

# Package ‘FCSlib’

February 12, 2020

**Type** Package

**Title** A Collection of Fluorescence Fluctuation Spectroscopy Methods

**Version** 1.1.0

**Date** 2020-02-12

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**Description** A set of tools for fluorescence fluctuation spectroscopy data analysis performance is provided in this package.

It includes techniques such as single-point fluorescence correlation spectroscopy, autocorrelation and pair correlation functions, number & brightness (raster line scan) and a novel, recently developed method by Hinde and co-workers, pair correlation of molecular brightness (doi:10.1038/ncomms11047).

A set of simulations and real experimental data is used for the examples of each function provided in this package.

For an in-depth description of the basics behind each function here included and a detailed step-by-step guide on how to use them on your own data, please refer to the Supplementary Material file provided at (<[https://github.com/RPintoC/FCSlib\\_Sup\\_Data](https://github.com/RPintoC/FCSlib_Sup_Data)>).

**License** GPL-3

**Depends** R(>= 3.5.0), tiff

**Suggests** fields

**RoxygenNote** 7.0.2

**Encoding** UTF-8

**LazyData** true

**NeedsCompilation** no

**Repository** CRAN

**Date/Publication** 2020-02-12 07:20:03 UTC

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Cy5_100nM	<i>Experimental data of free Cy5 molecules diffusing in water at a concentration of 100 nM.</i>
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### Description

A dataset containing a vector of a single-point, periodical acquisition.

### Usage

```
data(Cy5_100nM)
```

### Format

A data frame with 50000 rows and 2 variables

### Details

- t is a vector Tau.
- f is a time point of aquisition.

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fcs	<i>Fluorescence Correlation Spectroscopy</i>
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### Description

Calculates either the auto-correlation or cross-correlation between vectors x and y, returning a correlation function.

### Usage

```
fcs(x , y = NULL, nPoints = 25000, pcf = FALSE)
```

**Arguments**

x	Numeric vector of length N.
y	Numeric vector of length N.
nPoints	The size of the sub-vectors in which the input vectors will be divided. This number must be less than N/2.
pcf	A boolean parameter to determine if an alternate version of the correlation function is used for the calculation of de pCF and pComb functions.

**Details**

Fluorescence correlation spectroscopy (FCS) is a technique with high spatial and temporal resolution used to analyze the kinetics of particles diffusing at low concentrations. The detected fluorescence intensity as a function of time is:  $F(t)$ .

The correlation function is computed as the normalized autocorrelation function,  $G(\tau) = \langle \Delta F(t) \Delta F(t+\tau) \rangle / (\langle F(t) \rangle^2)$  to the collected data set, where  $t$  refers to a time point of fluorescence acquisition, and  $\tau$  refers to the temporal delay between acquisitions and  $\langle \dots \rangle$  indicates average.

The correlation between  $\Delta F(t) = F(t) - \langle F(t) \rangle$  and  $\Delta F(t+\tau) = F(t+\tau) - \langle F(t) \rangle$  is calculated for a range of delay times. For temporal acquisitions as FCS point,  $x$  takes the value of  $F(t)$  and  $y = \text{NULL}$ . For cross-correlation experiments between two fluorescent signals  $x = F1(t)$  and  $y = F2(t)$ , as channels, the correlation function is:  $G(\tau) = \langle \Delta F1(t) \Delta F2(t+\tau) \rangle / (\langle F1(t) \rangle \langle F2(t) \rangle)$ .

The function separate the original vector in sub-vectors of same length (n-points), then calculate an autocorrelation function form each sub-vector. The final result will be an average of all the autocorrelation functions.

**Value**

A numeric vector  $G$  containing either the autocorrelation for the input vector  $x$ , or the cross-correlation between  $x$  and  $y$  vectors, with a length of  $nPoints$ .

**Note**

The argument  $nPoints$  must be smaller than the total number of temporal observations  $N$ , it is recommended to set  $nPoints = 2^n$ , with  $n = 2, \dots, \text{infinity}$ .

