

# Package ‘ccfindR’

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( $\geq$  0.7)

**RdMacros** Rdpack

**biocViews** Transcriptomics, SingleCell, ImmunoOncology, Bayesian,  
Clustering

**Description** A collection of tools for cancer genomic data clustering analyses, including those for single cell RNA-seq. Cell clustering and feature gene selection analysis employ Bayesian (and maximum likelihood) non-negative matrix factorization (NMF) algorithm. Input data set consists of RNA count matrix, gene, and cell bar code annotations. Analysis outputs are factor matrices for multiple ranks and marginal likelihood values for each rank. The package includes utilities for downstream analyses, including meta-gene identification, visualization, and construction of rank-based trees for clusters.

**License** GPL ( $\geq$  2)

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---

|                |                                      |
|----------------|--------------------------------------|
| assignCelltype | <i>Cell type assignment via GSEA</i> |
|----------------|--------------------------------------|

---

## Description

Computes GSEA enrichment score of marker sets in meta gene list

## Usage

```
assignCelltype(obj, rank, gset, gene_names = NULL, p = 0,
  remove.na = FALSE, p.value = FALSE, nperm = 1000,
  progress.bar = TRUE, grp.prefix = c("IG"))
```

## Arguments

|            |                                                        |
|------------|--------------------------------------------------------|
| obj        | Object of class scNMFSets.                             |
| rank       | Rank to examine                                        |
| gset       | List of gene sets to be used as markers                |
| gene_names | Names of genes to be used for meta-gene identification |
| p          | Enrichment score exponent.                             |

|                           |                                                               |
|---------------------------|---------------------------------------------------------------|
| <code>remove.na</code>    | Remove gene sets with no overlap                              |
| <code>p.value</code>      | Estimate p values using permutation                           |
| <code>nperm</code>        | No. of permutation replicates                                 |
| <code>progress.bar</code> | Display progress bar for p value computation                  |
| <code>grp.prefix</code>   | Gene name prefix to search for with wildcard matches in query |

## Details

If `obj` is of class `scNMFSet`, it computes meta gene list using [meta\\_gene.cv](#). Otherwise, `obj` is expected to be a data frame of the same structure as the output of `meta_gene.cv`; the number of rows same as the total number of metagenes per cluster, three columns per each cluster (gene name, meta-gene score, and coefficient of variation). The argument `gset` is a list of gene sets to be checked for enrichment in each cluster meta gene list. The enrichment score is computed using the GSEA algorithm (Subramanian et al. 2005).

## Value

Matrix of enrichment score statistics with cell types in rows and clusters in columns

## References

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP (2005). “Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.” *Proc. Natl. Acad. Sci., USA*, **102**(43), 15545–15550. doi:[10.1073/pnas.0506580102](#).

## Examples

```
dir <- system.file('extdata',package='ccfindR')
pbmc <- read_10x(dir)
pbmc <- vb_factorize(pbmc, ranks=5)
meta <- meta_gene.cv(pbmc,rank=5, gene_names=rowData(pbmc)[,2])
markers <- list('B cell'=c('CD74','IG','HLA'),
               'CD8+ T'=c('CD8A','CD8B','GZMK','CCR7','LTB'),
               'CD4+ T'=c('CD3D','CD3E','IL7R','LEF1'),
               'NK'=c('GNLY','NKG7','GZMA','GZMH'),
               'Macrophage'=c('S100A8','S100A9','CD14','LYZ','CFD'))
gsea <- assignCelltype(meta, rank=5, gset=markers, grp.prefix=c('IG','HLA'))
gsea
```

---

basis

*Basis matrices in an Object*

---

## Description

Retrieve or set the basis matrices `W` from factorization in an object

**Usage**

```
basis(object)
```

**Arguments**

object                      Object of class scNMFSets

**Details**

After factorization, basis matrices corresponding to each rank value are stored as elements of a list, which is in slot basis of object of class scNMFSets. basis(object) will return the list of matrices. basis(object) <- value can be used to modify it.

**Value**

Either NULL or a list of same length as ranks(object), whose elements are basis matrices derived from factorization under each rank value.

**Examples**

```
s <- scNMFSets(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s,ranks=seq(2,4))
basis(s)[[1]]
```

---

basis,scNMFSets-method    *Basis matrix accessor*

---

**Description**

Basis matrix accessor

**Usage**

```
## S4 method for signature 'scNMFSets'
basis(object)
```

**Arguments**

object                      Object containing basis matrix

**Value**

List of basis matrices

---

basis<-                                      *Generics for basis matrix assignment*

---

### Description

Access and modify basis matrices

### Usage

```
basis(object) <- value
```

### Arguments

|        |                                |
|--------|--------------------------------|
| object | Object of class scNMFSet       |
| value  | Basis matrix to be substituted |

### Value

Input object with updated basis matrices

### Examples

```
set.seed(1)
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
basis(s)[[1]] <- apply(basis(s)[[1]],seq(1,2),round,digits=3)
basis(s)
```

---

basis<-,scNMFSet-method  
                                            *Modify basis matrices*

---

### Description

Access and modify basis matrices

### Usage

```
## S4 replacement method for signature 'scNMFSet'
basis(object) <- value
```

### Arguments

|        |                                |
|--------|--------------------------------|
| object | Object of class scNMFSet       |
| value  | Basis matrix to be substituted |

**Value**

Input object with updated basis matrices

**Examples**

```
set.seed(1)
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
basis(s)[[1]] <- apply(basis(s)[[1]],c(1,2),round,digits=3)
basis(s)
```

---

build\_tree

*Build tree connecting clusters at different ranks*

---

**Description**

Build tree connecting clusters at different ranks

**Usage**

```
build_tree(object, rmax)
```

**Arguments**

|        |                                            |
|--------|--------------------------------------------|
| object | Object of class scNMFSet                   |
| rmax   | Maximum rank at which tree branching stops |

**Value**

List containing the tree structure

**Examples**

```
set.seed(1)
x <- simulate_whx(nrow=50,ncol=100,rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s,ranks=seq(2,8),nrun=5)
tree <- build_tree(s,rmax=5)
tree
```

ccfindR

*ccfindR: Cancer Clone Finder***Description**

This package contains tools and utilities for cell-type discovery using single-cell transcriptomic data while evaluating significance of the depth of clustering (Woo et al. 2019).

**References**

Woo J, Winterhoff BJ, Starr TK, Aliferis C, Wang J (2019). “De novo prediction of cell-type complexity in single-cell RNA-seq and tumor microenvironments.” *Life Sci. Alliance*, **2**, e201900443. <http://dx.doi.org/10.26508/lsa.201900443>.

cell\_map

*Plot heatmap of clustering coefficient matrix***Description**

Retrieve a coefficient matrix H derived from factorization by rank value and generate heatmap of its elements.

