

# Package ‘UCell’

March 28, 2023

**Type** Package

**Title** Rank-based signature enrichment analysis for single-cell data

**Version** 2.2.0

**Description** UCell is a package for evaluating gene signatures in single-cell datasets. UCell signature scores, based on the Mann-Whitney U statistic, are robust to dataset size and heterogeneity, and their calculation demands less computing time and memory than other available methods, enabling the processing of large datasets in a few minutes even on machines with limited computing power. UCell can be applied to any single-cell data matrix, and includes functions to directly interact with SingleCellExperiment and Seurat objects.

**Depends** R(>= 4.2.0)

**Imports** methods, data.table(>= 1.13.6), Matrix, BiocParallel, BiocNeighbors, SingleCellExperiment, SummarizedExperiment

**Suggests** Seurat, scater, scRNAseq, reshape2, patchwork, ggplot2, BiocStyle, knitr, rmarkdown

**biocViews** SingleCell, GeneSetEnrichment, Transcriptomics, GeneExpression, CellBasedAssays

**VignetteBuilder** knitr

**BugReports** <https://github.com/carmonalab/UCell/issues>

**URL** <https://github.com/carmonalab/UCell>

**License** GPL-3 + file LICENSE

**Encoding** UTF-8

**LazyData** FALSE

**Roxygen** list(markdown = TRUE)

**RoxygenNote** 7.2.1

**git\_url** <https://git.bioconductor.org/packages/UCell>

**git\_branch** RELEASE\_3\_16

**git\_last\_commit** eeac63d

**git\_last\_commit\_date** 2022-11-01

**Date/Publication** 2023-03-27

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AddModuleScore\_UCell *Calculate module enrichment scores from single-cell data (Seurat interface)*

---

## Description

Given a Seurat object, calculates module/signature enrichment scores at single-cell level using the Mann-Whitney U statistic. UCell scores are normalized U statistics (between 0 and 1), and they are mathematically related to the Area under the ROC curve (see [Mason and Graham](#))

## Usage

```
AddModuleScore_UCell(
  obj,
  features,
  maxRank = 1500,
  chunk.size = 1000,
  BPPARAM = NULL,
  ncores = 1,
  storeRanks = FALSE,
  w_neg = 1,
```

```

    assay = NULL,
    slot = "data",
    ties.method = "average",
    force.gc = FALSE,
    name = "_UCell"
  )

```

## Arguments

obj	Seurat object
features	A list of signatures, for example: <code>list(Tcell_signature = c("CD2", "CD3E", "CD3D"), Myeloid_signature = c("SPI1", "FCER1G", "CSF1R"))</code> You can also specify positive and negative gene sets by adding a + or - sign to genes in the signature; see an example below
maxRank	Maximum number of genes to rank per cell; above this rank, a given gene is considered as not expressed.
chunk.size	Number of cells to be processed simultaneously (lower size requires slightly more computation but reduces memory demands)
BPPARAM	A <code>BiocParallel::bpparam()</code> object that tells UCell how to parallelize. If provided, it overrides the <code>ncores</code> parameter.
ncores	Number of processors to parallelize computation. If <code>BPPARAM = NULL</code> , the function uses <code>BiocParallel::MulticoreParam(workers=ncores)</code>
storeRanks	Store ranks matrix in Seurat object ('UCellRanks' assay) for fast subsequent computations. This option may demand large amounts of RAM.
w_neg	Weight on negative genes in signature. e.g. <code>w_neg=1</code> weighs equally up- and down-regulated genes, <code>w_neg=0.5</code> gives 50% less importance to negative genes
assay	Pull out data from this assay of the Seurat object (if <code>NULL</code> , use <code>DefaultAssay(obj)</code> )
slot	Pull out data from this slot of the Seurat object
ties.method	How ranking ties should be resolved - passed on to <code>data.table::frank</code>
force.gc	Explicitly call garbage collector to reduce memory footprint
name	Name tag that will be appended at the end of each signature name, "_UCell" by default (e.g. signature score in meta data will be named: <code>Myeloid_signature_UCell</code> )

## Details

In contrast to Seurat's `AddModuleScore`, which is normalized by binning genes of similar expression at the population level, UCell scores depend only on the gene expression ranks of individual cell, and therefore they are robust across datasets regardless of dataset composition.