**Author(s)**

Raul Pinto Camara, Adan O. Guerrero

**References**

R.A. Migueles-Ramirez, A.G. Velasco-Felix, R. Pinto-Cámara, C.D. Wood, A. Guerrero. Fluorescence fluctuation spectroscopy in living cells. *Microscopy and imaging science: practical approaches to applied research and education*, 138-151,2017.

**See Also**

[gcf](#)

## Examples

```

### Load the FCSlib package

library(FCSlib)

# As an example, we will use data from experiment acquisition
# of free Cy5 molecules diffusing in water at a concentration of 100 nM.
# Use readfileTiff() function to read the fcs data in TIFF format.

f<-readfileTiff("Cy5_100nM.tif")

### Note that $f$ is a matrix of 2048 x 5000 x 1 dimensions.
# This is due to the fact that this single-point FCS experiment was collected
# at intervals of 2048 points each, with an acquisition time of 2  $\mu$  s.
# Let's now create a dataframe with the FCS data which here-and-after will be called Cy5.

acqTime = 2E-6
f<-as.vector(f)
time <- (1:length(f))*acqTime
Cy5<-data.frame(t = time, f)

### The first 100 ms of the time series are:

plot(Cy5[1:5000,], type = "l", xlab = "t(s)", ylab = "Fluorescence Intensity", main = "Cy5")

# The fcs() function receives three parameters: 'x' (mandatory),
# 'y' (optional) and 'nPoints' (optional), where x is the main signal to analyze,
# y is a secondary signal (for the case of cross-correlation instead of autocorrelation)
# and nPoints is the final length of the calculated correlation curve.
# This function divides the original N-size signal into sub-vectors with a size of nPoints*2.
# Once all the sub-vectors are analyzed, these are then averaged.
# To use the fcs() function type

g <- fcs(x = Cy5$f)

# The result of the function is assigned to the variable 'g',
# which contains the autocorrelation curve

length <- 1:length(g)
tau <- Cy5$t[length]
G<-data.frame(tau,g)
plot(G, log = "x", type = "l", xlab = expression(tau(s)), ylab = expression(G(tau)), main = "Cy5")

# It is important to remove the first point from the data,
# where  $G(\tau=0)$  it is not properly computed

G<-G[-1,]
plot(G, log = "x", type = "l", xlab = expression(tau(s)), ylab = expression(G(tau)), main = "Cy5")

# The variable 'nPoints' can be adjusted to better assess the transport phenomena
# in study (i.e. free diffusion in three dimensions in the case of this example) and

```

```
# for better understanding of the diffusive nature of the molecules.
# In this example 'nPoints' will be set to 2048.

g <- fcs(x = Cy5$f, nPoints = 2048)
length <- 1:length(g)
tau <- Cy5$t[length]
G <- data.frame(tau, g)
G <- G[-1, ]
plot(G, log = "x", type = "l", xlab = expression(tau(s)), ylab = expression(G(tau)), main = "Cy5")
```

---

fitFCS

*Fitting FCS Data*


---

### Description

Estimates the parameters based on a given equation, on the data generated with the fcs() function.

### Usage

```
fitFCS(data = parent.frame(), start, low = -Inf, up = Inf, type = "D3D", trace = TRUE)
```

### Arguments

data	data frame in which to evaluate the variables in formula and weights.
start	a named list or named numeric vector of starting estimates.
low, up	a named list or named numeric vector of lower and upper bounds, replicated to be as long as start. If unspecified, all parameters are assumed to be -Inf and Inf.
type	specification for the equation to model, is a character string. The default value is "D3D" equation for three-dimensional free diffusion. Another possible values are: "D2D" for two-dimensional free diffusion, "D2DT" for two-dimensional free diffusion with triplet excited state, and "D3DT" for three-dimensional free diffusion with triplet excited state.
trace	logical value that indicates whether the progress of the non-linear regression (nls) should be printed.

### Details

A transport model, containing physical information about the diffusive nature of the fluorophores, can be fitted to the autocorrelation data to obtain parameters such as the diffusion coefficient  $D$  and the number of molecules within the observation volume  $N$ .

