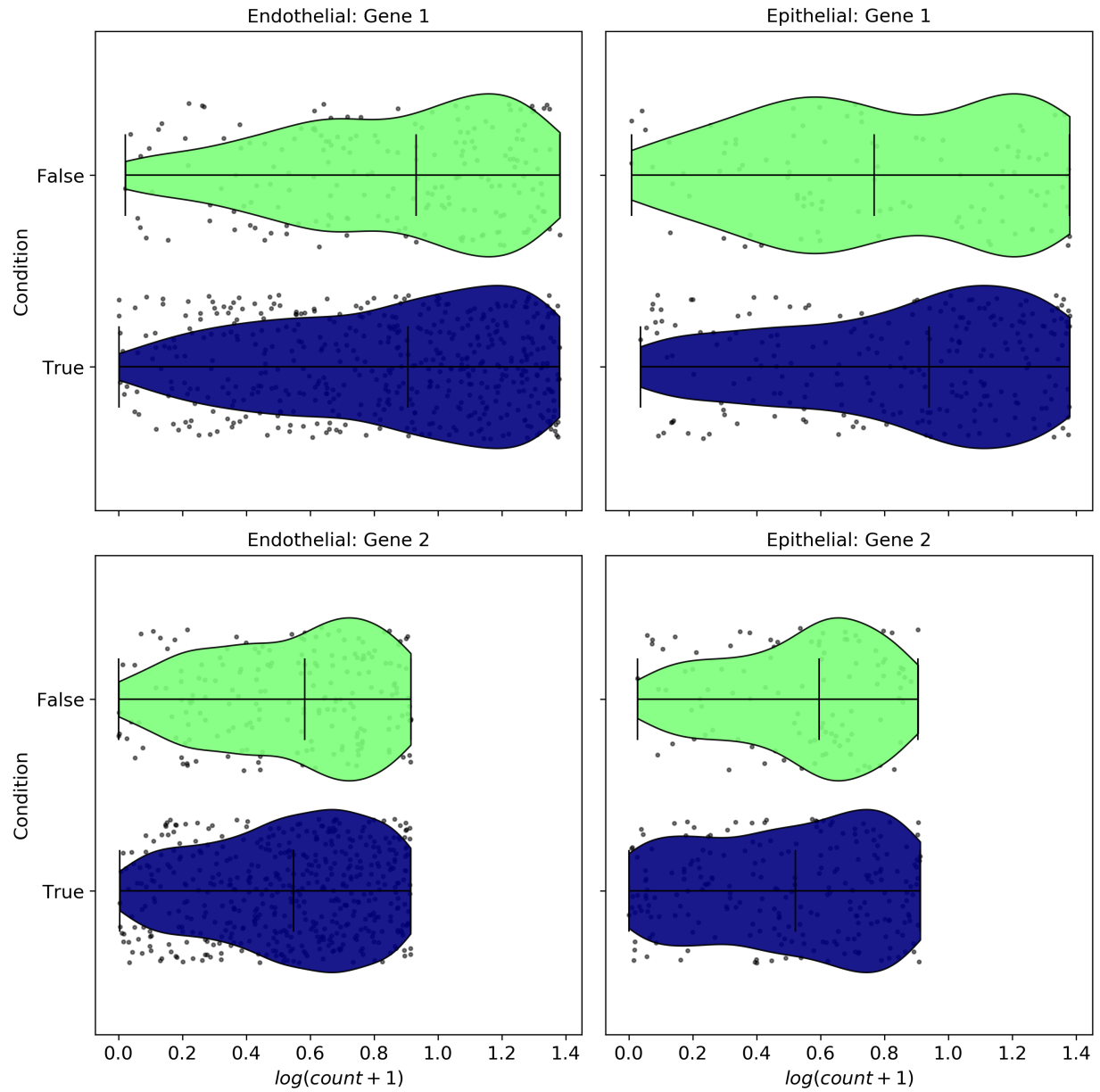
Usage: DCS.makeViolinPlot(data, [‘Numeric 1’, ‘Numeric 2’], dimPanels=‘Property A’, dimCategories=‘Property B’)