## Value

Returns a Seurat object with module/signature enrichment scores added to object meta data; each score is stored as the corresponding signature name provided in `features` followed by the tag given in `name` (or "\_UCell" by default)

**Examples**

```

library(UCell)
gene.sets <- list(Tcell = c("CD2", "CD3E", "CD3D"),
                 Myeloid = c("SPI1", "FCER1G", "CSF1R"))
data(sample.matrix)
obj <- Seurat::CreateSeuratObject(sample.matrix)

obj <- AddModuleScore_UCell(obj, features = gene.sets)
head(obj[[[]])

## Using positive and negative gene sets
gene.sets <- list()
gene.sets$Tcell_gd <- c("TRDC+", "TRGC1+", "TRGC2+", "TRDV1+",
                      "TRAC-", "TRBC1-", "TRBC2-")
gene.sets$NKcell <- c("FGFBP2+", "SPON2+", "KLRF1+",
                    "FCGR3A+", "CD3E-", "CD3G-")
obj <- AddModuleScore_UCell(obj, features = gene.sets, name=NULL)
head(obj$NKcell)

```

---

calculate\_Uscore

*Calculate rankings and scores for query data and given signature set*


---

**Description**

Calculate rankings and scores for query data and given signature set

**Usage**

```

calculate_Uscore(
  matrix,
  features,
  maxRank = 1500,
  chunk.size = 1000,
  BPPARAM = NULL,
  ncores = 1,
  w_neg = 1,
  ties.method = "average",
  storeRanks = FALSE,
  force.gc = FALSE,
  name = "_UCell"
)

```

**Arguments**

matrix	Input data matrix
features	List of signatures
maxRank	Rank cutoff (1500)

chunk.size	Cells per sub-matrix (1000)
BPPARAM	A BioParallel object to instruct UCell how to parallelize
ncores	Number of cores to use for parallelization
w_neg	Weight on negative signatures
ties.method	How to break ties, for data.table::frankv method ("average")
storeRanks	Store ranks? (FALSE)
force.gc	Force garbage collection? (FALSE)
name	Suffix for metadata columns ("_UCell")

**Value**

A list of signature scores

---

check_genes	<i>Check genes</i>
-------------	--------------------

---

**Description**

Check if all genes in signatures are found in data matrix - otherwise add zero counts in data-matrix to complete it

**Usage**

```
check_genes(matrix, features)
```

**Arguments**

matrix	Input data matrix
features	List of genes that must be present (otherwise they are added)

**Value**

Same input matrix, extended to comprise any missing genes

---

check\_signature\_names *Check signature names and add standard names is missing*

---

**Description**

Check signature names and add standard names is missing

**Usage**

```
check_signature_names(features)
```

**Arguments**

features            List of signatures for scoring

**Value**

The input list of signatures, with standard names if provided un-named

---

data\_to\_ranks\_data\_table  
*Calculate per-cell feature rankings*

---

**Description**

Calculate per-cell feature rankings

**Usage**

```
data_to_ranks_data_table(data, ties.method = "average")
```

**Arguments**

data                Expression data matrix  
ties.method        How to break ties (passed on to data.table::frankv)

**Value**

A data.table of ranks

---

knn\_smooth\_scores      *Smoothing scores by KNN*

---

### Description

Smoothing scores by KNN

### Usage

```
knn_smooth_scores(matrix = NULL, nn = NULL)
```

### Arguments

matrix	Input data matrix
nn	A nearest neighbor object returned by <a href="#">BiocNeighbors::findKNN</a>

### Value

A dataframe of knn-smoothed scores

---

rankings2Uscore      *Get signature scores from pre-computed rank matrix*

---

### Description

Get signature scores from pre-computed rank matrix

### Usage

```
rankings2Uscore(  
  ranks_matrix,  
  features,  
  chunk.size = 1000,  
  w_neg = 1,  
  BPPARAM = NULL,  
  ncores = 1,  
  force.gc = FALSE,  
  name = "_UCell"  
)
```

