

# Package ‘scater’

November 12, 2019

**Type** Package

**Version** 1.15.4

**Date** 2019-11-10

**License** GPL-3

**Title** Single-Cell Analysis Toolkit for Gene Expression Data in R

**Description** A collection of tools for doing various analyses of single-cell RNA-seq gene expression data, with a focus on quality control and visualization.

**Depends** SingleCellExperiment, ggplot2

**Imports** BiocGenerics, SummarizedExperiment, Matrix, ggbeeswarm, grid, DelayedArray, DelayedMatrixStats, methods, S4Vectors, stats, utils, viridis, Rcpp, BiocNeighbors, BiocSingular, BiocParallel

**Suggests** BiocStyle, BiocFileCache, biomaRt, beachmat, cowplot, destiny, knitr, scRNAseq, robustbase, rmarkdown, Rtsne, uwot, testthat, pheatmap, Biobase, limma, DropletUtils

**VignetteBuilder** knitr

**biocViews** ImmunoOncology, SingleCell, RNASeq, QualityControl, Preprocessing, Normalization, Visualization, DimensionReduction, Transcriptomics, GeneExpression, Sequencing, Software, DataImport, DataRepresentation, Infrastructure, Coverage

**LinkingTo** Rcpp, beachmat

**SystemRequirements** C++11

**BuildResaveData** no

**RoxygenNote** 6.1.1

**NeedsCompilation** yes

**URL** <http://bioconductor.org/packages/scater/>

**BugReports** <https://support.bioconductor.org/>

**git\_url** <https://git.bioconductor.org/packages/scater>

**git\_branch** master

**git\_last\_commit** 015579e

**git\_last\_commit\_date** 2019-11-10

**Date/Publication** 2019-11-11

**Author** Davis McCarthy [aut, cre],  
 Kieran Campbell [aut],  
 Aaron Lun [aut, ctb],  
 Quin Wills [aut],  
 Vladimir Kiselev [ctb]

**Maintainer** Davis McCarthy <davis@ebi.ac.uk>

## R topics documented:

addPerCellQC . . . . .	3
annotateBMFeatures . . . . .	4
bootstraps . . . . .	5
calculateAverage . . . . .	6
calculateCPM . . . . .	7
calculateDiffusionMap . . . . .	9
calculateFPKM . . . . .	11
calculateMDS . . . . .	12
calculatePCA . . . . .	13
calculateTPM . . . . .	15
calculateTSNE . . . . .	17
calculateUMAP . . . . .	19
defunct . . . . .	21
getExplanatoryPCs . . . . .	22
getVarianceExplained . . . . .	23
isOutlier . . . . .	24
librarySizeFactors . . . . .	26
logNormCounts . . . . .	27
mockSCE . . . . .	29
multiplot . . . . .	30
nexprs . . . . .	31
normalizeCounts . . . . .	32
norm_exprs . . . . .	34
numDetectedAcrossCells . . . . .	35
numDetectedAcrossFeatures . . . . .	37
perCellQCMetrics . . . . .	38
perFeatureQCMetrics . . . . .	41
plotColData . . . . .	43
plotDots . . . . .	44
plotExplanatoryPCs . . . . .	46
plotExplanatoryVariables . . . . .	47
plotExpression . . . . .	48
plotHeatmap . . . . .	50
plotHighestExprs . . . . .	52
plotPlatePosition . . . . .	53
plotReducedDim . . . . .	54
plotRLE . . . . .	56
plotRowData . . . . .	57
plotScater . . . . .	59
quickPerCellQC . . . . .	60
readSparseCounts . . . . .	61
Reduced dimension plots . . . . .	62

<i>addPerCellQC</i>	3
retrieveCellInfo . . . . .	64
retrieveFeatureInfo . . . . .	66
runColDataPCA . . . . .	67
scater-plot-args . . . . .	68
scater-red-dim-args . . . . .	69
SCESet . . . . .	71
sumCountsAcrossCells . . . . .	72
sumCountsAcrossFeatures . . . . .	74
uniquifyFeatureNames . . . . .	76
updateSCESet . . . . .	77
<b>Index</b>	<b>78</b>

---

<i>addPerCellQC</i>	<i>Add QC to an SE</i>
---------------------	------------------------

---

**Description**

Convenient utilities to compute QC metrics and add them to a [SummarizedExperiment](#)'s metadata.

**Usage**

```
addPerCellQC(x, ...)
addPerFeatureQC(x, ...)
```

**Arguments**

- x           A [SummarizedExperiment](#) object or one of its subclasses.
- ...         For addPerCellQC, further arguments to pass to [perCellQCMetrics](#).  
              For addPerFeatureQC, further arguments to pass to [perFeatureQCMetrics](#).

**Details**

These functions are simply wrappers around [perCellQCMetrics](#) and [perFeatureQCMetrics](#), respectively. The computed QC metrics are automatically appended onto the existing [colData](#) or [rowData](#). No protection is provided to avoid duplicated column names.

**Value**

An object like x but with the QC metrics added to the row or column metadata.

**Author(s)**

Aaron Lun

**See Also**

[perCellQCMetrics](#) and [perFeatureQCMetrics](#), which do the actual work.

**Examples**

```
example_sce <- mockSCE()
example_sce <- addPerCellQC(example_sce)
colData(example_sce)

example_sce <- addPerFeatureQC(example_sce)
rowData(example_sce)
```

---

annotateBMFeatures      *Get feature annotation information from Biomart*

---

**Description**

Use the **biomaRt** package to add feature annotation information to an [SingleCellExperiment](#).

**Usage**

```
annotateBMFeatures(ids, biomaRt = "ENSEMBL_MART_ENSEMBL",
  dataset = "mmusculus_gene_ensembl", id.type = "ensembl_gene_id",
  symbol.type, attributes = c(id.type, symbol.type, "chromosome_name",
  "gene_biotype", "start_position", "end_position"), filters = id.type,
  ...)

getBMFeatureAnnos(x, ids = rownames(x), ...)
```

**Arguments**

ids	A character vector containing feature identifiers.
biomaRt	String defining the biomaRt to be used, to be passed to <a href="#">useMart</a> .
dataset	String defining the dataset to use, to be passed to <a href="#">useMart</a> .
id.type	String specifying the type of identifier in ids.
symbol.type	String specifying the type of symbol to retrieve. If missing, this is set to "mgi_symbol" if dataset="mmusculus_gene_ensembl", or to "hgnc_symbol" if dataset="hsapiens_gene_ensembl".
attributes	Character vector defining the attributes to pass to <a href="#">getBM</a> .
filters	String defining the type of identifier in ids, to be used as a filter in <a href="#">getBM</a> .
...	For <a href="#">annotateBMFeatures</a> , further named arguments to pass to <code>biomaRt::useMart</code> . For <a href="#">getBMFeatureAnnos</a> , further arguments to pass to <a href="#">annotateBMFeatures</a> .
x	A <a href="#">SingleCellExperiment</a> object.

**Details**

These functions provide convenient wrappers around **biomaRt** to quickly obtain annotation in the required format.

**Value**

For [annotateBMFeatures](#), a [DataFrame](#) containing feature annotation, with one row per value in `ids`.

For [getBMFeatureAnnos](#), `x` is returned containing the output of [annotateBMFeatures](#) appended to its [rowData](#).

**Author(s)**

Aaron Lun, based on code by Davis McCarthy

**Examples**

```
## Not run:
# Making up Ensembl IDs for demonstration purposes.
mock_id <- paste0("ENSMUSG", sprintf("%011d", seq_len(1000)))
anno <- annotateBMFeatures(ids=mock_id)

## End(Not run)
```

---

bootstraps	<i>Accessor and replacement for bootstrap results in a <a href="#">SingleCellExperiment</a> object</i>
------------	--

---

**Description**

[SingleCellExperiment](#) objects can contain bootstrap expression values (for example, as generated by the kallisto software for quantifying feature abundance). These functions conveniently access and replace the 'bootstrap' elements in the assays slot with the value supplied, which must be an matrix of the correct size, namely the same number of rows and columns as the [SingleCellExperiment](#) object as a whole.

**Usage**

```
bootstraps(object)

bootstraps(object) <- value

## S4 method for signature 'SingleCellExperiment'
bootstraps(object)

## S4 replacement method for signature 'SingleCellExperiment,array'
bootstraps(object) <- value
```

**Arguments**

object	a <a href="#">SingleCellExperiment</a> object.
value	an array of class "numeric" containing bootstrap expression values

**Value**

If accessing bootstraps slot of an [SingleCellExperiment](#), then an array with the bootstrap values, otherwise an [SingleCellExperiment](#) object containing new bootstrap values.

**Author(s)**

Davis McCarthy

**Examples**

```
example_sce <- mockSCE()
bootstraps(example_sce)
```

---

calculateAverage	<i>Calculate per-feature average counts</i>
------------------	---

---

**Description**

Calculate average counts per feature after normalizing observations using size factors.

**Usage**

```
calculateAverage(x, ...)

## S4 method for signature 'ANY'
calculateAverage(x, size_factors = NULL,
  subset_row = NULL, BPPARAM = SerialParam())

## S4 method for signature 'SummarizedExperiment'
calculateAverage(x, ...,
  exprs_values = "counts")

## S4 method for signature 'SingleCellExperiment'
calculateAverage(x, size_factors = NULL,
  ...)
```

**Arguments**

x	A numeric matrix of counts where features are rows and Alternatively, a <a href="#">SummarizedExperiment</a> or a <a href="#">SingleCellExperiment</a> containing such counts.
...	For the generic, arguments to pass to specific methods. For the SummarizedExperiment method, further arguments to pass to the ANY method. For the SingleCellExperiment method, further arguments to pass to the SummarizedExperiment method.
size_factors	A numeric vector containing size factors. If NULL, these are calculated or extracted from x.
subset_row	A vector specifying the subset of rows of object for which to return a result.
BPPARAM	A BiocParallelParam object specifying whether the calculations should be parallelized.
exprs_values	A string specifying the assay of x containing the count matrix.

## Details

The size-adjusted average count is defined by dividing each count by the size factor and taking the average across cells. All sizes factors are scaled so that the mean is 1 across all cells, to ensure that the averages are interpretable on the same scale of the raw counts.

If no size factors are supplied, they are determined automatically:

- For count matrices and [SummarizedExperiment](#) inputs, the sum of counts for each cell is used to compute a size factor via the [librarySizeFactors](#) function.
- For [SingleCellExperiment](#) instances, the function searches for [sizeFactors](#) from `x`. If none are available, it defaults to library size-derived size factors.

If `size_factors` are supplied, they will override any size factors present in `x`.

## Value

A numeric vector of average count values with same length as number of features (or the number of features in `subset_row` if supplied).

## Author(s)

Aaron Lun

## See Also

[librarySizeFactors](#), for the default calculation of size factors.

[logNormCounts](#), for the calculation of normalized expression values.

## Examples

```
example_sce <- mockSCE()
ave_counts <- calculateAverage(example_sce)
summary(ave_counts)
```

---

calculateCPM	<i>Calculate counts per million (CPM)</i>
--------------	---

---

## Description

Calculate count-per-million (CPM) values from the count data.

## Usage

```
calculateCPM(x, ...)

## S4 method for signature 'ANY'
calculateCPM(x, size_factors = NULL, subset_row = NULL)

## S4 method for signature 'SummarizedExperiment'
calculateCPM(x, ...,
  exprs_values = "counts")
```

```
## S4 method for signature 'SingleCellExperiment'
calculateCPM(x, size_factors = NULL,
  ...)
```

### Arguments

x	A numeric matrix of counts where features are rows and cells are columns. Alternatively, a <a href="#">SummarizedExperiment</a> or a <a href="#">SingleCellExperiment</a> containing such counts.
...	For the generic, arguments to pass to specific methods. For the SummarizedExperiment method, further arguments to pass to the ANY method. For the SingleCellExperiment method, further arguments to pass to the SummarizedExperiment method.
size_factors	A numeric vector containing size factors to adjust the library sizes. If NULL, the library sizes are used directly.
subset_row	A vector specifying the subset of rows of x for which to return a result.
exprs_values	A string or integer scalar specifying the assay of x containing the count matrix.

### Details

If `size_factors` are provided or available in `x`, they are used to define the effective library sizes. This is done by scaling all size factors such that the mean factor is equal to the mean sum of counts across all features. The effective library sizes are then used as the denominator of the CPM calculation.

### Value

A numeric matrix of CPM values.

### Author(s)

Aaron Lun

### See Also

[normalizeCounts](#), on which this function is based.

### Examples

```
example_sce <- mockSCE()
cpm(example_sce) <- calculateCPM(example_sce)
str(cpm(example_sce))
```



---

calculateDiffusionMap *Create a diffusion map from cell-level data*

---

### Description

Produce a diffusion map for the cells, based on the data in a `SingleCellExperiment` object.

### Usage

```
calculateDiffusionMap(x, ...)

## S4 method for signature 'ANY'
calculateDiffusionMap(x, ncomponents = 2, ntop = 500,
  subset_row = NULL, scale = FALSE, transposed = FALSE, ...)

## S4 method for signature 'SummarizedExperiment'
calculateDiffusionMap(x, ...,
  exprs_values = "logcounts")

## S4 method for signature 'SingleCellExperiment'
calculateDiffusionMap(x, ...,
  exprs_values = "logcounts", dimred = NULL, n_dimred = NULL)

runDiffusionMap(x, ..., altexp = NULL, name = "DiffusionMap")
```

### Arguments

x	For <code>calculateDiffusionMap</code> , a numeric matrix of log-expression values where rows are features and columns are cells. Alternatively, a <a href="#">SummarizedExperiment</a> or <a href="#">SingleCellExperiment</a> containing such a matrix. For <code>runDiffusionMap</code> , a <a href="#">SingleCellExperiment</a> object.
...	For the <code>calculateDiffusionMap</code> generic, additional arguments to pass to specific methods. For the ANY method, additional arguments to pass to <a href="#">DiffusionMap</a> . For the <code>SummarizedExperiment</code> and <code>SingleCellExperiment</code> methods, additional arguments to pass to the ANY method. For <code>runDiffusionMap</code> , additional arguments to pass to <code>calculateDiffusionMap</code> .
ncomponents	Numeric scalar indicating the number of UMAP dimensions to obtain.
ntop	Numeric scalar specifying the number of features with the highest variances to use for PCA, see <code>?"scater-red-dim-args"</code> .
subset_row	Vector specifying the subset of features to use for PCA, see <code>?"scater-red-dim-args"</code> .
scale	Logical scalar, should the expression values be standardised? See <code>?"scater-red-dim-args"</code> for details.
transposed	Logical scalar, is x transposed with cells in rows? See <code>?"scater-red-dim-args"</code> for details.
exprs_values	Integer scalar or string indicating which assay of x contains the expression values, see <code>?"scater-red-dim-args"</code> .
dimred	String or integer scalar specifying the existing dimensionality reduction results to use, see <code>?"scater-red-dim-args"</code> .

n_dimred	Integer scalar or vector specifying the dimensions to use if dimred is specified, see <code>?scater-red-dim-args</code> .
altexp	String or integer scalar specifying an alternative experiment to use to compute the PCA, see <code>?scater-red-dim-args</code> .
name	String specifying the name to be used to store the result in the reducedDims of the output.

### Details

The function `DiffusionMap` is used internally to compute the diffusion map. The behaviour of `DiffusionMap` seems to be non-deterministic, in a manner that is not responsive to any `set.seed` call. The reason for this is unknown.

### Value

For `calculateDiffusionMap`, a matrix is returned containing the diffusion map coordinates for each cell (row) and dimension (column).

For `runDiffusionMap`, a modified `x` is returned that contains the diffusion map coordinates in `reducedDim(x, name)`.

### Author(s)

Aaron Lun, based on code by Davis McCarthy

### References

Haghverdi L, Buettner F, Theis FJ (2015). Diffusion maps for high-dimensional single-cell analysis of differentiation data. *Bioinformatics* 31(18), 2989-2998.

### See Also

`DiffusionMap`, to perform the underlying calculations.

`plotDiffusionMap`, to quickly visualize the results.

`?scater-red-dim-args`, for a full description of various options.

### Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

example_sce <- runDiffusionMap(example_sce)
reducedDimNames(example_sce)
head(reducedDim(example_sce))
```

---

calculateFPKM	<i>Calculate FPKMs</i>
---------------	------------------------

---

### Description

Calculate fragments per kilobase of exon per million reads mapped (FPKM) values from the feature-level counts.

### Usage

```
calculateFPKM(x, lengths, ..., subset_row = NULL)
```

### Arguments

x	A numeric matrix of counts where features are rows and cells are columns. Alternatively, a <a href="#">SummarizedExperiment</a> or a <a href="#">SingleCellExperiment</a> containing such counts.
lengths	Numeric vector providing the effective length for each feature in x.
...	Further arguments to pass to <a href="#">calculateCPM</a> .
subset_row	A vector specifying the subset of rows of x for which to return a result.

### Value

A numeric matrix of FPKM values.

### Author(s)

Aaron Lun, based on code by Davis McCarthy

### See Also

[calculateCPM](#), for the initial calculation of CPM values.

### Examples

```
example_sce <- mockSCE()
eff_len <- runif(nrow(example_sce), 500, 2000)
fout <- calculateFPKM(example_sce, eff_len)
str(fout)
```

---

calculateMDS	<i>Perform MDS on cell-level data</i>
--------------	---------------------------------------

---

### Description

Perform multi-dimensional scaling (MDS) on cells, based on the data in a `SingleCellExperiment` object.

### Usage

```
calculateMDS(x, ...)

