

Package ‘SingleCellExperiment’

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Description Defines a S4 class for storing data from single-cell experiments. This includes specialized methods to store and retrieve spike-in information, dimensionality reduction coordinates and size factors for each cell, along with the usual metadata for genes and libraries.

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altExps	<i>Alternative Experiment methods</i>
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Description

In some experiments, different features must be normalized differently or have different row-level metadata. Typical examples would be for spike-in transcripts in plate-based experiments and antibody or CRISPR tags in CITE-seq experiments. These data cannot be stored in the main assays of the [SingleCellExperiment](#) itself. However, it is still desirable to store these features *somewhere* in the [SingleCellExperiment](#). This simplifies book-keeping in long workflows and ensure that samples remain synchronised.

To facilitate this, the [SingleCellExperiment](#) class allows for “alternative Experiments”. Nested [SummarizedExperiment](#)-class objects are stored inside the [SingleCellExperiment](#) object `x`, in a manner that guarantees that the nested objects have the same columns in the same order as those in `x`. Methods are provided to enable convenient access to and manipulation of these alternative Experiments. Each alternative Experiment should contain experimental data and row metadata for a distinct set of features.

Getters

In the following examples, `x` is a [SingleCellExperiment](#) object.

`altExp(x, e, withColData=FALSE)`: Retrieves a [SummarizedExperiment](#) containing alternative features (rows) for all cells (columns) in `x`. `e` is either a string specifying the name of the alternative Experiment in `x` to retrieve, or a numeric scalar specifying the index of the desired Experiment. If `withColData=TRUE`, the column metadata of the output object is set to `colData(x)`.

`altExpNames(x)`: Returns a character vector containing the names of all alternative Experiments in `x`. This is guaranteed to be of the same length as the number of results, though the names may not be unique.

`altExps(x, withColData=FALSE)`: Returns a named [List](#) of matrices containing one or more [SummarizedExperiment](#) objects. Each object has the same number of columns. If `withColData=TRUE`, the column metadata of each output object is set to `colData(x)`.

Single-object setter

`altExp(x, e) <- value` will add or replace an alternative Experiment in a [SingleCellExperiment](#) object `x`. The value of `e` determines how the result is added or replaced:

- If `e` is missing, `value` is assigned to the first result. If the result already exists, its name is preserved; otherwise it is given a default name "unnamed1".
- If `e` is a numeric scalar, it must be within the range of existing results, and `value` will be assigned to the result at that index.
- If `e` is a string and a result exists with this name, `value` is assigned to that result. Otherwise a new result with this name is appended to the existing list of results.

`value` is expected to be a `SummarizedExperiment` object with number of columns equal to `ncol(x)`. Alternatively, if `value` is `NULL`, the alternative Experiment at `e` is removed from the object.

Other setters

In the following examples, `x` is a [SingleCellExperiment](#) object.

`altExps(x) <- value`: Replaces all alternative Experiments in `x` with those in `value`. The latter should be a list-like object containing any number of `SummarizedExperiment` objects with number of columns equal to `ncol(x)`.

If `value` is named, those names will be used to name the alternative Experiments in `x`. Otherwise, unnamed results are assigned default names prefixed with "unnamed".

If `value` is `NULL`, all alternative Experiments in `x` are removed.

`altExpNames(x) <- value`: Replaces all names for alternative Experiments in `x` with a character vector `value`. This should be of length equal to the number of results currently in `x`.

`removeAltExps(x)` will remove all alternative Experiments from `x`. This has the same effect as `altExps(x) <- NULL` but may be more convenient as it directly returns a `SingleCellExperiment`.

Author(s)

Aaron Lun

See Also

[splitAltExps](#), for a convenient way of adding alternative Experiments from existing features.

[swapAltExp](#), to swap the main and alternative Experiments.

Examples

```
example(SingleCellExperiment, echo=FALSE) # Using the class example
dim(counts(sce))

# Mocking up some alternative Experiments.
se1 <- SummarizedExperiment(matrix(rpois(1000, 5), ncol=ncol(se)))
rowData(se1)$stuff <- sample(LETTERS, nrow(se1), replace=TRUE)
se2 <- SummarizedExperiment(matrix(rpois(500, 5), ncol=ncol(se)))
rowData(se2)$blah <- sample(letters, nrow(se2), replace=TRUE)

# Setting the alternative Experiments.
altExp(sce, "spike-in") <- se1
altExp(sce, "CRISPR") <- se2
```

```
# Getting alternative Experimental data.
altExpNames(sce)
altExp(sce, "spike-in")
altExp(sce, 2)

# Setting alternative Experimental data.
altExpNames(sce) <- c("ERCC", "Ab")
altExp(sce, "ERCC") <- se1[1:2,]
```

colLabels

Get or set column labels

Description

Get or set column labels in an instance of a [SingleCellExperiment](#) class. Labels are expected to represent information about the the biological state of each cell.