**Usage**

```
cell_map(object, rank, main = "Cells", ...)
```

**Arguments**

|        |                                                                                                                                                                                                        |
|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| object | Object of class scNMFSet.                                                                                                                                                                              |
| rank   | Rank value for which the cell map is to be displayed. The object must contain the corresponding slot: one element of <code>coeff(object)[[k]]</code> for which <code>ranks(object)[[k]]==rank</code> . |
| main   | Title of plot.                                                                                                                                                                                         |
| ...    | Other arguments to be passed to <a href="#">heatmap</a> , <a href="#">image</a> , and <a href="#">plot</a> .                                                                                           |

**Value**

NULL

**Examples**

```

set.seed(1)
x <- simulate_data(nfeatures=10, nsamples=c(20, 20, 60))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(100)
s <- scNMFSets(count=x, rowData=seq_len(10), colData=seq_len(100))
s <- vb_factorize(s, ranks=seq(2, 5))
plot(s)
cell_map(s, rank=3)

```

---

|            |                                   |
|------------|-----------------------------------|
| cluster_id | <i>Assign cells into clusters</i> |
|------------|-----------------------------------|

---

**Description**

Use factorization results in an object to assign cells into clusters.

**Usage**

```
cluster_id(object, rank = 2)
```

**Arguments**

|        |                                                                 |
|--------|-----------------------------------------------------------------|
| object | Object of class scNMFSets                                       |
| rank   | Rank value whose factor matrices are to be used for assignment. |

**Value**

Vector of length equal to the number of cells containing cluster ID numbers of each cell.

**Examples**

```

set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSets(count=x$x)
s <- vb_factorize(s, ranks=seq(2, 8), nrun=5)
cid <- cluster_id(s, rank=5)
table(cid)

```



**Value**

List of coefficient matrices

---

coeff<-

*Generics for coefficient matrix assignment*


---

**Description**

Access and modify coefficient matrices

**Usage**

```
coeff(object) <- value
```

**Arguments**

|        |                                      |
|--------|--------------------------------------|
| object | Object of class scNMFSets            |
| value  | Coefficient matrix to be substituted |

**Value**

Input object with updated coefficient matrices

**Examples**

```
s <- scNMFSets(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
coeff(s)[[1]] <- apply(coeff(s)[[1]],c(1,2),round,digits=2)
coeff(s)
```

---

coeff<-, scNMFSets-method

*Modify coefficient matrices*


---

**Description**

Can be used to access and modify coefficient matrices

**Usage**

```
## S4 replacement method for signature 'scNMFSets'
coeff(object) <- value
```

**Arguments**

|        |                                      |
|--------|--------------------------------------|
| object | Object of class scNMFSet             |
| value  | Coefficient matrix to be substituted |

**Value**

Input object with updated coefficient matrices

**Examples**

```
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
coeff(s)[[1]] <- apply(coeff(s)[[1]],c(1,2),round,digits=2)
coeff(s)
```

---

colData,scNMFSet-method

*Sample annotation accessor*

---

**Description**

Sample annotation accessor

**Usage**

```
## S4 method for signature 'scNMFSet'
colData(x)
```

**Arguments**

|   |                                     |
|---|-------------------------------------|
| x | Object containing sample annotation |
|---|-------------------------------------|

**Value**

Column annotation DataFrame

**Examples**

```
library(S4Vectors)
x <- matrix(rpois(n=12,lambda=3),4,3)
rownames(x) <- seq_len(4)
colnames(x) <- c('a','b','c')
s <- scNMFSet(count=x,rowData=seq_len(4),colData=c('a','b','c'))
cols <- DataFrame(tissue=c('tissue1','tissue1','tissue2'))
rownames(cols) <- c('a','b','c')
colData(s) <- cols
s
```

---

```
colData<- ,scNMFSet,ANY-method
```

*Cell annotation assignment*

---

**Description**

Cell annotation assignment

**Usage**

```
## S4 replacement method for signature 'scNMFSet,ANY'
colData(x) <- value
```

**Arguments**

|       |                                   |
|-------|-----------------------------------|
| x     | Object containing cell annotation |
| value | DataFrame to be substituted       |

**Value**

Updated column annotation

**Examples**

```
library(S4Vectors)
x <- matrix(rpois(n=12,lambda=3),4,3)
rownames(x) <- seq_len(4)
colnames(x) <- c('a','b','c')
s <- scNMFSet(count=x,rowData=seq_len(4),colData=c('a','b','c'))
cols <- DataFrame(tissue=c('tissue1','tissue1','tissue2'))
rownames(cols) <- c('a','b','c')
colData(s) <- cols
s
```

---

```
counts,scNMFSet-method
```

*Accessor for count matrix*

---

**Description**

Accessor for count matrix

**Usage**

```
## S4 method for signature 'scNMFSet'
counts(object)
```

**Arguments**

object            Object containing count matrix

**Value**

Count matrix

**Examples**

```
s <- scNMFSet(count = matrix(rpois(n=12,lambda=3),3,4))
counts(s)
```

---

```
counts<-,scNMFSet-method
```

*Assignment of count matrix*

---

**Description**

Count matrix can be modified

**Usage**

```
## S4 replacement method for signature 'scNMFSet'
counts(object) <- value
```

**Arguments**

object            Object containing count  
value            Matrix-like object for replacement

**Value**

Object with updated count

**Examples**

```
mat <- matrix(rpois(n=12,lambda=3),3,4)
s <- scNMFSet(count = mat)
counts(s) <- mat^2
counts(s)
```

---

|        |                                 |
|--------|---------------------------------|
| dbasis | <i>Basis SD matrix accessor</i> |
|--------|---------------------------------|

---

**Description**

Basis SD matrix accessor

**Usage**

```
dbasis(object)
```

**Arguments**

|        |                                 |
|--------|---------------------------------|
| object | Object containing dbasis matrix |
|--------|---------------------------------|

**Value**

List of dbasis matrices

---

|                                 |
|---------------------------------|
| dbasis, scNMFSets-method        |
| <i>Basis SD matrix accessor</i> |

---

**Description**

Basis SD matrix accessor

**Usage**

```
## S4 method for signature 'scNMFSets'  
dbasis(object)
```

**Arguments**

|        |                                                        |
|--------|--------------------------------------------------------|
| object | Object containing basis standard deviation (SD) matrix |
|--------|--------------------------------------------------------|

**Value**

List of dbasis matrices

---

|          |                                   |
|----------|-----------------------------------|
| dbasis<- | <i>Basis SD matrix assignment</i> |
|----------|-----------------------------------|

---

**Description**

Basis SD matrix assignment

**Usage**

```
dbasis(object) <- value
```

**Arguments**

|        |                                 |
|--------|---------------------------------|
| object | Object containing dbasis matrix |
| value  | List for assignment             |

**Value**

Updated object

---

|                           |                               |
|---------------------------|-------------------------------|
| dbasis<- ,scNMFSet-method | <i>Modify dbasis matrices</i> |
|---------------------------|-------------------------------|

---

**Description**

Access and modify dbasis matrices

**Usage**

```
## S4 replacement method for signature 'scNMFSet'  
dbasis(object) <- value
```

**Arguments**

|        |                                   |
|--------|-----------------------------------|
| object | Object of class scNMFSet          |
| value  | Basis SD matrix to be substituted |

**Value**

Modified object

---

|        |                                 |
|--------|---------------------------------|
| dcoeff | <i>Coeff SD matrix accessor</i> |
|--------|---------------------------------|

---

**Description**

Coeff SD matrix accessor

**Usage**

```
dcoeff(object)
```

**Arguments**

|        |                                 |
|--------|---------------------------------|
| object | Object containing dcoeff matrix |
|--------|---------------------------------|