Example with synthetic data:

```
data = pd.DataFrame({'Celltype': np.where((np.random.rand(1000)>0.7), 'Epithelial',
↳ 'Endothelial'),
                    'Condition': np.random.rand(1000)>0.3,
                    'Gene 1': np.log(np.random.rand(1000)*3 + 1),
                    'Gene 2': np.log(np.random.rand(1000)*1.5 + 1)})

DCS = DigitalCellSorter.DigitalCellSorter()
DCS.makeViolinPlot(data, ['Gene 1', 'Gene 2'],
                    dimPanels='Celltype', dimCategories='Condition',
                    title='{name}: {gene}', ylabel='Condition',
                    pointsSize=5, fontsize=12)
```

Example output:



GENERIC FUNCTIONS

A set of generic tools grouped here are used by the Class of DigitalCellSorter.

Submodule GenericFunctions

General functions for convenience of use

Functions:

<code>extractFromZipOfGz(filepath[, ...])</code>	
<code>getElapsedTime(start)</code>	Print total elapsed time (in minutes) elapsed from the reference point
<code>getStartTime()</code>	Get time (in seconds) elapsed from the epoch
<code>read(fileName[, compressed, jsonFormat])</code>	Unpickle object from a (binary) file
<code>timeMark()</code>	Print total time elapsed from the beginning of the process from which the function is called
<code>write(data, fileName[, compressed, jsonFormat])</code>	Pickle object into a (binary) file

write (*data, fileName, compressed=False, jsonFormat=False*)

Pickle object into a (binary) file

Parameters: data: any Python object, e.g. list, dictionary, file, method, variable, etc. fileName: path and name of the file to store binary data in

Returns: None

Usage: data = [['A', 'B', 'C'], pd.DataFrame()] write(data, os.path.join('some dir 1', 'some dir 2', 'File with my data'))

read (*fileName, compressed=False, jsonFormat=False*)

Unpickle object from a (binary) file

Parameters: fileName: path and name of the file with binary data stored in

Returns: Data stored in the provided file

Usage: read(os.path.join('some dir 1', 'some dir 2', 'File with my data'))

timeMark ()

Print total time elapsed from the beginning of the process from which the function is called

Parameters: None

Returns: None

Usage: timeMark()

getStartTime ()

Get time (in seconds) elapsed from the epoch

Parameters: None

Returns: Time (in seconds)

Usage: start = getStartTime()

getElapsedTime (*start*)

Print total elapsed time (in minutes) elapsed from the reference point

Parameters:

start: float or int Reference time (in seconds)

Returns: None

Usage: getElapsedTime(start)

extractFromZipOfGz (*filepath, removeDownloadedZipFile=False*)

DEPENDENCIES

This graph was generated with **Python** module dependency visualization tool `pydeps`, see [GitHub](#), by running the following (after installation of the necessary components):

```
pydeps DigitalCellSorter --reverse --max-bacon=2 --cluster --max-cluster-size=6 --min-  
↪cluster-size=2 -T=png -o=docs/DigitalCellSorter_pydeps_current.png
```


DATA PREPARATION

8.1 Output from kallisto-bustools (kp-python)

In this example we use raw sequencing data stored in `.fastq` format, from 1000 PBMC, the data can be accessed at https://support.10xgenomics.com/single-cell-gene-expression/datasets/3.0.0/pbmc_1k_v3

Note: This is by no means a tutorial for processing scRNA-seq data. We only demonstrate the workflow of connecting upstream analysis software and DCS.