Usage

```
## S4 method for signature 'SingleCellExperiment'
colLabels(x, onAbsence = "none")

## S4 replacement method for signature 'SingleCellExperiment'
colLabels(x, ...) <- value
```

Arguments

x	A SingleCellExperiment object.
onAbsence	String indicating an additional action to take when labels are absent: nothing ("none"), a warning ("warn") or an error ("error").
...	Additional arguments, currently ignored.
value	Any vector-like object of length equal to <code>ncol(object)</code> , containing labels for all cells.

Details

A frequent task in single-cell data analyses is to label cells with some annotation, e.g., cluster identities, predicted cell type classifications and so on. In a [SummarizedExperiment](#), the `colData` represents the ideal place for such annotations, which can be easily set and retrieved with standard methods, e.g., `x$label <- my.labels`.

That said, it is desirable to have some informal standardization of the name of the column used to store these annotations as this makes it easier to programmatically set sensible defaults for retrieval of the labels in downstream functions. To this end, the `colLabels` function will get or set labels from the "label" field of the `colData`. This considers the use case where there is a "primary" set of labels that represents the default grouping of cells in downstream analyses.

To illustrate, let's say we have a downstream function that accepts a `SingleCellExperiment` object and requires labels. When defining our function, we can set `colLabels(x)` as the default value for our label argument. This pattern is useful as it accommodates on-the-fly changes to a secondary set of labels in `x` without requiring the user to run `colLabels(x) <- second.labels`, while facilitating convenient use of the primary labels by default.

For developers, `onAbsence` is provided to make it easier to mandate that `x` actually has labels. This avoids silent NULL values that flow to the rest of the function and make debugging difficult.

Value

For `colLabels`, a vector or equivalent is returned containing label assignments for all cells. If no labels are available, a NULL is returned (and/or a warning or error, depending on `onAbsence`).

For `colLabels<-`, a modified `x` is returned with labels in its `colData`.

Author(s)

Aaron Lun

See Also

[SingleCellExperiment](#), for the underlying class definition.

Examples

```
example(SingleCellExperiment, echo=FALSE) # Using the class example
colLabels(sce) <- sample(LETTERS, ncol(sce), replace=TRUE)
colLabels(sce)
```

Combining LEMs

LEM combining methods

Description

Methods to combine `LinearEmbeddingMatrix` objects.

Usage

```
## S4 method for signature 'LinearEmbeddingMatrix'
rbind(..., deparse.level=1)
```

```
## S4 method for signature 'LinearEmbeddingMatrix'
cbind(..., deparse.level=1)
```

Arguments

... One or more `LinearEmbeddingMatrix` objects.

`deparse.level` An integer scalar; see `?base::cbind` for a description of this argument.

Details

For `rbind`, `LinearEmbeddingMatrix` objects are combined row-wise, i.e., rows in successive objects are appended to the first object. This corresponds to adding more samples to the first object. Note that `featureLoadings` and `factorData` will only be taken from the first element in the list; no checks are performed to determine whether they are consistent or not across objects.

For `cbind`, `LinearEmbeddingMatrix` objects are combined columns-wise, i.e., columns in successive objects are appended to the first object. This corresponds to adding more factors to the first object. `featureLoadings` will also be combined column-wise across objects, provided that the number of features is the same across objects. Similarly, `factorData` will be combined row-wise across objects.

Combining objects with and without row names will result in the removal of all row names; similarly for column names. Duplicate row names are currently supported by duplicate column names are not, and will be de-duplicated appropriately.

Value

A `LinearEmbeddingMatrix` object containing all rows/columns of the supplied objects.

Author(s)

Aaron Lun

Examples

```
example(LinearEmbeddingMatrix, echo=FALSE) # using the class example
rbind(lm, lm)
cbind(lm, lm)
```

defunct

Defunct methods

Description

Defunct methods in the **SingleCellExperiment** package.

Named size factors

The class now only supports one set of size factors, accessible via `sizeFactors`. This represents a simplification of the class and removes a difficult part of the API (that had to deal with both `NULL` and strings to specify the size factor set of interest).

Spike-ins

It is recommended to handle spike-ins and other “alternative” features via `altExps`.

Author(s)

Aaron Lun

Getter/setter methods *LinearEmbeddingMatrix* getters/setters

Description

Getter/setter methods for the `LinearEmbeddingMatrix` class.

