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, xtable

biocViews FlowCytometry

LazyLoad yes

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`flowQB-package` *Automatic Q, B and CVintrinsic calculations*

Description

We developed an automated approach to determine a cytometer's detection efficiency (Q) and background illumination (B) independent of a pre-defined bead set, based on Kmeans clustering and quadratic regression methods. We used a quadratic formulation for modelling Q and B that offers more physical insight about cytometry sensitivity than its truncated linear version, by considering the term CVintrinsic as part of the problem to solve. Using Kmeans in place of manual gating enabled an automated analysis to calculate Q, B and CVintrinsic quantities objectively and in a time-efficient manner. This flowQB package is a R implementation of the theoretical description of Q, B and CVintrinsic calculations in our manuscript "Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity".

Details

Package:	flowQB
Type:	Package
Version:	1.3.3
Date:	2011-11-15
License:	
LazyLoad:	yes

A number of generic functions are used to conduct an automatic Q, B and CVintrinsic calculations, the following functions are considered as introductory:

Function ReadDD reads the given FCS file and removes doublet events in the channel of interest(Chang). The ReadDD function returns a 2D array having the mean flourescent intensities(MFI) of the singlet events, first column is for the channel of interest and the second column is for the companion channel to be used to facilitate the identification of the bead sub-populations.

Function KmeansMeanSD takes the 2D MFI array and generates a number of clusters and return their MFI means and SDs.

Function MFI2MESF converts the obtained MFI means to MESF means. For instance, the MFI output of KmeansMeanSD are converted to MESF values with an option to correct the SDs.

Functions lrMESF and qrMESF are the linear and quadratic regressions which use the obtained Means and Variances to calculate the Q and B values.

Function DiscriminantExamination uses the values in the output of the function qrMESF to estimate the discriminant of the resulting quadratic equation and can be used as an additional interpretation tool to aid in understanding cytometer sensitivity.

An advanced processing is in the vignettes AdvancedflowQBNIH2 and AdvancedflowQBNIH3 and have a detailed illustration how to use the functions: gPS, rPS, MultilevelgPS, MultilevelrPS and qrWEIGHTEDMESF. For this introductory processing, examples are provided here and in the vignette IntroductoryflowQBNIH.

Author(s)

Faysal El Khettabi

References

Faysal El Khettabi et al. 2013, 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

```
rm(list=ls(all=TRUE))
library("flowQB")
File= system.file("extdata","NIH.fcs",package="flowQB")

# Doublet Discriminations
   DD=96
# Reading the FCS file, the Forward Scattering Area 1 and 2
# are used to obtain singlet events with DD=96%.
# The processing returns a 2D singlet events for the channel
# of interest 5 with the companion channel Side Scattering 3.
   t2D=ReadDD(File,1,2,DD,5,3)
# Function KmeansMeanSD returns the MFI Means and SDs of the 8 clusters.
   MeansSDs=KmeansMeanSD(t2D,8,500,200,1)
# CVs calculation
   CVs=MeansSDs[,2]/MeansSDs[,1]
# MESF calculation,
   p=357217.00/7102
# Linear Q and B Calculation.
# MFIs are converted to MESFs and SDs are corrected using the beads in cluster 8.
   l2D=MFI2MESF(MeansSDs,p,CVs[8])
# Linear regression
```

```
# Peaks associated to cluster  3 to  cluster 5 are used
# to compute the linear regression coefficients.
   LQB=lrMESF(l2D,3,5)
   print("Linear QB")
   print(LQB)
# Quadratic Q and B Calculation.
# MFIs are converted to MESFs and SDs are NOT corrected.
   Q2D=MFI2MESF(MeansSDs,p,0)
# Peaks associated to cluster  3 to  cluster 6 are used to compute
# the quadratic regression coefficients.
   QQB=qrMESF(Q2D,3,6)
   print("Quadratic QB")
   print(QQB)
```

`DiscriminantExamination`

DiscriminantExamination

Description

DiscriminantExamination uses the values in the output of the function qrMESF to estimate the discriminant of the resulting quadratic equation and can be used as an additional interpretation tool to aid in understanding cytometer sensitivity.