## S4 method for signature 'ANY'
calculateMDS(x, ncomponents = 2, ntop = 500,
  subset_row = NULL, scale = FALSE, transposed = FALSE,
  method = "euclidean")

## S4 method for signature 'SummarizedExperiment'
calculateMDS(x, ...,
  exprs_values = "logcounts")

## S4 method for signature 'SingleCellExperiment'
calculateMDS(x, ...,
  exprs_values = "logcounts", dimred = NULL, n_dimred = NULL)

runMDS(x, ..., altexp = NULL, name = "MDS")
```

### Arguments

<code>x</code>	For <code>calculateMDS</code> , a numeric matrix of log-expression values where rows are features and columns are cells. Alternatively, a <a href="#">SummarizedExperiment</a> or <a href="#">SingleCellExperiment</a> containing such a matrix. For <code>runMDS</code> , a <a href="#">SingleCellExperiment</a> object.
<code>...</code>	For the <code>calculateMDS</code> generic, additional arguments to pass to specific methods. For the <code>SummarizedExperiment</code> and <code>SingleCellExperiment</code> methods, additional arguments to pass to the <code>ANY</code> method. For <code>runMDS</code> , additional arguments to pass to <code>calculateMDS</code> .
<code>ncomponents</code>	Numeric scalar indicating the number of MDS dimensions to obtain.
<code>ntop</code>	Numeric scalar specifying the number of features with the highest variances to use for PCA, see <code>?"scatter-red-dim-args"</code> .
<code>subset_row</code>	Vector specifying the subset of features to use for PCA, see <code>?"scatter-red-dim-args"</code> .
<code>scale</code>	Logical scalar, should the expression values be standardised? See <code>?"scatter-red-dim-args"</code> for details.
<code>transposed</code>	Logical scalar, is <code>x</code> transposed with cells in rows? See <code>?"scatter-red-dim-args"</code> for details.
<code>method</code>	String specifying the type of distance to be computed between cells.
<code>exprs_values</code>	Integer scalar or string indicating which assay of <code>x</code> contains the expression values, see <code>?"scatter-red-dim-args"</code> .

dimred	String or integer scalar specifying the existing dimensionality reduction results to use, see <code>?scater-red-dim-args</code> .
n_dimred	Integer scalar or vector specifying the dimensions to use if dimred is specified, see <code>?scater-red-dim-args</code> .
altexp	String or integer scalar specifying an alternative experiment to use to compute the PCA, see <code>?scater-red-dim-args</code> .
name	String specifying the name to be used to store the result in the reducedDims of the output.

### Details

The function `cmdscale` is used internally to compute the MDS components.

### Value

For `calculateMDS`, a matrix is returned containing the MDS coordinates for each cell (row) and dimension (column).

For `runMDS`, a modified `x` is returned that contains the MDS coordinates in `reducedDim(x, name)`.

### Author(s)

Aaron Lun, based on code by Davis McCarthy

### See Also

`cmdscale`, to perform the underlying calculations.

`plotMDS`, to quickly visualize the results.

`?scater-red-dim-args`, for a full description of various options.

### Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

example_sce <- runMDS(example_sce)
reducedDimNames(example_sce)
head(reducedDim(example_sce))
```

---

calculatePCA

*Perform PCA on expression data*

---

### Description

Perform a principal components analysis (PCA) on cells, based on the expression data in a `SingleCellExperiment` object.

**Usage**

```

calculatePCA(x, ...)

## S4 method for signature 'ANY'
calculatePCA(x, ncomponents = 50, ntop = 500,
  subset_row = NULL, scale = FALSE, transposed = FALSE,
  BSPARAM = bsparam(), BPPARAM = SerialParam())

## S4 method for signature 'SummarizedExperiment'
calculatePCA(x, ...,
  exprs_values = "logcounts")

## S4 method for signature 'SingleCellExperiment'
calculatePCA(x, ...,
  exprs_values = "logcounts", dimred = NULL, n_dimred = NULL)

## S4 method for signature 'SingleCellExperiment'
runPCA(x, ..., altexp = NULL,
  name = "PCA")

```

**Arguments**

x	For calculatePCA, a numeric matrix of log-expression values where rows are features and columns are cells. Alternatively, a <a href="#">SummarizedExperiment</a> or <a href="#">SingleCellExperiment</a> containing such a matrix. For runPCA, a <a href="#">SingleCellExperiment</a> object containing such a matrix.
...	For the calculatePCA generic, additional arguments to pass to specific methods. For the SummarizedExperiment and SingleCellExperiment methods, additional arguments to pass to the ANY method. For runPCA, additional arguments to pass to calculatePCA.
ncomponents	Numeric scalar indicating the number of principal components to obtain.
ntop	Numeric scalar specifying the number of features with the highest variances to use for PCA, see <a href="#">?"scater-red-dim-args"</a> .
subset_row	Vector specifying the subset of features to use for PCA, see <a href="#">?"scater-red-dim-args"</a> .
scale	Logical scalar, should the expression values be standardised? See <a href="#">?"scater-red-dim-args"</a> for details.
transposed	Logical scalar, is x transposed with cells in rows? See <a href="#">?"scater-red-dim-args"</a> for details.
BSPARAM	A <a href="#">BiocSingularParam</a> object specifying which algorithm should be used to perform the PCA.
BPPARAM	A <a href="#">BiocParallelParam</a> object specifying whether the PCA should be parallelized.
exprs_values	Integer scalar or string indicating which assay of x contains the expression values, see <a href="#">?"scater-red-dim-args"</a> .
dimred	String or integer scalar specifying the existing dimensionality reduction results to use, see <a href="#">?"scater-red-dim-args"</a> .
n_dimred	Integer scalar or vector specifying the dimensions to use if dimred is specified, see <a href="#">?"scater-red-dim-args"</a> .
altexp	String or integer scalar specifying an alternative experiment to use to compute the PCA, see <a href="#">?"scater-red-dim-args"</a> .

name                   String specifying the name to be used to store the result in the `reducedDims` of the output.

### Details

Fast approximate SVD algorithms like `BSPARAM=IrlbaParam()` or `RandomParam()` use a random initialization, after which they converge towards the exact PCs. This means that the result will change slightly across different runs. For full reproducibility, users should call `set.seed` prior to running `runPCA` with such algorithms. (Note that this includes `BSPARAM=bsparam()`, which uses approximate algorithms by default.)

### Value

A `SingleCellExperiment` object containing the first `ncomponents` principal coordinates for each cell. By default, this is stored in the "PCA" entry of the `reducedDims`. The proportion of variance explained by each PC is stored as a numeric vector in the "percentVar" attribute of the reduced dimension matrix.

### Author(s)

Aaron Lun, based on code by Davis McCarthy

### See Also

`runPCA`, for the underlying calculations.  
`plotPCA`, to conveniently visualize the results.  
`?"scater-red-dim-args"`, for a full description of various options.

### Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

example_sce <- runPCA(example_sce)
reducedDimNames(example_sce)
head(reducedDim(example_sce))
```

---

calculateTPM

*Calculate TPMs*

---

### Description

Calculate transcripts-per-million (TPM) values for expression from feature-level counts.

### Usage

```
calculateTPM(x, ...)

## S4 method for signature 'ANY'
calculateTPM(x, lengths = NULL, ...)

## S4 method for signature 'SummarizedExperiment'
```

```
calculateTPM(x, ...,
             exprs_values = "counts")

## S4 method for signature 'SingleCellExperiment'
calculateTPM(x, lengths = NULL,
             size_factors = NULL, ...)
```

### Arguments

x	A numeric matrix of counts where features are rows and cells are columns. Alternatively, a <a href="#">SummarizedExperiment</a> or a <a href="#">SingleCellExperiment</a> containing such counts.
...	For the generic, arguments to pass to specific methods. For the ANY method, further arguments to pass to <a href="#">calculateCPM</a> . For the SummarizedExperiment method, further arguments to pass to the ANY method. For the SingleCellExperiment method, further arguments to pass to the SummarizedExperiment method.
lengths	Numeric vector providing the effective length for each feature in x. Alternatively NULL, see Details.
exprs_values	A string specifying the assay of x containing the count matrix.
size_factors	A numeric vector containing size factors to adjust the library sizes. If NULL, the library sizes are used directly.

### Details

For read count data, this function assumes uniform coverage along the (effective) length of the transcript. Thus, the number of transcripts for a gene is proportional to the read count divided by the transcript length. Here, the division is done before calculation of the library size to compute per-million values, where [calculateFPKM](#) will only divide by the length after library size normalization.

For UMI count data, this function should be run with `effective_length=NULL`, i.e., no division by the effective length. This is because the number of UMIs is a direct (albeit biased) estimate of the number of transcripts.

### Value

A numeric matrix of TPM values.

### Author(s)

Aaron Lun, based on code by Davis McCarthy

### See Also

[calculateCPM](#), on which this function is based.

### Examples

```
example_sce <- mockSCE()
eff_len <- runif(nrow(example_sce), 500, 2000)
tout <- calculateTPM(example_sce, lengths = eff_len)
str(tout)
```



---

calculateTSNE	<i>Perform t-SNE on cell-level data</i>
---------------	---

---

## Description

Perform t-stochastic neighbour embedding (t-SNE) for the cells, based on the data in a `SingleCellExperiment` object.

## Usage

```
calculateTSNE(x, ...)

## S4 method for signature 'ANY'
calculateTSNE(x, ncomponents = 2, ntop = 500,
  subset_row = NULL, scale = FALSE, transposed = FALSE,
  perplexity = NULL, normalize = TRUE, theta = 0.5, ...,
  external_neighbors = FALSE, BNPARAM = KknnParam(),
  BPPARAM = SerialParam())

## S4 method for signature 'SummarizedExperiment'
calculateTSNE(x, ...,
  exprs_values = "logcounts")

## S4 method for signature 'SingleCellExperiment'
calculateTSNE(x, ...,
  pca = is.null(dimred), exprs_values = "logcounts", dimred = NULL,
  n_dimred = NULL)

runTSNE(x, ..., altexp = NULL, name = "TSNE")
```

## Arguments

<code>x</code>	For <code>calculateTSNE</code> , a numeric matrix of log-expression values where rows are features and columns are cells. Alternatively, a <a href="#">SummarizedExperiment</a> or <a href="#">SingleCellExperiment</a> containing such a matrix. For <code>runTSNE</code> , a <a href="#">SingleCellExperiment</a> object.
<code>...</code>	For the <code>calculateTSNE</code> generic, additional arguments to pass to specific methods. For the ANY method, additional arguments to pass to <code>Rtsne</code> . For the <code>SummarizedExperiment</code> and <code>SingleCellExperiment</code> methods, additional arguments to pass to the ANY method. For <code>runTSNE</code> , additional arguments to pass to <code>calculateTSNE</code> .
<code>ncomponents</code>	Numeric scalar indicating the number of t-SNE dimensions to obtain.
<code>ntop</code>	Numeric scalar specifying the number of features with the highest variances to use for PCA, see <code>?"scater-red-dim-args"</code> .
<code>subset_row</code>	Vector specifying the subset of features to use for PCA, see <code>?"scater-red-dim-args"</code> .
<code>scale</code>	Logical scalar, should the expression values be standardised? See <code>?"scater-red-dim-args"</code> for details.
<code>transposed</code>	Logical scalar, is <code>x</code> transposed with cells in rows? See <code>?"scater-red-dim-args"</code> for details.

perplexity	Numeric scalar defining the perplexity parameter, see <a href="#">?Rtsne</a> for more details.
normalize	Logical scalar indicating if input values should be scaled for numerical precision, see <a href="#">normalize_input</a> .
theta	Numeric scalar specifying the approximation accuracy of the Barnes-Hut algorithm, see <a href="#">Rtsne</a> for details.
external_neighbors	Logical scalar indicating whether a nearest neighbors search should be computed externally with <a href="#">findKNN</a> .
BNPARAM	A <a href="#">BiocNeighborParam</a> object specifying the neighbor search algorithm to use when <code>external_neighbors=TRUE</code> .
BPPARAM	A <a href="#">BiocParallelParam</a> object specifying how the neighbor search should be parallelized when <code>external_neighbors=TRUE</code> .
exprs_values	Integer scalar or string indicating which assay of <code>x</code> contains the expression values, see <a href="#">?"scater-red-dim-args"</a> .
pca	Logical scalar indicating whether a PCA step should be performed inside <a href="#">Rtsne</a> .
dimred	String or integer scalar specifying the existing dimensionality reduction results to use, see <a href="#">?"scater-red-dim-args"</a> .
n_dimred	Integer scalar or vector specifying the dimensions to use if <code>dimred</code> is specified, see <a href="#">?"scater-red-dim-args"</a> .
altexp	String or integer scalar specifying an alternative experiment to use to compute the PCA, see <a href="#">?"scater-red-dim-args"</a> .
name	String specifying the name to be used to store the result in the <code>reducedDims</code> of the output.

## Details

The function [Rtsne](#) is used internally to compute the t-SNE. Note that the algorithm is not deterministic, so different runs of the function will produce differing results. Users are advised to test multiple random seeds, and then use [set.seed](#) to set a random seed for replicable results.

The value of the perplexity parameter can have a large effect on the results. By default, the function will set a “reasonable” perplexity that scales with the number of cells in `x`. (Specifically, it is the number of cells divided by 5, capped at a maximum of 50.) However, it is often worthwhile to manually try multiple values to ensure that the conclusions are robust.

If `external_neighbors=TRUE`, the nearest neighbor search step will use a different algorithm to that in the [Rtsne](#) function. This can be parallelized or approximate to achieve greater speed for large data sets. The neighbor search results are then used for t-SNE via the [Rtsne\\_neighbors](#) function.

If `dimred` is specified, the PCA step of the [Rtsne](#) function is automatically turned off by default. This presumes that the existing dimensionality reduction is sufficient such that an additional PCA is not required.

## Value

For [calculateTSNE](#), a numeric matrix is returned containing the t-SNE coordinates for each cell (row) and dimension (column).

For [runTSNE](#), a modified `x` is returned that contains the t-SNE coordinates in [reducedDim\(x, name\)](#).

## Author(s)

Aaron Lun, based on code by Davis McCarthy

## References

van der Maaten LJP, Hinton GE (2008). Visualizing High-Dimensional Data Using t-SNE. *J. Mach. Learn. Res.* 9, 2579-2605.

## See Also

[Rtsne](#), for the underlying calculations.

[plotTSNE](#), to quickly visualize the results.

?["scater-red-dim-args"](#), for a full description of various options.

## Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

example_sce <- runTSNE(example_sce)
reducedDimNames(example_sce)
head(reducedDim(example_sce))
```

---

calculateUMAP

*Perform UMAP on cell-level data*

---

## Description

Perform uniform manifold approximation and projection (UMAP) for the cells, based on the data in a `SingleCellExperiment` object.

## Usage

```
calculateUMAP(x, ...)

## S4 method for signature 'ANY'
calculateUMAP(x, ncomponents = 2, ntop = 500,
  subset_row = NULL, scale = FALSE, transposed = FALSE, pca = if
  (transposed) NULL else 50, n_neighbors = 15, ...,
  external_neighbors = FALSE, BNPARAM = KmknnParam(),
  BPPARAM = SerialParam())

## S4 method for signature 'SummarizedExperiment'
calculateUMAP(x, ...,
  exprs_values = "logcounts")

## S4 method for signature 'SingleCellExperiment'
calculateUMAP(x, ..., pca = if
  (!is.null(dimred)) NULL else 50, exprs_values = "logcounts",
  dimred = NULL, n_dimred = NULL)

runUMAP(x, ..., altexp = NULL, name = "UMAP")
```

**Arguments**

x	For calculateUMAP, a numeric matrix of log-expression values where rows are features and columns are cells. Alternatively, a <a href="#">SummarizedExperiment</a> or <a href="#">SingleCellExperiment</a> containing such a matrix. For runTSNE, a <a href="#">SingleCellExperiment</a> object containing such a matrix.
...	For the calculateUMAP generic, additional arguments to pass to specific methods. For the ANY method, additional arguments to pass to <a href="#">umap</a> . For the SummarizedExperiment and SingleCellExperiment methods, additional arguments to pass to the ANY method. For runUMAP, additional arguments to pass to calculateUMAP.
ncomponents	Numeric scalar indicating the number of UMAP dimensions to obtain.
ntop	Numeric scalar specifying the number of features with the highest variances to use for PCA, see <code>?"scater-red-dim-args"</code> .
subset_row	Vector specifying the subset of features to use for PCA, see <code>?"scater-red-dim-args"</code> .
scale	Logical scalar, should the expression values be standardised? See <code>?"scater-red-dim-args"</code> for details.
transposed	Logical scalar, is x transposed with cells in rows? See <code>?"scater-red-dim-args"</code> for details.
pca	Integer scalar specifying how many PCs should be used as input into the UMAP algorithm. By default, no PCA is performed if the input is a dimensionality reduction result.
n_neighbors	Integer scalar, number of nearest neighbors to identify when constructing the initial graph.
external_neighbors	Logical scalar indicating whether a nearest neighbors search should be computed externally with <a href="#">findKNN</a> .
BNPARAM	A <a href="#">BiocNeighborParam</a> object specifying the neighbor search algorithm to use when <code>external_neighbors=TRUE</code> .
BPPARAM	A <a href="#">BiocParallelParam</a> object specifying how the neighbor search should be parallelized when <code>external_neighbors=TRUE</code> .
exprs_values	Integer scalar or string indicating which assay of x contains the expression values, see <code>?"scater-red-dim-args"</code> .
dimred	String or integer scalar specifying the existing dimensionality reduction results to use, see <code>?"scater-red-dim-args"</code> .
n_dimred	Integer scalar or vector specifying the dimensions to use if <code>dimred</code> is specified, see <code>?"scater-red-dim-args"</code> .
altexp	String or integer scalar specifying an alternative experiment to use to compute the PCA, see <code>?"scater-red-dim-args"</code> .
name	String specifying the name to be used to store the result in the <code>reducedDims</code> of the output.

**Details**

The function [umap](#) is used internally to compute the UMAP. Note that the algorithm is not deterministic, so different runs of the function will produce differing results. Users are advised to test multiple random seeds, and then use `set.seed` to set a random seed for replicable results.

If `external_neighbors=TRUE`, the nearest neighbor search is conducted using a different algorithm to that in the [umap](#) function. This can be parallelized or approximate to achieve greater speed for large data sets. The neighbor search results are then used directly to create the UMAP embedding.

**Value**

For `calculateUMAP`, a matrix is returned containing the UMAP coordinates for each cell (row) and dimension (column).

For `runUMAP`, a modified `x` is returned that contains the UMAP coordinates in `reducedDim(x, name)`.

**Author(s)**

Aaron Lun

**References**

McInnes L, Healy J, Melville J (2018). UMAP: uniform manifold approximation and projection for dimension reduction. arXiv.

**See Also**

`umap`, for the underlying calculations.