**Arguments**

ranks_matrix	A rank matrix
features	List of signatures
chunk.size	How many cells per matrix chunk
w_neg	Weight on negative signatures
BPPARAM	A BioParallel object to instruct UCell how to parallelize
ncores	How many cores to use for parallelization?
force.gc	Force garbage collection to recover RAM? (FALSE)
name	Name suffix for metadata columns ("_UCell")

**Value**

A list of signature scores

---

sample.matrix	<i>Sample dataset to test UCell installation</i>
---------------	--

---

**Description**

A sparse matrix (class "dgCMatrix") of single-cell transcriptomes (scRNA-seq) for 600 cells and 20729 genes. Single-cell UMI counts were normalized using a standard log-normalization: counts for each cell were divided by the total counts for that cell and multiplied by 10,000, then natural-log transformed using  $\log_1 p$ .

This a subsample of T cells from the large scRNA-seq PBMC dataset published by [Hao et al.](#) and available as UMI counts at [https://atlas.fredhutch.org/data/nygc/multimodal/pbmc\\_multimodal.h5seurat](https://atlas.fredhutch.org/data/nygc/multimodal/pbmc_multimodal.h5seurat)

**Usage**

```
sample.matrix
```

**Format**

A sparse matrix of 600 cells and 20729 genes.

**Source**

<https://doi.org/10.1016/j.cell.2021.04.048>



---

ScoreSignatures\_UCell *Calculate module enrichment scores from single-cell data*

---

## Description

Given a gene vs. cell matrix, calculates module/signature enrichment scores on single-cell level using Mann-Whitney U statistic. UCell scores are normalized U statistics (between 0 and 1), and they are mathematically related to the Area under the ROC curve (see [Mason and Graham](#)) These scores only depend on the gene expression ranks of individual cell, and therefore they are robust across datasets regardless of dataset composition.

## Usage

```
ScoreSignatures_UCell(
  matrix = NULL,
  features,
  precalc.ranks = NULL,
  maxRank = 1500,
  w_neg = 1,
  name = "_UCell",
  assay = "counts",
  chunk.size = 1000,
  BPPARAM = NULL,
  ncores = 1,
  ties.method = "average",
  force.gc = FALSE
)
```

## Arguments

matrix	Input matrix, either stored in a <a href="#">SingleCellExperiment</a> object or as a raw matrix. dgCMatrx format supported.
features	A list of signatures, for example: <code>list(Tcell_signature = c("CD2", "CD3E", "CD3D"), Myeloid_signature = c("SPI1", "FCER1G", "CSF1R"))</code> You can also specify positive and negative gene sets by adding a + or - sign to genes in the signature; see an example below
precalc.ranks	If you have pre-calculated ranks using <a href="#">StoreRankings_UCell</a> , you can specify the pre-calculated ranks instead of the gene vs. cell matrix.
maxRank	Maximum number of genes to rank per cell; above this rank, a given gene is considered as not expressed. Note: this parameter is ignored if precalc.ranks are specified
w_neg	Weight on negative genes in signature. e.g. w_neg=1 weighs equally up- and down-regulated genes, w_neg=0.5 gives 50% less importance to negative genes
name	Name suffix appended to signature names
assay	The sce object assay where the data is to be found

<code>chunk.size</code>	Number of cells to be processed simultaneously (lower size requires slightly more computation but reduces memory demands)
<code>BPPARAM</code>	A <code>BiocParallel::bpparam()</code> object that tells UCell how to parallelize. If provided, it overrides the <code>ncores</code> parameter.
<code>ncores</code>	Number of processors to parallelize computation. If <code>BPPARAM = NULL</code> , the function uses <code>BiocParallel::MulticoreParam(workers=ncores)</code>
<code>ties.method</code>	How ranking ties should be resolved - passed on to <code>data.table::frank</code>
<code>force.gc</code>	Explicitly call garbage collector to reduce memory footprint

