Package: scFastDE (via r-universe)

June 28, 2026

Type Package

Title Fast Donor-Weighted Pseudo-Bulk Differential Expression for scRNA-seq

Version 0.99.3

Description scFastDE provides fast, donor-weighted pseudo-bulk differential expression analysis for multi-donor single-cell RNA-seq experiments. Unlike existing tools that loop over genes serially, scFastDE uses vectorised sparse matrix operations across all genes simultaneously, achieving 10-50x speed gains on large datasets. Donors are weighted by the square root of their cell count, giving principled influence to well-represented donors without discarding donors with few cells. Paired experimental designs (same donors in multiple conditions) are automatically detected; pseudo-bulk is then aggregated per donor-condition pair and a blocking model accounts for inter-donor variation. A sparse pseudo-bulk guard automatically handles cell types where some donors fall below a minimum cell threshold. All functions operate natively on SingleCellExperiment objects.

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URL https://github.com/SubhadipJana1409/scFastDE

BugReports https://github.com/SubhadipJana1409/scFastDE/issues

biocViews SingleCell, DifferentialExpression, StatisticalMethod, Transcriptomics, GeneExpression, Sequencing, ATACSeq, CellBasedAssays, WorkflowStep

Encoding UTF-8

Depends R (>= 4.5.0)

Imports SingleCellExperiment, SummarizedExperiment, S4Vectors, BiocParallel, Matrix, limma, methods, stats, ggplot2, rlang, utils

Suggests BiocStyle, knitr, rmarkdown, testthat (>= 3.0.0), scuttle, withr

VignetteBuilder knitr

Config/testthat/edition 3

Config/roxygen2/version 8.0.0

Config/pak/sysreqs zlib1g-dev

Repository https://bioc.r-universe.dev

Date/Publication 2026-06-04 09:20:04 UTC

RemoteUrl https://github.com/bioc/scFastDE

RemoteRef HEAD

RemoteSha 543071ed85a3d260095cf95942c55c4df5d8fcc8

Contents

`scFastDE-package` *scFastDE: Fast Donor-Weighted Pseudo-Bulk DE for scRNA-seq*

Description

scFastDE provides fast, donor-weighted pseudo-bulk differential expression analysis for multi-donor single-cell RNA-seq experiments. Unlike existing tools that test genes serially in a loop, scFastDE uses vectorised sparse matrix operations across all genes simultaneously, giving 10-50x speed gains on large datasets (30k+ genes, 100k+ cells).

Key innovations

Vectorised gene testing All genes are tested simultaneously via matrix algebra — no per-gene loop.

Donor cell-count weighting Each sample's pseudo-bulk profile is weighted by `sqrt(n_cells)`, giving principled influence to well-represented samples.

Paired design auto-detection When the same donors appear in multiple conditions, `fastDE` automatically aggregates per donor-condition pair and uses a blocking model (`~ 0 + condition + donor`) to account for inter-donor variation.

Sparse pseudo-bulk guard Donors with fewer than `min_cells` cells per cell type are flagged and optionally removed before aggregation.

Main functions

`filterSparseDonors` Remove donors below minimum cell threshold per cell type.

`fastPseudobulk` Build donor-weighted pseudo-bulk matrix from a SingleCellExperiment. Supports per-donor or per-donor-condition aggregation.

`fastDE` Run vectorised DE across all genes. Auto-detects paired vs unpaired designs.

`plotDEResults` Volcano plot of DE results.

Author(s)

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References

Crowell HL et al. (2020). muscat detects subpopulation-specific state transitions from multi-sample multi-condition single-cell transcriptomics data. *Nature Communications*, 11, 6077.

See Also

Useful links:

- https://github.com/SubhadipJana1409/scFastDE
- Report bugs at https://github.com/SubhadipJana1409/scFastDE/issues

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`deTable` *Accessor for DE table in a FDEResult*

Description

Returns the per-gene differential expression statistics `DataFrame` from a `FDEResult` object.

Usage

```
deTable(x, ...)

## S4 method for signature 'FDEResult'
deTable(x, ...)
```

Arguments

x	A FDEResult object.
...	Additional arguments (not used).

Value

A DataFrame with columns logFC, AveExpr, t, P.Value, adj.P.Val, and B.

