Introduction to Splatter

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Splatter logo

Welcome to Splatter! Splatter is an R package for the simple simulation of single-cell RNA sequencing data. This vignette gives an overview and introduction to Splatter's functionality.

1 Installation

Splatter can be installed from Bioconductor:

```
if (!requireNamespace("BiocManager", quietly=TRUE))
    install.packages("BiocManager")
BiocManager::install("splatter")
```

To install the most recent development version from Github use:

```
BiocManager::install("Oshlack/splatter", dependencies = TRUE,
         build_vignettes = TRUE)
```

2 Quickstart

Assuming you already have a matrix of count data similar to that you wish to simulate there are two simple steps to creating a simulated data set with Splatter. Here is an example using the example dataset in the `scater` package:

```
# Load package
library(splatter)

# Load example data
library(scater)
data("sc_example_counts")
# Estimate parameters from example data
params <- splatEstimate(sc_example_counts)
# Simulate data using estimated parameters
sim <- splatSimulate(params)
```

```
## Getting parameters...

## Creating simulation object...

## Simulating library sizes...

## Simulating gene means...

## Simulating BCV...

## Simulating counts...

## Simulating dropout (if needed)...

## Done!
```

These steps will be explained in detail in the following sections but briefly the first step takes a dataset and estimates simulation parameters from it and the second step takes those parameters and simulates a new dataset.

3 The Splat simulation

Before we look at how we estimate parameters let's first look at how Splatter simulates data and what those parameters are. We use the term 'Splat' to refer to the Splatter's own simulation and differentiate it from the package itself. The core of the Splat model is a gamma-Poisson distribution used to generate a gene by cell matrix of counts. Mean expression levels for each gene are simulated from a gamma distribution (https://en.wikipedia.org/wiki/Gamma_distribution) and the Biological Coefficient of Variation is used to enforce a mean-variance trend before counts are simulated from a Poisson distribution (https://en.wikipedia.org/wiki/Poisson_distribution). Splat also allows you to simulate expression outlier genes (genes with mean expression outside the gamma distribution) and dropout (random knock out of counts based on mean expression). Each cell is given an expected library size (simulated from a log-normal distribution) that makes it easier to match to a given dataset.

Splat can also simulate differential expression between groups of different types of cells or differentiation paths between different cells types where expression changes in a continuous way. These are described further in the simulating counts section.

4 The `SplatParams` object

All the parameters for the Splat simulation are stored in a `SplatParams` object. Let's create a new one and see what it looks like.

```
params <- newSplatParams()
params
```

```
## A Params object of class SplatParams
## Parameters can be (estimable) or [not estimable], 'Default' o
r  'NOT DEFAULT'
##
## Global:
## (Genes)  (Cells)   [Seed]
##   10000      100   138572
##
## 28 additional parameters
##
## Batches:
##     [Batches]  [Batch Cells]     [Location]        [Scale]
##             1            100            0.1            0.1
##
## Mean:
##  (Rate)  (Shape)
##     0.3      0.6
##
## Library size:
## (Location)     (Scale)      (Norm)
##         11         0.2       FALSE
##
## Exprs outliers:
## (Probability)     (Location)        (Scale)
##          0.05              4            0.5
##
## Groups:
##      [Groups]  [Group Probs]
##             1              1
##
## Diff expr:
## [Probability]    [Down Prob]     [Location]        [Scale]
##           0.1            0.5            0.1            0.4
##
## BCV:
## (Common Disp)          (DoF)
##           0.1             60
##
## Dropout:
##     [Type]  (Midpoint)     (Shape)
##       none           0          -1
##
## Paths:
##         [From]         [Steps]          [Skew]    [Non-linea
r]
##              0             100             0.5             0.
1
## [Sigma Factor]
##            0.8
```

As well as telling us what type of object we have ("A `Params` object of class `SplatParams`") and showing us the values of the parameter this output gives us some extra information. We can see which parameters can be estimated by the `splatEstimate` function (those in parentheses), which can't be estimated (those in brackets) and which have been changed from their default values (those in ALL CAPS). For more details about the parameters of the Splat simulation refer to the Splat parameters vignette (splat_params.html).

4.1 Getting and setting

If we want to look at a particular parameter, for example the number of genes to simulate, we can extract it using the `getParam` function:

```
getParam(params, "nGenes")
```

```
## [1] 10000
```

Alternatively, to give a parameter a new value we can use the `setParam` function:

```
params <- setParam(params, "nGenes", 5000)
getParam(params, "nGenes")
```

```
## [1] 5000
```

If we want to extract multiple parameters (as a list) or set multiple parameters we can use the `getParams` or `setParams` functions:

```
# Set multiple parameters at once (using a list)
params <- setParams(params, update = list(nGenes = 8000, mean.ra
te = 0.5))
# Extract multiple parameters as a list
getParams(params, c("nGenes", "mean.rate", "mean.shape"))
```

```
## $nGenes
## [1] 8000
##
## $mean.rate
## [1] 0.5
##
## $mean.shape
## [1] 0.6
```

```
# Set multiple parameters at once (using additional arguments)
params <- setParams(params, mean.shape = 0.5, de.prob = 0.2)
params
```

```
## A Params object of class SplatParams
## Parameters can be (estimable) or [not estimable], 'Default' o
r  'NOT DEFAULT'
##
## Global:
## (GENES)  (Cells)   [Seed]
##    8000      100   138572
##
## 28 additional parameters
##
## Batches:
##     [Batches]  [Batch Cells]     [Location]        [Scale]
##             1            100            0.1            0.1
##
## Mean:
##  (RATE)  (SHAPE)
##     0.5      0.5
##
## Library size:
## (Location)     (Scale)      (Norm)
##         11         0.2       FALSE
##
## Exprs outliers:
## (Probability)     (Location)        (Scale)
##          0.05              4            0.5
##
## Groups:
##      [Groups]  [Group Probs]
##             1              1
##
## Diff expr:
## [PROBABILITY]    [Down Prob]     [Location]        [Scale]
##           0.2            0.5            0.1            0.4
##
## BCV:
## (Common Disp)          (DoF)
##           0.1             60
##
## Dropout:
##     [Type]  (Midpoint)     (Shape)
##       none           0          -1
##
## Paths:
##         [From]         [Steps]          [Skew]    [Non-linea
r]
##              0             100             0.5             0.
1
## [Sigma Factor]
##            0.8
```

The parameters with have changed are now shown in ALL CAPS to indicate that they been changed form the default.