`plotUMAP`, to quickly visualize the results.

`?"scater-red-dim-args"`, for a full description of various options.

**Examples**

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

example_sce <- runUMAP(example_sce)
reducedDimNames(example_sce)
head(reducedDim(example_sce))
```

---

defunct

*Defunct functions*

---

**Description**

Functions that have passed on to the function afterlife. Their successors are also listed.

**Usage**

```
calculateQCMetrics(...)

## S4 method for signature 'SingleCellExperiment'
normalize(object, ...)

centreSizeFactors(...)
```

**Arguments**

`object, ...` Ignored arguments.

**Details**

calculateQCMetrics is succeeded by [perCellQCMetrics](#) and [perFeatureQCMetrics](#).

normalize is succeeded by [logNormCounts](#).

centreSizeFactors has no replacement - the **SingleCellExperiment** is removing support for multiple size factors, so this function is now trivial.

**Value**

All functions error out with a defunct message pointing towards its descendent (if available).

**Author(s)**

Aaron Lun

**Examples**

```
try(calculateQCMetrics())
```

---

getExplanatoryPCs	<i>Per-PC variance explained by a variable</i>
-------------------	--

---

**Description**

Compute, for each principal component, the percentage of variance that is explained by one or more variables of interest.

**Usage**

```
getExplanatoryPCs(x, dimred = "PCA", n_dimred = 10, ...)
```

**Arguments**

x	A <a href="#">SingleCellExperiment</a> object containing dimensionality reduction results.
dimred	String or integer scalar specifying the field in <code>reducedDims(x)</code> that contains the PCA results.
n_dimred	Integer scalar specifying the number of the top principal components to use.
...	Additional arguments passed to <a href="#">getVarianceExplained</a> .

**Details**

This function computes the percentage of variance in PC scores that is explained by variables in the sample-level metadata. It allows identification of important PCs that are driven by known experimental conditions, e.g., treatment, disease. PCs correlated with technical factors (e.g., batch effects, library size) can also be detected and removed prior to further analysis.

By default, the function will attempt to use pre-computed PCA results in object. This is done by taking the top `n_dimred` PCs from the matrix specified by `dimred`. If these are not available or if `rerun=TRUE`, the function will rerun the PCA using [runPCA](#); however, this mode is deprecated and users are advised to explicitly call `runPCA` themselves.

**Value**

A matrix containing the percentage of variance explained by each factor (column) and for each PC (row).

**Author(s)**

Aaron Lun

**See Also**

[plotExplanatoryPCs](#), to plot the results.

[getVarianceExplained](#), to compute the variance explained.

**Examples**

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)
example_sce <- runPCA(example_sce)

r2mat <- getExplanatoryPCs(example_sce)
```

---

getVarianceExplained *Per-gene variance explained by a variable*

---

**Description**

Compute, for each gene, the percentage of variance that is explained by one or more variables of interest.

**Usage**

```
getVarianceExplained(x, ...)

## S4 method for signature 'ANY'
getVarianceExplained(x, variables, subset_row = NULL,
  chunk = 1000)

## S4 method for signature 'SummarizedExperiment'
getVarianceExplained(x,
  variables = NULL, ..., exprs_values = "logcounts")
```

**Arguments**

**x** A numeric matrix of expression values, usually log-transformed and normalized. Alternatively, a [SummarizedExperiment](#) containing such a matrix.

**...** For the generic, arguments to be passed to specific methods. For the `SummarizedExperiment` method, arguments to be passed to the ANY method.

variables	A <a href="#">DataFrame</a> or data.frame containing one or more variables of interest. This should have number of rows equal to the number of columns in x. For the SummarizedExperiment method, this can also be a character vector specifying column names of colData(x) to use; or NULL, in which case all columns in colData(x) are used.
subset_row	A vector specifying the subset of rows of x for which to return a result.
chunk	Integer scalar specifying the chunk size for chunk-wise processing. Only affects the speed/memory usage trade-off.
exprs_values	String or integer scalar specifying the expression values for which to compute the variance.

### Details

This function computes the percentage of variance in gene expression that is explained by variables in the sample-level metadata. It allows problematic factors to be quickly identified, as well as the genes that are most affected.

### Value

A numeric matrix containing the percentage of variance explained by each factor (column) and for each gene (row).

### Author(s)

Aaron Lun

### See Also

[getExplanatoryPCs](#), which calls this function.  
[plotExplanatoryVariables](#), to plot the results.

### Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

r2mat <- getVarianceExplained(example_sce)
```

---

isOutlier

*Identify outlier values*

---

### Description

Convenience function to determine which values in a numeric vector are outliers based on the median absolute deviation (MAD).

### Usage

```
isOutlier(metric, nmads = 3, type = c("both", "lower", "higher"),
  log = FALSE, subset = NULL, batch = NULL, min_diff = NA)
```



**Arguments**

<code>metric</code>	Numeric vector of values.
<code>nmads</code>	A numeric scalar, specifying the minimum number of MADs away from median required for a value to be called an outlier.
<code>type</code>	String indicating whether outliers should be looked for at both tails ("both"), only at the lower tail ("lower") or the upper tail ("higher").
<code>log</code>	Logical scalar, should the values of the metric be transformed to the log10 scale before computing MADs?
<code>subset</code>	Logical or integer vector, which subset of values should be used to calculate the median/MAD? If NULL, all values are used.
<code>batch</code>	Factor of length equal to <code>metric</code> , specifying the batch to which each observation belongs. A median/MAD is calculated for each batch, and outliers are then identified within each batch.
<code>min_diff</code>	A numeric scalar indicating the minimum difference from the median to consider as an outlier. The outlier threshold is defined from the larger of <code>nmads</code> MADs and <code>min_diff</code> , to avoid calling many outliers when the MAD is very small. Ignored if NA.

**Details**

Lower and upper thresholds are stored in the "threshold" attribute of the returned vector. This is a numeric vector of length 2 when `batch=NULL` for the threshold on each side. Otherwise, it is a matrix with one named column per level of batch and two rows (one per threshold).

Missing values trigger a warning and are automatically ignored during estimation of the median and MAD. The corresponding entries of the output vector are also set to NA values.

**Value**

A logical vector of the same length as the `metric` argument, specifying the observations that are considered as outliers.

**Author(s)**

Aaron Lun

**Examples**

```
example_sce <- mockSCE()
stats <- perCellQCMetrics(example_sce)

str(isOutlier(stats$sum))
str(isOutlier(stats$sum, type="lower"))
str(isOutlier(stats$sum, type="higher"))

str(isOutlier(stats$sum, log=TRUE))

b <- sample(LETTERS[1:3], ncol(example_sce), replace=TRUE)
str(isOutlier(stats$sum, log=TRUE, batch=b))
```

---

librarySizeFactors      *Compute library size factors*

---

### Description

Define per-cell size factors from the library sizes (i.e., total sum of counts per cell).

### Usage

```
librarySizeFactors(x, ...)

## S4 method for signature 'ANY'
librarySizeFactors(x, subset_row = NULL)

## S4 method for signature 'SummarizedExperiment'
librarySizeFactors(x,
  exprs_values = "counts", ...)

computeLibraryFactors(x, ..., altexp = NULL)
```

### Arguments

x	For librarySizeFactors, a numeric matrix of counts with one row per feature and column per cell. Alternatively, a <a href="#">SummarizedExperiment</a> or <a href="#">SingleCellExperiment</a> containing such counts. For computeLibraryFactors, only a <a href="#">SingleCellExperiment</a> is accepted.
...	For the librarySizeFactors generic, arguments to pass to specific methods. For the SummarizedExperiment method, further arguments to pass to the ANY method. For computeLibraryFactors, further arguments to pass to librarySizeFactors.
subset_row	A vector specifying whether the size factors should be computed from a subset of rows of x.
exprs_values	String or integer scalar indicating the assay of x containing the counts.
altexp	String or integer scalar indicating which (if any) alternative experiment should be used to provide the counts to compute the size factors.

### Details

Library sizes are converted into size factors by scaling them so that their mean across cells is unity. This ensures that the normalized values are still on the same scale as the raw counts.

Preserving the scale is useful for interpretation of operations on the normalized values, e.g., the pseudo-count used in [logNormCounts](#) can actually be considered an additional read/UMI. This is important for ensuring that the effect of the pseudo-count decreases with increasing sequencing depth.

Setting altexp is occasionally useful for computing size factors from spike-in transcripts and using them on the count matrix for endogenous genes (stored in the main experiment).

**Value**

For librarySizeFactors, a numeric vector of size factors is returned for all methods.

For computeLibraryFactors, a numeric vector is also returned for the ANY and SummarizedExperiment methods. For the SingleCellExperiment method, x is returned containing the size factors in sizeFactors(x).

**Author(s)**

Aaron Lun

**See Also**

[logNormCounts](#), where these size factors are used by default.

**Examples**

```
example_sce <- mockSCE()
summary(librarySizeFactors(example_sce))
```

---

logNormCounts

*Compute log-normalized expression values*


---

**Description**

Compute log-transformed normalized expression values from a count matrix in a [SingleCellExperiment](#) object.

**Usage**

```
logNormCounts(x, ...)

## S4 method for signature 'SummarizedExperiment'
logNormCounts(x, size_factors = NULL,
  log = TRUE, pseudo_count = 1, center_size_factors = TRUE, ...,
  exprs_values = "counts", name = NULL)

## S4 method for signature 'SingleCellExperiment'
logNormCounts(x, size_factors = NULL,
  log = TRUE, pseudo_count = 1, center_size_factors = TRUE, ...,
  exprs_values = "counts", use_altexprs = FALSE, name = NULL)
```

**Arguments**

x	A <a href="#">SingleCellExperiment</a> or <a href="#">SummarizedExperiment</a> object containing a count matrix.
...	For the generic, additional arguments passed to specific methods. For the methods, additional arguments passed to <a href="#">normalizeCounts</a> .
size_factors	A numeric vector of cell-specific size factors. Alternatively NULL, in which case the size factors are extracted or computed from x.
log	Logical scalar indicating whether normalized values should be log2-transformed.

pseudo_count	Numeric scalar specifying the pseudo_count to add when log-transforming expression values.
center_size_factors	Logical scalar indicating whether size factors should be centered at unity before being used.
exprs_values	A string or integer scalar specifying the assay of x containing the count matrix.
name	String containing an assay name for storing the output normalized values. Defaults to "logcounts" when log=TRUE and "normcounts" otherwise.
use_altexprs	Logical scalar indicating whether normalization should be performed for alternative experiments in x. Alternatively, a character vector specifying the names of the alternative experiments to be normalized. Alternatively, NULL in which case alternative experiments are not used.

### Details

This function is a convenience wrapper around [normalizeCounts](#). It returns a [SingleCellExperiment](#) or [SummarizedExperiment](#) containing the normalized values in a separate assay. This makes it easier to perform normalization by avoiding book-keeping errors during a long analysis workflow.

If x is a [SingleCellExperiment](#) that contains alternative experiments, normalized values can be computed and stored within each alternative experiment by setting use\_altexprs appropriately. By default, use\_altexprs=FALSE to avoid problems from attempting to library size-normalize alternative experiments that have zero total counts for some cells.

If size\_factors=NULL, size factors are obtained separately for each nested experiment following the rules in [normalizeCounts](#). However, if size\_factors is supplied, it will override any size factors available in the alternative experiments.

### Value

x is returned containing the (log-)normalized expression values in an additional assay named as name.

If x is a [SingleCellExperiment](#), the size factors used for normalization are stored in [sizeFactors](#). These are centered if center\_size\_factors=TRUE.

If x contains alternative experiments and use\_altexprs=TRUE, each of the alternative experiments in x will also contain an additional assay. This can be limited to particular [altExps](#) entries by specifying them in use\_altexprs.

### Author(s)

Aaron Lun, based on code by Davis McCarthy

### See Also

[normalizeCounts](#), which is used to compute the normalized expression values.

### Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)
assayNames(example_sce)
```

---

mockSCE	<i>Mock up a SingleCellExperiment</i>
---------	---------------------------------------

---

### Description

Mock up a [SingleCellExperiment](#) containing simulated data, for use in documentation examples.

### Usage

```
mockSCE(ncells = 200, ngenes = 2000, nspikes = 100)
```

### Arguments

ncells	Integer scalar, number of cells to simulate.
ngenes	Integer scalar, number of genes to simulate.
nspikes	Integer scalar, number of spike-in transcripts to simulate.

### Details

Users should set a seed to obtain reproducible results from this function.

### Value

A [SingleCellExperiment](#) object containing a count matrix in the "counts" assay, a set of simulated [colData](#) fields, and spike-in data in the "Spikes" field of [altExps](#).

### Author(s)

Aaron Lun

### See Also

[SingleCellExperiment](#), for the constructor.

### Examples

```
set.seed(1000)
sce <- mockSCE()
sce
```

---

`multiplot`*Multiple plot function for ggplot2 plots*

---

### Description

Place multiple `ggplot` plots on one page.

### Usage

```
multiplot(..., plotlist = NULL, cols = 1, layout = NULL)
```

### Arguments

<code>...</code>	One or more <code>ggplot</code> objects.
<code>plotlist</code>	A list of <code>ggplot</code> objects, as an alternative to <code>...</code>
<code>cols</code>	A numeric scalar giving the number of columns in the layout.
<code>layout</code>	A matrix specifying the layout. If present, <code>cols</code> is ignored.

### Details

If the layout is something like `matrix(c(1, 2, 3, 3), nrow=2, byrow=TRUE)`, then:

- plot 1 will go in the upper left;
- plot 2 will go in the upper right;
- and plot 3 will go all the way across the bottom.

There is no way to tweak the relative heights or widths of the plots with this simple function. It was adapted from [http://www.cookbook-r.com/Graphs/Multiple\\_graphs\\_on\\_one\\_page\\_\(ggplot2\)/](http://www.cookbook-r.com/Graphs/Multiple_graphs_on_one_page_(ggplot2)/)

### Value

A `ggplot` object.

### Examples

```
library(ggplot2)

## This example uses the ChickWeight dataset, which comes with ggplot2
## First plot
p1 <- ggplot(ChickWeight, aes(x = Time, y = weight, colour = Diet, group = Chick)) +
  geom_line() +
  ggtitle("Growth curve for individual chicks")
## Second plot
p2 <- ggplot(ChickWeight, aes(x = Time, y = weight, colour = Diet)) +
  geom_point(alpha = .3) +
  geom_smooth(alpha = .2, size = 1) +
  ggtitle("Fitted growth curve per diet")

## Third plot
p3 <- ggplot(subset(ChickWeight, Time == 21), aes(x = weight, colour = Diet)) +
  geom_density() +
```

```

  ggtitle("Final weight, by diet")
## Fourth plot
p4 <- ggplot(subset(ChickWeight, Time == 21), aes(x = weight, fill = Diet)) +
  geom_histogram(colour = "black", binwidth = 50) +
  facet_grid(Diet ~ .) +
  ggtitle("Final weight, by diet") +
  theme(legend.position = "none")      # No legend (redundant in this graph)

## Combine plots and display
multiplot(p1, p2, p3, p4, cols = 2)

```

---

nexprs

*Count the number of non-zero counts per cell or feature*


---

## Description

Counting the number of non-zero counts in each row (per feature) or column (per cell), without constructing an intermediate logical matrix.

## Usage

```

nexprs(x, ...)

## S4 method for signature 'ANY'
nexprs(x, byrow = FALSE, detection_limit = 0,
  subset_row = NULL, subset_col = NULL, BPPARAM = SerialParam())

## S4 method for signature 'SummarizedExperiment'
nexprs(x, ..., exprs_values = "counts")

```

## Arguments

x	A numeric matrix of counts where features are rows and cells are columns. Alternatively, a <a href="#">SummarizedExperiment</a> containing such counts.
...	For the generic, further arguments to pass to specific methods. For the SummarizedExperiment method, further arguments to pass to the ANY method.
byrow	Logical scalar indicating whether to count the number of detected cells per feature. If FALSE, the function will count the number of detected features per cell.
detection_limit	Numeric scalar providing the value above which observations are deemed to be expressed.
subset_row	Logical, integer or character vector indicating which rows (i.e. features) to use.
subset_col	Logical, integer or character vector indicating which columns (i.e., cells) to use.
BPPARAM	A <a href="#">BiocParallelParam</a> object specifying whether the calculations should be parallelized.
exprs_values	String or integer specifying the assay of x to obtain the count matrix from.

**Value**

An integer vector containing counts per gene or cell, depending on the provided arguments.

**Author(s)**

Aaron Lun

**See Also**

[numDetectedAcrossFeatures](#) and [numDetectedAcrossCells](#), to do this calculation for each group of features or cells, respectively.

**Examples**

```
example_sce <- mockSCE()

nexprs(example_sce)[1:10]
nexprs(example_sce, byrow = TRUE)[1:10]
```

---

normalizeCounts

*Compute normalized expression values*

---

**Description**

Compute (log-)normalized expression values by dividing counts for each cell by the corresponding size factor.

**Usage**

```
normalizeCounts(x, ...)

## S4 method for signature 'ANY'
normalizeCounts(x, size_factors = NULL, log = TRUE,
  pseudo_count = 1, center_size_factors = TRUE, subset_row = NULL,
  downsample = FALSE, down_target = NULL, down_prop = 0.01)

## S4 method for signature 'SummarizedExperiment'
normalizeCounts(x, ...,
  exprs_values = "counts")

## S4 method for signature 'SingleCellExperiment'
normalizeCounts(x, size_factors = NULL,
  ...)
```

**Arguments**

**x** A numeric matrix-like object containing counts for cells in the columns and features in the rows.  
Alternatively, a [SingleCellExperiment](#) or [SummarizedExperiment](#) object containing such a count matrix.



...	For the generic, arguments to pass to specific methods. For the SummarizedExperiment method, further arguments to pass to the ANY or <a href="#">DelayedMatrix</a> methods. For the SingleCellExperiment method, further arguments to pass to the SummarizedExperiment method.
size_factors	A numeric vector of cell-specific size factors. Alternatively NULL, in which case the size factors are extracted or computed from x.
log	Logical scalar indicating whether normalized values should be log2-transformed.
pseudo_count	Numeric scalar specifying the pseudo_count to add when log-transforming expression values.
center_size_factors	Logical scalar indicating whether size factors should be centered at unity before being used.
subset_row	A vector specifying the subset of rows of x for which to return a result.
downsample	Logical scalar indicating whether downsampling should be performed prior to scaling and log-transformation.
down_target	Numeric scalar specifying the downsampling target when downsample=TRUE. If NULL, this is defined by down_prop and a warning is emitted.
down_prop	Numeric scalar between 0 and 1 indicating the quantile to use to define the downsampling target when downsample=TRUE.
exprs_values	A string or integer scalar specifying the assay of x containing the count matrix.

