

# Package ‘COTAN’

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**Type** Package

**Title** COexpression Tables ANalysis

**Version** 2.5.6

**Description** Statistical and computational method to analyze the co-expression of gene pairs at single cell level. It provides the foundation for single-cell gene interactome analysis. The basic idea is studying the zero UMI counts' distribution instead of focusing on positive counts; this is done with a generalized contingency tables framework. COTAN can effectively assess the correlated or anti-correlated expression of gene pairs. It provides a numerical index related to the correlation and an approximate p-value for the associated independence test. COTAN can also evaluate whether single genes are differentially expressed, scoring them with a newly defined global differentiation index. Moreover, this approach provides ways to plot and cluster genes according to their co-expression pattern with other genes, effectively helping the study of gene interactions and becoming a new tool to identify cell-identity marker genes.

**URL** <https://github.com/seriph78/COTAN>

**BugReports** <https://github.com/seriph78/COTAN/issues>

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ClustersList	Clusters <i>utilities</i>
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**Description**

Handle *clusterization* <-> *clusters* list conversions, *clusters* grouping and merge

**Usage**

```
toClustersList(clusters)

fromClustersList(
  clustersList,
  elemNames = vector(mode = "character"),
  throwOnOverlappingClusters = TRUE
)

groupByClustersList(elemNames, clustersList, throwOnOverlappingClusters = TRUE)

groupByClusters(clusters)

mergeClusters(clusters, names, mergedName = "")

multiMergeClusters(clusters, namesList, mergedNames = NULL)
```

**Arguments**

<code>clusters</code>	A named vector or factor that defines the <i>clusters</i>
<code>clustersList</code>	A named list whose elements define the various clusters
<code>elemNames</code>	A list of names to which associate a cluster
<code>throwOnOverlappingClusters</code>	When TRUE, in case of overlapping clusters, the function <code>fromClustersList</code> and <code>groupByClustersList</code> will throw. This is the default. When FALSE, instead, in case of overlapping clusters, <code>fromClustersList</code> will return the last cluster to which each element belongs, while <code>groupByClustersList</code> will return a vector of positions that is longer than the given <code>elemNames</code>
<code>names</code>	A list of <i>clusters</i> names to be merged
<code>mergedName</code>	The name of the new merged clusters
<code>namesList</code>	A list of lists of <i>clusters</i> names to be respectively merged
<code>mergedNames</code>	The names of the new merged <i>clusters</i>

**Details**

`toClustersList()` given a *clusterization*, creates a list of *clusters* (i.e. for each *cluster*, which elements compose the *cluster*)

`fromClustersList()` given a list of *clusters* returns a *clusterization* (i.e. a named vector that for each element indicates to which cluster it belongs)

`groupByClusters()` given a *clusterization* returns a permutation, such that using the permutation on the input the *clusters* are grouped together

groupByClustersList() given the elements' names and a list of *clusters* returns a permutation, such that using the permutation on the given names the *clusters* are grouped together.

mergeClusters() given a *clusterization*, creates a new one where the given *clusters* are merged.

multiMergeClusters() given a *clusterization*, creates a new one where the given sets of *clusters* are merged.

## Value

toClustersList() returns a list of clusters

fromClustersList() returns a clusterization. If the given elemNames contain values not present in the clustersList, those will be marked as "-1"

groupByClusters() and groupByClustersList() return a permutation that groups the clusters together. For each cluster the positions are guaranteed to be in increasing order. In case, all elements not corresponding to any cluster are grouped together as the last group

mergeClusters() returns a new *clusterization* with the wanted *clusters* being merged. If less than 2 *cluster* names were passed the function will emit a warning and return the initial *clusterization*

multiMergeClusters() returns a new *clusterization* with the wanted *clusters* being merged by consecutive iterations of [mergeClusters\(\)](#) on the given namesList

## Examples

```
## create a clusterization
clusters <- paste0("",sample(7, 100, replace = TRUE))
names(clusters) <- paste0("E_",formatC(1:100, width = 3, flag = "0"))

## create a clusters list from a clusterization
clustersList <- toClustersList(clusters)
head(clustersList, 1)

## recreate the clusterization from the cluster list
clusters2 <- fromClustersList(clustersList, names(clusters))
all.equal(factor(clusters), clusters2)

cl1Size <- length(clustersList[["1"]])

## establish the permutation that groups clusters together
perm <- groupByClusters(clusters)
!is.unsorted(head(names(clusters)[perm],cl1Size))
head(clusters[perm], cl1Size)

## it is possible to have the list of the element names different
## from the names in the clusters list
selectedNames <- paste0("E_",formatC(11:110, width = 3, flag = "0"))
perm2 <- groupByClustersList(selectedNames, toClustersList(clusters))
all.equal(perm2[91:100], c(91:100))

## is is possible to merge a few clusters together
clustersMerged <- mergeClusters(clusters, names = c("7", "2"),
                               mergedName = "7_2")
sum(table(clusters)[c(2, 7)]) == table(clustersMerged)[["7_2"]]

## it is also possible to do multiple merges at once!
## Note the default new clusters' names
clustersMerged2 <-
```

```

multiMergeClusters(clusters2, namesList = list(c("2", "7"),
                                              c("1", "3", "5")))
table(clustersMerged2)

```

---

COTAN-class

*Definition of the COTAN class*


---

### Description

Definition of the COTAN class

### Slots

raw dgMatrix - the raw UMI count matrix  $n \times m$  (gene number  $\times$  cell number)

genesCoex dspMatrix - the correlation of COTAN between genes,  $n \times n$

cellsCoex dspMatrix - the correlation of COTAN between cells,  $m \times m$

metaDataset data.frame

metaCells data.frame

clustersCoex a list of COEX data.frames for each clustering in the metaCells

---

COTAN-Legacy

*Handle legacy scCOTAN-class and related symmetric matrix <-> vector conversions*


---

### Description

A class and some functions related to the V1 version of the COTAN package

### Usage

```
vec2mat_rfast(x, genes = "all")
```

```
mat2vec_rfast(mat)
```

### Arguments

x a list formed by two arrays: genes with the unique gene names and values with all the values.

genes an array with all wanted genes or the string "all". When equal to "all" (the default), it recreates the entire matrix.

mat a square (possibly symmetric) matrix with all genes as row and column names.

**Details**

Define the legacy `scCOTAN`-class

This is a legacy function related to old `scCOTAN` objects. Use the more appropriate `Matrix::dspMatrix` type for similar functionality.

`mat2vec_rfast` converts a compacted symmetric matrix (that is an array) into a symmetric matrix.

This is a legacy function related to old `scCOTAN` objects. Use the more appropriate `Matrix::dspMatrix` type for similar functionality.

`vec2mat_rfast` converts a symmetric matrix into a compacted symmetric matrix. It will forcibly make its argument symmetric.

**Value**

a `scCOTAN` object

`mat2vec_rfast` returns a list formed by two arrays:

- "genes" with the unique gene names,
- "values" with all the values.

`vec2mat_rfast` returns the reconstructed symmetric matrix

**Slots**

`raw` ANY. To store the raw data matrix

`raw.norm` ANY. To store the raw data matrix divided for the cell efficiency estimated (`nu`)

`coex` ANY. The coex matrix

`nu` vector.

`lambda` vector.

`a` vector.

`hk` vector.

`n_cells` numeric.

`meta` data.frame.

`yes_yes` ANY. Unused and deprecated. Kept for backward compatibility only

`clusters` vector.

`cluster_data` data.frame.

**Examples**

```
v <- list("genes" = paste0("gene_", c(1:9)), "values" = c(1:45))
```

```
M <- vec2mat_rfast(v)
all.equal(rownames(M), v[["genes"]])
all.equal(colnames(M), v[["genes"]])
```

```
genes <- paste0("gene_", sample.int(ncol(M), 3))
```

```
m <- vec2mat_rfast(v, genes)
all.equal(rownames(m), v[["genes"]])
all.equal(colnames(m), genes)
```

```
v2 <- mat2vec_rfast(M)
all.equal(v, v2)
```

---

COTANObjectCreation    COTAN *shortcuts*

---

## Description

These functions create a COTAN object and/or also run all the necessary steps until the genes' COEX matrix is calculated.

## Usage

```
COTAN(raw = "ANY")

## S4 method for signature 'COTAN'
proceedToCoex(
  objCOTAN,
  calcCoex = TRUE,
  optimizeForSpeed = TRUE,
  deviceStr = "cuda",
  cores = 1L,
  saveObj = TRUE,
  outDir = "."
)

automaticCOTANObjectCreation(
  raw,
  GEO,
  sequencingMethod,
  sampleCondition,
  calcCoex = TRUE,
  optimizeForSpeed = TRUE,
  deviceStr = "cuda",
  cores = 1L,
  saveObj = TRUE,
  outDir = "."
)
```

## Arguments

raw	a matrix or dataframe with the raw counts
objCOTAN	a newly created COTAN object
calcCoex	a Boolean to determine whether to calculate the genes' COEX or stop just before at the <a href="#">estimateDispersionBisection()</a> step
optimizeForSpeed	Boolean; when TRUE COTAN tries to use the torch library to run the matrix calculations. Otherwise, or when the library is not available will run the slower legacy code

deviceStr	On the torch library enforces which device to use to run the calculations. Possible values are "cpu" to use the system <i>CPU</i> , "cuda" to use the system <i>GPUs</i> or something like "cuda:0" to restrict to a specific device
cores	number of cores to use. Default is 1.
saveObj	Boolean flag; when TRUE saves intermediate analyses and plots to file
outDir	an existing directory for the analysis output.
GEO	a code reporting the GEO identification or other specific dataset code
sequencingMethod	a string reporting the method used for the sequencing
sampleCondition	a string reporting the specific sample condition or time point.

### Details

Constructor of the class COTAN

proceedToCoex() takes a newly created COTAN object (or the result of a call to dropGenesCells()) and runs [calculateCoex\(\)](#)

automaticCOTANObjectCreation() takes a raw dataset, creates and initializes a COTAN object and runs [proceedToCoex\(\)](#)

### Value

a COTAN object

proceedToCoex() returns the updated COTAN object with genes' COEX calculated. If asked to, it will also store the object, along all relevant clean-plots, in the output directory.

automaticCOTANObjectCreation() returns the new COTAN object with genes' COEX calculated. When asked, it will also store the object, along all relevant clean-plots, in the output directory.

### Examples

```
data("test.dataset")
obj <- COTAN(raw = test.dataset)

#
# In case one needs to run more steps to clean the dataset
# the following might apply
if (FALSE) {
  objCOTAN <- initializeMetaDataset(objCOTAN,
                                    GEO = "test",
                                    sequencingMethod = "artificial",
                                    sampleCondition = "test dataset")
#
# doing all the cleaning...
#
# in case the genes' `COEX` is not needed it can be skipped
# (e.g. when calling [cellsUniformClustering()])
objCOTAN <- proceedToCoex(objCOTAN, calcCoex = FALSE,
                          cores = 6L, optimizeForSpeed = TRUE,
                          deviceStr = "cuda", saveObj = FALSE)
}
```



```
## Otherwise it is possible to run all at once.
objCOTAN <- automaticCOTANObjectCreation(
  raw = test.dataset,
  GEO = "code",
  sequencingMethod = "10X",
  sampleCondition = "mouse_dataset",
  calcCoex = TRUE,
  saveObj = FALSE,
  outDir = tempdir(),
  cores = 6L)
```

---

Datasets

*Data-sets*

---

## Description

Simple data-sets included in the package

## Usage

```
data(raw.dataset)
```

```
data(ERCCraw)
```

```
data(test.dataset)
```

```
data(test.dataset.clusters1)
```

```
data(test.dataset.clusters2)
```

```
data(vignette.split.clusters)
```

```
data(vignette.merge.clusters)
```

## Format

`raw.dataset` is a data frame with 2000 genes and 815 cells

`ERCCraw` is a data.frame

`test.dataset` is a data.frame with 600 genes and 1200 cells

`test.dataset.clusters1` is a character array

`test.dataset.clusters2` is a character array

`vignette.split.clusters` is a factor

`vignette.merge.clusters` is a factor

**Details**

`raw.dataset` is a sub-sample of a real *scRNA-seq* data-set

ERCCRaw dataset

`test.dataset` is an artificial data set obtained by sampling target negative binomial distributions on a set of 600 genes on 2 two cells *clusters* of 600 cells each. Each *clusters* has its own set of parameters for the distributions even, but a fraction of the genes has the same expression in both *clusters*.