Examples

```
library(S4Vectors)
de <- DataFrame(logFC = c(1.2, -0.5), P.Value = c(0.001, 0.5),
                adj.P.Val = c(0.01, 0.8))
pb <- matrix(rpois(20, 10), nrow = 2, ncol = 10)
rownames(pb) <- paste0("Gene", 1:2)
colnames(pb) <- paste0("Donor", 1:10)
obj <- FDEResult(de, pb, setNames(sqrt(1:10), paste0("Donor", 1:10)))
deTable(obj)
```

---

donorWeights *Accessor for donor weights in a FDEResult*

Description

Returns the per-donor weight vector from a FDEResult object.

Usage

```
donorWeights(x, ...)

## S4 method for signature 'FDEResult'
donorWeights(x, ...)
```

Arguments

x	A FDEResult object.
...	Additional arguments (not used).

Value

A named numeric vector of donor weights.

Examples

```
library(S4Vectors)
de <- DataFrame(logFC = c(1.2, -0.5), P.Value = c(0.001, 0.5),
                adj.P.Val = c(0.01, 0.8))
pb <- matrix(rpois(20, 10), nrow = 2, ncol = 10)
rownames(pb) <- paste0("Gene", 1:2)
colnames(pb) <- paste0("Donor", 1:10)
obj <- FDEResult(de, pb, setNames(sqrt(1:10), paste0("Donor", 1:10)))
donorWeights(obj)
```

`fastDE` *Fast Vectorised Differential Expression*

Description

Runs differential expression analysis across all genes simultaneously using a weighted limma-voom linear model. Unlike tools that loop over genes serially, `fastDE` passes the entire pseudo-bulk matrix to limma, which uses LAPACK routines to fit all gene models in one vectorised call.

The function automatically detects whether the experimental design is **paired** (same donors in multiple conditions) or **unpaired** (each donor in one condition only) and builds the appropriate linear model:

- **Paired:** pseudo-bulk is aggregated per donor-condition pair, and a `~ 0 + condition + donor` model accounts for donor-level variation.
- **Unpaired:** pseudo-bulk is aggregated per donor, and a `~ 0 + condition` model is used.

Usage

```
fastDE(
  sce,
  donor = NULL,
  cell_type = NULL,
  condition = NULL,
  target_type = NULL,
  contrast = NULL,
  min_cpm = 1,
  min_donors = 2L,
  min_cells = 10L,
  BPPARAM = SerialParam()
)
```

Arguments

`sce`	A `SingleCellExperiment` object with raw counts in `assay(sce, "counts")`.
`donor`	A `character(1)` naming the donor column in `colData(sce)`.
`cell_type`	A `character(1)` naming the cell type column in `colData(sce)`.

condition	A character(1) naming the condition column in colData(sce) (e.g. "treatment", "disease"). Must have exactly two unique values.
target_type	A character(1) specifying which cell type to test. Must be present in colData(sce)[[cell_type]].
contrast	A character(1) specifying the contrast in limma syntax, e.g. "treat - ctrl". If NULL, the second level minus the first level is used. Default: NULL.
min_cpm	A numeric(1) minimum CPM for a gene to be considered expressed. Default: 1.
min_donors	An integer(1) minimum number of samples in which a gene must exceed min_cpm. Default: 2.
min_cells	An integer(1) minimum cells per donor before pseudo-bulk aggregation. Passed to filterSparseDonors. Default: 10.
BPPARAM	A BiocParallelParam object. Default: SerialParam().

Details

The analysis pipeline is:

1. Filter lowly-expressed genes (CPM > min_cpm in at least min_donors samples).
2. Apply limma::voom with sample cell-count weights.
3. Fit a weighted linear model with the appropriate design.
4. Extract the contrast of interest with limma::makeContrasts.
5. Apply empirical Bayes moderation with limma::eBayes.
6. Return all results as a FDEResult object.

Value

A FDEResult object containing:

- deTable: per-gene statistics (logFC, AveExpr, t, P.Value, adj.P.Val, B).
- pseudobulk: the aggregated count matrix.
- donorWeights: the per-sample sqrt(n_cells) weights.
- params: the analysis parameters.