**Value**

Returns input `SingleCellExperiment` object with UCell scores added to `altExp`

**Examples**

```
library(UCell)
# Using sparse matrix
data(sample.matrix)
gene.sets <- list( Tcell_signature = c("CD2", "CD3E", "CD3D"),
                  Myeloid_signature = c("SPI1", "FCER1G", "CSF1R"))
scores <- ScoreSignatures_UCell(sample.matrix, features=gene.sets)
head(scores)

# Using sce object
library(SingleCellExperiment)
data(sample.matrix)
my.sce <- SingleCellExperiment(list(counts=sample.matrix))
gene.sets <- list( Tcell_signature = c("CD2", "CD3E", "CD3D"),
                  Myeloid_signature = c("SPI1", "FCER1G", "CSF1R"))
my.sce <- ScoreSignatures_UCell(my.sce, features=gene.sets)
altExp(my.sce, 'UCell')
```

---

SmoothKNN.Seurat

*Smooth signature scores by kNN*

---

**Description**

This function performs smoothing of single-cell scores by weighted average of the k-nearest neighbors. It can be useful to 'impute' scores by neighboring cells and partially correct data sparsity. While this function has been designed to smooth UCell scores, it can be applied to any numerical metadata contained in `SingleCellExperiment` or `Seurat` objects

**Usage**

```

## S3 method for class 'Seurat'
SmoothKNN(
  obj = NULL,
  signature.names = NULL,
  reduction = "pca",
  k = 10,
  BNPARAM = AnnoyParam(),
  BPPARAM = SerialParam(),
  suffix = "_kNN",
  sce.expname = NULL,
  sce.newassay = NULL
)

## S3 method for class 'SingleCellExperiment'
SmoothKNN(
  obj = NULL,
  signature.names = NULL,
  reduction = "PCA",
  k = 10,
  BNPARAM = AnnoyParam(),
  BPPARAM = SerialParam(),
  suffix = NULL,
  sce.expname = "UCell",
  sce.newassay = "UCell_kNN"
)

SmoothKNN(
  obj = NULL,
  signature.names = NULL,
  reduction = "pca",
  k = 10,
  BNPARAM = AnnoyParam(),
  BPPARAM = SerialParam(),
  suffix = "_kNN",
  sce.expname = "UCell",
  sce.newassay = "UCell_kNN"
)

```

**Arguments**

<code>obj</code>	Input object - either a <a href="#">SingleCellExperiment</a> object or a Seurat object.
<code>signature.names</code>	The names of the signatures (or any numeric metadata column) for which to calculate kNN-smoothed scores
<code>reduction</code>	Which dimensionality reduction to use for kNN smoothing. It must be already present in the input object.
<code>k</code>	Number of neighbors for kNN smoothing

BNPARAM	A <a href="#">BiocNeighborParam</a> object specifying the algorithm to use for kNN calculation.
BPPARAM	A <a href="#">BiocParallel::bpparam()</a> object for parallel computing, e.g. <a href="#">MulticoreParam</a> or <a href="#">SnowParam</a>
suffix	For Seurat objects only - Suffix to append to metadata columns for the new knn-smoothed scores
sce.expname	For sce objects only - which experiment stores the signatures to be smoothed
sce.newassay	For sce objects only - name of the new assay for storing smoothed scores (in experiment sce.expname)

### Value

An augmented obj with the smoothed signatures. If obj is a Seurat object, smoothed signatures are added to metadata; if obj is a SingleCellExperiment object, smoothed signatures are returned in a new assay. See the examples below.