## Details

Normalized expression values are computed by dividing the counts for each cell by the size factor for that cell. This aims to remove cell-specific scaling biases, e.g., due to differences in sequencing coverage or capture efficiency. If log=TRUE, log-normalized values are calculated by adding pseudo\_count to the normalized count and performing a log2 transformation.

If no size factors are supplied, they are determined automatically from x:

- For count matrices and [SummarizedExperiment](#) inputs, the sum of counts for each cell is used to compute a size factor via the [librarySizeFactors](#) function.
- For [SingleCellExperiment](#) instances, the function searches for [sizeFactors](#) from x. If none are available, it defaults to library size-derived size factors.

If size\_factors are supplied, they will override any size factors present in x.

If center\_size\_factors=TRUE, size factors are centred at unity prior to calculation of normalized expression values. This means that the computed expression values can be interpreted as being on the same scale as log-counts, and that the value of pseudo\_count can be interpreted as being on the same scale as the counts. It also ensures that abundances are roughly comparable between features normalized with different sets of size factors.

## Value

A matrix-like object of (log-)normalized expression values.

### Downsampling instead of scaling

If `downsample=TRUE`, counts for each cell are randomly downsampled according to their size factors prior to log-transformation. This is occasionally useful for avoiding artifacts caused by scaling count data with a strong mean-variance relationship. Each cell is downsampled according to the ratio between `down_target` and that cell's size factor. (Cells with size factors below the target are not downsampled and are directly scaled by this ratio.) If `log=TRUE`, a log-transformation is also performed after adding `pseudo_count` to the downsampled counts.

Note that the normalized expression values in this mode cannot be interpreted as being on the same abundance as the original counts, but instead have abundance equivalent to counts after downsampling to the target size factor. This motivates the use of a fixed `down_target` to ensure that expression values are comparable across different `normalizeCounts` calls. We automatically set `down_target` to the 1st percentile of size factors across all cells involved in the analysis, but this is only appropriate if the resulting expression values are only compared within the same call to `normalizeCounts`. If expression values are to be compared across multiple calls (e.g., in `modelGeneVarWithSpikes` or `multiBatchNorm`), `down_target` should be manually set to a constant target value that can be considered a low size factor in every call.

### Author(s)

Aaron Lun

### See Also

[logNormCounts](#), which wraps this function for convenient use with `SingleCellExperiment` instances.  
[downsampleMatrix](#), to perform the downsampling.

### Examples

```
example_sce <- mockSCE()
normed <- normalizeCounts(example_sce)
str(normed)
```

---

norm_exprs	<i>Additional accessors for the typical elements of a SingleCellExperiment object.</i>
------------	--

---

### Description

Convenience functions to access commonly-used assays of the `SingleCellExperiment` object.

### Usage

```
norm_exprs(object)

norm_exprs(object) <- value

stand_exprs(object)

stand_exprs(object) <- value

fpkm(object)
```

```
fpm(object) <- value
```

### Arguments

**object** SingleCellExperiment class object from which to access or to which to assign assay values. Namely: "exprs", "norm\_exprs", "stand\_exprs", "fpm". The following are imported from [SingleCellExperiment](#): "counts", "normcounts", "logcounts", "cpm", "tpm".

**value** a numeric matrix (e.g. for exprs)

### Value

a matrix of normalised expression data  
 a matrix of standardised expression data  
 a matrix of FPKM values  
 A matrix of numeric, integer or logical values.

### Author(s)

Davis McCarthy

### Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)
head(logcounts(example_sce)[,1:10])
head(exprs(example_sce)[,1:10]) # identical to logcounts()

norm_exprs(example_sce) <- log2(calculateCPM(example_sce) + 1)

stand_exprs(example_sce) <- log2(calculateCPM(example_sce) + 1)

tpm(example_sce) <- calculateTPM(example_sce, lengths = 5e4)

cpm(example_sce) <- calculateCPM(example_sce)

fpm(example_sce)
```

---

numDetectedAcrossCells

*Number of detected expression values per group of cells*

---

### Description

Computes the number of detected expression values (default defined as non-zero counts) for each feature in each group of cells.

**Usage**

```

numDetectedAcrossCells(x, ...)

## S4 method for signature 'ANY'
numDetectedAcrossCells(x, ids, average = FALSE,
  subset_row = NULL, subset_col = NULL, ..., BPPARAM = SerialParam())

## S4 method for signature 'SummarizedExperiment'
numDetectedAcrossCells(x, ...,
  exprs_values = "counts")

```

**Arguments**

x	A numeric matrix of counts where features are rows and cells are columns. Alternatively, a <a href="#">SummarizedExperiment</a> containing such counts.
...	For the generic, further arguments to pass to specific methods. For the SummarizedExperiment method, further arguments to pass to the ANY method. For the ANY method, further arguments to pass to the <a href="#">nexprs</a> function.
ids	A vector of length ncol(x), specifying the group assignment for each cell.
average	Logical scalar indicating whether the proportion of non-zero counts in each group should be computed instead.
subset_row	A vector specifying the rows to use. Defaults to all rows.
subset_col	A vector specifying the columns to use. Defaults to all cells with non-NA entries of ids.
BPPARAM	A <a href="#">BiocParallelParam</a> object specifying whether the calculations should be parallelized.
exprs_values	String or integer specifying the assay of x to obtain the count matrix from.

**Value**

An integer or numeric matrix containing the number or proportion of detected expression values for each feature (row) in each group of cells (column).

**Author(s)**

Aaron Lun

**See Also**

[nexprs](#), on which this function is based.

**Examples**

```

example_sce <- mockSCE()

ids <- sample(LETTERS[1:5], ncol(example_sce), replace=TRUE)
bycol <- numDetectedAcrossCells(example_sce, ids)
head(bycol)

```

---

 numDetectedAcrossFeatures

*Number of detected expression values per group of features*


---

### Description

Computes the number of detected expression values (default defined as non-zero counts) for each cell in each group of features.

### Usage

```
numDetectedAcrossFeatures(x, ...)

## S4 method for signature 'ANY'
numDetectedAcrossFeatures(x, ids, average = FALSE,
  subset_row = NULL, subset_col = NULL, ..., BPPARAM = SerialParam())

## S4 method for signature 'SummarizedExperiment'
numDetectedAcrossFeatures(x, ...,
  exprs_values = "counts")
```

### Arguments

x	A numeric matrix of counts where features are rows and cells are columns. Alternatively, a <a href="#">SummarizedExperiment</a> containing such counts.
...	For the generic, further arguments to pass to specific methods. For the SummarizedExperiment method, further arguments to pass to the ANY method. For the ANY method, further arguments to pass to the <a href="#">nexprs</a> function.
ids	A vector of length nrow(x), specifying the group assignment for each feature.
average	Logical scalar indicating whether the proportion of non-zero counts in each group should be computed instead.
subset_row	A vector specifying the rows to use. Defaults to all rows with non-NA entries of ids.
subset_col	A vector specifying the columns to use. Defaults to all columns.
BPPARAM	A <a href="#">BiocParallelParam</a> object specifying whether the calculations should be parallelized.
exprs_values	String or integer specifying the assay of x to obtain the count matrix from.

### Value

An integer or numeric matrix containing the number of detected expression values in each group of features (row) and cell (column).

### Author(s)

Aaron Lun

**See Also**

[nexprs](#), on which this function is based.

**Examples**

```
example_sce <- mockSCE()

ids <- sample(paste0("GENE_", 1:100), nrow(example_sce), replace=TRUE)
byrow <- numDetectedAcrossFeatures(example_sce, ids)
head(byrow[,1:10])
```

---

perCellQCMetrics      *Per-cell quality control metrics*

---

**Description**

Compute per-cell quality control metrics for a count matrix or a [SingleCellExperiment](#).

**Usage**

```
perCellQCMetrics(x, ...)

## S4 method for signature 'ANY'
perCellQCMetrics(x, subsets = NULL, percent_top = c(50,
  100, 200, 500), detection.limit = 0, BPPARAM = SerialParam(),
  flatten = TRUE)

## S4 method for signature 'SummarizedExperiment'
perCellQCMetrics(x, ...,
  exprs_values = "counts")

## S4 method for signature 'SingleCellExperiment'
perCellQCMetrics(x, subsets = NULL,
  percent_top = c(50, 100, 200, 500), ..., flatten = TRUE,
  exprs_values = "counts", use_altexprs = TRUE)
```

**Arguments**

x	A numeric matrix of counts with cells in columns and features in rows. Alternatively, a <a href="#">SummarizedExperiment</a> or <a href="#">SingleCellExperiment</a> object containing such a matrix.
...	For the generic, further arguments to pass to specific methods. For the <a href="#">SummarizedExperiment</a> and <a href="#">SingleCellExperiment</a> methods, further arguments to pass to the ANY method.
subsets	A named list containing one or more vectors (a character vector of feature names, a logical vector, or a numeric vector of indices), used to identify interesting feature subsets such as ERCC spike-in transcripts or mitochondrial genes.
percent_top	An integer vector. Each element is treated as a number of top genes to compute the percentage of library size occupied by the most highly expressed genes in each cell.

detection.limit	A numeric scalar specifying the lower detection limit for expression.
BPPARAM	A BiocParallelParam object specifying whether the QC calculations should be parallelized.
flatten	Logical scalar indicating whether the nested <a href="#">DataFrames</a> in the output should be flattened.
exprs_values	A string or integer scalar indicating which assays in the x contains the count matrix.
use_altexprs	Logical scalar indicating whether QC statistics should be computed for alternative Experiments in x. If TRUE, statistics are computed for all alternative experiments. Alternatively, an integer or character vector specifying the alternative Experiments to use to compute QC statistics. Alternatively NULL, in which case alternative experiments are not used.

## Details

This function calculates useful QC metrics for identification and removal of potentially problematic cells. Obvious per-cell metrics are the sum of counts (i.e., the library size) and the number of detected features. The percentage of counts in the top features also provides a measure of library complexity.

If subsets is specified, these statistics are also computed for each subset of features. This is useful for investigating gene sets of interest, e.g., mitochondrial genes, Y chromosome genes. These statistics are stored as nested [DataFrames](#) in the subsets field of the output. For example, if the input subsets contained "Mito" and "Sex", the output would look like:

```
output
|-- sum
|-- detected
|-- percent_top
+-- subsets
  |-- Mito
  |   |-- sum
  |   |-- detected
  |   +-- percent
  +-- Sex
      |-- sum
      |-- detected
      +-- percent
```

Here, the percent field contains the percentage of each cell's count sum assigned to each subset.

If use\_altexprs is TRUE, the same statistics are computed for each alternative experiment in x. This can also be an integer or character vector specifying the alternative Experiments to use. These statistics are also stored as nested [DataFrames](#), this time in the altexprs field of the output. For example, if x contained the alternative Experiments "Spike" and "Ab", the output would look like:

```
output
|-- sum
|-- detected
|-- percent_top
+-- altexprs
```

```

| |-- Spike
| | |-- sum
| | |-- detected
| | +-- percent.total
| +-- Ab
|     |-- sum
|     |-- detected
|     +-- percent.total
+-- total

```

The `total` field contains the total sum of counts for each cell across the main and alternative Experiments. The `percent` field contains the percentage of the total count in each alternative Experiment for each cell.

If `flatten=TRUE`, the nested DataFrames are flattened by concatenating the column names with underscores. This means that, say, the `subsets$Mito$sum` nested field becomes the top-level `subsets_Mito_sum` field. A flattened structure is more convenient for end-users performing interactive analyses, but less convenient for programmatic access as artificial construction of strings is required.

## Value

A [DataFrame](#) of QC statistics where each row corresponds to a column in `x`. This contains the following fields:

- `sum`: numeric, the sum of counts for each cell.
- `detected`: numeric, the number of observations above `detection.limit`.

If `flatten=FALSE`, the DataFrame will contain the additional columns:

- `percent_top`: numeric matrix, the percentage of counts assigned to the `percent_topage` of most highly expressed genes. Each column of the matrix corresponds to an entry of the sorted `percent_top`, in increasing order.
- `subsets`: A nested DataFrame containing statistics for each subset, see [Details](#).
- `altexps`: A nested DataFrame containing statistics for each alternative experiment, see [Details](#). This is only returned for the `SingleCellExperiment` method.
- `total`: numeric, the total sum of counts for each cell across main and alternative Experiments. This is only returned for the `SingleCellExperiment` method.

If `flatten=TRUE`, nested matrices and DataFrames are flattened to remove the hierarchical structure from the output DataFrame.

## Author(s)

Aaron Lun

## See Also

[addPerCellQC](#), to add the QC metrics to the column metadata.



**Examples**

```

example_sce <- mockSCE()
stats <- perCellQCMetrics(example_sce)
stats

# With subsets.
stats2 <- perCellQCMetrics(example_sce, subsets=list(Mito=1:10),
  flatten=FALSE)
stats2$subsets

# With alternative Experiments.
pretend.spike <- ifelse(seq_len(nrow(example_sce)) < 10, "Spike", "Gene")
alt_sce <- splitAltExps(example_sce, pretend.spike)
stats3 <- perCellQCMetrics(alt_sce, flatten=FALSE)
stats3$altexps

```

---

perFeatureQCMetrics    *Per-feature quality control metrics*

---

**Description**

Compute per-feature quality control metrics for a count matrix or a [SummarizedExperiment](#).

**Usage**

```

perFeatureQCMetrics(x, ...)

## S4 method for signature 'ANY'
perFeatureQCMetrics(x, subsets = NULL,
  detection_limit = 0, BPPARAM = SerialParam(), flatten = TRUE)

## S4 method for signature 'SummarizedExperiment'
perFeatureQCMetrics(x, ...,
  exprs_values = "counts")

```

**Arguments**

x	A numeric matrix of counts with cells in columns and features in rows. Alternatively, a <a href="#">SummarizedExperiment</a> object containing such a matrix.
...	For the generic, further arguments to pass to specific methods. For the <code>SummarizedExperiment</code> and <code>SingleCellExperiment</code> methods, further arguments to pass to the ANY method.
subsets	A named list containing one or more vectors (a character vector of cell names, a logical vector, or a numeric vector of indices), used to identify interesting sample subsets such as negative control wells.
detection_limit	A numeric scalar specifying the lower <code>detection_limit</code> for expression.
BPPARAM	A <code>BiocParallelParam</code> object specifying whether the QC calculations should be parallelized.

flatten	Logical scalar indicating whether the nested <a href="#">DataFrames</a> in the output should be flattened.
exprs_values	A string or integer scalar indicating which assays in the x contains the count matrix.

## Details

This function calculates useful QC metrics for features, including the mean across all cells and the number of expressed features (i.e., counts above the `detection_limit`).

If `subsets` is specified, the same statistics are computed for each subset of cells. This is useful for obtaining statistics for cell sets of interest, e.g., negative control wells. These statistics are stored as nested [DataFrames](#) in the output. For example, if `subsets` contained "empty" and "cellpool", the output would look like:

```
output
|-- mean
|-- detected
+-- subsets
  |-- empty
  |   |-- mean
  |   |-- detected
  |   +-- ratio
  +-- cellpool
      |-- mean
      |-- detected
      +-- ratio
```

The `ratio` field contains the ratio of the mean within each subset to the mean across all cells.

If `flatten=TRUE`, the nested [DataFrames](#) are flattened by concatenating the column names with underscores. This means that, say, the `subsets$empty$mean` nested field becomes the top-level `subsets_empty_mean` field. A flattened structure is more convenient for end-users performing interactive analyses, but less convenient for programmatic access as artificial construction of strings is required.

## Value

A [DataFrame](#) of QC statistics where each row corresponds to a row in `x`. This contains the following fields:

- `mean`: numeric, the mean counts for each feature.
- `detected`: numeric, the percentage of observations above `detection_limit`.

If `flatten=FALSE`, the output [DataFrame](#) also contains the `subsets` field. This a nested [DataFrame](#) containing per-feature QC statistics for each subset of columns.

If `flatten=TRUE`, `subsets` is flattened to remove the hierarchical structure.

## Author(s)

Aaron Lun

## See Also

[addPerFeatureQC](#), to add the QC metrics to the row metadata.

**Examples**

```

example_sce <- mockSCE()
stats <- perFeatureQCMetrics(example_sce)
stats

# With subsets.
stats2 <- perFeatureQCMetrics(example_sce, subsets=list(Empty=1:10))
stats2$subsets

```

---

plotColData

*Plot column metadata*


---

**Description**

Plot column-level (i.e., cell) metadata in an `SingleCellExperiment` object.

**Usage**

```

plotColData(object, y, x = NULL, colour_by = NULL, shape_by = NULL,
  size_by = NULL, by_exprs_values = "logcounts",
  other_fields = list(), ...)

```

**Arguments**

object	A <a href="#">SingleCellExperiment</a> object containing expression values and experimental information.
y	String specifying the column-level metadata field to show on the y-axis. Alternatively, an <a href="#">AsIs</a> vector or data.frame, see <a href="#">?retrieveCellInfo</a> .
x	String specifying the column-level metadata to show on the x-axis. Alternatively, an <a href="#">AsIs</a> vector or data.frame, see <a href="#">?retrieveCellInfo</a> . If NULL, nothing is shown on the x-axis.
colour_by	Specification of a column metadata field or a feature to colour by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
shape_by	Specification of a column metadata field or a feature to shape by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
size_by	Specification of a column metadata field or a feature to size by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
by_exprs_values	A string or integer scalar specifying which assay to obtain expression values from, for use in point aesthetics - see <a href="#">?retrieveCellInfo</a> for details.
other_fields	Additional cell-based fields to include in the data.frame, see <a href="#">?"scater-plot-args"</a> for details.
...	Additional arguments for visualization, see <a href="#">?"scater-plot-args"</a> for details.

**Details**

If *y* is continuous and *x*=NULL, a violin plot is generated. If *x* is categorical, a grouped violin plot will be generated, with one violin for each level of *x*. If *x* is continuous, a scatter plot will be generated.

If *y* is categorical and *x* is continuous, horizontal violin plots will be generated. If *x* is missing or categorical, rectangle plots will be generated where the area of a rectangle is proportional to the number of points for a combination of factors.

**Value**

A `ggplot` object.

**Author(s)**

Davis McCarthy, with modifications by Aaron Lun

**Examples**

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)
colData(example_sce) <- cbind(colData(example_sce),
  perCellQCMetrics(example_sce))

plotColData(example_sce, y = "detected", x = "sum",
  colour_by = "Mutation_Status") + scale_x_log10()

plotColData(example_sce, y = "detected", x = "sum",
  colour_by = "Mutation_Status", size_by = "Gene_0001",
  shape_by = "Treatment") + scale_x_log10()

plotColData(example_sce, y = "Treatment", x = "sum",
  colour_by = "Mutation_Status") + scale_y_log10() # flipped violin.

plotColData(example_sce, y = "detected",
  x = "Cell_Cycle", colour_by = "Mutation_Status")
```

---

plotDots

*Create a dot plot of expression values*

---

**Description**

Create a dot plot of expression values for a grouping of cells, where the size and color of each dot represents the proportion of detected expression values and the average expression, respectively, for each feature in each group of cells.