The fitFCS() function uses the 'Non-linear Least Squares' function to fit a physical model into a data set. There are four possible models to be fit:

"D2D" for two-dimensional diffusion

"D2DT" for two-dimensional diffusion with triplet state

"D3D" for three-dimensional diffusion

"D3DT" for three-dimensional diffusion with triplet state

Inside the equations for each model,  $\gamma$  a geometric factor that depends on the illumination profile. For confocal excitation its magnitude approaches  $\gamma = 1/\sqrt{8} \approx 0.35$ . The diffusion time is defined as  $\tau_D = s^2/4D$ , where  $s$  and  $u$  are the radius and the half-length of the focal volume, respectively. The parameter  $u$  is usually expressed as  $u = ks$ , with  $k$  being the eccentricity of the focal volume; for confocal excitation  $k \approx 3$ . The fraction of molecules in the triplet state is  $B$ , and  $\tau_B$  is a time constant for the triplet state.

### Value

A nls object (from nls).

### Author(s)

Raul Pinto Camara

### See Also

[nls](#), [fcs](#)

### Examples

```
# Load the FCSlib package

library(FCSlib)

g <- fcs(x = Cy5_100nM$f, nPoints = 2048)
len <- 1:length(g)
tau <- Cy5_100nM$t[len]
G <- data.frame(tau, g)
G <- G[-1,]

# Once the correlation curve 'g' has been generated,
# a data frame containing known parameters must be then defined

df <- data.frame(G, s = 0.27, k = 3)
head(df)

# The radius of the focal volume must be computed experimentally.
# For this example, we choose a  $s = 0.27 \mu\text{m}$ 
# Then, three lists that contain the initial values of the data,
# as well as the upper and lower limits of these values, must be defined.
# The input values here are the expected values for the real experimental data
# to be very similar or close to, so that the function calculates them accurately.
# Initial values:

start <- list(D = 100, G0 = 0.1)
up <- list(D = 1E3, G0 = 10)
low <- list(D = 1E-1, G0 = 1E-2)

# Once the known parameters are defined, we now proceed to use the fitFCS() function.
```

```

# The result will be a nls object

modelFCS <- fitFCS(df, start, low, up, type = "D3D", trace = F)
# summary(modelFCS)

# By using the predict() function, the object generated in the previous step
# is transformed into a vector that contains the curve fitted by the desired model.

fit <- predict(modelFCS, tau)

# Finally, use the following command to obtain the resulting graph,
# where the blue line corresponds to the fitted data and the black surface
# corresponds to the unfitted

plot (G, log = "x", type = "l", xlab = expression(tau(s)),
      ylab = expression(G(tau)), main = "Cy5")
lines(fit~G$tau, col = "blue")

# To acquire access to the physical coefficients of the model type

s<-summary(modelFCS)
s$coefficients[,1]

```

---

gcf

*General Correlation Function*


---

### Description

Performs either the auto-correlation or cross-correlation between vectors  $x$  and  $y$ , returning a correlation function.

### Usage

```
gcf(x, y, xmean = 1, ymean = 1, c = 0)
```

### Arguments

$x$	A numerical signal with dimensions $M \times N \times Z$ .
$y$	A numerical signal with dimensions $M \times N \times Z$ .
$xmean$	The mean value of the signal $x$ .
$ymean$	The mean value of the signal $y$ .
$c$	A numeric variable to restrict the correlation to positives values.

## Details

The number of emission events per unit time is determined and used to generate autocorrelation and cross-correlation curves from the intensity traces  $F(t)$  and the fluctuations  $\delta F(t) = F(t) - \langle F(t) \rangle$ . The auto-correlation function of the collected data set, is computed as the normalized auto-correlation function, when  $y=x$ . The general auto-correlation function is defined as:  $G(\tau) = (\delta F(t) \delta F(t+\tau)) / (\langle F(t) \rangle \langle F(t) \rangle)$ , where  $t$  refers to a time point of fluorescence acquisition, and  $\tau$  refers to the temporal delay between acquisitions.  $\langle \dots \rangle$  is the temporal average of  $F(t)$ ; and  $\delta F(t) = F(t) - \langle F(t) \rangle$ ,  $\delta F(t+\tau) = F(t+\tau) - \langle F(t) \rangle$ .