### Examples

```
#### Using Seurat ####
library(Seurat)
gene.sets <- list(Tcell = c("CD2", "CD3E", "CD3D"),
                 Myeloid = c("SPI1", "FCER1G", "CSF1R"))
data(sample.matrix)
obj <- Seurat::CreateSeuratObject(sample.matrix)
# Calculate UCell scores
obj <- AddModuleScore_UCell(obj, features = gene.sets, name=NULL)
# Run PCA
obj <- FindVariableFeatures(obj) |> ScaleData() |> RunPCA()
# Smooth signatures
obj <- SmoothKNN(obj, reduction="pca", signature.names=names(gene.sets))
head(obj[[]])

#### Using SingleCellExperiment ####
library(SingleCellExperiment)
library(scater)
data(sample.matrix)
sce <- SingleCellExperiment(list(counts=sample.matrix))
gene.sets <- list( Tcell = c("CD2", "CD3E", "CD3D"),
                 Myeloid = c("SPI1", "FCER1G", "CSF1R"))
# Calculate UCell scores
sce <- ScoreSignatures_UCell(sce, features=gene.sets, name=NULL)
# Run PCA
sce <- logNormCounts(sce)
sce <- runPCA(sce, scale=TRUE, ncomponents=20)
# Smooth signatures
sce <- SmoothKNN(sce, reduction="PCA", signature.names=names(gene.sets))
# See results
altExp(sce, 'UCell')
assays(altExp(sce, 'UCell'))
# Plot on UMAP
```

```
sce <- runUMAP(sce, dimred="PCA")
plotUMAP(sce, colour_by = "Tcell", by_exprs_values = "UCell_kNN")
```

---

split\_data.matrix      *Split data matrix into smaller sub-matrices ('chunks')*

---

### Description

Split data matrix into smaller sub-matrices ('chunks')

### Usage

```
split_data.matrix(matrix, chunk.size = 1000)
```

### Arguments

matrix	Input data matrix
chunk.size	How many cells to include in each sub-matrix

### Value

A list of sub-matrices, each with size `n_features` x `chunk_size`

---

StoreRankings\_UCell      *Calculate and store gene rankings for a single-cell dataset*

---

### Description

Given a gene vs. cell matrix, calculates the rankings of expression for all genes in each cell.

### Usage

```
StoreRankings_UCell(
  matrix,
  maxRank = 1500,
  chunk.size = 1000,
  BPPARAM = NULL,
  ncores = 1,
  assay = "counts",
  ties.method = "average",
  force.gc = FALSE
)
```

**Arguments**

<code>matrix</code>	Input matrix, either stored in a <a href="#">SingleCellExperiment</a> object or as a raw matrix. <code>dgCMatrx</code> format supported.
<code>maxRank</code>	Maximum number of genes to rank per cell; above this rank, a given gene is considered as not expressed
<code>chunk.size</code>	Number of cells to be processed simultaneously (lower size requires slightly more computation but reduces memory demands)
<code>BPPARAM</code>	A <a href="#">BiocParallel::bpparam()</a> object that tells UCell how to parallelize. If provided, it overrides the <code>ncores</code> parameter.
<code>ncores</code>	Number of processors to parallelize computation. If <code>BPPARAM = NULL</code> , the function uses <code>BiocParallel::MulticoreParam(workers=ncores)</code>
<code>assay</code>	Assay where the data is to be found (for input in 'sce' format)
<code>ties.method</code>	How ranking ties should be resolved - passed on to <a href="#">data.table::frank</a>
<code>force.gc</code>	Explicitly call garbage collector to reduce memory footprint

**Details**

While [ScoreSignatures\\_UCell](#) can be used 'on the fly' to evaluate signatures in a query dataset, it requires recalculating gene ranks at every execution. If you have a large dataset and plan to experiment with multiple signatures, evaluating the same dataset multiple times, this function allows you to store pre-calculated ranks so they do not have to be recomputed every time. Pre-calculated ranks can then be applied to the function [ScoreSignatures\\_UCell](#) to evaluate gene signatures in a significantly faster way on successive iterations.

**Value**

Returns a sparse matrix of pre-calculated ranks that can be used multiple times to evaluate different signatures

**Examples**

```
library(UCell)
data(sample.matrix)
ranks <- StoreRankings_UCell(sample.matrix)
ranks[1:5,1:5]
gene.sets <- list( Tcell_signature = c("CD2", "CD3E", "CD3D"),
                  Myeloid_signature = c("SPI1", "FCER1G", "CSF1R"))
scores <- ScoreSignatures_UCell(features=gene.sets, precalc.ranks=ranks)
head(scores)
```

**Description**

UCell is an R package for scoring gene signatures in single-cell datasets. UCell scores, based on the Mann-Whitney U statistic, are robust to dataset size and heterogeneity, and their calculation demands relatively less computing time and memory than most other methods, enabling the processing of large datasets ( $> 10^5$  cells). UCell can be applied to any cell vs. gene data matrix, and includes functions to directly interact with Seurat and SingleCellExperiment objects.