Download the data and unpack the `.tar` file (~5.17 GB):

```
wget https://cf.10xgenomics.com/samples/cell-exp/3.0.0/pbmc_1k_v3/pbmc_1k_v3_fastqs.  
→tar  
tar -xvf pbmc_1k_v3_fastqs.tar
```

To process sequencing data one could use `kallisto bus` tool to generate BUS file following by `bustools count` to generate count matrices from a BUS file. However, we prefer to use `kb-python`, a package that wraps the `kallisto` and `bustools` single-cell RNA-seq workflow (Bray, N. L., Pimentel, H., Melsted, P., & Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nature biotechnology*, 34(5), 525) `kb-python` can be installed with `pip`.

```
kb count -i kallisto_index/homo_sapiens/transcriptome.idx \  
-g kallisto_index/homo_sapiens/transcripts_to_genes.txt \  
-x 10xv3 \  
--filter \  
-t 4 \  
pbmc_1k_v3_fastqs/pbmc_1k_v3_S1_L001_R1_001.fastq.gz \  
pbmc_1k_v3_fastqs/pbmc_1k_v3_S1_L001_R2_001.fastq.gz \  
pbmc_1k_v3_fastqs/pbmc_1k_v3_S1_L002_R1_001.fastq.gz \  
pbmc_1k_v3_fastqs/pbmc_1k_v3_S1_L002_R2_001.fastq.gz
```

Output from kb count command above [2020-11-20 14:32:51,136] INFO Using index `kallisto_index/homo_sapiens/transcriptome.idx` to generate BUS file to `.` from

[2020-11-20 14:32:51,136] INFO `pbmc_1k_v3_fastqs/pbmc_1k_v3_S1_L001_R1_001.fastq.gz`

[2020-11-20 14:32:51,136] INFO `pbmc_1k_v3_fastqs/pbmc_1k_v3_S1_L001_R2_001.fastq.gz`

[2020-11-20 14:32:51,136] INFO `pbmc_1k_v3_fastqs/pbmc_1k_v3_S1_L002_R1_001.fastq.gz`

[2020-11-20 14:32:51,136] INFO `pbmc_1k_v3_fastqs/pbmc_1k_v3_S1_L002_R2_001.fastq.gz`

[2020-11-20 14:36:33,477] INFO Sorting BUS file `./output.bus` to `./tmp/output.s.bus`

```
[2020-11-20 14:36:57,118] INFO Whitelist not provided
[2020-11-20 14:36:57,118] INFO Copying pre-packaged 10XV3 whitelist to .
[2020-11-20 14:36:57,675] INFO Inspecting BUS file ./tmp/output.s.bus
[2020-11-20 14:37:07,641] INFO Correcting BUS records in ./tmp/output.s.bus to ./tmp/output.s.c.bus with whitelist
./10xv3_whitelist.txt
[2020-11-20 14:37:29,264] INFO Sorting BUS file ./tmp/output.s.c.bus to ./output.unfiltered.bus
[2020-11-20 14:37:47,478] INFO Generating count matrix ./counts_unfiltered/cells_x_genes from BUS file ./out-
put.unfiltered.bus
[2020-11-20 14:37:59,662] INFO Filtering with bustools
[2020-11-20 14:37:59,662] INFO Generating whitelist ./filter_barcodes.txt from BUS file ./output.unfiltered.bus
[2020-11-20 14:37:59,790] INFO Correcting BUS records in ./output.unfiltered.bus to ./tmp/output.unfiltered.c.bus
with whitelist ./filter_barcodes.txt
[2020-11-20 14:38:14,344] INFO Sorting BUS file ./tmp/output.unfiltered.c.bus to ./output.filtered.bus
[2020-11-20 14:38:30,918] INFO Generating count matrix ./counts_filtered/cells_x_genes from BUS file ./out-
put.filtered.bus
```

The output directory that we are interested in is `counts_filtered/`. Rename it:

```
mv counts_filtered/ kb_1k_PBMC_output/
```

This will generate counts data in the directory `kb_1k_PBMC_output/`.

