# Package: conos (via r-universe)

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**Title** Clustering on Network of Samples

**Version** 1.5.4

**Description** Wires together large collections of single-cell RNA-seq datasets, which allows for both the identification of recurrent cell clusters and the propagation of information between datasets in multi-sample or atlas-scale collections. 'Conos' focuses on the uniform mapping of homologous cell types across heterogeneous sample collections. For instance, users could investigate a collection of dozens of peripheral blood samples from cancer patients combined with dozens of controls, which perhaps includes samples of a related tissue such as lymph nodes. This package interacts with data available through the 'conosPanel' package, which is available in a 'drat' repository. To access this data package, see the instructions at <https://github.com/kharchenkolab/conos>. The size of the 'conosPanel' package is approximately 12 MB.

**License** GPL-3

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`basicSeuratProc` *Create and preprocess a Seurat object*

---

## Description

Create and preprocess a Seurat object

## Usage

```
basicSeuratProc(
  count.matrix,
  vars.to.regress = NULL,
  verbose = TRUE,
  do.par = TRUE,
  n.pcs = 100,
  cluster = TRUE,
  tsne = TRUE,
  umap = FALSE
)
```

## Arguments

| | |
|---|---|
| `count.matrix` | gene count matrix |
| `vars.to.regress` | variables to regress with Seurat (default=NULL) |
| `verbose` | boolean Verbose mode (default=TRUE) |
| `do.par` | boolean Use parallel processing for regressing out variables faster (default=TRUE) |
| `n.pcs` | numeric Number of principal components (default=100) |
| `cluster` | boolean Whether to perform clustering (default=TRUE) |
| `tsne` | boolean Whether to construct tSNE embedding (default=TRUE) |
| `umap` | boolean Whether to construct UMAP embedding, works only for Seurat v2.3.1 or higher (default=FALSE) |

## Value

Seurat object

`bestClusterThresholds` *Find threshold of cluster detectability*

## Description

For a given clustering, walks the walktrap result tree to find a subtree with max(min(sens,spec)) for each cluster, where sens is sensitivity, spec is specificity

## Usage

```
bestClusterThresholds(res, clusters, clmerges = NULL)
```

## Arguments

| | |
|---|---|
| `res` | walktrap result object (igraph) |
| `clusters` | cluster factor |
| `clmerges` | integer matrix of cluster merges (default=NULL). If NULL, the function treeJaccard() performs calculation without it. |

## Value

a list of $thresholds - per cluster optimal detectability values, and $node - internal node id (merge row) where the optimum was found

`bestClusterTreeThresholds`
*Find threshold of cluster detectability in trees of clusters*

## Description

For a given clustering, walks the walktrap (of clusters) result tree to find a subtree with max(min(sens,spec)) for each cluster, where sens is sensitivity, spec is specificity

## Usage

```
bestClusterTreeThresholds(res, leaf.factor, clusters, clmerges = NULL)
```

## Arguments

| | |
|---|---|
| `res` | walktrap result object (igraph) where the nodes were clusters |
| `leaf.factor` | a named factor describing cell assignments to the leaf nodes (in the same order as res$names) |
| `clusters` | cluster factor |
| `clmerges` | integer matrix of cluster merges (default=NULL). If NULL, the function treeJaccard() performs calculation without it. |

## Value

a list of $thresholds - per cluster optimal detectability values, and $node - internal node id (merge row) where the optimum was found

---

`buildWijMatrix` *Rescale the weights in an edge matrix to match a given perplexity.*

---

## Description

Rescale the weights in an edge matrix to match a given perplexity.

## Usage

```
buildWijMatrix(x, threads = NULL, perplexity = 50)

## S3 method for class 'TsparseMatrix'
buildWijMatrix(x, threads = NULL, perplexity = 50)

## S3 method for class 'CsparseMatrix'
buildWijMatrix(x, threads = NULL, perplexity = 50)
```

## Arguments

| | |
|---|---|
| `x` | A sparse matrix |
| `threads` | numeric The maximum number of threads to spawn. Determined automatically if `NULL` (default=NULL) |
| `perplexity` | numeric Given perplexity (default=50) |

## Value

A `list` with the following components:

**'dist'** An [N,K] matrix of the distances to the nearest neighbors.

**'id'** An [N,K] matrix of the node indexes of the neartest neighbors. Note that this matrix is 1-indexed, unlike most other matrices in this package.

**'k'** The number of nearest neighbors.

Conos *Conos R6 class*

## Description

The class encompasses sample collections, providing methods for calculating and visualizing joint graph and communities.

## Public fields

`samples` list of samples (Pagoda2 or Seurat objects)

`pairs` pairwise alignment results

`graph` alignment graph

`clusters` list of clustering results named by clustering type

`expression.adj` adjusted expression values

`embeddings` list of joint embeddings

`embedding` joint embedding

`n.cores` number of cores

`misc` list with unstructured additional info

`override.conos.plot.theme` boolean Whether to override the conos plot theme

## Methods

### Public methods:

- `Conos$new()`
- `Conos$addSamples()`
- `Conos$buildGraph()`
- `Conos$getDifferentialGenes()`
- `Conos$findCommunities()`
- `Conos$plotPanel()`
- `Conos$embedGraph()`
- `Conos$plotClusterStability()`
- `Conos$plotGraph()`
- `Conos$correctGenes()`
- `Conos$propagateLabels()`
- `Conos$getClusterCountMatrices()`
- `Conos$getDatasetPerCell()`
- `Conos$getJointCountMatrix()`
- `Conos$clone()`

**Method** `new()`**:** initialize Conos class

*Usage:*

```
Conos$new(
  x,
  ...,
  n.cores = parallel::detectCores(logical = FALSE),
  verbose = TRUE,
  override.conos.plot.theme = FALSE
)
```

*Arguments:*

`x` a named list of pagoda2 or Seurat objects (one per sample)

`...` additional parameters upon initializing Conos

`n.cores` numeric Number of cores to use (default=parallel::detectCores(logical=FALSE))

`verbose` boolean Whether to provide verbose output (default=TRUE)

`override.conos.plot.theme` boolean Whether to reset plot settings to the ggplot2 default (default=FALSE)

*Returns:* a new 'Conos' object

*Examples:*

```
con <- Conos$new(small_panel.preprocessed, n.cores=1)
```

**Method** `addSamples()`**:** Initialize or add a set of samples to the conos panel. Note: this will simply add samples, but will not update graph, clustering, etc.

*Usage:*

```
Conos$addSamples(x, replace = FALSE, verbose = FALSE)
```

*Arguments:*

`x` a named list of pagoda2 or Seurat objects (one per sample)

`replace` boolean Whether the existing samples should be purged before adding new ones (default=FALSE)

`verbose` boolean Whether to provide verbose output (default=FALSE)

*Returns:* invisible view of the full sample list

**Method** `buildGraph()`**:** Build the joint graph that encompasses all the samples, establishing weighted inter-sample cell-to-cell links

*Usage:*

```
Conos$buildGraph(
  k = 15,
  k.self = 10,
  k.self.weight = 0.1,
  alignment.strength = NULL,
  space = "PCA",
  matching.method = "mNN",
  metric = "angular",
  k1 = k,
  data.type = "counts",
  l2.sigma = 1e+05,
```

```
  var.scale = TRUE,
  ncomps = 40,
  n.odgenes = 2000,
  matching.mask = NULL,
  exclude.samples = NULL,
  common.centering = TRUE,
  verbose = TRUE,
  base.groups = NULL,
  append.global.axes = TRUE,
  append.decoys = TRUE,
  decoy.threshold = 1,
  n.decoys = k * 2,
  score.component.variance = FALSE,
  snn = FALSE,
  snn.quantile = 0.9,
  min.snn.jaccard = 0,
  min.snn.weight = 0,
  snn.k.self = k.self,
  balance.edge.weights = FALSE,
  balancing.factor.per.cell = NULL,
  same.factor.downweight = 1,
  k.same.factor = k,
  balancing.factor.per.sample = NULL
)
```

*Arguments:*

`k` integer integer Size of the inter-sample neighborhood (default=15)

`k.self` integer Size of the with-sample neighborhoods (default=10).

`k.self.weight` numeric Weight multiplier on the intra-sample edges relative to inter-sample edges (default=0.1)

`alignment.strength` numeric Alignment strength (default=NULL will result in alignment.strength=0)

`space` character Reduced expression space used to establish putative alignments between pairs of samples (default='PCA'). Currently supported spaces are: — "CPCA" Common principal component analysis — "JNMF" Joint NMF — "genes" Gene expression space (log2 transformed) — "PCA" Principal component analysis — "CCA" Canonical correlation analysis — "PMA" (Penalized Multivariate Analysis <https://cran.r-project.org/web/packages/PMA/index.html>)

`matching.method` character Matching method (default='mNN'). Currently supported methods are "NN" (nearest neighbors) or "mNN" (mututal nearest neighbors).