Usage

```
## S4 method for signature 'LinearEmbeddingMatrix'  
sampleFactors(x, withDimnames=TRUE)  
  
## S4 replacement method for signature 'LinearEmbeddingMatrix'  
sampleFactors(x) <- value  
  
## S4 method for signature 'LinearEmbeddingMatrix'  
featureLoadings(x, withDimnames=TRUE)  
  
## S4 replacement method for signature 'LinearEmbeddingMatrix'  
featureLoadings(x) <- value  
  
## S4 method for signature 'LinearEmbeddingMatrix'  
factorData(x)  
  
## S4 replacement method for signature 'LinearEmbeddingMatrix'  
factorData(x) <- value  
  
## S4 method for signature 'LinearEmbeddingMatrix'  
as.matrix(x, ...)  
  
## S4 method for signature 'LinearEmbeddingMatrix'  
dim(x)  
  
## S4 method for signature 'LinearEmbeddingMatrix'  
dimnames(x)  
  
## S4 replacement method for signature 'LinearEmbeddingMatrix'  
dimnames(x) <- value  
  
## S4 method for signature 'LinearEmbeddingMatrix'  
x$name  
  
## S4 replacement method for signature 'LinearEmbeddingMatrix'  
x$name <- value
```

Arguments

<code>x</code>	A <code>LinearEmbeddingMatrix</code> object.
<code>value</code>	An appropriate value to assign to the relevant slot.
<code>withDimnames</code>	A logical scalar indicating whether dimension names should be attached to the returned object.

name A string specifying a field of the factorData slot.
 ... Further arguments, ignored.

Details

Any value to assign to `sampleFactors` and `featureLoadings` should be matrix-like objects, while `factorData` should be a `DataFrame` - see [LinearEmbeddingMatrix](#) for details.

The `as.matrix` method will return the matrix of sample factors, consistent with the fact that the `LinearEmbeddingMatrix` mimics a sample-factor matrix. However, unlike the `sampleFactors` method, this is always guaranteed to return an ordinary R matrix, even if an alternative representation was stored in the slot. This ensures consistency with `as.matrix` methods for other matrix-like S4 classes.

For assignment to `dimnames`, a list of length 2 should be used containing vectors of row and column names.

Value

For the getter methods `sampleFactors`, `featureLoadings` and `factorData`, the value of the slot with the same name is returned. For the corresponding setter methods, a `LinearEmbeddingMatrix` is returned with modifications to the named slot.

For `dim`, the dimensions of the `sampleFactors` slot are returned in an integer vector of length 2. For `dimnames`, a list of length 2 containing the row and column names is returned. For `as.matrix`, an ordinary matrix derived from `sampleFactors` is returned.

For `$`, the value of the named field of the `factorData` slot is returned. For `$<-`, a `LinearEmbeddingMatrix` is returned with the modified field in `factorData`.

Author(s)

Keegan Korthauer, Davide Risso and Aaron Lun

See Also

[LinearEmbeddingMatrix](#)

Examples

```
example(LinearEmbeddingMatrix, echo=FALSE) # Using the class example

sampleFactors(lem)
sampleFactors(lem) <- sampleFactors(lem) * -1

featureLoadings(lem)
featureLoadings(lem) <- featureLoadings(lem) * -1

factorData(lem)
factorData(lem)$whee <- 1

nrow(lem)
ncol(lem)
colnames(lem) <- LETTERS[seq_len(ncol(lem))]
as.matrix(lem)
```

LinearEmbeddingMatrix *LinearEmbeddingMatrix class*

Description

A description of the LinearEmbeddingMatrix class for storing low-dimensional embeddings from linear dimensionality reduction methods.

Usage

```
LinearEmbeddingMatrix(sampleFactors = matrix(nrow = 0, ncol = 0),  
  featureLoadings = matrix(nrow = 0, ncol = 0), factorData = NULL,  
  metadata = list())
```

Arguments

sampleFactors	A matrix-like object of sample embeddings, where rows are samples and columns are factors.
featureLoadings	A matrix-like object of feature loadings, where rows are features and columns are factors.
factorData	A DataFrame containing factor-level information, with one row per factor.
metadata	An optional list of arbitrary content describing the overall experiment.

Details

The LinearEmbeddingMatrix class is a matrix-like object that supports `dim`, `dimnames` and `as.matrix`. It is designed for the storage of results from linear dimensionality reduction methods like principal components analysis (PCA), factor analysis and non-negative matrix factorization.

The `sampleFactors` slot is intended to store The low-dimensional representation of the samples, such as the principal coordinates from PCA. The feature loadings contributing to each factor are stored in `featureLoadings`, and should have the same number of columns as `sampleFactors`. The `factorData` stores additional factor-level information, such as the percentage of variance explained by each factor, and should have the same number of rows as `sampleFactors`.

The intended use of this class is to allow PCA and other results to be stored in the `reducedDims` slot of a `SingleCellExperiment` object. This means that feature loadings remain attached to the embedding, allowing it to be used in downstream analyses.

Value

A LinearEmbeddingMatrix object is returned from the constructor.

Author(s)

Aaron Lun, Davide Risso and Keegan Korthauer

Examples

```
lem <- LinearEmbeddingMatrix(matrix(rnorm(1000), ncol=5),  
  matrix(runif(20000), ncol=5))  
lem
```

Miscellaneous LEM *Miscellaneous LEM methods*

Description

Various methods for the LinearEmbeddingMatrix class.