**Usage**

```
plotDots(object, features, group = NULL, exprs_values = "logcounts",
  detection_limit = 0, low_color = "white", high_color = "red",
  max_ave = NULL, max_detected = NULL, other_fields = list(),
  by_exprs_values = exprs_values)
```

**Arguments**

object	A <a href="#">SingleCellExperiment</a> object.
features	A character vector of feature names to show as rows of the dot plot.
group	Specification of a column metadata field or a feature to show as columns. Alternatively, an <a href="#">AsIs</a> vector, see <a href="#">?retrieveCellInfo</a> for details.
exprs_values	A string or integer scalar specifying which assay in assays(object) to obtain expression values from.
detection_limit	Numeric scalar providing the value above which observations are deemed to be expressed. This is also used as the
low_color	String specifying the color to use for low expression. This is also used as the background color, see Details.
high_color	String specifying the color to use for high expression.
max_ave	Numeric value specifying the cap on the average expression.
max_detected	Numeric value specifying the cap on the proportion of detected expression values.
other_fields	Additional feature-based fields to include in the data.frame, see <a href="#">?"scatter-plot-args"</a> for details. Note that any <a href="#">AsIs</a> vectors or data.frames must be of length equal to nrow(object), not features.
by_exprs_values	A string or integer scalar specifying which assay to obtain expression values from, to use when extracting values according to each entry of other_fields.

**Details**

This implements a **Seurat**-style “dot plot” that creates a dot for each feature (row) in each group of cells (column). The proportion of detected expression values and the average expression for each feature in each group of cells is visualized efficiently using the size and colour, respectively, of each dot.

We impose two restrictions - the low end of the color scale must correspond to the detection limit, and the color at this end of the scale must be the same as the background color. These ensure that the visual cues from low average expression or low detected proportions are consistent, as both will result in a stronger low\_color. (In the latter case, the reduced size of the dot means that the background color dominates.)

If these restrictions are violated, visualization can be misleading due to the difficulty of simultaneously interpreting both size and color. For example, if we colored by z-score on a conventional blue-white-red color axis, a gene that is downregulated in a group of cells would show up as a small blue dot. If the background color was also white, this might be mistaken for a gene that is not downregulated at all. On the other hand, any other background color would effectively require consideration of two color axes as expression decreases.

We can also cap the color and size scales at max\_ave and max\_detected, respectively. This aims to preserve resolution for low-abundance genes by preventing domination of the scales by high-abundance features.

**Value**

A [ggplot](#) object containing a dot plot.

**Author(s)**

Aaron Lun

**See Also**[plotExpression](#) and [plotHeatmap](#), for alternatives to visualizing group-level expression values.**Examples**

```
sce <- mockSCE()
sce <- logNormCounts(sce)
plotDots(sce, features=rownames(sce)[1:10], group="Cell_Cycle")
```

---

plotExplanatoryPCs      *Plot the explanatory PCs for each variable*

---

**Description**

Plot the explanatory PCs for each variable

**Usage**

```
plotExplanatoryPCs(object, nvars_to_plot = 10, npcs_to_plot = 50,
  theme_size = 10, ...)
```

**Arguments**

object	A SingleCellExperiment object containing expression values and experimental information. Alternatively, a matrix containing the output of <a href="#">getExplanatoryPCs</a> .
nvars_to_plot	Integer scalar specifying the number of variables with the greatest explanatory power to plot. This can be set to Inf to show all variables.
npcs_to_plot	Integer scalar specifying the number of PCs to plot.
theme_size	numeric scalar providing base font size for ggplot theme.
...	Parameters to be passed to <a href="#">getExplanatoryPCs</a> .

**Details**

A density plot is created for each variable, showing the R-squared for each successive PC (up to npcs\_to\_plot PCs). Only the nvars\_to\_plot variables with the largest maximum R-squared across PCs are shown.

If object is a SingleCellExperiment object, [getExplanatoryPCs](#) will be called to compute the variance in expression explained by each variable in each gene. Users may prefer to run [getExplanatoryPCs](#) manually and pass the resulting matrix as object, in which case the R-squared values are used directly.

**Value**

A ggplot object.

**Examples**

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)
example_sce <- runPCA(example_sce)

plotExplanatoryPCs(example_sce)
```

---

```
plotExplanatoryVariables
```

*Plot explanatory variables ordered by percentage of variance explained*

---

**Description**

Plot explanatory variables ordered by percentage of variance explained

**Usage**

```
plotExplanatoryVariables(object, nvars_to_plot = 10,
  min_marginal_r2 = 0, theme_size = 10, ...)
```

**Arguments**

object	A SingleCellExperiment object containing expression values and experimental information. Alternatively, a matrix containing the output of <a href="#">getVarianceExplained</a> .
nvars_to_plot	Integer scalar specifying the number of variables with the greatest explanatory power to plot. This can be set to Inf to show all variables.
min_marginal_r2	Numeric scalar specifying the minimal value required for median marginal R-squared for a variable to be plotted. Only variables with a median marginal R-squared strictly larger than this value will be plotted.
theme_size	Numeric scalar specifying the font size to use for the plotting theme
...	Parameters to be passed to <a href="#">getVarianceExplained</a> .

**Details**

A density plot is created for each variable, showing the distribution of R-squared across all genes. Only the `nvars_to_plot` variables with the largest median R-squared across genes are shown. Variables are also only shown if they have median R-squared values above `min_marginal_r2`.

If `object` is a SingleCellExperiment object, [getVarianceExplained](#) will be called to compute the variance in expression explained by each variable in each gene. Users may prefer to run [getVarianceExplained](#) manually and pass the resulting matrix as `object`, in which case the R-squared values are used directly.

**Value**

A ggplot object.

**Examples**

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)
plotExplanatoryVariables(example_sce)
```

---

plotExpression	<i>Plot expression values for all cells</i>
----------------	---

---

**Description**

Plot expression values for a set of features (e.g. genes or transcripts) in a SingleExperiment object, against a continuous or categorical covariate for all cells.

**Usage**

```
plotExpression(object, features, x = NULL, exprs_values = "logcounts",
  log2_values = FALSE, colour_by = NULL, shape_by = NULL,
  size_by = NULL, by_exprs_values = exprs_values, xlab = NULL,
  feature_colours = TRUE, one_facet = TRUE, ncol = 2,
  scales = "fixed", other_fields = list(), ...)
```

**Arguments**

object	A SingleCellExperiment object containing expression values and other meta-data.
features	A character vector or a list specifying the features to plot. If a list is supplied, each entry of the list can be a string, an AsIs-wrapped vector or a data.frame - see <a href="#">?retrieveCellInfo</a> .
x	Specification of a column metadata field or a feature to show on the x-axis, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
exprs_values	A string or integer scalar specifying which assay in assays(object) to obtain expression values from.
log2_values	Logical scalar, specifying whether the expression values be transformed to the log2-scale for plotting (with an offset of 1 to avoid logging zeroes).
colour_by	Specification of a column metadata field or a feature to colour by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
shape_by	Specification of a column metadata field or a feature to shape by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
size_by	Specification of a column metadata field or a feature to size by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
by_exprs_values	A string or integer scalar specifying which assay to obtain expression values from, for use in point aesthetics - see the exprs_values argument in <a href="#">?retrieveCellInfo</a> .
xlab	String specifying the label for x-axis. If NULL (default), x will be used as the x-axis label.
feature_colours	Logical scalar indicating whether violins should be coloured by feature when x and colour_by are not specified and one_facet=TRUE.



one_facet	Logical scalar indicating whether grouped violin plots for multiple features should be put onto one facet. Only relevant when x=NULL.
ncol	Integer scalar, specifying the number of columns to be used for the panels of a multi-facet plot.
scales	String indicating whether should multi-facet scales be fixed ("fixed"), free ("free"), or free in one dimension ("free_x", "free_y"). Passed to the scales argument in the <code>facet_wrap</code> when multiple facets are generated.
other_fields	Additional cell-based fields to include in the data.frame, see <code>?"scatter-plot-args"</code> for details.
...	Additional arguments for visualization, see <code>?"scatter-plot-args"</code> for details.

### Details

This function plots expression values for one or more features. If x is not specified, a violin plot will be generated of expression values. If x is categorical, a grouped violin plot will be generated, with one violin for each level of x. If x is continuous, a scatter plot will be generated.

If multiple features are requested and x is not specified and one\_facet=TRUE, a grouped violin plot will be generated with one violin per feature. This will be coloured by feature if colour\_by=NULL and feature\_colours=TRUE, to yield a more aesthetically pleasing plot. Otherwise, if x is specified or one\_facet=FALSE, a multi-panel plot will be generated where each panel corresponds to a feature. Each panel will be a scatter plot or (grouped) violin plot, depending on the nature of x.

Note that this assumes that the expression values are numeric. If not, and x is continuous, horizontal violin plots will be generated. If x is missing or categorical, rectangle plots will be generated where the area of a rectangle is proportional to the number of points for a combination of factors.

### Value

A ggplot object.

### Author(s)

Davis McCarthy, with modifications by Aaron Lun

### Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

## default plot
plotExpression(example_sce, rownames(example_sce)[1:15])

## plot expression against an x-axis value
plotExpression(example_sce, c("Gene_0001", "Gene_0004"),
  x="Mutation_Status")
plotExpression(example_sce, c("Gene_0001", "Gene_0004"),
  x="Gene_0002")

## add visual options
plotExpression(example_sce, rownames(example_sce)[1:6],
  colour_by = "Mutation_Status")
plotExpression(example_sce, rownames(example_sce)[1:6],
  colour_by = "Mutation_Status", shape_by = "Treatment",
  size_by = "Gene_0010")
```

```
## plot expression against expression values for Gene_0004
plotExpression(example_sce, rownames(example_sce)[1:4],
               "Gene_0004", show_smooth = TRUE)
```

---

plotHeatmap

*Plot heatmap of gene expression values*


---

### Description

Create a heatmap of expression values for each cell and specified features in a `SingleCellExperiment` object.

### Usage

```
plotHeatmap(object, features, columns = NULL,
            exprs_values = "logcounts", center = FALSE, zlim = NULL,
            symmetric = FALSE, color = NULL, colour_columns_by = NULL,
            order_columns_by = NULL, by_exprs_values = exprs_values,
            show_colnames = FALSE, cluster_cols = is.null(order_columns_by), ...)
```

### Arguments

object	A <code>SingleCellExperiment</code> object.
features	A character vector of row names, a logical vector or integer vector of indices specifying rows of object to show in the heatmap.
columns	A vector specifying the subset of columns in object to show as columns in the heatmap. Also specifies the column order if <code>cluster_cols=FALSE</code> and <code>order_columns_by=NULL</code> . By default, all columns are used.
exprs_values	A string or integer scalar indicating which assay of object should be used as expression values for colouring in the heatmap.
center	A logical scalar indicating whether each row should have its mean expression centered at zero prior to plotting.
zlim	A numeric vector of length 2, specifying the upper and lower bounds for the expression values. This winsorizes the expression matrix prior to plotting (but after centering, if <code>center=TRUE</code> ). If <code>NULL</code> , it defaults to the range of the expression matrix.
symmetric	A logical scalar specifying whether the default <code>zlim</code> should be symmetric around zero. If <code>TRUE</code> , the maximum absolute value of <code>zlim</code> will be computed and multiplied by <code>c(-1, 1)</code> to redefine <code>zlim</code> .
color	A vector of colours specifying the palette to use for mapping expression values to colours. This defaults to the default setting in <a href="#">pheatmap</a> .
colour_columns_by	A list of values specifying how the columns should be annotated with colours. Each entry of the list can be any acceptable input to the <code>by</code> argument in <a href="#">?retrieveCellInfo</a> . A character vector can also be supplied and will be treated as a list of strings.

order\_columns\_by

A list of values specifying how the columns should be ordered. Each entry of the list can be any acceptable input to the by argument in [?retrieveCellInfo](#). A character vector can also be supplied and will be treated as a list of strings. This argument is automatically appended to colour\_columns\_by.

by\_exprs\_values

A string or integer scalar specifying which assay to obtain expression values from, for colouring of column-level data - see the exprs\_values argument in [?retrieveCellInfo](#).

show\_colnames, cluster\_cols, ...

Additional arguments to pass to [pheatmap](#).

## Details

Setting center=TRUE is useful for examining log-fold changes of each cell's expression profile from the average across all cells. This avoids issues with the entire row appearing a certain colour because the gene is highly/lowly expressed across all cells.

Setting zlim preserves the dynamic range of colours in the presence of outliers. Otherwise, the plot may be dominated by a few genes, which will "flatten" the observed colours for the rest of the heatmap.

Setting order\_columns\_by is useful for automatically ordering the heatmap by one or more factors of interest, e.g., cluster identity. This the need to set colour\_columns\_by, cluster\_cols and columns to achieve the same effect.

## Value

A heatmap is produced on the current graphics device. The output of [pheatmap](#) is invisibly returned.

## Author(s)

Aaron Lun

## See Also

[pheatmap](#)

## Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

plotHeatmap(example_sce, features=rownames(example_sce)[1:10])

plotHeatmap(example_sce, features=rownames(example_sce)[1:10],
             center=TRUE, symmetric=TRUE)

plotHeatmap(example_sce, features=rownames(example_sce)[1:10],
             colour_columns_by=c("Mutation_Status", "Cell_Cycle"))
```

---

plotHighestExprs      *Plot the highest expressing features*

---

### Description

Plot the features with the highest average expression across all cells, along with their expression in each individual cell.

### Usage

```
plotHighestExprs(object, n = 50, colour_cells_by = NULL,
  drop_features = NULL, exprs_values = "counts",
  by_exprs_values = exprs_values, feature_names_to_plot = NULL,
  as_percentage = TRUE)
```

### Arguments

object	A SingleCellExperiment object.
n	A numeric scalar specifying the number of the most expressed features to show.
colour_cells_by	Specification of a column metadata field or a feature to colour by, see <a href="#">?retrieveCellInfo</a> for possible values.
drop_features	A character, logical or numeric vector indicating which features (e.g. genes, transcripts) to drop when producing the plot. For example, spike-in transcripts might be dropped to examine the contribution from endogenous genes.
exprs_values	A integer scalar or string specifying the assay to obtain expression values from.
by_exprs_values	A string or integer scalar specifying which assay to obtain expression values from, for use in colouring - see <a href="#">?retrieveCellInfo</a> for details.
feature_names_to_plot	String specifying which row-level metadata column contains the feature names. Alternatively, an <a href="#">AsIs</a> -wrapped vector or a data.frame, see <a href="#">?retrieveFeatureInfo</a> for possible values. Default is NULL, in which case rownames(object) are used.
as_percentage	logical scalar indicating whether percentages should be plotted. If FALSE, the raw exprs_values are shown instead.

### Details

This function will plot the percentage of counts accounted for by the top n most highly expressed features across the dataset. Each row on the plot corresponds to a feature and is sorted by average expression (denoted by the point). The distribution of expression across all cells is shown as tick marks for each feature. These ticks can be coloured according to cell-level metadata, as specified by colour\_cells\_by.

### Value

A [ggplot](#) object.

**Examples**

```
example_sce <- mockSCE()
colData(example_sce) <- cbind(colData(example_sce),
  perCellQCMetrics(example_sce))

plotHighestExprs(example_sce, colour_cells_by="detected")
plotHighestExprs(example_sce, colour_cells_by="Mutation_Status")
```

---

plotPlatePosition      *Plot cells in plate positions*

---

**Description**

Plots cells in their position on a plate, coloured by metadata variables or feature expression values from a SingleCellExperiment object.

**Usage**

```
plotPlatePosition(object, plate_position = NULL, colour_by = NULL,
  size_by = NULL, shape_by = NULL, by_exprs_values = "logcounts",
  add_legend = TRUE, theme_size = 24, point_alpha = 0.6,
  point_size = 24, other_fields = list())
```

**Arguments**

object	A SingleCellExperiment object.
plate_position	A character vector specifying the plate position for each cell (e.g., A01, B12, and so on, where letter indicates row and number indicates column). If NULL, the function will attempt to extract this from object\$plate_position. Alternatively, a list of two factors ("row" and "column") can be supplied, specifying the row (capital letters) and column (integer) for each cell in object.
colour_by	Specification of a column metadata field or a feature to colour by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
size_by	Specification of a column metadata field or a feature to size by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
shape_by	Specification of a column metadata field or a feature to shape by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
by_exprs_values	A string or integer scalar specifying which assay to obtain expression values from, for use in point aesthetics - see the exprs_values argument in <a href="#">?retrieveCellInfo</a> .
add_legend	Logical scalar specifying whether a legend should be shown.
theme_size	Numeric scalar, see <a href="#">?"scater-plot-args"</a> for details.
point_alpha	Numeric scalar specifying the transparency of the points, see <a href="#">?"scater-plot-args"</a> for details.
point_size	Numeric scalar specifying the size of the points, see <a href="#">?"scater-plot-args"</a> for details.
other_fields	Additional cell-based fields to include in the data.frame, see <a href="#">?"scater-plot-args"</a> for details.