`metric` character Distance metric to measure similarity (default='angular'). Currenlty supported metrics are "angular" and "L2".

`k1` numeric Neighborhood radius for identifying mutually-matching neighbors (default=k). Note that k1 must be greater than or equal to k, i.e. k1>=k. Increasing k1 beyond k will lead to more aggressive alignment of distinct subpopulations (i.e. increased alignment strengths).

`data.type` character Type of data type in the input pagoda2 objects within r.n (default='counts').

`l2.sigma` numeric L2 distances get transformed as exp(-d/sigma) using this value (default=1e5)

`var.scale` boolean Whether to use common variance scaling (default=TRUE). If TRUE, use geometric means for variance, as we're trying to focus on the common variance components. See scaledMatricesP2() code.

ncomps integer Number of components (default=40)

n.odgenes integer Number of overdispersed genes to be used in each pairwise alignment (default=2000)

matching.mask an optional matrix explicitly specifying which pairs of samples should be compared (a symmetrical matrix of logical values with row and column names corresponding to sample names). (default=NULL). By default, comparisons between all paris are allowed. The argument can be used to exclude comparisons across certain pairs of samples (e.g. techincal replicates, which are expected to show very high similarity).

exclude.samples optional list of sample names that should be excluded from the alignment and the resulting graph (default=NULL)

common.centering boolean When calculating reduced expression space for a given sample pair, whether the expression of genes should be centered using the mean from both samples (TRUE) or using the mean within each sample (FALSE) (default=TRUE)

verbose boolean Whether to provide verbose output (default=TRUE)

base.groups an optional factor on cells specifying previously-obtained cell grouping to be used for adjusting the sample alignment (default: NULL). Specifically, cell clusters specfiieid by the base.groups can be used to i) calculate global expression axes which are appended to the overall set of eigenvectors, ii) adding decoy cells.

append.global.axes boolean Whether to project samples on global expression axes, as defined by pre-defined (typically crude) set of cell subpopulations as specified by the base.gruops parameter (default=TRUE, but works only if base.groups is specified)

append.decoys boolean Whether to use pre-defined cell groups (specified by base.groups) to append decoy cells to the samples which are otherwise lacking any of the pre-specified cell groups (default=TRUE, but works only if base.groups is specified). The decoy cells can reduce the number of erroneous matches in highly heterogeneous sample collections, where some of the samples lack entire cell subpopulations which are found in other samples. The approach only works if the base.groups (typically a crude clustering of top-level cell types) can be established with a reasonable confidence.

decoy.threshold integer Minimal number of cells of a given cell type that should exist in a given sample (according to base.groups) to avoid addition of decoy cells to that sample for the purposes of alignment (default=1)

n.decoys integer Number of decoy cells that should be added to a sample that had less than decoy.threshold cells of a given cell type (default=k*2)

score.component.variance boolean Whether to score the amount of total variance explained by different components (default=FALSE as it takes extra time to calculate)

snn boolean Whether to transform the joint graph by computing a shared nearest neighborhood graph (analogous to Seurat 3), further weighting the edges between two matched cells based on the similarity (measured by Jaccard coefficient) of all of their predicted neighbors (across all of the samples) (default: FALSE)

snn.quantile numeric Specifies how the shared neighborhood graph transformation will determine final edge weights. If snn.quantile=NULL, the edge weight will be simply equal to the Jaccard coefficient of the neighborhoods. If snn.quantile is a vector of two numeric values (p1, p2), they will be treated as quantile probabilities, and quantile values (q1,q2) on the set of all Jaccard coefficients (for all edges) will be determiend. The edge weights will then be reset, so that edges with Jaccard coefficients below or equal to q1 will be set to 0, and those with coefficients >=q2 will be set to 1. The rest of the weights will be mapped uniformly from [q1,q2]->[0,1] range. If a single numeric value is supplied, it will

be treated as a symmetric quantile probability (i.e. snn.quantile=0.8 is equivalent to specifying snn.quantile=c(1-0.8,0.8)). (default: 0.9)

`min.snn.jaccard` numeric Minimum Jaccard coefficient required for a shared neighborhood graph edge (default: 0). The edges with Jaccard coefficients below this threshold will be removed (i.e. weight set to 0)

`min.snn.weight` numeric Shared nearest neighbor procedure will adjust the weights of the edges, and even eliminate some of the edges (by setting their weight to zero). The min.snn.weight parameter allows to set a minimal adjusted edge weight, so that the edge weight is never reduced beyond this level (and hence never deleted) (default: 0 - no adjustments)

`snn.k.self` integer Size of the within-sample neighorhood to be used in shared nearest neighbor calculations (default=k.self)

`balance.edge.weights` boolean Whether to balance edge weights to control for a cell- or sample- specific factor (default=FALSE)

`balancing.factor.per.cell` A per-cell factor (discrete factor, named with cell names) specifying a design difference should be controlled for by adjusting edge weights in the joint graph (default=NULL)

`same.factor.downweight` numeric Optional weighting factor for edges connecting cells with the same cell factor level per cell balancing (default=1.0)

`k.same.factor` integer An neighborhood size that should be used when aligning samples of the same balancing.factor.per.sample level. Setting a value smaller than k will lead to reduction of alingment strenth within the sample batches (default=k)

`balancing.factor.per.sample` A covariate factor per sample that should be controlled for by adjusting edge weights in the joint graph (default=NULL)

*Returns:* joint graph to be used for downstream analysis

*Examples:*

```
con <- Conos$new(small_panel.preprocessed, n.cores=1)
con$buildGraph(k=10, k.self=5, space='PCA', ncomps=10, n.odgenes=20, matching.method='mNN',
    metric='angular', score.component.variance=TRUE, verbose=TRUE)
```

**Method** `getDifferentialGenes()`**:** Calculate genes differentially expressed between cell clusters. Estimates base mean, z-score, p-values, specificity, precision, expressionFraction, AUC (if append.auc=TRUE)

*Usage:*

```
Conos$getDifferentialGenes(
  clustering = NULL,
  groups = NULL,
  z.threshold = 3,
  upregulated.only = FALSE,
  verbose = TRUE,
  append.specificity.metrics = TRUE,
  append.auc = TRUE
)
```

*Arguments:*

clustering character Name of the clustering to use (see names(con$clusters)) for the value of the groups factor (default: NULL - if groups are not specified, the first clustering will be used)

groups a cell factor (a factor named with cell names) specifying clusters of cells to be compared (one against all). To compare two cell clusters against each other, simply pass a factor containing only two levels (default: NULL, see clustering)

z.threshold numeric Minimum absolute value of a Z score for which the genes should be reported (default=3.0).

upregulated.only boolean If TRUE, will report only genes significantly upregulated in each cluster; otherwise both up- and down-regulated genes will be reported (default=FALSE)

verbose boolean Whether to provide verbose output (default=TRUE)

append.specificity.metrics boolean Whether to append specificity metrics (default=TRUE)

append.auc boolean Whether to append AUC scores (default=TRUE)

*Returns:* list of DE results; each is a data frame with rows corresponding to the differentially expressed genes, and columns listing log2 fold change (M), signed Z scores (both raw and adjusted for mulitple hypothesis using BH correction), optional specificty/sensitivity and AUC metrics.

**Method** `findCommunities()`**:** Find cell clusters (as communities on the joint graph)

*Usage:*

```
Conos$findCommunities(
  method = leiden.community,
  min.group.size = 0,
  name = NULL,
  test.stability = FALSE,
  stability.subsampling.fraction = 0.95,
  stability.subsamples = 100,
  verbose = TRUE,
  cls = NULL,
  sr = NULL,
  ...
)
```

*Arguments:*

method community detection method (igraph syntax) (default=leiden.community)

min.group.size numeric Minimal allowed community size (default=0)

name character Optional name of the clustering result (will default to the algorithm name) (default=NULL will try to obtain the name from the community detection method, or will use 'community' as a default)

test.stability boolean Whether to test stability of community detection (default=FALSE)

stability.subsampling.fraction numeric Fraction of clusters to subset (default=0.95). Must be within range [0, 1].

stability.subsamples integer Number of subsampling iterations (default=100)

verbose boolean Whether to provide verbose output (default=TRUE)

cls optional pre-calculated community result (may be useful for stability testing) (default: NULL)

`sr` optional pre-calculated subsampled community results (useful for stability testing) (default: NULL)

`...` extra parameters are passed to the specified community detection method

*Returns:* invisible list containing identified communities (groups) and the full community detection result (result); The results are stored in $clusters$name slot in the conos object. Each such slot contains an object with elements: $results which stores the raw output of the community detection method, and $groups which is a factor on cells describing the resulting clustering. The later can be used, for instance, in plotting: con$plotGraph(groups=con$clusters$leiden$groups). If test.stability==TRUE, then the result object will also contain a $stability slot.