Usage

```
DiscriminantExamination(Q, B, sigmaS2)
```

Arguments

`Q`	Q: the first value in the output of the function qrMESF.
`B`	B: the second value in the output of the function qrMESF.
`sigmaS2`	sigmaS2: the fourth value in the output of the function qrMESF.

Details

It examines the discriminant, Delta = c1^2 -4*c0*c2, of the quadratic equation, c0+c1*MESF+c2*MESF^2.

There are two possible scenarios: Delta >= 0 or Delta < 0. The coefficients (c0,c1,c2) are derived from the output of the function qrMESF.

Value

Output c0, c1, c2 and Delta values.

If Delta >= 0, the larger the variation product of (sigmaE2) and (sigmaS2), the lower the upper bound on the detection efficiency Q.

If Delta < 0, the lower the variation product of (sigmaE2) and (sigmaS2), the greater the upper bound on the detection efficiency Q.

Author(s)

Faysal El Khettabi \ Terry Fox Laboratory \ British Columbia Cancer Agency \ Vancouver, BC, Canada \ E-mail: fkhettabi@bccrc.ca

References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

The function qrMESF in flowQB.

Examples

```
rm(list=ls(all=TRUE))
library("flowQB")
File= system.file("extdata","NIH.fcs",package="flowQB")

# Doublet Discriminations
P=96

# Reading the FCS file, the Forward Scattering Area 1 and 2
# are used to obtain singlet events with P=96%.
# The processing returns a 2D singlet events for the channel
# of interest, index 5, with the companion channel Side Scattering, index 3.

# Usage of the function ReadDD
t2D=ReadDD(File,1,2,P,5,3)
summary(t2D)

# Usage of the function KmeansMeanSD
MFIMeansSDs=KmeansMeanSD(t2D,8,500,200,1)
# Function KmeansMeanSD returns the MFI Means and SDs of the 8 clusters.
MFIMeansSDs

# For MESF calculation, the constant conversion between MFI and MESF is set to:
p=357217.00/7102

# MFIs are converted to MESFs and SDs are not corrected as we set IllCorrCV=0.
# Usage of the function MFI2MESF
MFI2MSEF=MFI2MESF(MFIMeansSDs,p,0)
MFI2MSEF
# Note that MESF: MESF Mean and MESFV: MESF Variance (SD^2).


# Quadratic Q and B Calculation.
# Peaks associated to cluster  3 to  cluster 6 are used to compute
# the quadratic regression coefficients.

# Usage of the function qrMESF
QQB=qrMESF(MFI2MSEF,3,6)
```

```
print("Quadratic Q & B")
OV=c(Q=as.numeric(QQB[1]), B=as.numeric(QQB[2]), Rsquared=as.numeric(round(QQB[3], 2)), sigmaS2=as.numeric((QQB[
print(OV)
# Note  c1 =1/Q, c0= B/Q and c2 = sigmaS2

# Discriminant of the Quadratic Equation
Coefs <- DiscriminantExamination(as.numeric(QQB[1]),as.numeric(QQB[2]),as.numeric(QQB[4]))

Delta=Coefs[4]

if(Delta >= 0)
{
cat(paste("The sign of the discriminant is positive with the value",round(Delta,2),"\n"))
cat("The larger the variation product of (sigmaE2) and (sigmaS2), \n")
cat("the lower the upper bound on the detection efficiency Q.\n")
}

if(Delta < 0)
{
cat(paste("The sign of the discriminant is negative with the value",round(Delta,2),"\n"))
cat("The lower the variation product of (sigmaE2) and (sigmaS2), \n")
cat("the greater the upper bound on the detection efficiency Q. \n")
}
```

---

gPS *gPS Process Kmeans and fit the data to a Gaussian distribution using MASS package.*

Description

This function uses Gaussian distribution fitting to extract the statistics for the peaks found by Kmeans. This function is usefull when a marker is associated to SSC to form a 2D processing using Kmeans. This function process Kmeans assuming nClusters peaks to be detected and output their statistics with the associated number of events.