**Details**

This function expects plate positions to be given in a character format where a letter indicates the row on the plate and a numeric value indicates the column. Each cell has a plate position such as "A01", "B12", "K24" and so on. From these plate positions, the row is extracted as the letter, and the column as the numeric part. Alternatively, the row and column identities can be directly supplied by setting `plate_position` as a list of two factors.

**Value**

A ggplot object.

**Author(s)**

Davis McCarthy, with modifications by Aaron Lun

**Examples**

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

## define plate positions
example_sce$plate_position <- paste0(
  rep(LETTERS[1:5], each = 8),
  rep(formatC(1:8, width = 2, flag = "0"), 5)
)

## plot plate positions
plotPlatePosition(example_sce, colour_by = "Mutation_Status")

plotPlatePosition(example_sce, shape_by = "Treatment",
  colour_by = "Gene_0004")

plotPlatePosition(example_sce, shape_by = "Treatment", size_by = "Gene_0001",
  colour_by = "Cell_Cycle")
```

---

plotReducedDim

*Plot reduced dimensions*

---

**Description**

Plot cell-level reduced dimension results stored in a SingleCellExperiment object.

**Usage**

```
plotReducedDim(object, dimred, ncomponents = 2, percentVar = NULL,
  colour_by = NULL, shape_by = NULL, size_by = NULL,
  by_exprs_values = "logcounts", text_by = NULL, text_size = 5,
  text_colour = "black", label_format = c("%s %i", "(%i%)"),
  other_fields = list(), ...)
```

**Arguments**

object	A SingleCellExperiment object.
dimred	A string or integer scalar indicating the reduced dimension result in reducedDims(object) to plot.
ncomponents	A numeric scalar indicating the number of dimensions to plot, starting from the first dimension. Alternatively, a numeric vector specifying the dimensions to be plotted.
percentVar	A numeric vector giving the proportion of variance in expression explained by each reduced dimension. Only expected to be used in PCA settings, e.g., in the <a href="#">plotPCA</a> function.
colour_by	Specification of a column metadata field or a feature to colour by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
shape_by	Specification of a column metadata field or a feature to shape by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
size_by	Specification of a column metadata field or a feature to size by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
by_exprs_values	A string or integer scalar specifying which assay to obtain expression values from, for use in point aesthetics - see the exprs_values argument in <a href="#">?retrieveCellInfo</a> .
text_by	String specifying the column metadata field with which to add text labels on the plot. This must refer to a categorical field, i.e., coercible into a factor. Alternatively, an <a href="#">AsIs</a> vector or data.frame, see <a href="#">?retrieveCellInfo</a> .
text_size	Numeric scalar specifying the size of added text.
text_colour	String specifying the colour of the added text.
label_format	Character vector of length 2 containing format strings to use for the axis labels. The first string expects a string containing the result type (e.g., "PCA") and an integer containing the component number, while the second string shows the rounded percentage of variance explained and is only relevant when this information is provided in object.
other_fields	Additional cell-based fields to include in the data.frame, see <a href="#">?"scatter-plot-args"</a> for details.
...	Additional arguments for visualization, see <a href="#">?"scatter-plot-args"</a> for details.

**Details**

If ncomponents is a scalar equal to 2, a scatterplot of the first two dimensions is produced. If ncomponents is greater than 2, a pairs plots for the top dimensions is produced.

Alternatively, if ncomponents is a vector of length 2, a scatterplot of the two specified dimensions is produced. If it is of length greater than 2, a pairs plot is produced containing all pairwise plots between the specified dimensions.

The text\_by option will add factor levels as labels onto the plot, placed at the median coordinate across all points in that level. This is useful for annotating position-related metadata (e.g., clusters) when there are too many levels to distinguish by colour. It is only available for scatterplots.

**Value**

A ggplot object

**Author(s)**

Davis McCarthy, with modifications by Aaron Lun

**Examples**

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

example_sce <- runPCA(example_sce, ncomponents=5)
plotReducedDim(example_sce, "PCA")
plotReducedDim(example_sce, "PCA", colour_by="Cell_Cycle")
plotReducedDim(example_sce, "PCA", colour_by="Gene_0001")

plotReducedDim(example_sce, "PCA", ncomponents=5)
plotReducedDim(example_sce, "PCA", ncomponents=5, colour_by="Cell_Cycle",
  shape_by="Treatment")
```

---

plotRLE

*Plot relative log expression*

---

**Description**

Produce a relative log expression (RLE) plot of one or more transformations of cell expression values.

**Usage**

```
plotRLE(object, exprs_values = "logcounts", exprs_logged = TRUE,
  style = "minimal", legend = TRUE, ordering = NULL,
  colour_by = NULL, by_exprs_values = exprs_values, ...)
```

**Arguments**

object	A SingleCellExperiment object.
exprs_values	A string or integer scalar specifying the expression matrix in object to use.
exprs_logged	A logical scalar indicating whether the expression matrix is already log-transformed. If not, a log2-transformation (+1) will be performed prior to plotting.
style	String defining the boxplot style to use, either "minimal" (default) or "full"; see Details.
legend	Logical scalar specifying whether a legend should be shown.
ordering	A vector specifying the ordering of cells in the RLE plot. This can be useful for arranging cells by experimental conditions or batches.
colour_by	Specification of a column metadata field or a feature to colour by, see the by argument in <a href="#">?retrieveCellInfo</a> for possible values.
by_exprs_values	A string or integer scalar specifying which assay to obtain expression values from, for use in point aesthetics - see the exprs_values argument in <a href="#">?retrieveCellInfo</a> .
...	further arguments passed to <a href="#">geom_boxplot</a> when style="full".



## Details

Relative log expression (RLE) plots are a powerful tool for visualising unwanted variation in high dimensional data. These plots were originally devised for gene expression data from microarrays but can also be used on single-cell expression data. RLE plots are particularly useful for assessing whether a procedure aimed at removing unwanted variation (e.g., scaling normalisation) has been successful.

If style is “full”, the usual **ggplot2** boxplot is created for each cell. Here, the box shows the inter-quartile range and whiskers extend no more than 1.5 times the IQR from the hinge (the 25th or 75th percentile). Data beyond the whiskers are called outliers and are plotted individually. The median (50th percentile) is shown with a white bar. This approach is detailed and flexible, but can take a long time to plot for large datasets.

If style is “minimal”, a Tufte-style boxplot is created for each cell. Here, the median is shown with a circle, the IQR in a grey line, and “whiskers” (as defined above) for the plots are shown with coloured lines. No outliers are shown for this plot style. This approach is more succinct and faster for large numbers of cells.

## Value

A ggplot object

## Author(s)

Davis McCarthy, with modifications by Aaron Lun

## References

Gandolfo LC, Speed TP (2017). RLE plots: visualising unwanted variation in high dimensional data. *arXiv*.

## Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

plotRLE(example_sce, colour_by = "Mutation_Status", style = "minimal")

plotRLE(example_sce, colour_by = "Mutation_Status", style = "full",
         outlier.alpha = 0.1, outlier.shape = 3, outlier.size = 0)
```

---

plotRowData

*Plot row metadata*

---

## Description

Plot row-level (i.e., gene) metadata from a SingleCellExperiment object.

## Usage

```
plotRowData(object, y, x = NULL, colour_by = NULL, shape_by = NULL,
            size_by = NULL, by_exprs_values = "logcounts",
            other_fields = list(), ...)
```

**Arguments**

object	A SingleCellExperiment object containing expression values and experimental information.
y	String specifying the column-level metadata field to show on the y-axis. Alternatively, an <a href="#">AsIs</a> vector or data.frame, see <a href="#">?retrieveFeatureInfo</a> .
x	String specifying the column-level metadata to show on the x-axis. Alternatively, an <a href="#">AsIs</a> vector or data.frame, see <a href="#">?retrieveFeatureInfo</a> . If NULL, nothing is shown on the x-axis.
colour_by	Specification of a row metadata field or a cell to colour by, see <a href="#">?retrieveFeatureInfo</a> for possible values.
shape_by	Specification of a row metadata field or a cell to shape by, see <a href="#">?retrieveFeatureInfo</a> for possible values.
size_by	Specification of a row metadata field or a cell to size by, see <a href="#">?retrieveFeatureInfo</a> for possible values.
by_exprs_values	A string or integer scalar specifying which assay to obtain expression values from, for use in point aesthetics - see <a href="#">?retrieveFeatureInfo</a> for details.
other_fields	Additional feature-based fields to include in the data.frame, see <a href="#">?"scatter-plot-args"</a> for details.
...	Additional arguments for visualization, see <a href="#">?"scatter-plot-args"</a> for details.

**Details**

If y is continuous and x=NULL, a violin plot is generated. If x is categorical, a grouped violin plot will be generated, with one violin for each level of x. If x is continuous, a scatter plot will be generated.

If y is categorical and x is continuous, horizontal violin plots will be generated. If x is missing or categorical, rectangle plots will be generated where the area of a rectangle is proportional to the number of points for a combination of factors.

**Value**

A [ggplot](#) object.

**Examples**

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)
rowData(example_sce) <- cbind(rowData(example_sce),
  perFeatureQCMetrics(example_sce))

plotRowData(example_sce, y="detected", x="mean") +
  scale_x_log10()
```

---

plotScater

*Plot an overview of expression for each cell*


---

### Description

Plot the relative proportion of the library size that is accounted for by the most highly expressed features for each cell in a `SingleCellExperiment` object.

### Usage

```
plotScater(x, nfeatures = 500, exprs_values = "counts",
  colour_by = NULL, by_exprs_values = exprs_values, block1 = NULL,
  block2 = NULL, ncol = 3, line_width = 1.5, theme_size = 10)
```

### Arguments

<code>x</code>	A <a href="#">SingleCellExperiment</a> object.
<code>nfeatures</code>	Numeric scalar indicating the number of top-expressed features to show <code>n</code> in the plot.
<code>exprs_values</code>	String or integer scalar indicating which assay of object should be used to obtain the expression values for this plot.
<code>colour_by</code>	Specification of a column metadata field or a feature to colour by, see the <code>by</code> argument in <a href="#">?retrieveCellInfo</a> for possible values. The curve for each cell will be coloured according to this specification.
<code>by_exprs_values</code>	A string or integer scalar specifying which assay to obtain expression values from, for use in point aesthetics - see the <code>exprs_values</code> argument in <a href="#">?retrieveCellInfo</a> .
<code>block1</code>	String specifying the column-level metadata field by which to separate the cells into separate panels in the plot. Alternatively, an <a href="#">AsIs</a> vector or data.frame, see <a href="#">?retrieveCellInfo</a> . Default is <code>NULL</code> , in which case there is no blocking.
<code>block2</code>	Same as <code>block1</code> , providing another level of blocking.
<code>ncol</code>	Number of columns to use for <a href="#">facet_wrap</a> if only one block is defined.
<code>line_width</code>	Numeric scalar specifying the line width.
<code>theme_size</code>	Numeric scalar specifying the font size to use for the plotting theme.

### Details

For each cell, the features are ordered from most-expressed to least-expressed. The cumulative proportion of the total expression for the cell is computed across the top `nfeatures` features. These plots can flag cells with a very high proportion of the library coming from a small number of features; such cells are likely to be problematic for downstream analyses.

Using the `colour` and `blocking` arguments can flag overall differences in cells under different experimental conditions or affected by different batch and other variables. If only one of `block1` and `block2` are specified, each panel corresponds to a separate level of the specified blocking factor. If both are specified, each panel corresponds to a combination of levels.

### Value

A [ggplot](#) object.

**Author(s)**

Davis McCarthy, with modifications by Aaron Lun

**Examples**

```
example_sce <- mockSCE()
plotScater(example_sce)
plotScater(example_sce, exprs_values = "counts", colour_by = "Cell_Cycle")
plotScater(example_sce, block1 = "Treatment", colour_by = "Cell_Cycle")
```

---

quickPerCellQC

*Quick cell-level QC*

---

**Description**

A convenient utility that identifies low-quality cells based on frequently used QC metrics.

**Usage**

```
quickPerCellQC(df, lib_size = "sum", n_features = "detected",
  percent_subsets = NULL, ...)
```

**Arguments**

df	A <a href="#">DataFrame</a> containing per-cell QC statistics, as computed by <a href="#">perCellQCMetrics</a> .
lib_size	String specifying the column of df containing the library size for each cell.
n_features	String specifying the column of df containing the number of detected features per cell.
percent_subsets	String specifying the column(s) of df containing the percentage of counts in subsets of “control features”.
...	Further arguments to pass to <a href="#">isOutlier</a> .

**Details**

This function simply calls [isOutlier](#) on the various QC metrics in df.

- For `lib_size`, small outliers are detected on the log-scale to remove cells with low library sizes.
- For `n_features`, small outliers are detected on the log-scale to remove cells with few detected features.
- For each field in `percent_subsets`, large outliers are detected on the original scale. This aims to remove cells with high spike-in or mitochondrial content.

Users can change the number of MADs used to define an outlier or specify batches by passing appropriate arguments to ...

**Value**

A [DataFrame](#) with one row per cell and containing columns of logical vectors. Each column specifies a reason for why a cell was considered to be low quality, with the final discard column indicating whether the cell should be discarded.

**Author(s)**

Aaron Lun

**See Also**

[perCellQCMetrics](#), for calculation of these metrics.

[isOutlier](#), to identify outliers with a MAD-based approach.

**Examples**

```
example_sce <- mockSCE()
df <- perCellQCMetrics(example_sce, subsets=list(Mito=1:100))

discarded <- quickPerCellQC(df, percent_subsets=c(
  "subsets_Mito_percent", "altexps_Spikes_percent"))
colSums(as.data.frame(discarded))
```

---

readSparseCounts

*Read sparse count matrix from file*


---

**Description**

Reads a sparse count matrix from file containing a dense tabular format.

**Usage**

```
readSparseCounts(file, sep = "\t", quote = NULL, comment.char = "",
  row.names = TRUE, col.names = TRUE, ignore.row = 0L,
  skip.row = 0L, ignore.col = 0L, skip.col = 0L, chunk = 1000L)
```

**Arguments**

file	A string containing a file path to a count table, or a connection object opened in read-only text mode.
sep	A string specifying the delimiter between fields in file.
quote	A string specifying the quote character, e.g., in column or row names.
comment.char	A string specifying the comment character after which values are ignored.
row.names	A logical scalar specifying whether row names are present.
col.names	A logical scalar specifying whether column names are present.
ignore.row	An integer scalar specifying the number of rows to ignore at the start of the file, <i>before</i> the column names.
skip.row	An integer scalar specifying the number of rows to ignore at the start of the file, <i>after</i> the column names.

<code>ignore.col</code>	An integer scalar specifying the number of columns to ignore at the start of the file, <i>before</i> the column names.
<code>skip.col</code>	An integer scalar specifying the number of columns to ignore at the start of the file, <i>after</i> the column names.
<code>chunk</code>	A integer scalar indicating the chunk size to use, i.e., number of rows to read at any one time.

### Details

This function provides a convenient method for reading dense arrays from flat files into a sparse matrix in memory. Memory usage can be further improved by setting `chunk` to a smaller positive value.

The `ignore.*` and `skip.*` parameters allow irrelevant rows or columns to be skipped. Note that the distinction between the two parameters is only relevant when `row.names=FALSE` (for skipping/ignoring columns) or `col.names=FALSE` (for rows).

### Value

A `dgCMatrix` containing double-precision values (usually counts) for each row (gene) and column (cell).

### Author(s)

Aaron Lun

### See Also

[read.table](#), [readMM](#)

### Examples

```
outfile <- tempfile()
write.table(data.frame(A=1:5, B=0, C=0:4, row.names=letters[1:5]),
            file=outfile, col.names=NA, sep="\t", quote=FALSE)

readSparseCounts(outfile)
```

---

Reduced dimension plots

*Plot specific reduced dimensions*

---

### Description

Wrapper functions to create plots for specific types of reduced dimension results in a `SingleCellExperiment` object.

**Usage**

```
plotPCASCE(object, ..., ncomponents = 2)

plotTSNE(object, ..., ncomponents = 2)

plotUMAP(object, ..., ncomponents = 2)

plotDiffusionMap(object, ..., ncomponents = 2)

plotMDS(object, ..., ncomponents = 2)

## S4 method for signature 'SingleCellExperiment'
plotPCA(object, ..., ncomponents = 2)
```

**Arguments**

object	A <code>SingleCellExperiment</code> object.
...	Additional arguments to pass to <a href="#">plotReducedDim</a> .
ncomponents	Numeric scalar indicating the number of dimensions components to (calculate and) plot. This can also be a numeric vector, see <a href="#">?plotReducedDim</a> for details.

**Details**

Each function is a convenient wrapper around [plotReducedDim](#) that searches the `reducedDims` slot for an appropriately named dimensionality reduction result:

- "PCA" for `plotPCA`
- "TSNE" for `plotTSNE`
- "DiffusionMap" for `plotDiffusionMap`
- "MDS" for "plotMDS"
- "UMAP" for "plotUMAP"

Its only purpose is to streamline workflows to avoid the need to specify the `dimred` argument.

**Value**

A [ggplot](#) object.

**Author(s)**

Davis McCarthy, with modifications by Aaron Lun

**See Also**

[runPCA](#), [runDiffusionMap](#), [runTSNE](#), [runMDS](#), and [runUMAP](#), for the functions that actually perform the calculations.

[plotReducedDim](#), for the underlying plotting function.

## Examples

```

example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)
example_sce <- runPCA(example_sce)

## Examples plotting PC1 and PC2
plotPCA(example_sce)
plotPCA(example_sce, colour_by = "Cell_Cycle")
plotPCA(example_sce, colour_by = "Cell_Cycle", shape_by = "Treatment")

## Examples plotting more than 2 PCs
plotPCA(example_sce, ncomponents = 4, colour_by = "Treatment",
        shape_by = "Mutation_Status")

## Same for TSNE:
example_sce <- runTSNE(example_sce)
plotTSNE(example_sce, colour_by="Mutation_Status")

## Same for DiffusionMaps:
example_sce <- runDiffusionMap(example_sce)
plotDiffusionMap(example_sce)

## Same for MDS plots:
example_sce <- runMDS(example_sce)
plotMDS(example_sce)

```

---

retrieveCellInfo	<i>Cell-based data retrieval</i>
------------------	----------------------------------

---

## Description

Retrieves a per-cell (meta)data field from a [SingleCellExperiment](#) based on a single keyword, typically for use in visualization functions.