*Examples:*

```
con <- Conos$new(small_panel.preprocessed, n.cores=1)
con$buildGraph(k=10, k.self=5, space='PCA', ncomps=10, n.odgenes=20, matching.method='mNN',
    metric='angular', score.component.variance=TRUE, verbose=TRUE)
con$findCommunities(method = igraph::walktrap.community, steps=5)
```

**Method** `plotPanel()`**:** Plot panel of individual embeddings per sample with joint coloring

*Usage:*

```
Conos$plotPanel(
  clustering = NULL,
  groups = NULL,
  colors = NULL,
  gene = NULL,
  use.local.clusters = FALSE,
  plot.theme = NULL,
  use.common.embedding = FALSE,
  embedding = NULL,
  adj.list = NULL,
  ...
)
```

*Arguments:*

`clustering` character Name of the clustering to use (see names(con$clusters)) for the value of the groups factor (default=NULL - if groups are not specified, the first clustering will be used)

`groups` a cell factor (a factor named with cell names) specifying clusters of cells to be compared (one against all). To compare two cell clusters against each other, simply pass a factor containing only two levels (default=NULL, see clustering)

`colors` a color factor (named with cell names) use for cell coloring

`gene` show expression of a gene

`use.local.clusters` boolean Whether clusters should be taken from the individual samples; otherwise joint clusters in the conos object will be used (see clustering) (default=FALSE).

`plot.theme` string Theme for the plot, passed to plotSamples() (default=NULL)

`use.common.embedding` boolean Whether a joint embedding in the conos object should be used (or embeddings determined for the individual samples) (default=FALSE)

`embedding` (default=NULL) If a character value is passed, it is interpreted as an embedding name (a name of a joint embedding in conos when use.commmon.embedding=TRUE, or a

name of an embedding within the individual objects when use.common.embedding=FALSE). If a matrix is passed, it is interpreted as an actual embedding (then first two columns are interpreted as x/y coordinates, row names must be cell names). If NULL, the default embedding will be used.

`adj.list` an optional list of additional ggplot2 directions to apply (default=NULL)

`...` Additional parameters passed to plotSamples(), plotEmbeddings(), sccore::embeddingPlot().

*Returns:* cowplot grid object with the panel of plots

**Method** `embedGraph()`**:** Generate an embedding of a joint graph

*Usage:*

```
Conos$embedGraph(
  method = "largeVis",
  embedding.name = method,
  M = 1,
  gamma = 1,
  alpha = 0.1,
  perplexity = NA,
  sgd_batches = 1e+08,
  seed = 1,
  verbose = TRUE,
  target.dims = 2,
  ...
)
```

*Arguments:*

`method` Embedding method (default='largeVis'). Currently 'largeVis' and 'UMAP' are supported.

`embedding.name` character Optional name of the name of the embedding set by user to store multiple embeddings (default: method name)

`M` numeric (largeVis) The number of negative edges to sample for each positive edge to be used (default=1)

`gamma` numeric (largeVis) The strength of the force pushing non-neighbor nodes apart (default=1)

`alpha` numeric (largeVis) Hyperparameter used in the default distance function, $1/(1+\alpha\dot{||}y_i - y_j||^2)$ (default=0.1). The function relates the distance between points in the low-dimensional projection to the likelihood that the two points are nearest neighbors. Increasing $\alpha$ tends to push nodes and their neighbors closer together; decreasing $\alpha$ produces a broader distribution. Setting $\alpha$ to zero enables the alternative distance function. $\alpha$ below zero is meaningless.

`perplexity` (largeVis) The perplexity passed to largeVis (default=NA)

`sgd_batches` (largeVis) The number of edges to process during SGD (default=1e8). Defaults to a value set based on the size of the dataset. If the parameter given is between `0` and `1`, the default value will be multiplied by the parameter.

`seed` numeric Random seed for the largeVis algorithm (default=1)

`verbose` boolean Whether to provide verbose output (default=TRUE)

`target.dims` numeric Number of dimensions for the reduction (default=2). Higher dimensions can be used to generate embeddings for subsequent reductions by other methods, such as tSNE

... additional arguments, passed to UMAP embedding (run ?conos:::embedGraphUmap for more info)

**Method** `plotClusterStability()`**:** Plot cluster stability statistics.

*Usage:*

```
Conos$plotClusterStability(clustering = NULL, what = "all")
```

*Arguments:*

`clustering` string Name of the clustering result to show (default=NULL)

`what` string Show a specific plot (ari - adjusted rand index, fjc - flat Jaccard, hjc - hierarchical Jaccard, dend - cluster dendrogram, all - everything except 'dend') (default='all')

*Returns:* cluster stability statistics

**Method** `plotGraph()`**:** Plot joint graph

*Usage:*

```
Conos$plotGraph(
  color.by = "cluster",
  clustering = NULL,
  embedding = NULL,
  groups = NULL,
  colors = NULL,
  gene = NULL,
  plot.theme = NULL,
  subset = NULL,
  ...
)
```

*Arguments:*

`color.by` character A shortcut to color the plot by 'cluster' or by 'sample' (default: 'cluster'). If any other string is input, an error is thrown.

`clustering` a character name of the clustering to use (see names(con$clusters)) for the value of the groups factor (default: NULL - if groups are not specified, the first clustering will be used)

`embedding` A character name of an embedding, or a matrix of the actual embedding (rownames should correspond to cells, first to columns to x/y coordinates). If NULL (default: NULL), the latest generated embedding will be used

`groups` a cell factor (a factor named with cell names) specifying clusters of cells to be compared (one against all). To compare two cell clusters against each other, simply pass a factor containing only two levels (default: NULL, see clustering)

`colors` a color factor (named with cell names) use for cell coloring (default=NULL)

`gene` Show expression of a gene (default=NULL)

`plot.theme` Theme for the plot, passed to sccore::embeddingPlot() (default=NULL)

`subset` A subset of cells to show (default: NULL - shows all the cells)

... Additional parameters passed to sccore::embeddingPlot()

*Returns:* ggplot2 plot of joint graph

**Method** `correctGenes()`**:** Smooth expression of genes to minimize the batch effect between samples Use diffusion of expression on graph with the equation dv = exp(-a * (v + b))

*Usage:*

```
Conos$correctGenes(
  genes = NULL,
  n.od.genes = 500,
  fading = 10,
  fading.const = 0.5,
  max.iters = 15,
  tol = 0.005,
  name = "diffusion",
  verbose = TRUE,
  count.matrix = NULL,
  normalize = TRUE
)
```

*Arguments:*

`genes` List of genes to be smooothed smoothing (default=NULL will smooth top n.od.genes overdispersed genes)

`n.od.genes` numeric If 'genes' is NULL, top n.od.genes of overdispersed genes are taken across all samples (default=500)

`fading` numeric Level of fading of expression change from distance on the graph (parameter 'a' of the equation) (default=10)

`fading.const` numeric Minimal penalty for each new edge during diffusion (parameter 'b' of the equation) (default=0.5)

`max.iters` numeric Maximal number of diffusion iterations (default=15)

`tol` numeric Tolerance after which the diffusion stops (default=5e-3)

`name` string Name to save the correction (default='diffusion')

`verbose` boolean Verbose mode (default=TRUE)

`count.matrix` Alternative gene count matrix to correct (rows: genes, columns: cells; has to be dense matrix). Default: joint count matrix for all datasets.

`normalize` boolean Whether to normalize values (default=TRUE)

*Returns:* smoothed expression of the input genes

**Method** `propagateLabels()`**:** Estimate labeling distribution for each vertex, based on a partial labeling of the cells. There are two methods used for the propagation to calculate the distribution of labels: "solver" and "diffusion". * "diffusion" (default) will estimate the labeling distribution for each vertex, based on provided labels using a random walk. * "solver" will propagate labels using the algorithm described by Zhu, Ghahramani, Lafferty (2003) <http://mlg.eng.cam.ac.uk/zoubin/papers/zgl.pdf> Confidence values are then calculated by taking the maximum value from this distribution of labels, for each cell.

