Package 'Seqtometry'

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Title Signature scoring for single cell analysis

Version 1.0.0

URL https://github.com/HawigerLab/Seqtometry

BugReports https://github.com/HawigerLab/Seqtometry/issues

Description
This package provides functions used in Seqtometry (Kousnetsov et al. 2024), a method for analyzing single cell (scRNA-seq or scATAC-seq) data via signature (gene set) enrichment scores. The Seqtometry scores may be useful for annotating or characterizing cells, either in a flow cytometry like workflow (where scores are standalone features used for progressive partitoning as described in the Seqtometry publication) or in a cluster-based workflow (as features of clusters). The exported impute function (a port of Python's MAGIC-impute, van Dijk et al. 2018), may also be useful for single cell analysis on its own.

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`Seqtometry-package` *Signature scoring for single cell analysis*

Description

This package provides functions used in Seqtometry (Kousnetsov et al. 2024), a method for analyzing single cell (scRNA-seq or scATAC-seq) data via signature (gene set) enrichment scores. The Seqtometry scores may be useful for annotating or characterizing cells, either in a flow cytometry like workflow (where scores are standalone features used for progressive partitoning as demonstrated in the Seqtometry publication) or in a cluster-based workflow (as features of clusters). The exported impute function (a port of Python's MAGIC-impute, van Dijk et al. 2018), may also be useful for single cell analysis on its own.

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.apply_diff_op *Perform data diffusion*

Description

Apply diffusion operator to data in order to perform imputation.

Usage

```
.apply_diff_op(gex, pcs, aff, dft, t_max, tol, exact_solver)
```

Arguments

gex	**matrix or Matrix** Gene expression matrix
pcs	**matrix** Principal components matrix
aff	**dgCMatrix** Markov affinity matrix
dft	**NULL or integer(1)** Diffusion time
t_max	**integer(1)** Maximum diffusion time
tol	**numeric(1)** Tolerance for Procrustes disparity
exact_solver	**logical(1)** Perform imputation in gene space

Value

list(matrix, integer(1)) Imputed matrix and diffusion time used

.calc_diff_op *Compute diffusion operator*

Description

Calculate graph diffusion operator (Markov affinity matrix).

Usage

```
.calc_diff_op(pcs, knn, ka, dist_metric)
```

Arguments

pcs	**matrix** Principal components matrix (used for kNN search)
knn	**integer(1)** Number of nearest neighbors to search for
ka	**integer(1)** Number of nearest neighbors to use for adaptive kernel
dist_metric	**character(1)** Type of metric to use for distance calculations during kNN search

Value

dgCMatrix Markov affinity matrix

.calc_pca *PCA wrapper*

Description

Calculate leading principal components (via truncated singular value decomposition).

Usage

```
.calc_pca(gex, npc, scale)
```

Arguments

gex	**matrix or Matrix** Gene expression matrix (without any zero variance genes)
npc	**numeric(1)** Number of leading principal components to compute
scale	**logical(1)** Whether to scale genes to unit variance

Value

list PC loading/rotation matrices as well as centering/scaling vectors

.check_params *Parameter validation*

Description

Checks that all parameters used in `impute` are valid.

Usage

```
.check_params(args)
```

Arguments

args	**list** The arguments to the magic_impute function

Value

NULL (but stops execution for invalid parameters)

.gene_indices *Finds row indices of signature genes*

Description

For converting from character to integer based indexing

Usage

```
.gene_indices(mat, gss)
```

Arguments

mat	**matrix-like** Gene expression data (genes x cells)
gss	**named list of character** Signature genes (with same nomenclature system as mat)

Value

integer 0-based indices (for passing to Rcpp function) of signature genes

.invert_pca *PCA inversion*

Description

Reverses operations done for PCA: back-rotation, unscaling, and uncentering.

Usage

```
.invert_pca(pcs, rot, ctr, sdv, low_mem)
```

Arguments

pcs	**matrix** The principal components (scaled left singular vectors)
rot	**matrix** The rotation matrix (right singular vectors)
ctr	**integer** The centering vector
sdv	**integer or NULL** The scaling vector (or NULL if no scaling was applied)
low_mem	**logical(1)** Whether to use delayed operations to reduce memory usage

Value

matrix or DelayedMatrix `rot %*% t(pcs) * sdv + ctr`

.minmax_scale *Minmax transform*

Description

Scales input vector to unit range

Usage

```
.minmax_scale(x)
```

Arguments

x	**numeric** Values to be scaled

Value

numeric Minmax transformed values

.normalize *LogCP10K transform*

Description

Simple normalization method for scRNA-seq data.

Usage

```
.normalize(gex)
```

Arguments

gex	**matrix or Matrix** Gene expression matrix (cells x genes)

Value

matrix or Matrix Transformed (normalized) matrix

.procrustes *Procrustes disparity*

Description

Calculates symmetric Procrustes distance (adapted from MATLAB procrustes).

Usage

```
.procrustes(x, y)
```

Arguments

x	**matrix**
y	**matrix**

Value

numeric(1) Procrustes disparity between input matrices

impute *MAGIC imputation (van Dijk et al. 2018)*

Description

Calculates a graph diffusion operator for the given input matrix and applies it to produce an imputed matrix.