Usage

```
## S4 method for signature 'LinearEmbeddingMatrix'  
show(object)
```

Arguments

object A LinearEmbeddingMatrix object.

Details

The show method will print out information about the data contained in object. This includes the number of samples, the number of factors, the number of genes and the fields available in factorData.

Value

A message is printed to screen describing the data stored in object.

Author(s)

Davide Risso

See Also

[LinearEmbeddingMatrix](#)

Examples

```
example(LinearEmbeddingMatrix, echo=FALSE) # Using the class example  
show(lem)
```

reducedDims *Reduced dimensions methods*

Description

Methods to get or set dimensionality reduction results in a [SingleCellExperiment](#) object. These are typically used to store and retrieve low-dimensional representations of single-cell datasets. Each row of a reduced dimension result is expected to correspond to a column of the SingleCellExperiment object.

Getters

In the following examples, `x` is a [SingleCellExperiment](#) object.

`reducedDim(x, type, withDimnames=TRUE)`: Retrieves a matrix (or matrix-like object) containing reduced dimension coordinates for cells (rows) and dimensions (columns). `type` is either a string specifying the name of the dimensionality reduction result in `x` to retrieve, or a numeric scalar specifying the index of the desired result. If `withDimnames=TRUE`, row names of the output matrix are replaced with the column names of `x`.

`reducedDimNames(x)`: Returns a character vector containing the names of all dimensionality reduction results in `x`. This is guaranteed to be of the same length as the number of results, though the names may not be unique.

`reducedDims(x, withDimnames=TRUE)`: Returns a named [List](#) of matrices containing one or more dimensionality reduction results. Each result is a matrix (or matrix-like object) with the same number of rows. If `withDimnames=TRUE`, row names of each matrix are replaced with the column names of `x`.

Single-result setter

`reducedDim(x, type) <- value` will add or replace a dimensionality reduction result in a [SingleCellExperiment](#) object `x`. The value of `type` determines how the result is added or replaced:

- If `type` is missing, `value` is assigned to the first result. If the result already exists, its name is preserved; otherwise it is given a default name "unnamed1".
- If `type` is a numeric scalar, it must be within the range of existing results, and `value` will be assigned to the result at that index.
- If `type` is a string and a result exists with this name, `value` is assigned to that result. Otherwise a new result with this name is append to the existing list of results.

`value` is expected to be a matrix or matrix-like object with number of rows equal to `ncol(x)`. Alternatively, if `value` is `NULL`, the result corresponding to `type` is removed from the object.

Other setters

In the following examples, `x` is a [SingleCellExperiment](#) object.

`reducedDims(x) <- value`: Replaces all dimensionality reduction results in `x` with those in `value`. The latter should be a list-like object containing any number of matrices or matrix-like objects with number of rows equal to `ncol(x)`.

If `value` is named, those names will be used to name the dimensionality reduction results in `x`. Otherwise, unnamed results are assigned default names prefixed with "unnamed".

If `value` is `NULL`, all dimensionality reduction results in `x` are removed.

`reducedDimNames(x) <- value`: Replaces all names for dimensionality reduction results in `x` with a character vector `value`. This should be of length equal to the number of results currently in `x`.

Author(s)

Aaron Lun and Kevin Rue-Albrecht

Examples

```

example(SingleCellExperiment, echo=FALSE)
reducedDim(sce, "PCA")
reducedDim(sce, "tSNE")
reducedDims(sce)

reducedDim(sce, "PCA") <- NULL
reducedDims(sce)

reducedDims(sce) <- SimpleList()
reducedDims(sce)

```

SCE-assays

*Named assay getters and setters***Description**

These are methods for getting or setting `assay(sce, i=X, ...)` where `sce` is a [SingleCellExperiment](#) object and `X` is the name of the method. For example, `counts` will get or set `X="counts"`. This provides some convenience for users as well as encouraging standardization of assay names across packages.

Available methods

In the following code snippets, `x` is a [SingleCellExperiment](#) object, `value` is a matrix-like object with the same dimensions as `x`, and `...` are further arguments passed to `assay` (for the getter) or `assay<-` (for the setter).

`counts(x, ...)`, `counts(x, ...) <- value`: Get or set a matrix of raw count data, e.g., number of reads or transcripts.

`normcounts(x, ...)`, `normcounts(x, ...) <- value`: Get or set a matrix of normalized values on the same scale as the original counts. For example, counts divided by cell-specific size factors that are centred at unity.

`logcounts(x, ...)`, `logcounts(x, ...) <- value`: Get or set a matrix of log-transformed counts or count-like values. In most cases, this will be defined as log-transformed `normcounts`, e.g., using log base 2 and a pseudo-count of 1.

`cpm(x, ...)`, `cpm(x, ...) <- value`: Get or set a matrix of counts-per-million values. This is the read count for each gene in each cell, divided by the library size of each cell in millions.