*Usage:*

```
Conos$propagateLabels(labels, method = "diffusion", ...)
```

*Arguments:*

`labels` Input labels

`method` type of propagation. Either 'diffusion' or 'solver'. 'solver' gives better result but has bad asymptotics, so is inappropriate for datasets > 20k cells. (default='diffusion')

`...` additional arguments for conos:::propagateLabels* functions

*Returns:* list with three fields: * labels = matrix with distribution of label probabilities for each vertex by rows. * uncertainty = 1 - confidence values * label.distribution = the distribution of labels calculated using either the methods "diffusion" or "solver"

**Method** `getClusterCountMatrices()`**:** Calculate pseudo-bulk expression matrices for clusters (by adding up, for each gene, all of the molecules detected for all cells in a given cluster in a given sample)

*Usage:*

```
Conos$getClusterCountMatrices(
  clustering = NULL,
  groups = NULL,
  common.genes = TRUE,
  omit.na.cells = TRUE
)
```

*Arguments:*

`clustering` string Name of the clustering to use

`groups` a factor on cells to use for coloring

`common.genes` boolean Whether to bring individual sample matrices to a common gene list (default=TRUE)

`omit.na.cells` boolean If set to FALSE, the resulting matrices will include a first column named 'NA' that will report total molecule counts for all of the cells that were not covered by the provided factor. (default=TRUE)

*Returns:* a list of per-sample uniform dense matrices with rows being genes, and columns being clusters

**Method** `getDatasetPerCell()`**:** applies 'getCellNames()' on all samples

*Usage:*

```
Conos$getDatasetPerCell()
```

*Returns:* list of cellnames for all samples

*Examples:*

```
con <- Conos$new(small_panel.preprocessed, n.cores=1)
con$getDatasetPerCell()
```

**Method** `getJointCountMatrix()`**:** Retrieve joint count matrices

*Usage:*

```
Conos$getJointCountMatrix(raw = FALSE)
```

*Arguments:*

`raw` boolean If TRUE, return merged "raw" count matrices, using function getRawCountMatrix(). Otherwise, return the merged count matrices, using getCountMatrix(). (default=FALSE)

*Returns:* list of merged count matrices

*Examples:*

```
con <- Conos$new(small_panel.preprocessed, n.cores=1)
con$getJointCountMatrix()
```

**Method** `clone()`**:** The objects of this class are cloneable with this method.

*Usage:*

```
Conos$clone(deep = FALSE)
```

*Arguments:*

deep Whether to make a deep clone.

## Examples

```
## ------------------------------------------------
## Method `Conos$new`
## ------------------------------------------------

con <- Conos$new(small_panel.preprocessed, n.cores=1)


## ------------------------------------------------
## Method `Conos$buildGraph`
## ------------------------------------------------

con <- Conos$new(small_panel.preprocessed, n.cores=1)
con$buildGraph(k=10, k.self=5, space='PCA', ncomps=10, n.odgenes=20, matching.method='mNN',
    metric='angular', score.component.variance=TRUE, verbose=TRUE)



## ------------------------------------------------
## Method `Conos$findCommunities`
## ------------------------------------------------

con <- Conos$new(small_panel.preprocessed, n.cores=1)
con$buildGraph(k=10, k.self=5, space='PCA', ncomps=10, n.odgenes=20, matching.method='mNN',
    metric='angular', score.component.variance=TRUE, verbose=TRUE)
con$findCommunities(method = igraph::walktrap.community, steps=5)


## ------------------------------------------------
## Method `Conos$getDatasetPerCell`
## ------------------------------------------------

con <- Conos$new(small_panel.preprocessed, n.cores=1)
con$getDatasetPerCell()


## ------------------------------------------------
## Method `Conos$getJointCountMatrix`
## ------------------------------------------------

con <- Conos$new(small_panel.preprocessed, n.cores=1)
con$getJointCountMatrix()
```

## convertToPagoda2 *Convert Conos object to Pagoda2 object*

### Description

Convert Conos object to Pagoda2 object

### Usage

```
convertToPagoda2(con, n.pcs = 100, n.odgenes = 2000, verbose = TRUE, ...)
```

### Arguments

| | |
|---|---|
| con | Conos object |
| n.pcs | numeric Number of principal components (default=100) |
| n.odgenes | numeric Number of overdispersed genes (default=2000) |
| verbose | boolean Whether to give verbose output (default=TRUE) |
| ... | parameters passed to Pagoda2$new() |

### Value

pagoda2 object

## edgeMat<- *Set edge matrix edgeMat with certain values on sample*

### Description

Set edge matrix edgeMat with certain values on sample

Access edgeMat from sample

### Usage

```
edgeMat(sample) <- value

## S4 replacement method for signature 'Pagoda2'
edgeMat(sample) <- value

## S4 replacement method for signature 'seurat'
edgeMat(sample) <- value

## S4 replacement method for signature 'Seurat'
edgeMat(sample) <- value
```

```
edgeMat(sample)

## S4 method for signature 'Pagoda2'
edgeMat(sample)

## S4 method for signature 'seurat'
edgeMat(sample)

## S4 method for signature 'Seurat'
edgeMat(sample)
```

## Arguments

| | |
|---|---|
| sample | sample from which to access edge matrix edgeMat |
| value | values to set with edgeMat<- |

---

`estimateWeightEntropyPerCell` *Estimate entropy of edge weights per cell according to the specified factor. Can be used to visualize alignment quality according to this factor.*

---

## Description

Estimate entropy of edge weights per cell according to the specified factor. Can be used to visualize alignment quality according to this factor.

## Usage

```
estimateWeightEntropyPerCell(con, factor.per.cell)
```

## Arguments

| | |
|---|---|
| con | conos object |
| factor.per.cell | some factor, which group cells, such as sample or a specific condition |

## Value

entropy of edge weights per cell

## findSubcommunities *Increase resolution for a specific set of clusters*

### Description

Increase resolution for a specific set of clusters

### Usage

```
findSubcommunities(
  con,
  target.clusters,
  clustering = NULL,
  groups = NULL,
  method = leiden.community,
  ...
)
```

### Arguments

| | |
|---|---|
| con | conos object |
| target.clusters | clusters for which the resolution should be increased |
| clustering | name of clustering in the conos object to use. Either 'clustering' or 'groups' must be provided (default=NULL). |
| groups | set of clusters to use. Ignored if 'clustering' is not NULL (default=NULL). |
| method | function, used to find communities (default=leiden.community). |
| ... | additional params passed to the community function |

### Value

set of clusters with increased resolution

## getBetweenCellTypeCorrectedDE *Compare two cell types across the entire panel*

### Description

Compare two cell types across the entire panel

## Usage

```
getBetweenCellTypeCorrectedDE(
  con.obj,
  sample.groups = NULL,
  groups = NULL,
  cooks.cutoff = FALSE,
  refgroup = NULL,
  altgroup = NULL,
  min.cell.count = 10,
  independent.filtering = FALSE,
  cluster.sep.chr = "<!!>",
  return.details = TRUE,
  only.paired = TRUE,
  correction = NULL,
  ref.level = NULL
)
```

## Arguments

| | |
|---|---|
| con.obj | conos object |
| sample.groups | a named list of two character vectors specifying the app groups to compare |
| groups | factor describing cell grouping |
| cooks.cutoff | cooksCutoff parameter for DESeq2 |
| refgroup | cell type to compare to be used as reference |
| altgroup | cell type to compare to |
| min.cell.count | minimum number of cells per celltype/sample combination to keep |
| independent.filtering | independentFiltering parameter for DESeq2 |
| cluster.sep.chr | character string of length 1 specifying a delimiter to separate cluster and app names |
| return.details | logical, return detailed results |
| only.paired | only keep samples that that both cell types above the min.cell.count threshold |
| correction | fold change corrections per genes |
| ref.level | reference level on the basis of which the correction was calculated |

## Value

Returns either a DESeq2::results() object, or if return.details=TRUE, returns a list of the DESeq2::results(), the samples from the panel to use in this comparison, refgroups, altgroup, and samplegroups

`getBetweenCellTypeDE` *Compare two cell types across the entire panel*

## Description

Compare two cell types across the entire panel

## Usage

```
getBetweenCellTypeDE(
  con.obj,
  groups = NULL,
  sample.groups = NULL,
  cooks.cutoff = FALSE,
  refgroup = NULL,
  altgroup = NULL,
  min.cell.count = 10,
  independent.filtering = FALSE,
  cluster.sep.chr = "<!!>",
  return.details = TRUE,
  only.paired = TRUE,
  remove.na = TRUE
)
```