`test.dataset.clusters1` is the *clusterization* obtained running `cellsUniformClustering()` on the `test.dataset`

`test.dataset.clusters2` is the *clusterization* obtained running `mergeUniformCellsClusters()` on the `test.dataset` using the previous *clusterization*

`vignette.split.clusters` is the clusterization obtained running `cellsUniformClustering()` on the vignette dataset (mouse cortex E17.5, GEO: GSM2861514)

`vignette.merge.clusters` is the clusterization obtained running `mergeUniformCellsClusters()` on the vignette dataset (mouse cortex E17.5, GEO: GSM2861514) using the previous *clusterization*

**Source**

GEO GSM2861514

ERCC

---

getColorsVector

*getColorsVector*

---

**Description**

This function returns a list of colors based on the `brewer.pal()` function

**Usage**

```
getColorsVector(numNeededColors = 0L)
```

**Arguments**

`numNeededColors`

The number of returned colors. If omitted it returns all available colors

**Details**

The colors are taken from the `brewer.pal.info()` sets with Set1, Set2, Set3 placed first.

**Value**

an array of RGB colors of the wanted size

**Examples**

```
colorsVector <- getColorsVector(17)
```

---

getGDI,COTAN-method      *Calculations of genes statistics*

---

### Description

A collection of functions returning various statistics associated to the genes. In particular the *discrepancy* between the expected probabilities of zero and their actual occurrences, both at single gene level or looking at genes' pairs

To make the GDI more specific, it may be desirable to restrict the set of genes against which GDI is computed to a selected subset, with the recommendation to include a consistent fraction of cell-identity genes, and possibly focusing on markers specific for the biological question of interest (for instance neural cortex layering markers). In this case we denote it as *Local Differentiation Index* (LDI) relative to the selected subset.

### Usage

```
## S4 method for signature 'COTAN'
getGDI(objCOTAN)

## S4 method for signature 'COTAN'
storeGDI(objCOTAN, genesGDI)

genesCoexSpace(objCOTAN, primaryMarkers, numGenesPerMarker = 25L)

establishGenesClusters(
  objCOTAN,
  groupMarkers,
  numGenesPerMarker = 25L,
  kCuts = 6L,
  distance = "cosine",
  hclustMethod = "ward.D2"
)

calculateGenesCE(objCOTAN)

calculateGDIGivenCorr(corr, numDegreesOfFreedom, rowsFraction = 0.05)

calculateGDI(objCOTAN, statType = "S", rowsFraction = 0.05)

calculatePValue(
  objCOTAN,
  statType = "S",
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character")
)

calculatePDI(
  objCOTAN,
  statType = "S",
  geneSubsetCol = vector(mode = "character"),
```

```
geneSubsetRow = vector(mode = "character")
)
```

### Arguments

objCOTAN	a COTAN object
genesGDI	the named genes' GDI array to store or the output data . frame of the function <a href="#">calculateGDI()</a>
primaryMarkers	A vector of primary marker names.
numGenesPerMarker	the number of correlated genes to keep as other markers (default 25)
groupMarkers	a named list with an element for each group comprised of one or more marker genes
kCuts	the number of estimated <i>cluster</i> (this defines the height for the tree cut)
distance	type of distance to use. Default is "cosine". Can be chosen among those supported by <a href="#">parallelDist::parDist()</a>
hclustMethod	default is "ward.D2" but can be any method defined by <a href="#">stats::hclust()</a> function
corr	a matrix object, possibly a subset of the columns of the full symmetric matrix
numDegreesOfFreedom	a int that determines the number of degree of freedom to use in the $\chi^2$ test
rowsFraction	The fraction of rows that will be averaged to calculate the GDI. Defaults to 5%
statType	Which statistics to use to compute the p-values. By default it will use the "S" (Pearson's $\chi^2$ test) otherwise the "G" (G-test)
geneSubsetCol	an array of genes. It will be put in columns. If left empty the function will do it genome-wide.
geneSubsetRow	an array of genes. It will be put in rows. If left empty the function will do it genome-wide.

### Details

[getGDI\(\)](#) extracts the genes' **GDI** array as it was stored by the method [storeGDI\(\)](#)

[storeGDI\(\)](#) stored and already calculated genes' GDI array in a COTAN object. It can be retrieved using the method [getGDI\(\)](#)

[genesCoexSpace\(\)](#) calculates genes groups based on the primary markers and uses them to prepare the genes' COEX space data . frame.

[establishGenesClusters\(\)](#) perform the genes' clustering based on a pool of gene markers, using the genes' COEX space

[calculateGenesCE\(\)](#) is used to calculate the discrepancy between the expected probability of zero and the observed zeros across all cells for each gene as *cross-entropy*:  $-\sum_c \mathbb{1}_{X_c=0} \log(p_c) - \mathbb{1}_{X_c \neq 0} \log(1 - p_c)$  where  $X_c$  is the observed count and  $p_c$  the probability of zero

[calculateGDIGivenCorr\(\)](#) produces a vector with the *GDI* for each column based on the given correlation matrix, using the *Pearson's  $\chi^2$  test*

[calculateGDI\(\)](#) produces a data . frame with the *GDI* for each gene based on the COEX matrix

[calculatePValue\(\)](#) computes the p-values for genes in the COTAN object. It can be used genome-wide or by setting some specific genes of interest. By default it computes the *p-values* using the S statistics ( $\chi^2$ )

[calculatePDI\(\)](#) computes the p-values for genes in the COTAN object using [calculatePValue\(\)](#) and takes their log ( $-\log(\cdot)$ ) to calculate the genes' *Pair Differential Index*



## Description

These are the functions and methods used to calculate the **COEX** matrices according to the COTAN model. From there it is possible to calculate the associated *pValue* and the *GDI (Global Differential Expression)*

The **COEX** matrix is defined by following formula:

$$\frac{\sum_{i,j \in \{Y, N\}} (-1)^{\#\{i,j\}} \frac{O_{ij} - E_{ij}}{1 \vee E_{ij}}}{\sqrt{n \sum_{i,j \in \{Y, N\}} \frac{1}{1 \vee E_{ij}}}}$$

where  $O$  and  $E$  are the observed and expected contingency tables and  $n$  is the relevant numerosity (the number of genes/cells depending on given actOnCells flag).

The formula can be more effectively implemented as:

$$\sqrt{\frac{1}{n} \sum_{i,j \in \{Y, N\}} \frac{1}{1 \vee E_{ij}}} (O_{YY} - E_{YY})$$

once one notices that  $O_{ij} - E_{ij} = (-1)^{\#\{i,j\}} r$  for some constant  $r$  for all  $i, j \in \{Y, N\}$ .

The latter follows from the fact that the relevant marginal sums of the expected contingency tables were enforced to match the marginal sums of the observed ones.

The new implementation of the function relies on the `torch` package. This implies that it is potentially able to use the system GPU to run the heavy duty calculations required by this method. However installing the `torch` package on a system can be *finicky*, so we tentatively provide a short help page [Installing\\_torch](#) hoping that it will help...

## Usage

```
getMu(objCOTAN)

## S4 method for signature 'COTAN'
getGenesCoex(
  objCOTAN,
  genes = vector(mode = "character"),
  zeroDiagonal = TRUE,
  ignoreSync = FALSE
)

## S4 method for signature 'COTAN'
getCellsCoex(
  objCOTAN,
  cells = vector(mode = "character"),
  zeroDiagonal = TRUE,
  ignoreSync = FALSE
)
```

```
## S4 method for signature 'COTAN'
isCoexAvailable(objCOTAN, actOnCells = FALSE, ignoreSync = FALSE)

## S4 method for signature 'COTAN'
dropGenesCoex(objCOTAN)

## S4 method for signature 'COTAN'
dropCellsCoex(objCOTAN)

calculateLikelihoodOfObserved(objCOTAN)

observedContingencyTablesYY(
  objCOTAN,
  actOnCells = FALSE,
  asDspMatrices = FALSE
)

observedPartialContingencyTablesYY(
  objCOTAN,
  columnsSubset,
  zeroOne = NULL,
  actOnCells = FALSE
)

observedContingencyTables(objCOTAN, actOnCells = FALSE, asDspMatrices = FALSE)

observedPartialContingencyTables(
  objCOTAN,
  columnsSubset,
  zeroOne = NULL,
  actOnCells = FALSE
)

expectedContingencyTablesNN(
  objCOTAN,
  actOnCells = FALSE,
  asDspMatrices = FALSE,
  optimizeForSpeed = TRUE
)

expectedPartialContingencyTablesNN(
  objCOTAN,
  columnsSubset,
  probZero = NULL,
  actOnCells = FALSE,
  optimizeForSpeed = TRUE
)

expectedContingencyTables(
  objCOTAN,
  actOnCells = FALSE,
```

```

    asDspMatrices = FALSE,
    optimizeForSpeed = TRUE
  )

  expectedPartialContingencyTables(
    objCOTAN,
    columnsSubset,
    probZero = NULL,
    actOnCells = FALSE,
    optimizeForSpeed = TRUE
  )

  contingencyTables(objCOTAN, g1, g2)

  ## S4 method for signature 'COTAN'
  calculateCoex(
    objCOTAN,
    actOnCells = FALSE,
    returnPPFract = FALSE,
    optimizeForSpeed = TRUE,
    deviceStr = "cuda"
  )

  calculatePartialCoex(
    objCOTAN,
    columnsSubset,
    probZero = NULL,
    zeroOne = NULL,
    actOnCells = FALSE,
    optimizeForSpeed = TRUE
  )

  calculateS(
    objCOTAN,
    geneSubsetCol = vector(mode = "character"),
    geneSubsetRow = vector(mode = "character")
  )

  calculateG(
    objCOTAN,
    geneSubsetCol = vector(mode = "character"),
    geneSubsetRow = vector(mode = "character")
  )

```

### Arguments

objCOTAN	a COTAN object
genes	The given genes' names to select the wanted COEX columns. If missing all columns will be returned. When not empty a proper result is provided by calculating the partial COEX matrix on the fly
zeroDiagonal	When TRUE sets the diagonal to zero.



ignoreSync	When TRUE ignores whether the lambda/nu/dispersion have been updated since the COEX matrix was calculated.
cells	The given cells' names to select the wanted COEX columns. If missing all columns will be returned. When not empty a proper result is provided by calculating the partial COEX matrix on the fly
actOnCells	Boolean; when TRUE the function works for the cells, otherwise for the genes
asDspMatrices	Boolean; when TRUE the function will return only packed dense symmetric matrices
columnsSubset	a sub-set of the columns of the matrices that will be returned
zeroOne	the raw count matrix projected to 0 or 1. If not given the appropriate one will be calculated on the fly
optimizeForSpeed	Boolean; deprecated: always TRUE
probZero	is the expected <b>probability of zero</b> for each gene/cell pair. If not given the appropriate one will be calculated on the fly
g1	a gene
g2	another gene
returnPPFract	Boolean; when TRUE the function returns the fraction of genes/cells pairs for which the <i>expected contingency table</i> is smaller than 0.5. Default is FALSE
deviceStr	On the torch library enforces which device to use to run the calculations. Possible values are "cpu" to use the system CPU, "cuda" to use the system GPUs or something like "cuda:0" to restrict to a specific device
geneSubsetCol	an array of genes. It will be put in columns. If left empty the function will do it genome-wide.
geneSubsetRow	an array of genes. It will be put in rows. If left empty the function will do it genome-wide.