For temporal acquisitions such as point FCS,  $x$  and  $y$  are  $F(t)$ . The cross-correlation function between two channels of fluorescent signals,  $x = F1(t)$  and  $y = F2(t)$ , the cross-correlation function is defined as:  $G(\tau) = (\delta F1(t) \delta F2(t+\tau)) / (\langle F1(t) \rangle \langle F2(t) \rangle)$ , where  $x_{\text{mean}} = \langle F1(t) \rangle$  and  $y_{\text{mean}} = \langle F2(t) \rangle$  are the mean values of the fluorescent signals.

## Value

G A numerical signal with dimension  $N' \times M' \times Z'$

## Author(s)

Raul Pinto Camara.

## References

Siegel, A. P., Hays, N. M., & Day, R. N. (2013). Unraveling transcription factor interactions with heterochromatin protein 1 using fluorescence lifetime imaging microscopy and fluorescence correlation spectroscopy. *Journal of biomedical optics*, 18(2), 025002.

## See Also

[fcs](#), [convolve](#)

## Examples

```
# Load the FCSlib package

library(FCSlib)

# As an example, we will use data from experiment acquisition
# of free Cy5 molecules diffusing in water at a concentration of 100 nM.

oldpar <- par(no.readonly = TRUE)
g <- gcf(x = Cy5_100nM$f, y = Cy5_100nM$f, xmean = mean(Cy5_100nM$f), ymean = mean(Cy5_100nM$f))
length <- 1:length(g)
par(mfrow=c(1,1))
plot(y = g, x = Cy5_100nM$t[length], log = 'x', type = 'l',
     xlab = expression(tau(mu~s)), ylab = expression(G(tau)),
     main = "Cy5 100nM")
par(oldpar)
```



---

nblinc	<i>Number &amp; Brightness (Single Image)</i>
--------	---

---

**Description**

Performs the Number and Brightness Analysis (N&B) on an image

**Usage**

```
nblinc(img, sigma0 = 0, offset = 0, S = 1, wSigma = 0)
```

**Arguments**

img	The image to analyze.
sigma0	Variance of the optical system readout noise
offset	Constant number that depends on the optical system configuration. Signal values smaller than the offset should be considered zero.
S	Proportionality factor S. Indicates the ratio between the amount of incident photons in the detector and those converted to an electronic signal.
wSigma	Time window at which the running average is calculated

**Details**

The Number and Brightness (N&B) method is a time-independent technique that provides an estimate of molecular concentration and aggregation state (or stoichiometry), based on the statistical moments of the fluorescence intensity fluctuations. In other words, this tool allows to distinguish between two or more homo-oligomeric states of a molecule present in a given region in the sample (Brightness) while also providing a direct indicator of the molecule's relative abundance (Number). The intensity of the fluorescence signal is mostly due to the mere presence of fluorophores in the media, affected by the fluorophore quantum yield, the sensitivity of the detector and the photophysical characteristics of the optical instrumentation. The average particle number and brightness are calculated directly from the mean value  $\langle k \rangle$  and variance ( $\sigma^2$ ) of the fluorescence intensity data (image) for a given pixel as follows:  $N = (\langle k \rangle^2) / (\sigma^2)$  and  $B = (\sigma^2) / \langle k \rangle$

**Value**

A list containing two vectors, the Brightness and the Number of the image.

**Author(s)**

Raul Pinto Camara.

**See Also**

[var](#), [mean](#)