See Also

fastPseudobulk, filterSparseDonors, plotDEResults

Examples

```
library(SingleCellExperiment)

set.seed(42)
n_genes <- 200
counts <- matrix(rpois(n_genes * 60, 8), nrow = n_genes, ncol = 60)
rownames(counts) <- paste0("Gene", seq_len(n_genes))
colnames(counts) <- paste0("Cell", seq_len(60))
```

```
# Inject DE signal into first 10 genes for treatment group
counts[1:10, 31:60] <- counts[1:10, 31:60] * 3L

sce <- SingleCellExperiment(assays = list(counts = counts))
sce$donor     <- rep(paste0("D", 1:6), each = 10)
sce$cell_type <- "Tcell"
sce$condition <- rep(c("ctrl", "treat"), each = 30)

result <- fastDE(sce,
                 donor       = "donor",
                 cell_type   = "cell_type",
                 condition   = "condition",
                 target_type = "Tcell",
                 min_cells   = 5)
result
head(deTable(result))
```

`fastPseudobulk` *Build Donor-Weighted Pseudo-Bulk Profiles*

Description

Aggregates single-cell counts into pseudo-bulk profiles for a specified cell type, using vectorised sparse matrix operations.

When `condition` is provided, aggregation is performed per donor-condition pair (one column per sample, e.g. `D1_ctrl`, `D1_stim`). This is the correct approach for paired experimental designs where the same donors contribute cells under multiple conditions.

When `condition` is `NULL` (default), aggregation is performed per donor only (backward-compatible behaviour for unpaired designs).

Each sample's weight equals `sqrt(n_cells)`, giving more influence to well-represented samples while not discarding those with fewer cells.

Usage

```
fastPseudobulk(
  sce,
  donor = NULL,
  cell_type = NULL,
  target_type = NULL,
  condition = NULL,
  assay_name = "counts"
)
```

Arguments

sce	A SingleCellExperiment object with raw counts in assay(sce, "counts").
donor	A character(1) naming the donor column in colData(sce).
cell_type	A character(1) naming the cell type column in colData(sce).
target_type	A character(1) specifying which cell type to aggregate. Must be a value present in colData(sce)[[cell_type]].
condition	A character(1) naming the condition column in colData(sce). When provided, aggregation is done per donor-condition pair (required for paired designs). Default: NULL (aggregate per donor only).
assay_name	A character(1) name of the assay to aggregate. Default: "counts".

Details

Pseudo-bulk aggregation sums the raw counts for all cells belonging to each donor (or donor-condition pair) within a given cell type:

$$PB_{g,s} = \sum_{c \in \text{sample}_s, \text{type}_t} X_{g,c}$$

Sample weights are then computed as:

$$w_s = \sqrt{n_{s,t}}$$

where $n_{s,t}$ is the number of cells for sample s in cell type t.

The aggregation uses sparse matrix column-sums grouped by sample, avoiding a slow R-level loop.

Value

A list with elements:

pseudobulk A matrix of aggregated counts, rows = genes, columns = samples.

sample_weights A named numeric vector of per-sample weights (sqrt(n_cells)).

sample_ncells A named integer vector giving the raw cell count per sample.

sample_info A data.frame with columns sample_id, donor, and (if applicable) condition — one row per pseudo-bulk sample.

For backward compatibility, donor_weights and donor_ncells are also provided as aliases when condition is NULL.

See Also

filterSparseDonors, fastDE

Examples

```
library(SingleCellExperiment)

set.seed(42)
counts <- matrix(rpois(500 * 60, 5), nrow = 500, ncol = 60)
rownames(counts) <- paste0("Gene", seq_len(500))
colnames(counts) <- paste0("Cell", seq_len(60))
sce <- SingleCellExperiment(assays = list(counts = counts))
sce$donor     <- rep(paste0("D", 1:6), each = 10)
sce$cell_type <- "Tcell"
sce$condition <- rep(c("ctrl", "treat"), each = 30)

# Unpaired (aggregate by donor only)
pb <- fastPseudobulk(sce, donor = "donor",
                     cell_type = "cell_type",
                     target_type = "Tcell")
dim(pb$pseudobulk)

# Paired (aggregate by donor x condition)
pb2 <- fastPseudobulk(sce, donor = "donor",
                      cell_type = "cell_type",
                      target_type = "Tcell",
                      condition = "condition")
dim(pb2$pseudobulk)
pb2$sample_info
```

`FDEResult` *Constructor for FDEResult*

Description

Create a new `FDEResult` object.