Usage

```
impute(
  gex,
  transpose = TRUE,
  do_norm = FALSE,
  pca = NULL,
  npc = 100L,
  scale = TRUE,
  knn = 16L,
  ka = 6L,
  dist_metric = "euclidean",
  dft = NULL,
  t_max = 16L,
  tol = 0.001,
  exact_solver = TRUE,
  conserve_memory = FALSE,
```

```
  env_ret = FALSE,
  verbose = FALSE
)
```

Arguments

gex	**matrix or Matrix** Gene expression values (that has passed quality control).
transpose	**logical(1)** Whether to transpose gex (make it cells x genes) prior to downstream operations.
do_norm	**logical(1)** Whether to perform LogCP10K normalization on gex.
pca	**matrix (cells x PCs) or NULL** Precomputed principal component matrix (or NULL to derive it from gex).
npc	**integer(1)** Number of principal components (min = 1) to calculate.
scale	**logical(1)** Whether to scale columns of input matrix to unit variance prior to PCA.
knn	**integer(1)** Number of nearest neighbors (min = 2) to consider during distance calculation.
ka	**integer(1)** Number of nearest neighbors (min = 2, max <= knn) to use for the adaptive kernel.
dist_metric	**character(1)** Type of metric to use for distance calculations during kNN search.
dft	**NULL or integer(1)** Automatic (NULL) or user-defined (integer) diffusion time (min = 1, max = 16).
t_max	**integer(1)** Maximum diffusion time to test when using automatic diffusion time (min = 1, max = 16).
tol	**numeric(1)** Threshold for Procrustes disparity (min = 0, max = 1) between successive diffusion times.
exact_solver	**logical(1)** Whether to perform imputation in gene space (TRUE) or PCA space (FALSE).
conserve_memory	**logical(1)** Whether to avoid allocating a large dense matrix when `exact_solver = FALSE`.
env_ret	**logical(1)** Return all variables in the environment (TRUE) or just the imputed matrix (FALSE).
verbose	**logical(1)** Whether to print messages at different major parts of the algorithm.

Value

matrix-like or list If `env_ret = FALSE`, then just the imputed matrix. Otherwise the function environment as a list containing all parameters (possibly modified) as well as

- imp **matrix or DelayedMatrix** Imputed matrix.
- aff **dgCMatrix** Markov affinity matrix (graph diffusion operator).
- pca **list** Possibly computed (if pca was NULL), yielding a four element list, where:
  - x **matrix (cells x PCs)** The principal components matrix (scaled left singular vectors).

- `v` **matrix (genes x PCs)** The rotation matrix (right singular vectors).
- `center` **integer (cells)** The centering vector.
- `scale` **integer (cells) or NULL** The scaling vector (or NULL if no scaling was applied).

Examples

```
box::use(
  TENxPBMCData[TENxPBMCData],
  SingleCellExperiment[rowData, logcounts],
  scuttle[quickPerCellQC, logNormCounts],
  scater[runUMAP, plotReducedDim],
  patchwork[wrap_plots])

# PBMC data, basic processing pipeline
dat <- TENxPBMCData(dataset = "pbmc3k")
dimnames(dat) <- list(
  rowData(dat)[["Symbol_TENx"]],
  dat[["Barcode"]])
dat <- dat |>
  quickPerCellQC() |>
  logNormCounts() |>
  runUMAP()

# MAGIC imputation
imp <- logcounts(dat) |>
  as("dgCMatrix") |>
  impute()

# Visualize unimputed versus imputed expression
# on UMAP plots for a gene of interest (GOI)
goi <- "CD19"
dat[["Imputed_GOI"]] <- imp[goi, ]
p1 <- plotReducedDim(dat, "UMAP", color_by = goi)
p2 <- plotReducedDim(dat, "UMAP", color_by = "Imputed_GOI")
wrap_plots(p1, p2, ncol = 2)
```

---

score *Seqtometry scoring (Kousnetsov et al. 2024)*

Description

Computes signature scores (a weighted KS-like statistic) for single cell expression data

Usage

```
score(mat, signatures, minmax = TRUE)
```

Arguments

mat	**matrix, Matrix, or DelayedMatrix** Gene expression data (genes x cells)
signatures	**named list of character** Signature genes (with same nomenclature system used in mat)
minmax	**logical(1)** Whether to perform minmax transform on scoring results (default: TRUE)

Value

data.table Single cell scores (cells x signatures) for each signature, where cell barcodes are stored in the "id" column

Examples

```
box::use(
  TENxPBMCData[TENxPBMCData],
  SingleCellExperiment[rowData, logcounts],
  scuttle[quickPerCellQC, logNormCounts],
  scater[runUMAP, plotReducedDim],
  patchwork[wrap_plots])

# PBMC data, basic processing pipeline
dat <- TENxPBMCData(dataset = "pbmc3k")
dimnames(dat) <- list(
  rowData(dat)[["Symbol_TENx"]],
  dat[["Barcode"]])
dat <- dat |>
  quickPerCellQC() |>
  logNormCounts() |>
  runUMAP()

# MAGIC imputation
imp <- logcounts(dat) |>
  as("dgCMatrix") |>
  impute()

# Score with a B cell signature (gene set)
options(future.globals.maxSize = 1024^3)
b_cell_sig <- list("B_cell" = c("CD19", "MS4A1", "CD79A", "CD79B"))
dat[["B cell signature"]] <- Seqtometry::score(imp, b_cell_sig)[["B_cell"]]

# Visualize a hallmark B cell gene versus a B cell signature score
p1 <- plotReducedDim(dat, "UMAP", color_by = "CD19")
p2 <- plotReducedDim(dat, "UMAP", color_by = "B cell signature")
wrap_plots(p1, p2, ncol = 2)
```

wks *Helper function for performing a weighted Kolmogorov-Smirnov-like procedure.*

Description

Helper function for performing a weighted Kolmogorov-Smirnov-like procedure.

Usage

```
wks(gex, gss, mus, sds)
```

Arguments

gex	numeric: normalized gene expression values
gss	list: indices of genes in each gene set
mus	numeric: means of all genes
sds	numeric: standard deviations of all genes

Value

Modified Kuiper statistic (sum of minimal and maximal deviations during running sum)

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