

# Package ‘splatter’

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**Type** Package

**Title** Simple Simulation of Single-cell RNA Sequencing Data

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**Description** Splatter is a package for the simulation of single-cell RNA sequencing count data. It provides a simple interface for creating complex simulations that are reproducible and well-documented. Parameters can be estimated from real data and functions are provided for comparing real and simulated datasets.

**License** GPL-3 + file LICENSE

**LazyData** TRUE

**Depends** R (>= 3.4), scater

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**BugReports** <https://github.com/Oshlack/splatter/issues>

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---

addFeatureStats      *Add feature statistics*

---

### Description

Add additional feature statistics to an SCESet object

### Usage

```
addFeatureStats(sce, value = c("counts", "cpm", "tpm", "fpkm"), log = FALSE,
  offset = 1, no.zeros = FALSE)
```

### Arguments

sce	SCESet to add feature statistics to.
value	the expression value to calculate statistics for. Options are "counts", "cpm", "tpm" or "fpkm". The values need to exist in the given SCESet.
log	logical. Whether to take log2 before calculating statistics.
offset	offset to add to avoid taking log of zero.
no.zeros	logical. Whether to remove all zeros from each feature before calculating statistics.

### Details

Currently adds the following statistics: mean, variance, coefficient of variation, median and median absolute deviation. Statistics are added to the fData slot and are named Stat[Log]Value[No0] where Log and No0 are added if those arguments are true. UpperCamelCase is used to differentiate these columns from those added by scater.

### Value

SCESet with additional feature statistics

---

addGeneLengths      *Add gene lengths*

---

### Description

Add gene lengths to an SCESet object

### Usage

```
addGeneLengths(sce, method = c("generate", "sample"), loc = 7.9,
  scale = 0.7, lengths = NULL)
```

**Arguments**

sce	SCESet to add gene lengths to.
method	Method to use for creating lengths.
loc	Location parameter for the generate method.
scale	Scale parameter for the generate method.
lengths	Vector of lengths for the sample method.

**Details**

This function adds simulated gene lengths to the `fData` slot of an `SCESet` object that can be used for calculating length normalised expression values such as TPM or FPKM. The `generate` simulates lengths using a (rounded) log-normal distribution, with the default `loc` and `scale` parameters based on human coding genes. Alternatively the `sample` method can be used which randomly samples lengths (with replacement) from a supplied vector.

**Value**

SCESet with added gene lengths

**Examples**

```
# Default generate method
sce <- simpleSimulate()
sce <- addGeneLengths(sce)
head(fData(sce))
# Sample method (human coding genes)
## Not run:
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
library(GenomicFeatures)
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
tx.lens <- transcriptLengths(txdb, with.cds_len = TRUE)
tx.lens <- tx.lens[tx.lens$cds_len > 0, ]
gene.lens <- max(splitAsList(tx.lens$tx_len, tx.lens$gene_id))
sce <- addGeneLengths(sce, method = "sample", lengths = gene.lens)

## End(Not run)
```

---

bridge

*Brownian bridge*


---

**Description**

Calculate a smoothed Brownian bridge between two points. A Brownian bridge is a random walk with fixed end points.

**Usage**

```
bridge(x = 0, y = 0, N = 5, n = 100, sigma.fac = 0.8)
```

**Arguments**

x	starting value.
y	end value.
N	number of steps in random walk.
n	number of points in smoothed bridge.
sigma.fac	multiplier specifying how extreme each step can be.

**Value**

Vector of length n following a path from x to y.

---

compareSCESets	<i>Compare SCESet objects</i>
----------------	-------------------------------

---

**Description**

Combine the data from several SCESet objects and produce some basic plots comparing them.

**Usage**

```
compareSCESets(sces, point.size = 0.1, point.alpha = 0.1, fits = TRUE,
               colours = NULL)
```

**Arguments**

sces	named list of SCESet objects to combine and compare.
point.size	size of points in scatter plots.
point.alpha	opacity of points in scatter plots.
fits	whether to include fits in scatter plots.
colours	vector of colours to use for each dataset.

**Details**

The returned list has three items:

**FeatureData** Combined feature data from the provided SCESets.

**PhenoData** Combined pheno data from the provided SCESets.

**Plots** Comparison plots

**Means** Boxplot of mean distribution.

**Variances** Boxplot of variance distribution.

**MeanVar** Scatter plot with fitted lines showing the mean-variance relationship.

**LibraySizes** Boxplot of the library size distribution.

**ZerosGene** Boxplot of the percentage of each gene that is zero.

**ZerosCell** Boxplot of the percentage of each cell that is zero.

**MeanZeros** Scatter plot with fitted lines showing the mean-dropout relationship.

The plots returned by this function are created using [ggplot](#) and are only a sample of the kind of plots you might like to consider. The data used to create these plots is also returned and should be in the correct format to allow you to create further plots using [ggplot](#).

**Value**

List containing the combined datasets and plots.

**Examples**

```
sim1 <- splatSimulate(nGenes = 1000, groupCells = 20)
sim2 <- simpleSimulate(nGenes = 1000, nCells = 20)
comparison <- compareSCESets(list(Splat = sim1, Simple = sim2))
names(comparison)
names(comparison$Plots)
```

---

diffSCESets

*Diff SCESet objects*


---

**Description**

Combine the data from several SCESet objects and produce some basic plots comparing them to a reference.

**Usage**

```
diffSCESets(sces, ref, point.size = 0.1, point.alpha = 0.1, fits = TRUE,
  colours = NULL)
```

**Arguments**

sces	named list of SCESet objects to combine and compare.
ref	string giving the name of the SCESet to use as the reference
point.size	size of points in scatter plots.
point.alpha	opacity of points in scatter plots.
fits	whether to include fits in scatter plots.
colours	vector of colours to use for each dataset.

**Details**

This function aims to look at the differences between a reference SCESet and one or more others. It requires each SCESet to have the same dimensions. Properties are compared by ranks, for example when comparing the means the values are ordered and the differences between the reference and another dataset plotted. A series of Q-Q plots are also returned.