**Value**

List of dcoeff matrices

---

|                          |                                       |
|--------------------------|---------------------------------------|
| dcoeff, scNMFSets-method | <i>Coefficient SD matrix accessor</i> |
|--------------------------|---------------------------------------|

---

**Description**

Coefficient SD matrix accessor

**Usage**

```
## S4 method for signature 'scNMFSets'  
dcoeff(object)
```

**Arguments**

|        |                                                              |
|--------|--------------------------------------------------------------|
| object | Object containing coefficient standard deviation (SD) matrix |
|--------|--------------------------------------------------------------|

**Value**

List of dcoeff matrices

---

|          |                                   |
|----------|-----------------------------------|
| dcoeff<- | <i>Coeff SD matrix assignment</i> |
|----------|-----------------------------------|

---

**Description**

Coeff SD matrix assignment

**Usage**

```
dcoeff(object) <- value
```

**Arguments**

|        |                                 |
|--------|---------------------------------|
| object | Object containing dcoeff matrix |
| value  | List for assignment             |

**Value**

Updated object

---

|                               |
|-------------------------------|
| dcoeff<- , scNMFSets-method   |
| <i>Modify dcoeff matrices</i> |

---

**Description**

Access and modify dcoeff matrices

**Usage**

```
## S4 replacement method for signature 'scNMFSets'  
dcoeff(object) <- value
```

**Arguments**

|        |                                   |
|--------|-----------------------------------|
| object | Object of class scNMFSets         |
| value  | Coeff SD matrix to be substituted |

**Value**

Updated object

---

|           |                                         |
|-----------|-----------------------------------------|
| factorize | <i>Maximum likelihood factorization</i> |
|-----------|-----------------------------------------|

---

## Description

Performs single or multiple rank NMF factorization of count matrix using maximum likelihood

## Usage

```
factorize(object, ranks = 2, nrun = 20, randomize = FALSE,
          nsmpl = 1, verbose = 2, progress.bar = TRUE, Itmax = 10000,
          ncnn.step = 40, criterion = "likelihood", linkage = "average",
          Tol = 1e-05, store.connectivity = FALSE)
```

## Arguments

|                    |                                                                                                                                                                                                                      |
|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| object             | scNMFSets object containing count matrix.                                                                                                                                                                            |
| ranks              | Rank for factorization; can be a vector of multiple values.                                                                                                                                                          |
| nrun               | No. of runs with different initial guess.                                                                                                                                                                            |
| randomize          | Boolean; if TRUE, input matrix is randomized.                                                                                                                                                                        |
| nsmpl              | No. of randomized samples to average over.                                                                                                                                                                           |
| verbose            | The verbosity level: 3, each iteration output printed; 2, each run output printed; 1, each randomized sample output printed; 0, silent.                                                                              |
| progress.bar       | Display progress bar when nrun > 1 and verbose = 1.                                                                                                                                                                  |
| Itmax              | Maximum no. of iteration.                                                                                                                                                                                            |
| ncnn.step          | Minimum no. of steps with no change in connectivity matrix to achieve convergence.                                                                                                                                   |
| criterion          | If 'likelihood', iteration stops when fractional changes in likelihood is below tolerance Tol. If criterion = 'connectivity', iteration stops when connectivity matrix does not change for at least ncnn.step steps. |
| linkage            | Method to be sent to hclust in calculating cophenetic correlation.                                                                                                                                                   |
| Tol                | Tolerance for checking convergence with criterion = 'likelihood'.                                                                                                                                                    |
| store.connectivity | Returns a list also containing connectivity data.                                                                                                                                                                    |

## Details

The main input is the scNMFSets object with count matrix. This function performs non-negative factorization and fills in the empty slots basis, coeff, and ranks.

When run with multiple values of ranks, factorization is repeated for each rank and the slot measure contains quality measures of the ranks. The quality measure likelihood is negative the KL distance of the fit to the target. With nrun > 1, the likelihood is the maximum among all runs.

The quality measure dispersion is the scalar measure of how far the connectivity matrix is from 0, 1. With increasing nrun, dispersion decreases from 1. nrun should be chosen such that dispersion does not change appreciably. With randomization, count matrix of object is shuffled. nsmp1 can be used to average over multiple permutations. This averaging applies to each quality measure under a given rank.

### Value

Object of class scNMFSet with factorization slots filled.

### Examples

```
set.seed(1)
x <- simulate_data(nfeatures=10, nsamples=c(20, 20, 60, 40, 30))
s <- scNMFSet(count=x)
s <- factorize(s, ranks=seq(2, 8), nrun=5)
plot(s)
```

---

feature\_map

*Plot heatmap of basis matrix*


---

### Description

Generate heatmap of features derived from factorization of count data.

### Usage

```
feature_map(object, basis.matrix = NULL, rank, markers = NULL,
  subtract.mean = TRUE, log = TRUE, max.per.cluster = 10,
  feature.names = NULL, perm = NULL, main = "Feature map",
  cscale = NULL, cex.cluster = 1, cex.feature = 0.5, mar = NULL,
  ...)
```

### Arguments

|               |                                                                                                                                                                             |
|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| object        | Object of class scNMFSet.                                                                                                                                                   |
| basis.matrix  | Basis matrix can be supplied instead of object.                                                                                                                             |
| rank          | Rank value for which the gene map is to be displayed. The object must contain the corresponding slot (one element of basis(object)[[k]] for which ranks(object)[[k]]==rank. |
| markers       | Vector of gene names containing markers to be included in addition to the meta-genes. All entries of rowData(object) matching them will be added to the metagene list.      |
| subtract.mean | Process each rows of basis matrix W by standardization using the mean of elements within the row.                                                                           |
| log           | If TRUE, subtract.mean uses geometric mean and division. Otherwise, use arithmetic mean and subtraction.                                                                    |

|                 |                                                                                    |
|-----------------|------------------------------------------------------------------------------------|
| max.per.cluster | Maximum number of metagenes per cluster.                                           |
| feature.names   | Names to be used in the plot for features.                                         |
| perm            | Permutation of cluster IDs.                                                        |
| main            | Main title.                                                                        |
| cscale          | Colors for heatmap.                                                                |
| cex.cluster     | Cluster ID label size.                                                             |
| cex.feature     | Feature ID label size.                                                             |
| mar             | Margins for graphics: :par.                                                        |
| ...             | Other arguments to be passed to <a href="#">image</a> , and <a href="#">plot</a> . |

### Details

This function uses `image()` and is more flexible than `gene_map`.