We can also set parameters directly when we call `newSplatParams`:

```
params <- newSplatParams(lib.loc = 12, lib.scale = 0.6)
getParams(params, c("lib.loc", "lib.scale"))
```

```
## $lib.loc
## [1] 12
##
## $lib.scale
## [1] 0.6
```

5 Estimating parameters

Splat allows you to estimate many of it's parameters from a data set containing counts using the `splatEstimate` function.

```
# Check that sc_example counts is an integer matrix
class(sc_example_counts)
```

```
## [1] "matrix"
```

```
typeof(sc_example_counts)
```

```
## [1] "integer"
```

```
# Check the dimensions, each row is a gene, each column is a cel
l
dim(sc_example_counts)
```

```
## [1] 2000   40
```

```
# Show the first few entries
sc_example_counts[1:5, 1:5]
```

```
##           Cell_001 Cell_002 Cell_003 Cell_004 Cell_005
## Gene_0001        0      123        2        0        0
## Gene_0002      575       65        3     1561     2311
## Gene_0003        0        0        0        0     1213
## Gene_0004        0        1        0        0        0
## Gene_0005        0        0       11        0        0
```

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

Here we estimated parameters from a counts matrix but `splatEstimate` can also take a `SingleCellExperiment` object. The estimation process has the following steps:

1. Mean parameters are estimated by fitting a gamma distribution to the mean expression levels.
2. Library size parameters are estimated by fitting a log-normal distribution to the library sizes.
3. Expression outlier parameters are estimated by determining the number of outliers and fitting a log-normal distribution to their difference from the median.
4. BCV parameters are estimated using the `estimateDisp` function from the `edgeR` package.

5. Dropout parameters are estimated by checking if dropout is present and fitting a logistic function to the relationship between mean expression and proportion of zeros.

For more details of the estimation procedures see `?splatEstimate`.

6 Simulating counts

Once we have a set of parameters we are happy with we can use `splatSimulate` to simulate counts. If we want to make small adjustments to the parameters we can provide them as additional arguments, alternatively if we don't supply any parameters the defaults will be used:

```
sim <- splatSimulate(params, nGenes = 1000)
```

```
## Getting parameters...
```

```
## Creating simulation object...
```

```
## Simulating library sizes...
```

```
## Simulating gene means...
```

```
## Simulating BCV...
```

```
## Simulating counts...
```

```
## Simulating dropout (if needed)...
```

```
## Done!
```

```
sim
```

```
## class: SingleCellExperiment
## dim: 1000 40
## metadata(1): Params
## assays(6): BatchCellMeans BaseCellMeans ... TrueCounts counts
## rownames(1000): Gene1 Gene2 ... Gene999 Gene1000
## rowData names(4): Gene BaseGeneMean OutlierFactor GeneMean
## colnames(40): Cell1 Cell2 ... Cell39 Cell40
## colData names(3): Cell Batch ExpLibSize
## reducedDimNames(0):
## spikeNames(0):
```

Looking at the output of `splatSimulate` we can see that `sim` is `SingleCellExperiment` object with 1000 features (genes) and 40 samples (cells). The main part of this object is a features by samples matrix containing the simulated counts (accessed using `counts`), although it can also hold other expression measures such as FPKM or TPM. Additionally a `SingleCellExperiment` contains phenotype information about each cell

(accessed using `colData`) and feature information about each gene (accessed using `rowData`). Splatter uses these slots, as well as `assays`, to store information about the intermediate values of the simulation.

```
# Access the counts
counts(sim)[1:5, 1:5]
```

```
##       Cell1 Cell2 Cell3 Cell4 Cell5
## Gene1    11     0     0     0  4814
## Gene2     0     0     0     0     0
## Gene3  1848   210     6     1  1096
## Gene4     0     0     0     0     0
## Gene5     0     0    27    29    80
```

```
# Information about genes
head(rowData(sim))
```

```
## DataFrame with 6 rows and 4 columns
##           Gene        BaseGeneMean OutlierFactor            G
eneMean
##       <factor>           <numeric>     <numeric>           <n
umeric>
## Gene1    Gene1     162.27961350832             1     162.2796
1350832
## Gene2    Gene2 0.00394586265413722             1 0.0039458626
5413722
## Gene3    Gene3    27.0488594070323             1    27.048859
4070323
## Gene4    Gene4 0.00893408360914568             1 0.0089340836
0914568
## Gene5    Gene5     43.218226825995             1     43.21822
6825995
## Gene6    Gene6   0.436957397662496             1   0.43695739
7662496
```

```
# Information about cells
head(colData(sim))
```

```
## DataFrame with 6 rows and 3 columns
##           Cell       Batch       ExpLibSize
##       <factor> <character>        <numeric>
## Cell1    Cell1      Batch1  633156.33789341
## Cell2    Cell2      Batch1 543355.825769202
## Cell3    Cell3      Batch1 361636.014794991
## Cell4    Cell4      Batch1 273146.845309571
## Cell5    Cell5      Batch1 762131.522140643
## Cell6    Cell6      Batch1 1111265.22540117
```

```
# Gene by cell matrices
names(assays(sim))
```

```
## [1] "BatchCellMeans" "BaseCellMeans"  "BCV"            "CellM
eans"
## [5] "TrueCounts"     "counts"
```

```
# Example of cell means matrix
assays(sim)$CellMeans[1:5, 1:5]
```

