Package 'flowQB'

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Type Package

Title Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity: Q, B and CVinstrinsic calculations.

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Description flowQB is a fully automated R Bioconductor package to calculate automatically the detector efficiency (Q), optical background (B) and intrinsic CV of the beads.

Imports Biobase, graphics,methods, flowCore,stats,MASS

License Artistic-2.0

Suggests MASS, flowCore

biocViews FlowCytometry

LazyLoad yes

NeedsCompilation no

R topics documented:

`flowQB-package` *Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity: Q, B and CV-instrinsic calculations.*

Description

flowQB is a fully automated R Bioconductor package to calculate automatically the detector efficiency (Q), optical background (B) and intrinsic CV of the beads.

Details

Package:	flowQB
Type:	Package
Version:	1.0
Date:	2011-11-15
License:	
LazyLoad:	yes

BEADflowQBCalculation: This function is used for the bead FCS file to determine the singlet events. These singlet events are clustered for the channels of interest to determine the raw statitics for the regression and the generation of the regression's coefficients, Q and B values.

LEDflowQBCalculation: This function is used for the LED FCS files to determine, for the channels of interest, the raw statitics for the regression and the generation of the regression's coefficients, Q and B values.

These functions generate the results as a list, the first element of the list is for Raw Statistics and the second element of the list is for the coefficients, Q and B values.

Raw Statistics uses 3 approaches, Robust Statistics, Density estimation assuming a Gaussian distribution (MASS package) and the second 'extremevalues' package used to determine the raw statistics without outliers: 1) Number of events in each peak: NE 2) MFI associated to Robust Statistics: mfiRS 3) MFI associated to Gauss estimation using MASS: mfiGS 4) MFI associated to Gauss estimation using 'extremevalues': mfino 5) Standard deviation associated to Robust Statistics: mfirSD 6) Standard deviation associated to Gauss estimation using MASS: mfiGS 6) Standard deviation associated to Gauss estimation using 'extremevalues': mfiSDno 7) Number of events in each peak without outliers: Nesno.ORD

For each approach (StatsProcedure) and channel (MARKER), the coefficients (c0,c1,c2) and (Q,B) are listed with their associated (Pvalue, Std-Error).

Author(s)

Faysal EL Khettabi and Wayne Moore

References

Faysal El Khettabi et al. 2014, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

J. Wood, Fundamental Flow Cytometer Properties Governing Sensitivity and Resolution, Cytometry 33, (1998), p.~ 260 - 6. E. Chase and R. Hoffman, Resolution of Dimly Fluorescent Particles: a Practical Measure of Fluorescence Sensitivity, Cytometry 33 (1998), p.~ 267-279. R. Hoffman and J. Wood, Characterization of Flow Cytometer Instrument Sensitivity, Current Protocols in Cytometry, Chapter 1: Unit 1.20 (2007). A. Gaigalas and L. Wang, Approaches to Quantitation in Flow Cytometry, in Standardization and Quality Assurance in Fluorescence Measurements II Springer Series on Fluorescence (2008), Volume 6, Part D, 371-398.

Examples

```
if(1==0)
{
rm(list=ls(all=TRUE))
library("flowQB")
library(flowCore)
# TO READ FCS FILE, the flowcore library is needed
# Where is the file and its name?
fpath = system.file("extdata","FCSSMALLEVENTS.fcs",package="flowQB")
# fpath="/home/fkhettabi/Desktop/newflowQB2014/flowQB/inst/extdata/FCSSMALLEVENTS.fcs"
# Name of the FCS file
file=fpath
# Singlet Parameter:
# R: gate by an ellipse of radius R on the scatter data
R=2
# width: a parameter used to find the lower bound (default width at half maximum) and the upper bound (default .5)
width=.5
# fraction: a parameter used to find the densest fraction of cells (default .1).
fraction=.1
# Number of the peaks in the data
numberoftotalepeaks=8
# To define the indices of the FS and SS.
# NOW GO THE THE INPUT
fcs <- read.FCS(file)
names(fcs)
# Scatters FS & SS are:
scatters=c(2,5)
# Channels of interest, you need to select at least two channels, for instance:
CHANNELTOBEPROCESSED=c(8,9,36,37,38,39)
names(fcs)[CHANNELTOBEPROCESSED]
nClusters=numberoftotalepeaks
#####################################################
### PEAKS TO BE USED IN THE REGRESSION
#####################################################
pi=1
pf=7
```

```
output=""
RESULTS=BEADflowQBCalculation(file,R,width,fraction,numberoftotalepeaks,scatters,CHANNELTOBEPROCESSED,pi,pf,0,
}

if( 1==0)
{
rm(list=ls(all=TRUE))
library("flowQB")
NAMEOFTHEFILES=c("935295.fcs","935297.fcs","935299.fcs","935301.fcs","935303.fcs","935305.fcs")
# GET THE FILES WITH THEIR PATHS
# bulid "input"
input=rep("",length(NAMEOFTHEFILES))
for( i in 1:length(NAMEOFTHEFILES))
{
input[i] = system.file("extdata",NAMEOFTHEFILES[i],package="flowQB")
}

# TO READ FCS FILE, the flowcore library is needed
library(flowCore)
# read one file to have the name of the channels of interest!
fcs <- read.FCS(input[1])
orgdata=data.frame(exprs(fcs))
# Channels of interest, for instance:
CHANNELTOBEPROCESSEDx=c(8,9,36,37,38,39)
CHANNELTOBEPROCESSED = names(orgdata)[CHANNELTOBEPROCESSEDx]
# Minimum MFI to consider for the events.
minF=0
# Maximum MFI to consider for the events.
maxF=100000
RESULTS=LEDflowQBCalculation(input,CHANNELTOBEPROCESSED,minF,maxF)
}
```

`BEADflowQBCalculation` *QB Calculation for Beads*

Description

This function is used for the bead FCS file to determine the singlet events. These singlet events are clustered for the channels of interest to determine the raw statitics for the regression and the generation of the regression's coefficients, Q and B values.