If object contains multiple ranks, only the requested rank's basis matrix *W* will be displayed. As in `gene_map`, the features displayed in rows are selected by "max" scheme

### Value

NULL

### Examples

```
set.seed(1)
x <- simulate_data(nfeatures=10,nsamples=c(20,20,60))
rownames(x) <- seq_len(10)

set.seed(1)
x <- simulate_data(nfeatures=10,nsamples=c(20,20,60))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(100)
s <- scNMFSet(count=x,rowData=seq_len(10), colData=seq_len(100))
s <- vb_factorize(s,ranks=seq(2,5))
plot(s)
feature_map(s, rank=3)
```

---

filter\_cells

*Filter cells with quality control criteria*

---

### Description

Remove low quality cell entries from object

### Usage

```
filter_cells(object, umi.min = 0, umi.max = Inf, plot = TRUE,
  remove.zeros = TRUE)
```

**Arguments**

|              |                                                                                                     |
|--------------|-----------------------------------------------------------------------------------------------------|
| object       | scNMFSets object                                                                                    |
| umi.min      | Minimum UMI count for cell filtering                                                                |
| umi.max      | Maximum UMI count for cell filtering                                                                |
| plot         | If TRUE, the UMI count distribution of all cells will be displayed. Cells selected are colored red. |
| remove.zeros | Remove rows/columns containing zeros only                                                           |

**Details**

Takes as input scNMFSets object and plots histogram of UMI counts for each cell. Optionally, cells are filtered using minimum and maximum UMI counts. The resulting object is returned after removing empty rows and columns, if any.

**Value**

scNMFSets object with cells filtered.

**Examples**

```
set.seed(1)
s <- scNMFSets(matrix(stats::rpois(n=1200,lambda=3),40,30))
s <- filter_cells(s,umi.min=10^2.0,umi.max=10^2.1)
```

---

|              |                                                   |
|--------------|---------------------------------------------------|
| filter_genes | <i>Filter genes with quality control criteria</i> |
|--------------|---------------------------------------------------|

---

**Description**

Select genes with high relative variance in count data for further analysis

**Usage**

```
filter_genes(object, markers = NULL, vmr.min = 0,
  min.cells.expressed = 0, max.cells.expressed = Inf,
  rescue.genes = FALSE, progress.bar = TRUE, save.memory = FALSE,
  plot = TRUE, log = "xy", cex = 0.5)
```

**Arguments**

|                     |                                                                                                                               |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------|
| object              | scNMFSets object.                                                                                                             |
| markers             | A vector containing marker genes to be selected. All rows in rowData that contain columns matching this set will be selected. |
| vmr.min             | Minimum variance-to-mean ratio for gene filtering.                                                                            |
| min.cells.expressed | Minimum no. of cells expressed for gene filtering.                                                                            |

|                     |                                                                                                                                                           |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| max.cells.expressed | Maximum no. of cells expressed for gene filtering.                                                                                                        |
| rescue.genes        | Selected additional genes whose (non-zero) count distributions have at least one mode.                                                                    |
| progress.bar        | Display progress of mode-gene scan or VMR calculation with save.memory = TRUE.                                                                            |
| save.memory         | For a very large number of cells, calculate VMR row by row while avoiding calls to as.matrix(). Progress bar will be displayed unless progress.bar=FALSE. |
| plot                | Plot the distribution of no. of cells expressed vs. VMR.                                                                                                  |
| log                 | Axis in log-scale, c('x', 'y', 'xy').                                                                                                                     |
| cex                 | Symbol size for each gene in the plot.                                                                                                                    |

### Details

Takes as input scNMFSet object and scatterplot no. of cells expressed versus VMR (variance-to-mean ratio) for each gene. Optionally, genes are filtered using minimum VMR together with a range of no. of cells expressed.

### Value

Object of class scNMFSet.

### Examples

```
set.seed(1)
s <- scNMFSet(matrix(stats::rpois(n=1200,lambda=3),40,30))
s <- filter_genes(s,vmr.min=1.0,min.cells.expressed=28,
  rescue.genes=FALSE)
```

---

|          |                                        |
|----------|----------------------------------------|
| gene_map | <i>Plot heatmap of metagene matrix</i> |
|----------|----------------------------------------|

---

### Description

Generate heatmap of metagenes derived from factorization of count data.

### Usage

```
gene_map(object, rank, markers = NULL, subtract.mean = TRUE,
  log = TRUE, max.per.cluster = 10, Colv = NA, gene.names = NULL,
  main = "Genes", col = NULL, ...)
```

**Arguments**

|                              |                                                                                                                                                                                                        |
|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>object</code>          | Object of class <code>scNMFSet</code> .                                                                                                                                                                |
| <code>rank</code>            | Rank value for which the gene map is to be displayed. The object must contain the corresponding slot (one element of <code>basis(object)[[k]]</code> for which <code>ranks(object)[[k]]==rank</code> . |
| <code>markers</code>         | Vector of gene names containing markers to be included in addition to the metagenes. All entries of <code>rowData(object)</code> matching them will be added to the metagene list.                     |
| <code>subtract.mean</code>   | Process each rows of basis matrix <code>W</code> by standardization using the mean of elements within the row.                                                                                         |
| <code>log</code>             | If TRUE, <code>subtract.mean</code> uses geometric mean and division. Otherwise, use arithmetic mean and subtraction.                                                                                  |
| <code>max.per.cluster</code> | Maximum number of metagenes per cluster.                                                                                                                                                               |
| <code>Colv</code>            | NA suppresses reordering and dendrogram of clusters along the column. See <a href="#">heatmap</a> .                                                                                                    |
| <code>gene.names</code>      | Names to be used in the plot for genes.                                                                                                                                                                |
| <code>main</code>            | Title of plot.                                                                                                                                                                                         |
| <code>col</code>             | Colors for the cluster panels on the left and top.                                                                                                                                                     |
| <code>...</code>             | Other arguments to be passed to <a href="#">heatmap</a> , <a href="#">image</a> , and <a href="#">plot</a> .                                                                                           |

**Details**

Wrapper for [heatmap](#) to display metagenes and associated basis matrix element magnitudes. Factorization results inside an object specified by its rank value will be retrieved, and metagene sets identified from clusters.

If object contains multiple ranks, only the requested rank's basis matrix `W` will be displayed. The genes displayed in rows are selected by "max" scheme [Carmona-Saez, BMC Bioinformatics (2006), <https://doi.org/10.1186/1471-2105-7-54>]: for each cluster (`k` in `1:ncol`), rows of `W` are sorted by decreasing order of `W[,k]`. Marker genes for `k` are those among the top `nmarker` for which `W[,k]` is maximum within each row.

**Value**

NULL

**Examples**

```
set.seed(1)
x <- simulate_data(nfeatures=10,nsamples=c(20,20,60))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(100)
s <- scNMFSet(count=x,rowData=seq_len(10), colData=seq_len(100))
s <- vb_factorize(s,ranks=seq(2,5))
plot(s)
gene_map(s, rank=3)
```

measure

*Factorization measures in an Object***Description**

Retrieve or set factorization measures in an object

**Usage**

```
measure(object)
```

**Arguments**

`object`            Object of class `scNMFSets`.

**Details**

Factorization under multiple rank values lead to measures stored in a data frame inside a slot `measure`. In maximum likelihood using `factorize`, this set of quality measures include dispersion and cophenetic coefficients for each rank. In Bayesian factorization using `vb_factorize`, log evidence for each rank is stored. `measure(object)` will return the data frame. `measure(object) <- value` can be used to modify it.

**Value**

Either `NULL` or a data frame containing measures.