The returned list has five items:

Reference The SCESet used as the reference.

FeatureData Combined feature data from the provided SCESets.

PhenoData Combined pheno data from the provided SCESets.

Plots Difference plots

Means Boxplot of mean differences.

Variances Boxplot of variance differences.

MeanVar Scatter plot showing the difference from the reference variance across expression ranks.

LibrarySizes Boxplot of the library size differences.  
 ZerosGene Boxplot of the differences in the percentage of each gene that is zero.  
 ZerosCell Boxplot of the differences in the percentage of each cell that is zero.  
 MeanZeros Scatter plot showing the difference from the reference percentage of zeros across expression ranks.  
 QQPlots Quantile-Quantile plots  
   Means Q-Q plot of the means.  
   Variances Q-Q plot of the variances.  
   LibrarySizes Q-Q plot of the library sizes.  
   ZerosGene Q-Q plot of the percentage of zeros per gene.  
   ZerosCell Q-Q plot of the percentage of zeros per cell.

The plots returned by this function are created using [ggplot](#) and are only a sample of the kind of plots you might like to consider. The data used to create these plots is also returned and should be in the correct format to allow you to create further plots using [ggplot](#).

### Value

List containing the combined datasets and plots.

### Examples

```

sim1 <- splatSimulate(nGenes = 1000, groupCells = 20)
sim2 <- simpleSimulate(nGenes = 1000, nCells = 20)
difference <- diffSCESets(list(Splat = sim1, Simple = sim2), ref = "Simple")
names(difference)
names(difference$Plots)

```

---

expandParams	<i>Expand parameters</i>
--------------	--------------------------

---

### Description

Expand the parameters that can be vectors so that they are the same length as the number of groups.

### Usage

```

expandParams(object, ...)

## S4 method for signature 'LunParams'
expandParams(object)

## S4 method for signature 'SplatParams'
expandParams(object)

```

### Arguments

object            object to expand.  
 ...                additional arguments.

### Value

Expanded object.

---

getLNormFactors	<i>Get log-normal factors</i>
-----------------	-------------------------------

---

**Description**

Randomly generate multiplication factors from a log-normal distribution.

**Usage**

```
getLNormFactors(n.facs, sel.prob, neg.prob, fac.loc, fac.scale)
```

**Arguments**

n.facs	Number of factors to generate.
sel.prob	Probability that a factor will be selected to be different from 1.
neg.prob	Probability that a selected factor is less than one.
fac.loc	Location parameter for the log-normal distribution.
fac.scale	Scale factor for the log-normal distribution.

**Value**

Vector containing generated factors.

---

getParam	<i>Get a parameter</i>
----------	------------------------

---

**Description**

Accessor function for getting parameter values.

**Usage**

```
getParam(object, name)

## S4 method for signature 'Params'
getParam(object, name)
```

**Arguments**

object	object to get parameter from.
name	name of the parameter to get.

**Value**

The extracted parameter value

**Examples**

```
params <- newSimpleParams()
getParam(params, "nGenes")
```



---

getParams	<i>Get parameters</i>
-----------	-----------------------

---

**Description**

Get multiple parameter values from a Params object.

**Usage**

```
getParams(params, names)
```

**Arguments**

params	Params object to get values from.
names	vector of names of the parameters to get.

**Value**

List with the values of the selected parameters.

**Examples**

```
params <- newSimpleParams()
getParams(params, c("nGenes", "nCells", "mean.rate"))
```

---

getPathOrder	<i>Get path order</i>
--------------	-----------------------

---

**Description**

Identify the correct order to process paths so that preceding paths have already been simulated.

**Usage**

```
getPathOrder(path.from)
```

**Arguments**

path.from	vector giving the path endpoints that each path originates from.
-----------	--

**Value**

Vector giving the order to process paths in.

---

`listSims`*List simulations*

---

**Description**

List all the simulations that are currently available in Splatter with a brief description.

**Usage**

```
listSims(print = TRUE)
```

**Arguments**

`print` logical. Whether to print to the console.

**Value**

Invisibly returns a data.frame containing the information that is displayed.

**Examples**

```
listSims()
```

---

`logistic`*Logistic function*

---

**Description**

Implementation of the logistic function

**Usage**

```
logistic(x, x0, k)
```

**Arguments**

`x` value to apply the function to.  
`x0` midpoint parameter. Gives the centre of the function.  
`k` shape parameter. Gives the slope of the function.

**Value**

Value of logistic function with given parameters

---

lun2Estimate	<i>Estimate Lun2 simulation parameters</i>
--------------	--

---

## Description

Estimate simulation parameters for the Lun2 simulation from a real dataset.

## Usage

```
lun2Estimate(counts, plates, params = newLun2Params(), min.size = 200,
             verbose = TRUE, BPPARAM = SerialParam())
```

```
## S3 method for class 'SCESet'
lun2Estimate(counts, plates, params = newLun2Params(),
             min.size = 200, verbose = TRUE, BPPARAM = SerialParam())
```

```
## S3 method for class 'matrix'
lun2Estimate(counts, plates, params = newLun2Params(),
             min.size = 200, verbose = TRUE, BPPARAM = SerialParam())
```

## Arguments

counts	either a counts matrix or an SCESet object containing count data to estimate parameters from.
plates	integer vector giving the plate that each cell originated from.
params	Lun2Params object to store estimated values in.
min.size	minimum size of clusters when identifying group of cells in the data.
verbose	logical. Whether to show progress messages.
BPPARAM	A <a href="#">BiocParallelParam</a> instance giving the parallel back-end to be used. Default is <a href="#">SerialParam</a> which uses a single core.

## Details

See [Lun2Params](#) for more details on the parameters.

## Value

LunParams object containing the estimated parameters.