## Details

getMu() calculates the vector  $\mu = \lambda \times \nu^T$

getGenesCoex() extracts a complete (or a partial after genes dropping) genes' COEX matrix from the COTAN object.

getCellsCoex() extracts a complete (or a partial after cells dropping) cells' COEX matrix from the COTAN object.

isCoexAvailable() allows to query whether the relevant COEX matrix from the COTAN object is available to use

dropGenesCoex() drops the genesCoex member from the given COTAN object

dropCellsCoex() drops the cellsCoex member from the given COTAN object

calculateLikelihoodOfObserved() gives for each cell and each gene the likelihood of the observed zero/one data

observedContingencyTablesYY() calculates observed *Yes/Yes* field of the contingency table

observedPartialContingencyTablesYY() calculates observed *Yes/Yes* field of the contingency table

observedContingencyTables() calculates the observed contingency tables. When the parameter asDspMatrices == TRUE, the method will effectively throw away the lower half from the returned

observedYN and observedNY matrices, but, since they are transpose one of another, the full information is still available.

observedPartialContingencyTables() calculates the observed contingency tables.

expectedContingencyTablesNN() calculates the expected *No/No* field of the contingency table

expectedPartialContingencyTablesNN() calculates the expected *No/No* field of the contingency table

expectedContingencyTables() calculates the expected values of contingency tables. When the parameter `asDspMatrices == TRUE`, the method will effectively throw away the lower half from the returned `expectedYN` and `expectedNY` matrices, but, since they are transpose one of another, the full information is still available.

expectedPartialContingencyTables() calculates the expected values of contingency tables, restricted to the specified column sub-set

contingencyTables() returns the observed and expected contingency tables for a given pair of genes. The implementation runs the same algorithms used to calculate the full observed/expected contingency tables, but restricted to only the relevant genes and thus much faster and less memory intensive

calculateCoex() estimates and stores the COEX matrix in the `cellCoex` or `genesCoex` field depending on given `actOnCells` flag. It also calculates the percentage of *problematic* genes/cells pairs. A pair is *problematic* when one or more of the expected counts were significantly smaller than 1 ( $< 0.5$ ). These small expected values signal that scant information is present for such a pair.

calculatePartialCoex() estimates a sub-section of the COEX matrix in the `cellCoex` or `genesCoex` field depending on given `actOnCells` flag. It also calculates the percentage of *problematic* genes/cells pairs. A pair is *problematic* when one or more of the expected counts were significantly smaller than 1 ( $< 0.5$ ). These small expected values signal that scant information is present for such a pair.

calculateS() calculates the statistics **S** for genes contingency tables. It always has the diagonal set to zero.

calculateG() calculates the statistics *G-test* for genes contingency tables. It always has the diagonal set to zero. It is proportional to the genes' presence mutual information.

## Value

getMu() returns the mu matrix

getGenesCoex() returns the genes' COEX values

getCellsCoex() returns the cells' COEX values

isCoexAvailable() returns whether relevant COEX matrix has been calculated and, in case, if it is still aligned to the estimators.

dropGenesCoex() returns the updated COTAN object

dropCellsCoex() returns the updated COTAN object

calculateLikelihoodOfObserved() returns a `data.frame` with the likelihood of the observed zero/one

observedContingencyTablesYY() returns a list with:

- `observedYY` the *Yes/Yes* observed contingency table as matrix
- `observedY` the full *Yes* observed vector

observedPartialContingencyTablesYY() returns a list with:

- observedYY the *Yes/Yes* observed contingency table as matrix, restricted to the selected columns as named list with elements
- observedY the full *Yes* observed vector

observedContingencyTables() returns the observed contingency tables as named list with elements:

- "observedNN"
- "observedNY"
- "observedYN"
- "observedYY"

observedPartialContingencyTables() returns the observed contingency tables, restricted to the selected columns, as named list with elements:

- "observedNN"
- "observedNY"
- "observedYN"
- "observedYY"

expectedContingencyTablesNN() returns a list with:

- expectedNN the *No/No* expected contingency table as matrix
- expectedN the *No* expected vector

expectedPartialContingencyTablesNN() returns a list with:

- expectedNN the *No/No* expected contingency table as matrix, restricted to the selected columns, as named list with elements
- expectedN the full *No* expected vector

expectedContingencyTables() returns the expected contingency tables as named list with elements:

- "expectedNN"
- "expectedNY"
- "expectedYN"
- "expectedYY"

expectedPartialContingencyTables() returns the expected contingency tables, restricted to the selected columns, as named list with elements:

- "expectedNN"
- "expectedNY"
- "expectedYN"
- "expectedYY"

contingencyTables() returns a list containing the observed and expected contingency tables

calculateCoex() returns the updated COTAN object

calculatePartialCoex() returns the asked section of the COEX matrix

calculateS() returns the S matrix

calculateG() returns the G matrix

**Note**

The sum of the matrices returned by the function `observedContingencyTables()` and `expectedContingencyTables()` will have the same value on all elements. This value is the number of genes/cells depending on the parameter `actOnCells` being TRUE/FALSE.

**See Also**

[ParametersEstimations](#) for more details.

[Installing\\_torch](#) about the torch package

**Examples**

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- initializeMetaDataset(objCOTAN, GEO = "test_GEO",
                                sequencingMethod = "distribution_sampling",
                                sampleCondition = "reconstructed_dataset")

objCOTAN <- clean(objCOTAN)

objCOTAN <- estimateDispersionBisection(objCOTAN, cores = 6L)

## Now the `COTAN` object is ready to calculate the genes' `COEX`

## mu <- getMu(objCOTAN)
## observedY <- observedContingencyTablesYY(objCOTAN, asDspMatrices = TRUE)
obs <- observedContingencyTables(objCOTAN, asDspMatrices = TRUE)

## expectedN <- expectedContingencyTablesNN(objCOTAN, asDspMatrices = TRUE)
exp <- expectedContingencyTables(objCOTAN, asDspMatrices = TRUE)

objCOTAN <- calculateCoex(objCOTAN, actOnCells = FALSE)

stopifnot(isCoexAvailable(objCOTAN))
genesCoex <- getGenesCoex(objCOTAN)
genesSample <- sample(getNumGenes(objCOTAN), 10)
partialGenesCoex <- calculatePartialCoex(objCOTAN, genesSample,
                                         actOnCells = FALSE)

identical(partialGenesCoex,
          getGenesCoex(objCOTAN, getGenes(objCOTAN)[sort(genesSample)]))

## S <- calculateS(objCOTAN)
## G <- calculateG(objCOTAN)
## pValue <- calculatePValue(objCOTAN)
gdiDF <- calculateGDI(objCOTAN)
objCOTAN <- storeGDI(objCOTAN, genesGDI = gdiDF)

## Touching any of the lambda/nu/dispersino parameters invalidates the `COEX`
## matrix and derivatives, so it can be dropped it from the `COTAN` object
objCOTAN <- dropGenesCoex(objCOTAN)
stopifnot(!isCoexAvailable(objCOTAN))

objCOTAN <- estimateDispersionNuBisection(objCOTAN, cores = 6L)

## Now the `COTAN` object is ready to calculate the cells' `COEX`
```

```

## In case one need to caclualte both it is more sensible to run the above
## before any `COEX` evaluation

g1 <- getGenes(objCOTAN)[sample(getNumGenes(objCOTAN), 1)]
g2 <- getGenes(objCOTAN)[sample(getNumGenes(objCOTAN), 1)]
tables <- contingencyTables(objCOTAN, g1 = g1, g2 = g2)
tables

objCOTAN <- calculateCoex(objCOTAN, actOnCells = TRUE)
stopifnot(isCoexAvailable(objCOTAN, actOnCells = TRUE, ignoreSync = TRUE))
cellsCoex <- getCellsCoex(objCOTAN)

cellsSample <- sample(getNumCells(objCOTAN), 10)
partialCellsCoex <- calculatePartialCoex(objCOTAN, cellsSample,
                                         actOnCells = TRUE)

identical(partialCellsCoex, cellsCoex[, sort(cellsSample)])

objCOTAN <- dropCellsCoex(objCOTAN)
stopifnot(!isCoexAvailable(objCOTAN, actOnCells = TRUE))

lh <- calculateLikelihoodOfObserved(objCOTAN)

```

---

HandleMetaData

*Handling meta-data in COTAN objects*


---

## Description

Much of the information stored in the COTAN object is compacted into three data.frames:

- "metaDataset" - contains all general information about the data-set
- "metaGenes" - contains genes' related information along the lambda and dispersion vectors and the fully-expressed flag
- "metaCells" - contains cells' related information along the nu vector, the fully-expressing flag, the *clusterizations* and the *conditions*

## Usage

```

## S4 method for signature 'COTAN'
getMetadataDataset(objCOTAN)

## S4 method for signature 'COTAN'
getMetadataElement(objCOTAN, tag)

## S4 method for signature 'COTAN'
getMetadataGenes(objCOTAN)

## S4 method for signature 'COTAN'
getMetadataCells(objCOTAN)

## S4 method for signature 'COTAN'

```

```

getDims(objCOTAN)

datasetTags()

## S4 method for signature 'COTAN'
initializeMetaDataset(objCOTAN, GEO, sequencingMethod, sampleCondition)

## S4 method for signature 'COTAN'
addElementToMetaDataset(objCOTAN, tag, value)

getColumnFromDF(df, colName)

setColumnInDF(df, colToSet, colName, rowNames = vector(mode = "character"))

```

### Arguments

objCOTAN	a COTAN object
tag	the new information tag
GEO	a code reporting the GEO identification or other specific data-set code
sequencingMethod	a string reporting the method used for the sequencing
sampleCondition	a string reporting the specific sample condition or time point
value	a value (or an array) containing the information
df	the data.frame
colName	the name of the new or existing column in the data.frame
colToSet	the column to add
rowNames	when not empty, if the input data.frame has no real row names, the new row names of the resulting data.frame

### Details

getMetadataDataset() extracts the meta-data stored for the current data-set.

getMetadataElement() extracts the value associated with the given tag if present or an empty string otherwise.

getMetadataGenes() extracts the meta-data stored for the genes

getMetadataCells() extracts the meta-data stored for the cells

getDims() extracts the sizes of all slots of the COTAN object

datasetTags() defines a list of short names associated to an enumeration. It also defines the relative long names as they appear in the meta-data

initializeMetaDataset() initializes meta-data data-set

addElementToMetaDataset() is used to add a line of information to the meta-data data.frame. If the tag was already used it will update the associated value(s) instead

getColumnFromDF() is a function to extract a column from a data.frame, while keeping the rowNames as vector names

setColumnInDF() is a function to append, if missing, or resets, if present, a column into a data.frame, whether the data.frame is empty or not. The given rowNames are used only in the case the data.frame has only the default row numbers, so this function cannot be used to override row names

**Value**

`getMetadataDataset()` returns the meta-data `data.frame`  
`getMetadataElement()` returns a string with the relevant value  
`getMetadataGenes()` returns the genes' meta-data `data.frame`  
`getMetadataCells()` returns the cells' meta-data `data.frame`  
`getDims()` returns a named list with the sizes of the slots  
`datasetTags()` a named character array with the standard labels used in the `metaDataset` of the COTAN objects  
`initializeMetaDataset()` returns the given COTAN object with the updated `metaDataset`  
`addElementToMetaDataset()` returns the updated COTAN object  
`getColumnFromDF()` returns the column in the `data.frame` as named array, NULL if the wanted column is not available  
`setColumnInDF()` returns the updated, or the newly created, `data.frame`

**Examples**