**Examples**

```

### Load the FCSlib package

library(FCSlib)

# As an example, we will use a data set that corresponds
# to a population of Venus dimers and hexamers diffusing in HEK-293 cells.
# Use the readFileTiff() function to extract the information from the '.tiff' files.

# rawtif <- "<path>/V2V6.tif"

V2V6 <- readFileTiff(rawtif)
V2V6 <- V2V6[, , 1]

# To compute the apparent Number and Brightness with the nblines() function, type:

nbv2v6 <- nblines(V2V6)
pixelSize = 0.05
r<- (1:dim(V2V6)[1])*pixelSize
par(mar = c(5, 5, 3, 5))
plot(nbv2v6$B~r, type = "l", col = "blue", axes = F, ann = F)
mtext(side = 2, text = axTicks(2), at = axTicks(2), col = "blue", line = 1, las = 1)
mtext(side = 2, text = "B", line = 3, col = "blue", las = 1)
axis(1)
mtext(side = 1, text = expression(r(mu*m)), line = 3, las = 1)
par(new = T)
plot(nbv2v6$N, type = "l", col = "red", axes = F, ann = F)
mtext(side = 4, text = axTicks(4), at = axTicks(4), col = "red", line = 1, las = 1)
mtext(side = 4, text = "N", line = 3, col = "red", las = 1)

### To compute the real Number $n$ and Brightness $e$ type:

par(mar = c(5, 5, 3, 5))
nbv2v6 <- nblines(img = V2V6, S=3.5, sigma0 = 1, offset = 0)
plot(nbv2v6$epsilon~r, type = "l", ylim = c(0,3), col = "blue", axes = F, ann = F)
mtext(side = 2, text = axTicks(2), at = axTicks(2), col = "blue", line = 1, las = 1)
mtext(side = 2, text = expression(epsilon), line = 3, col = "blue", las = 1)
axis(1)
mtext(side = 1, text = expression(r(mu*m)), line = 3, las = 1)
par(new = T)
plot(nbv2v6$n, type = "l", col = "red", ylim = c(0,30), axes = F, ann = F)
mtext(side = 4, text = axTicks(4), at = axTicks(4), col = "red", line = 1, las = 1)
mtext(side = 4, text = "n", line = 3, col = "red", las = 1)

```

**Description**

Calculates the correlation between the pixel  $i$  and pixel  $i + dr$ .

**Usage**

```
pcf(img, nPoints = 1000, type = "d", dr = 1)
```

**Arguments**

img	The image to analyze
nPoints	The size of the sub-vectors in which the input vectors will be divided. This number must be less than $N/2$ .
type	Possible values are 'l' and 'c'. Set to 'l' by default
dr	Distance between pixel at which the correlation is calculated. For a value of $\text{delta}_r = 3$ , the columns are correlated as follows: (1,4), (2,5), ..., (n-3, n), with n being the last column.

**Details**

The pair correlation function (pCF) analyzes data of a periodically scanned line, measuring the time it takes a particle to go from one pixel to another, i.e. calculates the spatial cross-correlation function between pixels.  $G(\tau, \text{deltar}) = \langle F(t,0) F(t + \tau, \text{deltar}) \rangle / \langle F(t,0) \rangle \langle F(t, \text{deltar}) \rangle - 1$

**Value**

An image depicting the correlation between the pixel  $i$  and pixel  $i + dr$ .

**Author(s)**

Raul Pinto Camara.

**See Also**

[fcs](#), [pcomb](#)

**Examples**

```
### Load the FCSlib package

library(FCSlib)

### As an example, we will use a data set that corresponds to a population of Venus dimers
# diffusing in HEK-293 cells. Use the readfileTiff() function to extract the information
# from the '.tiff' files.

dmv2 <- data.matrix(V2)
pB <- pcf(dmv2, nPoints = 2500, dr = 10, type = 'd')

### Plot the result
```

```

library(fields)
di <- dim(pB)
tau <- (1:(di[2]))
image.plot( x = 1:di[1], y = log10(tau), z = pB, main = "Column Distance 10",
xlab = "Pixel", ylab = "Logarithmic tau",
cex.lab = 1.2, cex.main = 1.2, cex.axis = 1)

### Pair Correlation Function (Autocorrelation mode)
dmv2 <- data.matrix(v2DataSet)
pB <- pcf(dmv2, nPoints = 5000, type = 'a')

### Plot the result
library("fields")
di <- dim(pB)
tau <- (1:(di[2]))
image.plot( x = 1:di[1], y = log10(tau), z = pB, main = "Autocorrelation",
xlab = "Pixel", ylab = "Logarithmic tau",
cex.lab = 1.2, cex.main = 1.2, cex.axis = 1)

### Pair Correlation Function (Fixed column mode)
dmv2 <- data.matrix(v2DataSet)
pB <- pcf(dmv2, nPoints = 5000, dr = 10, type = 'c')

### Plot the result
library("fields")
di <- dim(pB)
tau <- (1:(di[2]))
image.plot( x = 1:di[1], y = log10(tau), z = pB, main = "Fixed Colum 10",
xlab = "Pixel", ylab = "Logarithmic tau",
cex.lab = 1.2, cex.main = 1.2, cex.axis = 1)

```

---

pcomb

*Pair Correlation of Molecular Brightness*


---

## Description

Performs the pair correlation of molecular brightness (pCOMB) analysis.