8.2 Output from CellRanger

Here we use CellRanger-processed data stored in `.mtx` format, from 1000 PBMC, the data can be accessed at https://support.10xgenomics.com/single-cell-gene-expression/datasets/3.0.0/pbmc_1k_v3

Download the data and unpack the `.tar.gz` file (~9 MB):

```
wget https://cf.10xgenomics.com/samples/cell-exp/3.0.0/pbmc_1k_v3/pbmc_1k_v3_filtered_
↪feature_bc_matrix.tar.gz
tar -xzf pbmc_1k_v3_filtered_feature_bc_matrix.tar.gz && mv filtered_feature_bc_
↪matrix/ cellranger_1k_PBMC_output/
```

These two commands will prepare the processed counts data in the directory `cellranger_1k_PBMC_output/`.

8.3 Import from kallisto-bustools (kp-python)

```
import DigitalCellSorter
from DigitalCellSorter.core import readMTXdata

# Read the MTX data
df = readMTXdata(dataDir='kb_1k_PBMC_output/', origin='kb-python')

# (Optional) Convert gene names to HUGO
DCS = DigitalCellSorter.DigitalCellSorter()
DCS.prepare(df)
DCS.convert('ensembl', 'hugo')

# Check the DCS data
print(DCS.df_expr)
```

8.4 Import from CellRanger

```
import DigitalCellSorter
from DigitalCellSorter.core import readMTXdata

# Read the MTX data
df = readMTXdata(dataDir='cellranger_1k_PBMC_output/', origin='cellranger')

# (Optional) Convert gene names to HUGO
DCS = DigitalCellSorter.DigitalCellSorter()
DCS.prepare(df)
DCS.convert('ensembl', 'hugo')

# Check the DCS data
print(DCS.df_expr)
```

8.5 Function readMTXdata

Function to read data in MTX format (see usage examples above).

readMTXdata (*dataDir*, *origin*, *fileMatrix=None*, *fileBarcodes=None*, *fileGenes=None*, *headerRows=None*, *sampleName=None*, *stripGeneVersions=True*, *saveData=True*, *dropGeneDuplicates=True*, *dropCellDuplicates=True*)

Read MTX format into pandas DataFrame compatible with DCS input format

Parameters

dataDir: str Path to gene expression counts data

origin: str

Name of the software where the data was generated. Supported options are: 'kb-python' for kallisto-bustools 'cellranger' for cellRanger

fileMatrix: str, Default None Name of the matrix file

fileBarcodes: str, Default None Name of the cell barcodes file

fileGenes: str, Default None Name of the genes file

headerRows: list, Default None List of rows in matrix file to skip

sampleName: str, Default None Name of the data sample to include in the batch level

stripGeneVersions: boolean, Default True Remove ensembl gene version. E.g.
“ENSG00000236246.1” -> “ENSG00000236246”

saveData: boolean, Default True Whether to save data in hdf format. If True then the data is saved to a compressed hdf at the same location as matrix data

dropGeneDuplicates: boolean, Default True Whether to remove gene duplicates (keep first)

dropBarcodeDuplicates: boolean, Default True Whether to remove barcode duplicates (keep first)

Returns:

pandas.DataFrame Table that has genes in rows and cells in columns

Usage: df = readMTX(dataDir='filtered_feature_bc_matrix/', origin='cellranger') #df =
readMTX(dataDir='counts_filtered/', origin='kb-python')

DCS = DigitalCellSorter.DigitalCellSorter() DCS.prepare(df) DCS.convert('ensembl', 'hugo')
print(DCS.df_expr)

8.6 Human Cell Atlas tools

Set of generic tools for retrieving, loading, and preparation of [Human Cell Atlas \(HCA\)](#) datasets is contained in this module.