**Examples**

```
s <- scNMFSets(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s,ranks=seq(2,4))
measure(s)
```

measure, scNMFSets-method

*Rank measure accessor***Description**

Rank measure accessor

**Usage**

```
## S4 method for signature 'scNMFSets'
measure(object)
```

**Arguments**

object            Object containing measure

**Value**

Data frame of measure

---

measure<-                      *Generics for factorization measure assignment*

---

**Description**

Can be used to access and modify factorization measure

**Usage**

```
measure(object) <- value
```

**Arguments**

object            Object of class scNMFSet  
value            Measure to be substituted

**Value**

Input object with updated measure

**Examples**

```
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
measure(s)[,-1] <- apply(measure(s)[,-1], c(1,2), round,digits=3)
measure(s)
```

---

measure<- ,scNMFSet-method  
                                *Modify factorization measure*

---

**Description**

Can be used to access and modify factorization measure

**Usage**

```
## S4 replacement method for signature 'scNMFSet'
measure(object) <- value
```

**Arguments**

|        |                           |
|--------|---------------------------|
| object | Object of class scNMFSet  |
| value  | Measure to be substituted |

**Value**

Input object with updated measure

**Examples**

```
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
measure(s)[,-1] <- apply(measure(s)[,-1], c(1,2), round,digits=3)
measure(s)
```

---

|              |                                |
|--------------|--------------------------------|
| meta_gene.cv | <i>Meta gene table with CV</i> |
|--------------|--------------------------------|

---

**Description**

Generates meta gene table with coefficient of variation

**Usage**

```
meta_gene.cv(object = NULL, rank, basis.matrix = NULL, dbasis = NULL,
  max.per.cluster = 100, gene_names = NULL, subtract.mean = TRUE,
  log = TRUE, cv.max = Inf)
```

**Arguments**

|                 |                                                                |
|-----------------|----------------------------------------------------------------|
| object          | Main object containing factorization outcome                   |
| rank            | Rank for which meta gene is to be found                        |
| basis.matrix    | Basis matrix to work with. Only necessary when object is NULL. |
| dbasis          | Variance of basis matrix. Only necessary when object is NULL.  |
| max.per.cluster | Maximum meta genes per cluster.                                |
| gene_names      | Name of genes. If NULL, will be taken from row names.          |
| subtract.mean   | Standardize magnitudes of basis elements by subtracting mean   |
| log             | Use geometric mean.                                            |
| cv.max          | Upper bound for CV in selecting meta genes.                    |

**Value**

Data frame with meta genes and their CV in each column.

**Examples**

```
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s, ranks=seq(2,8), nrun=5)
plot(s)
meta_gene.cv(s, rank=5)
```

---

|            |                                         |
|------------|-----------------------------------------|
| meta_genes | <i>Find metagenes from basis matrix</i> |
|------------|-----------------------------------------|

---

**Description**

Retrieve a basis matrix from an object and find metagenes.

**Usage**

```
meta_genes(object, rank, basis.matrix = NULL, max.per.cluster = 10,
  gene_names = NULL, subtract.mean = TRUE, log = TRUE)
```

**Arguments**

|                 |                                                                                                |
|-----------------|------------------------------------------------------------------------------------------------|
| object          | Object of class scNMFSet.                                                                      |
| rank            | Rank value for which metagenes are to be found.                                                |
| basis.matrix    | Instead of an object containing basis matrices, the matrix itself can be provided.             |
| max.per.cluster | Maximum number of metagenes per cluster.                                                       |
| gene_names      | Names of genes to replace row names of basis matrix.                                           |
| subtract.mean   | Standardize the matrix elements with means within each row.                                    |
| log             | Use geometric mean and division instead of arithmetic mean and subtraction with subtract.mean. |

**Value**

List of vectors each containing metagene names of clusters.

**Examples**

```
set.seed(1)
x <- simulate_data(nfeatures=10, nsamples=c(20,20,60))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(100)
s <- scNMFSet(count=x, rowData=seq_len(10), colData=seq_len(100))
s <- vb_factorize(s, ranks=seq(2,5))
meta_genes(s, rank=4)
```

---

|        |                                                                 |
|--------|-----------------------------------------------------------------|
| newick | <i>Generate Newick format tree string from tree list object</i> |
|--------|-----------------------------------------------------------------|

---

**Description**

Generate Newick format tree string from tree list object

**Usage**

```
newick(tree, parent = "1.1", string = "")
```

**Arguments**

|        |                                                  |
|--------|--------------------------------------------------|
| tree   | Tree list object from <a href="#">build_tree</a> |
| parent | Parent ID                                        |
| string | Newick string of parent tree                     |

**Value**

String of newick tree

**Examples**

```
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSets(x$x)
s <- vb_factorize(s, ranks=seq(2,8), nrun=5)
tree <- build_tree(s, rmax=5)
nw <- newick(tree=tree)
nw
```

---

|                 |                             |
|-----------------|-----------------------------|
| normalize_count | <i>Normalize count data</i> |
|-----------------|-----------------------------|

---

**Description**

Rescale count matrix entries such that all cells have the same library size.

**Usage**

```
normalize_count(object)
```

**Arguments**

|        |                   |
|--------|-------------------|
| object | scNMFSets object. |
|--------|-------------------|

**Details**

For analysis purposes, it is sometimes useful to rescale integer count data into floats such that all cells have the same median counts. This function will calculate the median of all UMI counts of cells (total number of RNAs derived from each cell). All count data are then rescaled such that cells have uniform UMI count equal to the median.

**Value**

scNMFSets object with normalized count data.

**Examples**

```
library(Matrix)
set.seed(1)
s <- scNMFSets(count=matrix(rpois(n=1200,lambda=3),40,30))
colMeans(counts(s))
s <- normalize_count(s)
colMeans(counts(s))
```

---

|              |                               |
|--------------|-------------------------------|
| optimal_rank | <i>Determine optimal rank</i> |
|--------------|-------------------------------|

---

**Description**

Takes as main argument scNMFSets object containing factorized output and estimate the optimal rank.

**Usage**

```
optimal_rank(object, df = 10, BF.threshold = 3, type = NULL,
             m = NULL)
```

**Arguments**

|              |                                                                                                                                                                                    |
|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| object       | scNMFSets object containing factorization output, or data frame containing the rank-evidence profile.                                                                              |
| df           | Degrees of freedom for split fit. Upper bound is the total number of data points (number of rank values scanned).                                                                  |
| BF.threshold | Bayes factor threshold for statistical threshold.                                                                                                                                  |
| type         | c(1,2). Type 1 is where there is a clear maximum. Type 2 is where marginal likelihood reaches a maximal level and stays constant. If omitted, the type will be inferred from data. |
| m            | Number of features (e.g., genes) in the count matrix. Only necessary when object is of type data.frame.                                                                            |

## Details

The input object is used along with Bayes factor threshold to determine the heterogeneity type (1 or 2) and the optimal rank. If  $\text{evidence}(\text{rank } 1)/\text{evidence}(\text{rank } 2) > \text{BF.threshold}$ , rank 1 is favorable than rank 2.

## Value

List containing type and ropt (optimal rank).

## Examples

```
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s, ranks=seq(2,8), nrun=5)
plot(s)
optimal_rank(s)
```

---

|            |                                         |
|------------|-----------------------------------------|
| plot_genes | <i>Plot gene variance distributions</i> |
|------------|-----------------------------------------|

---

## Description

Gene variance to mean ratio and the number of expressing cells are plotted.