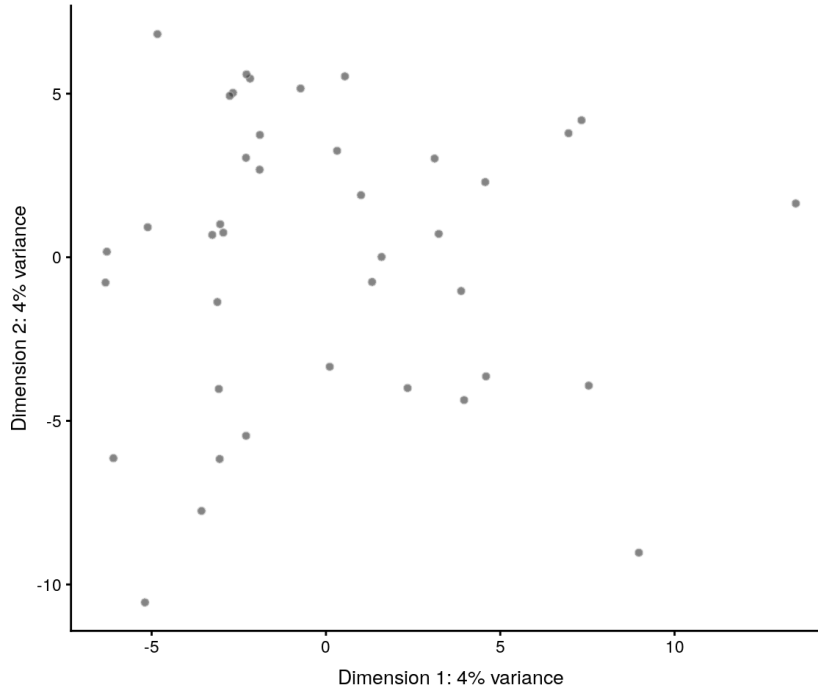
```
##              Cell1        Cell2         Cell3        Cell4
Cell5
## Gene1 1.656039e+01 9.782325e-05  7.783061e-19 1.550924e-15 4.
785735e+03
## Gene2 4.884689e-23 1.469951e-58  3.569772e-10 1.902942e-58 6.
634616e-32
## Gene3 1.850411e+03 2.161880e+02  6.841197e+00 1.690751e+00 1.
095851e+03
## Gene4 2.167214e-43 1.058928e-27 3.464428e-109 1.379729e-56 1.
953030e-03
## Gene5 5.842502e-03 1.104395e-03  2.863923e+01 3.535881e+01 7.
466149e+01
```

An additional (big) advantage of outputting a `SingleCellExperiment` is that we get immediate access to other analysis packages, such as the plotting functions in `scater`. For example we can make a PCA plot:

```
# Use scater to calculate logcounts
sim <- normalize(sim)
```

```
## Warning in .local(object, ...): using library sizes as size f
actors
```

```
# Plot PCA
plotPCA(sim)
```



(**NOTE:** Your values and plots may look different as the simulation is random and produces different results each time it is run.)

For more details about the `SingleCellExperiment` object refer to the [vignette] SCE-vignette (https://bioconductor.org/packages/devel/bioc/vignettes/SingleCellExperiment/inst/doc/intro.ht

For information about what you can do with `scater` refer to the `scater` documentation and vignette (https://bioconductor.org/packages/release/bioc/vignettes/scater/inst/doc/vignette.html).

The `splatSimulate` function outputs the following additional information about the simulation:

- **Cell information (`colData`)**
  - `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.
- **Gene information (`rowData`)**
  - `Gene` - Unique gene identifier.
  - `BaseGeneMean` - The base expression level for that gene.
  - `OutlierFactor` - Expression outlier factor for that gene (1 is not an outlier).
  - `GeneMean` - Expression level after applying outlier factors.
  - `DEFac[Group]` - The differential expression factor for each gene in a particular group (1 is not differentially expressed).
  - `GeneMean[Group]` - Expression level of a gene in a particular group after applying differential expression factors.
- **Gene by cell information (`assays`)**
  - `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 counts have been dropped in which cells.

Values that have been added by Splatter are named using `UpperCamelCase` to separate them from the `underscore_naming` used by `scater` and other packages. For more information on the simulation see `?splatSimulate` .

6.1 Simulating groups

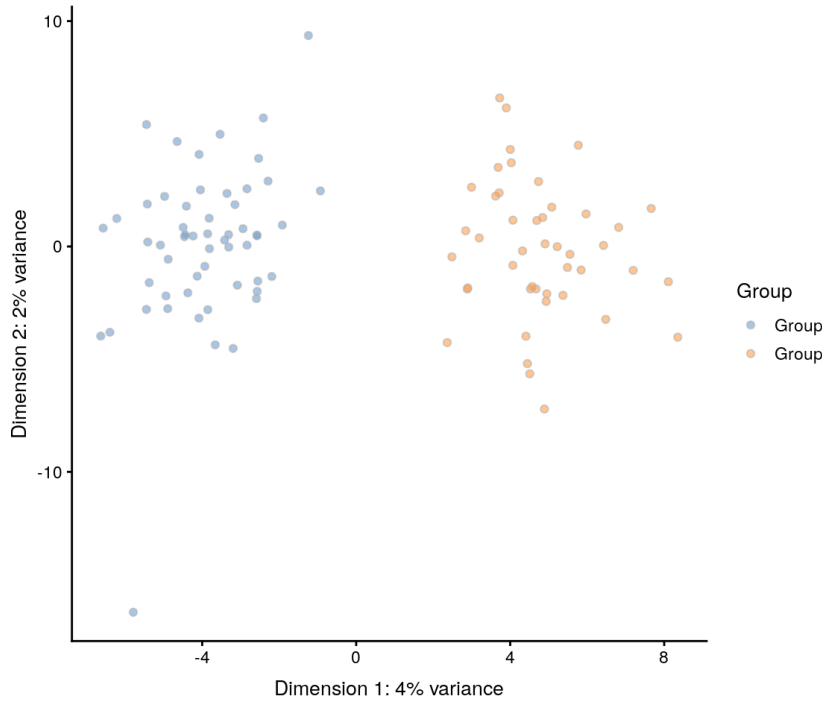
So far we have only simulated a single population of cells but often we are interested in investigating a mixed population of cells and looking to see what cell types are present or what differences there are between them. Splatter is able to simulate these situations by changing the `method` argument Here we are going to simulate two groups, by specifying the `group.prob` parameter and setting the `method` parameter to `"groups"` :