Example:

```
import os
import DigitalCellSorter.ReadPrepareDataHCA as prep

# Example URL of a relatively small dataset of scRNA-seq of human pancreas
url = "https://data.humancellatlas.org/project-assets/project-matrices/cddab57b-6868-
↳4be4-806f-395ed9dd635a.homo_sapiens.mtx.zip"

# Path of directories where the data will be placed
extractPath = os.path.join(os.path.join(os.path.dirname(__file__), ''), 'data', os.
↳path.splitext(os.path.basename(url))[0])

# Download data and unpack it to a specified directory
prep.getHCAdataByURL(url, extractPath)

# Record *.h5 files of individual donor IDs
IDs = prep.recordFilesOfIndividualDonors(extractPath, organName='islet of Langerhans')

# Load ready-to-use dataset of the first donor ID
df = prep.getDataframeByDonorID(extractPath, IDs[0])

# Print the shape of just loaded dataset
print(df.shape)
```

Submodule ReadPrepareDataHCA

Functions:

<code>PrepareDataOnePatient_PREVIEW_DATASET(...)</code>	Prepare data from Human Cell Atlas (HCA) preview dataset h5 data file.
<code>getDataframeByDonorID(extractPath, donorID)</code>	Get pandas.DataFrame by Donor ID
<code>getHCadataByURL(url, extractPath[, extractData])</code>	Download and extract data from Human Cell Atlas Portal
<code>prepareDemo5kData(dir)</code>	
<code>recordFilesOfIndividualDonors(extractPath[, ...])</code>	Record h5 files of HCA individual donors in a dataset

getHCadataByURL (*url, extractPath, extractData=True*)

Download and extract data from Human Cell Atlas Portal

Parameters:

url: **str** URL of the data of interest

extractPath: **str** Path where to save and extract data to

extractData: **boolean, Default True** Whether to extract downloaded data

Returns: None

Usage: getHCadataByURL(url, extractPath)

recordFilesOfIndividualDonors (*extractPath, organName=None, donorIDcolumn='donor_organism.provenance.document_id', organColumn='derived_organ_parts_label', useHogoGeneNames=True*)

Record h5 files of HCA individual donors in a dataset

Parameters:

extractPath: **str** Path of directories where HCA matrix files were downloaded and extracted. See function getHCadataByURL() for detail.

organName: **str, Default None** Name of the organ name. E.g. 'pancreas', 'bone marrow', etc.

donorIDcolumn: **str, Default donor_organism.provenance.document_id** Column with unique IDs of donors in the file. Another option is 'specimen_from_organism.provenance.document_id' IDs at samples level is needed.

organColumn: **str, Default 'derived_organ_parts_label'** 'derived_organ_label'

'derived_organ_parts_label' This option is ignored when organName parameter is None.

useHogoGeneNames: **boolean, Default True** Whether to use HUGO gene names.

Returns:

list List of donor IDs

Usage: recordFilesOfIndividualDonors(extractPath, organName='retina')

getDataframeByDonorID (*extractPath, donorID*)

Get pandas.DataFrame by Donor ID

Parameters:

extractPath: **str** Path of directories where HCA matrix files were downloaded and extracted. See function getHCadataByURL() for detail.

donorID: **str** Donor ID.

Returns:

pandas.DataFrame Matrix corresponding to the Donor ID

Usage: `getDataframeByDonorID(extractPath, donorID)`

PrepareDataOnePatient_PREVIEW_DATASET (*filename, patient, saveFolderName, useAllData=True, cellsLimitToUse=1000, randomlySample=True, randomSeed=0*)

Prepare data from Human Cell Atlas (HCA) preview dataset h5 data file. The user can download the file `ica_bone_marrow_h5.h5` from <https://preview.data.humancellatlas.org/> (Raw Counts Matrix - Bone Marrow) and place in folder `data`. The file is ~485Mb and contains all 378000 cells from 8 bone marrow donors (BM1-BM8). Note: this data file is no longer available at HCA data server, however, some users may have a copy of it and need to extract data from it.