## Usage

```
pcomb(img, nPoints = 25000, type = "d", dr = 1, w = 100, pcf = TRUE)
```

## Arguments

img	The image to analyze.
nPoints	The size of the sub-vectors in which the input vectors will be divided. This number must be less than N/2.
type	By defaultl 'd'. The possible values are 'd', 'a' and 'c'

dr	Is the distance between the two columns that will be correlated. For a value of $\text{deltar} = 3$ , the columns are correlated as follows: (1,4), (2,5), ..., (n-3, n), with n as the last column.
w	Range value that is used to calculate the brightness in the image.
pcf	A boolean variable that determines whether the pcf analysis is performed on the brightness carpet or not.

### Details

With the Pair Correlation of Molecular Brightness (pCOMB) method, one can distinguish between different homo-oligomeric species of the same molecule coexisting in the same microenvironment, while separately and specifically tracking each species' mobility across the cellular compartments. This technique amplifies the signal from the brightest species present and filters the dynamics of the extracted oligomeric population based on arrival time between two locations. This method is suitable for mapping the impact oligomerization on transcription factor dynamics. The resulting intensity fluctuations, pCF, are transformed into brightness fluctuations using  $B = (\text{sigma}^2)/\text{mean}$ , and the pair correlation analysis is then performed on the brightness fluctuations along the line scan, at a distance ( $\text{delta}(r)$ ).

If the pcf is set as FALSE the pComb data will not be generated and will be NULL. In order to generate that data the pcf function must be used on the BCarpet data.

### Value

A list containing the Brightness Carpet and the Pair Correlation of that carpet

### Author(s)

Raul Pinto Camara.

### See Also

[fcs](#), [pcf](#)

### Examples

```
### Load the FCSlib package

library(FCSlib)

# As an example, we will use a data set that corresponds to a population of Venus dimers
# diffusing in HEK-293 cells. Use the readFileTiff() function to extract the information
# from the '.tiff' files.

dmv2 <- data.matrix(V2)
pC <- pcomb(dmv2[1:32,1:2001], nPoints = 1000, type = 'd', dr = 10, w = 2, pcf = FALSE)
dmv2 <- data.matrix(v2DataSet)
pC <- pcomb(dmv2, nPoints = 5000, type = 'd', dr = 10, w = 100)
di <- dim(pC$pComb)
tau <- (1:(di[2]))
```

```
# Plot the result
library("fields")
image.plot( x = 1:di[1], y = log10(tau), z = pC$pComb, main = "pComb",
xlab = "Pixel", ylab = "Logarithmic tau",
cex.lab = 1.2, cex.main = 1.2, cex.axis = 1)
```

---

readFileTiff

*Read File Tiff*

---

### Description

Reads a TIFF file and converts it into a 2D-array. If the file contains multiple pages, a 3D-array will be then returned.

### Usage

```
readFileTiff(filename, invert = TRUE)
```

### Arguments

filename	Either name of the file to read from or a raw vector representing the TIFF file content.
invert	If set to TRUE then the order of the data will be reversed. Default TRUE.

### Details

Read a TIFF file image using readTIFF and converts it to a matrix with n-dimensions.

### Value

A matrix containing the image data.

### Note

This function must be used in order to extract the information from the TIFF files needed to test the functions in this package. The TIFF file must be grayscale.

### Author(s)

Adan O. Guerrero Cardenas.

### See Also

[readTIFF](#) [writeFileTiff](#)

**Examples**

```
raw <- readFileTiff(FileName)
```

---

simplifyFCS

*Simplify FCS*

---

**Description**

Reduces the amount of data in a data set without altering its overall structure

**Usage**

```
simplifyFCS(g, tau)
```

**Arguments**

**g** A vector containing the FCS data analysis  
**tau** A vector that represents the time frame between data acquisitions

**Details**

Allows to significantly reduce the points of the autocorrelation vector, maintaining its overall structure and allowing to further adjust physical models while obtaining consistent results.