## Arguments

| | |
|---|---|
| `con.obj` | conos object |
| `groups` | factor describing cell grouping (default=NULL) |
| `sample.groups` | a named list of two character vectors specifying the app groups to compare (default=NULL) |
| `cooks.cutoff` | boolean cooksCutoff parameter for DESeq2 (default=FALSE) |
| `refgroup` | cell type to compare to be used as reference (default=NULL) |
| `altgroup` | cell type to compare to be used as ALT against refgroup (default=NULL) |
| `min.cell.count` | numeric Minimum number of cells per celltype/sample combination to keep (default=10) |
| `independent.filtering` | boolean Whether to use independentFiltering parameter for DESeq2 (default=FALSE) |
| `cluster.sep.chr` | character string of length 1 specifying a delimiter to separate cluster and app names (default='<!!>') |
| `return.details` | boolean Return detailed results (default=TRUE) |
| `only.paired` | boolean Only keep samples that that both cell types above the min.cell.count threshold (default=TRUE) |
| `remove.na` | boolean If TRUE, remove NAs from DESeq calculations (default=TRUE) |

### Value

Returns either a DESeq2::results() object, or if return.details=TRUE, returns a list of the DESeq2::results(), the samples from the panel to use in this comparison, refgroups, altgroup, and samplegroups

---

`getCellNames` *Access cell names from sample*

---

### Description

Access cell names from sample

### Usage

```
getCellNames(sample)

## S4 method for signature 'Pagoda2'
getCellNames(sample)

## S4 method for signature 'seurat'
getCellNames(sample)

## S4 method for signature 'Seurat'
getCellNames(sample)

## S4 method for signature 'Conos'
getCellNames(sample)
```

### Arguments

| | |
|---|---|
| `sample` | sample from which to cell names |

---

`getClustering` *Access clustering from sample*

---

### Description

Access clustering from sample

### Usage

```
getClustering(sample, type)

## S4 method for signature 'Pagoda2'
getClustering(sample, type)

## S4 method for signature 'seurat'
getClustering(sample, type)

## S4 method for signature 'Seurat'
getClustering(sample, type)

## S4 method for signature 'Conos'
getClustering(sample, type)
```

### Arguments

| | |
|---|---|
| sample | sample from which to get the clustering |
| type | character Type of clustering to get |

---

## getCountMatrix *Access count matrix from sample*

---

### Description

Access count matrix from sample

### Usage

```
getCountMatrix(sample, transposed = FALSE)

## S4 method for signature 'Pagoda2'
getCountMatrix(sample, transposed = FALSE)

## S4 method for signature 'seurat'
getCountMatrix(sample, transposed = FALSE)

## S4 method for signature 'Seurat'
getCountMatrix(sample, transposed = FALSE)
```

### Arguments

| | |
|---|---|
| sample | sample from which to get the count matrix |
| transposed | boolean Whether the count matrix should be transposed (default=FALSE) |

## getEmbedding *Access embedding from sample*

### Description

Access embedding from sample

### Usage

```
getEmbedding(sample, type)

## S4 method for signature 'Pagoda2'
getEmbedding(sample, type)

## S4 method for signature 'seurat'
getEmbedding(sample, type)

## S4 method for signature 'Seurat'
getEmbedding(sample, type)

## S4 method for signature 'Conos'
getEmbedding(sample, type)
```

### Arguments

| | |
|---|---|
| sample | sample from which to get the embedding |
| type | character Type of embedding to get |

## getGeneExpression *Access gene expression from sample*

### Description

Access gene expression from sample

### Usage

```
getGeneExpression(sample, gene)

## S4 method for signature 'Pagoda2'
getGeneExpression(sample, gene)

## S4 method for signature 'Conos'
getGeneExpression(sample, gene)
```

```
## S4 method for signature 'Seurat'
getGeneExpression(sample, gene)

## S4 method for signature 'seurat'
getGeneExpression(sample, gene)
```

## Arguments

| | |
|---|---|
| sample | sample from which to access gene expression |
| gene | character vector Genes to access |

---

`getGenes` *Access genes from sample*

---

## Description

Access genes from sample

## Usage

```
getGenes(sample)

## S4 method for signature 'Pagoda2'
getGenes(sample)

## S4 method for signature 'seurat'
getGenes(sample)

## S4 method for signature 'Seurat'
getGenes(sample)

## S4 method for signature 'Conos'
getGenes(sample)
```

## Arguments

| | |
|---|---|
| sample | sample from which to get genes |

## getOverdispersedGenes *Access overdispersed genes from sample*

### Description

Access overdispersed genes from sample

### Usage

```
getOverdispersedGenes(sample, n.odgenes = 1000)

## S4 method for signature 'Pagoda2'
getOverdispersedGenes(sample, n.odgenes = NULL)

## S4 method for signature 'seurat'
getOverdispersedGenes(sample, n.odgenes = NULL)

## S4 method for signature 'Seurat'
getOverdispersedGenes(sample, n.odgenes = NULL)

## S4 method for signature 'Conos'
getOverdispersedGenes(sample, n.odgenes = NULL)
```

### Arguments

| | |
|---|---|
| sample | sample from which to overdispereed genes |
| n.odgenes | numeric Number of overdisperesed genes to get |

## getPca *Access PCA from sample*

### Description

Access PCA from sample

### Usage

```
getPca(sample)

## S4 method for signature 'Pagoda2'
getPca(sample)

## S4 method for signature 'seurat'
getPca(sample)

## S4 method for signature 'Seurat'
getPca(sample)
```

### Arguments

| | |
|---|---|
| sample | sample from which to access PCA |

---

`getPerCellTypeDE` *Do differential expression for each cell type in a conos object between the specified subsets of apps*

---

### Description

Do differential expression for each cell type in a conos object between the specified subsets of apps

### Usage

```
getPerCellTypeDE(
  con.obj,
  groups = NULL,
  sample.groups = NULL,
  cooks.cutoff = FALSE,
  ref.level = NULL,
  min.cell.count = 10,
  remove.na = TRUE,
  max.cell.count = Inf,
  test = "LRT",
  independent.filtering = FALSE,
  n.cores = 1,
  cluster.sep.chr = "<!!>",
  return.details = TRUE
)
```

### Arguments

| | |
|---|---|
| con.obj | conos object |
| groups | factor specifying cell types (default=NULL) |
| sample.groups | a list of two character vector specifying the app groups to compare (default=NULL) |
| cooks.cutoff | boolean cooksCutoff for DESeq2 (default=FALSE) |
| ref.level | the reference level of the sample.groups against which the comparison should be made (default=NULL). If NULL, will pick the first one. |
| min.cell.count | integer Minimal number of cells per cluster for a sample to be taken into account in a comparison (default=10) |
| remove.na | boolean If TRUE, remove NAs from DESeq calculations, which often arise as comparisons not possible (default=TRUE) |
| max.cell.count | maximal number of cells per cluster per sample to include in a comparison (useful for comparing the number of DE genes between cell types) (default=Inf) |
| test | which DESeq2 test to use (options: "LRT" or "Wald") (default="LRT") |

independent.filtering
: boolean independentFiltering for DESeq2 (default=FALSE)

n.cores
: numeric Number of cores (default=1)

cluster.sep.chr
: character string of length 1 specifying a delimiter to separate cluster and app names (default='<!!>')

return.details
: boolean Whether to return verbose details (default=TRUE)

## Value

A list of differential expression results for every cell type

---

`getRawCountMatrix` *Access raw count matrix from sample*

---

## Description

Access raw count matrix from sample

## Usage

```
getRawCountMatrix(sample, transposed = FALSE)

## S4 method for signature 'Pagoda2'
getRawCountMatrix(sample, transposed = FALSE)

## S4 method for signature 'seurat'
getRawCountMatrix(sample, transposed = FALSE)

## S4 method for signature 'Seurat'
getRawCountMatrix(sample, transposed = FALSE)

## S4 method for signature 'Conos'
getRawCountMatrix(sample, transposed = FALSE)
```

## Arguments

sample
: sample from which to get the raw count matrix

transposed
: boolean Whether the raw count matrix should be transposed (default=FALSE)

## getSampleNamePerCell *Retrieve sample names per cell*

### Description

Retrieve sample names per cell

### Usage

```
getSampleNamePerCell(samples)
```

### Arguments

| | |
|---|---|
| samples | list of samples |

### Value

list of sample names getSampleNamePerCell(small_panel.preprocessed)