Usage

```
BEADflowQBCalculation(file, R, width, fraction, numberoftotalepeaks, scatters, CHANNELTOBEPROCESSED,
```

Arguments

file	Name of the FCS file.
R	Gate by an ellipse of radius R on the scatter data.
width	Parameter used to find the lower bound (default width at half maximum) and the upper bound (default .5).
fraction	Parameter used to find the densest fraction of cells (default .1).
numberoftotalepeaks	Global Number of the peaks in the data.
scatters	Indices of Forward Scatter (FS) and Side scatter (SS) in the bead FCS file.
CHANNELTOBEPROCESSED	Indices of channels of interest in the bead FCS file.
pi	Dimmest peak to be used in the regression.
pf	Last peak to be used in the regression.
Viz	Parameter to control the visualization of Kmeans results only if Viz=1, a subfolder called flowQB in the output folder has subfolder "PLOTSKMEANS_BEADS_" with the visualizations.
output	Folder where the Kmeans image results should be stored.

Details

The function generates the results as a list, the first is for RAW STATISTICS and the second is for THE COEFFICIENTS.

Value

Q and B values with their "Pvalue" & "Std-Error" are in the second list with the coefficients.

Author(s)

Faysal El Khettabi et al.

References

Faysal El Khettabi et al. 2014, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

J. Wood, Fundamental Flow Cytometer Properties Governing Sensitivity and Resolution, Cytometry 33, (1998), p.~ 260 - 6.

E. Chase and R. Hoffman, Resolution of Dimly Fluorescent Particles: a Practical Measure of Fluorescence Sensitivity, Cytometry 33 (1998), p.~ 267-279.

R. Hoffman and J. Wood, Characterization of Flow Cytometer Instrument Sensitivity, Current Protocols in Cytometry, Chapter 1: Unit 1.20 (2007).

A. Gaigalas and L. Wang, Approaches to Quantitation in Flow Cytometry, in Standardization and Quality Assurance in Fluorescence Measurements II Springer Series on Fluorescence (2008), Volume 6, Part D, 371-398.

`find_peak` *Peak Detection*

Description

The determination of the peak uses the densest fraction (default fraction = .1) of events, the lower bound and the upper bound (default width = .5)

Usage

```
find_peak(data, width = 0.5, fraction = 0.1)
```

Arguments

`data`	FCS file.
`width`	Parameter used to find the lower bound (default width at half maximum) and the upper bound (default .5).
`fraction`	Parameter used to find the densest fraction of cells (default .1).

Details

This function will be used with the function scatter_gate.

Value

Peaks for the scatters.

Author(s)

Wayne Moore

References

Wayne Moore et al. 2014, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

LEDflowQBCalculation *QB Calculation for LEDs*

Description

This function is used for the LED FCS files to determine, for the channels of interest, the raw statitics for the regression and the generation of the regression's coefficients, Q and B values.

Usage

```
LEDflowQBCalculation(input, CHANNELTOBEPROCESSED, minF, maxF)
```

Arguments

input	Folder where the LED FCS files is stored.
CHANNELTOBEPROCESSED	Indices of channels of interest in the LED FCS files.
minF	Minimum MFI to consider for the peaks.
maxF	Maximum MFI to consider for the peaks.

Details

The function generates the results as a list, the first element of the list is for raw statistics and the second element of the list is for the coefficients.

Value

Q and B values with their "Pvalue" & "Std-Error" are in the second list with the coefficients.

Author(s)

Faysal El Khettabi et al.

References

Faysal El Khettabi et al. 2014, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

J. Wood, Fundamental Flow Cytometer Properties Governing Sensitivity and Resolution, Cytometry 33, (1998), p.~ 260 - 6.

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A. Gaigalas and L. Wang, Approaches to Quantitation in Flow Cytometry, in Standardization and Quality Assurance in Fluorescence Measurements II Springer Series on Fluorescence (2008), Volume 6, Part D, 371-398.

`scatter_gate` *Singlets Detection*

Description

Gate by an ellipse of radius R on the scatter data.

Usage

```
scatter_gate(fcs, scatters, R = 1)
```

Arguments

`fcs`	FCS file.
`scatters`	Indices of the Forward scatter and Side Scatter in the FCS file.
`R`	Gate by an ellipse of radius R on the scatter data.

Details

Singlets detection using Forward scatter and Side Scatter in the FCS file.

Value

A new FCS file with only singlets.

Author(s)

Wayne Moore

References

Wayne Moore et al. 2014, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

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