## Examples

```
## Not run:
data("sc_example_counts")
data("sc_example_cell_info")
plates <- factor(sc_example_cell_info$Mutation_Status)
params <- lun2Estimate(sc_example_counts, plates, min.size = 20)
params

## End(Not run)
```

Lun2Params

*The Lun2Params class***Description**

S4 class that holds parameters for the Lun simulation.

**Parameters**

The Lun2 simulation uses the following parameters:

nGenes The number of genes to simulate.

nCells The number of cells to simulate.

[seed] Seed to use for generating random numbers.

**Gene parameters** gene.params A data.frame containing gene parameters with two columns: Mean (mean expression for each gene) and Disp (dispersion for each gene).

zi.params A data.frame containing zero-inflated gene parameters with three columns: Mean (mean expression for each gene), Disp (dispersion for each, gene), and Prop (zero proportion for each gene).

[nPlates] The number of plates to simulate.

**Plate parameters** plate.ingroup Character vector giving the plates considered to be part of the "ingroup".

plate.mod Plate effect modifier factor. The plate effect variance is divided by this value.

plate.var Plate effect variance.

**Cell parameters** cell.plates Factor giving the plate that each cell comes from.

cell.libSizes Library size for each cell.

cell.libMod Modifier factor for library sizes. The library sizes are multiplied by this value.

**Differential expression parameters** de.nGenes Number of differentially expressed genes.

de.fc Fold change for differentially expressed genes.

The parameters not shown in brackets can be estimated from real data using [lun2Estimate](#). For details of the Lun2 simulation see [lun2Simulate](#).

lun2Simulate

*Lun2 simulation***Description**

Simulate single-cell RNA-seq count data using the method described in Lun and Marioni "Overcoming confounding plate effects in differential expression analyses of single-cell RNA-seq data".

**Usage**

```
lun2Simulate(params = newLun2Params(), zinb = FALSE, verbose = TRUE, ...)
```

**Arguments**

params	Lun2Params object containing simulation parameters.
zinb	logical. Whether to use a zero-inflated model.
verbose	logical. Whether to print progress messages
...	any additional parameter settings to override what is provided in params.

**Details**

The Lun2 simulation uses a negative-binomial distribution where the means and dispersions have been sampled from a real dataset (using `lun2Estimate`). The other core feature of the Lun2 simulation is the addition of plate effects. Differential expression can be added between two groups of plates (an "ingroup" and all other plates). Library size factors are also applied and optionally a zero-inflated negative-binomial can be used.

If the number of genes to simulate differs from the number of provided gene parameters or the number of cells to simulate differs from the number of library sizes the relevant parameters will be sampled with a warning. This allows any number of genes or cells to be simulated regardless of the number in the dataset used in the estimation step but has the downside that some genes or cells may be simulated multiple times.

**Value**

SCSESet containing simulated counts.

**References**

Lun ATL, Marioni JC. Overcoming confounding plate effects in differential expression analyses of single-cell RNA-seq data. bioRxiv (2016).

Paper: [dx.doi.org/10.1101/073973](https://doi.org/10.1101/073973)

Code: <https://github.com/MarioniLab/PlateEffects2016>

**Examples**

```
sim <- lun2Simulate()
```

---

lunEstimate

*Estimate Lun simulation parameters*

---

**Description**

Estimate simulation parameters for the Lun simulation from a real dataset.

**Usage**

```
lunEstimate(counts, params = newLunParams())

## S3 method for class 'SCSESet'
lunEstimate(counts, params = newLunParams())

## S3 method for class 'matrix'
lunEstimate(counts, params = newLunParams())
```

**Arguments**

counts	either a counts matrix or an SCESet object containing count data to estimate parameters from.
params	LunParams object to store estimated values in.

**Details**

The nGenes and nCells parameters are taken from the size of the input data. No other parameters are estimated. See [LunParams](#) for more details on the parameters.

**Value**

LunParams object containing the estimated parameters.

**Examples**

```
data("sc_example_counts")
params <- lunEstimate(sc_example_counts)
params
```

---

LunParams

*The LunParams class*


---

**Description**

S4 class that holds parameters for the Lun simulation.

**Parameters**

The Lun simulation uses the following parameters:

nGenes The number of genes to simulate.

nCells The number of cells to simulate.

[nGroups] The number of groups to simulate.

[groupCells] Vector giving the number of cells in each simulation group/path.

[seed] Seed to use for generating random numbers.

**Mean parameters** [mean.shape] Shape parameter for the mean gamma distribution.

[mean.rate] Rate parameter for the mean gamma distribution.

**Counts parameters** [count.disp] The dispersion parameter for the counts negative binomial distribution.

**Differential expression parameters** [de.nGenes] The number of genes that are differentially expressed in each group

[de.upProp] The proportion of differentially expressed genes that are up-regulated in each group

[de.upFC] The fold change for up-regulated genes

[de.downFC] The fold change for down-regulated genes

The parameters not shown in brackets can be estimated from real data using [lunEstimate](#). For details of the Lun simulation see [lunSimulate](#).

---

lunSimulate	<i>Lun simulation</i>
-------------	-----------------------

---

### Description

Simulate single-cell RNA-seq count data using the method described in Lun, Bach and Marioni "Pooling across cells to normalize single-cell RNA sequencing data with many zero counts".

### Usage

```
lunSimulate(params = newLunParams(), verbose = TRUE, ...)
```

### Arguments

params	LunParams object containing Lun simulation parameters.
verbose	logical. Whether to print progress messages.
...	any additional parameter settings to override what is provided in params.

### Details

The Lun simulation generates gene mean expression levels from a gamma distribution with `shape = mean.shape` and `rate = mean.rate`. Counts are then simulated from a negative binomial distribution with `mu = means` and `size = 1 / bcv.common`. In addition each cell is given a size factor ( $2^{\text{rnorm}(n\text{Cells}, \text{mean} = 0, \text{sd} = 1)}$ ) and differential expression can be simulated with fixed fold changes.

See [LunParams](#) for details of the parameters.

### Value

SCESet object containing the simulated counts and intermediate values.