Usage

```
FDEResult(deTable, pseudobulk, donorWeights, params = list())
```

Arguments

`deTable`	A `DataFrame` of per-gene DE statistics.
`pseudobulk`	A `matrix` of pseudo-bulk counts.
`donorWeights`	A named `numeric` vector of donor weights.
`params`	A `list` of analysis parameters.

Value

A `FDEResult` object.

Examples

```
library(S4Vectors)
de <- DataFrame(
    logFC     = c(1.2, -0.5, 0.8),
    P.Value   = c(0.001, 0.5, 0.02),
    adj.P.Val = c(0.01, 0.8, 0.05)
)
pb <- matrix(rpois(30, 10), nrow = 3, ncol = 10)
rownames(pb) <- paste0("Gene", 1:3)
colnames(pb) <- paste0("Donor", 1:10)
obj <- FDEResult(
    deTable      = de,
    pseudobulk   = pb,
    donorWeights = setNames(sqrt(1:10), paste0("Donor", 1:10)),
    params       = list(cell_type = "T_cells", condition = "group")
)
obj
```

`FDEResult-class` *FDEResult: Fast DE Result Container*

Description

An S4 class to store the output of `fastDE`. Slots hold per-gene DE statistics, the pseudo-bulk matrix used, donor weights, and the analysis parameters.

Slots

`deTable` A `DataFrame` with one row per gene containing columns `logFC`, `AveExpr`, `t`, `P.Value`, `adj.P.Val`, and `B`.

`pseudobulk` A `matrix` of pseudo-bulk counts (genes x donors) used as input to the DE model.

`donorWeights` A named `numeric` vector of per-donor weights (sqrt of cell count) used in the linear model.

`params` A `list` of analysis parameters including `cell_type`, `condition`, `donor`, `min_cells`.

`filterSparseDonors` *Filter Donors with Too Few Cells per Cell Type*

Description

Removes or flags donors that have fewer than `min_cells` cells for a given cell type. When forming pseudo-bulk profiles, donors with very few cells produce highly variable, unreliable aggregated counts that can inflate false-positive DE calls. This function provides a principled pre-filtering step before calling `fastPseudobulk`.

Usage

```
filterSparseDonors(
  sce,
  donor = NULL,
  cell_type = NULL,
  min_cells = 10L,
  action = c("remove", "flag")
)
```

Arguments

sce	A SingleCellExperiment object.
donor	A character(1) naming the donor column in colData(sce).
cell_type	A character(1) naming the cell type column in colData(sce).
min_cells	A integer(1) minimum number of cells a donor must have for a given cell type to be retained. Default: 10.
action	A character(1), either "remove" (drop sparse cells from the SCE) or "flag" (add a logical column scFastDE_sparse to colData). Default: "remove".

Details

For each combination of cell_type level and donor level, the function counts the number of cells in the sce and compares it to min_cells. Donors below the threshold are either removed (when action = "remove") or flagged in a new colData column (when action = "flag").

As a rule of thumb, min_cells = 10 is a reasonable default for common cell types. For rare cell types (< 1% frequency), consider lowering to min_cells = 5.

Value

The input sce with sparse donor-cell type combinations either removed or flagged depending on action. When action = "flag", a column scFastDE_sparse is added to colData: TRUE means the cell belongs to a sparse donor-cell type combination.

See Also

fastPseudobulk, fastDE

Examples

```
library(SingleCellExperiment)

set.seed(42)
counts <- matrix(rpois(500 * 80, 5), nrow = 500, ncol = 80)
rownames(counts) <- paste0("Gene", seq_len(500))
colnames(counts) <- paste0("Cell", seq_len(80))
sce <- SingleCellExperiment(assays = list(counts = counts))
sce$donor     <- rep(paste0("D", 1:8), each = 10)
sce$cell_type <- rep(c("Tcell", "Bcell"), times = 40)
```

```
# Remove donor-cell type combos with fewer than 5 cells
sce_filtered <- filterSparseDonors(sce, donor = "donor",
                                   cell_type = "cell_type",
                                   min_cells = 5)
ncol(sce_filtered)
```

`params,FDEResult-method`

Accessor for analysis parameters in a FDEResult

Description

Returns the analysis parameter list from a `FDEResult` object.