`tpm(x, ...)`, `tpm(x, ...) <- value`: Get or set a matrix of transcripts-per-million values. This is the number of transcripts for each gene in each cell, divided by the total number of transcripts in that cell (in millions).

`weights(x, ...)`, `weights(x, ...) <- value`: Get or set a matrix of weights, e.g., observational weights to be used in differential expression analysis.

Author(s)

Aaron Lun

See Also

[assay](#) and [assay<-](#), for the wrapped methods.

Examples

```
example(SingleCellExperiment, echo=FALSE) # Using the class example
counts(sce) <- matrix(rnorm(nrow(sce)*ncol(sce)), ncol=ncol(sce))
dim(counts(sce))

# One possible way of computing normalized "counts"
sf <- 2^rnorm(ncol(sce))
sf <- sf/mean(sf)
normcounts(sce) <- t(t(counts(sce))/sf)
dim(normcounts(sce))

# One possible way of computing log-counts
logcounts(sce) <- log2(normcounts(sce)+1)
dim(normcounts(sce))
```

SCE-combine

Combining or subsetting SingleCellExperiment objects

Description

An overview of methods to combine multiple [SingleCellExperiment](#) objects by row or column, or to subset a [SingleCellExperiment](#) by row or column. These methods are useful for ensuring that all data fields remain synchronized when cells or genes are added or removed.

Combining

In the following code snippets, `...` contains one or more [SingleCellExperiment](#) objects.

`rbind(..., deparse.level=1)`: Returns a [SingleCellExperiment](#) where all objects in `...` are combined row-wise, i.e., rows in successive objects are appended to the first object.

Refer to `?rbind, SummarizedExperiment-method` for details on how metadata is combined in the output object. Refer to `?rbind` for the interpretation of `deparse.level`.

Note that all objects in `...` must have the exact same values for `reducedDims` and `altExps`. Any `sizeFactors` should either be `NULL` or contain the same values across objects.

`cbind(..., deparse.level=1)`: Returns a [SingleCellExperiment](#) where all objects in `...` are combined column-wise, i.e., columns in successive objects are appended to the first object.

Each object `x` in `...` must have the same values of `reducedDimNames(x)` (though they can be unordered). Dimensionality reduction results with the same name across objects will be combined row-wise to create the corresponding entry in the output object.

Each object `x` in `...` must have the same values of `altExpNames(x)` (though they can be unordered). Alternative Experiments with the same name across objects will be combined column-wise to create the corresponding entry in the output object.

`sizeFactors` should be either set to `NULL` in all objects, or set to a numeric vector in all objects.

Refer to `?cbind, SummarizedExperiment-method` for details on how metadata is combined in the output object. Refer to `?cbind` for the interpretation of `deparse.level`.

Subsetting

In the following code snippets, `x` is a `SingleCellExperiment` object.

`x[i, j, ..., drop=TRUE]`: Returns a `SingleCellExperiment` containing the specified rows `i` and columns `j`.

`i` and `j` can be a logical, integer or character vector of subscripts, indicating the rows and columns respectively to retain. Either can be missing, in which case subsetting is only performed in the specified dimension. If both are missing, no subsetting is performed.

Arguments in `...` and `drop` are passed to to `[, SummarizedExperiment-method]`.

`x[i, j, ...] <- value`: Replaces all data for rows `i` and columns `j` with the corresponding fields in a `SingleCellExperiment` value.

`i` and `j` can be a logical, integer or character vector of subscripts, indicating the rows and columns respectively to replace. Either can be missing, in which case replacement is only performed in the specified dimension. If both are missing, `x` is replaced entirely with `value`.

If `j` is specified, `value` is expected to have the same name and order of `reducedDimNames` and `altExpNames` as `x`. If `sizeFactors` is set for `x`, it should also be set for `value`.

Arguments in `...` are passed to the corresponding `SummarizedExperiment` method.

Author(s)

Aaron Lun

Examples

```
example(SingleCellExperiment, echo=FALSE) # using the class example

# Combining:
rbind(sce, sce)
cbind(sce, sce)

# Subsetting:
sce[1:10,]
sce[,1:5]

sce2 <- sce
sce2[1:10,] <- sce[11:20,]

# Can also use subset()
sce$WHEE <- sample(LETTERS, ncol(sce), replace=TRUE)
subset(sce, , WHEE=="A")

# Can also use split()
split(sce, sample(LETTERS, nrow(sce), replace=TRUE))
```

Description

Methods to get or set internal fields from the `SingleCellExperiment` class. These functions are intended for package developers who want to add protected fields to a `SingleCellExperiment`. They should *not* be used by ordinary users of the **SingleCellExperiment** package.

Getters

In the following code snippets, `x` is a [SingleCellExperiment](#).

`int_elementMetadata(x)`: Returns a [DataFrame](#) of internal row metadata, with number of rows equal to `nrow(x)`. This is analogous to the user-visible [colData](#).

`int_colData(x)`: Returns a [DataFrame](#) of internal column metadata, with number of rows equal to `ncol(x)`. This is analogous to the user-visible [rowData](#).