## Usage

```

retrieveCellInfo(x, by, search = c("colData", "assays", "altExps"),
                exprs_values = "logcounts")

```

## Arguments

x	A <a href="#">SingleCellExperiment</a> object.
by	A string specifying the field to extract (see Details). Alternatively, a data.frame, <a href="#">DataFrame</a> or an <a href="#">AsIs</a> vector.
search	Character vector specifying the types of data or metadata to use.
exprs_values	String or integer scalar specifying the assay from which expression values should be extracted.



## Details

Given an [AsIs](#)-wrapped vector in `by`, this function will directly return the vector values as `value`, while `name` is set to an empty string. For `data.frame` or `DataFrame` instances with a single column, this function will return the vector from that column as `value` and the column name as `name`. This allows downstream visualization functions to accommodate arbitrary inputs for adjusting aesthetics.

Given a character string in `by`, this function will:

1. Search `colData` for a column named `by`, and return the corresponding field as the output value. We do not consider nested elements within the `colData`.
2. Search `assay(x, exprs_values)` for a row named `by`, and return the expression vector for this feature as the output value.
3. Search each alternative experiment in `altExps(x)` for a row names `by`, and return the expression vector for this feature at `exprs_values` as the output value.

Any match will cause the function to return without considering later possibilities. The search can be modified by changing the presence and ordering of elements in `search`.

If there is a name clash that results in retrieval of an unintended field, users should explicitly set `by` to a `data.frame`, `DataFrame` or `AsIs`-wrapped vector containing the desired values. Developers can also consider setting `search` to control the fields that are returned.

## Value

A list containing `name`, a string with the name of the extracted field (usually identically to `by`); and `value`, a vector of length equal to `ncol(x)` containing per-cell (meta)data values. If `by=NULL` or was not found in `x`, both `name` and `value` are set to `NULL`.

## Author(s)

Aaron Lun

## See Also

[plotColData](#), [plotReducedDim](#), [plotExpression](#), [plotPlatePosition](#), and most other cell-based plotting functions.

## Examples

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)

retrieveCellInfo(example_sce, "Cell_Cycle")
retrieveCellInfo(example_sce, "Gene_0001")

arbitrary.field <- rnorm(ncol(example_sce))
retrieveCellInfo(example_sce, I(arbitrary.field))
retrieveCellInfo(example_sce, data.frame(stuff=arbitrary.field))
```

---

retrieveFeatureInfo    *Feature-based data retrieval*

---

### Description

Retrieves a per-feature (meta)data field from a [SingleCellExperiment](#) based on a single keyword, typically for use in visualization functions.

### Usage

```
retrieveFeatureInfo(x, by, search = c("rowData", "assays"),
  exprs_values = "logcounts")
```

### Arguments

x	A <a href="#">SingleCellExperiment</a> object.
by	A string specifying the field to extract (see Details). Alternatively, a data.frame, <a href="#">DataFrame</a> or an <a href="#">AsIs</a> vector.
search	Character vector specifying the types of data or metadata to use.
exprs_values	String or integer scalar specifying the assay from which expression values should be extracted.

### Details

Given a [AsIs](#)-wrapped vector in `by`, this function will directly return the vector values as `value`, while `name` is set to an empty string. For `data.frame` or `DataFrame` instances with a single column, this function will return the vector from that column as `value` and the column name as `name`. This allows downstream visualization functions to accommodate arbitrary inputs for adjusting aesthetics.

Given a character string in `by`, this function will:

1. Search `rowData` for a column named `by`, and return the corresponding field as the output value. We do not consider nested elements within the `rowData`.
2. Search `assay(x, exprs_values)` for a column named `by`, and return the expression vector for this feature as the output value.

Any match will cause the function to return without considering later possibilities. The search can be modified by changing the presence and ordering of elements in `search`.

If there is a name clash that results in retrieval of an unintended field, users should explicitly set `by` to a `data.frame`, `DataFrame` or [AsIs](#)-wrapped vector containing the desired values. Developers can also consider setting `search` to control the fields that are returned.

### Value

A list containing `name`, a string with the name of the extracted field (usually identically to `by`); and `value`, a vector of length equal to `ncol(x)` containing per-feature (meta)data values. If `by=NULL` or was not found in `x`, both `name` and `value` are set to `NULL`.

### Author(s)

Aaron Lun

**See Also**

[plotRowData](#) and other feature-based plotting functions.

**Examples**

```
example_sce <- mockSCE()
example_sce <- logNormCounts(example_sce)
rowData(example_sce)$blah <- sample(LETTERS,
  nrow(example_sce), replace=TRUE)

str(retrieveFeatureInfo(example_sce, "blah"))
str(retrieveFeatureInfo(example_sce, "Cell_001"))

arbitrary.field <- rnorm(nrow(example_sce))
str(retrieveFeatureInfo(example_sce, I(arbitrary.field)))
str(retrieveFeatureInfo(example_sce, data.frame(stuff=arbitrary.field)))
```

---

runColDataPCA

*Perform PCA on column metadata*


---

**Description**

Perform a principal components analysis (PCA) on cells, based on the column metadata in a `SingleCellExperiment` object.

**Usage**

```
runColDataPCA(x, ncomponents = 2, variables = NULL, scale = TRUE,
  outliers = FALSE, BSPARAM = ExactParam(), BPPARAM = SerialParam(),
  name = "PCA_coldata")
```

**Arguments**

<code>x</code>	A <a href="#">SingleCellExperiment</a> object.
<code>ncomponents</code>	Numeric scalar indicating the number of principal components to obtain.
<code>variables</code>	List of strings or a character vector indicating which variables in <code>colData(x)</code> to use. If a list, each entry can also be an <a href="#">AsIs</a> vector or a <code>data.frame</code> , as described in <a href="#">?retrieveCellInfo</a> .
<code>scale</code>	Logical scalar, should the expression values be standardised so that each feature has unit variance? This will also remove features with standard deviations below $1e-8$ .
<code>outliers</code>	Logical indicating whether outliers should be detected based on PCA coordinates.
<code>BSPARAM</code>	A <a href="#">BiocSingularParam</a> object specifying which algorithm should be used to perform the PCA.
<code>BPPARAM</code>	A <a href="#">BiocParallelParam</a> object specifying whether the PCA should be parallelized.
<code>name</code>	String specifying the name to be used to store the result in the <code>reducedDims</code> of the output.

## Details

This function performs PCA on variables from the column-level metadata instead of the gene expression matrix. Doing so can be occasionally useful when other forms of experimental data are stored in the `colData`, e.g., protein intensities from FACs or other cell-specific phenotypic information.

This function is particularly useful for identifying low-quality cells based on QC metrics with `outliers=TRUE`. This uses an “outlyingness” measure computed by `adjOutlyingness` in the **robustbase** package. Outliers are defined those cells with outlyingness values more than 5 MADs above the median, using `isOutlier`.

## Value

A `SingleCellExperiment` object containing the first `ncomponent` principal coordinates for each cell. By default, these are stored in the `"PCA_coldata"` entry of the `reducedDims` slot. The proportion of variance explained by each PC is stored as a numeric vector in the `"percentVar"` attribute.

If `outliers=TRUE`, the output `colData` will also contain a logical `outlier` field. This specifies the cells that correspond to the identified outliers.

## Author(s)

Aaron Lun, based on code by Davis McCarthy

## See Also

`runPCA`, for the corresponding method operating on expression data.

## Examples

```
example_sce <- mockSCE()
qc.df <- perCellQCMetrics(example_sce, subset=list(Mito=1:10))
colData(example_sce) <- cbind(colData(example_sce), qc.df)

# Can supply names of colData variables to 'variables',
# as well as AsIs-wrapped vectors of interest.
example_sce <- runColDataPCA(example_sce, variables=list(
  "sum", "detected", "subsets_Mito_percent", "altexps_Spikes_percent"
))
reducedDimNames(example_sce)
head(reducedDim(example_sce))
```

---

scatter-plot-args

*General visualization parameters*

---

## Description

**scatter** functions that plot points share a number of visualization parameters, which are described on this page.

### Aesthetic parameters

- `add_legend`: Logical scalar, specifying whether a legend should be shown. Defaults to TRUE.
- `theme_size`: Integer scalar, specifying the font size. Defaults to 10.
- `point_alpha`: Numeric scalar in [0, 1], specifying the transparency. Defaults to 0.6.
- `point_size`: Numeric scalar, specifying the size of the points. Defaults to NULL.
- `jitter_type`: String to define how points are to be jittered in a violin plot. This is either with random jitter on the x-axis ("jitter") or in a "beeswarm" style (if "swarm", default). The latter usually looks more attractive, but for datasets with a large number of cells, or for dense plots, the jitter option may work better.

### Distributional calculations

- `show_median`: Logical, should the median of the distribution be shown for violin plots? Defaults to FALSE.
- `show_violin`: Logical, should the outline of a violin plot be shown? Defaults to TRUE.
- `show_smooth`: Logical, should a smoother be fitted to a scatter plot? Defaults to FALSE.
- `show_se`: Logical, should standard errors for the fitted line be shown on a scatter plot when `show_smooth=TRUE`? Defaults to TRUE.

### Miscellaneous fields

Additional fields can be added to the `data.frame` passed to [ggplot](#) by setting the `other_fields` argument. This allows users to easily incorporate additional metadata for use in further **ggplot** operations.

The `other_fields` argument should be character vector where each string is passed to [retrieveCellInfo](#) (for cell-based plots) or [retrieveFeatureInfo](#) (for feature-based plots). Alternatively, `other_fields` can be a named list where each element is of any type accepted by [retrieveCellInfo](#) or [retrieveFeatureInfo](#). This includes [AsIs](#)-wrapped vectors, `data.frames` or [DataFrames](#).

Each additional column of the output `data.frame` will be named according to the name returned by [retrieveCellInfo](#) or [retrieveFeatureInfo](#). If these clash with inbuilt names (e.g., X, Y, `colour_by`), a warning will be raised and the additional column will not be added to avoid overwriting an existing column.

### See Also

[plotColData](#), [plotRowData](#), [plotReducedDim](#), [plotExpression](#), [plotPlatePosition](#), and most other plotting functions.

---

scater-red-dim-args     *Dimensionality reduction options*

---

### Description

An overview of the common options for dimensionality reduction methods in **scater**. The following sections consider an input `x` to the various `run*` methods, where `x` can be a numeric matrix or a [SingleCellExperiment](#).

### Feature selection

This section is relevant if `x` is a numeric matrix of (log-)expression values with features in rows and cells in columns; or if `x` is a [SingleCellExperiment](#) and `dimred=NULL`. In the latter, the expression values are obtained from the assay specified by `exprs_values`.

The `subset_row` argument specifies the features to use in a dimensionality reduction algorithm. This can be set to any user-defined vector containing, e.g., highly variable features or genes in a pathway of interest. It can be a character vector of row names, an integer vector of row indices or a logical vector.

If `subset_row=NULL`, the `ntop` features with the largest variances are used instead. This literally computes the variances from the expression values without considering any mean-variance trend. Note that the value of `ntop` is ignored if `subset_row` is specified.

If `scale=TRUE`, the expression values for each feature are standardized so that their variance is unity. This will also remove features with standard deviations below  $1e-8$ .

### Using reduced dimensions

This section is relevant if `x` is a [SingleCellExperiment](#) and `dimred` is not `NULL`.

All dimensionality reduction methods can be applied on existing dimensionality reduction results in `x` by setting `dimred`. This is typically used to run non-linear algorithms like t-SNE or UMAP on the PCA results. It may also be desirable in cases where the existing reduced dimensions are computed from *a priori* knowledge (e.g., gene set scores). In such cases, further reduction with PCA could be used to compress the data.

The matrix of existing reduced dimensions is taken from `reducedDims(x, dimred)`. By default, all dimensions are used to compute the second set of reduced dimensions. If `n_dimred` is also specified, only the first `n_dimred` columns are used. Alternatively, `n_dimred` can be an integer vector specifying the column indices of the dimensions to use.

When `dimred` is specified, no additional feature selection or standardization is performed. This means that any settings of `ntop`, `subset_row` and `scale` are ignored.

### Transposed inputs

This section is relevant if `x` is a numeric matrix and `transposed=TRUE`, such that cells are the rows and the various dimensions are the columns.

Here, the aim is to allow users to manually pass in dimensionality reduction results without needing to wrap them in a [SingleCellExperiment](#). As such, no feature selection or standardization is performed, i.e., `ntop`, `subset_row` and `scale` are ignored.

### Alternative experiments

This section is relevant if `x` is a [SingleCellExperiment](#) and `altexp` is not `NULL`.

If `altexp` is specified, the method is run on data from an alternative [SummarizedExperiment](#) nested within `x`. This is useful for performing dimensionality reduction on other features stored in `altExp(x, altexp)`, e.g., antibody tags.

Setting `altexp` with `exprs_values` will use the specified assay from the alternative [SummarizedExperiment](#). If the alternative is a [SingleCellExperiment](#), setting `dimred` will use the specified dimensionality reduction results from the alternative. This option will also interact as expected with `n_dimred`.

Note that the output is still stored in the `reducedDims` of the output [SingleCellExperiment](#). It is advisable to use a different name to distinguish this from PCA results obtained from the main experiment's assay values.

**Author(s)**

Aaron Lun

**See Also**

These arguments are used throughout [runPCA](#), [runTSNE](#), [runUMAP](#), [runMDS](#) and [runDiffusionMap](#).

---

SCESet

*The "Single Cell Expression Set" (SCESet) class*

---

**Description**

S4 class and the main class used by scater to hold single cell expression data. SCESet extends the basic Bioconductor ExpressionSet class.

**Details**

This class is initialized from a matrix of expression values.

Methods that operate on SCESet objects constitute the basic scater workflow.

**Slots**

**logExprsOffset:** Scalar of class "numeric", providing an offset applied to expression data in the 'exprs' slot when undergoing log2-transformation to avoid trying to take logs of zero.

**lowerDetectionLimit:** Scalar of class "numeric", giving the lower limit for an expression value to be classified as "expressed".

**cellPairwiseDistances:** Matrix of class "numeric", containing pairwise distances between cells.

**featurePairwiseDistances:** Matrix of class "numeric", containing pairwise distances between features.

**reducedDimension:** Matrix of class "numeric", containing reduced-dimension coordinates for cells (generated, for example, by PCA).

**bootstraps:** Array of class "numeric" that can contain bootstrap estimates of the expression or count values.

**sc3:** List containing results from consensus clustering from the SC3 package.

**featureControlInfo:** Data frame of class "AnnotatedDataFrame" that can contain information/metadata about sets of control features defined for the SCESet object. bootstrap estimates of the expression or count values.

**References**

Thanks to the Monocle package ([github.com/cole-trapnell-lab/monocle-release/](https://github.com/cole-trapnell-lab/monocle-release/)) for their CellDataSet class, which provided the inspiration and template for SCESet.

---

sumCountsAcrossCells *Sum counts across sets of cells*

---

### Description

Sum together expression values (by default, counts) for each set of cells and for each feature.

### Usage

```
sumCountsAcrossCells(x, ...)

aggregateAcrossCells(x, ...)

## S4 method for signature 'ANY'
sumCountsAcrossCells(x, ids, subset_row = NULL,
  subset_col = NULL, average = FALSE, BPPARAM = SerialParam())

## S4 method for signature 'SummarizedExperiment'
sumCountsAcrossCells(x, ...,
  exprs_values = "counts")

## S4 method for signature 'SummarizedExperiment'
aggregateAcrossCells(x, ids, ...,
  coldata_merge = NULL, use_exprs_values = "counts")

## S4 method for signature 'SingleCellExperiment'
aggregateAcrossCells(x, ids, ...,
  subset_row = NULL, coldata_merge = NULL,
  use_exprs_values = "counts", use_alt_exps = TRUE)
```

### Arguments

x	For <code>sumCountsAcrossCells</code> , a numeric matrix of counts containing features in rows and cells in columns. Alternatively, a <a href="#">SummarizedExperiment</a> object containing such a count matrix. For <code>aggregateAcrossCells</code> , a <a href="#">SingleCellExperiment</a> or <code>SummarizedExperiment</code> containing a count matrix.
...	For the generics, further arguments to be passed to specific methods. For the <code>sumCountsAcrossCells SummarizedExperiment</code> method, further arguments to be passed to the ANY method. For <code>aggregateAcrossCells</code> , further arguments to be passed to <code>sumCountsAcrossCells</code> .
ids	A factor specifying the set to which each cell in x belongs. Alternatively, a <a href="#">DataFrame</a> of such vectors or factors, in which case each unique combination of levels defines a set.
subset_row	An integer, logical or character vector specifying the features to use. Defaults to all features. For the <a href="#">SingleCellExperiment</a> method, this argument will not affect alternative Experiments, where summation is always performed for all features (or not at all, depending on <code>use_alt_exps</code> ).



subset_col	An integer, logical or character vector specifying the cells to use. Defaults to all cells with non-NA entries of <code>ids</code> .
average	Logical scalar indicating whether the average should be computed instead of the sum.
BPPARAM	A <a href="#">BiocParallelParam</a> object specifying whether summation should be parallelized.
exprs_values	A string or integer scalar specifying the assay of <code>x</code> containing the matrix of counts (or any other expression quantity that can be meaningfully summed).
coldata_merge	A named list of functions specifying how each column metadata field should be aggregated. For any unspecified field, metadata is retained for the first instance of a cell from each set in <code>ids</code> . If NULL, the first instance is retained for all fields.
use_exprs_values	A character or integer vector specifying the assay(s) of <code>x</code> containing count matrices.
use_altexprs	Logical scalar indicating whether aggregation should be performed for alternative experiments in <code>x</code> . Alternatively, a character vector specifying the names of the alternative experiments to be aggregated.

## Details

This function provides a convenient method for aggregating counts across multiple columns for each feature. A typical application would be to sum counts across all cells in each cluster to obtain “pseudo-bulk” samples for further analysis.

The behaviour of this function is equivalent to that of [colsum](#). However, this function can operate on any matrix representation in object; can do so in a parallelized manner for large matrices without resorting to block processing; and can natively support combinations of multiple factors in `ids`.

Any NA values in `ids` are implicitly ignored and will not be considered during summation. This may be useful, e.g., to remove undesirable cells by setting their entries in `ids` to NA. Alternatively, we can explicitly select the cells of interest with `subset_col`.

Setting `average=TRUE` will compute the average in each set rather than the sum. This is particularly useful if `x` contains expression values that have already been normalized in some manner, as computing the average avoids another round of normalization to account for differences in the size of each set.

## Value

For `sumCountsAcrossCells` with a factor `ids`, a count matrix is returned with one column per level of `ids`. For each feature, counts for all cells in the same set are summed together. Columns are ordered by `levels(ids)`.

For `sumCountsAcrossCells` with a `DataFrame` `ids`, a `SummarizedExperiment` is returned containing a similar count matrix in the first assay. Each column corresponds to a unique combination of levels in `ids` and contains the sum of counts for all cells with that combination. The identities of the levels for each column are reported in the [colData](#).

For `aggregateAcrossCells`, a `SummarizedExperiment` of the same class as `x` is returned, containing summed matrices generated by `sumCountsAcrossCell` on all assays specified by `use_exprs_values`. By default, column metadata is retained for the first instance of a cell from each set in `ids`, but this behavior can be customized by supplying appropriate functions to `coldata_merge`. If `ids` is a `DataFrame`, the combination of levels corresponding to each column is also reported in the column metadata.

**Author(s)**

Aaron Lun

**Examples**

```
example_sce <- mockSCE()
ids <- sample(LETTERS[1:5], ncol(example_sce), replace=TRUE)

out <- sumCountsAcrossCells(example_sce, ids)
head(out)

batches <- sample(1:3, ncol(example_sce), replace=TRUE)
out2 <- sumCountsAcrossCells(example_sce,
  DataFrame(label=ids, batch=batches))
out2

# Using another column metadata merge strategy.
example_sce$stuff <- runif(ncol(example_sce))
example_merged <- aggregateAcrossCells(example_sce, ids,
  coldata_merge=list(stuff=sum))
```

---

sumCountsAcrossFeatures

*Sum counts across feature sets*

---

**Description**

Sum together expression values (by default, counts) for each feature set in each cell.

**Usage**

```
sumCountsAcrossFeatures(x, ...)