```

data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

objCOTAN <- initializeMetaDataset(objCOTAN, GEO = "test_GEO",
                                sequencingMethod = "distribution_sampling",
                                sampleCondition = "reconstructed_dataset")

objCOTAN <- addElementToMetaDataset(objCOTAN, "Test",
                                    c("These are ", "some values"))

dataSetInfo <- getMetadataDataset(objCOTAN)

numInitialCells <- getMetadataElement(objCOTAN, "cells")

metaGenes <- getMetadataGenes(objCOTAN)

metaCells <- getMetadataCells(objCOTAN)

allSizes <- getDims(objCOTAN)

```

---

HandlingClusterizations

*Handling cells' clusterization and related functions*


---

**Description**

These functions manage the *clusterizations* and their associated *cluster* COEX `data.frames`.

A *clusterization* is any partition of the cells where to each cell it is assigned a **label**; a group of cells with the same label is called *cluster*.

For each *cluster* is also possible to define a COEX value for each gene, indicating its increased or decreased expression in the *cluster* compared to the whole background. A `data.frame` with

these values listed in a column for each *cluster* is stored separately for each *clusterization* in the `clustersCoex` member.

The formulae for this *In/Out* COEX are similar to those used in the `calculateCoex()` method, with the **role** of the second gene taken by the *In/Out* status of the cells with respect to each *cluster*.

## Usage

```
## S4 method for signature 'COTAN'
estimateNuLinearByCluster(objCOTAN, clName = "", clusters = NULL)

## S4 method for signature 'COTAN'
getClusterizations(objCOTAN, dropNoCoex = FALSE, keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getClusterizationName(objCOTAN, clName = "", keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getClusterizationData(objCOTAN, clName = "")

getClusters(objCOTAN, clName = "")

## S4 method for signature 'COTAN'
getClustersCoex(objCOTAN)

## S4 method for signature 'COTAN'
addClusterization(
  objCOTAN,
  clName,
  clusters,
  coexDF = data.frame(),
  override = FALSE
)

## S4 method for signature 'COTAN'
addClusterizationCoex(objCOTAN, clName, coexDF)

## S4 method for signature 'COTAN'
dropClusterization(objCOTAN, clName)

DEAOnClusters(objCOTAN, clName = "", clusters = NULL)

pValueFromDEA(coexDF, numCells, adjustmentMethod = "none")

logFoldChangeOnClusters(
  objCOTAN,
  clName = "",
  clusters = NULL,
  floorLambdaFraction = 0.05
)

distancesBetweenClusters(
  objCOTAN,
```



```
    clName = "",
    clusters = NULL,
    coexDF = NULL,
    useDEA = TRUE,
    distance = NULL
  )

UMAPPlot(
  df,
  clusters = NULL,
  elements = NULL,
  title = "",
  colors = NULL,
  numNeighbors = 0L,
  minPointsDist = NA
)

cellsUMAPPlot(
  objCOTAN,
  clName = "",
  clusters = NULL,
  dataMethod = "",
  genesSel = "HVG_Seurat",
  numGenes = 2000L,
  colors = NULL,
  numNeighbors = 0L,
  minPointsDist = NA
)

clustersDeltaExpression(objCOTAN, clName = "", clusters = NULL)

clustersMarkersHeatmapPlot(
  objCOTAN,
  groupMarkers = list(),
  clName = "",
  clusters = NULL,
  kCuts = 3L,
  condNameList = NULL,
  conditionsList = NULL
)

clustersSummaryData(
  objCOTAN,
  clName = "",
  clusters = NULL,
  condName = "",
  conditions = NULL
)

clustersSummaryPlot(
  objCOTAN,
  clName = "",
```

```

clusters = NULL,
condName = "",
conditions = NULL,
plotTitle = ""
)

clustersTreePlot(
  objCOTAN,
  kCuts,
  clName = "",
  clusters = NULL,
  useDEA = TRUE,
  distance = NULL,
  hclustMethod = "ward.D2"
)

findClustersMarkers(
  objCOTAN,
  n = 10L,
  markers = NULL,
  clName = "",
  clusters = NULL,
  coexDF = NULL,
  adjustmentMethod = "bonferroni"
)

geneSetEnrichment(clustersCoex, groupMarkers = list())