## greedyModularityCut *Performs a greedy top-down selective cut to optmize modularity*

### Description

Performs a greedy top-down selective cut to optmize modularity

### Usage

```
greedyModularityCut(
  wt,
  N,
  leaf.labels = NULL,
  minsize = 0,
  minbreadth = 0,
  flat.cut = TRUE
)
```

### Arguments

| | |
|---|---|
| wt | walktrap result |
| N | numeric Number of top greedy splits to take |
| leaf.labels | leaf sample label factor, for breadth calculations - must be a named factor containing all wt$names, or if wt$names is null, a factor listing cells in the same order as wt leafs (default=NULL) |
| minsize | numeric Minimum size of the branch (in number of leafs) (default=0) |

| | |
|---|---|
| minbreadth | numeric Minimum allowed breadth of a branch (measured as normalized entropy) (default=0) |
| flat.cut | boolean Whether to simply take a flat cut (i.e. follow provided tree; default=TRUE). Does no observe minsize/minbreadth restrictions |

## Value

list(hclust - hclust structure of the derived tree, leafContent - binary matrix with rows corresponding to old leaves, columns to new ones, deltaM - modularity increments)

---

# p2app4conos *Utility function to generate a pagoda2 app from a conos object*

---

## Description

Utility function to generate a pagoda2 app from a conos object

## Usage

```
p2app4conos(
  conos,
  cdl = NULL,
  metadata = NULL,
  filename = "conos_app.bin",
  save = TRUE,
  n.cores = 1,
  n.odgenes = 3000,
  nPcs = 100,
  k = 30,
  perplexity = 50,
  log.scale = TRUE,
  trim = 10,
  keep.genes = NULL,
  min.cells.per.gene = 0,
  min.transcripts.per.cell = 100,
  get.largevis = TRUE,
  get.tsne = TRUE,
  make.geneknn = TRUE,
  go.env = NULL,
  cell.subset = NULL,
  max.cells = Inf,
  additional.embeddings = NULL,
  test.pathway.overdispersion = FALSE,
  organism = NULL,
  return.details = FALSE
)
```

## Arguments

| | |
|---|---|
| conos | Conos object |
| cdl | list Optional list of raw matrices (so that gene merging doesn't have to be redone) (default=NULL) |
| metadata | list Optional list of (named) metadata factors (default=NULL) |
| filename | string Name of the *.bin file to seralize for the pagoda2 application if save=TRUE (default='conos_app.bin') |
| save | boolean Save serialized *bin file specified in filename (default=TRUE) |
| n.cores | integer Number of cores (default=1) |
| n.odgenes | numeric Number of top overdispersed genes to use (dfault=3e3). From pagoda2::basicP2proc(). |
| nPcs | numeric Number of PCs to use (default=100). From pagoda2::basicP2proc(). |
| k | numeric Default number of neighbors to use in kNN graph (default=30). From pagoda2::basicP2proc(). |
| perplexity | numeric Perplexity to use in generating tSNE and largeVis embeddings (default=50). From pagoda2::basicP2proc(). |
| log.scale | boolean Whether to use log scale normalization (default=TRUE). From pagoda2::basicP2proc(). |
| trim | numeric Number of cells to trim in winsorization (default=10). From pagoda2::basicP2proc(). |
| keep.genes | optional set of genes to keep from being filtered out (even at low counts) (default=NULL). From pagoda2::basicP2proc(). |
| min.cells.per.gene | numeric Minimal number of cells required for gene to be kept (unless listed in keep.genes) (default=0). From pagoda2::basicP2proc(). |
| min.transcripts.per.cell | numeric Minimumal number of molecules/reads for a cell to be admitted (default=100). From pagoda2::basicP2proc(). |
| get.largevis | boolean Whether to caluclate largeVis embedding (default=TRUE). From pagoda2::basicP2proc(). |
| get.tsne | boolean Whether to calculate tSNE embedding (default=TRUE). From pagoda2::basicP2proc(). |
| make.geneknn | boolean Whether pre-calculate gene kNN (for gene search) (default=TRUE). From pagoda2::basicP2proc(). |
| go.env | GO environment for the organism of interest (default=NULL) |
| cell.subset | string Cells to subset with the conos embedding conos$embedding. If NULL, uses all cells via rownames(conos$embedding) (default=NULL) |
| max.cells | numeric Limit to the cells that are included in the conos. If Inf, there is no limit (default=Inf) |
| additional.embeddings | list Additional embeddings to add to conos for the pagoda2 app (default=NULL) |
| test.pathway.overdispersion | boolean Find all IDs using GO category against either org.Hs.eg.db ('hs') or org.Mm.eg.db ('mm') (default=FALSE |
| organism | string Organism of interest, either 'hs' (Homo sapiens) or 'mm' (Mus musculus, i.e. mouse) (default=NULL). Only used if test.pathway.overdispersion is TRUE. If NULL and test.pathway.overdispersion=TRUE, then 'hs' is used. |

`return.details` boolean If TRUE, return list of p2 application, pagoda2 object, list of raw matrices, and cell names. If FALSE, simply return pagoda2 app object. (default=FALSE)

## Value

pagoda2 app object

---

`plotClusterBarplots` *Plots barplots per sample of composition of each pagoda2 application based on selected clustering*

---

## Description

Plots barplots per sample of composition of each pagoda2 application based on selected clustering

## Usage

```
plotClusterBarplots(
  conos.obj = NULL,
  clustering = NULL,
  groups = NULL,
  sample.factor = NULL,
  show.entropy = TRUE,
  show.size = TRUE,
  show.composition = TRUE,
  legend.height = 0.2
)
```

## Arguments

| | |
|---|---|
| `conos.obj` | A conos object (default=NULL) |
| `clustering` | name of clustering in the current object (default=NULL) |
| `groups` | arbitrary grouping of cells (to use instead of the clustering) (default=NULL) |
| `sample.factor` | a factor describing cell membership in the samples (or some other category) (default=NULL). This will default to samples if not provided. |
| `show.entropy` | boolean Whether to include entropy barplot (default=TRUE) |
| `show.size` | boolean Whether to include size barplot (default=TRUE) |
| `show.composition` | boolean Whether to include composition barplot (default=TRUE) |
| `legend.height` | numeric Relative hight of the legend panel (default=0.2) |

## Value

a ggplot object

## plotClusterBoxPlotsByAppType

*Generate boxplot per cluster of the proportion of cells in each celltype*

### Description

Generate boxplot per cluster of the proportion of cells in each celltype

### Usage

```
plotClusterBoxPlotsByAppType(
  conos.obj,
  clustering = NULL,
  apptypes = NULL,
  return.details = FALSE
)
```

### Arguments

| | |
|---|---|
| conos.obj | conos object |
| clustering | name of the clustering to use (default=NULL) |
| apptypes | a factor specifying how to group the samples (default=NULL) |
| return.details | boolean If TRUE return a list with the plot and the summary data.frame (default=FALSE) |

### Value

Boxplot per cluster of the proportion of cells in each celltype

## plotComponentVariance

*Plot fraction of variance explained by the successive reduced space components (PCA, CPCA)*

### Description

Requires buildGraph() or updatePairs() to be ran first with the argument score.component.variance=TRUE.

### Usage

```
plotComponentVariance(
  conos.obj,
  space = "PCA",
  plot.theme = ggplot2::theme_bw()
)
```

### Arguments

| | |
|---|---|
| conos.obj | conos object |
| space | character Reduction space to be analyzed (currently, component variance scoring is only supported by PCA and CPCA) (default='PCA') |
| plot.theme | ggplot theme (default=ggplot2::theme_bw()). Refer to <https://ggplot2.tidyverse.org/reference/ggtheme.h for more details. |

### Value

ggplot

---

## plotDEheatmap *Plot a heatmap of differential genes*

---

### Description

Plot a heatmap of differential genes

### Usage

```
plotDEheatmap(
  con,
  groups,
  de = NULL,
  min.auc = NULL,
  min.specificity = NULL,
  min.precision = NULL,
  n.genes.per.cluster = 10,
  additional.genes = NULL,
  exclude.genes = NULL,
  labeled.gene.subset = NULL,
  expression.quantile = 0.99,
  pal = colorRampPalette(c("dodgerblue1", "grey95", "indianred1"))(1024),
  ordering = "-AUC",
  column.metadata = NULL,
  show.gene.clusters = TRUE,
  remove.duplicates = TRUE,
  column.metadata.colors = NULL,
  show.cluster.legend = TRUE,
  show_heatmap_legend = FALSE,
  border = TRUE,
  return.details = FALSE,
  row.label.font.size = 10,
  order.clusters = FALSE,
  split = FALSE,
  split.gap = 0,
```

```
  cell.order = NULL,
  averaging.window = 0,
  max.cells = Inf,
  ...
)
```