(**NOTE:** We have also set the `verbose` argument to `FALSE` to stop Splatter printing progress messages.)

```
sim.groups <- splatSimulate(group.prob = c(0.5, 0.5), method =
"groups",
                            verbose = FALSE)
sim.groups <- normalize(sim.groups)
```

```
## Warning in .local(object, ...): using library sizes as size f
actors
```

```
plotPCA(sim.groups, colour_by = "Group")
```



As we have set both the group probabilities to 0.5 we should get approximately equal numbers of cells in each group (around 50 in this case). If we wanted uneven groups we could set `group.prob` to any set of probabilities that sum to 1.

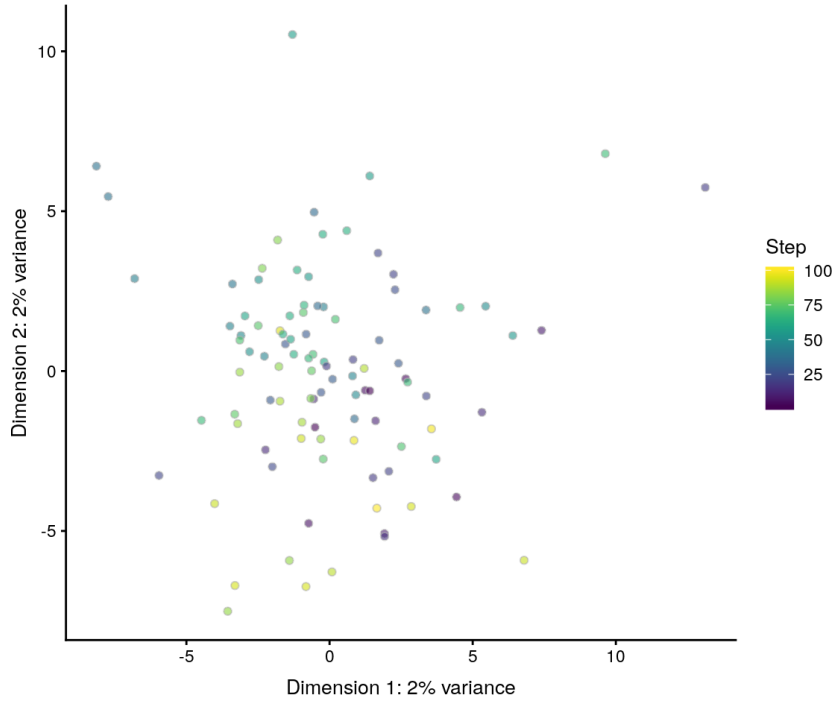
6.2 Simulating paths

The other situation that is often of interest is a differentiation process where one cell type is changing into another. Splatter approximates this process by simulating a series of steps between two groups and randomly assigning each cell to a step. We can create this kind of simulation using the `"paths"` method.

```
sim.paths <- splatSimulate(method = "paths", verbose = FALSE)
sim.paths <- normalize(sim.paths)
```

```
## Warning in .local(object, ...): using library sizes as size f
actors
```

```
plotPCA(sim.paths, colour_by = "Step")
```



Here the colours represent the "step" of each cell or how far along the differentiation path it is. We can see that the cells with dark colours are more similar to the originating cell type and the light coloured cells are closer to the final, differentiated, cell type. By setting additional parameters it is possible to simulate more complex process (for example multiple mature cell types from a single progenitor).

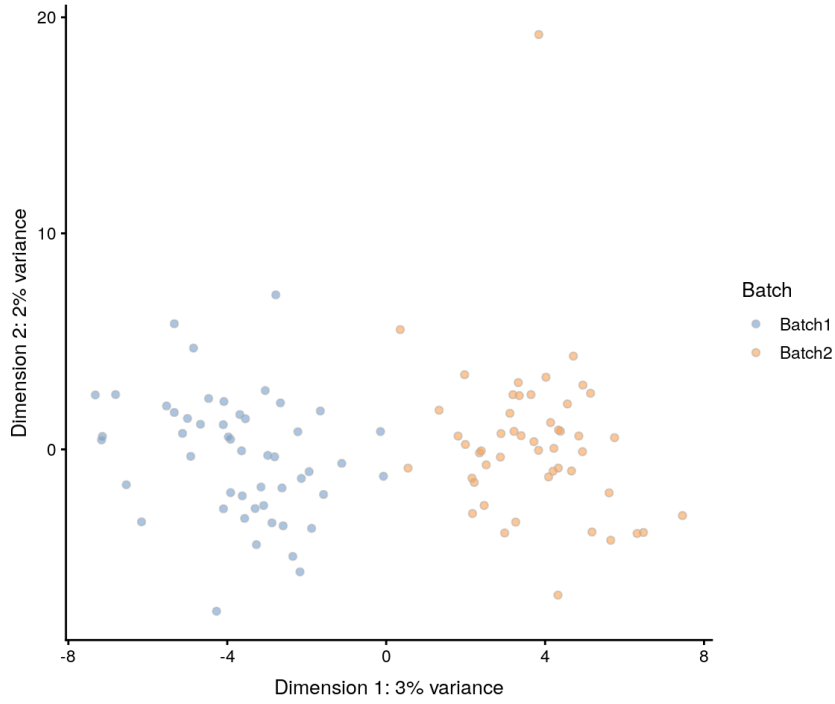
6.3 Batch effects

Another factor that is important in the analysis of any sequencing experiment are batch effects, technical variation that is common to a set of samples processed at the same time. We apply batch effects by telling Splatter how many cells are in each batch:

```
sim.batches <- splatSimulate(batchCells = c(50, 50), verbose = F
ALSE)
sim.batches <- normalize(sim.batches)
```

```
## Warning in .local(object, ...): using library sizes as size f
actors
```

```
plotPCA(sim.batches, colour_by = "Batch")
```