reorderClusterization(
  objCOTAN,
  clName = "",
  clusters = NULL,
  coexDF = NULL,
  reverse = FALSE,
  keepMinusOne = TRUE,
  useDEA = TRUE,
  distance = NULL,
  hclustMethod = "ward.D2"
)

```

### Arguments

<code>objCOTAN</code>	a COTAN object
<code>clName</code>	The name of the <i>clusterization</i> . If not given the last available <i>clusterization</i> will be used, as it is probably the most significant!
<code>clusters</code>	A <i>clusterization</i> to use. If given it will take precedence on the one indicated by <code>clName</code>
<code>dropNoCoex</code>	When TRUE drops the names from the <i>clusterizations</i> with empty associated coex <code>data.frame</code>
<code>keepPrefix</code>	When TRUE returns the internal name of the <i>clusterization</i> : the one with the <code>CL_</code> prefix.

coexDF	a data.frame where each column indicates the COEX for each of the <i>clusters</i> of the <i>clusterization</i>
override	When TRUE silently allows overriding data for an existing <i>clusterization</i> name. Otherwise the default behavior will avoid potential data losses
numCells	the number of overall cells in all <i>clusters</i>
adjustmentMethod	<i>p-value</i> multi-test adjustment method. Defaults to "bonferroni"; use "none" for no adjustment
floorLambdaFraction	Indicates the lower bound to the average count sums inside or outside the cluster for each gene as fraction of the relevant lambda parameter. Default is 5%
useDEA	Boolean indicating whether to use the <i>DEA</i> to define the distance; alternatively it will use the average <i>Zero-One</i> counts, that is faster but less precise.
distance	type of distance to use. Default is "cosine" for <i>DEA</i> and "euclidean" for <i>Zero-One</i> . Can be chosen among those supported by <code>parallelDist::parDist()</code>
df	The data.frame to plot. It must have a row names containing the given elements
elements	a named list of elements to label. Each array in the list will be shown with a different color
title	a string giving the plot title. Will default to UMAP Plot if not specified
colors	an array of colors to use in the plot. If not sufficient colors are given it will complete the list using colors from <code>getColorVector()</code>
numNeighbors	Overrides the <code>n_neighbors</code> value from <code>umap.defaults</code>
minPointsDist	Overrides the <code>min_dist</code> value from <code>umap.defaults</code>
dataMethod	selects the method to use to create the data.frame to pass to the <code>UMAPPlot()</code> . The following methods calculate, for each cell, a statistic for each gene based on available data/model. The following methods are supported: <ul style="list-style-type: none"> <li>• "NuNorm" uses the <i><math>\nu</math>-normalized</i> counts</li> <li>• "LogNormalized" uses the <i>log-normalized</i> counts. The default method</li> <li>• "Likelihood" uses the likelihood of observed presence/absence of each gene</li> <li>• "LogLikelihood" uses the likelihood of observed presence/absence of each gene</li> <li>• "Binarized" uses the binarized data matrix</li> <li>• "AdjBinarized" uses the binarized data matrix where ones and zeros are replaced by the per-gene estimated probability of zero and its complement respectively</li> </ul>
genesSel	Decides whether and how to perform gene-selection. It can be a string indicating one of the following methods or a straight list of genes. The following methods are supported: <ul style="list-style-type: none"> <li>• "HGDI" Will pick-up the genes with highest <b>GDI</b>. Since it requires an available COEX matrix it will fall-back to "HVG" when the matrix is not available</li> <li>• "HVG" Will pick-up the genes with the highest variability. The default method.</li> <li>• "None" Will use all genes</li> </ul>
numGenes	the number of genes to select using the above method. Will be ignored when no selection have been asked or when an explicit list of genes was passed in

groupMarkers	an optional named list with an element for each group comprised of one or more marker genes
kCuts	the number of estimated <i>cluster</i> (this defines the height for the tree cut)
condNameList	a list of <i>conditions</i> ' names to be used for additional columns in the final plot. When none are given no new columns will be added using data extracted via the function <code>clustersSummaryData()</code>
conditionsList	a list of <i>conditions</i> to use. If given they will take precedence on the ones indicated by <code>condNameList</code>
condName	The name of a condition in the COTAN object to further separate the cells in more sub-groups. When no condition is given it is assumed to be the same for all cells (no further sub-divisions)
conditions	The <i>conditions</i> to use. If given it will take precedence on the one indicated by <code>condName</code> that will only indicate the relevant column name in the returned <code>data.frame</code>
plotTitle	The title to use for the returned plot
hclustMethod	It defaults is "ward.D2" but can be any of the methods defined by the <code>stats::hclust()</code> function.
n	the number of extreme COEX values to return
markers	a list of marker genes
clustersCoex	the COEX <code>data.frame</code>
reverse	a flag to the output order
keepMinusOne	a flag to decide whether to keep the cluster "-1" (representing the non-clustered cells) untouched

## Details

`estimateNuLinearByCluster()` does a linear estimation of nu: cells' counts averages normalized *cluster* by *cluster*

`getClusterizations()` extracts the list of the *clusterizations* defined in the COTAN object.

`getClusterizationName()` normalizes the given *clusterization* name or, if none were given, returns the name of last available *clusterization* in the COTAN object. It can return the *clusterization internal name* if needed

`getClusterizationData()` extracts the asked *clusterization* and its associated COEX `data.frame` from the COTAN object

`getClusters()` extracts the asked *clusterization* from the COTAN object

`getClustersCoex()` extracts the full `clusterCoex` member list

`addClusterization()` adds a *clusterization* to the current COTAN object, by adding a new column in the `metaCells data.frame` and adding a new element in the `clustersCoex` list using the passed in COEX `data.frame` or an empty `data.frame` if none were passed in.

`addClusterizationCoex()` adds a *clusterization* COEX `data.frame` to the current COTAN object. It requires the named *clusterization* to be already present.

`dropClusterization()` drops a *clusterization* from the current COTAN object, by removing the corresponding column in the `metaCells data.frame` and the corresponding COEX `data.frame` from the `clustersCoex` list.

`DEAOnClusters()` is used to run the Differential Expression analysis using the COTAN contingency tables on each *cluster* in the given *clusterization*

pValueFromDEA() is used to convert to *p-value* the Differential Expression analysis using the COTAN contingency tables on each *cluster* in the given *clusterization*

logFoldChangeOnClusters() is used to get the log difference of the expression levels for each *cluster* in the given *clusterization* against the rest of the data-set

distancesBetweenClusters() is used to obtain a distance between the clusters. Depending on the value of the useDEA flag will base the distance on the *DEA* columns or the averages of the *Zero-One* matrix.

UMAPPlot() plots the given data.frame containing genes information related to clusters after applying the `umap::umap()` transformation

cellsUMAPPlot() returns a ggplot2 plot where the given *clusters* are placed on the base of their relative distance. Also if needed calculates and stores the DEA of the relevant *clusterization*.

clustersDeltaExpression() estimates the change in genes' expression inside the *cluster* compared to the average situation in the data set.

clustersMarkersHeatmapPlot() returns the heatmap plot of a summary score for each *cluster* and each gene marker list in the given *clusterization*. It also returns the numerosity and percentage of each *cluster* on the right and a gene *clusterization* dendrogram on the left (as returned by the function `geneSetEnrichment()`) that allows to estimate which markers groups are more or less expressed in each *cluster* so it is easier to derive the *clusters'* cell types.

clustersSummaryData() calculates various statistics about each cluster (with an optional further condition to separate the cells).

clustersSummaryPlot() calculates various statistics about each cluster via `clustersSummaryData()` and puts them together into a plot.

clustersTreePlot() returns the dendrogram plot where the given *clusters* are placed on the base of their relative distance. Also if needed calculates and stores the DEA of the relevant *clusterization*.

findClustersMarkers() takes in a COTAN object and a *clusterization* and produces a data.frame with the *n* most positively enriched and the *n* most negatively enriched genes for each *cluster*. The function also provides whether and the found genes are in the given markers list or not. It also returns the *adjusted p-value* for multi-tests using the `stats::p.adjust()`

geneSetEnrichment() returns a cumulative score of enrichment in a *cluster* over a gene set. In formulae it calculates  $\frac{1}{n} \sum_i (1 - e^{-\theta X_i})$ , where the  $X_i$  are the positive values from `DEAOnClusters()` and  $\theta = -\frac{1}{0.1} \ln(0.25)$

reorderClusterization() takes in a *clusterizations* and reorder its labels so that in the new order near labels indicate near clusters according to a *DEA* (or *Zero-One*) based distance

## Value

estimateNuLinearByCluster() returns the updated COTAN object

getClusterizations() returns a vector of *clusterization* names, usually without the CL\_ prefix

getClusterizationName() returns the normalized *clusterization* name or NULL if no *clusterizations* are present

getClusterizationData() returns a list with 2 elements:

- "clusters" the named cluster labels array
- "coex" the associated COEX data.frame. This will be an **empty** data.frame when not specified for the relevant *clusterization*

getClusters() returns the named cluster labels array

`getClustersCoex()` returns the list with a COEX data.frame for each *clusterization*. When not empty, each data.frame contains a COEX column for each *cluster*.

`addClusterization()` returns the updated COTAN object

`addClusterizationCoex()` returns the updated COTAN object

`dropClusterization()` returns the updated COTAN object

`DEAOnClusters()` returns the co-expression data.frame for the genes in each *cluster*

`pValueFromDEA()` returns a data.frame containing the *p-values* corresponding to the given COEX adjusted for *multi-test*

`logFoldChangeOnClusters()` returns the log-expression-change data.frame for the genes in each *cluster*

`distancesBetweenClusters()` returns a dist object

`UMAPPlot()` returns a ggplot2 object

`cellsUMAPPlot()` returns a list with 2 objects:

- "plot" a ggplot2 object representing the umap plot
- "cellsPCA" the data.frame PCA used to create the plot

`clustersDeltaExpression()` returns a data.frame with the weighted discrepancy of the expression of each gene within the *cluster* against model expectations

`clustersMarkersHeatmapPlot()` returns a list with:

- "heatmapPlot" the complete heatmap plot
- "dataScore" the data.frame with the score values

`clustersSummaryData()` returns a data.frame with the following statistics: The calculated statistics are:

- "clName" the *cluster labels*
- "condName" the relevant condition (that sub-divides the *clusters*)
- "CellNumber" the number of cells in the group
- "MeanUDE" the average "UDE" in the group of cells
- "MedianUDE" the median "UDE" in the group of cells
- "ExpGenes25" the number of genes expressed in at the least 25% of the cells in the group
- "ExpGenes" the number of genes expressed at the least once in any of the cells in the group
- "CellPercentage" fraction of the cells with respect to the total cells

`clustersSummaryPlot()` returns a list with a data.frame and a ggplot objects

- "data" contains the data,
- "plot" is the returned plot

`clustersTreePlot()` returns a list with 2 objects:

- "dend" a ggplot2 object representing the dendrogram plot
- "objCOTAN" the updated COTAN object

`findClustersMarkers()` returns a data.frame containing n genes for each *cluster* scoring top/bottom COEX scores. The data.frame also contains:

- "CL" the cluster

- "Gene" the gene
- "Score" the COEX score of the gene
- "adjPVal" the *p-values* associated to the COEX adjusted for *multi-testing*
- "DEA" the differential expression of the gene
- "IsMarker" whether the gene is among the given markers
- "logFoldCh" the *log-fold-change* of the gene expression inside versus outside the cluster from `logFoldChangeOnClusters()`

`geneSetEnrichment()` returns a `data.frame` with the cumulative score

`reorderClusterization()` returns a list with 2 elements:

- "clusters" the newly reordered cluster labels array
- "coex" the associated COEX `data.frame`
- "permMap" the reordering mapping

### Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- proceedToCoex(objCOTAN, cores = 6L, calcCoex = FALSE,
                          optimizeForSpeed = TRUE, saveObj = FALSE)

data("test.dataset.clusters1")
clusters <- test.dataset.clusters1

coexDF <- DEAOnClusters(objCOTAN, clusters = clusters)

groupMarkers <- list(G1 = c("g-000010", "g-000020", "g-000030",
                           "g-000150", "g-000160", "g-000170"),
                    G2 = c("g-000300", "g-000330", "g-000450",
                           "g-000460", "g-000470"),
                    G3 = c("g-000510", "g-000530", "g-000550",
                           "g-000570", "g-000590"))

geneClusters <- rep(1:3, each = 240)[1:600]
names(geneClusters) <- getGenes(objCOTAN)

umapPlot <- UMAPPlot(coexDF, clusters = NULL, elements = groupMarkers)
plot(umapPlot)

objCOTAN <- addClusterization(objCOTAN, clName = "first_clusterization",
                             clusters = clusters, coexDF = coexDF)

lfcDF <- logFoldChangeOnClusters(objCOTAN, clusters = clusters)
umapPlot2 <- UMAPPlot(lfcDF, clusters = geneClusters)
plot(umapPlot2)

objCOTAN <- estimateNuLinearByCluster(objCOTAN, clusters = clusters)

clSummaryPlotAndData <-
  clustersSummaryPlot(objCOTAN, clName = "first_clusterization",
                     plotTitle = "first clusterization")
plot(clSummaryPlotAndData[["plot"]])
```

```

if (FALSE) {
  objCOTAN <- dropClusterization(objCOTAN, "first_clusterization")
}

clusterizations <- getClusterizations(objCOTAN, dropNoCoex = TRUE)
stopifnot(length(clusterizations) == 1)

cellsUmapPlotAndDF <- cellsUMAPPlot(objCOTAN, dataMethod = "LogNormalized",
                                   clName = "first_clusterization",
                                   genesSel = "HVG_Seurat")
plot(cellsUmapPlotAndDF[["plot"]])

enrichment <- geneSetEnrichment(clustersCoex = coexDF,
                                groupMarkers = groupMarkers)

clHeatmapPlotAndData <- clustersMarkersHeatmapPlot(objCOTAN, groupMarkers)

conditions <- as.integer(substring(getCells(objCOTAN), 3L))
conditions <- factor(ifelse(conditions <= 600, "L", "H"))
names(conditions) <- getCells(objCOTAN)

clHeatmapPlotAndData2 <-
  clustersMarkersHeatmapPlot(objCOTAN, groupMarkers, kCuts = 2,
                             condNameList = list("High/Low"),
                             conditionsList = list(conditions))

clName <- getClusterizationName(objCOTAN)

clusterDataList <- getClusterizationData(objCOTAN, clName = clName)

clusters <- getClusters(objCOTAN, clName = clName)

allClustersCoexDF <- getClustersCoex(objCOTAN)

deltaExpression <- clustersDeltaExpression(objCOTAN, clusters = clusters)

summaryData <- clustersSummaryData(objCOTAN)

treePlotAndObj <- clustersTreePlot(objCOTAN, 2)
objCOTAN <- treePlotAndObj[["objCOTAN"]]
plot(treePlotAndObj[["dend"]])

clMarkers <- findClustersMarkers(objCOTAN, markers = list(),
                                clusters = clusters)

```

---

HandlingConditions      *Handling cells' conditions and related functions*

---

## Description

These functions manage the *conditions*.

A *condition* is a set of **labels** that can be assigned to cells: one **label** per cell. This is especially useful in cases when the data-set is the result of merging multiple experiments' raw data



**Usage**

```

## S4 method for signature 'COTAN'
getAllConditions(objCOTAN, keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getConditionName(objCOTAN, condName = "", keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getCondition(objCOTAN, condName = "")

normalizeNameAndLabels(objCOTAN, name = "", labels = NULL, isCond = FALSE)

## S4 method for signature 'COTAN'
addCondition(objCOTAN, condName, conditions, override = FALSE)

## S4 method for signature 'COTAN'
dropCondition(objCOTAN, condName)

```

**Arguments**

objCOTAN	a COTAN object
keepPrefix	When TRUE returns the internal name of the <i>condition</i> : the one with the COND_ prefix.
condName	the name of an existing <i>condition</i> .
name	the name of the <i>clusterization/condition</i> . If not given the last available <i>clusterization</i> will be used, or no <i>conditions</i>
labels	a <i>clusterization/condition</i> to use. If given it will take precedence on the one indicated by name
isCond	a Boolean to indicate whether the function is dealing with <i>clusterizations</i> FALSE or <i>conditions</i> TRUE
conditions	a (factors) array of <i>condition labels</i>
override	When TRUE silently allows overriding data for an existing <i>condition</i> name. Otherwise the default behavior will avoid potential data losses

**Details**

getAllConditions() extracts the list of the *conditions* defined in the COTAN object.

getConditionName() normalizes the given *condition* name or, if none were given, returns the name of last available *condition* in the COTAN object. It can return the *condition internal name* if needed

getCondition() extracts the asked *condition* from the COTAN object

normalizeNameAndLabels() takes a pair of name/labels and normalize them based on the available information in the COTAN object

addCondition() adds a *condition* to the current COTAN object, by adding a new column in the metaCells data.frame

dropCondition() drops a *condition* from the current COTAN object, by removing the corresponding column in the metaCells data.frame

**Value**

getAllConditions() returns a vector of *conditions* names, usually without the COND\_ prefix

getConditionName() returns the normalized *condition* name or NULL if no *conditions* are present

getCondition() returns a named factor with the *condition*

normalizeNameAndLabels() returns a list with:

- "name" the relevant name
- "labels" the relevant *clusterization/condition*

addCondition() returns the updated COTAN object

dropCondition() returns the updated COTAN object

**Examples**

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

cellLine <- rep(c("A", "B"), getNumCells(objCOTAN) / 2)
names(cellLine) <- getCells(objCOTAN)
objCOTAN <- addCondition(objCOTAN, condName = "Line", conditions = cellLine)

##objCOTAN <- dropCondition(objCOTAN, "Genre")

conditionsNames <- getAllConditions(objCOTAN)

condName <- getConditionName(objCOTAN)

condition <- getCondition(objCOTAN, condName = condName)
isa(condition, "factor")

nameAndCond <- normalizeNameAndLabels(objCOTAN, name = condName,
                                     isCond = TRUE)
isa(nameAndCond[["labels"]], "factor")
```

---

HeatmapPlots

*Heatmap Plots*


---

**Description**

These functions create heatmap COEX plots.

**Usage**

