# Package 'detrendr'

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<b>Description</b> Image series affected by bleaching must be corrected by 'detrending' prior to the performance of quantitative analysis. 'detrendr' is for correctly detrending images. It uses Nolan's algorithm (Nolan et al., 2017 <doi:10.1093 bioinformatics="" btx434="">).</doi:10.1093>
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apply\_on\_pillars

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

### **Description**

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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).

### 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"))
```

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#### **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 spectroscopy) calculation (like cross-correlated number and brightness or autocorrelation), 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 Robin Hood 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**

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"))
```

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#### **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 spectroscopy) calculation (like cross-correlated number and brightness or autocorrelation), 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 Robin Hood 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.

#### 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**

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)
```

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#### **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]).

#### 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**

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 *exponential filtering* 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% of the maximum weight may be ignored. The default 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 spectroscopy) calculation (like cross-correlated number and brightness or autocorrelation), choose 'FCS'. The difference is that if purpose is 'FFS', the time

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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 *Robin Hood* 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 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**

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 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 var(img[y, x, ch, ]) / mean(img[y, x, ch, ])
```

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#### **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,
    msg = TRUE)

dir_detrend_rh(folder_path = ".", swaps = "auto", thresh = NULL,
    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)

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 algorithm to automatically find a suitable value for this parameter (recommended). For multi-channel images, it is possible to have a different swaps for each channel 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.
msg	Receive messages to tell you how the processing of the directory is going? Default 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.

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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 spectroscopy) calculation (like cross-correlated number and brightness or autocorrelation), 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 *Robin Hood* 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 *tau* parameter for *exponential filtering* 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.

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#### **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.

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.

### 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.	
S	Ö	

### Description

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

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#### Usage

```
img_detrend_robinhood(img, swaps = "auto")
img_detrend_rh(img, swaps = "auto")
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 *Robin Hood* detrending. Set this to "auto" (the default) 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 swaps for each channel by specifying swaps as a vector or list.

1

The length parameter for *boxcar* 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 spectroscopy) calculation (like cross-correlated number and brightness or autocorrelation), 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 *Robin Hood* 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 tau parameter for exponential filtering 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 *exponential filtering* 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% of the maximum weight may be ignored. The default value of this parameter is sensible and its value should not be set to anything else without good reason.

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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.

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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>
```

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pillar-stats	Get the sums/means/medians/variances of pillars of an ijtiff_img-style
	array.

### **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, ]).
```

### **Examples**

```
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)</pre>
```

rfromboxes

Randomly draw balls from boxes.

### **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.

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#### 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.

### **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)
```

### 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.

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