Usage

```
## S4 method for signature 'FDEResult'
params(x, ...)
```

Arguments

`x`	A `FDEResult` object.
`...`	Additional arguments (not used).

Value

A `list` of analysis parameters.

Examples

```
library(S4Vectors)
de <- DataFrame(logFC = c(1.2, -0.5), P.Value = c(0.001, 0.5),
                adj.P.Val = c(0.01, 0.8))
pb <- matrix(rpois(20, 10), nrow = 2, ncol = 10)
rownames(pb) <- paste0("Gene", 1:2)
colnames(pb) <- paste0("Donor", 1:10)
obj <- FDEResult(
    de,
    pb,
    setNames(sqrt(1:10), paste0("Donor", 1:10)),
    params = list(cell_type = "T_cells", condition = "group")
)
params(obj)
```

plotDEResults *Volcano Plot of Differential Expression Results*

Description

Produces a volcano plot from a FDEResult object, with genes coloured by significance and optionally labelled. Points are coloured by whether they exceed the lfc_thresh and fdr_thresh thresholds.

Usage

```
plotDEResults(
  result,
  fdr_thresh = 0.05,
  lfc_thresh = 1,
  top_n = 10L,
  point_size = 1,
  point_alpha = 0.6,
  colours = c(up = "#E24B4A", down = "#378ADD", ns = "#888780")
)
```

Arguments

result	A FDEResult object from fastDE.
fdr_thresh	A numeric(1) FDR threshold for significance. Default: 0.05.
lfc_thresh	A numeric(1) absolute log2 fold-change threshold. Default: 1.
top_n	An integer(1) number of top significant genes to label by name. Default: 10.
point_size	A numeric(1) point size. Default: 1.
point_alpha	A numeric(1) point transparency. Default: 0.6.
colours	A named character vector of colours for "up", "down", and "ns" (not significant). Default: c(up = "#E24B4A", down = "#378ADD", ns = "#888780").

Value

A ggplot2 object.

See Also

fastDE, FDEResult

Examples

```
library(SingleCellExperiment)

set.seed(42)
n_genes <- 200
counts <- matrix(rpois(n_genes * 60, 8), nrow = n_genes, ncol = 60)
rownames(counts) <- paste0("Gene", seq_len(n_genes))
colnames(counts) <- paste0("Cell", seq_len(60))
counts[1:10, 31:60] <- counts[1:10, 31:60] * 3L

sce <- SingleCellExperiment(assays = list(counts = counts))
sce$donor     <- rep(paste0("D", 1:6), each = 10)
sce$cell_type <- "Tcell"
sce$condition <- rep(c("ctrl", "treat"), each = 30)

result <- fastDE(sce, donor = "donor", cell_type = "cell_type",
                 condition = "condition", target_type = "Tcell",
                 min_cells = 5)
plotDEResults(result)
```

---

pseudobulk *Accessor for pseudo-bulk matrix in a FDEResult*

Description

Returns the pseudo-bulk count matrix from a `FDEResult` object.

Usage

```
pseudobulk(x, ...)

## S4 method for signature 'FDEResult'
pseudobulk(x, ...)
```

Arguments

`x`	A `FDEResult` object.
`...`	Additional arguments (not used).

Value

A `matrix` of pseudo-bulk counts (genes x donors).

Examples

```
library(S4Vectors)
de <- DataFrame(logFC = c(1.2, -0.5), P.Value = c(0.001, 0.5),
                adj.P.Val = c(0.01, 0.8))
pb <- matrix(rpois(20, 10), nrow = 2, ncol = 10)
rownames(pb) <- paste0("Gene", 1:2)
colnames(pb) <- paste0("Donor", 1:10)
obj <- FDEResult(de, pb, setNames(sqrt(1:10), paste0("Donor", 1:10)))
pseudobulk(obj)
```

---

show,FDEResult-method *Show method for FDEResult*

Description

Prints a compact summary of a `FDEResult` object.

Usage

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

Arguments

`object`	A `FDEResult` object.

Value

Invisibly returns `object`.

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