Parameters:

filename: **str** Path and name of the file to store binary data in

patient: **str** Identifier of the patient: 'BM1', 'BM2', 'BM3', 'BM4', 'BM5', 'BM6', 'BM7' or 'BM8'

saveFolderName: **str** Path where to save prepared data file

useAllData: **boolean, Default True** Whether to use all data or a subset

cellsLimitToUse: **int, Default 1000** Number of cells to use if `useAllData=False`

randomlySample: **boolean, Default True** Whether to sample cell randomly or pick top number

randomSeed: **int, Default 0** Random seed

Returns: None

Usage: `PrepareDataOnePatient(os.path.join('data', 'ica_bone_marrow_h5.h5'), 'BM1', os.path.join('data', ''), useAllData=False, cellsLimitToUse=5000)`

prepareDemo5kData (*dir*)

INPUT DATA FORMAT

Gene Expression Data Format

The input gene expression data is expected in one of the following formats:

1. Spreadsheet of comma-separated values `csv` containing condensed matrix in a form ('cell', 'gene', 'expr'). If there are batches in the data the matrix has to be of the form ('batch', 'cell', 'gene', 'expr'). Columns order can be arbitrary.

cell	gene	expr
C1	G1	3
C1	G2	2
C1	G3	1
C2	G1	1
C2	G4	5
...

or:

batch	cell	gene	expr
batch0	C1	G1	3
batch0	C1	G2	2
batch0	C1	G3	1
batch1	C2	G1	1
batch1	C2	G4	5
...

2. Spreadsheet of comma-separated values `csv` where rows are genes, columns are cells with gene expression counts. If there are batches in the data the spreadsheet the first row should be 'batch' and the second 'cell'.

cell	C1	C2	C3	C4
G1		3	1	7
G2	2	2		2
G3	3	1		5
G4	10		5	4
...

or:

batch	batch0	batch0	batch1	batch1
cell	C1	C2	C3	C4
G1		3	1	7
G2	2	2		2
G3	3	1		5
G4	10		5	4
...

3. Pandas DataFrame where axis 0 is genes and axis 1 are cells. If the are batched in the data then the index of axis 1 should have two levels, e.g. ('batch', 'cell'), with the first level indicating patient, batch or experiment where that cell was sequenced, and the second level containing cell barcodes for identification.

```
df = pd.DataFrame(data=[[2,np.nan],[3,8],[3,5],[np.nan,1]],
                  index=['G1','G2','G3','G4'],
                  columns=pd.MultiIndex.from_arrays(['batch0','batch1'],['C1','C2']),
                  names=['batch', 'cell'])
```

4. Pandas Series where index should have two levels, e.g. ('cell', 'gene'). If there are batched in the data the first level should be indicating patient, batch or experiment where that cell was sequenced, the second level cell barcodes for identification and the third level gene names.

```
se = pd.Series(data=[1,8,3,5,5],
               index=pd.MultiIndex.from_arrays(['batch0','batch0','batch1','batch1',
               ↪ 'batch1'],
               ['C1','C1','C1','C2','C2'],
               ['G1','G2','G3','G1','G4']), names=[
               ↪ 'batch', 'cell', 'gene'])
```

Any of the data types outlined above need to be prepared/validated with a function `prepare()`.