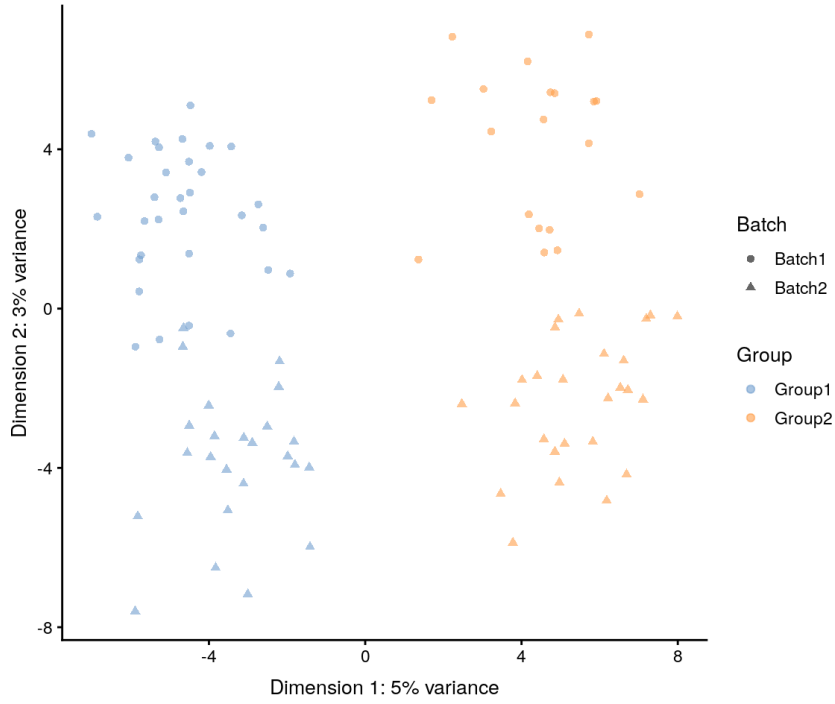


This looks at lot like when we simulated groups and that is because the process is very similar. The difference is that batch effects are applied to all genes, not just those that are differentially expressed, and the effects are usually smaller. By combining groups and batches we can simulate both unwanted variation that we aren't interested in (batch) and the wanted variation we are looking for (group):

```
sim.groups <- splatSimulate(batchCells = c(50, 50), group.prob =
c(0.5, 0.5),
                            method = "groups", verbose = FALSE)
sim.groups <- normalize(sim.groups)
```

```
## Warning in .local(object, ...): using library sizes as size f
actors
```

```
plotPCA(sim.groups, shape_by = "Batch", colour_by = "Group")
```



Here we see that the effects of the group (first component) are stronger than the batch effects (second component) but by adjusting the parameters we could made the batch effects dominate.

6.4 Convenience functions

Each of the Splatter simulation methods has it's own convenience function. To simulate a single population use `splatSimulateSingle()` (equivalent to `splatSimulate(method = "single")`), to simulate groups use `splatSimulateGroups()` (equivalent to `splatSimulate(method = "groups")`) or to simulate paths use `splatSimulatePaths()` (equivalent to `splatSimulate(method = "paths")`).

7 Other simulations

As well as it's own Splat simulation method the Splatter package contains implementations of other single-cell RNA-seq simulations that have been published or wrappers around simulations included in other packages. To see all the available simulations run the `listSims()` function:

```
listSims()
```

```
## Splatter currently contains 13 simulations
##
## Splat (splat)
## DOI: 10.1186/s13059-017-1305-0    GitHub: Oshlack/splatter
## The Splat simulation generates means from a gamma distributio
n, adjusts them for BCV and generates counts from a gamma-poisso
n. Dropout and batch effects can be optionally added.
##
## Splat Single (splatSingle)
## DOI: 10.1186/s13059-017-1305-0    GitHub: Oshlack/splatter
## The Splat simulation with a single population.
##
## Splat Groups (splatGroups)
## DOI: 10.1186/s13059-017-1305-0    GitHub: Oshlack/splatter
## The Splat simulation with multiple groups. Each group can hav
e it's own differential expression probability and fold change d
istribution.
##
## Splat Paths (splatPaths)
## DOI: 10.1186/s13059-017-1305-0    GitHub: Oshlack/splatter
## The Splat simulation with differentiation paths. Each path ca
n have it's own length, skew and probability. Genes can change i
n non-linear ways.
##
## Simple (simple)
## DOI: 10.1186/s13059-017-1305-0    GitHub: Oshlack/splatter
## A simple simulation with gamma means and negative binomial co
unts.
##
## Lun (lun)
## DOI: 10.1186/s13059-016-0947-7    GitHub: MarioniLab/Deconvol
ution2016
## Gamma distributed means and negative binomial counts. Cells a
re given a size factor and differential expression can be simula
ted with fixed fold changes.
##
## Lun 2 (lun2)
## DOI: 10.1093/biostatistics/kxw055     GitHub: MarioniLab/Plat
eEffects2016
## Negative binomial counts where the means and dispersions have
been sampled from a real dataset. The core feature of the Lun 2
simulation is the addition of plate effects. Differential expres
sion can be added between two groups of plates and optionally a
zero-inflated negative-binomial can be used.
##
## scDD (scDD)
## DOI: 10.1186/s13059-016-1077-y    GitHub: kdkorthauer/scDD
## The scDD simulation samples a given dataset and can simulate
differentially expressed and differentially distributed genes be
tween two conditions.
##
## BASiCS (BASiCS)
## DOI: 10.1371/journal.pcbi.1004333     GitHub: catavallejos/BA
SiCS
## The BASiCS simulation is based on a bayesian model used to de
convolve biological and technical variation and includes spike-i
ns and batch effects.
##
## mfa (mfa)
## DOI: 10.12688/wellcomeopenres.11087.1     GitHub: kieranrcamp
bell/mfa
```

```
## The mfa simulation produces a bifurcating pseudotime trajecto
ry. This can optionally include genes with transient changes in
expression and added dropout.
##
## PhenoPath (pheno)
## DOI: 10.1101/159913   GitHub: kieranrcampbell/phenopath
## The PhenoPath simulation produces a pseudotime trajectory wit
h different types of genes.
##
## ZINB-WaVE (zinb)
## DOI: 10.1101/125112   GitHub: drisso/zinbwave
## The ZINB-WaVE simulation simulates counts from a sophisticate
d zero-inflated negative-binomial distribution including cell an
d gene-level covariates.
##
## SparseDC (sparseDC)
## DOI: 10.1093/nar/gkx1113      GitHub: cran/SparseDC
## The SparseDC simulation simulates a set of clusters across tw
o conditions, where some clusters may be present in only one con
dition.
```