Usage

```
gPS(TSSINGLETS, OSSINGLETS, nClusters)
```

Arguments

TSSINGLETS	TSSINGLETS table having the LOGICLE events VALUES of the selected marker including SSC in the first Column.
OSSINGLETS	OSSINGLETS table having the ORIGINAL events VALUES of the selected marker including SSC in the first Column.
nClusters	Number of the peaks to detect.

Details

Use logicle transformation to transform the data. Second identify bead singlets. Select the marker with SSC in the first Column. Store the 2D data in TSSINGLETS and the no-transformed 2D data in OSSINGLETS.

Value

The probability distribution fitting of the peak data events to a Gaussian distribution is used for peak statistics extraction, the fitting parameters means and standard deviations are the estimations of MFIs and SDs values with the associated number of events.

Note

This function is a 2D processing of selected marker with SSC. A general function is given in the function MultilevelgPS.

Author(s)

Faysal el Khettabi

References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

See MultilevelgPS function

Examples

```
# The vignettes $AdvancedflowQBNIH2.Rnw$ and $AdvancedflowQBNIH3.Rnw$ have a detailed illustration how to use this
```

`KmeansMeanSD` *KmeansMeanSD*

Description

Function KmeansMeanSD takes the 2D array generated by the function ReadDD and be clustered into a number of clusters and returns their Means and SDs.

Usage

```
KmeansMeanSD(transformed2Darray, nClusters, nstart, itermax, Vis)
```

Arguments

transformed2Darray	2D array.
nClusters	Number of clusters
nstart	See R kmeans.
itermax	See R kmeans.
Vis	If 0 no visualization, if 1 with visualization.

Details

These MFI Means and SDs will be first converted to MESF Means and SDs using the function MFI2MESF.

Value

Output for each cluster the statistics: MFI Mean and standard deviation (SD).

References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

kmeans in R software.

Examples

```
rm(list=ls(all=TRUE))
library("flowQB")
File= system.file("extdata","NIH.fcs",package="flowQB")

#  Now FCS file is in data folder as a temp file.
#  This file will be removed at the end of the illustration.

# Doublet Discriminations
P=96

# Reading the FCS file, the Forward Scattering Area 1 and 2
# are used to obtain singlet events with P=96%.
# The processing returns a 2D singlet events for the channel
# of interest, index 5, with the companion channel Side Scattering, index 3.

# Usage of the function ReadDD
t2D=ReadDD(File,1,2,P,5,3)
summary(t2D)

# Usage of the function KmeansMeanSD
```

```
MFIMeansSDs=KmeansMeanSD(t2D,8,500,200,1)
# Function KmeansMeanSD returns the MFI Means and SDs of the 8 clusters.
MFIMeansSDs
```

KmeansMedianrSD *KmeansMedianrSD*

Description

Function KmeansMedianrSD takes a given 2D array and generates a number of clusters and returns their Medians and robust standard deviations (rSDs).

Usage

```
KmeansMedianrSD(transformed2Darray, nClusters, nstart, itermax, Vis)
```

Arguments

transformed2Darray	2D array.
nClusters	Number of clusters
nstart	See R kmeans.
itermax	See R kmeans.
Vis	If 0 no visualization, if 1 with visualization.

Details

See the manuscript in the references.

Value

Output for each cluster the robust statistics: Medians and robust standard deviations (rSDs).

Author(s)

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References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

kmeans in R software.

Examples

```
# see Examples.
```

`lrMESF` *lrMESF*

Description

Function lrMESF uses the Means and Variance in terms of the MESF values to conduct a linear regression. The obtained coefficients are used to calculate the Q and B values.

