

Package ‘nucim’

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Title Nucleome Imaging Toolbox

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Depends R (>= 3.0.0), EBImage, bioimager (>= 1.1.4)

Imports fields, parallel, stringr

SystemRequirements tiff fftw libcurl openssl

Description Tools for 4D nucleome imaging.

Quantitative analysis of the 3D nuclear landscape recorded with super-resolved fluorescence microscopy.

See Volker J. Schmid, Marion Cremer, Thomas Cremer (2017) <[doi:10.1016/j.ymeth.2017.03.013](https://doi.org/10.1016/j.ymeth.2017.03.013)>.

License GPL-3

URL <https://bioimaginggroup.github.io/nucim/>

RoxygenNote 7.3.3

Encoding UTF-8

Suggests knitr, rmarkdown, R.rsp

VignetteBuilder knitr, R.rsp

BugReports <https://github.com/bioimaginggroup/nucim/issues>

NeedsCompilation no

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barplot_with_interval *Barplot with Intervals*

Description

Barplot with Intervals

Usage

```
barplot_with_interval(
  x,
  method = "minmax",
  qu = c(0, 1),
  ylim = NULL,
  horiz = FALSE,
  border = NA,
  ...
)
```

Arguments

x	matrix
method	method for intervals: "minmax" (default), "quantile" or "sd"
qu	vector of two quantiles for method="quantile"
ylim	limits for y axis. Default:NULL is ylim=c(0,max(interval))
horiz	boolean: horizontal bars?
border	border parameter forwarded to barplot, default: NA is nor border
...	additional parameters forwarded to barplot

Value

plot

barplot_with_interval_23

Barplot with Intervals for two or three bars beside

Description

Barplot with Intervals for two or three bars beside

Usage

```
barplot_with_interval_23(x, method = "minmax", qu = c(0, 1), ylim = NULL, ...)
```

Arguments

x	array
method	method for intervals: "minmax" (default), "quantile" or "sd"
qu	vector of two quantiles for method="quantile"
ylim	limits for y axis. Default:NULL is ylim=c(0,max(interval))
...	additional parameters forwarded to barplot

Value

plot

class.neighbours *Class neighbourhood distribution*

Description

Class neighbourhood distribution

Usage

```
class.neighbours(img, N, N.max = 7, cores = 1)
```

Arguments

img	Class image
N	which class
N.max	maximum class (default: 7)
cores	number of cores used in parallel (needs parallel package)

Value

vector of length N.max

class.neighbours.folder
class.neighbours.folder

Description

class.neighbours.folder

Usage

```
class.neighbours.folder(inputfolder, outputfolder, N = 7)
```

Arguments

inputfolder	Input folder
outputfolder	Output folder
N	Max class #'

Value

plots

classify	<i>Classify DAPI</i>
----------	----------------------

Description

Classify DAPI

Usage

```
classify(blue, mask, N, beta = 0.1, z = 1/3, silent = TRUE)
```

Arguments

blue	DAPI channel (image)
mask	mask (image)
N	number of classes
beta	smoothing parameter used in potts model (default: 0.1)
z	scaling parameter: size of voxel in X-/Y-direction divided by the size of voxel in Z-direction (slice scaling parameter: size of voxel in X-/Y-direction divided by the size of voxel in Z-direction (slice thickness))
silent	boolean. Should algorithm be silent?

Value

image with classes

classify.folder	<i>Classify DAPI</i>
-----------------	----------------------

Description

Classify DAPI

Usage

```
classify.folder(f, N, beta = 0.1, output = paste0("class", N), cores = 1)
```

Arguments

f	folder
N	number of classes
beta	beta parameter used in bioimager::segment()
output	output folder
cores	number of cores used in parallel (needs parallel package)

Value

results in "output" and "output"-n

classify.single	<i>Classify DAPI</i>
-----------------	----------------------

Description

These functions are provided for compatibility with older version of the nucim package. They may eventually be completely removed.

Usage

```
classify.single(...)
```

Arguments

... parameters for classify

Value

image with classes

classify.table	<i>Count classes in classified image</i>
----------------	--

Description

Count classes in classified image

Usage

```
classify.table(class, N)
```

Arguments

class	classes image
N	number of classes

Value

table with number of voxels per class

colors.in.classes *Compute colors in classes distribution*

Description

Compute colors in classes distribution

Usage

```
colors.in.classes(  
  classes,  
  color1,  
  color2 = NULL,  
  mask = array(TRUE, dim(classes)),  
  N = max(classes, na.rm = TRUE),  
  type = "tresh",  
  thresh1 = NULL,  
  thresh2 = NULL,  
  sd1 = 2,  
  sd2 = 2,  
  col1 = "green",  
  col2 = "red",  
  test = FALSE,  
  plot = TRUE,  
  beside = TRUE,  
  ylim = NULL,  
  verbose = FALSE,  
  ...  
)
```