Each simulation has it's own prefix which gives the name of the functions associated with that simulation. For example the prefix for the simple simulation is `simple` so it would store it's parameters in a `SimpleParams` object that can be created using `newSimpleParams()` or estimated from real data using `simpleEstimate()`. To simulate data using that simulation you would use `simpleSimulate()`. Each simulation returns a `SingleCellExperiment` object with intermediate values similar to that returned by `splatSimulate()`. For more detailed information on each simulation see the appropriate help page (eg. `?simpleSimulate` for information on how the simple simulation works or `? lun2Estimate` for details of how the Lun 2 simulation estimates parameters) or refer to the appropriate paper or package.

8 Other expression values

Splatter is designed to simulate count data but some analysis methods expect other expression values, particularly length-normalised values such as TPM or FPKM. The `scater` package has functions for adding these values to a `SingleCellExperiment` object but they require a length for each gene. The `addGeneLengths` function can be used to simulate these lengths:

```
sim <- simpleSimulate(verbose = FALSE)
sim <- addGeneLengths(sim)
head(rowData(sim))
```

```
## DataFrame with 6 rows and 3 columns
##            Gene          GeneMean    Length
##        <factor>         <numeric> <numeric>
## Gene1     Gene1  3.28104748809824      5210
## Gene2     Gene2  2.70699225514288      2618
## Gene3     Gene3  5.02153797531669      4864
## Gene4     Gene4 0.932123671863265      1269
## Gene5     Gene5  1.54033300781193      1746
## Gene6     Gene6  1.99567718581344      5139
```

We can then use `scater` to calculate TPM:

```
tpm(sim) <- calculateTPM(sim, rowData(sim)$Length)
tpm(sim)[1:5, 1:5]
```

```
##           Cell1     Cell2     Cell3     Cell4     Cell5
## Gene1   0.00000  92.24158  30.00788 273.11117  30.16607
## Gene2 120.80264 122.37806 238.87101 301.95000 300.16277
## Gene3   0.00000 131.73757 192.85495  97.51296 193.87158
## Gene4   0.00000 126.23552   0.00000 249.17418 123.84967
## Gene5  90.56738  91.74850  89.54243   0.00000 270.04335
```

The default method used by `addGeneLengths` to simulate lengths is to generate values from a log-normal distribution which are then rounded to give an integer length. The parameters for this distribution are based on human protein coding genes but can be adjusted if needed (for example for other species). Alternatively lengths can be sampled from a provided vector (see `?addGeneLengths` for details and an example).

9 Comparing simulations and real data

One thing you might like to do after simulating data is to compare it to a real dataset, or compare simulations with different parameters or models. Splatter provides a function `compareSCEs` that aims to make these comparisons easier. As the name suggests this function takes a list of `SingleCellExperiment` objects, combines the datasets and produces some plots comparing them. Let's make two small simulations and see how they compare.

```
sim1 <- splatSimulate(nGenes = 1000, batchCells = 20, verbose =
FALSE)
sim2 <- simpleSimulate(nGenes = 1000, nCells = 20, verbose = FAL
SE)
comparison <- compareSCEs(list(Splat = sim1, Simple = sim2))

names(comparison)
```

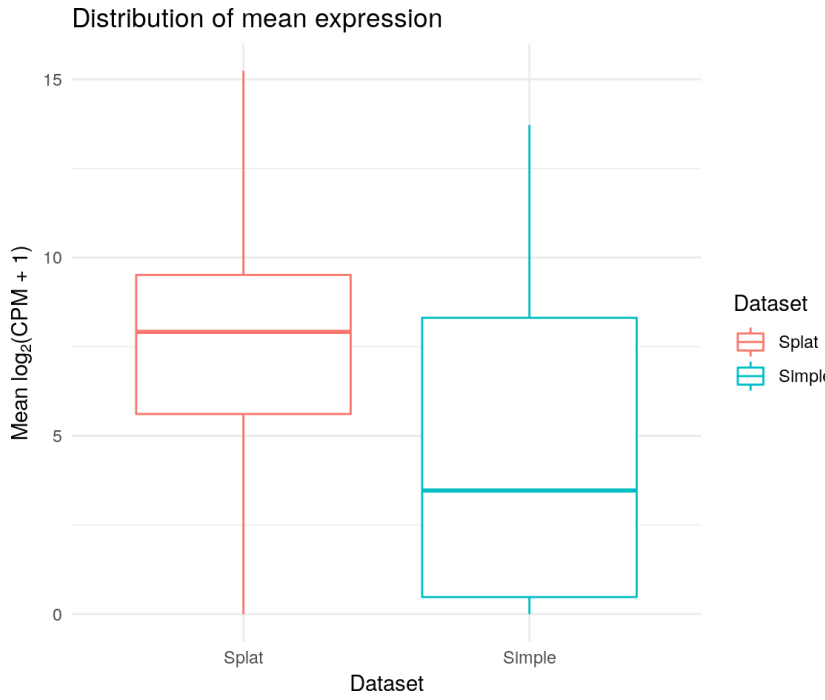
```
## [1] "RowData" "ColData" "Plots"
```

```
names(comparison$Plots)
```

```
## [1] "Means"        "Variances"    "MeanVar"      "LibrarySize
s"
## [5] "ZerosGene"    "ZerosCell"    "MeanZeros"
```