**Value**

A vector of the FCS data with reduced length

**Author(s)**

Adan O. Guerrero

**See Also**

[gcf](#), [var](#), [mean](#)

**Examples**

```
f <- Cy5_100nM$f
acqTime <- 2E-6
f <- as.vector(f)
time <- (1:length(f))*acqTime
cy5 <- data.frame(t = time, f)

len <- 1:length(g)
```

```
tau <- cy5$t[1:n]
G <- data.frame(tau,g)#' g <- fcs(x = cy5$f)

sfcs <- simplifyFCS(G$g, G$tau)
plot(df$g~df$tau, log = "x", type = "l",
      xlab = expression(tau(s)),
      ylab = expression(G(tau)), main = "Cy5")
```

---

smoothCarpet

*Smooth Carpet (Single Image)*

---

### Description

Generates a smooth carpet.

### Usage

```
smoothCarpet(img, dfV = 0, dfH = 0)
```

### Arguments

img	The image to analyze.
dfV	The desired equivalent number of degrees of freedom in the vertical axis.
dfH	The desired equivalent number of degrees of freedom in the horizontal axis.

### Details

The smoothCarpet function makes use of the smooth.spline method to smooth the vertical and horizontal axes of an image. The magnitude of the smoothing depends on the degrees of freedom set for and vertical ('dfV') and horizontal ('dfH') axes of the image.

### Value

Smooth Carpet A smooth image.

### Author(s)

Raul Pinto Camara.

### See Also

[pcomb](#), [smooth.spline](#)



## Examples

```
### Load the FCslib package

library(FCslib)

### As an example, we will use a data set that corresponds to a population of Venus dimers
# diffusing in HEK-293 cells. Use the readfileTiff() function to extract the information
# from the '.tiff' files.

v2 <- data.matrix(V2)
nbv2 <- nblines(img = v2, S=3.5, sigma0 = 1, offset = 0, wSigma = 100);
sC <- smoothCarpet(img = nbv2$number, dfV = 5, dfH = 5)
```

---

V2

*Experimental data of Venus dimers dynamics in HEK-293 cells.*

---

## Description

This data set consists on a raster line scan performed over HEK-293 cells expressing dimers of the fluorescent protein Venus, also known as SEYFP-F46L. The scan line is 64 pixels long, and the scanning direction is from the cytoplasm to the nucleus, across the nuclear envelope. A pixel size of 50 nm was used, as well as a pixel dwell time of 12.5 us and a line scan time of 1.925 ms. Fluorescence excitation was provided by a 488 nm laser at 0.1 Fluorescence intensity data was collected using the photon-counting mode in an Olympus FV1000 Upright BX61WI confocal microscope.

## Usage

```
data(V2)
```

## Format

A data frame with 64 rows and 25000 columns

## Details

In order to use the data in pcfData, the data.matrix() must be used to transform the data set into a matrix, and can be used in the examples.

---

writeFileTiff	<i>Write File Tiff</i>
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---

**Description**

Create a TIFF file from a 3D-array.

**Usage**

```
writeFileTiff(img, file.name, invert = TRUE, bits.per.sample = NULL)
```

**Arguments**

<code>img</code>	Either an image or a list of images. An image is a real matrix or array of three dimensions.
<code>file.name</code>	Either the name of the file or the name of a raw vector.
<code>invert</code>	If set to TRUE then the order of the data will be reversed. Default TRUE.
<code>bits.per.sample</code>	Number of bits per sample (numeric scalar). Supported values in this version are 8, 16, and 32.

**Details**

Create a TIFF file using `writeTIFF`, converting a 2D-array. If the file contains multiple pages, a 3D-array is turned into a 2D-array to implement the aforementioned function.

**Value**

None.

**Author(s)**

Adan O. Guerrero Cardenas.

**References**

None

**See Also**

[writeTIFF](#) [readFileTiff](#)

**Examples**

```
imagsave <- array(data = 1:10, dim = c(100,100,10))
writeFileTiff(imagsave, paste(tempdir(), "/image_Test.tif", sep = ""))
```

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