Usage

```
lrMESF(MESFmeanssd2, Peak1, Peak2)
```

Arguments

`MESFmeanssd2`	MESF 2D data having means and Variances.
`Peak1`	First peak to consider.
`Peak2`	Last peak to consider.

Details

The detector efficiency (Q) and optical background (B) are derived from the obtained linear regression coefficients when the SDs are corrected for illumination.

Value

Output linear regression detector efficiency (Q) and optical background (B).

Author(s)

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References

Faysal El Khettabi et al. 2013, 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).

Examples

```
rm(list=ls(all=TRUE))
rm(list=ls(all=TRUE))
library("flowQB")
File= system.file("extdata","NIH.fcs",package="flowQB")

# Doublet Discriminations
P=96

# Reading the FCS file, the Forward Scattering Area 1 and 2
# are used to obtain singlet events with P=96%.
# The processing returns a 2D singlet events for the channel
# of interest, index 5, with the companion channel Side Scattering, index 3.

# Usage of the function ReadDD
t2D=ReadDD(File,1,2,P,5,3)
summary(t2D)

# Usage of the function KmeansMeanSD
MFIMeansSDs=KmeansMeanSD(t2D,8,500,200,1)
# Function KmeansMeanSD returns the MFI Means and SDs of the 8 clusters.
MFIMeansSDs

# For MESF calculation, the constant conversion between MFI and MESF is set to:
p=357217.00/7102

# MFIs are converted to MESFs and SDs are not corrected as we set,
IllCorrCV=MFIMeansSDs[8,2]/MFIMeansSDs[8,1] #SDs are corrected using the beads in cluster 8.

# Usage of the function MFI2MESF
MFI2MSEF=MFI2MESF(MFIMeansSDs,p,IllCorrCV)
MFI2MSEF
# Note that MESF: MESF Mean and MESFV: MESF Variance (SD^2).

# Linear regression
# Peaks associated to cluster  3 to  cluster 5 are used
# to compute the linear regression coefficients.

# Usage of the function lrMESF
LQB=lrMESF(MFI2MSEF,3,5)
print("Linear QB")
```

```
OV=c(Q=as.numeric(LQB[1]), B=as.numeric(LQB[2]), Rsquared=as.numeric(round(LQB[3], 2)))
print(OV)
# Note  c1 =1/Q, c0= B/Q
```

MFI2MESF *MFI2MESF*

Description

MFI2MESF converts the MFI means to MESF means.

Usage

```
MFI2MESF(MeansSDs, p, IllCorrCV)
```

Arguments

MeansSDs	2D array, first column MFI means , second column the associated SDs.
p	Constant Conversion between MFI and MESF
IllCorrCV	Value to be used to correct the SDs.

Details

The output will be used as input in the functions lrMESF or qrMESF to calculate the detector efficiency (Q) and optical background (B).

Value

Convert MFI to MSEF, the output is a 2D array having the MESF Means (MESF) and the associated MESF Variances (MESFV).

Author(s)

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References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

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.

See Also

The functions lrMESF and qrMESF in flowQB.

Examples

```
rm(list=ls(all=TRUE))
rm(list=ls(all=TRUE))
library("flowQB")

File= system.file("extdata","NIH.fcs",package="flowQB")

# Doublet Discriminations
P=96

# Reading the FCS file, the Forward Scattering Area 1 and 2
# are used to obtain singlet events with P=96%.
# The processing returns a 2D singlet events for the channel
# of interest, index 5, with the companion channel Side Scattering, index 3.

library("flowQB")

# Usage of the function ReadDD
t2D=ReadDD(File,1,2,P,5,3)
summary(t2D)

# Usage of the function KmeansMeanSD
MFIMeansSDs=KmeansMeanSD(t2D,8,500,200,1)
# Function KmeansMeanSD returns the MFI Means and SDs of the 8 clusters.
MFIMeansSDs

# For MESF calculation, the constant conversion between MFI and MESF is set to:
p=357217.00/7102

# MFIs are converted to MESFs and SDs are not corrected as we set IllCorrCV=0.
# Usage of the function MFI2MESF
MFI2MSEF=MFI2MESF(MFIMeansSDs,p,0)
MFI2MSEF
# Note that MESF: MESF Mean and MESFV: MESF Variance (SD^2).
```

