Package 'detrendr'

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Type Package

Title Detrend Images

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Description Detrend fluorescence microscopy image series for fluorescence fluctuation and correlation spectroscopy ('FCS' and 'FFS') analysis. This package contains functionality published in a 2016 paper <doi:10.1093/bioinformatics/btx434> but it has been extended since then with the Robin Hood algorithm and thus contains unpublished work.

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URL https://rorynolan.github.io/detrendr,

https://www.github.com/rorynolan/detrendr#readme

BugReports https://www.github.com/rorynolan/detrendr/issues

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apply_on_pillars *Apply a function to each pillar of a 3-dimensional array.*

Description

Define a 'pillar' of a 3-dimensional array as pillar i, j off array arr being arr[i,j,]. This function applies a specified function to each pillar.

Usage

apply_on_pillars(arr3d, FUN)

Arguments

arr3d	A 3-dimensional array.
FUN	A function which takes a vector as input and, for a given input length, outputs a vector of constant length (can be 1).
	(con co constant rengen (con co r))

best_degree

Value

If FUN is returning length 1 vectors, a matrix whereby mat[i,j] = FUN(arr3d[i,j,]). If FUN is returning vectors of length l > 1, a 3-dimensional array whereby arr[i,j,] = FUN(arr3d[i,j,]).

best_degree

Find the best polynomial degree for polynomial detrending.

Description

Use Nolan's algorithm to find the ideal polynomial degree for polynomial detrending.

Usage

```
best_degree(img, parallel = FALSE, purpose = c("FCS", "FFS"))
```

Arguments

img	A 4-dimensional array in the style of an ijtiff_img (indexed by img[y,x,channel,frame]) or a 3-dimensional array which is a single channel of an ijtiff_img (indexed by img[y,x,frame]).
parallel	Would you like to use multiple cores to speed up this function? If so, set the number of cores here, or to use all available cores, use parallel = TRUE.
purpose	What type of calculation do you intend to perform on the detrended image? If it is an FFS (fluorescence fluctuation spectroscopy) calculation (like number and brightness), choose 'FFS'. If it is an FCS (fluorescence correlation spec- troscopy) calculation (like cross-correlated number and brightness or autocor- relation), choose 'FCS'. The difference is that if purpose is 'FFS', the time series is corrected for non-stationary mean and variance, whereas if purpose is 'FCS', the time series is corrected for non-stationary mean only. purpose is not required for <i>Robin Hood</i> detrending.

Value

If no detrend is necessary, this function returns NA. If a detrend is required, this function returns a natural number which is the ideal polynomial degree for polynomial detrending. If there are multiple channels, the function returns a vector, one degree parameter for each channel.

References

Rory Nolan, Luis A. J. Alvarez, Jonathan Elegheert, Maro Iliopoulou, G. Maria Jakobsdottir, Marina Rodriguez-Muñoz, A. Radu Aricescu, Sergi Padilla-Parra; nandb—number and brightness in R with a novel automatic detrending algorithm, Bioinformatics, https://doi.org/10.1093/bioinformatics/btx434.

Examples

```
## Not run:
## These examples are not run on CRAN because they take too long.
## You can still try them for yourself.
img <- ijtiff::read_tif(system.file("extdata", "bleached.tif",
    package = "detrendr"
))
best_degree(img, parallel = 2)
## End(Not run)
```

best_l

Find the best length parameter for boxcar detrending.

Description

Use Nolan's algorithm to find the ideal length parameter for boxcar detrending. Boxcar detrending is also referred to as 'running average'.

Usage

```
best_l(img, parallel = FALSE, purpose = c("FCS", "FFS"))
```

Arguments

img	A 4-dimensional array in the style of an ijtiff_img (indexed by img[y,x,channel,frame]) or a 3-dimensional array which is a single channel of an ijtiff_img (indexed by img[y,x,frame]).
parallel	Would you like to use multiple cores to speed up this function? If so, set the number of cores here, or to use all available cores, use parallel = TRUE.
purpose	What type of calculation do you intend to perform on the detrended image? If it is an FFS (fluorescence fluctuation spectroscopy) calculation (like number and brightness), choose 'FFS'. If it is an FCS (fluorescence correlation spec- troscopy) calculation (like cross-correlated number and brightness or autocor- relation), choose 'FCS'. The difference is that if purpose is 'FFS', the time series is corrected for non-stationary mean and variance, whereas if purpose is 'FCS', the time series is corrected for non-stationary mean only. purpose is not required for <i>Robin Hood</i> detrending.