`int_metadata(x)`: Returns a list of internal metadata, analogous to the user-visible [metadata](#).

It may occasionally be useful to return both the visible and the internal `colData` in a single [DataFrame](#). This is facilitated by the following methods:

`rowData(x, ..., internal=FALSE)`: Returns a [DataFrame](#) of the user-visible row metadata. If `internal=TRUE`, the internal row metadata is added column-wise to the user-visible metadata. A warning is emitted if the user-visible metadata column names overlap with the internal fields. Any arguments in `...` are passed to [rowData, SummarizedExperiment-method](#).

`colData(x, ..., internal=FALSE)`: Returns a [DataFrame](#) of the user-visible column metadata. If `internal=TRUE`, the internal column metadata is added column-wise to the user-visible metadata. A warning is emitted if the user-visible metadata column names overlap with the internal fields. Any arguments in `...` are passed to [colData, SummarizedExperiment-method](#).

Setters

In the following code snippets, `x` is a [SingleCellExperiment](#).

`int_elementMetadata(x) <- value`: Replaces the internal row metadata with `value`, a [DataFrame](#) with number of rows equal to `nrow(x)`. This is analogous to the user-visible [colData<-](#).

`int_colData(x) <- value`: Replaces the internal column metadata with `value`, a [DataFrame](#) with number of rows equal to `ncol(x)`. This is analogous to the user-visible [rowData<-](#).

`int_metadata(x) <- value`: Replaces the internal metadata with `value`, analogous to the user-visible [metadata<-](#).

Comments

The internal metadata fields allow easy and extensible storage of additional elements that are parallel to the rows or columns of a [SingleCellExperiment](#) class. This avoids the need to specify new slots and adjust the subsetting/combining code for a new data element. For example, [altExps](#) and [reducedDims](#) are implemented as fields in the internal column metadata.

That these elements are internal is important as this ensures that the implementation details are abstracted away. Any user interaction with these internal fields should be done via the designated getter and setter methods, e.g., [reducedDim](#) and friends for retrieving or modifying reduced dimensions. This provides developers with more freedom to change the internal representation without breaking user code.

Package developers intending to use these methods to store their own content should read the development vignette for guidance.

Author(s)

Aaron Lun

See Also

[colData](#), [rowData](#) and [metadata](#) for the user-visible equivalents.

Examples

```
example(SingleCellExperiment, echo=FALSE) # Using the class example
int_metadata(sce)$whee <- 1
```

SCE-miscellaneous *Miscellaneous SingleCellExperiment methods*

Description

Miscellaneous methods for the [SingleCellExperiment](#) class that do not fit in any other documentation category.

Available methods

In the following code snippets, `x` and `object` are [SingleCellExperiment](#) objects.

`show(object)`: Print a message to screen describing the contents of `object`.

`objectVersion(x)`: Return the version of the package with which `x` was constructed.

`sizeFactors(object)`: Return a numeric vector of size factors of length equal to `ncol(object)`.
If no size factors are available in `object`, return `NULL` instead.

`sizeFactors(object) <- value`: Replace the size factors with `value`, usually expected to be a numeric vector or vector-like object. Alternatively, `value` can be `NULL` in which case any size factors in `object` are removed.

Author(s)

Aaron Lun

See Also

[updateObject](#), where `objectVersion` is used.

Examples

```
example(SingleCellExperiment, echo=FALSE) # Using the class example

show(sce)

objectVersion(sce)

# Setting/getting size factors.
sizeFactors(sce) <- runif(ncol(sce))
sizeFactors(sce)

sizeFactors(sce) <- NULL
sizeFactors(sce)
```

SingleCellExperiment-class

The SingleCellExperiment class

Description

The SingleCellExperiment class is designed to represent single-cell sequencing data. It inherits from the [RangedSummarizedExperiment](#) class and is used in the same manner. In addition, the class supports storage of dimensionality reduction results (e.g., PCA, t-SNE) via [reducedDims](#), and storage of alternative feature types (e.g., spike-ins) via [altExps](#).

Usage

```
SingleCellExperiment(..., reducedDims = list(), altExps = list())
```

Arguments

...	Arguments passed to the SummarizedExperiment constructor to fill the slots of the base class.
reducedDims	A list of any number of matrix-like objects containing dimensionality reduction results, each of which should have the same number of rows as the output SingleCellExperiment object.
altExps	A list of any number of SummarizedExperiment objects containing alternative Experiments, each of which should have the same number of columns as the output SingleCellExperiment object.

Details

In this class, rows should represent genomic features (e.g., genes) while columns represent samples generated from single cells. As with any [SummarizedExperiment](#) derivative, different quantifications (e.g., counts, CPMs, log-expression) can be stored simultaneously in the [assays](#) slot, and row and column metadata can be attached using [rowData](#) and [colData](#), respectively.