### References

Lun ATL, Bach K, Marioni JC. Pooling across cells to normalize single-cell RNA sequencing data with many zero counts. *Genome Biology* (2016).

Paper: [dx.doi.org/10.1186/s13059-016-0947-7](https://doi.org/10.1186/s13059-016-0947-7)

Code: <https://github.com/MarioniLab/Deconvolution2016>

### Examples

```
sim <- lunSimulate()
```

---

makeCompPanel                      *Make comparison panel*

---

### Description

Combine the plots from compareSCESets into a single panel.

### Usage

```
makeCompPanel(comp, title = "Comparison", labels = c("Means", "Variance",
  "Mean-variance relationship", "Library size", "Zeros per gene",
  "Zeros per cell", "Mean-zeros relationship"))
```

### Arguments

comp	list returned by <a href="#">compareSCESets</a> .
title	title for the panel.
labels	vector of labels for each of the seven plots.

### Value

Combined panel plot

### Examples

```
## Not run:
sim1 <- splatSimulate(nGenes = 1000, groupCells = 20)
sim2 <- simpleSimulate(nGenes = 1000, nCells = 20)
comparison <- compareSCESets(list(Splat = sim1, Simple = sim2))
panel <- makeCompPanel(comparison)

## End(Not run)
```

---

makeDiffPanel                      *Make difference panel*

---

### Description

Combine the plots from diffSCESets into a single panel.

### Usage

```
makeDiffPanel(diff, title = "Difference comparison", labels = c("Means",
  "Variance", "Library size", "Zeros per cell", "Zeros per gene",
  "Mean-variance relationship", "Mean-zeros relationship"))
```



**Arguments**

diff            list returned by [diffSCESets](#).  
title           title for the panel.  
labels          vector of labels for each of the seven sections.

**Value**

Combined panel plot

**Examples**

```
## Not run:  
sim1 <- splatSimulate(nGenes = 1000, groupCells = 20)  
sim2 <- simpleSimulate(nGenes = 1000, nCells = 20)  
difference <- diffSCESets(list(Splat = sim1, Simple = sim2), ref = "Simple")  
panel <- makeDiffPanel(difference)  
  
## End(Not run)
```

---

makeOverallPanel	<i>Make overall panel</i>
------------------	---------------------------

---

**Description**

Combine the plots from [compSCESets](#) and [diffSCESets](#) into a single panel.

**Usage**

```
makeOverallPanel(comp, diff, title = "Overall comparison",  
  row.labels = c("Means", "Variance", "Mean-variance relationship",  
  "Library size", "Zeros per cell", "Zeros per gene",  
  "Mean-zeros relationship"))
```

**Arguments**

comp            list returned by [compareSCESets](#).  
diff            list returned by [diffSCESets](#).  
title           title for the panel.  
row.labels      vector of labels for each of the seven rows.

**Value**

Combined panel plot

## Examples

```
## Not run:
sim1 <- splatSimulate(nGenes = 1000, groupCells = 20)
sim2 <- simpleSimulate(nGenes = 1000, nCells = 20)
comparison <- compSCEsets(list(Splat = sim1, Simple = sim2))
difference <- diffSCEsets(list(Splat = sim1, Simple = sim2), ref = "Simple")
panel <- makeOverallPanel(comparison, difference)

## End(Not run)
```

---

newParams

*New Params*

---

## Description

Create a new Params object. Functions exist for each of the different Params subtypes.

## Usage

```
newLun2Params(...)
newLunParams(...)
newSCDDParams(...)
newSimpleParams(...)
newSplatParams(...)
```

## Arguments

... additional parameters passed to [setParams](#).

## Value

New Params object.

## Examples

```
params <- newSimpleParams()
params <- newSimpleParams(nGenes = 200, nCells = 10)
```

---

Params	<i>The Params virtual class</i>
--------	---------------------------------

---

**Description**

Virtual S4 class that all other Params classes inherit from.

**Parameters**

The Params class defines the following parameters:

[nGenes] The number of genes to simulate.

[nCells] The number of cells to simulate.

seed Seed to use for generating random numbers.

The parameters shown in brackets can be estimated from real data.

---

rbindMatched	<i>Bind rows (matched)</i>
--------------	----------------------------

---

**Description**

Bind the rows of two data frames, keeping only the columns that are common to both.

**Usage**

```
rbindMatched(df1, df2)
```

**Arguments**

df1 first data.frame to bind.

df2 second data.frame to bind.

**Value**

data.frame containing rows from df1 and df2 but only common columns.

---

scDDEstimate	<i>Estimate scDD simulation parameters</i>
--------------	--

---

### Description

Estimate simulation parameters for the scDD simulation from a real dataset.

### Usage

```
scDDEstimate(counts, conditions, params = newSCDDParams())

## S3 method for class 'SCESet'
scDDEstimate(counts, conditions, params = newSCDDParams())

## S3 method for class 'matrix'
scDDEstimate(counts, conditions, params = newSCDDParams())
```

### Arguments

counts	either a counts matrix or an SCESet object containing count data to estimate parameters from.
conditions	Vector giving the condition that each cell belongs to. Conditions can be 1 or 2.
params	SCDDParams object to store estimated values in.

### Details

This function is just a wrapper around [preprocess](#) that takes the output and converts it to a SCDDParams object. See [preprocess](#) for details.

### Value

SCDDParams object containing the estimated parameters.

### Examples

```
data("sc_example_counts")
conditions <- sample(1:2, ncol(sc_example_counts), replace = TRUE)
params <- scDDEstimate(sc_example_counts, conditions)
params
```

---

SCDDParams	<i>The SCDDParams class</i>
------------	-----------------------------

---

### Description

S4 class that holds parameters for the scDD simulation.

## Parameters

The SCDD simulation uses the following parameters:

[nGenes] The number of genes to simulate (not used).

nCells The number of cells to simulate in each condition.

[seed] Seed to use for generating random numbers.

SCdat [SummarizedExperiment](#) containing real data.