Value

If no detrend is necessary, this function returns NA. If a detrend is required, this function returns a natural number which is the ideal length parameter for boxcar detrending. If there are multiple channels, the function returns a vector, one 1 parameter for each channel.

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best_swaps

References

Rory Nolan, Luis A. J. Alvarez, Jonathan Elegheert, Maro Iliopoulou, G. Maria Jakobsdottir, Marina Rodriguez-Muñoz, A. Radu Aricescu, Sergi Padilla-Parra; nandb—number and brightness in R with a novel automatic detrending algorithm, Bioinformatics, https://doi.org/10.1093/bioinformatics/btx434.

Examples

```
## Not run:
## These examples are not run on CRAN because they take too long.
## You can still try them for yourself.
img <- ijtiff::read_tif(system.file("extdata", "bleached.tif",
    package = "detrendr"
))
best_l(img, parallel = 2, purpose = "FFS")
## End(Not run)
```

best_swaps

Find the best swaps parameter for Robin Hood detrending.

Description

Use Nolan's algorithm to find the ideal swaps parameter for Robin Hood detrending.

Usage

```
best_swaps(img, quick = FALSE)
```

Arguments

img	A 4-dimensional array in the style of an ijtiff_img (indexed by img[y,x,channel,frame]) or a 3-dimensional array which is a single channel of an ijtiff_img (indexed by img[y,x,frame]).
quick	If FALSE (the default), the swap finding routine is run several times to get a consensus for the best parameter. If TRUE, the swap finding routine is run only once.

Value

A natural number. The ideal swaps parameter for boxcar detrending. If there are multiple channels, the function returns a vector, one swaps parameter for each channel.

Examples

```
## Not run:
## These examples are not run on CRAN because they take too long.
## You can still try them for yourself.
img <- ijtiff::read_tif(system.file("extdata", "bleached.tif",
    package = "detrendr"
))
best_swaps(img)
## End(Not run)
```

best_tau

```
Find the best tau parameter for exponential smoothing detrending.
```

Description

Use Nolan's algorithm to find the ideal tau parameter for exponential smoothing detrending.

Usage

```
best_tau(img, cutoff = 0.05, parallel = FALSE, purpose = c("FCS", "FFS"))
```

Arguments

img	A 4-dimensional array in the style of an ijtiff_img (indexed by img[y,x,channel,frame]) or a 3-dimensional array which is a single channel of an ijtiff_img (indexed by img[y,x,frame]).
cutoff	In <i>exponential filtering</i> detrending, for the weighted average, every point gets a weight. This can slow down the computation massively. However, many of the weights will be approximately zero. With cutoff, we say that any point with weight less than or equal to cutoff times the maximum weight may be ignored; so with cutoff = 0.05 , any weight less than 5\ value of this parameter is sensible and its value should not be set to anything else without good reason.
parallel	Would you like to use multiple cores to speed up this function? If so, set the number of cores here, or to use all available cores, use parallel = TRUE.
purpose	What type of calculation do you intend to perform on the detrended image? If it is an FFS (fluorescence fluctuation spectroscopy) calculation (like number and brightness), choose 'FFS'. If it is an FCS (fluorescence correlation spec- troscopy) calculation (like cross-correlated number and brightness or autocor- relation), choose 'FCS'. The difference is that if purpose is 'FFS', the time series is corrected for non-stationary mean and variance, whereas if purpose is 'FCS', the time series is corrected for non-stationary mean only. purpose is not required for <i>Robin Hood</i> detrending.

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Value

If no detrend is necessary, this function returns NA. If a detrend is required, this function returns a natural number which is the ideal tau parameter for exponential smoothing detrending. If there are multiple channels, the function returns a vector, one tau parameter for each channel.

References

Rory Nolan, Luis A. J. Alvarez, Jonathan Elegheert, Maro Iliopoulou, G. Maria Jakobsdottir, Marina Rodriguez-Muñoz, A. Radu Aricescu, Sergi Padilla-Parra; nandb—number and brightness in R with a novel automatic detrending algorithm, Bioinformatics, https://doi.org/10.1093/bioinformatics/btx434.

