Package 'flowAI'

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Title Au	tomatic and interactive quality control for flow cytometry		
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Author (Gianni Monaco, Hao Chen		
con	escription The package is able to perform an automatic or interactive quality control on FCS data acquired using flow cytometry instruments. By evaluating three different properties: 1) flow rate, 2) signal acquisition, 3) dynamic range, the quality control enables the detection and removal of anomalies.		
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R topi	ics documented:		
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Bcells

flowSet of B cells.

Description

This data set contain three flowFrame objects created by subsetting three FCS files of an Aging study made in Singapore. The samples were stained with a panel aimed to identify B cell subpopulations. The data is stored as a flowSet object, a class implemented in the flowCore package to handle FCS files in R.

Usage

```
data(Bcells)
```

Format

A flowSet containing 3 flowFrames

flow_auto_qc

Automatic quality control of flow cytometry data.

Description

For a set of FCS files, flow_auto_qc performs a complete and automatic quality control. It consists in the detection and removal of anomalies by checking three properties of flow cytometry: 1) flow rate, 2) signal acquisition, 3) dynamic range.

Usage

```
flow_auto_qc(fcsfiles, remove_from = "all", output = 1,
   timeCh = NULL, second_fractionFR = 0.1, alphaFR = 0.01,
   decompFR = TRUE, ChExcludeFS = c("FSC", "SSC"),
   outlier_binsFS = FALSE, pen_valueFS = 500, max_cptFS = 3,
   ChExcludeFM = c("FSC", "SSC"), sideFM = "both", neg_valuesFM = 1,
   html_report = "_QC", mini_report = "QCmini", fcs_QC = "_QC",
   fcs_highQ = FALSE, fcs_lowQ = FALSE, folder_results = "resultsQC")
```

Arguments

fcsfiles

It can be a character vector with the filenames of the FCS files, a flowSet or a flowFrame.

remove_from

Select from which of the three steps the anomalies have to be excluded in the high quality FCS file. The default option "all" removes the anomalies from all the three steps. Alternatively, you can use: "FR_FS", "FR_FM", "FS_FM", "FR", "FS", "FM", to remove the anomalies only on a subset of the steps where FR stands for the flow rate, FS stands for signal acquisition and FM stands for dynamic range.

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output Set it to 1 to return a flowFrame or a flowSet with high quality events only. Set

it to 2 to return a flowFrame or a flowSet with an additional parameter where the low quality events have a value higher than 10,000. Set it to 3 to return a list with the IDs of low quality cells. Set it to any other value if no R object has to

be returned. Default is 1.

timeCh Character string corresponding to the name of the Time Channel in the set of

FCS files. By default is NULL and the name is retrieved automatically.

 $second_fractionFR$

The fraction of a second that is used to split the time channel in order to recreate the flow rate. Set it to "timestep" if you wish to recreate the flow rate at the maximum resolution allowed by the flow cytometry instrument. Usually, the timestep corresponds to 0.01, however, to shorten the running time of the analysis the fraction used by default is 0.1, corresponding to 1/10 of a second.

alphaFR The level of statistical significance used to accept anomalies detected by the

ESD method. The default value is 0.01.

decompFR Logical indicating whether the flow rate should be decomposed in the trend and

cyclical components. Default is TRUE and the ESD outlier detection will be executed on the trend component penalized by the magnitude of the cyclical component. If it is FALSE the ESD outlier detection will be executed on the

original flow rate.

ChexcludeFS Character vector with the names or name patterns of the channels that you want

to exclude from the signal acquisition check. The default option, c("FSC", "SSC"), excludes the scatter parameters. If you want to include all the parameters in the

analysis use NULL.

outlier_binsFS logical indicating whether outlier bins (not events) have to be removed before

the changepoint detection of the signal acquisition check. The default is FALSE.

pen_valueFS The value of the penalty for the changepoint detection algorithm. This can be

a numeric value or text giving the formula to use; for instance, you can use the character string "1.5*log(n)", where n indicates the number of cells in the FCS file. The higher the penalty value the less strict is the detection of the anomalies.

The default is 500.

max_cptFS The maximum number of changepoints that can be detected for each channel.

The default is 3.

ChExcludeFM Character vector with the names or name patterns of the channels that you want

to exclude from the signal acquisition check. The default option, c("FSC", "SSC"), excludes the scatter parameters. If you want to include all the parameters in the

analysis use NULL.

sideFM Select whether the dynamic range check has to be executed on both limits, the

 $upper\ limit\ or\ the\ lower\ limit.\ Use\ one\ of\ the\ options:\ "both"\ ,\ "upper"\ ,\ "lower".$

The default is "both".

neg_valuesFM Scalar indicating the method to use for the removal of the anomalies from the

lower limit of the dynamic range. Use 1 to remove negative outliers or use 2 to

truncate the negative values to the cut-off indicated in the FCS file.

html_report Suffix to be added to the FCS filename to name the HTML report of the quality

control. The default is "_QC". If you do not want to generate a report use FALSE.

mini_report Name for the TXT file containing the percentage of anomalies detected in the set

of FCS files analyzed. The default is "_QCmini". If you do not want to generate

the mini report use FALSE.

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fcs_QC	Suffix to be added for the filename of the new FCS containing a new parameter where the low quality events only have a value higher than 10,000. The default is "_QC". If you do not want to generate the high quality FCS file use FALSE.
fcs_highQ	Suffix to be added for the filename of the new FCS containing only the events that passed the quality control. The default is FALSE and hence the high quality FCS file is not generated.
fcs_lowQ	Suffix to be added for the filename of the new FCS containing only the events that did not pass the quality control. The default is FALSE and hence the low quality FCS file is not generated.
folder_results	Character string used to name the directory that contains the results. The default is "resultsQC". If you intend to return the results in the working directory use FALSE.

Value

A complete quality control is performed on flow cytometry data in FCS format. By default the analysis returns:

- 1. a flowFrame or flowSet object containing new FCS files with only high quality events and a directory named *resultsQC* containing:
- 1. a set of new FCS files with a new parameter to gate out the low quality events a value larger than 10,000 is assigned to them only,
- 2. a set of HTML reports, one for each FCS file, that include graphs and table indicating where the anomalies were detected,
- 3. a single TXT file reporting the percentage of events removed in each FCS file.

Author(s)

Gianni Monaco, Chen Hao

Examples

```
## a sample dataset as flowSet object
data(Bcells)

## quality control on a flowFrame object
resQC <- flow_auto_qc(Bcells[[1]], html_report = FALSE, mini_report = FALSE, fcs_QC = FALSE, folder_results =</pre>
```

 $flow_iQC$

Interactive quality control of Flow Cytometry Data

Description

The call of the flow_iQC function opens a Shiny application that allows to perfom a complete and interactive quality control of an FCS file. The framework of the interactive quality control is complementary to the automatic one of the flow_auto_qc function. Hence, the anomalies are manually selected from the evaluation of three main properties of flow cytometry: 1) flow rate, 2) signal acquisition, 3) dynamic range.

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Usage

flow_iQC()

Author(s)

Chen Hao, Gianni Monaco

Examples

```
if (interactive()) flowAI::flow_iQC()
```

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