Arguments

classes	Image of classes
color1	Image of first color
color2	Image of second color
mask	Image mask
N	Maximum number of classes
type	Type of spot definition, see details
thresh1	Threshold for first color image
thresh2	Threshold for second color image
sd1	For automatic threshold, that is: $\text{mean}(\text{color1}) + \text{sd1} * \text{sd}(\text{color1})$
sd2	For automatic threshold of color2
col1	Name of color 1
col2	Name of color 2

test	Compute tests: "Wilcoxon" for Wilcoxon rank-sum (Mann-Whitney U), chisq for Chi-squared test
plot	Plot barplots
beside	a logical value. If FALSE, the columns of height are portrayed as stacked bars, and if TRUE the columns are portrayed as juxtaposed bars.
ylim	limits for the y axis (plot)
verbose	verbose mode
...	additional plotting parameters

Details

Type of spot definitions: "thresh" or "t": Threshold based (threshold can be given by thresh1/2 or automatically derived) "voxel" or "v": Spots are given as binary voxel mask "intensity" or "i": Voxels are weighted with voxel intensity. Intensity is scaled to [0,1] after subtracting thresh1/2 (or automatic threshold)

Value

Table of classes with color 1 (and 2)

colors.in.classes.folder

Compute colors in classes distribution for folders

Description

Compute colors in classes distribution for folders

Usage

```
colors.in.classes.folder(
  path,
  color1,
  color2 = NULL,
  N = 7,
  type = "intensity",
  thresh1 = NULL,
  thresh2 = NULL,
  sd1 = 2,
  sd2 = 2,
  col1 = "green",
  col2 = "red",
  cores = 1,
  verbose = FALSE
)
```

Arguments

path	Path to root folder
color1	Image of first color
color2	Image of second color
N	Maximum number of classes
type	Type of spot definition, see details
thresh1	Threshold for first color image
thresh2	Threshold for second color image
sd1	For automatic threshold, that is: $\text{mean}(\text{color1}) + \text{sd1} * \text{sd}(\text{color1})$
sd2	For automatic threshold of color2
col1	Name of color 1
col2	Name of color 2
cores	Number of cores used in parallel, cores=1 implies no parallelization
verbose	verbose mode

Value

Results are in folder colorsinclasses

compute.distance2border

Compute distance to border of classes

Description

Compute distance to border of classes

Usage

```
compute.distance2border(  
  f,  
  color,  
  N,  
  from.spots = FALSE,  
  output = "dist2border",  
  cores = 1  
)
```

Arguments

f	folder of classes images
color	folder of color images ("spots-"color for spots images)
N	which class
from.spots	Logical.
output	output folder
cores	number of parallel cores which can be used

Value

images in output-"color"-"N

dapimask	<i>Mask DAPI in kernel</i>
----------	----------------------------

Description

Mask DAPI in kernel

Usage

```
dapimask(
  img,
  size = NULL,
  voxelsize = NULL,
  thresh = "auto",
  silent = TRUE,
  cores = 1
)
```

Arguments

img	DAPI channel image (3d)
size	size of img in microns
voxelsize	size of voxel in microns
thresh	threshold for intensity. Can be "auto": function will try to find automatic threshold
silent	Keep silent?
cores	number of cores available for parallel computing

Value

mask image, array with same dimension as img.

dapimask.file *Automatic DAPI mask segmentation for files*

Description

Automatic DAPI mask segmentation for files

Usage

```
dapimask.file(  
    file,  
    folder = "blue",  
    voxelsize = NULL,  
    size = NULL,  
    silent = FALSE,  
    cores = 1  
)
```

Arguments

file	file to read
folder	with
voxelsize	real size of voxel (in microns), if NULL (default), look in folder XYZmic
size	real size of image (in microns), if NULL (default), look in folder XYZmic
silent	Keep silent?
cores	Number of cores available for parallel computing

Value

nothing, DAPI mask image will be saved to dapimask/

dapimask.folder *Automatic DAPI mask segmentation for folder*

Description

Automatic DAPI mask segmentation for folder

Usage

```
dapimask.folder(  
    path,  
    folder = "blue",  
    voxelsize = NULL,  
    size = NULL,  
    cores = 1  
)
```

Arguments

path	path to folder with DAPI
folder	folder with DAPI images
voxelsize	real size of voxel (in microns), if NULL (default), look in folder XYZmic
size	real size of image (in microns), if NULL (default), look in folder XYZmic
cores	number of cores to use in parallel (need parallel package)