Examples

```
## Not run:
## These examples are not run on CRAN because they take too long.
## You can still try them for yourself.
img <- ijtiff::read_tif(system.file("extdata", "bleached.tif",
    package = "detrendr"
))[, , 1, ]
best_tau(img, parallel = 2)
## End(Not run)
```

brightness_pillars Get the brightness of pillars of a 3d array.

Description

For an ijtiff_img-style array img (indexed as img[y,x,channel,frame]), 3-dimensional array mat3d, pillar xy of channel ch is defined as img[y,x,ch,]. This function computes the brightness, of each pillar.

Usage

```
brightness_pillars(img, parallel = FALSE)
```

Arguments

img	A 4-dimensional array in the style of an ijtiff_img (indexed by img[y,x,channel,frame]) or a 3-dimensional array which is a single channel of an ijtiff_img (indexed by
	<pre>img[y,x,frame]).</pre>
parallel	Would you like to use multiple cores to speed up this function? If so, set the number of cores here, or to use all available cores, use parallel = TRUE.

Value

An ijtiff_img-style array arr with one frame. arr[y,x,ch,1] is equal to var(img[y,x,ch,]) / mean(img[y,x,ch,]).

Examples

```
aaa <- array(1:16, dim = c(2, 2, 4))
brightness_pillars(aaa)</pre>
```

detrend-directory *Detrend all TIFF images in an entire folder.*

Description

Batch processing. Apply any of the available detrending routines to detrend all of the TIFF images in a folder, saving the detrended images as TIFF files in the same folder.

Usage

```
dir_detrend_robinhood(
  folder_path = ".",
  swaps = "auto",
  thresh = NULL,
 quick = FALSE,
 msg = TRUE
)
dir_detrend_rh(
  folder_path = ".",
  swaps = "auto",
  thresh = NULL,
  quick = FALSE,
 msg = TRUE
)
dir_detrend_boxcar(
  folder_path = ".",
 1,
 purpose = c("FCS", "FFS"),
  thresh = NULL,
 parallel = FALSE,
 msg = TRUE
)
dir_detrend_exp(
  folder_path = ".",
  tau,
  purpose = c("FCS", "FFS"),
  thresh = NULL,
 parallel = FALSE,
 msg = TRUE
)
```

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```
dir_detrend_polynom(
  folder_path = ".",
  degree,
  purpose = c("FCS", "FFS"),
  thresh = NULL,
  parallel = FALSE,
  msg = TRUE
)
```

Arguments

folder_path	The path (relative or absolute) to the folder you wish to process.
swaps	The number of swaps (giving of one count from rich to poor) to perform during the <i>Robin Hood</i> detrending. Set this to "auto" (the default) to use Nolan's algo- rithm to automatically find a suitable value for this parameter (recommended). For multi-channel images, it is possible to have a different swaps for each chan- nel by specifying swaps as a vector or list.
thresh	The threshold or thresholding method (see autothresholdr::mean_stack_thresh()) to use on the image prior to detrending.
quick	If FALSE (the default), the swap finding routine is run several times to get a consensus for the best parameter. If TRUE, the swap finding routine is run only once.
msg	Receive messages to tell you how the processing of the directory is going? De- fault is yes.
1	The length parameter for <i>boxcar</i> detrending. The size of the sliding window will be $2 * 1 + 1$. This must be a positive integer. Set this to "auto" to use Nolan's algorithm to automatically find a suitable value for this parameter (recommended). For multi-channel images, it is possible to have a different 1 for each channel by specifying 1 as a vector or list.
purpose	What type of calculation do you intend to perform on the detrended image? If it is an FFS (fluorescence fluctuation spectroscopy) calculation (like number and brightness), choose 'FFS'. If it is an FCS (fluorescence correlation spec- troscopy) calculation (like cross-correlated number and brightness or autocor- relation), choose 'FCS'. The difference is that if purpose is 'FFS', the time series is corrected for non-stationary mean and variance, whereas if purpose is 'FCS', the time series is corrected for non-stationary mean only. purpose is not required for <i>Robin Hood</i> detrending.
parallel	Would you like to use multiple cores to speed up this function? If so, set the number of cores here, or to use all available cores, use parallel = TRUE.
tau	The <i>tau</i> parameter for <i>exponential filtering</i> detrending. This must be a positive number. Set this to "auto" to use Nolan's algorithm to automatically find a suitable value for this parameter (recommended). For multi-channel images, it is possible to have a different tau for each channel by specifying tau as a vector or list.

degree The degree of the polynomial to use for the polynomial detrending. This must be a positive integer. Set this to "auto" to use Nolan's algorithm to automatically find a suitable value for this parameter (recommended). For multi-channel images, it is possible to have a different degree for each channel by specifying degree as a vector or list.

Details

These functions include a thresholding option, unlike their non-batch processing counterparts which they wrap (i.e. img_detrend_boxcar, img_detrend_exp and img_detrend_polynom). This is because, when working interactively, it's easy to threshold and then detrend, but for batch processing, it's not so easy to efficiently do one after the other, so it's nice to have that taken care of should you want it.

Value

Silently, a character vector of the paths to the detrended images.

Examples