[nDE] Number of DE genes to simulate.

[nDP] Number of DP genes to simulate.

[nDM] Number of DM genes to simulate.

[nDB] Number of DB genes to simulate.

[nEE] Number of EE genes to simulate.

[nEP] Number of EP genes to simulate.

[sd.range] Interval for fold change standard deviations.

[modeFC] Values for DP, DM and DB mode fold changes.

[varInflation] Variance inflation factors for each condition. If all equal to 1 will be set to NULL (default)

[condition] String giving the column that represents biological group of interest

The parameters not shown in brackets can be estimated from real data using [scDDEstimate](#). See [simulateSet](#) for more details of the parameters. For details of the Splatter implementation of the scDD simulation see [scDDSimulate](#).

---

scDDSimulate

*scDD simulation*

---

## Description

Simulate counts using the scDD method.

## Usage

```
scDDSimulate(params = newSCDDParams(), plots = FALSE, plot.file = NULL,
  verbose = TRUE, BPPARAM = SerialParam(), ...)
```

## Arguments

params	SCDDParams object containing simulation parameters.
plots	logical. whether to generate scDD fold change and validation plots.
plot.file	File path to save plots as PDF.
verbose	logical. Whether to print progress messages
BPPARAM	A <a href="#">BiocParallelParam</a> instance giving the parallel back-end to be used. Default is <a href="#">SerialParam</a> which uses a single core.
...	any additional parameter settings to override what is provided in params.

## Details

This function is just a wrapper around `simulateSet` that takes a `SCDDParams`, runs the simulation then converts the output to an `SCESet` object. See `simulateSet` for more details of how the simulation works.

## Value

SCESet containing simulated counts

## References

Korthauer KD, Chu L-F, Newton MA, Li Y, Thomson J, Stewart R, et al. A statistical approach for identifying differential distributions in single-cell RNA-seq experiments. *Genome Biology* (2016).

Paper: [10.1186/s13059-016-1077-y](https://doi.org/10.1186/s13059-016-1077-y)

Code: <https://github.com/kdkorthauer/scDD>

## Examples

```
## Not run:  
sim <- scDDSimulate()  
  
## End(Not run)
```

---

setParam

*Set a parameter*

---

## Description

Function for setting parameter values.

## Usage

```
setParam(object, name, value)  
  
## S4 method for signature 'Lun2Params'  
setParam(object, name, value)  
  
## S4 method for signature 'LunParams'  
setParam(object, name, value)  
  
## S4 method for signature 'Params'  
setParam(object, name, value)  
  
## S4 method for signature 'SCDDParams'  
setParam(object, name, value)  
  
## S4 method for signature 'SplatParams'  
setParam(object, name, value)
```

**Arguments**

object	object to set parameter in.
name	name of the parameter to set.
value	value to set the parameter to.

**Value**

Object with new parameter value.

**Examples**

```
params <- newSimpleParams()
setParam(params, "nGenes", 100)
```

---

setParams                      *Set parameters*

---

**Description**

Set multiple parameters in a Params object.

**Usage**

```
setParams(params, update = NULL, ...)
```

**Arguments**

params	Params object to set parameters in.
update	list of parameters to set where names(update) are the names of the parameters to set and the items in the list are values.
...	additional parameters to set. These are combined with any parameters specified in update.

**Details**

Each parameter is set by a call to [setParam](#). If the same parameter is specified multiple times it will be set multiple times. Parameters can be specified using a list via update (useful when collecting parameter values in some way) or individually (useful when setting them manually), see examples.

**Value**

Params object with updated values.

## Examples

```
params <- newSimpleParams()
params
# Set individually
params <- setParams(params, nGenes = 1000, nCells = 50)
params
# Set via update list
params <- setParams(params, list(mean.rate = 0.2, mean.shape = 0.8))
params
```

---

setParamsUnchecked	<i>Set parameters UNCHECKED</i>
--------------------	---------------------------------

---

## Description

Set multiple parameters in a Params object.

## Usage

```
setParamsUnchecked(params, update = NULL, ...)
```

## Arguments

params	Params object to set parameters in.
update	list of parameters to set where names(update) are the names of the parameters to set and the items in the list are values.
...	additional parameters to set. These are combined with any parameters specified in update.

## Details

Each parameter is set by a call to [setParam](#). If the same parameter is specified multiple times it will be set multiple times. Parameters can be specified using a list via update (useful when collecting parameter values in some way) or individually (useful when setting them manually), see examples. **THE FINAL OBJECT IS NOT CHECKED FOR VALIDITY!**

## Value

Params object with updated values.



---

setParamUnchecked	<i>Set a parameter UNCHECKED</i>
-------------------	----------------------------------

---

**Description**

Function for setting parameter values. THE OUTPUT IS NOT CHECKED FOR VALIDITY!

**Usage**

```
setParamUnchecked(object, name, value)
```

```
## S4 method for signature 'Params'  
setParamUnchecked(object, name, value)
```

**Arguments**

object	object to set parameter in.
name	name of the parameter to set.
value	value to set the parameter to.

**Value**

Object with new parameter value.

---

showPP	<i>Show pretty print</i>
--------	--------------------------

---

**Description**

Function used for pretty printing params object.

**Usage**

```
showPP(params, pp)
```

**Arguments**

params	object to show.
pp	list specifying how the object should be displayed.

**Value**

Print params object to console

---

simpleEstimate	<i>Estimate simple simulation parameters</i>
----------------	--

---

### Description

Estimate simulation parameters for the simple simulation from a real dataset.

### Usage

```
simpleEstimate(counts, params = newSimpleParams())

## S3 method for class 'SCESet'
simpleEstimate(counts, params = newSimpleParams())

## S3 method for class 'matrix'
simpleEstimate(counts, params = newSimpleParams())
```

### Arguments

counts	either a counts matrix or an SCESet object containing count data to estimate parameters from.
params	SimpleParams object to store estimated values in.