## Usage

```
plot_genes(object, vmr = NULL, ncexpr = NULL, selected_genes = NULL,
  variable_genes = NULL, mode_genes = NULL, marker_genes = NULL,
  save.memory = FALSE, progress.bar = TRUE, log = "xy", cex = 0.5)
```

## Arguments

|                |                                                                                                   |
|----------------|---------------------------------------------------------------------------------------------------|
| object         | Object containing count data                                                                      |
| vmr            | Variance to mean ratio (VMR)                                                                      |
| ncexpr         | Number of cells expressing each gene                                                              |
| selected_genes | Logical vector specifying genes selected                                                          |
| variable_genes | Logical vector specifying genes with high VMR                                                     |
| mode_genes     | Logical vector specifying genes with nonzero modes                                                |
| marker_genes   | Logical vector specifying marker genes                                                            |
| save.memory    | If TRUE, calculate VMR using slower method to save memory. Not used when gene lists are supplied. |
| progress.bar   | Display progress bar for VMR calculation. Not used when gene lists are supplied.                  |
| log            | Axis in log-scale, c('x', 'y', 'xy').                                                             |
| cex            | Symbol size for genes (supplied to plot()).                                                       |

**Details**

This function can be called separately or is also called within [filter\\_genes](#) by default. In the latter case, parameters other than `object` will have been already filled. If called separately with NULL gene lists, VMR is recalculated but gene selection is not done.

**Value**

NULL

**Examples**

```
set.seed(1)
s <- scNMFSet(matrix(stats::rpois(n=1200,lambda=3),40,30))
plot_genes(s)
```

---

plot\_tree

*Plot cluster tree*

---

**Description**

Visualize the output of [build\\_tree](#) as a dendrogram.

**Usage**

```
plot_tree(tree, direction = "rightwards", cex = 0.7, ...)
```

**Arguments**

|                        |                                                                         |
|------------------------|-------------------------------------------------------------------------|
| <code>tree</code>      | List containing tree structure. Output from <a href="#">build_tree</a>  |
| <code>direction</code> | <code>c('rightwards', 'downwards')</code> ; the direction of dendrogram |
| <code>cex</code>       | Font size of edge/tip labels                                            |
| <code>...</code>       | Other parameters to <a href="#">plot.phylo</a>                          |

**Details**

Uses [plot.phylo](#) to visualize cluster tree.

**Value**

NULL

**Examples**

```
set.seed(1)
x <- simulate_whx(nrow=50,ncol=100,rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s,ranks=seq(2,8),nrun=5)
tree <- build_tree(s,rmax=5)
plot_tree(tree)
```

---

|       |                                 |
|-------|---------------------------------|
| ranks | <i>Rank values in an Object</i> |
|-------|---------------------------------|

---

**Description**

Retrieve or set the rank values in an object

**Usage**

```
ranks(object)
```

**Arguments**

object                      Object of class scNMFSets.

**Details**

Ranks for which factorization has been performed are stored in slot ranks of scNMFSets object. ranks(object) will return the rank vector. ranks(object) <- value can be used to modify it.

**Value**

Either NULL or vector.

**Examples**

```
s <- scNMFSets(matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s,ranks=seq(2,4))
ranks(s)
```

---

ranks,scNMFSets-method    *Rank accessor*

---

**Description**

Rank accessor

**Usage**

```
## S4 method for signature 'scNMFSets'
ranks(object)
```

**Arguments**

object                      Object containing rank values

**Value**

Vector of rank values

---

ranks<-                      *Generics for ranks assignment*

---

### Description

Replace ranks slot of scNMFSet object

### Usage

```
ranks(object) <- value
```

### Arguments

|        |                                        |
|--------|----------------------------------------|
| object | Object of class scNMFSet               |
| value  | Rank values (vector) to be substituted |

### Value

Input object with updated ranks

### Examples

```
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=seq(2,3))
ranks(s) <- c('two','three')
ranks(s)
```

---

ranks<-,scNMFSet-method  
*Modify ranks*

---

### Description

Replace ranks slot of scNMFSet object

### Usage

```
## S4 replacement method for signature 'scNMFSet'
ranks(object) <- value
```

### Arguments

|        |                                        |
|--------|----------------------------------------|
| object | Object of class scNMFSet               |
| value  | Rank values (vector) to be substituted |

**Value**

Input object with updated ranks

**Examples**

```
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=seq(2,3))
ranks(s) <- c('two','three')
ranks(s)
```

---

read\_10x

---

*Read 10x data and generate scNMF object*


---

**Description**

Read count, gene, and barcode annotation data in 10x format and create an object of class scNMFSet.

**Usage**

```
read_10x(dir, count = "matrix.mtx", genes = "genes.tsv",
  barcodes = "barcodes.tsv", remove.zeros = TRUE)
```

**Arguments**

|              |                                          |
|--------------|------------------------------------------|
| dir          | Name of directory containing data files. |
| count        | Name of count matrix file.               |
| genes        | Name of gene annotation file.            |
| barcodes     | Name of cell annotation file.            |
| remove.zeros | If TRUE, empty rows/columns are removed. |

**Details**

Files for count, genes, and barcodes are assumed to be present in dir. Count data are in sparse "Matrix Market" format (<https://math.nist.gov/MatrixMarket/formats.html>).

**Value**

Object of class scNMFSet

**Examples**

```
library(S4Vectors)
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
rowData(s) <- DataFrame(seq_len(4))
colData(s) <- DataFrame(seq_len(3))
write_10x(s,dir='.')
s <- read_10x(dir='.')
s
```

---

|              |                                                             |
|--------------|-------------------------------------------------------------|
| remove_zeros | <i>Remove rows or columns that are empty from an object</i> |
|--------------|-------------------------------------------------------------|

---

**Description**

Remove rows or columns that are empty from an object

**Usage**

```
remove_zeros(object)
```

**Arguments**

|        |                        |
|--------|------------------------|
| object | Object containing data |
|--------|------------------------|

**Value**

Object with empty rows/columns removed

**Examples**

```
set.seed(1)
x <- matrix(rpois(n=100,lambda=0.1),10,10)
s <- scNMFSet(count=x,remove.zeros=FALSE)
s2 <- remove_zeros(s)
s2
```

---

|             |                                             |
|-------------|---------------------------------------------|
| rename_tips | <i>Rename tips of trees with cell types</i> |
|-------------|---------------------------------------------|

---

**Description**

Rename tips of trees with cell types

**Usage**

```
rename_tips(tree, rank, tip.labels)
```

**Arguments**

|            |                                                  |
|------------|--------------------------------------------------|
| tree       | List containing tree                             |
| rank       | Rank value of which tip names are to be replaced |
| tip.labels | Vector of new names for tips                     |

**Value**

List containing tree with updated tip labels

**Examples**

```

set.seed(1)
x <- simulate_whx(nrow=50,ncol=100,rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s,ranks=seq(2,8),nrun=5)
tree <- build_tree(s,rmax=5)
tree <- rename_tips(tree,rank=5,tip.labels=letters[seq_len(5)])
tree

```

---

rowData,scNMFSet-method

*Feature annotation accessor*


---

**Description**

Feature annotation accessor

**Usage**

```

## S4 method for signature 'scNMFSet'
rowData(x)

```

**Arguments**

x                      Object containing data

**Value**

DataFrame of feature annotation

**Examples**

```

x <- matrix(rpois(n=12,lambda=3),4,3)
rownames(x) <- seq_len(4)
colnames(x) <- seq_len(3)
s <- scNMFSet(count=x,rowData=seq_len(4),colData=seq_len(3))
rowData(s)