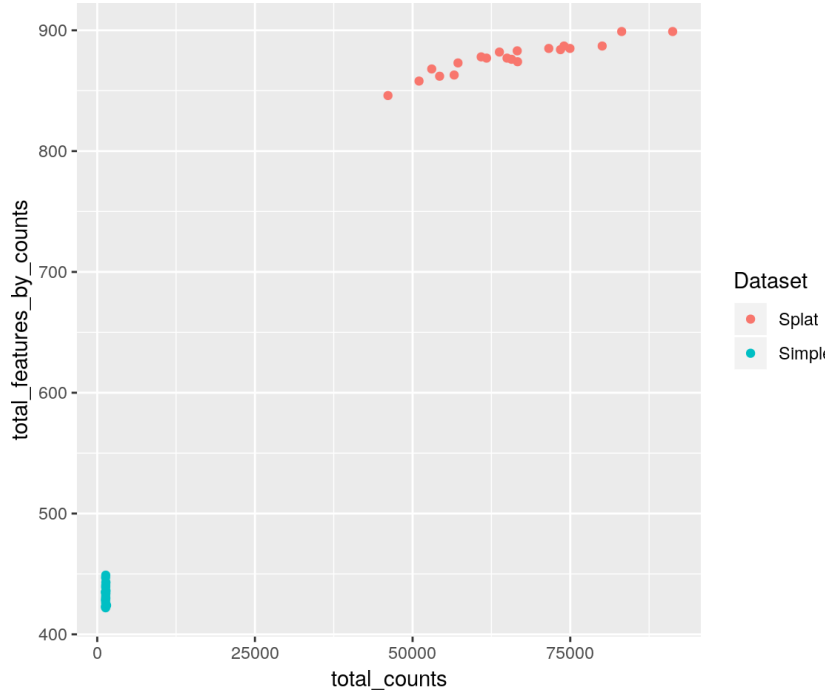
The returned list has three items. The first two are the combined datasets by gene (`RowData`) and by cell (`ColData`) and the third contains some comparison plots (produced using `ggplot2`), for example a plot of the distribution of means:

```
comparison$Plots$Means
```



These are only a few of the plots you might want to consider but it should be easy to make more using the returned data. For example, we could plot the number of expressed genes against the library size:

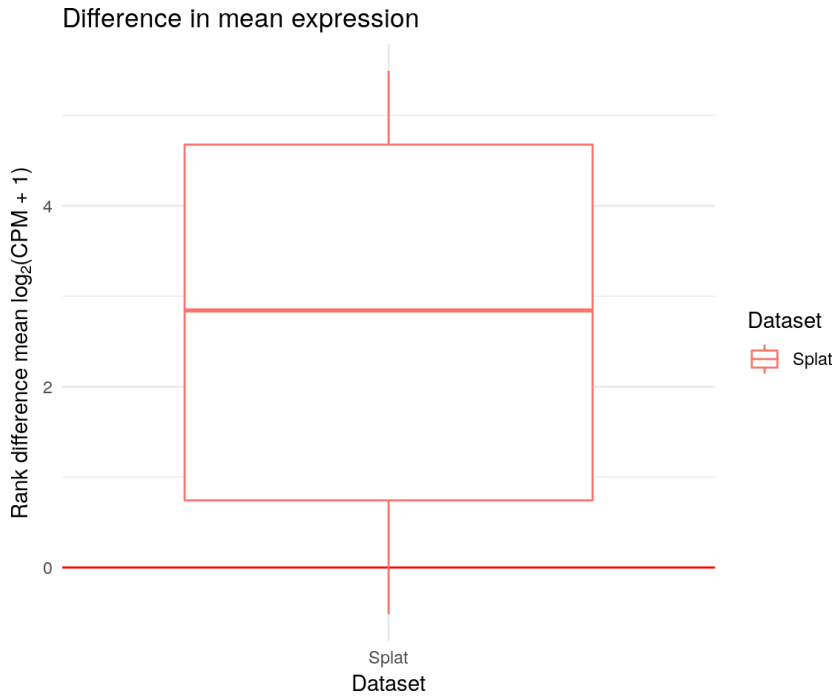
```
library("ggplot2")
ggplot(comparison$ColData,
       aes(x = total_counts, y = total_features_by_counts, colou
r = Dataset)) +
    geom_point()
```



9.1 Comparing differences

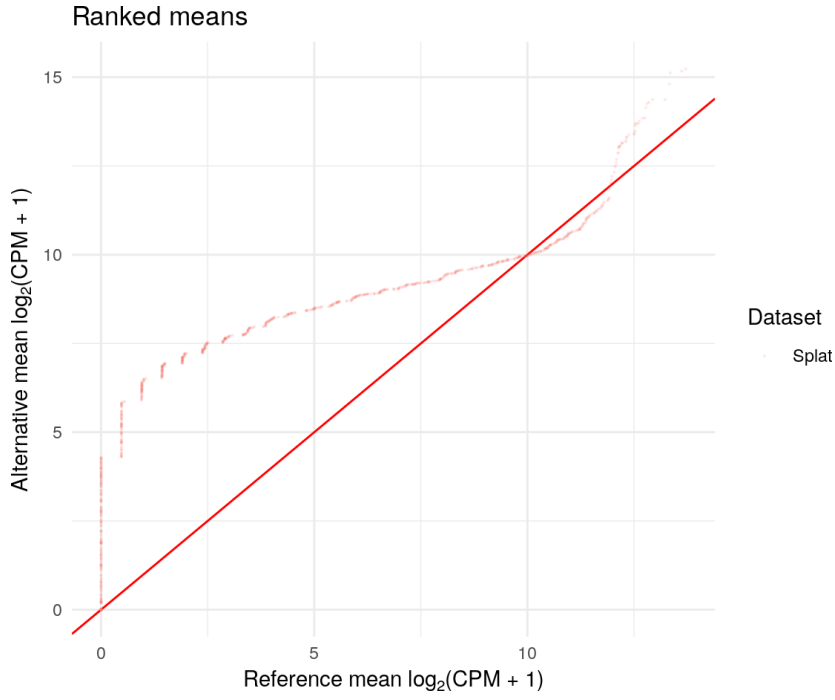
Sometimes instead of visually comparing datasets it may be more interesting to look at the differences between them. We can do this using the `diffSCEs` function. Similar to `compareSCEs` this function takes a list of `SingleCellExperiment` objects but now we also specify one to be a reference. A series of similar plots are returned but instead of showing the overall distributions they demonstrate differences from the reference.

```
difference <- diffSCEs(list(Splat = sim1, Simple = sim2), ref =
"Simple")
difference$Plots$Means
```