Value

nothing, results are in folder dapimask

find.spots.file	<i>Detects spots for one file</i>
-----------------	-----------------------------------

Description

Detects spots for one file

Usage

```
find.spots.file(
  file,
  dir,
  color,
  thresh = NULL,
  thresh.auto = FALSE,
  thresh.quantile = 0.9,
  filter = NULL,
  cores = 1
)
```

Arguments

file	file
dir	directory for results
color	which color, images have to be in folder with color name
thresh	threshold
thresh.auto	Logical. Find threshold automatically?
thresh.quantile	numeric. use simple
filter	2d-filter to use before spot detection
cores	number of cores to use in parallel (with parallel package only)

Value

spot images in spot-color/, number of spots as txt files in spot-color/

find.spots.folder	<i>Detects spots</i>
-------------------	----------------------

Description

Detects spots

Usage

```
find.spots.folder(
  f,
  color,
  thresh = 1,
  thresh.auto = TRUE,
  filter = NULL,
  cores = 1
)
```

Arguments

f	path to folder
color	which color, images have to be in folder with color name
thresh	threshold
thresh.auto	Logical. Find threshold automatically?
filter	2d-filter to use before spot detection
cores	number of cores to use in parallel (with parallel package only)

Value

spot images in spot-color/, number of spots as txt files in spot-color/

heatmap.color	<i>Heatmap colors for n classes</i>
---------------	-------------------------------------

Description

Heatmap colors for n classes

Usage

```
heatmap.color(n)
```

Arguments

n	number of colors.
---	-------------------

Examples

```
barplot(8:1,col=heatmap.color(8))
```

heatmap7	<i>Heatmap colors for 7 classes</i>
----------	-------------------------------------

Description

Heatmap colors for 7 classes

Usage

```
heatmap7(...)
```

Arguments

... parameters are ignored.

Examples

```
barplot(7:1,col=heatmap7())
```

nearestClassDistances.folder	<i>Find all distances to next neighbour of all classes for folders</i>
------------------------------	--

Description

Find all distances to next neighbour of all classes for folders

Usage

```
nearestClassDistances.folder(  
  path,  
  N = 7,  
  voxelsize = NULL,  
  add = FALSE,  
  cores = 1  
)
```

Arguments

path	path to folder
N	number of classes, default: 7
voxelsize	real size of voxels (in microns), if NULL (default), look in folder XYZmic
add	if TRUE, only process images which have not been processed before (i.e. have been added to classN)
cores	number of cores to use in parallel (needs parallel package if cores>1)

Value

nothing, results are in folder distances in RData format

plot_classify.folder *Plot barplot for classified images in a folder*

Description

Plot barplot for classified images in a folder

Usage

```
plot_classify.folder(
  path,
  N = 7,
  cores = 1,
  col = grDevices::grey(0.7),
  method = "sd"
)
```

Arguments

path	path to folder
N	number of classes, default: 7
cores	number of cores to use in parallel (needs parallel package if cores>1)
col	color of bars, either one or a vector of hex RGB characters
method	method for error bars ("sd", "minmax", "quartile")

Value

plots

`plot_colors.in.classes.folder`*Plot for colors in classes distribution for folders*

Description

Plot for colors in classes distribution for folders

Usage

```
plot_colors.in.classes.folder(path, col1 = "green", col2 = "red")
```

Arguments

path	path to folder
col1	color of channel 1
col2	color of channel 2

Value

plot

`plot_nearestClassDistances.folder`*Plots all distances to next neighbour of all classes for folders*

Description

Plots all distances to next neighbour of all classes for folders

Usage

```
plot_nearestClassDistances.folder(  
  path,  
  N = 7,  
  cores = 1,  
  method = "quantile",  
  qu = 0.01  
)
```

Arguments

path	path to folder
N	number of classes, default: 7
cores	number of cores to use in parallel (needs parallel package if cores>1)
method	method for summarizing distances, either "min" or "quantile"
qu	quantile for method="quantile", default: 0.01

Value

plots

splitchannel	<i>Split RGB channels</i>
--------------	---------------------------

Description

Split RGB channels

Usage

```
splitchannel(img, preprocess = TRUE)
```

Arguments

img	rgb image
preprocess	logical. Should preprocessing be applied?

Value

list with red, green, blue channels and size in microns.

splitchannels	<i>Split RGB images into channels and pixel size information</i>
---------------	--

Description

These functions are provided for compatibility with older version of the nucim package. They may eventually be completely removed.