`MultilevelgPS` *MultilevelgPS Process multi-level Kmeans and fit the data to a Gaussian distribution using MASS package.*

Description

This function uses Gaussian distribution fitting to extract the statistics for the peaks found by multi-level Kmeans. This function is usefull when a number of markers are processed as a multidimensional space to detect the assocaited peaks for each marker in the data. This function process Kmeans assuming nClusters peaks to be defined and output their statistics with the associated number of events.

Usage

```
MultilevelgPS(TSSINGLETS, OSSINGLETS, nClusters)
```

Arguments

TSSINGLETS	TSSINGLETS table having the LOGICLE events VALUES of the selected markers.
OSSINGLETS	OSSINGLETS table having the ORIGINAL events VALUES of the selected markers.
nClusters	Number of the peaks to detect.

Details

Use logicle transformation to transform the data. Second identify bead singlets. Select the markers to be processed. Store the data of the selected markers in TSSINGLETS and the no-transformed data in OSSINGLETS.

Value

The probability distribution fitting of the peak data events to a Gaussian distribution is used for peak statistics extraction, the fitting parameters means and standard deviations are the estimations of MFIs and SDs values with the associated number of events.

Note

This function is a multi-level processing as the function gPS without using the SSC.

Author(s)

Faysal el Khettabi

References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

See gPS function

Examples

```
# The vignettes $AdvancedflowQBNIH2.Rnw$ and $AdvancedflowQBNIH3.Rnw$ have a detailed illustration how to use this
```

`MultilevelrPS` *MultilevelrPS Process multi-level Kmeans and extract the Robust statistics.*

Description

This function uses Robust statistics for the peaks found by multi-level Kmeans. This function is usefull when a number of markers are processed as a multidimensional space to detect the assocaited peaks for each marker in the data. This function process a multi-level Kmeans assuming nClusters peaks to be detected and output their statistics with the associated number of events.

Usage

```
MultilevelrPS(TSSINGLETS, OSSINGLETS, nClusters)
```

Arguments

`TSSINGLETS`	TSSINGLETS table having the LOGICLE events VALUES of the selected markers.
`OSSINGLETS`	OSSINGLETS table having the ORIGINAL events VALUES of the selected markers.
`nClusters`	Number of the peaks to detect.

Details

Use logicle transformation to transform the data. Second identify bead singlets. Select the markers to be processed. Store the data of the selected markers in TSSINGLETS and the no-transformed data in OSSINGLETS.

Value

Robust statistics with the associated number of events.

Note

This function is a multi-level processing as the function rPS without using the SSC.

Author(s)

Faysal el Khettabi

References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

See rPS function

Examples

```
# The vignettes $AdvancedflowQBNIH2.Rnw$ and $AdvancedflowQBNIH3.Rnw$ have a detailed illustration how to use this
```

`NIH.fcs` *NIH data*

Description

To illustrate Q and B calculations.

Usage

```
NIH.fcs
```

Format

fcs

Source

NIH

References

NIH

`qrMESF` *qrMESF*

Description

qrMESF uses the Means and variances in terms of the MESF values to conduct a quadratic regression

Usage

```
qrMESF(MESFmeanssd2, Peak1, Peak2)
```

Arguments

`MESFmeanssd2`	MESF 2D data having means and Variances.
`Peak1`	First peak to consider.
`Peak2`	Last peak to consider.

Details

The detector efficiency (Q) and optical background (B) are derived from the obtained quadratic regression coefficients.

Value

Output quadratic regression detector efficiency (Q) and optical background (B).

Author(s)

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References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

lrMESF function.