### Details

The nGenes and nCells parameters are taken from the size of the input data. The mean parameters are estimated by fitting a gamma distribution to the library size normalised mean expression level using `fitdist`. See [SimpleParams](#) for more details on the parameters.

### Value

SimpleParams object containing the estimated parameters.

### Examples

```
data("sc_example_counts")
params <- simpleEstimate(sc_example_counts)
params
```

---

SimpleParams	<i>The SimpleParams class</i>
--------------	-------------------------------

---

### Description

S4 class that holds parameters for the simple simulation.

**Parameters**

The simple simulation uses the following parameters:

nGenes The number of genes to simulate.

nCells The number of cells to simulate.

[seed] Seed to use for generating random numbers.

mean.shape The shape parameter for the mean gamma distribution.

mean.rate The rate parameter for the mean gamma distribution.

[count.disp] The dispersion parameter for the counts negative binomial distribution.

The parameters not shown in brackets can be estimated from real data using [simpleEstimate](#). For details of the simple simulation see [simpleSimulate](#).

---

simpleSimulate	<i>Simple simulation</i>
----------------	--------------------------

---

**Description**

Simulate counts from a simple negative binomial distribution without simulated library sizes, differential expression etc.

**Usage**

```
simpleSimulate(params = newSimpleParams(), verbose = TRUE, ...)
```

**Arguments**

params	SimpleParams object containing simulation parameters.
verbose	logical. Whether to print progress messages
...	any additional parameter settings to override what is provided in params.

**Details**

Gene means are simulated from a gamma distribution with `shape = mean.shape` and `rate = mean.rate`. Counts are then simulated from a negative binomial distribution with `mu = means` and `size = 1 / counts.disp`. See [SimpleParams](#) for more details of the parameters.

**Value**

SCESet containing simulated counts

**Examples**

```
sim <- simpleSimulate()
# Override default parameters
sim <- simpleSimulate(nGenes = 1000, nCells = 50)
```

---

splatEstBCV

*Estimate Splat Biological Coefficient of Variation parameters*


---

### Description

Parameters are estimated using the [estimateDisp](#) function in the edgeR package.

### Usage

```
splatEstBCV(counts, params)
```

### Arguments

counts	counts matrix to estimate parameters from.
params	SplatParams object to store estimated values in.

### Details

The [estimateDisp](#) function is used to estimate the common dispersion and prior degrees of freedom. See [estimateDisp](#) for details. When estimating parameters on simulated data we found a broadly linear relationship between the true underlying common dispersion and the edgeR estimate, therefore we apply a small correction,  $disp = 0.1 + 0.25 * edgeR.disp$ .

### Value

SplatParams object with estimated values.

---

splatEstDropout

*Estimate Splat dropout parameters*


---

### Description

Estimate the midpoint and shape parameters for the logistic function used when simulating dropout.

### Usage

```
splatEstDropout(norm.counts, params)
```

### Arguments

norm.counts	library size normalised counts matrix.
params	SplatParams object to store estimated values in.

### Details

Logistic function parameters are estimated by fitting a logistic function to the relationship between  $\log_2$  mean gene expression and the proportion of zeros in each gene. See [nls](#) for details of fitting.

### Value

SplatParams object with estimated values.

---

splatEstimate	<i>Estimate Splat simulation parameters</i>
---------------	---

---

**Description**

Estimate simulation parameters for the Splat simulation from a real dataset. See the individual estimation functions for more details on how this is done.

**Usage**

```
splatEstimate(counts, params = newSplatParams())
```

```
## S3 method for class 'SCESet'
splatEstimate(counts, params = newSplatParams())
```

```
## S3 method for class 'matrix'
splatEstimate(counts, params = newSplatParams())
```

**Arguments**

counts	either a counts matrix or an SCESet object containing count data to estimate parameters from.
params	SplatParams object to store estimated values in.

**Value**

SplatParams object containing the estimated parameters.

**See Also**

[splatEstMean](#), [splatEstLib](#), [splatEstOutlier](#), [splatEstBCV](#), [splatEstDropout](#)

**Examples**

```
data("sc_example_counts")
params <- splatEstimate(sc_example_counts)
params
```

---

splatEstLib	<i>Estimate Splat library size parameters</i>
-------------	---

---

**Description**

A log-normal distribution is fitted to the library sizes and the estimated parameters are added to the params object. See [fitdist](#) for details on the fitting.

**Usage**

```
splatEstLib(counts, params)
```

**Arguments**

counts            counts matrix to estimate parameters from.  
 params            splatParams object to store estimated values in.

**Value**

splatParams object with estimated values.

---

splatEstMean	<i>Estimate Splat mean parameters</i>
--------------	---------------------------------------

---

**Description**

Estimate rate and shape parameters for the gamma distribution used to simulate gene expression means.

**Usage**

```
splatEstMean(norm.counts, params)
```

**Arguments**

norm.counts      library size normalised counts matrix.  
 params            SplatParams object to store estimated values in.

**Details**

Parameter for the gamma distribution are estimated by fitting the mean normalised counts using [fitdist](#). The 'maximum goodness-of-fit estimation' method is used to minimise the Cramer-von Mises distance. This can fail in some situations, in which case the 'method of moments estimation' method is used instead. Prior to fitting the means are winsorized by setting the top and bottom 10 percent of values to the 10th and 90th percentiles.

**Value**

SplatParams object with estimated values.

---

splatEstOutlier	<i>Estimate Splat expression outlier parameters</i>
-----------------	---

---

**Description**

Parameters are estimated by comparing means of individual genes to the median mean expression level.

**Usage**

```
splatEstOutlier(norm.counts, params)
```

**Arguments**

norm.counts      library size normalised counts matrix.  
 params            SplatParams object to store estimated values in.

**Details**

Expression outlier genes are detected using the Median Absolute Deviation (MAD) from median method. If the log<sub>2</sub> mean expression of a gene is greater than two MADs above the median log<sub>2</sub> mean expression it is designated as an outlier. The proportion of outlier genes is used to estimate the outlier probability. Factors for each outlier gene are calculated by dividing mean expression by the median mean expression. A log-normal distribution is then fitted to these factors in order to estimate the outlier factor location and scale parameters using [fitdist](#).