```
## Not run:
setwd(tempdir())
file.copy(
    c(
      system.file("extdata", "bleached.tif", package = "detrendr"),
      system.file("img", "2ch_ij.tif", package = "ijtiff")
    ),
    "."
)
dir_detrend_robinhood(thresh = "huang")
dir_detrend_boxcar(l = "auto", thresh = "tri", purpose = "FFS")
dir_detrend_exp(tau = "auto", thresh = "tri", purpose = "FFS")
dir_detrend_polynom(degree = "auto", thresh = "huang", purpose = "FFS")
## End(Not run)
```

detrended_img Detrended image class.

Description

A detrended_img is a 4-dimensional array of positive integers in the style of an ijtiff_img (indexed by img[y,x,channel,frame]) which is the result of a detrending routine. It has 4 attributes:

method The detrending method used. This must be one of "boxcar", "exponential" or "polynomial".

- parameter The value of the parameter used. This will be the 1, tau or degree parameter for the respective methods.
- auto A boolean that is TRUE if the parameter was found automatically or FALSE if it was manually selected.

detrending

purpose Either "FCS" or "FFS" to denote whether the detrending was done for the purpose of fluorescence correlation spectroscopy or fluorescence fluctuation spectroscopy calculations respectively. purpose is not required for *Robin Hood* detrending.

Usage

```
detrended_img(img, method, parameter, auto, purpose = NULL)
```

Arguments

img	The detrended image series. A 4-dimensional array of non-negative integers in the style of an ijtiff_img, or a 3-dimensional array of non-negative integers which represents a single channel of an ijtiff_img-style array (indexed by img[y,x,frame]).
method	The method used. One of "robinhood", "boxcar", "exponential" or "polynomial".
parameter	A number. The detrend parameter used. One per channel.
auto	Logical. Was automatic detrending used? One per channel.
purpose	Either "FCS" or "FFS". Was the image detrended for the purpose of doing FCS or FFS calculations? See detrending. purpose is not required for <i>Robin Hood</i> detrending.

Details

Sometimes when detrending, you can get slight negative values in the detrended image. These values should really just be zero, so this constructor function sets negative values of img to zero.

Value

An object of class detrended_img.

detrending Detrend images.

Description

Correct images for bleaching (or any other effect that introduces an unwanted trend) by detrending.

Usage

```
img_detrend_robinhood(img, swaps = "auto", quick = FALSE)
img_detrend_rh(img, swaps = "auto", quick = FALSE)
img_detrend_boxcar(img, l, purpose = c("FCS", "FFS"), parallel = FALSE)
img_detrend_exp(
    img,
```