Examples

```
rm(list=ls(all=TRUE))
rm(list=ls(all=TRUE))
library("flowQB")
File= system.file("extdata","NIH.fcs",package="flowQB")

# Doublet Discriminations
P=96

# Reading the FCS file, the Forward Scattering Area 1 and 2
# are used to obtain singlet events with P=96%.
# The processing returns a 2D singlet events for the channel
# of interest, index 5, with the companion channel Side Scattering, index 3.

library("flowQB")

# Usage of the function ReadDD
t2D=ReadDD(File,1,2,P,5,3)
summary(t2D)

# Usage of the function KmeansMeanSD
MFIMeansSDs=KmeansMeanSD(t2D,8,500,200,1)
# Function KmeansMeanSD returns the MFI Means and SDs of the 8 clusters.
MFIMeansSDs

# For MESF calculation, the constant conversion between MFI and MESF is set to:
p=357217.00/7102
```

```
# MFIs are converted to MESFs and SDs are not corrected as we set IllCorrCV=0.
# Usage of the function MFI2MESF
MFI2MSEF=MFI2MESF(MFIMeansSDs,p,0)
MFI2MSEF
# Note that MESF: MESF Mean and MESFV: MESF Variance (SD^2).


# Quadratic Q and B Calculation.
# Peaks associated to cluster  3 to  cluster 6 are used to compute
# the quadratic regression coefficients.

# Usage of the function qrMESF
QQB=qrMESF(MFI2MSEF,3,6)
print("Quadratic Q & B")
OV=c(Q=as.numeric(QQB[1]), B=as.numeric(QQB[2]), Rsquared=as.numeric(round(QQB[3], 2)), sigmaS2=as.numeric((QQB[
print(OV)
# Note  c1 =1/Q, c0= B/Q and c2 = sigmaS2
```

---

qrWEIGHTEDMESF *qrMESF*

Description

qrWEIGHTEDMESF uses the Means and variances in terms of the MESF values to conduct a weighted quadratic regression

Usage

```
qrWEIGHTEDMESF(MESFmeanssd2, Peak1, Peak2,Givenweights)
```

Arguments

MESFmeanssd2	MESF 2D data having means and Variances.
Peak1	First peak to consider.
Peak2	Last peak to consider.
Givenweights	Givenweights: a vector of weights to be used in the fitting process,

Details

The detector efficiency (Q) and optical background (B) are derived from the obtained quadratic regression coefficients.

Value

Output quadratic regression detector efficiency (Q) and optical background (B).

Author(s)

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References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

lrMESF function.

Examples

```
#  The vignettes $AdvancedflowQBNIH2.Rnw$ and $AdvancedflowQBNIH3.Rnw$ have a detailed illustration how to use this

rm(list=ls(all=TRUE))
rm(list=ls(all=TRUE))
library("flowQB")
File= system.file("extdata","NIH.fcs",package="flowQB")

# Doublet Discriminations
P=96

# Reading the FCS file, the Forward Scattering Area 1 and 2
# are used to obtain singlet events with P=96%.
# The processing returns a 2D singlet events for the channel
# of interest, index 5, with the companion channel Side Scattering, index 3.

library("flowQB")

# Usage of the function ReadDD
t2D=ReadDD(File,1,2,P,5,3)
summary(t2D)

# Usage of the function KmeansMeanSD
MFIMeansSDs=KmeansMeanSD(t2D,8,500,200,1)
# Function KmeansMeanSD returns the MFI Means and SDs of the 8 clusters.
MFIMeansSDs

# For MESF calculation, the constant conversion between MFI and MESF is set to:
p=357217.00/7102

# MFIs are converted to MESFs and SDs are not corrected as we set IllCorrCV=0.
# Usage of the function MFI2MESF
MFI2MSEF=MFI2MESF(MFIMeansSDs,p,0)
MFI2MSEF
# Note that MESF: MESF Mean and MESFV: MESF Variance (SD^2).
```

```
# Quadratic Q and B Calculation.
# Peaks associated to cluster  3 to  cluster 6 are used to compute
# the quadratic regression coefficients.
# For illustration, the given-weights are all equal to one for the four peaks.
Givenweights=rep(1,4)
# Usage of the function qrMESF
QQB=qrWEIGHTEDMESF(MFI2MSEF,3,6,Givenweights)
print("Quadratic Q & B")
OV=c(Q=as.numeric(QQB[1]), B=as.numeric(QQB[2]), Rsquared=as.numeric(round(QQB[3], 2)), sigmaS2=as.numeric((QQB[
print(OV)
# Note  c1 =1/Q, c0= B/Q and c2 = sigmaS2
```