```
singleHeatmapDF(objCOTAN, genesLists, sets, pValueThreshold = 0.01)
```

```
heatmapPlot(
  objCOTAN = NULL,
  genesLists,
  sets = NULL,
  pValueThreshold = 0.01,
```

```

    conditions = NULL,
    dir = "."
)

genesHeatmapPlot(
  objCOTAN,
  primaryMarkers,
  secondaryMarkers = vector(mode = "character"),
  pValueThreshold = 0.01,
  symmetric = TRUE
)

cellsHeatmapPlot(objCOTAN, cells = NULL, clusters = NULL)

plotTheme(plotKind = "common", textSize = 14L)

```

### Arguments

<code>objCOTAN</code>	a COTAN object
<code>genesLists</code>	A list of genes' arrays. The first array defines the genes in the columns
<code>sets</code>	A numeric array indicating which fields in the previous list should be used. Defaults to all fields
<code>pValueThreshold</code>	The p-value threshold. Default is 0.01
<code>conditions</code>	An array of prefixes indicating the different files
<code>dir</code>	The directory in which are all COTAN files (corresponding to the previous prefixes)
<code>primaryMarkers</code>	A set of genes plotted as rows
<code>secondaryMarkers</code>	A set of genes plotted as columns
<code>symmetric</code>	A Boolean: default TRUE. When TRUE the union of <code>primaryMarkers</code> and <code>secondaryMarkers</code> is used for both rows and column genes
<code>cells</code>	Which cells to plot (all if no argument is given)
<code>clusters</code>	Use this clusterization to select/reorder the cells to plot
<code>plotKind</code>	a string indicating the plot kind
<code>textSize</code>	axes and strip text size (default=14)

### Details

`singleHeatmapDF()` creates the heatmap data.frame of one COTAN object

`heatmapPlot()` creates the heatmap of one or more COTAN objects

`genesHeatmapPlot()` is used to plot an *heatmap* made using only some genes, as markers, and collecting all other genes correlated with these markers with a p-value smaller than the set threshold. Than all relations are plotted. Primary markers will be plotted as groups of rows. Markers list will be plotted as columns.

`cellsHeatmapPlot()` creates the heatmap plot of the cells' COEX matrix

`plotTheme()` returns the appropriate theme for the selected plot kind. Supported kinds are: "common", "pca", "genes", "UDE", "heatmap", "GDI", "UMAP", "size-plot"

**Value**

singleHeatmapDF() returns a data.frame  
 heatmapPlot() returns a ggplot2 object  
 genesHeatmapPlot() returns a ggplot2 object  
 cellsHeatmapPlot() returns the cells' COEX *heatmap* plot  
 plotTheme() returns a ggplot2::theme object

**See Also**

[ggplot2::theme\(\)](#) and [ggplot2::ggplot\(\)](#)

**Examples**

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- clean(objCOTAN)
objCOTAN <- estimateDispersionNuBisection(objCOTAN, cores = 6L)
objCOTAN <- calculateCoex(objCOTAN, actOnCells = FALSE)
objCOTAN <- calculateCoex(objCOTAN, actOnCells = TRUE)

## some genes
primaryMarkers <- c("g-000010", "g-000020", "g-000030")

## an example of named list of different gene set
groupMarkers <- list(G1 = primaryMarkers,
                     G2 = c("g-000300", "g-000330"),
                     G3 = c("g-000510", "g-000530", "g-000550",
                             "g-000570", "g-000590"))

hPlot <- heatmapPlot(objCOTAN, pValueThreshold = 0.05,
                    genesLists = groupMarkers, sets = 2L:3L)
plot(hPlot)

ghPlot <- genesHeatmapPlot(objCOTAN, primaryMarkers = primaryMarkers,
                          secondaryMarkers = groupMarkers,
                          pValueThreshold = 0.05, symmetric = FALSE)
plot(ghPlot)

clusters <- c(rep_len("1", getNumCells(objCOTAN)/2),
             rep_len("2", getNumCells(objCOTAN)/2))
names(clusters) <- getCells(objCOTAN)

chPlot <- cellsHeatmapPlot(objCOTAN, clusters = clusters)
## plot(chPlot)

theme <- plotTheme("pca")
```

**Description**

A brief explanation of how to install the torch package on WSL2 (Windows Subsystem for Linux), but it might work the same for other Linux systems. Naturally it makes a difference whether one wants to install support only for the CPU or also have the system GPU at the ready!

The main resources to install torch is <https://torch.mlverse.org/docs/articles/installation.html> or <https://cran.r-project.org/web/packages/torch/vignettes/installation.html>

**Details**

For the CPU-only support one need to ensure that also numeric libraries are installed, like BLAS and LAPACK and/or MKL if your CPU is from *Intel*. Otherwise torch will be stuck at using a single core for all computations.

For the GPU, currently only cuda devices are supported. Moreover only some specific versions of cuda (and corresponding cudnn) are effectively usable, so one needs to install them to actually use the GPU.

As of today only cuda 11.7 and 11.8 are supported, but check the torch documentation for more up-to-date information. Before downgrading your cuda version, please be aware that it is possible to maintain separate main versions of cuda at the same time on the system: that is one can have installed both 11.8 and a 12.4 cuda versions on the same system.

Below a link to install cuda 11.8 for WSL2 given: use a local installer to be sure the wanted cuda version is being installed, and not the latest one: [cuda 11.8 for WSL2](#)

**Description**

Logging is currently supported for all COTAN functions. It is possible to see the output on the terminal and/or on a log file. The level of output on terminal is controlled by the COTAN.LogLevel option while the logging on file is always at its maximum verbosity

**Usage**

```
setLoggingLevel(newLevel = 1L)
```

```
setLoggingFile(logFileName)
```

```
logThis(msg, logLevel = 2L, appendLF = TRUE)
```

**Arguments**

newLevel	the new default logging level. It defaults to 1
logFileName	the log file.
msg	the message to print
logLevel	the logging level of the current message. It defaults to 2
appendLF	whether to add a new-line character at the end of the message

## Details

`setLoggingLevel()` sets the COTAN logging level. It set the `COTAN.LogLevel` options to one of the following values:

- 0 - Always on log messages
- 1 - Major log messages
- 2 - Minor log messages
- 3 - All log messages

`setLoggingFile()` sets the log file for all COTAN output logs. By default no logging happens on a file (only on the console). Using this function COTAN will use the indicated file to dump the logs produced by all `logThis()` commands, independently from the log level. It stores the connection created by the call to `bzfile()` in the option: `COTAN.LogFile`

`logThis()` prints the given message string if the current log level is greater or equal to the given log level (it always prints its message on file if active). It uses `message()` to actually print the messages on the `stderr()` connection, so it is subject to `suppressMessages()`

## Value

`setLoggingLevel()` returns the old logging level or default level if not set yet.

`logThis()` returns TRUE if the message has been printed on the terminal

## Examples

```
setLoggingLevel(3) # for debugging purposes only

logFile <- file.path(".", "COTAN_Test1.log")
setLoggingFile(logFile)
logThis("Some log message")
setLoggingFile("") # closes the log file
file.remove(logFile)

logThis("LogLevel 0 messages will always show, ",
        logLevel = 0, appendLF = FALSE)
suppressMessages(logThis("unless all messages are suppressed",
                        logLevel = 0))
```

## Description

A set of function helper related to the statistical model underlying the COTAN package

**Usage**

```
funProbZero(dispersion, mu)
```

```
dispersionBisection(
  sumZeros,
  lambda,
  nu,
  threshold = 0.001,
  maxIterations = 100L
)
```

```
parallelDispersionBisection(
  genes,
  sumZeros,
  lambda,
  nu,
  threshold = 0.001,
  maxIterations = 100L
)
```

```
nuBisection(
  sumZeros,
  lambda,
  dispersion,
  initialGuess,
  threshold = 0.001,
  maxIterations = 100L
)
```

```
parallelNuBisection(
  cells,
  sumZeros,
  lambda,
  dispersion,
  initialGuess,
  threshold = 0.001,
  maxIterations = 100L
)
```

**Arguments**

dispersion	the estimated dispersion (a $n$ -sized vector)
mu	the lambda times nu values (a $n \times m$ matrix)
sumZeros	the number of genes not expressed in the relevant cell (a $m$ -sized vector)
lambda	the estimated lambda (a $n$ -sized vector)
nu	the estimated nu (a $m$ -sized vector)
threshold	minimal solution precision
maxIterations	max number of iterations (avoids infinite loops)
genes	names of the relevant genes
initialGuess	the initial guess for nu (a $m$ -sized vector)
cells	names of the relevant cells

## Details

funProbZero is a private function that gives the probability that a sample gene's reads are zero, given the dispersion and mu parameters.

Using  $d$  for disp and  $\mu$  for mu, it returns:  $(1 + d\mu)^{-\frac{1}{d}}$  when  $d > 0$  and  $\exp((d - 1)\mu)$  otherwise. The function is continuous in  $d = 0$ , increasing in  $d$  and decreasing in  $\mu$ . It returns 0 when  $d = -\infty$  or  $\mu = \infty$ . It returns 1 when  $\mu = 0$ .

dispersionBisection is a private function for the estimation of *dispersion* slot of a COTAN object via a bisection solver

The goal is to find a dispersion value that reduces to zero the difference between the number of estimated and counted zeros

parallelDispersionBisection is a private function invoked by `estimateDispersionBisection()` for the estimation of the dispersion slot of a COTAN object via a parallel bisection solver

The goal is to find a dispersion array that reduces to zero the difference between the number of estimated and counted zeros

nuBisection is a private function for the estimation of nu slot of a COTAN object via a bisection solver

The goal is to find a nu value that reduces to zero the difference between the number of estimated and counted zeros

parallelNuBisection is a private function invoked by `estimateNuBisection()` for the estimation of nu slot of a COTAN object via a parallel bisection solver

The goal is to find a nu array that reduces to zero the difference between the number of estimated and counted zeros

## Value

the probability matrix that a *read count* is identically zero

the dispersion value

the dispersion values

the nu value

the dispersion values

---

ParametersEstimations *Estimation of the COTAN model's parameters*

---

## Description

These functions are used to estimate the COTAN model's parameters. That is the average count for each gene (lambda) the average count for each cell (nu) and the dispersion parameter for each gene to match the probability of zero.

The estimator methods are named Linear if they can be calculated as a linear statistic of the raw data or Bisection if they are found via a parallel bisection solver.



**Usage**

```
## S4 method for signature 'COTAN'
estimateLambdaLinear(objCOTAN)

## S4 method for signature 'COTAN'
estimateNuLinear(objCOTAN)

## S4 method for signature 'COTAN'
estimateDispersionBisection(
  objCOTAN,
  threshold = 0.001,
  cores = 1L,
  maxIterations = 100L,
  chunkSize = 1024L
)

## S4 method for signature 'COTAN'
estimateNuBisection(
  objCOTAN,
  threshold = 0.001,
  cores = 1L,
  maxIterations = 100L,
  chunkSize = 1024L
)

## S4 method for signature 'COTAN'
estimateDispersionNuBisection(
  objCOTAN,
  threshold = 0.001,
  cores = 1L,
  maxIterations = 100L,
  chunkSize = 1024L,
  enforceNuAverageToOne = TRUE
)

## S4 method for signature 'COTAN'
estimateDispersionNuLminb(
  objCOTAN,
  threshold = 0.001,
  maxIterations = 50L,
  chunkSize = 1024L,
  enforceNuAverageToOne = TRUE
)

## S4 method for signature 'COTAN'
getNu(objCOTAN)

## S4 method for signature 'COTAN'
getLambda(objCOTAN)

## S4 method for signature 'COTAN'
getDispersion(objCOTAN)
```

```

estimatorsAreReady(objCOTAN)

getNuNormData(objCOTAN)

getLogNormData(objCOTAN)

getNormalizedData(objCOTAN, retLog = FALSE)

getProbabilityOfZero(objCOTAN)

```

### Arguments

objCOTAN	a COTAN object
threshold	minimal solution precision
cores	number of cores to use. Default is 1.
maxIterations	max number of iterations (avoids infinite loops)
chunkSize	number of genes to solve in batch in a single core. Default is 1024.
enforceNuAverageToOne	a Boolean on whether to keep the average nu equal to 1
retLog	When TRUE calls <a href="#">getLogNormData()</a> , calls <a href="#">getNuNormData()</a>

### Details

[estimateLambdaLinear\(\)](#) does a linear estimation of lambda (genes' counts averages)

[estimateNuLinear\(\)](#) does a linear estimation of nu (normalized cells' counts averages)

[estimateDispersionBisection\(\)](#) estimates the negative binomial dispersion factor for each gene (a). Determines the dispersion such that, for each gene, the probability of zero count matches the number of observed zeros. It assumes [estimateNuLinear\(\)](#) being already run.