## Arguments

| | |
|---|---|
| con | conos (or p2) object |
| groups | groups in which the DE genes were determined (so that the cells can be ordered correctly) |
| de | differential expression result (list of data frames) (default=NULL) |
| min.auc | optional minimum AUC threshold (default=NULL) |
| min.specificity | optional minimum specificity threshold (default=NULL) |
| min.precision | optional minimum precision threshold (default=NULL) |
| n.genes.per.cluster | numeric Number of genes to show for each cluster (default=10) |
| additional.genes | optional additional genes to include (the genes will be assigned to the closest cluster) (default=NULL) |
| exclude.genes | an optional list of genes to exclude from the heatmap (default=NULL) |
| labeled.gene.subset | a subset of gene names to show (instead of all genes) (default=NULL). Can be a vector of gene names, or a number of top genes (in each cluster) to show the names for. |
| expression.quantile | numeric Expression quantile to show (default=0.99) |
| pal | palette to use for the main heatmap (default=colorRampPalette(c('dodgerblue1','grey95','indianred1'))(1 |
| ordering | order by which the top DE genes (to be shown) are determined (default "-AUC") |
| column.metadata | additional column metadata, passed either as a data.frame with rows named as cells, or as a list of named cell factors (default=NULL). |
| show.gene.clusters | whether to show gene cluster color codes |
| remove.duplicates | remove duplicated genes (leaving them in just one of the clusters) |
| column.metadata.colors | a list of color specifications for additional column metadata, specified according to the HeatmapMetadata format. Use "clusters" slot to specify cluster colors. |
| show.cluster.legend | boolean Whether to show the cluster legend (default=TRUE) |
| show_heatmap_legend | boolean Whether to show the expression heatmap legend (default=FALSE) |

| | |
|---|---|
| `border` | boolean Whether to show borders around the heatmap and annotations (default=TRUE) |
| `return.details` | boolean If TRUE will return a list containing the heatmap (ha), but also raw matrix (x), expression list (expl) and other info to produce the heatmap on your own (default=FALSE). |
| `row.label.font.size` | numeric Font size for the row labels (default=10) |
| `order.clusters` | boolean Whether to re-order the clusters according to the similarity of the expression patterns (of the genes being shown) (default=FALSE) |
| `split` | boolean Whether to use arguments "row_split" and "column_split" in ComplexHeatmap::Heatmap() (default=FALSE). These arguments are categorical vectors used to split the rows/columns in the heatmap. |
| `split.gap` | numeric Value of millimeters "mm" to use for 'row_gap' and 'column_gap' (default=0). If split is FALSE, this argument is ignored. |
| `cell.order` | explicitly supply cell order (default=NULL) |
| `averaging.window` | numeric Optional window averaging between neighboring cells within each group (turned off by default) - useful when very large number of cells shown (requires zoo package) (default=0) |
| `max.cells` | numeric Maximum cells to include in any given group (default: Inf) |
| `...` | extra parameters are passed to ComplexHeatmap::Heatmap() call |

## Value

ComplexHeatmap::Heatmap object (see return.details param for other output)

---

`projectKNNs` *Project a distance matrix into a lower-dimensional space.*

---

## Description

Takes as input a sparse matrix of the edge weights connecting each node to its nearest neighbors, and outputs a matrix of coordinates embedding the inputs in a lower-dimensional space.

## Usage

```
projectKNNs(
  wij,
  dim = 2,
  sgd_batches = NULL,
  M = 5,
  gamma = 7,
  alpha = 1,
  rho = 1,
  coords = NULL,
```

```
  useDegree = FALSE,
  momentum = NULL,
  seed = NULL,
  threads = NULL,
  verbose = getOption("verbose", TRUE)
)
```

## Arguments

| | |
|---|---|
| wij | A symmetric sparse matrix of edge weights, in C-compressed format, as created with the Matrix package. |
| dim | numeric Number of dimensions for the projection space (default=2). |
| sgd_batches | The number of edges to process during SGD (default=NULL). Defaults to a value set based on the size of the dataset. If the parameter given is between 0 and 1, the default value will be multiplied by the parameter. |
| M | numeric Number of negative edges to sample for each positive edge (default=5). |
| gamma | numeric Strength of the force pushing non-neighbor nodes apart (default=7). |
| alpha | numeric Hyperparameter used in the default distance function, $1/(1+\alpha\dot{\|\|}y_i - y_j\|\|^2)$ (default=1). The function relates the distance between points in the low-dimensional projection to the likelihood that the two points are nearest neighbors. Increasing $\alpha$ tends to push nodes and their neighbors closer together; decreasing $\alpha$ produces a broader distribution. Setting $\alpha$ to zero enables the alternative distance function. $\alpha$ below zero is meaningless. |
| rho | numeric Initial learning rate (default=1) |
| coords | An initialized coordinate matrix (default=NULL). |
| useDegree | boolean Whether to use vertex degree to determine weights (default=FALSE). If TRUE, weights determined in negative sampling; if FALSE, weights determined by the sum of the vertex's edges. See Notes. |
| momentum | If not NULL (the default), SGD with momentum is used, with this multiplier, which must be between 0 and 1. Note that momentum can drastically speed-up training time, at the cost of additional memory consumed. |
| seed | numeric Random seed to be passed to the C++ functions (default=NULL). If NULL, sampled from hardware entropy pool. Note that if the seed is not NULL (the default), the maximum number of threads will be set to 1 in phases of the algorithm that would otherwise be non-deterministic. |
| threads | numeric The maximum number of threads to spawn (default=NULL). Determined automatically if NULL. |
| verbose | boolean Verbosity (default=getOption("verbose", TRUE)) |

## Details

The algorithm attempts to estimate a dim-dimensional embedding using stochastic gradient descent and negative sampling.

The objective function is:

$$O = \sum_{(i,j) \in E} w_{ij} (\log f(||p(e_{ij} = 1||) + \sum_{k=1}^{M} E_{jk\ P_n(j)} \gamma \log(1 - f(||p(e_{ij_k} - 1||)))$$

where $f()$ is a probabilistic function relating the distance between two points in the low-dimensional projection space, and the probability that they are nearest neighbors.

The default probabilistic function is $1/(1 + \alpha \dot{||} x||^2)$. If $\alpha$ is set to zero, an alternative probabilistic function, $1/(1 + \exp(x^2))$ will be used instead.

Note that the input matrix should be symmetric. If any columns in the matrix are empty, the function will fail.

## Value

A dense [N,D] matrix of the coordinates projecting the w_ij matrix into the lower-dimensional space.

## Note

If specified, `seed` is passed to the C++ and used to initialize the random number generator. This will not, however, be sufficient to ensure reproducible results, because the initial coordinate matrix is generated using the `R` random number generator. To ensure reproducibility, call `set.seed` before calling this function, or pass it a pre-allocated coordinate matrix.

The original paper called for weights in negative sampling to be calculated according to the degree of each vertex, the number of edges connecting to the vertex. The reference implementation, however, uses the sum of the weights of the edges to each vertex. In experiments, the difference was imperceptible with small (MNIST-size) datasets, but the results seems aesthetically preferrable using degree. The default is to use the edge weights, consistent with the reference implementation.