DEMO

The demo can be executed after installation of the DigitalCellSorter. In the terminal run the command, and follow the prompts:

```
python -m DigitalCellSorter
```

The demo script is detailed below:

```
import sys
sys.path.append("..")

import os
import urllib.request
import DigitalCellSorter
import DigitalCellSorter.ReadPrepareDataHCA as prep

if __name__ == '__main__':

    print('This is a large dataset demo.\nFor the "5k PBMC demo" run "python -m_
↳DigitalCellSorter"\n')

    here = os.path.dirname(__file__)

    url = "https://data.humancellatlas.org/project-assets/project-matrices/cc95ff89-
↳2e68-4a08-a234-480eca21ce79.homo_sapiens.mtx.zip"
    extractPath = os.path.join(here, 'data', os.path.splitext(os.path.
↳basename(url))[0])

    ## Download and unpack data
    #prep.getHCadataByURL(url, extractPath)

    # Call function recordFilesOfIndividualDonors to load the data from HCA Data_
↳Portal
    #id = prep.recordFilesOfIndividualDonors(extractPath, organName='bone marrow')[0]
    id = '085e737d-adb5-4597-bd54-5ebeda170038'

    # Get the data. The file will be downloaded from github if not found locally
    try:
        if not os.path.exists(extractPath):
            os.makedirs(extractPath)

        if not os.path.isfile(os.path.join(extractPath, 'dfDonorID %s.h5' % id)):
            print('Downloading 110 Mb data file (50000 cells)')
            temp = 'https://github.com/sdomanskyi/DigitalCellSorter/raw/master/data/
↳dfDonorID %s.h5' % id
```

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```

        urllib.request.urlretrieve(temp.replace(' ', '%20'), os.path.
↪ join(extractPath, 'dfDonorID %s.h5' % id))
    except Exception as exception:
        print('Could not download the file\n', exception)
        exit()

    # Load gene expression data from h5 file
    df_expr = prep.getDataframeByDonorID(extractPath, id)
    df_expr.columns.names = ['batch', 'cell']

    # Create an instance of class DigitalCellSorter.
    # Here we use Default parameter values for most of the parameters
    DCS = DigitalCellSorter.DigitalCellSorter(dataName='BM1', geneNamesType = 'ensembl
↪ ',
                                                saveDir=os.path.join(here, 'output',
↪ 'BM1', ''),
                                                geneListFileName='CIBERSORT_LM22_7')

    # Validate the expression data, so that it has correct form
    DCS.prepare(df_expr)

    # Delete df_expr as now DCS contains the master copy of it
    del df_expr

    # Process the expression data, i.e. quality control, dimensionality reduction,
↪ clustering
    DCS.process()

    # Load marker genes and annotate cells
    DCS.annotate()

    # Make plots of annotated data
    DCS.visualize()

    # Make CD19 gene expression plot
    for name in DCS.getHugoName('CD19'):
        DCS.makeIndividualGeneExpressionPlot(name)

    # Make CD33 gene expression plot
    for name in DCS.getHugoName('CD33'):
        DCS.makeIndividualGeneExpressionPlot(name)

    # Further analysis can be done on cell types of interest, e.g. here 'T cell' and
↪ 'B cell'.
    # Let's create a new instance of DigitalCellSorter to run "sub-analysis" with it.
    # It is important to disable Quality control, because the low quality cells have
    # already been identified and filtered with DCS.
    # Parameter dataName points to the location processed with DCS.
    DCSsub = DigitalCellSorter.DigitalCellSorter(dataName='BM1',
                                                nClusters=10,
                                                doQualityControl=False,
                                                layout='PHATE',
                                                subclusteringName='T cell')

    # Modify a few other attributes
    DCSsub.saveDir = os.path.join(here, 'output', 'BM1', 'subclustering T cell', '')
    DCSsub.geneListFileName = os.path.join(here, 'docs', 'examples', 'CIBERSORT_T_SUB.
↪ xlsx')

```

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```
# Get index of T cells
indexOfTcells = DCS.getCells(celltype='T cell')

# Get expression of these T cells using their index
df_expr = DCS.getExprOfCells(indexOfTcells)

# Insert expression data into DCSSub
DCSSub.prepare(df_expr)

# Process subtype 'T cell'
DCSSub.process(dataIsNormalized=True)

# Load marker genes and annotate cells
DCSSub.annotate()

# Make plots of annotated data
DCSSub.visualize()
```


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