[estimateNuBisection\(\)](#) estimates the nu vector of a COTAN object by bisection. It determines the nu parameters such that, for each cell, the probability of zero counts matches the number of observed zeros. It assumes [estimateDispersionBisection\(\)](#) being already run. Since this breaks the assumption that the average nu is one, it is recommended not to run this in isolation but use [estimateDispersionNuBisection\(\)](#) instead.

[estimateDispersionNuBisection\(\)](#) estimates the dispersion and nu field of a COTAN object by running sequentially a bisection for each parameter.

[estimateDispersionNuNlminb\(\)](#) estimates the nu and dispersion parameters to minimize the discrepancy between the observed and expected probability of zero. It uses the [stats::nlminb\(\)](#) solver, but since the joint parameters have too high dimensionality, it converges too slowly to be actually useful in real cases.

[getNu\(\)](#) extracts the nu array (normalized cells' counts averages)

[getLambda\(\)](#) extracts the lambda array (mean expression for each gene)

[getDispersion\(\)](#) extracts the dispersion array

[estimatorsAreReady\(\)](#) checks whether the estimators arrays lambda, nu, dispersion are available

[getNuNormData\(\)](#) extracts the  $\nu$ -normalized count table (i.e. where each column is divided by nu) and returns it

[getLogNormData\(\)](#) extracts the log-normalized count table (i.e. where each column is divided by the [getCellsSize\(\)](#)), takes its  $\log_{10}$  and returns it.

`getNormalizedData()` is deprecated: please use `getNuNormData()` or `getLogNormData()` directly as appropriate

`getProbabilityOfZero()` gives for each cell and each gene the probability of observing zero reads

## Value

`estimateLambdaLinear()` returns the updated COTAN object

`estimateNuLinear()` returns the updated COTAN object

`estimateDispersionBisection()` returns the updated COTAN object

`estimateNuBisection()` returns the updated COTAN object

`estimateDispersionNuBisection()` returns the updated COTAN object

`estimateDispersionNuNlminb()` returns the updated COTAN object

`getNu()` returns the nu array

`getLambda()` returns the lambda array

`getDispersion()` returns the dispersion array

`estimatorsAreReady()` returns a boolean specifying whether all three arrays are non-empty

`getNuNormData()` returns the  *$\nu$ -normalized* count data.frame

`getLogNormData()` returns a data.frame after applying the formula  $\log_{10}(10^4 * x + 1)$  to the raw counts normalized by *cells-size*

`getNormalizedData()` returns a data.frame

`getProbabilityOfZero()` returns a data.frame with the probabilities of zero

## Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

objCOTAN <- estimateLambdaLinear(objCOTAN)
lambda <- getLambda(objCOTAN)

objCOTAN <- estimateNuLinear(objCOTAN)
nu <- getNu(objCOTAN)

objCOTAN <- estimateDispersionBisection(objCOTAN, cores = 6L)
dispersion <- getDispersion(objCOTAN)

objCOTAN <- estimateDispersionNuBisection(objCOTAN, cores = 6L,
                                          enforceNuAverageToOne = TRUE)
nu <- getNu(objCOTAN)
dispersion <- getDispersion(objCOTAN)

nuNorm <- getNuNormData(objCOTAN)

logNorm <- getLogNormData(objCOTAN)

logNorm <- getNormalizedData(objCOTAN, retLog = TRUE)

probZero <- getProbabilityOfZero(objCOTAN)
```

RawDataCleaning

*Raw data cleaning***Description**

These methods are to be used to clean the raw data. That is drop any number of genes/cells that are too sparse or too present to allow proper calibration of the COTAN model.

We call genes that are expressed in all cells *Fully-Expressed* while cells that express all genes in the data are called *Fully-Expressing*. In case it has been made quite easy to exclude the flagged genes/cells in the user calculations.

**Usage**

```
## S4 method for signature 'COTAN'
flagNotFullyExpressedGenes(objCOTAN)

## S4 method for signature 'COTAN'
flagNotFullyExpressingCells(objCOTAN)

## S4 method for signature 'COTAN'
getFullyExpressedGenes(objCOTAN)

## S4 method for signature 'COTAN'
getFullyExpressingCells(objCOTAN)

## S4 method for signature 'COTAN'
findFullyExpressedGenes(objCOTAN, cellsThreshold = 0.99)

## S4 method for signature 'COTAN'
findFullyExpressingCells(objCOTAN, genesThreshold = 0.99)

## S4 method for signature 'COTAN'
dropGenesCells(
  objCOTAN,
  genes = vector(mode = "character"),
  cells = vector(mode = "character")
)

ECDPlot(objCOTAN, yCut)

## S4 method for signature 'COTAN'
clean(
  objCOTAN,
  cellsCutoff = 0.003,
  genesCutoff = 0.002,
  cellsThreshold = 0.99,
  genesThreshold = 0.99
)

cleanPlots(objCOTAN, includePCA = TRUE)
```

```

cellSizePlot(objCOTAN, splitPattern = " ", numCol = 2L)

genesSizePlot(objCOTAN, splitPattern = " ", numCol = 2L)

mitochondrialPercentagePlot(
  objCOTAN,
  splitPattern = " ",
  numCol = 2L,
  genePrefix = "^MT-"
)

scatterPlot(objCOTAN, splitPattern = " ", numCol = 2L, splitSamples = FALSE)

```

### Arguments

objCOTAN	a COTAN object
cellsThreshold	any gene that is expressed in more cells than threshold times the total number of cells will be marked as <b>fully-expressed</b> . Default threshold is 0.99 (99.0%)
genesThreshold	any cell that is expressing more genes than threshold times the total number of genes will be marked as <b>fully-expressing</b> . Default threshold is 0.99 (99.0%)
genes	an array of gene names
cells	an array of cell names
yCut	y threshold of library size to drop
cellsCutoff	clean() will delete from the raw data any gene that is expressed in less cells than threshold times the total number of cells. Default cutoff is 0.003 (0.3%)
genesCutoff	clean() will delete from the raw data any cell that is expressing less genes than threshold times the total number of genes. Default cutoff is 0.002 (0.2%)
includePCA	a Boolean flag to determine whether to calculate the <i>PCA</i> associated with the normalized matrix. When TRUE the first four elements of the returned list will be NULL
splitPattern	Pattern used to extract, from the column names, the sample field (default " ")
numCol	Once the column names are split by splitPattern, the column number with the sample name (default 2)
genePrefix	Prefix for the mitochondrial genes (default "^MT-" for Human, mouse "^mt-")
splitSamples	Boolean. Whether to plot each sample in a different panel (default FALSE)

### Details

flagNotFullyExpressedGenes() returns a Boolean array with TRUE for those genes that are not fully-expressed.

flagNotFullyExpressingCells() returns a Boolean vector with TRUE for those cells that are not expressing all genes

getFullyExpressedGenes() returns the genes expressed in all cells of the dataset

getFullyExpressingCells() returns the cells that did express all genes of the dataset

findFullyExpressedGenes() determines the fully-expressed genes inside the raw data

findFullyExpressingCells() determines the cells that are expressing all genes in the dataset

`dropGenesCells()` removes an array of genes and/or cells from the current COTAN object.

`ECDPlot()` plots the empirical distribution function of library sizes (UMI number). It helps to define where to drop "cells" that are simple background signal.

`clean()` is the main method that can be used to check and clean the dataset. It will discard any genes that has less than 3 non-zero counts per thousand cells and all cells expressing less than 2 per thousand genes. It also produces and stores the estimators for  $\nu$  and  $\lambda$

`cleanPlots()` creates the plots associated to the output of the `clean()` method.

`cellSizePlot()` plots the raw library size for each cell and sample.

`genesSizePlot()` plots the raw gene number (reads > 0) for each cell and sample

`mitochondrialPercentagePlot()` plots the raw library size for each cell and sample.

`scatterPlot()` creates a plot that check the relation between the library size and the number of genes detected.

## Value

`flagNotFullyExpressedGenes()` returns a Booleans array with TRUE for genes that are not fully-expressed

`flagNotFullyExpressingCells()` returns an array of Booleans with TRUE for cells that are not expressing all genes

`getFullyExpressedGenes()` returns an array containing all genes that are expressed in all cells

`getFullyExpressingCells()` returns an array containing all cells that express all genes

`findFullyExpressedGenes()` returns the given COTAN object with updated **fully-expressed** genes' information

`findFullyExpressingCells()` returns the given COTAN object with updated **fully-expressing** cells' information

`dropGenesCells()` returns a completely new COTAN object with the new raw data obtained after the indicated genes/cells were expunged. All remaining data is dropped too as no more relevant with the restricted matrix. Exceptions are:

- the meta-data for the data-set that gets kept unchanged
- the meta-data of genes/cells that gets restricted to the remaining elements. The columns calculated via estimate and find methods are dropped too

`ECDPlot()` returns an ECD plot

`clean()` returns the updated COTAN object

`cleanPlots()` returns a list of ggplot2 plots:

- "pcaCells" is for pca cells
- "pcaCellsData" is the data of the pca cells (can be plotted)
- "genes" is for B group cells' genes
- "UDE" is for cells' UDE against their pca
- "nu" is for cell  $\nu$
- "zoomedNu" is the same but zoomed on the left and with an estimate for the low  $\nu$  threshold that defines problematic cells

`cellSizePlot()` returns the violin-boxplot plot

`genesSizePlot()` returns the violin-boxplot plot

`mitochondrialPercentagePlot()` returns a list with:

- "plot" a violin-boxplot object
- "sizes" a sizes data.frame

scatterPlot() returns the scatter plot

## Examples

```
library(zeallot)

data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

genes.to.rem <- getGenes(objCOTAN)[grep('^MT', getGenes(objCOTAN))]
cells.to.rem <- getCells(objCOTAN)[which(getCellsSize(objCOTAN) == 0)]
objCOTAN <- dropGenesCells(objCOTAN, genes.to.rem, cells.to.rem)

objCOTAN <- clean(objCOTAN)

objCOTAN <- findFullyExpressedGenes(objCOTAN)
goodPos <- flagNotFullyExpressedGenes(objCOTAN)

objCOTAN <- findFullyExpressingCells(objCOTAN)
goodPos <- flagNotFullyExpressingCells(objCOTAN)

feGenes <- getFullyExpressedGenes(objCOTAN)

feCells <- getFullyExpressingCells(objCOTAN)