```

---

```
rowData<-,scNMFSet-method
```

*Gene annotation assignment*

---

**Description**

Gene annotation assignment

**Usage**

```
## S4 replacement method for signature 'scNMFSet'
rowData(x) <- value
```

**Arguments**

|       |                                               |
|-------|-----------------------------------------------|
| x     | Object containing data                        |
| value | DataFrame of row annotation to be substituted |

**Value**

Row annotation DataFrame

---

```
scNMFSet
```

*Create scNMFSet object*

---

**Description**

Object derived from [SingleCellExperiment](#)

**Usage**

```
scNMFSet(count = NULL, ..., remove.zeros = TRUE)
```

**Arguments**

|              |                                                          |
|--------------|----------------------------------------------------------|
| count        | Count matrix                                             |
| ...          | Other parameters of <a href="#">SingleCellExperiment</a> |
| remove.zeros | Remove empty rows and columns                            |

**Value**

Object of class scNMFSet.

**Examples**

```
count <- matrix(rpois(n=12,lambda=2),4,3)
s <- scNMFSet(count=count)
s
```

scNMFSets-class

*Class scNMFSets for storing input data and results***Description**

S4 class derived from [SingleCellExperiment](#) that can store single-cell count matrix, gene and cell annotation data frames, and factorization factors as well as quality measures for rank determination.

**Usage**

```
## S4 method for signature 'scNMFSets,ANY'
plot(x)
```

**Arguments**

x                      Object containing measure

**Value**

Object of class scNMFSets

NULL

**Methods (by generic)**

- `plot`: Plot measures of an object. For quality measures derived from maximum likelihood inference, dispersion and cophenetic will be plotted separately.  
For measure derived from Bayesian inference, log evidence as a function of rank values will be plotted.

**Slots**

`assays` Named list for count matrix counts.

`rowData` `DataFrame` for gene (feature) names and annotations in columns.

`colData` `DataFrame` for cell IDs and other annotations in columns (e.g., barcodes, cell types).

`ranks` Vector for rank values for which factorization has been performed.

`basis` List (of length equal to that of `ranks`) of basis matrices **W** from factorization; dimension `nrow` x `rank`, where `nrow` is no. of rows in count.

`coeff` List (of length equal to that of `ranks`) of coefficient matrices **H** from factorization; dimension `rank` x `ncol`, where `ncol` is no. of columns in count.

`measure` Data frame of factorization quality measures for each rank (likelihood and dispersion).

Other slots inherited from `SingleCellExperiment` class are not explicitly used.

**Examples**

```

library(S4Vectors)
# toy matrix
ngenes <- 8
ncells <- 5
mat <- matrix(rpois(n=ngenes*ncells,lambda=3),ngenes,ncells)

abc <- letters[seq_len(ngenes)]
ABC <- LETTERS[seq_len(ncells)]
genes <- DataFrame(gene_id=abc)
cells <- DataFrame(cell_id=ABC)
rownames(mat) <- rownames(genes) <- abc
colnames(mat) <- rownames(cells) <- ABC

# create scNMFSet object
s <- scNMFSet(count=mat,rowData=genes,colData=cells)
# alternative ways
s2 <- scNMFSet(count=mat)
s2 <- scNMFSet(assays=list(counts=mat))

# show dimensions
dim(s)

# show slots
rowData(s)

# modify slots
colData(s) <- DataFrame(cell_id=seq_len(ncells),
                        cell_type=c(rep('tissue1',2),
                                   rep('tissue2',ncells-2)))
colData(s)

```

---

show,scNMFSet-method    *Display object*

---

**Description**

Display the class and dimension of an object

Object name itself on command line or (show(object)) will display class and dimensionality

**Usage**

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

**Arguments**

object                      Object of class scNMFSet

**Value**

NULL

**Examples**

```
s <- scNMFSet(matrix(rpois(n=12,lambda=3),4,3))
show(s)
```

---

|               |                                                  |
|---------------|--------------------------------------------------|
| simulate_data | <i>Generate simulated data for factorization</i> |
|---------------|--------------------------------------------------|

---

**Description**

Use one of two schemes to generate simulated data suitable for testing factorization.

**Usage**

```
simulate_data(nfeatures, nsamples, generate.factors = FALSE,
              nfactor = 10, alpha0 = 0.5, shuffle = TRUE)
```

**Arguments**

|                  |                                                                                                                                                                                                              |
|------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| nfeatures        | Number of features $m$ (e.g., genes).                                                                                                                                                                        |
| nsamples         | Vector of sample sizes in each cluster. Rank $r$ is equal to the length of this vector. Sum of elements is the total sample size $n$ .                                                                       |
| generate.factors | Generate factor matrices $W$ and $H$ , each with dimension $n \times r$ and $r \times n$ . If FALSE, factor matrices are not used and count data are generated directly from $r$ multinomials for $m$ genes. |
| nfactor          | Total RNA count of multinomials for each cluster with <code>generate.factors = FALSE</code> . Small <code>nfactor</code> will yield sparse count matrix.                                                     |
| alpha0           | Variance parameter of Dirichlet distribution from which multinomial probabilities are sampled with <code>generate.factors = FALSE</code> .                                                                   |
| shuffle          | Randomly permute rows and columns of count matrix.                                                                                                                                                           |

**Details**

In one scheme (`generate.factors = TRUE`), simulated factor matrices  $W$  and  $H$  are used to build count data  $X = WH$ . In the second scheme, factor matrices are not used and  $X$  is sampled directly from  $r$  (rank requested) sets of multinomial distributions.

**Value**

If `generate.factors = TRUE`, list of components  $w$  (basis matrix,  $nfeatures \times rank$ ),  $h$  (coefficient matrix,  $rank \times ncells$ , where  $ncells$  is equal to  $n$ , the sum of `nsamples`), and  $x$ , a matrix of Poisson deviates with mean  $W \times H$ . If `generate.factors = FALSE`, only the count matrix  $x$  is in the list.

**Examples**

```
set.seed(1)
x <- simulate_data(nfeatures=10, nsamples=c(20, 20, 60, 40, 30))
s <- scNMFSet(x)
s
```

---

simulate\_whx

---

*Simulate factor matrices and data using priors*


---

**Description**

Under Bayesian formulation, use prior distributions of factor matrices and generate simulated data

**Usage**

```
simulate_whx(nrow, ncol, rank, aw = 0.1, bw = 1, ah = 0.1, bh = 1)
```

**Arguments**

|      |                                                               |
|------|---------------------------------------------------------------|
| nrow | Number of features (genes).                                   |
| ncol | Number of cells (samples).                                    |
| rank | Rank (ncol of W, nrow of H).                                  |
| aw   | Shape parameter of basis prior.                               |
| bw   | Mean of basis prior. Scale parameter is equal to aw/bw.       |
| ah   | Shape parameter of coefficient prior.                         |
| bh   | Mean of coefficient prior. Scale parameter is equal to ah/bh. |

**Details**

Basis W and coefficient matrices H are sampled from gamma distributions (priors) with shape (aw, ah) and mean (bw, bh) parameters. Count data X are sampled from Poisson distribution with mean values given by WH.