```
tau,
cutoff = 0.05,
purpose = c("FCS", "FFS"),
parallel = FALSE
)
```

img_detrend_polynom(img, degree, purpose = c("FCS", "FFS"), parallel = FALSE)

Arguments

img	A 4-dimensional array in the style of an ijtiff_img (indexed by img[y,x,channel,frame]) or a 3-dimensional array which is a single channel of an ijtiff_img (indexed by img[y,x,frame]).
swaps	The number of swaps (giving of one count from rich to poor) to perform during the <i>Robin Hood</i> detrending. Set this to "auto" (the default) to use Nolan's algo- rithm to automatically find a suitable value for this parameter (recommended). For multi-channel images, it is possible to have a different swaps for each chan- nel by specifying swaps as a vector or list.
quick	If FALSE (the default), the swap finding routine is run several times to get a consensus for the best parameter. If TRUE, the swap finding routine is run only once.
1	The length parameter for <i>boxcar</i> detrending. The size of the sliding window will be $2 * 1 + 1$. This must be a positive integer. Set this to "auto" to use Nolan's algorithm to automatically find a suitable value for this parameter (recommended). For multi-channel images, it is possible to have a different 1 for each channel by specifying 1 as a vector or list.
purpose	What type of calculation do you intend to perform on the detrended image? If it is an FFS (fluorescence fluctuation spectroscopy) calculation (like number and brightness), choose 'FFS'. If it is an FCS (fluorescence correlation spec- troscopy) calculation (like cross-correlated number and brightness or autocor- relation), choose 'FCS'. The difference is that if purpose is 'FFS', the time series is corrected for non-stationary mean and variance, whereas if purpose is 'FCS', the time series is corrected for non-stationary mean only. purpose is not required for <i>Robin Hood</i> detrending.
parallel	Would you like to use multiple cores to speed up this function? If so, set the number of cores here, or to use all available cores, use parallel = TRUE.
tau	The <i>tau</i> parameter for <i>exponential filtering</i> detrending. This must be a positive number. Set this to "auto" to use Nolan's algorithm to automatically find a suitable value for this parameter (recommended). For multi-channel images, it is possible to have a different tau for each channel by specifying tau as a vector or list.
cutoff	In <i>exponential filtering</i> detrending, for the weighted average, every point gets a weight. This can slow down the computation massively. However, many of the weights will be approximately zero. With cutoff, we say that any point with weight less than or equal to cutoff times the maximum weight may be ignored; so with cutoff = 0.05 , any weight less than 5\ value of this parameter is sensible and its value should not be set to anything else without good reason.

detrending

degree The degree of the polynomial to use for the polynomial detrending. This must be a positive integer. Set this to "auto" to use Nolan's algorithm to automatically find a suitable value for this parameter (recommended). For multi-channel images, it is possible to have a different degree for each channel by specifying degree as a vector or list.

Details

There are 4 detrending methods available: *Robin Hood*, *boxcar*, *exponential filtering* and *polynomial*. *Robin Hood* is described in Nolan et al., 2018. The others are described in Nolan et al., 2017.

- *Robin Hood* is a method whereby counts are taken from frames with higher mean intensity and given directly to frames of lower intensity.
- *Boxcar* detrending with parameter l is a moving average detrending method using a sliding window of size 2l + 1.
- Exponential filtering detrending is a moving weighted average method where for parameter tau the weights are calculated as $\exp(-t/tau)$ where t is the distance from the point of interest.
- *Polynomial* detrending works by fitting a polynomial line to a series of points and then correcting the series to remove the trend detailed by this polynomial fit.

Value

The detrended image, an object of class detrended_img.

References

Rory Nolan, Luis A. J. Alvarez, Jonathan Elegheert, Maro Iliopoulou, G. Maria Jakobsdottir, Marina Rodriguez-Muñoz, A. Radu Aricescu, Sergi Padilla-Parra; nandb—number and brightness in R with a novel automatic detrending algorithm, Bioinformatics, https://doi.org/10.1093/bioinformatics/btx434.