**Value**

SplatParams object with estimated values.

---

SplatParams	<i>The SplatParams class</i>
-------------	------------------------------

---

**Description**

S4 class that holds parameters for the Splatter simulation.

**Parameters**

The Splatter simulation requires the following parameters:

nGenes The number of genes to simulate.

nCells The number of cells to simulate.

[nGroups] The number of groups or paths to simulate.

[groupCells] Vector giving the number of cells in each simulation group/path.

[seed] Seed to use for generating random numbers.

**Mean parameters** mean.shape Shape parameter for the mean gamma distribution.

mean.rate Rate parameter for the mean gamma distribution.

**Library size parameters** lib.loc Location (meanlog) parameter for the library size log-normal distribution.

lib.scale Scale (sdlog) parameter for the library size log-normal distribution.

**Expression outlier parameters** out.prob Probability that a gene is an expression outlier.

out.facLoc Location (meanlog) parameter for the expression outlier factor log-normal distribution.

out.facScale Scale (sdlog) parameter for the expression outlier factor log-normal distribution.

**Differential expression parameters** [de.prob] Probability that a gene is differentially expressed in a group. Can be a vector.

[de.loProb] Probability that a differentially expressed gene is down-regulated. Can be a vector.

[de.facLoc] Location (meanlog) parameter for the differential expression factor log-normal distribution. Can be a vector.

[de.facScale] Scale (sdlog) parameter for the differential expression factor log-normal distribution. Can be a vector.

**Biological Coefficient of Variation parameters** bcv.common Underlying common dispersion across all genes.

bcv.df Degrees of Freedom for the BCV inverse chi-squared distribution.

**Dropout parameters** dropout.present Logical. Whether to simulate dropout.

dropout.mid Midpoint parameter for the dropout logistic function.

dropout.shape Shape parameter for the dropout logistic function.

**Differentiation path parameters** [path.from] Vector giving the originating point of each path. This allows path structure such as a cell type which differentiates into an intermediate cell type that then differentiates into two mature cell types. A path structure of this form would have a "from" parameter of c(0, 1, 1) (where 0 is the origin). If no vector is given all paths will start at the origin.

[path.length] Vector giving the number of steps to simulate along each path. If a single value is given it will be applied to all paths.

[path.skew] Vector giving the skew of each path. Values closer to 1 will give more cells towards the starting population, values closer to 0 will give more cells towards the final population. If a single value is given it will be applied to all paths.

[path.nonlinearProb] Probability that a gene follows a non-linear path along the differentiation path. This allows more complex gene patterns such as a gene being equally expressed at the beginning and end of a path but lowly expressed in the middle.

[path.sigmaFac] Sigma factor for non-linear gene paths. A higher value will result in more extreme non-linear variations along a path.

The parameters not shown in brackets can be estimated from real data using [splatEstimate](#). For details of the Splatter simulation see [splatSimulate](#).

---

splatSimBCVMeans      *Simulate BCV means*

---

## Description

Simulate means for each gene in each cell that are adjusted to follow a mean-variance trend using Biological Coefficient of Variation taken from and inverse gamma distribution.

## Usage

```
splatSimBCVMeans(sim, params)
```

## Arguments

sim                    SCESet to add BCV means to.  
 params                SplatParams object with simulation parameters.

## Value

SCESet with simulated BCV means.



---

splatSimCellMeans	<i>Simulate cell means</i>
-------------------	----------------------------

---

**Description**

Simulate a gene by cell matrix giving the mean expression for each gene in each cell. Cells start with the mean expression for the group they belong to (when simulating groups) or cells are assigned the mean expression from a random position on the appropriate path (when simulating paths). The selected means are adjusted for each cell's expected library size.

**Usage**

```
splatSimSingleCellMeans(sim, params)
```

```
splatSimGroupCellMeans(sim, params)
```

```
splatSimPathCellMeans(sim, params)
```

**Arguments**

sim	SCESet to add cell means to.
params	SplatParams object with simulation parameters.

**Value**

SCESet with added cell means.

---

splatSimDE	<i>Simulate group differential expression</i>
------------	---

---

**Description**

Simulate differential expression. Differential expression factors for each group are produced using [getLNormFactors](#) and these are added along with updated means for each group. For paths care is taken to make sure they are simulated in the correct order.

**Usage**

```
splatSimGroupDE(sim, params)
```

```
splatSimPathDE(sim, params)
```

**Arguments**

sim	SCESet to add differential expression to.
params	splatParams object with simulation parameters.

**Value**

SCESet with simulated differential expression.

---

splatSimDropout	<i>Simulate dropout</i>
-----------------	-------------------------

---

**Description**

A logistic function is used to form a relationship between the expression level of a gene and the probability of dropout, giving a probability for each gene in each cell. These probabilities are used in a Bernoulli distribution to decide which counts should be dropped.

**Usage**

```
splatSimDropout(sim, params)
```

**Arguments**

sim	SCESet to add dropout to.
params	SplatParams object with simulation parameters.

**Value**

SCESet with simulated dropout and observed counts.

---

splatSimGeneMeans	<i>Simulate gene means</i>
-------------------	----------------------------

---

**Description**

Simulate gene means from a gamma distribution. Also simulates outlier expression factors. Genes with an outlier factor not equal to 1 are replaced with the median mean expression multiplied by the outlier factor.

**Usage**

```
splatSimGeneMeans(sim, params)
```

**Arguments**

sim	SCESet to add gene means to.
params	SplatParams object with simulation parameters.

**Value**

SCESet with simulated gene means.

---

splatSimLibSizes	<i>Simulate library sizes</i>
------------------	-------------------------------

---

**Description**

Simulate expected library sizes from a log-normal distribution

**Usage**

```
splatSimLibSizes(sim, params)
```

**Arguments**

sim	SCESet to add library size to.
params	SplatParams object with simulation parameters.