We also get a series of Quantile-Quantile plot that can be used to compare distributions.

```
difference$QQPlots$Means
```



9.2 Making panels

Each of these comparisons makes several plots which can be a lot to look at. To make this easier, or to produce figures for publications, you can make use of the functions `makeCompPanel`, `makeDiffPanel` and `makeOverallPanel`.

These functions combine the plots into a single panel using the `cowplot` package. The panels can be quite large and hard to view (for example in RStudio's plot viewer) so it can be better to output the panels and view them separately. Luckily `cowplot` provides a convenient function for saving the images. Here are some suggested parameters for outputting each of the panels:

```
# This code is just an example and is not run
panel <- makeCompPanel(comparison)
cowplot::save_plot("comp_panel.png", panel, nrow = 4, ncol = 3)

panel <- makeDiffPanel(difference)
cowplot::save_plot("diff_panel.png", panel, nrow = 3, ncol = 5)

panel <- makeOverallPanel(comparison, difference)
cowplot::save_plot("overall_panel.png", panel, ncol = 4, nrow =
7)
```

10 Citing Splatter

If you use Splatter in your work please cite our paper:

```
citation("splatter")
```

```
##
##   Zappia L, Phipson B, Oshlack A. Splatter: Simulation of
##   single-cell RNA sequencing data. Genome Biology. 2017;
##   doi:10.1186/s13059-017-1305-0
##
## A BibTeX entry for LaTeX users is
##
##   @Article{,
##     author = {Luke Zappia and Belinda Phipson and Alicia Oshl
ack},
##     title = {Splatter: simulation of single-cell RNA sequenci
ng data},
##     journal = {Genome Biology},
##     year = {2017},
##     url = {http://dx.doi.org/10.1186/s13059-017-1305-0},
##     doi = {10.1186/s13059-017-1305-0},
##   }
```

Session information

```
sessionInfo()
```

```
## R version 3.6.0 (2019-04-26)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 18.04.2 LTS
##
## Matrix products: default
## BLAS:   /home/biocbuild/bbs-3.9-bioc/R/lib/libRblas.so
## LAPACK: /home/biocbuild/bbs-3.9-bioc/R/lib/libRlapack.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=C
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils
datasets
## [8] methods   base
##
## other attached packages:
##  [1] scater_1.12.0               ggplot2_3.1.1
##  [3] splatter_1.8.0              SingleCellExperiment_1.6.0
##  [5] SummarizedExperiment_1.14.0 DelayedArray_0.10.0
##  [7] BiocParallel_1.18.0         matrixStats_0.54.0
##  [9] Biobase_2.44.0              GenomicRanges_1.36.0
## [11] GenomeInfoDb_1.20.0         IRanges_2.18.0
## [13] S4Vectors_0.22.0            BiocGenerics_0.30.0
## [15] BiocStyle_2.12.0
##
## loaded via a namespace (and not attached):
##  [1] viridis_0.5.1            edgeR_3.26.0
##  [3] BiocSingular_1.0.0       viridisLite_0.3.0
##  [5] splines_3.6.0            lsei_1.2-0
##  [7] DelayedMatrixStats_1.6.0 assertthat_0.2.1
##  [9] BiocManager_1.30.4       sp_1.3-1
## [11] GenomeInfoDbData_1.2.1   vipor_0.4.5
## [13] yaml_2.2.0               pillar_1.3.1
## [15] backports_1.1.4          lattice_0.20-38
## [17] limma_3.40.0             glue_1.3.1
## [19] digest_0.6.18            XVector_0.24.0
## [21] checkmate_1.9.1          colorspace_1.4-1
## [23] cowplot_0.9.4            htmltools_0.3.6
## [25] Matrix_1.2-17            plyr_1.8.4
## [27] pkgconfig_2.0.2          bookdown_0.9
## [29] zlibbioc_1.30.0          purrr_0.3.2
## [31] scales_1.0.0             tibble_2.1.1
## [33] withr_2.1.2              lazyeval_0.2.2
## [35] survival_2.44-1.1        magrittr_1.5
## [37] crayon_1.3.4             evaluate_0.13
## [39] MASS_7.3-51.4            beeswarm_0.2.3
## [41] tools_3.6.0              fitdistrplus_1.0-14
## [43] stringr_1.4.0            munsell_0.5.0
## [45] locfit_1.5-9.1           irlba_2.3.3
## [47] akima_0.6-2              compiler_3.6.0
## [49] rsvd_1.0.0               rlang_0.3.4
## [51] grid_3.6.0               RCurl_1.95-4.12
## [53] BiocNeighbors_1.2.0      bitops_1.0-6
## [55] labeling_0.3             rmarkdown_1.12
## [57] npsurv_0.4-0             gtable_0.3.0
```

```
## [59] R6_2.4.0                gridExtra_2.3
## [61] knitr_1.22              dplyr_0.8.0.1
## [63] stringi_1.4.3           ggbeeswarm_0.6.0
## [65] Rcpp_1.0.1              tidyselect_0.2.5
## [67] xfun_0.6
```