## Examples

```
## Not run:
data(CO2)
CO2$Plant <- as.integer(CO2$Plant)
CO2$Type <- as.integer(CO2$Type)
CO2$Treatment <- as.integer(CO2$Treatment)
co <- scale(as.matrix(CO2))
# Very small datasets often produce a warning regarding the alias table.  This is safely ignored.
suppressWarnings(vis <- largeVis(t(co), K = 20, sgd_batches = 1, threads = 2))
suppressWarnings(coords <- projectKNNs(vis$wij, threads = 2))
plot(t(coords))

## End(Not run)
```

rawMatricesWithCommonGenes *Get raw matrices with common genes*

## Description

Get raw matrices with common genes

## Usage

```
rawMatricesWithCommonGenes(con.obj, sample.groups = NULL)
```

## Arguments

| | |
|---|---|
| con.obj | Conos object |
| sample.groups | list of samples to select from Conos object, con.obj$samples (default=NULL) |

## Value

raw matrices subset with common genes

saveConosForScanPy *Save Conos object on disk to read it from ScanPy*

## Description

Save Conos object on disk to read it from ScanPy

## Usage

```
saveConosForScanPy(
  con,
  output.path,
  hdf5_filename,
  metadata.df = NULL,
  cm.norm = FALSE,
  pseudo.pca = FALSE,
  pca = FALSE,
  n.dims = 100,
  embedding = TRUE,
  alignment.graph = TRUE,
  verbose = FALSE
)
```

### Arguments

| | |
|---|---|
| con | conos object |
| output.path | path to a folder, where intermediate files will be saved |
| hdf5_filename | name of HDF5 written with ScanPy files. Note: the rhdf5 package is required |
| metadata.df | data.frame with additional metadata with rownames corresponding to cell ids, which should be passed to ScanPy (default=NULL) If NULL, only information about cell ids and origin dataset will be saved. |
| cm.norm | boolean Whether to include the matrix of normalised counts (default=FALSE). |
| pseudo.pca | boolean Whether to produce an emulated PCA by embedding the graph to a space with 'n.dims' dimensions and save it as a pseudoPCA (default=FALSE). |
| pca | boolean Whether to include PCA of all the samples (not batch corrected) (default=FALSE). |
| n.dims | numeric Number of dimensions for calculating PCA and/or pseudoPCA (default=100). |
| embedding | boolean Whether to include the current conos embedding (default=TRUE). |
| alignment.graph | boolean Whether to include graph of connectivities and distances (default=TRUE). |
| verbose | boolean Whether to use verbose mode (default=FALSE) |

### Value

AnnData object for ScanPy, saved to disk

### See Also

The rhdf5 package documentation here: <https://www.bioconductor.org/packages/release/bioc/html/rhdf5.html>

---

## saveDEasCSV *Save differential expression as table in *csv format*

---

### Description

Save differential expression as table in *csv format

### Usage

```
saveDEasCSV(de.results, saveprefix, gene.metadata = NULL)
```

### Arguments

| | |
|---|---|
| de.results | output of differential expression results, corrected or uncorrected |
| saveprefix | character prefix for output file |
| gene.metadata | gene metadta to include (default=NULL) |

## saveDEasJSON *Save differential expression results as JSON*

### Description

Save differential expression results as JSON

### Usage

```
saveDEasJSON(
  de.results = NULL,
  saveprefix = NULL,
  gene.metadata = NULL,
  cluster.sep.chr = "<!!>"
)
```

### Arguments

| | |
|---|---|
| de.results | differential expression results (default=NULL) |
| saveprefix | prefix for the differential expression output (default=NULL) |
| gene.metadata | data.frame with gene metadata (default=NULL) |
| cluster.sep.chr | character string of length 1 specifying a delimiter to separate cluster and app names (default='<!!>') |

### Value

JSON with DE results

## scanKModularity *Scan joint graph modularity for a range of k (or k.self) values Builds graph with different values of k (or k.self if scan.k.self=TRUE), evaluating modularity of the resulting multilevel clustering NOTE: will run evaluations in parallel using con$n.cores (temporarily setting con$n.cores to 1 in the process)*

### Description

Scan joint graph modularity for a range of k (or k.self) values Builds graph with different values of k (or k.self if scan.k.self=TRUE), evaluating modularity of the resulting multilevel clustering NOTE: will run evaluations in parallel using con$n.cores (temporarily setting con$n.cores to 1 in the process)

### Usage

```
scanKModularity(
  con,
  min = 3,
  max = 50,
  by = 1,
  scan.k.self = FALSE,
  omit.internal.edges = TRUE,
  verbose = TRUE,
  plot = TRUE,
  ...
)
```

### Arguments

| | |
|---|---|
| con | Conos object to test |
| min | numeric Minimal value of k to test (default=3) |
| max | numeric Value of k to test (default=50) |
| by | numeric Scan step (default=1) |
| scan.k.self | boolean Whether to test dependency on scan.k.self (default=FALSE) |
| omit.internal.edges | boolean Whether to omit internal edges of the graph (default=TRUE) |
| verbose | boolean Whether to provide verbose output (default=TRUE) |
| plot | boolean Whether to plot the output (default=TRUE) |
| ... | other parameters will be passed to con$buildGraph() |

### Value

a data frame with $k $m columns giving k and the corresponding modularity

---

## sgdBatches *Calculate the default number of batches for a given number of vertices and edges. The formula used is the one used by the 'largeVis' reference implementation. This is substantially less than the recommendation $E * 10000$ in the original paper.*

---

### Description

Calculate the default number of batches for a given number of vertices and edges. The formula used is the one used by the 'largeVis' reference implementation. This is substantially less than the recommendation $E * 10000$ in the original paper.

### Usage

```
sgdBatches(N, E = 150 * N/2)
```

### Arguments

| | |
|---|---|
| N | Number of vertices |
| E | Number of edges (default = 150*N/2) |

### Value

The recommended number of sgd batches.

### Examples

```
# Observe that increasing K has no effect on processing time
N <- 70000 # MNIST
K <- 10:250
plot(K, sgdBatches(rep(N, length(K)), N * K / 2))

# Observe that processing time scales linarly with N
N <- c(seq(from = 1, to = 10000, by = 100), seq(from = 10000, to = 10000000, by = 1000))
plot(N, sgdBatches(N))
```

---

## small_panel.preprocessed

*Small pre-processed data from Pagoda2, two samples, each dimension (1000, 100)*

---

### Description

Small pre-processed data from Pagoda2, two samples, each dimension (1000, 100)

### Usage

```
small_panel.preprocessed
```

### Format

An object of class `list` of length 2.

`stableTreeClusters` *Determine number of detectable clusters given a reference walktrap and a bunch of permuted walktraps*

## Description

Determine number of detectable clusters given a reference walktrap and a bunch of permuted walktraps

## Usage

```
stableTreeClusters(
  refwt,
  tests,
  min.threshold = 0.8,
  min.size = 10,
  n.cores = 30,
  average.thresholds = FALSE
)
```

## Arguments

| | |
|---|---|
| `refwt` | reference walktrap result |
| `tests` | a list of permuted walktrap results |
| `min.threshold` | numeric Min detectability threshold (default=0.8) |
| `min.size` | numeric Minimum cluster size (number of leafs) (default=10) |
| `n.cores` | numeric Number of cores (default=30) |
| `average.thresholds` | boolean Report a single number of detectable clusters for averaged detected thresholds (default=FALSE) (a list of detected clusters for each element of the tests list is returned by default) |

## Value

number of detectable stable clusters

`velocityInfoConos` *RNA velocity analysis on samples integrated with conos Create a list of objects to pass into gene.relative.velocity.estimates function from the velocyto.R package*

## Description

RNA velocity analysis on samples integrated with conos Create a list of objects to pass into gene.relative.velocity.estimates function from the velocyto.R package

## Usage

```
velocityInfoConos(
  cms.list,
  con,
  clustering = NULL,
  groups = NULL,
  n.odgenes = 2000,
  verbose = TRUE,
  min.max.cluster.average.emat = 0.2,
  min.max.cluster.average.nmat = 0.05,
  min.max.cluster.average.smat = 0.01
)
```

## Arguments

| | |
|---|---|
| cms.list | list of velocity files written out as cell.counts.matrices.rds files by running dropest with -V option |
| con | conos object (after creating an embedding and running leiden clustering) |
| clustering | name of clustering in the conos object to use (default=NULL). Either 'clustering' or 'groups' must be provided. |
| groups | set of clusters to use (default=NULL). Ignored if 'clustering' is not NULL. |
| n.odgenes | numeric Number of overdispersed genes to use for PCA (default=2000). |
| verbose | boolean Whether to use verbose mode (default=TRUE) |
| min.max.cluster.average.emat | Required minimum average expression count for emat, the spliced (exonic) count matrix (default=0.2). Note: no normalization is perfomed. See the parameter 'min.max.cluster.average' in the function 'filter.genes.by.cluster.expression.' |
| min.max.cluster.average.nmat | Required minimum average expression count for nmat, the unspliced (nascent) count matrix (default=0.05). Note: no normalization is perfomed. See the parameter 'min.max.cluster.average' in the function 'filter.genes.by.cluster.expression.' |
| min.max.cluster.average.smat | Required minimum average expression count for smat, the spanning read matrix (used in offset calculations) (default=0.01). Note: no normalization is perfomed. See the parameter 'min.max.cluster.average' in the function 'filter.genes.by.cluster.expression.' |

## Value

List with cell distances, combined spliced expression matrix, combined unspliced expression matrix, combined matrix of spanning reads, cell colors for clusters and embedding (taken from conos)

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