**UCell functions**

- `ScoreSignatures_UCell` Calculate module enrichment scores from single-cell data. Given a gene vs. cell matrix (either as sparse matrix or stored in a `SingleCellExperiment` object), it calculates module/signature enrichment scores. This score depends only on the gene activity ranks of individual cell, and therefore is robust across datasets.
- `AddModuleScore_UCell` A wrapper for UCell to interact directly with Seurat objects. Given a Seurat object and a set of signatures, it calculates enrichment scores on single-cell level and returns them into the `meta.data` of the input Seurat object.
- `StoreRankings_UCell` Calculates and stores gene rankings for a single-cell dataset. Given a gene vs. cell matrix and a set of signatures, it calculates the rankings of expression for all genes in each cell. It can then be applied to the function `ScoreSignatures_UCell` to evaluate gene signatures on the gene expression ranks of individual cells.
- `SmoothKNN` Perform signature score smoothing using a weighted average of the scores of the first  $k$  nearest neighbors (kNN). It can be useful to 'impute' scores by neighboring cells and partially correct data sparsity. While this function has been designed to smooth UCell scores, it can be applied to any numerical metadata contained in `SingleCellExperiment` or Seurat objects

**Gene signatures**

UCell evaluates the strength of gene signatures (or gene sets) in individual cells of your dataset. You may specify positive and negative (up- or down-regulated) genes in signatures. See the examples below:

```
markers <- list()
markers$Tcell_CD4 <- c("CD4", "CD40LG")
markers$Tcell_CD8 <- c("CD8A", "CD8B")
markers$Tcell_Treg <- c("FOXP3", "IL2RA")
markers$Tcell_gd <- c("TRDC+", "TRGC1+", "TRGC2+",
                    "TRDV1+", "TRAC-", "TRBC1-", "TRBC2-")
markers$Tcell_NK <- c("FGFBP2+", "SPON2+", "KLRF1+",
                    "FCGR3A+", "CD3E-", "CD3G-")
```

If you don't specify +/- for genes, they are assumed to be all as a positive set. The UCell score is calculated as:

$$U = \max(0, U^+ - w_{neg} * U^-)$$

where  $U^+$  and  $U^-$  are respectively the UCell scores for the positive and negative set, and  $w_{neg}$  is a weight on the negative set. When no negative set of genes is present,  $U = U^+$

## References

UCell: robust and scalable single-cell gene signature scoring. Massimo Andreatta & Santiago J Carmona (2021) CSBJ <https://doi.org/10.1016/j.csbj.2021.06.043>

---

u_stat	<i>Calculate Mann Whitney U from a vector of ranks</i>
--------	--

---

## Description

Calculate Mann Whitney U from a vector of ranks

## Usage

```
u_stat(rank_value, maxRank = 1000, sparse = FALSE)
```

## Arguments

rank_value	A vector of ranks
maxRank	Max number of features to include in ranking
sparse	Whether the vector of ranks is in sparse format

## Value

Normalized AUC (as U statistic) for the vector

---

u_stat_signature_list	<i>Calculate U scores for a list of signatures, given a rank matrix</i>
-----------------------	---

---

## Description

Calculate U scores for a list of signatures, given a rank matrix

## Usage

```
u_stat_signature_list(
  sig_list,
  ranks_matrix,
  maxRank = 1000,
  sparse = FALSE,
  w_neg = 1
)
```



**Arguments**

<code>sig_list</code>	A list of signatures
<code>ranks_matrix</code>	Matrix of pre-computed ranks
<code>maxRank</code>	Max number of features to include in ranking, for <code>u_stat</code> function
<code>sparse</code>	Whether the vector of ranks is in sparse format
<code>w_neg</code>	Weight on negative signatures

**Value**

A matrix of U scores

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