**Value**

SCESet with simulated library sizes.

---

splatSimTrueCounts	<i>Simulate true counts</i>
--------------------	-----------------------------

---

**Description**

Simulate a true counts matrix. Counts are simulated from a poisson distribution where Each gene in each cell has it's own mean based on the group (or path position), expected library size and BCV.

**Usage**

```
splatSimTrueCounts(sim, params)
```

**Arguments**

sim	SCESet to add true counts to.
params	SplatParams object with simulation parameters.

**Value**

SCESet with simulated true counts.

---

splatSimulate                      *Splat simulation*

---

### Description

Simulate count data from a fictional single-cell RNA-seq experiment using the Splat method.

### Usage

```
splatSimulate(params = newSplatParams(), method = c("single", "groups",
  "paths"), verbose = TRUE, ...)
```

```
splatSimulateSingle(params = newSplatParams(), verbose = TRUE, ...)
```

```
splatSimulateGroups(params = newSplatParams(), verbose = TRUE, ...)
```

```
splatSimulatePaths(params = newSplatParams(), verbose = TRUE, ...)
```

### Arguments

params	SplatParams object containing parameters for the simulation. See <a href="#">SplatParams</a> for details.
method	which simulation method to use. Options are "single" which produces a single population, "groups" which produces distinct groups (eg. cell types) or "paths" which selects cells from continuous trajectories (eg. differentiation processes).
verbose	logical. Whether to print progress messages.
...	any additional parameter settings to override what is provided in params.

### Details

Parameters can be set in a variety of ways. If no parameters are provided the default parameters are used. Any parameters in `params` can be overridden by supplying additional arguments through a call to [setParams](#). This design allows the user flexibility in how they supply parameters and allows small adjustments without creating a new `SplatParams` object. See examples for a demonstration of how this can be used.

The simulation involves the following steps:

1. Set up simulation object
2. Simulate library sizes
3. Simulate gene means
4. Simulate groups/paths
5. Simulate BCV adjusted cell means
6. Simulate true counts
7. Simulate dropout
8. Create final `SCESet` object

The final output is an `SCESet` object that contains the simulated counts but also the values for various intermediate steps. These are stored in the `phenoData` (for cell specific information), `featureData` (for gene specific information) or `assayData` (for gene by cell matrices) slots. This additional information includes:

phenoData **Cell** Unique cell identifier.

**Group** The group or path the cell belongs to.

**ExpLibSize** The expected library size for that cell.

**Step (paths only)** how far along the path each cell is.

featureData **Gene** Unique gene identifier.

**BaseGeneMean** The base expression level for that gene.

**OutlierFactor** Expression outlier factor for that gene. Values of 1 indicate the gene is not an expression outlier.

**GeneMean** Expression level after applying outlier factors.

**DEFac[Group ]** The differential expression factor for each gene in a particular group. Values of 1 indicate the gene is not differentially expressed.

**GeneMean[Group ]** Expression level of a gene in a particular group after applying differential expression factors.

**SigmaFac[Path ]** Factor applied to genes that have non-linear changes in expression along a path.

assayData **BaseCellMeans** The expression of genes in each cell adjusted for expected library size.

**BCV** The Biological Coefficient of Variation for each gene in each cell.

**CellMeans** The expression level of genes in each cell adjusted for BCV.

**TrueCounts** The simulated counts before dropout.

**Dropout** Logical matrix showing which values have been dropped in which cells.

Values that have been added by Splatter are named using CamelCase in order to differentiate them from the values added by Scater which uses underscore\_naming.

## Value

SCESet object containing the simulated counts and intermediate values.

## See Also

[splatSimLibSizes](#), [splatSimGeneMeans](#), [splatSimDE](#), [splatSimCellMeans](#), [splatSimBCVMeans](#), [splatSimTrueCounts](#), [splatSimDropout](#)

## Examples

```
# Simulation with default parameters
## Not run:
sim <- splatSimulate()
# Simulation with different number of genes
sim <- splatSimulate(nGenes = 1000)
# Simulation with custom parameters
params <- newSplatParams(nGenes = 100, mean.rate = 0.5)
sim <- splatSimulate(params)
# Simulation with adjusted custom parameters
sim <- splatSimulate(params, mean.rate = 0.6, out.prob = 0.2)
# Simulate groups
sim <- splatSimulate(method = "groups")
# Simulate paths
sim <- splatSimulate(method = "paths")

## End(Not run)
```

splatter

*splatter*

---

**Description**

**splatter** is a package for the well-documented and reproducible simulation of single-cell RNA-seq count data.

**Details**

As well as its own simulation model **splatter** provides functions for the estimation of model parameters.

**See Also**

Zappia L, Phipson B, Oshlack A. Splatter: Simulation Of Single-Cell RNA Sequencing Data. bioRxiv. 2017; doi:10.1101/133173

---

summariseDiff

*Summarise diffSCESets*

---

**Description**

Summarise the results of [diffSCESets](#). The various properties are sorted, differences calculated, the Median Absolute Deviation taken as the summary statistic and the ranks calculated.

**Usage**

```
summariseDiff(diff)
```

**Arguments**

`diff` Output from [diffSCESets](#)

**Value**

List with MADs, ranks and both combined in long format

**Examples**

```
sim1 <- splatSimulate(nGenes = 1000, groupCells = 20)
sim2 <- simpleSimulate(nGenes = 1000, nCells = 20)
difference <- diffSCESets(list(Splat = sim1, Simple = sim2), ref = "Simple")
summary <- summariseDiff(difference)
names(summary)
head(summary$Long)
```

---

`winsorize`*Winsorize vector*

---

**Description**

Set outliers in a numeric vector to a specified percentile.

**Usage**

```
winsorize(x, q)
```

**Arguments**

<code>x</code>	Numeric vector to winsorize
<code>q</code>	Percentile to set from each end

**Value**

Winsorized numeric vector

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