Usage

```
splitchannels(...)
```

Arguments

... parameters for splitchannels.folder

Value

Nothing, folders red, green, blue and XYZmic include separate channels and pixel size information

splitchannels.file *Split channels into files and extracts size in microns*

Description

Split channels into files and extracts size in microns

Usage

```
splitchannels.file(file, channels, rgb.folder, normalize = FALSE)
```

Arguments

file	file name
channels	e.g. c("red", "green", "blue")
rgb.folder	folder with file
normalize	boolean. Should we try to do normalization?

Value

files in `"/red/"`, `"/green/"`, `"/blue/"` and `"/XYZmic"`

splitchannels.folder *Split RGB images into channels and pixel size information*

Description

Split RGB images into channels and pixel size information

Usage

```
splitchannels.folder(
  path,
  channels = c("red", "green", "blue"),
  rgb.folder = "rgb",
  normalize = FALSE,
  cores = 1
)
```

Arguments

path	Path to root folder
channels	Vector of channels in images
rgb.folder	Folder with RGB images
normalize	boolean. Should we try to do normalization
cores	Number of cores used in parallel, cores=1 implies no parallelization

Value

Nothing, folders red, green, blue and XYZmic include separate channels and pixel size information

Examples

```
splitchannels.folder("./")
```

spots.combined	<i>Find spots using information from two channels</i>
----------------	---

Description

Find spots using information from two channels

Usage

```
spots.combined(
  red,
  green,
  mask,
  size = NULL,
  voxelsize = NULL,
  thresh.offset = 0.1,
  window = c(5, 5),
  min.sum.intensity = 2,
  max.distance = 0.5,
  use.brightest = FALSE,
  max.spots = NA,
  full.voxel = FALSE
)
```

Arguments

red	image
green	image
mask	image mask

size	size of img in microns
voxelsize	size of voxel in microns
thresh.offset	Thresh offset used in EBImage::thresh()
window	Half width and height of the moving rectangular window.
min.sum.intensity	spots smaller than min.sum.intensity are ignored
max.distance	use only spots with distance to other color spot smaller than max.distance
use.brightest	Logical; use only brightest in max.distance?
max.spots	maximum of spots (per channel), only when use brightest=TRUE
full.voxel	Logical; output contains full voxel instead of rgb intensities

Value

RGB image with spots will be written to output folder

spots.combined.file *Find spots using information from two channels*

Description

Find spots using information from two channels

Usage

```
spots.combined.file(
  file,
  size = NULL,
  voxelsize = NULL,
  folder = "./",
  thresh.offset = 0.1,
  min.sum.intensity = 2,
  max.distance = 0.5,
  use.brightest = FALSE,
  max.spots = 2,
  full.voxel = FALSE,
  output = "markers"
)
```

Arguments

file	File name
size	size of img in microns, if size and voxelsize are NULL, size is determined from folder XYZmic
voxelsize	size of voxel in microns

folder	Folder
thresh.offset	Thresh offset used in EBImage::thresh()
min.sum.intensity	spots smaller than min.sum.intensity are ignored
max.distance	use only spots with distance to other color spot smaller than max.distance
use.brightest	Logical; use only brightest in max.distance?
max.spots	maximum of spots (per channel), only when use brightest=TRUE
full.voxel	Logical; output contains full voxel instead of rgb intensities
output	output folder

Value

RGB image with spots will be written to output folder

spots.combined.folder *Find spots using information from two channels for folder*

Description

Find spots using information from two channels for folder

Usage

```
spots.combined.folder(
  path,
  size = NULL,
  voxelsize = NULL,
  thresh.offset = 0.1,
  min.sum.intensity = 2,
  max.distance = 0.5,
  use.brightest = FALSE,
  max.spots = 2,
  full.voxel = FALSE,
  output = "markers",
  cores = 1
)
```

Arguments

path	path to folder
size	size of img in microns, if size and voxelsize are NULL, size is determined from folder XYZmic
voxelsize	size of voxel in microns
thresh.offset	Thresh offset used in EBImage::thresh()

min.sum.intensity	spots smaller than min.sum.intensity are ignored
max.distance	use only spots with distance to other color spot smaller than max.distance
use.brightest	Logical; use only brightest in max.distance?
max.spots	maximum of spots (per channel), only when use brightest=TRUE
full.voxel	Logical; output contains full voxel instead of rgb intensities
output	output folder
cores	number of cores we can use of parallel computing (needs parallel package if cores>1)

Value

RGB image with spots will be written to output folder

t_colors.in.classes.folder

Test for colors in classes distribution for folders

Description

Test for colors in classes distribution for folders

Usage

```
t_colors.in.classes.folder(path, test = "Wilcoxon")
```

Arguments

path	path to folder
test	"Wilcoxon", "wilcox" or "U" for Wilcoxon rank-sum (Mann-Whitney U), chisq for Chi-squared test

Value

test results

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