

# Package ‘simpleSeg’

July 17, 2025

**Type** Package

**Title** A package to perform simple cell segmentation

**Description** Image segmentation is the process of identifying the borders of individual objects (in this case cells) within an image. This allows for the features of cells such as marker expression and morphology to be extracted, stored and analysed. simpleSeg provides functionality for user friendly, watershed based segmentation on multiplexed cellular images in R based on the intensity of user specified protein marker channels. simpleSeg can also be used for the normalization of single cell data obtained from multiple images.

**Version** 1.10.1

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**VignetteBuilder** knitr

**Encoding** UTF-8

**biocViews** Classification, Survival, SingleCell, Normalization, Spatial

**Imports** BiocParallel, EBImage, terra, stats, spatstat.geom, S4Vectors, grDevices, SummarizedExperiment, methods, cytomapper

**Suggests** BiocStyle, testthat (>= 3.0.0), knitr, ggplot2

**License** GPL-3

**RoxygenNote** 7.2.3

**Config/testthat/edition** 3

**BugReports** <https://github.com/SydneyBioX/simpleSeg/issues>

**URL** <https://sydneybiox.github.io/simpleSeg/>  
<https://github.com/SydneyBioX/simpleSeg>

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generateBPParam	<i>Utility function to generate BPPARM object.</i>
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Description

Utility function to generate BPPARM object.

Usage

generateBPParam(cores = 1)

Arguments

cores                      Desired number of cores for BPPARAM object.

Value

A BPPPARAM object.

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normalizeCells	<i>Normalizes and transforms cell data in preparation for clustering (accepts dataframe, SingleCellExperiment and SpatialExperiment).</i>
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Description

Normalizes and transforms cell data in preparation for clustering (accepts dataframe, SingleCellExperiment and SpatialExperiment).

**Usage**

```
normalizeCells(
  cells,
  markers = NULL,
  assayIn = NULL,
  assayOut = "norm",
  imageID = "imageID",
  transformation = NULL,
  method = NULL,
  cores = 1
)
```

**Arguments**

cells	A Dataframe of SingleCellExperiment or SpatialExperiment containing cells and features to be normalized/transformed
markers	A list containing the names of cell markers which will be normalized and/or transformed.
assayIn	If input is a SingleCellExperiment or SpatialExperiment with multiple assays, specify the assay to be normalized and/or transformed.
assayOut	If input is a SingleCellExperiment or SpatialExperiment, the new of the normalized data.
imageID	If input is a SingleCellExperiment or SpatialExperiment, this is the name of the image ID variable in order to stratify. cells correctly
transformation	The transformation/s to be performed, default is NULL, accepted values: 'asinh' and 'sqrt'.
method	The normalization method/s to be performed, default is NULL, accepted values: 'mean', 'minMax', 'trim99', 'PC1'.
cores	The number of cores for parallel processing.

**Value**

returns a dataframe with individual cells as rows and features as columns.

**Examples**

```
library(cytomapper)
data("pancreasSCE")
cells.normalized <- normalizeCells(
  cells = pancreasSCE,
  markers = c("CD99", "PIN", "CD8a", "CDH"),
  assayIn = "counts",
  assayOut = "normCounts",
  imageID = "ImageNb",
  transformation = "asinh",
  method = "trim99"
)
```

simpleSeg

*Perform simple segmentation of multiplexed cellular images***Description**

Perform simple segmentation of multiplexed cellular images

**Usage**

```
simpleSeg(
  image,
  nucleus,
  cellBody = "dilate",
  sizeSelection = 10,
  smooth = 1,
  transform = NULL,
  watershed = "intensity",
  tolerance = NULL,
  ext = 1,
  discSize = 3,
  tissue = NULL,
  pca = FALSE,
  cores = 1
)
```

**Arguments**

image	An image or list of images or CytoImageList to be read into the function.
nucleus	The marker or list of markers corresponding to the nuclei.
cellBody	Method of cytoplasm identification. Can be 'none', 'dilate', 'discModel' or the name of a dedicated cytoplasm marker
sizeSelection	Minimum pixels for an object to be recognized as a cell and not noise.
smooth	The amount of Gaussian smoothing to be applied to the image/s
transform	A transformation or list of transformations and normalizations to be performed prior to nuclei or cytoplasm identification. Accepted vales: "sqrt", "asinh", "norm99", "maxThresh" and "tissueMask". Tissue mask may be used when the sample does not take up the entirety of the image (typically a circular sample inside the image. When tissue mask is specified the background noise present outside the sample area is removed).
watershed	Method used to perform watershed. Accepted values: "intensity", "distance" or "combine".
tolerance	The minimum height of the object in the units of image intensity between its highest point (seed) and the point where it contacts another object (checked for every contact pixel). If the height is smaller than the tolerance, the object will be combined with one of its neighbors, which is the highest. Tolerance should

	be chosen according to the range of x. Default value is 1, which is a reasonable value if x comes from distmap.
ext	Radius of the neighborhood in pixels for the detection of neighboring objects. Higher value smooths out small objects.
discSize	The size of dilation around nuclei to create cell disc or capture cytoplasm
tissue	Channels to be used to create the tissue mask if specified in transforms.
pca	Whether to run PCA on aggregated nucleus markers in order to detect the cellular nuclei.
cores	The number of cores for parallel processing or a BPPARAM object

**Value**

A list of image masks

**Examples**

```
library(cytomapper)
data("pancreasImages")
masks <- simpleSeg(pancreasImages,
  nucleus = "H3",
  cellBody = "discModel",
  sizeSelection = 8,
  smooth = 1.2,
  transform = "sqrt",
  watershed = "combine",
  tolerance = 1, ext = 1,
  discSize = 3,
  cores = 5
)
```

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