

# Package ‘flowQB’

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**Type** Package

**Title** Flow cytometer sensitivity-Automatic Q and B Calculations.

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**Author** Faysal El Khettabi

**Maintainer** Faysal El Khettabi <faysal.el.khettabi@gmail.com>

**Description** flowQB is a fully automated R Bioconductor package to calculate automatically the detector efficiency (Q), optical background (B), and electronic noise.

**Imports** Biobase, graphics, methods, flowCore, stats

**License** Artistic-2.0

**Suggests** flowCore

**biocViews** FlowCytometry

**LazyLoad** yes

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**Description**

We implemented a set of generic functions for an automatic Q and B calculations: To estimate automatically the detector efficiency (Q), optical background (B), and electronic noise and to determine the optimal voltages for each fluorescence parameter when a series of voltages are applied to the photomultiplier tube (PMT) to setup the optimal separation and sensitivity.

The flowQB package is a R implementation of the theoretical description of Q and B calculation in our manuscript "Flow Cytometer Sensitivity: A Quadratic Model".

**Details**

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

Six generic functions are used to conduct an automatic Q and B calculation with the following utilities:

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 fluorescent 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.

Illustrative scripts are provided in examples.

**Author(s)**

Faysal El Khettabi

## References

F. El Khettabi et al. 2012, Flow Cytometer Sensitivity: A Quadratic Model 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*

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## 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,  $\Delta = c_1^2 - 4 \cdot c_0 \cdot c_2$ , of the quadratic equation,  $c_0 + c_1 \cdot \text{MESF} + c_2 \cdot \text{MESF}^2$ .

There are two possible scenarios:  $\Delta \geq 0$  or  $\Delta < 0$ . The coefficients ( $c_0, c_1, c_2$ ) are derived from the output of the function qrMESF.

## Value

Output  $c_0$ ,  $c_1$ ,  $c_2$  and  $\Delta$  values.

If  $\Delta \geq 0$ , the larger the variation product of ( $\sigma_{E2}$ ) and ( $\sigma_{S2}$ ), the lower the upper bound on the detection efficiency  $Q$ .

If  $\Delta < 0$ , the lower the variation product of ( $\sigma_{E2}$ ) and ( $\sigma_{S2}$ ), 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. 2012, Flow Cytometer Sensitivity: A Quadratic Model, to be submitted.

**See Also**

The function 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.

# 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.double(QQB[1]), B=as.double(QQB[2]), Rsquared=as.double(round(QQB[3], 2)), sigmaS2=as.double((QQB[4])))
print(OV)
# Note c1 =1/Q, c0= B/Q and c2 = sigmaS2

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

```

---

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 <i>kmeans</i> .
itermax	See R <i>kmeans</i> .
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).

**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. 2012, Flow Cytometer Sensitivity: A Quadratic Model, 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)**

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

**References**

F. El Khettabi, E. Ramadan and R. Brinkman, Flow Cytometer Sensitivity: a Quadratic Approach, 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)

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

### References

Faysal El Khettabi et al. 2012, Flow Cytometer Sensitivity: A Quadratic Model, 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.double(LQB[1]), B=as.double(LQB[2]), Rsquared=as.double(round(LQB[3], 2)))
print(OV)
# Note c1 =1/Q, c0= B/Q

```

**Description**

MFI2MESF converts the MFI means to MESF means.

**Usage**

```
MFI2MESF(MeansSDs, p, I11CorrCV)
```

**Arguments**

MeansSDs	2D array, first column MFI means , second column the associated SDs.
p	Constant Conversion between MFI and MESF
I11CorrCV	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 MEF, the output is a 2D array having the MESF Means (MESF) and the associated MESF Variances (MESFV).

**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. 2012, Flow Cytometer Sensitivity: A Quadratic Model, 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).

```

---

NIH.fcs

*NIH data*


---

### Description

To illustrate Q and B calculation.

### 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)**

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

**References**

Faysal El Khettabi et al. 2012, Flow Cytometer Sensitivity: A Quadratic Model, 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.double(QQB[1]), B=as.double(QQB[2]), Rsquared=as.double(round(QQB[3], 2)), sigmaS2=as.double((QQB[4])))
print(OV)
# Note c1 =1/Q, c0= B/Q and c2 = sigmaS2

```

---

ReadDD	<i>ReadDD</i>
--------	---------------

---

### 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 satisfying $P/100 < \text{Chan1DD}/\text{Chan2DD} \leq 2 \cdot P/100$ 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 fluorescent 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)

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

### References

Faysal El Khettabi et al. 2012, Flow Cytometer Sensitivity: A Quadratic Model, 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)
```



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