## These plots might help to identify genes/cells that need to be dropped
ecdPlot <- ECDPlot(objCOTAN, yCut = 100)
plot(ecdPlot)

# This creates many infomative plots useful to determine whether
# there is still something to drop...
# Here we use the tuple-like assignment feature of the `zeallot` package
c(pcaCellsPlot, ., genesPlot, UDEPlot, ., zNuPlot) %<-% cleanPlots(objCOTAN)
plot(pcaCellsPlot)
plot(UDEPlot)
plot(zNuPlot)

lsPlot <- cellSizePlot(objCOTAN)
plot(lsPlot)

gsPlot <- genesSizePlot(objCOTAN)
plot(gsPlot)

mitPercPlot <-
  mitochondrialPercentagePlot(objCOTAN, genePrefix = "g-0000")[[ "plot" ]]
plot(mitPercPlot)

scPlot <- scatterPlot(objCOTAN)
plot(scPlot)
```

## Description

These methods extract information out of a just created COTAN object. The accessors have **read-only** access to the object.

## Usage

```
## S4 method for signature 'COTAN'  
getRawData(objCOTAN)
```

```
## S4 method for signature 'COTAN'  
getNumCells(objCOTAN)
```

```
## S4 method for signature 'COTAN'  
getNumGenes(objCOTAN)
```

```
## S4 method for signature 'COTAN'  
getCells(objCOTAN)
```

```
## S4 method for signature 'COTAN'  
getGenes(objCOTAN)
```

```
## S4 method for signature 'COTAN'  
getZeroOneProj(objCOTAN)
```

```
## S4 method for signature 'COTAN'  
getCellsSize(objCOTAN)
```

```
## S4 method for signature 'COTAN'  
getNumExpressedGenes(objCOTAN)
```

```
## S4 method for signature 'COTAN'  
getGenesSize(objCOTAN)
```

```
## S4 method for signature 'COTAN'  
getNumOfExpressingCells(objCOTAN)
```

## Arguments

objCOTAN            a COTAN object

## Details

getRawData() extracts the raw count table.

getNumCells() extracts the number of cells in the sample ( $m$ )

getNumGenes() extracts the number of genes in the sample ( $n$ )

getCells() extract all cells in the dataset.



`getGenes()` extract all genes in the dataset.

`getZeroOneProj()` extracts the raw count table where any positive number has been replaced with 1

`getCellsSize()` extracts the cell raw library size.

`getNumExpressedGenes()` extracts the number of genes expressed for each cell. Exploits a feature of [Matrix::CsparseMatrix](#)

`getGenesSize()` extracts the genes raw library size.

`getNumOfExpressingCells()` extracts, for each gene, the number of cells that are expressing it. Exploits a feature of [Matrix::CsparseMatrix](#)

### Value

`getRawData()` returns the raw count sparse matrix

`getNumCells()` returns the number of cells in the sample ( $m$ )

`getNumGenes()` returns the number of genes in the sample ( $n$ )

`getCells()` returns a character array with the cells' names

`getGenes()` returns a character array with the genes' names

`getZeroOneProj()` returns the raw count matrix projected to 0 or 1

`getCellsSize()` returns an array with the library sizes

`getNumExpressedGenes()` returns an array with the library sizes

`getGenesSize()` returns an array with the library sizes

`getNumOfExpressingCells()` returns an array with the library sizes

### Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

rawData <- getRawData(objCOTAN)

numCells <- getNumCells(objCOTAN)

numGenes <- getNumGenes(objCOTAN)

cellsNames <- getCells(objCOTAN)

genesNames <- getGenes(objCOTAN)

zeroOne <- getZeroOneProj(objCOTAN)

cellsSize <- getCellsSize(objCOTAN)

numExpGenes <- getNumExpressedGenes(objCOTAN)

genesSize <- getGenesSize(objCOTAN)

numExpCells <- getNumOfExpressingCells(objCOTAN)
```

**Description**

This group of functions takes in input a COTAN object and handle the task of dividing the dataset into **Uniform Clusters**, that is *clusters* that have an homogeneous genes' expression. This condition is checked by calculating the GDI of the *cluster* and verifying that no more than a small fraction of the genes have their GDI level above the given GDIThreshold

**Usage**

```
GDIPlot(  
  objCOTAN,  
  genes,  
  condition = "",  
  statType = "S",  
  GDIThreshold = 1.43,  
  GDIIn = NULL  
)  
  
cellsUniformClustering(  
  objCOTAN,  
  GDIThreshold = 1.43,  
  ratioAboveThreshold = 0.01,  
  cores = 1L,  
  maxIterations = 25L,  
  optimizeForSpeed = TRUE,  
  deviceStr = "cuda",  
  initialClusters = NULL,  
  initialResolution = 0.8,  
  useDEA = TRUE,  
  distance = NULL,  
  hclustMethod = "ward.D2",  
  saveObj = TRUE,  
  outDir = "."  
)  
  
isClusterUniform(  
  GDIThreshold,  
  ratioAboveThreshold,  
  ratioQuantile,  
  fractionAbove,  
  usedGDIThreshold,  
  usedRatioAbove  
)  
  
checkClusterUniformity(  
  objCOTAN,  
  clusterName,  
  cells,
```

```

    GDIThreshold = 1.43,
    ratioAboveThreshold = 0.01,
    cores = 1L,
    optimizeForSpeed = TRUE,
    deviceStr = "cuda",
    saveObj = TRUE,
    outDir = "."
)

mergeUniformCellsClusters(
  objCOTAN,
  clusters = NULL,
  GDIThreshold = 1.43,
  ratioAboveThreshold = 0.01,
  batchSize = 0L,
  allCheckResults = data.frame(),
  cores = 1L,
  optimizeForSpeed = TRUE,
  deviceStr = "cuda",
  useDEA = TRUE,
  distance = NULL,
  hclustMethod = "ward.D2",
  saveObj = TRUE,
  outDir = "."
)

```

### Arguments

objCOTAN	a COTAN object
genes	a named list of genes to label. Each array will have different color.
condition	a string corresponding to the condition/sample (it is used only for the title).
statType	type of statistic to be used. Default is "S": Pearson's chi-squared test statistics. "G" is G-test statistics
GDIThreshold	the threshold level that discriminates uniform clusters. It defaults to 1.43
GDIIn	when the GDI data frame was already calculated, it can be put here to speed up the process (default is NULL)
ratioAboveThreshold	the fraction of genes allowed to be above the GDIThreshold. It defaults to 1%
cores	number of cores to use. Default is 1.
maxIterations	max number of re-clustering iterations. It defaults to 25
optimizeForSpeed	Boolean; when TRUE COTAN tries to use the torch library to run the matrix calculations. Otherwise, or when the library is not available will run the slower legacy code
deviceStr	On the torch library enforces which device to use to run the calculations. Possible values are "cpu" to use the system CPU, "cuda" to use the system GPUs or something like "cuda:0" to restrict to a specific device
initialClusters	an existing <i>clusterization</i> to use as starting point: the <i>clusters</i> deemed <b>uniform</b> will be kept and the rest processed as normal

initialResolution	a number indicating how refined are the clusters before checking for <b>uniformity</b> . It defaults to 0.8, the same as <code>Seurat::FindClusters()</code>
useDEA	Boolean indicating whether to use the <i>DEA</i> to define the distance; alternatively it will use the average <i>Zero-One</i> counts, that is faster but less precise.
distance	type of distance to use. Default is "cosine" for <i>DEA</i> and "euclidean" for <i>Zero-One</i> . Can be chosen among those supported by <code>parallelDist::parDist()</code>
hclustMethod	It defaults is "ward.D2" but can be any of the methods defined by the <code>stats::hclust()</code> function.
saveObj	Boolean flag; when TRUE saves intermediate analyses and plots to file
outDir	an existing directory for the analysis output. The effective output will be paced in a sub-folder.
ratioQuantile	the GDI quantile corresponding to the usedRatioAbove
fractionAbove	the fraction of genes above the usedGDIThreshold
usedGDIThreshold	the threshold level actually used to calculate fourth argument
usedRatioAbove	the fraction of genes actually used to calculate the third argument
clusterName	the tag of the <i>cluster</i>
cells	the cells belonging to the <i>cluster</i>
clusters	The <i>clusterization</i> to merge. If not given the last available <i>clusterization</i> will be used, as it is probably the most significant!
batchSize	Number pairs to test in a single round. If none of them succeeds the merge stops. Defaults to $2(\#cl)^{2/3}$
allCheckResults	An optional data.frame with the results of previous checks about the merging of clusters. Useful to restart the <i>merging</i> process after an interruption.

## Details

`GDIPlot()` directly evaluates and plots the GDI for a sample.

`cellsUniformClustering()` finds a **Uniform clusterizations** by means of the GDI. Once a preliminary *clusterization* is obtained from the Seurat-package methods, each *cluster* is checked for **uniformity** via the function `checkClusterUniformity()`. Once all *clusters* are checked, all cells from the **non-uniform** clusters are pooled together for another iteration of the entire process, until all *clusters* are deemed **uniform**. In the case only a few cells are left out ( $\leq 50$ ), those are flagged as "-1" and the process is stopped.

`isClusterUniform()` takes in the current thresholds and used them to check whether the calculated cluster parameters are sufficient to determine whether the cluster is **uniform** and in the positive scenario the corresponding answer

`checkClusterUniformity()` takes a COTAN object and a cells' *cluster* and checks whether the latter is **uniform** by GDI. The function runs COTAN to check whether the GDI is lower than the given `GDIThreshold` (1.43) for all but at the most `ratioAboveThreshold` (1%) genes. If the GDI results to be too high for too many genes, the *cluster* is deemed **non-uniform**.

`mergeUniformCellsClusters()` takes in a **uniform clusterization** and iteratively checks whether merging two *near clusters* would form a **uniform cluster** still. Multiple thresholds will be used from 1.37 up to the given one in order to prioritize merge of the best fitting pairs.

This function uses the *cosine distance* to establish the *nearest clusters pairs*. It will use the `checkClusterUniformity()` function to check whether the merged *clusters* are **uniform**. The function will stop once no *tested pairs* of clusters are mergeable after testing all pairs in a single batch



```
deviceStr = "cuda",
initialResolution = 0.8,
GDIThreshold = 1.46, saveObj = FALSE)

clusters <- splitList[["clusters"]]

firstCluster <- getCells(objCOTAN)[clusters %in% clusters[[1L]]]
firstClusterIsUniform <-
  checkClusterUniformity(objCOTAN, GDIThreshold = 1.46,
    ratioAboveThreshold = 0.01,
    cluster = clusters[[1L]], cells = firstCluster,
    cores = 6L, optimizeForSpeed = TRUE,
    deviceStr = "cuda", saveObj = FALSE)[["isUniform"]]

objCOTAN <- addClusterization(objCOTAN,
  clName = "split",
  clusters = clusters)

objCOTAN <- addClusterizationCoex(objCOTAN,
  clName = "split",
  coexDF = splitList[["coex"]])

identical(reorderClusterization(objCOTAN)[["clusters"]], clusters)

mergedList <- mergeUniformCellsClusters(objCOTAN,
  GDIThreshold = 1.43,
  ratioAboveThreshold = 0.02,
  batchSize = 2L,
  clusters = clusters,
  cores = 6L,
  optimizeForSpeed = TRUE,
  deviceStr = "cpu",
  distance = "cosine",
  hclustMethod = "ward.D2",
  saveObj = FALSE)

objCOTAN <- addClusterization(objCOTAN,
  clName = "merged",
  clusters = mergedList[["clusters"]],
  coexDF = mergedList[["coex"]])

identical(reorderClusterization(objCOTAN), mergedList)
```

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