ReadDD *ReadDD*

Description

Function ReadDD reads a given FCS file and remove doublet events in the channel of interest(ChanGiven).

Usage

```
ReadDD(File, Chan1DD, Chan2DD, P, ChanGiven, ChanCompanion)
```

Arguments

File	Path of the file to be read.
Chan1DD	Number(index) of the first channel to be used in detecting singlet events.
Chan2DD	Number(index) of the second channel to be used in detecting singlet events.
P	Doublet discrimination level, only events satistfying P/100 < Chan1DD/Chan2DD <= 2-P/100 are extracted by the function ReadDD and they are singlet events. P can be a number between 50 to 99.
ChanGiven	Number(index) of the channel of interest.
ChanCompanion	Number(index) of the companion channel to be used to facilitate the identification of the bead sub-populations.

Details

The returned 2D array will be clustered using the function KmeansMeanSD.

Value

The ReadDD function returns a 2D array having the mean flourescent intensities(MFI) of the singlet events, first column is for the channel of interest(ChanGiven) and the second column is for the companion channel(ChanCompanion) to be used to facilitate the identification of the bead sub-populations.

Note

It reads only a FCS file.

Author(s)

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References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

flowCore package.

Examples

```
rm(list=ls(all=TRUE))
rm(list=ls(all=TRUE))
library("flowQB")
File= system.file("extdata","NIH.fcs",package="flowQB")

#  Now FCS file is in data folder as a temp file.
#  This file will be removed at the end of the illustration.

# Doublet Discriminations
P=96

# Reading the FCS file, the Forward Scattering Area 1 and 2
# are used to obtain singlet events with P=96%.
# The processing returns a 2D singlet events for the channel
# of interest, index 5, with the companion channel Side Scattering, index 3.

# Usage of the function ReadDD
t2D=ReadDD(File,1,2,P,5,3)
summary(t2D)
```

---

rPS *rPS*

Description

This function uses Robust Statistics to extract the statistics for the peaks found by Kmeans. This function process Kmeans assuming nClusters peaks to be defined and output their Robust Statistics.

Usage

```
rPS(TSSINGLETS, OSSINGLETS, nClusters)
```

Arguments

TSSINGLETS	TSSINGLETS table having the LOGICLE events VALUES of the selected marker including SSC in the first Column.
OSSINGLETS	OSSINGLETS table having the ORIGINAL events VALUES of the selected marker including SSC in the first Column.
nClusters	Number of the peaks to define.

Details

Use logicle transformation to transform the data. Second identify bead singlets. Select the marker with SSC in the first Column. Store the 2D data in TSSINGLETS and the no-transformed 2D data in OSSINGLETS.

Value

The robust statistics and the number of events of each peak are returned.

Note

This function is a 2D processing of selected marker with SSC. A general function is given in the function MultilevelrPS.

Author(s)

Faysal el Khettabi

References

Faysal El Khettabi et al. 2013, Automated Quadratic Characterization of Flow Cytometer Instrument Sensitivity, to be submitted.

See Also

See MultilevelrPS function

Examples

```
# The vignettes $AdvancedflowQBNIH2.Rnw$ and $AdvancedflowQBNIH3.Rnw$ have a detailed illustration how to use this
```

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