**Value**

List with elements w, h, and x, each containing basis, coefficient, and count matrices.

**Examples**

```
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(count=x$x)
s <- vb_factorize(s, ranks=seq(2, 8), nrun=5)
plot(s)
```

vb\_factorize

*Bayesian NMF inference of count matrix***Description**

Perform variational Bayes NMF and store factor matrices in object

**Usage**

```
vb_factorize(object, ranks = 2, nrun = 1, verbose = 2,
  progress.bar = TRUE, initializer = "random", Itmax = 10000,
  hyper.update = rep(TRUE, 4), gamma.a = 1, gamma.b = 1,
  Tol = 1e-05, hyper.update.n0 = 10, hyper.update.dn = 1,
  connectivity = TRUE, fudge = NULL, ncores = 1, useC = TRUE,
  unif.stop = TRUE)
```

**Arguments**

|                 |                                                                                                                                                                                                                          |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| object          | scNMFSet object containing count matrix.                                                                                                                                                                                 |
| ranks           | Rank for factorization; can be a vector of multiple values.                                                                                                                                                              |
| nrun            | No. of runs with different initial guesses.                                                                                                                                                                              |
| verbose         | The verbosity level: 3, each iteration output printed; 2, each run output printed; 1, each randomized sample output printed; 0, silent.                                                                                  |
| progress.bar    | Display progress bar with verbose = 1 for multiple runs.                                                                                                                                                                 |
| initializer     | If 'random', randomized initial conditions; 'svd2' for singular value decomposed initial condition.                                                                                                                      |
| Itmax           | Maximum no. of iteration.                                                                                                                                                                                                |
| hyper.update    | Vector of four logicals, each indicating whether hyperparameters c(aw, bw, ah, bh) should be optimized.                                                                                                                  |
| gamma.a         | Gamma distribution shape parameter.                                                                                                                                                                                      |
| gamma.b         | Gamma distribution mean. These two parameters are used for fixed hyperparameters with hyper.update elements FALSE.                                                                                                       |
| Tol             | Tolerance for terminating iteration.                                                                                                                                                                                     |
| hyper.update.n0 | Initial number of steps in which hyperparameters are fixed.                                                                                                                                                              |
| hyper.update.dn | Step intervals for hyperparameter updates.                                                                                                                                                                               |
| connectivity    | If TRUE, connectivity and dispersion will be calculated after each run. Can be turned off to save memory.                                                                                                                |
| fudge           | Small positive number used as lower bound for factor matrix elements to avoid singularity. If fudge = NULL (default), it will be replaced by <code>.Machine\$double.eps</code> . Can be set to 0 to skip regularization. |
| ncores          | Number of processors (cores) to run. If ncores > 1, parallelization is attempted.                                                                                                                                        |
| useC            | Use C++ version of updates for speed.                                                                                                                                                                                    |
| unif.stop       | Terminate if any of columns in basis matrix is uniform.                                                                                                                                                                  |

### Details

The main input is the `scNMFSet` object with count matrix. This function performs non-negative factorization using Bayesian algorithm and gamma priors. Slots `basis`, `coeff`, and `ranks` are filled.

When run with multiple values of ranks, factorization is repeated for each rank and the slot `measure` contains log evidence and optimal hyperparameters for each rank. With `nrun > 1`, the solution with the maximum log evidence is stored for a given rank.

### Value

Object of class `scNMFSet` with factorization slots filled.

### Examples

```
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s, ranks=seq(2,8), nrun=5)
plot(s)
```

---

|                                 |                           |
|---------------------------------|---------------------------|
| <code>visualize_clusters</code> | <i>Visualize clusters</i> |
|---------------------------------|---------------------------|

---

### Description

Use tSNE to generate two-dimensional map of coefficient matrix.

### Usage

```
visualize_clusters(object, rank, verbose = FALSE, cex = 1,
  cex.names = 0.7, ...)
```

### Arguments

|                        |                                                  |
|------------------------|--------------------------------------------------|
| <code>object</code>    | scNMF object.                                    |
| <code>rank</code>      | Rank value to extract from object.               |
| <code>verbose</code>   | Print tSNE messages.                             |
| <code>cex</code>       | Symbol size in tSNE plot                         |
| <code>cex.names</code> | Font size of labels in count barplot.            |
| <code>...</code>       | Other parameters to send to <code>Rtsne</code> . |

### Details

It retrieves a coefficient matrix `H` from an object and use its elements to assign each cell into clusters. t-Distributed Stochastic Neighbor Embedding (t-SNE; <https://lvdmaaten.github.io/tsne/>) is used to visualize the clustering in 2D. Also plotted is the distribution of cell counts for all clusters.

**Value**

NULL

**Examples**

```
set.seed(1)
x <- simulate_data(nfeatures=10,nsamples=c(20,20,60,40,30))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(170)
s <- scNMFSets(count=x,rowData=seq_len(10),colData=seq_len(170))
s <- vb_factorize(s,ranks=seq(2,5))
visualize_clusters(s,rank=5)
```

write\_10x

*Write 10x data files***Description**

Use an object and write count and annotation files in 10x format.

**Usage**

```
write_10x(object, dir, count = "matrix.mtx", genes = "genes.tsv",
  barcodes = "barcodes.tsv", quote = FALSE)
```

**Arguments**

|          |                                                 |
|----------|-------------------------------------------------|
| object   | Object of class scNMFSets containing count data |
| dir      | Directory where files are to be written.        |
| count    | File name for count matrix.                     |
| genes    | File name for gene annotation.                  |
| barcodes | File name for cell annotation.                  |
| quote    | Suppress quotation marks in output files.       |

**Value**

NULL

**Examples**

```
set.seed(1)
x <- matrix(rpois(n=12,lambda=3),4,3)
rownames(x) <- seq_len(4)
colnames(x) <- seq_len(3)
s <- scNMFSets(count=x,rowData=seq_len(4),colData=seq_len(3))
write_10x(s,dir='.')
```

---

|            |                                   |
|------------|-----------------------------------|
| write_meta | <i>Write meta genes to a file</i> |
|------------|-----------------------------------|

---

**Description**

Write a csv file of meta gene lists from input list

**Usage**

```
write_meta(meta, file)
```

**Arguments**

|      |                                           |
|------|-------------------------------------------|
| meta | List of meta genes output from meta_genes |
| file | Output file name                          |

**Value**

NULL

**Examples**

```
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s, ranks=seq(2,8), nrun=5)
plot(s)
m <- meta_genes(s, rank=5)
write_meta(m, file='meta.csv')
```

---

|                               |                                   |
|-------------------------------|-----------------------------------|
| [,scNMFSet,ANY,ANY,ANY-method | <i>Subsetting scNMFSet object</i> |
|-------------------------------|-----------------------------------|

---

**Description**

Subsetting scNMFSet object

**Usage**

```
## S4 method for signature 'scNMFSet,ANY,ANY,ANY'
x[i, j]
```

**Arguments**

|   |                        |
|---|------------------------|
| x | Object to be subsetted |
| i | row index              |
| j | column index           |

**Value**

Subsetted object

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