The [reducedDims](#) and [altExps](#) concepts are the main extensions of the SingleCellExperiment class. This enables formalized representation of data structures that are commonly encountered during single-cell data analysis. Readers are referred to the specific documentation pages for more details.

A SingleCellExperiment can also be created by coercing from a [SummarizedExperiment](#) or [Ranged-SummarizedExperiment](#) instance.

Value

A SingleCellExperiment object.

Author(s)

Aaron Lun and Davide Risso

See Also

[reducedDims](#), for representation of dimensionality reduction results.
[altExps](#), for representation of data for alternative feature sets.
[sizeFactors](#), to store size factors for normalization.
[?"SCE-combine"](#), to combine or subset a `SingleCellExperiment` object.
[?"SCE-internals"](#), for developer use.

Examples

```
ncells <- 100
u <- matrix(rpois(20000, 5), ncol=ncells)
v <- log2(u + 1)

pca <- matrix(runif(ncells*5), ncells)
tsne <- matrix(rnorm(ncells*2), ncells)

sce <- SingleCellExperiment(assays=list(counts=u, logcounts=v),
  reducedDims=SimpleList(PCA=pca, tSNE=tsne))
sce

## coercion from SummarizedExperiment
se <- SummarizedExperiment(assays=list(counts=u, logcounts=v))
as(se, "SingleCellExperiment")
```

sizeFactors

*Size factor methods***Description**

Gets or sets the size factors for all cells in a `SingleCellExperiment` object.

Usage

```
## S4 method for signature 'SingleCellExperiment'
sizeFactors(object, onAbsence = "none")

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

Arguments

object	A <code>SingleCellExperiment</code> object.
onAbsence	String indicating an additional action to take when size factors are absent: nothing ("none"), a warning ("warn") or an error ("error").
...	Additional arguments, currently ignored.
value	A numeric vector of length equal to <code>ncol(object)</code> , containing size factors for all cells.

Details

A size factor is a scaling factor used to divide the raw counts of a particular cell to obtain normalized expression values, thus allowing downstream comparisons between cells that are not affected by differences in library size or total RNA content. The `sizeFactors` methods can be used to get or set size factors for all cells in a `SingleCellExperiment` object.

When setting size factors, the values are stored in the `colData` as the `sizeFactors` field. This name is chosen for general consistency with other packages (e.g., **DESeq2**) and to allow the size factors to be easily extracted from the `colData` for use as covariates.

For developers, `onAbsence` is provided to make it easier to mandate that object has size factors. This avoids silent NULL values that flow to the rest of the function and make debugging difficult.

Value

For `sizeFactors`, a numeric vector is returned containing size factors for all cells. If no size factors are available, a NULL is returned (and/or a warning or error, depending on `onAbsence`).

For `sizeFactors<-`, a modified object is returned with size factors in its `colData`.

Author(s)

Aaron Lun

See Also

[SingleCellExperiment](#), for the underlying class definition.

`librarySizeFactors` from the **scater** package or `computeSumFactors` from the **scran** package, as examples of functions that compute the size factors.

Examples

```
example(SingleCellExperiment, echo=FALSE) # Using the class example
sizeFactors(sce) <- runif(ncol(sce))
sizeFactors(sce)
```

splitAltExps

Split off alternative features

Description

Split a [SingleCellExperiment](#) based on the feature type, creating alternative Experiments to hold features that are not in the majority set.

Usage

```
splitAltExps(x, f, ref = NULL)
```

Arguments

<code>x</code>	A SingleCellExperiment object.
<code>f</code>	A character vector or factor of length equal to <code>nrow(x)</code> , specifying the feature type of each row.
<code>ref</code>	String indicating which level of <code>f</code> should be treated as the main set.

Details

This function provides a convenient way to create a `SingleCellExperiment` with alternative Experiments. For example, a `SingleCellExperiment` with rows corresponding to all features can be quickly split into endogenous genes (main) and other alternative features like spike-in transcripts and antibody tags.

By default, the most frequent level of `f` is treated as the `ref` if the latter is not specified.

Value

A `SingleCellExperiment` where each row corresponds to a feature in the main set. Each other feature type is stored as an alternative Experiment, accessible by `altExp`.

Author(s)

Aaron Lun

See Also

`altExp`, to access and manipulate the alternative Experiment fields.

Examples

```
example(SingleCellExperiment, echo=FALSE)
feat.type <- sample(c("endog", "ERCC", "CITE"), nrow(sce),
  replace=TRUE, p=c(0.8, 0.1, 0.1))

sce2 <- splitAltExps(sce, feat.type)
sce2
```

Description

Methods to subset `LinearEmbeddingMatrix` objects.

Usage

```
## S4 method for signature 'LinearEmbeddingMatrix,ANY,ANY'
x[i, j, ..., drop=TRUE]