## S4 method for signature 'ANY'
sumCountsAcrossFeatures(x, ids, average = FALSE,
  BPPARAM = SerialParam())

## S4 method for signature 'SummarizedExperiment'
sumCountsAcrossFeatures(x, ...,
  exprs_values = "counts")

aggregateAcrossFeatures(x, ids, ..., use_exprs_values = "counts")
```

**Arguments**

**x** For `sumCountsAcrossFeatures`, a numeric matrix of counts containing features in rows and cells in columns. Alternatively, a [SummarizedExperiment](#) object containing such a count matrix.  
For `aggregateAcrossFeatures`, a `SummarizedExperiment` containing a count matrix.

...	For the sumCountsAcrossFeatures generic, further arguments to be passed to specific methods. For the SummarizedExperiment method, further arguments to be passed to the ANY method. For aggregateAcrossFeatures, further arguments to be passed to sumCountsAcrossFeatures.
ids	A factor specifying the set to which each feature in x belongs. Alternatively, a list of integer or character vectors, where each vector specifies the indices or names of features in a set.
average	Logical scalar indicating whether the average should be computed instead of the sum.
BPPARAM	A <a href="#">BiocParallelParam</a> object specifying whether summation should be parallelized.
exprs_values	A string or integer scalar specifying the assay of x containing the matrix of counts (or any other expression quantity that can be meaningfully summed).
use_exprs_values	A character or integer vector specifying the assay(s) of x containing count matrices.

## Details

This function provides a convenient method for aggregating counts across multiple rows for each cell. Several possible applications are listed below:

- Using a list of genes in `ids`, we can obtain a summary expression value for all genes in one or more gene sets. This allows the activity of various pathways to be compared across cells.
- Genes with multiple mapping locations in the reference will often manifest as multiple rows with distinct Ensembl/Entrez IDs. These counts can be aggregated into a single feature by setting the shared identifier (usually the gene symbol) as `ids`.
- It is theoretically possible to aggregate transcript-level counts to gene-level counts with this function. However, it is often better to do so with dedicated functions (e.g., from the **tximport** or **tximeta** packages) that account for differences in length across isoforms.

The behaviour of this function is equivalent to that of `rowsum`. However, this function can operate on any matrix representation in object, and can do so in a parallelized manner for large matrices without resorting to block processing.

If `ids` is a factor, any NA values are implicitly ignored and will not be considered or reported. This may be useful, e.g., to remove undesirable feature sets by setting their entries in `ids` to NA.

Setting `average=TRUE` will compute the average in each set rather than the sum. This is particularly useful if `x` contains expression values that have already been normalized in some manner, as computing the average avoids another round of normalization to account for differences in the size of each set.

## Value

For `sumCountsAcrossFeatures`, a count matrix is returned with one row per level of `ids`. In each cell, counts for all features in the same set are summed together. Rows are ordered according to `levels(ids)`.

For `aggregateAcrossFeatures`, a `SummarizedExperiment` of the same class as `x` is returned, containing summed matrices generated by `sumCountsAcrossFeatures` on all assays in `use_exprs_values`. Row metadata is retained for the first instance of a feature from each set in `ids`.

**Author(s)**

Aaron Lun

**Examples**

```
example_sce <- mockSCE()
ids <- sample(LETTERS, nrow(example_sce), replace=TRUE)
out <- sumCountsAcrossFeatures(example_sce, ids)
dimnames(out)
```

---

uniquifyFeatureNames *Make feature names unique*

---

**Description**

Combine a user-interpretable feature name (e.g., gene symbol) with a standard identifier that is guaranteed to be unique and valid (e.g., Ensembl) for use as row names.

**Usage**

```
uniquifyFeatureNames(ID, names)
```

**Arguments**

ID	A character vector of unique identifiers.
names	A character vector of feature names.

**Details**

This function will attempt to use names if it is unique. If not, it will append the `_ID` to any non-unique value of names. Missing names will be replaced entirely by ID.

The output is guaranteed to be unique, assuming that ID is also unique. This can be directly used as the row names of a `SingleCellExperiment` object.

**Value**

A character vector of unique-ified feature names.

**Author(s)**

Aaron Lun

**Examples**

```
uniquifyFeatureNames(
  ID=paste0("ENSG000000000", 1:5),
  names=c("A", NA, "B", "C", "A")
)
```

---

updateSCESet	<i>Convert an SCESet object to a SingleCellExperiment object</i>
--------------	--

---

**Description**

Convert an SCESet object produced with an older version of the package to a SingleCellExperiment object compatible with the current version.

**Usage**

```
updateSCESet(object)
```

```
toSingleCellExperiment(object)
```

**Arguments**

object            an [SCESet](#) object to be updated

**Value**

a [SingleCellExperiment](#) object

**Examples**

```
## Not run:  
updateSCESet(example_sceset)  
  
## End(Not run)  
## Not run:  
toSingleCellExperiment(example_sceset)  
  
## End(Not run)
```

# Index

- addPerCellQC, [3](#), [40](#)
- addPerFeatureQC, [42](#)
- addPerFeatureQC (addPerCellQC), [3](#)
- aggregateAcrossCells
  - (sumCountsAcrossCells), [72](#)
- aggregateAcrossCells, SingleCellExperiment-method
  - (sumCountsAcrossCells), [72](#)
- aggregateAcrossCells, SummarizedExperiment-method
  - (sumCountsAcrossCells), [72](#)
- aggregateAcrossFeatures
  - (sumCountsAcrossFeatures), [74](#)
- altExp, [70](#)
- altExps, [28](#), [29](#), [65](#)
- annotateBMFeatures, [4](#)
- AsIs, [43](#), [45](#), [52](#), [55](#), [58](#), [59](#), [64–67](#), [69](#)
- assay, [65](#), [66](#)
  
- BiocNeighborParam, [18](#), [20](#)
- BiocParallelParam, [14](#), [18](#), [20](#), [31](#), [36](#), [37](#),  
[67](#), [73](#), [75](#)
- BiocSingularParam, [14](#), [67](#)
- bootstraps, [5](#)
- bootstraps, SingleCellExperiment-method
  - (bootstraps), [5](#)
- bootstraps<- (bootstraps), [5](#)
- bootstraps<-, SingleCellExperiment, array-method
  - (bootstraps), [5](#)
- bsparam, [15](#)
  
- calculateAverage, [6](#)
- calculateAverage, ANY-method
  - (calculateAverage), [6](#)
- calculateAverage, SingleCellExperiment-method
  - (calculateAverage), [6](#)
- calculateAverage, SummarizedExperiment-method
  - (calculateAverage), [6](#)
- calculateCPM, [7](#), [11](#), [16](#)
- calculateCPM, ANY-method (calculateCPM),  
[7](#)
- calculateCPM, SingleCellExperiment-method
  - (calculateCPM), [7](#)
- calculateCPM, SummarizedExperiment-method
  - (calculateCPM), [7](#)
- calculateDiffusionMap, [9](#)
- calculateDiffusionMap, ANY-method
  - (calculateDiffusionMap), [9](#)
- calculateDiffusionMap, SingleCellExperiment-method
  - (calculateDiffusionMap), [9](#)
- calculateDiffusionMap, SummarizedExperiment-method
  - (calculateDiffusionMap), [9](#)
- calculateFPKM, [11](#), [16](#)
- calculateMDS, [12](#)
- calculateMDS, ANY-method (calculateMDS),  
[12](#)
- calculateMDS, SingleCellExperiment-method
  - (calculateMDS), [12](#)
- calculateMDS, SummarizedExperiment-method
  - (calculateMDS), [12](#)
- calculatePCA, [13](#)
- calculatePCA, ANY-method (calculatePCA),  
[13](#)
- calculatePCA, SingleCellExperiment-method
  - (calculatePCA), [13](#)
- calculatePCA, SummarizedExperiment-method
  - (calculatePCA), [13](#)
- calculateQCMetrics (defunct), [21](#)
- calculateTPM, [15](#)
- calculateTPM, ANY-method (calculateTPM),  
[15](#)
- calculateTPM, SingleCellExperiment-method
  - (calculateTPM), [15](#)
- calculateTPM, SummarizedExperiment-method
  - (calculateTPM), [15](#)
- calculateTSNE, [17](#)
- calculateTSNE, ANY-method
  - (calculateTSNE), [17](#)
- calculateTSNE, SingleCellExperiment-method
  - (calculateTSNE), [17](#)
- calculateTSNE, SummarizedExperiment-method
  - (calculateTSNE), [17](#)
- calculateUMAP, [19](#)
- calculateUMAP, ANY-method
  - (calculateUMAP), [19](#)
- calculateUMAP, SingleCellExperiment-method
  - (calculateUMAP), [19](#)
- calculateUMAP, SummarizedExperiment-method
  - (calculateUMAP), [19](#)

- centreSizeFactors (defunct), 21
- cmdscale, 13
- colData, 3, 29, 65, 73
- colsum, 73
- computeLibraryFactors  
(librarySizeFactors), 26
- DataFrame, 4, 24, 39, 40, 42, 60, 61, 64, 66,  
69, 72
- defunct, 21
- DelayedMatrix, 33
- DiffusionMap, 9, 10
- downsampleMatrix, 34
- exprs (norm\_exprs), 34
- exprs, SingleCellExperiment-method,  
(norm\_exprs), 34
- exprs<-, SingleCellExperiment, ANY-method  
(norm\_exprs), 34
- facet\_wrap, 49, 59
- findKNN, 18, 20
- fpkm (norm\_exprs), 34
- fpkm<- (norm\_exprs), 34
- geom\_boxplot, 56
- getBM, 4
- getBMFeatureAnnos (annotateBMFeatures),  
4
- getExplanatoryPCs, 22, 24, 46
- getVarianceExplained, 22, 23, 23, 47
- getVarianceExplained, ANY-method  
(getVarianceExplained), 23
- getVarianceExplained, SummarizedExperiment-method  
(getVarianceExplained), 23
- ggplot, 30, 44, 45, 52, 58, 59, 63, 69
- isOutlier, 24, 60, 61, 68
- librarySizeFactors, 7, 26, 33
- librarySizeFactors, ANY-method  
(librarySizeFactors), 26
- librarySizeFactors, SummarizedExperiment-method  
(librarySizeFactors), 26
- logNormCounts, 7, 22, 26, 27, 27, 34
- logNormCounts, SingleCellExperiment-method  
(logNormCounts), 27
- logNormCounts, SummarizedExperiment-method  
(logNormCounts), 27
- mockSCE, 29
- modelGeneVarWithSpikes, 34
- multiBatchNorm, 34
- multiplot, 30
- nexprs, 31, 36–38
- nexprs, ANY-method (nexprs), 31
- nexprs, SummarizedExperiment-method  
(nexprs), 31
- norm\_exprs, 34
- norm\_exprs<- (norm\_exprs), 34
- normalize, SingleCellExperiment-method  
(defunct), 21
- normalize\_input, 18
- normalizeCounts, 8, 27, 28, 32
- normalizeCounts, ANY-method  
(normalizeCounts), 32
- normalizeCounts, SingleCellExperiment-method  
(normalizeCounts), 32
- normalizeCounts, SummarizedExperiment-method  
(normalizeCounts), 32
- numDetectedAcrossCells, 32, 35
- numDetectedAcrossCells, ANY-method  
(numDetectedAcrossCells), 35
- numDetectedAcrossCells, SummarizedExperiment-method  
(numDetectedAcrossCells), 35
- numDetectedAcrossFeatures, 32, 37
- numDetectedAcrossFeatures, ANY-method  
(numDetectedAcrossFeatures), 37
- numDetectedAcrossFeatures, SummarizedExperiment-method  
(numDetectedAcrossFeatures), 37
- perCellQCMetrics, 3, 22, 38, 60, 61
- perCellQCMetrics, ANY-method  
(perCellQCMetrics), 38
- perCellQCMetrics, SingleCellExperiment-method  
(perCellQCMetrics), 38
- perCellQCMetrics, SummarizedExperiment-method  
(perCellQCMetrics), 38
- perFeatureQCMetrics, 3, 22, 41
- perFeatureQCMetrics, ANY-method  
(perFeatureQCMetrics), 41
- perFeatureQCMetrics, SummarizedExperiment-method  
(perFeatureQCMetrics), 41
- pheatmap, 50, 51
- plotColData, 43, 65, 69
- plotDiffusionMap, 10
- plotDiffusionMap (Reduced dimension  
plots), 62
- plotDots, 44
- plotExplanatoryPCs, 23, 46
- plotExplanatoryVariables, 24, 47
- plotExpression, 46, 48, 65, 69
- plotHeatmap, 46, 50
- plotHighestExprs, 52
- plotMDS, 13
- plotMDS (Reduced dimension plots), 62
- plotPCA, 15, 55

- plotPCA (Reduced dimension plots), 62
- plotPCA, SingleCellExperiment-method  
(Reduced dimension plots), 62
- plotPCASCE (Reduced dimension plots), 62
- plotPlatePosition, 53, 65, 69
- plotReducedDim, 54, 63, 65, 69
- plotRLE, 56
- plotRLE, SingleCellExperiment-method  
(plotRLE), 56
- plotRowData, 57, 67, 69
- plotScater, 59
- plotTSNE, 19
- plotTSNE (Reduced dimension plots), 62
- plotUMAP, 21
- plotUMAP (Reduced dimension plots), 62
  
- quickPerCellQC, 60
  
- read.table, 62
- readMM, 62
- readSparseCounts, 61
- Reduced dimension plots, 62
- reducedDim, 10, 13, 18, 21
- reducedDims, 15, 63, 70
- retrieveCellInfo, 43, 45, 48, 50–53, 55, 56,  
59, 64, 67, 69
- retrieveFeatureInfo, 52, 58, 66, 69
- rowData, 3, 4, 66
- rowsum, 75
- Rtsne, 17–19
- Rtsne\_neighbors, 18
- runColDataPCA, 67
- runDiffusionMap, 63, 71
- runDiffusionMap  
(calculateDiffusionMap), 9
- runMDS, 63, 71
- runMDS (calculateMDS), 12
- runPCA, 15, 22, 63, 68, 71
- runPCA (calculatePCA), 13
- runPCA, SingleCellExperiment-method  
(calculatePCA), 13
- runTSNE, 63, 71
- runTSNE (calculateTSNE), 17
- runUMAP, 63, 71
- runUMAP (calculateUMAP), 19
  
- sc\_example\_cell\_info (mockSCE), 29
- sc\_example\_counts (mockSCE), 29
- scater-plot-args, 68
- scater-red-dim-args, 69
- SCESet, 71, 77
- SCESet-class (SCESet), 71
- set.seed, 10, 15, 18, 20
  
- SingleCellExperiment, 4–9, 11, 12, 14, 16,  
17, 20, 22, 26–29, 32–35, 38, 43, 45,  
59, 64, 66, 67, 69, 70, 72, 77
- sizeFactors, 7, 27, 28, 33
- stand\_exprs (norm\_exprs), 34
- stand\_exprs<- (norm\_exprs), 34
- sumCountsAcrossCells, 72
- sumCountsAcrossCells, ANY-method  
(sumCountsAcrossCells), 72
- sumCountsAcrossCells, SummarizedExperiment-method  
(sumCountsAcrossCells), 72
- sumCountsAcrossFeatures, 74
- sumCountsAcrossFeatures, ANY-method  
(sumCountsAcrossFeatures), 74
- sumCountsAcrossFeatures, SummarizedExperiment-method  
(sumCountsAcrossFeatures), 74
- SummarizedExperiment, 3, 6–9, 11, 12, 14,  
16, 17, 20, 23, 26–28, 31–33, 36–38,  
41, 70, 72, 74
  
- toSingleCellExperiment (updateSCESet),  
77
  
- umap, 20, 21
- uniquifyFeatureNames, 76
- updateSCESet, 77
- useMart, 4