```
## Not run:
## These examples are not run on CRAN because they take too long.
## You can still try them for yourself.
img <- ijtiff::read_tif(system.file("extdata", "bleached.tif",
    package = "detrendr"
))
corrected <- img_detrend_rh(img)
corrected <- img_detrend_boxcar(img, "auto", purpose = "fcs", parallel = 2)
corrected10 <- img_detrend_boxcar(img, 10, purpose = "fcs", parallel = 2)
corrected50 <- img_detrend_boxcar(img, 50, purpose = "fcs", parallel = 2)
corrected10 <- img_detrend_exp(img, "auto", purpose = "ffs", parallel = 2)
corrected10 <- img_detrend_exp(img, 10, purpose = "ffs", parallel = 2)
corrected10 <- img_detrend_exp(img, 10, purpose = "ffs", parallel = 2)
corrected50 <- img_detrend_exp(img, 50, purpose = "ffs", parallel = 2)
corrected50 <- img_detrend_exp(img, 50, purpose = "ffs", parallel = 2)
corrected <- img_detrend_polynom(img, "auto", purpose = "ffs", parallel = 2)
corrected2 <- img_detrend_polynom(img, 2, purpose = "ffs", parallel = 2)</pre>
```

mean_frames

End(Not run)

detrendr

detrendr: Image detrending in R.

Description

The detrendr package gives functions for detrending images, most often used for preprocessing in fluorescence fluctuation and correlation spectroscopy (FFS and FCS).

References

Rory Nolan, Luis A. J. Alvarez, Jonathan Elegheert, Maro Iliopoulou, G. Maria Jakobsdottir, Marina Rodriguez-Muñoz, A. Radu Aricescu, Sergi Padilla-Parra; nandb—number and brightness in R with a novel automatic detrending algorithm, Bioinformatics, https://doi.org/10.1093/bioinformatics/btx434.

mean_frames

Get the sums/means of frames in a 3-dimensional array.

Description

Frame i of a 3-dimensional array arr3d is defined as arr3d[,,i].

Usage

mean_frames(arr3d, na_rm = FALSE)

sum_frames(arr3d, na_rm = FALSE)

Arguments

arr3d	A 3-dimensional numeric array.
na_rm	Do you want NA values to be excluded from calculations?

Value

A numeric vector.

```
a <- array(seq_len(2^3), dim = rep(2, 3))
sum_frames(a)
mean_frames(a)</pre>
```

pillar-stats

Description

For an ijtiff_img-style array img (indexed as img[y,x,channel,frame]), pillar xy of channel ch is defined as img[y,x,ch,]. These functions compute the mean, median and variance of each pillar for each channel.

Usage

```
sum_pillars(img, parallel = FALSE)
mean_pillars(img, parallel = FALSE)
median_pillars(img, parallel = FALSE)
var_pillars(img, parallel = FALSE)
```

Arguments

img	A 4-dimensional array in the style of an ijtiff_img (indexed by img[y,x,channel,frame]) or a 3-dimensional array which is a single channel of an ijtiff_img (indexed by img[y,x,frame]).
parallel	Would you like to use multiple cores to speed up this function? If so, set the number of cores here, or to use all available cores, use parallel = TRUE.

Value

An ijtiff_img-style array arr with one frame. arr[y,x,ch,1] is equal to mean(img[y,x,ch,]), median(img[y,x,ch,]), or var(img[y,x,ch,]).

```
aaa <- array(seq_len(2^4), dim = rep(2, 4)) # a 2-channel, 2-frame array
sum_pillars(aaa)
mean_pillars(aaa)
median_pillars(aaa)
var_pillars(aaa)
```

```
rfromboxes
```

Description

Given a number of boxes with a specified number of balls in each, randomly draw a number of balls from these boxes, recording how many balls was drawn from each. An empty box cannot be drawn from.

Usage

```
rfromboxes(n, balls, weights = NULL)
```

Arguments

n	A natural number. The number of balls to draw.
balls	A vector of natural numbers. The number of balls in each box to begin with.
weights	A non-negative numeric vector the same length as balls. The relative probabil- ities of drawing a ball from each box. Default is each box is equally likely to be drawn from.

Value

A vector of natural numbers with the same length as balls. The number of balls drawn from each box.

See Also

rtoboxes

Examples

```
balls <- 1:10
rfromboxes(40, balls)
rfromboxes(40, balls, weights = c(rep(1, 9), 0))</pre>
```

rtoboxes

Randomly place balls in boxes.

Description

Given a number of boxes, randomly distribute n balls into these boxes.

Usage

```
rtoboxes(n, boxes, weights = NULL, capacities = NULL)
```

rtoboxes

Arguments

n	A natural number. The number of balls to put into the boxes.
boxes	A natural number. The number of boxes.
weights	A non-negative numeric vector. The relative probabilities of putting a ball in each box. Default is each box is equally likely.
capacities	A vector of natural numbers. The capacity of each box. Default is each box has infinite capacity.

Value

A vector of natural numbers with the same length as boxes. The number of balls placed in each box.

See Also

rfromboxes

```
rtoboxes(30, 7)
rtoboxes(30, 7, capacities = c(rep(1, 3), rep(7, 4)))
rtoboxes(30, 7,
    capacities = c(rep(1, 3), rep(70, 4)),
    weights = c(rep(0.1, 6), 1)
)
```

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