## S4 replacement method for signature
## 'LinearEmbeddingMatrix,ANY,ANY,LinearEmbeddingMatrix'
x[i, j] <- value
```

Arguments

<code>x</code>	A <code>LinearEmbeddingMatrix</code> object.
<code>i, j</code>	A vector of logical or integer subscripts, indicating the rows and columns to be subsetting for <code>i</code> and <code>j</code> , respectively.
<code>...</code>	Extra arguments that are ignored.
<code>drop</code>	A logical scalar indicating whether the result should be coerced to the lowest possible dimension.
<code>value</code>	A <code>LinearEmbeddingMatrix</code> object with number of rows equal to length of <code>i</code> (or that of <code>x</code> , if <code>i</code> is not specified). The number of columns must be equal to the length of <code>j</code> (or number of columns in <code>x</code> , if <code>j</code> is not specified).

Details

Subsetting yields a `LinearEmbeddingMatrix` object containing the specified rows (samples) and columns (factors). If column subsetting is performed, values of `featureLoadings` and `factorData` will be modified to retain only the selected factors.

If `drop=TRUE` and the subsetting would produce dimensions of length 1, those dimensions are dropped and a vector is returned directly from `sampleFactors`. This mimics the expected behaviour from a matrix-like object. Users should set `drop=FALSE` to ensure that a `LinearEmbeddingMatrix` is returned.

For subset replacement, if neither `i` or `j` are set, `x` will be effectively replaced by `value`. However, row and column names will *not* change, consistent with replacement in ordinary matrices.

Value

For `[],` a subsetting `LinearEmbeddingMatrix` object is returned.

For `[-,` a modified `LinearEmbeddingMatrix` object is returned.

Author(s)

Aaron Lun

See Also

[LinearEmbeddingMatrix-class](#)

Examples

```
example(LinearEmbeddingMatrix, echo=FALSE) # using the class example

lem[1:10,]
lem[,1:5]

lem2 <- lem
lem2[1:10,] <- lem[11:20,]
```

 swapAltExp

Swap main and alternative Experiments

Description

Swap the main Experiment for an alternative Experiment in a [SingleCellExperiment](#) object.

Usage

```
swapAltExp(x, name, saved = NULL, withColData = TRUE)
```

Arguments

x	A SingleCellExperiment object.
name	String or integer scalar specifying the alternative Experiment to use to replace the main Experiment.
saved	String specifying the name to use to save the original x as an alternative experiment in the output. If NULL, the original is not saved.
withColData	Logical scalar specifying whether the column metadata of x should be preserved in the output.

Details

During the course of an analysis, we may need to perform operations on each of the alternative Experiments in turn. This would require us to repeatedly call `altExp(x, name)` prior to running downstream functions on those Experiments. In such cases, it may be more convenient to switch the main Experiment with the desired alternative Experiments, allowing a particular section of the analysis to be performed on the latter by default.

For example, the initial phases of the analysis might use the entire set of features. At some point, we might want to focus only on a subset of features of interest, but we do not want to discard the rest of the features. This can be achieved by storing the subset as an alternative Experiment and swapping it with the main Experiment, as shown in the Examples below.

Value

A [SingleCellExperiment](#) derived from `altExp(x, name)`. This contains all alternative Experiments in `altExps(x)`, with an additional entry containing x if saved is specified. If `withColData=TRUE`, the column metadata is set to `colData(x)`.

Author(s)

Aaron Lun

See Also

[altExps](#), for a description of the alternative Experiment concept.

Examples

```

example(SingleCellExperiment, echo=FALSE) # using the class example

# Let's say we defined a subset of genes of interest.
# We can save the feature set as its own altExp.
hvgs <- 1:10
altExp(sce, "subset") <- sce[hvgs,]

# At some point, we want to do our analysis on the HVGs only,
# but we want to hold onto the other features for later reference.
sce <- swapAltExp(sce, name="subset", saved="all")
sce

# Once we're done, it is straightforward to switch back.
swapAltExp(sce, "all")

```

updateObject

Update a SingleCellExperiment object

Description

Update [SingleCellExperiment](#) objects to the latest version of the class structure. This is usually called by methods in the **SingleCellExperiment** package rather than by users or downstream packages.

Usage

```

## S4 method for signature 'SingleCellExperiment'
updateObject(object, ..., verbose = FALSE)

```

Arguments

object	A old SingleCellExperiment object.
...	Additional arguments that are ignored.
verbose	Logical scalar indicating whether a message should be emitted as the object is updated.

Details

This function updates the `SingleCellExperiment` to match changes in the internal class representation. Changes are as follows:

- Objects created before 1.7.1 are modified to include `altExps` and `reducedDims` fields in their internal column metadata. Reduced dimension results previously in the `reducedDims` slot are transferred to the `reducedDims` field.
- Objects created before 1.9.1 are modified so that the size factors are stored by `sizeFactors<-` in `colData` rather than `int_colData`.

Value

An updated version of object.

Author(s)

Aaron Lun

See Also

[objectVersion](#), which is used to determine if the object is up-to-date.

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