Package ‘flowDensity’

March 22, 2024

Type Package

Title Sequential Flow Cytometry Data Gating

Version 1.36.1

Date 2014-10-14

Author Mehrnoush Malek, M. Jafar Taghiyar

Maintainer Mehrnoush Malek <mehrmalek@gmail.com>

Description This package provides tools for automated sequential gating analogous to the manual gating strategy based on the density of the data.

Imports flowCore, graphics, flowViz (>= 1.42), car, polyclip, gplots, methods, stats, grDevices

License Artistic-2.0

biocViews Bioinformatics, FlowCytometry, CellBiology, Clustering, Cancer, FlowCytData, DataRepresentation, StemCell, DensityGating

Suggests knitr, rmarkdown

LazyLoad yes

VignetteBuilder knitr

SystemRequirements xml2, GNU make, C++11

git_url https://git.bioconductor.org/packages/flowDensity

git_branch RELEASE_3_18

git_last_commit e159b7d8

git_last_commit_date 2023-10-27

Repository Bioconductor 3.18

Date/Publication 2024-03-22
CellPopulation-class

Description

This class represents the output of `flowDensity(.)` function from flowDensity package.

Objects from the Class

Objects can be created by calls of the form `new("CellPopulation", ...)`. 

Slots

- `flow.frame`: Object of class "flowFrame" representing the flow cytometry data of the cell population
- `proportion`: Object of class "numeric" representing proportion of the cell population with respect to its parent cell population
- `cell.count`: Object of class "numeric" representing cell count of the cell population
- `channels`: Object of class "character" representing channel names corresponding to the 2 dimensions where the cell population is extracted
- `position`: Object of class "logical" representing position of the cell population in the 2-dimensional space
- `gates`: Object of class "numeric" representing thresholds on each channel used to gate the cell population
- `filter`: Object of class "matrix" representing boundary of the cell population using a convex polygon
- `index`: Object of class "numeric" representing indices of the data points in the cell population with respect to its parent cell population
**Methods**

- **flowDensity** signature(obj = "CellPopulation", channels = "ANY", position = "logical", singlet.gate = "missing"): ...
- **flowDensity** signature(obj = "CellPopulation", channels = "missing", position = "missing", singlet.gate = "logical"): ...
- **getflowFrame** signature(obj = "CellPopulation"): ...
- **plot** signature(x = "flowFrame", y = "CellPopulation"): ...

**Author(s)**

Jafar Taghiyar &lt;email: jtaghiyar@bccrc.ca»

**Examples**

showClass("CellPopulation")

---

**deGate  
1D density gating method**

**Description**

Find the best threshold for a single channel in flow cytometry data based on its density distribution.

**Usage**

degate(obj, channel, n.sd = 1.5, use.percentile = FALSE, percentile = NA, use.upper = FALSE, upper = NA, verbose = TRUE, twin.factor = .98, bimodal = F, after.peak = NA, alpha = 0.1, sd.threshold = FALSE, all.cuts = FALSE, tinypeak.removal = 1/25, adjust.dens = 1, count.lim = 20, magnitude = .3, slope.w = 4, seq.w = 4, spar = 0.4, ...)

**Arguments**

- **obj**
  - obj: a 'FlowFrame' object, 'CellPopulation' or 'GatingHierarchy'
- **channel**
  - a channel’s name or its corresponding index in the 'flow.frame'.
- **n.sd**
  - an integer coefficient for the standard deviation to determine the threshold based on the standard deviation if ‘sd.threshold’ is TRUE.
- **use.percentile**
  - if TRUE, forces to return the 'percentile'th threshold.
- **percentile**
  - A value in [0,1] that is used as the percentile. The default is NA. If set to a value(n) and use.percentile=F, it returns the n-th percentile, for 1-peak populations.
- **use.upper**
  - Logical. If TRUE, forces to return the inflection point based on the first (last) peak if upper=F (upper=T). Default value is set to 'FALSE'
- **upper**
  - if TRUE, finds the change in the slope at the tail of the density curve, if FALSE, finds it at the head. Default value is set to 'NA'.
verbose Logical. If TRUE, Prints a message if only one peak is found, or when inflection point is used to set the gates.
twin.factor a value in [0,1] that is used to exclude twinpeaks
bimodal Logical. If TRUE, it returns a cutoff that splits population closer to 50-50, when there are more than two peaks.
after.peak Logical. If TRUE, it returns a cutoff that is after the maximum peaks, when there are more than two peaks.
alpha a value in [0,1) specifying the significance of change in the slope being detected. This is by default 0.1, and typically need not be changed.
sd.threshold if TRUE, uses 'n.sd' times standard deviation as the threshold. Default value is set to 'FALSE'.
all.cuts if TRUE, returns all the identified cutoff points, i.e. potential thresholds for that channel. Default value is set to 'FALSE'.
tinypeak.removal A number in [0,1] to exclude/include tiny peaks in density distribution.
adjust.dens The smoothness of density in [0,Inf] to be used in density(.). The default value is 1 and should not be changed unless necessary
count.lim minimum limit for events count in order to calculate the threshold. Default is 20, returning NA as threshold.
magnitude A value between 0 and 1, for tracking a slope and reporting changes that are smaller than magnitude*peak_height
slope.w window.width for tracking slope. Default is 4, calculating a slope based on 4 points before and after the current point.
seq.w value used for making the sequence of density points, used in trackSlope.
spar value used in smooth.spline function, used in generating the density, default is 0.4.
... Extra arguments to be passed to smoothSpline function.

Details
deGate works for GatingHierarchy, flowFrame, CellPopulation object or a numeric vector of data. In case the input is a numeric vector, channel doesn’t need to provided, but the rest of arguments can be used to tune the outcome.

Value
an integer value (vector) of cutoff(s), i.e. threshold(s), on the specified channel

Author(s)
Mehrnoush Malek «mmalekes@bccrc.ca»

See Also
getflowFrame notSubFrame flowDensity
Examples

data_dir <- system.file("extdata", package = "flowDensity")
load(list.files(pattern = "sampleFCS_1", data_dir, full = TRUE))
# Find the threshold for CD20
cd19.gate <- deGate(f, channel="PerCP-Cy5-5-A")
# Gate out the CD20- populations using the notSubFrame
plotDens(f, c("APC-H7-A", "PerCP-Cy5-5-A"))
abline(h=cd19.gate, lty=3, col=2)

Description

Methods for function flowDensity in package flowDensity

Arguments

obj GatingHierarchy or CellPopulation object
channels a vector of two channel names or their corresponding indices.
position a vector of two logical values specifying the position of the cell subset of interest on the 2D plot.

... This can be used to pass one of the following arguments:

- 'use.percentile' if TRUE, returns the 'percentile'th threshold.
- 'percentile' a value in [0,1] that is used as the percentile if 'use.percentile' is TRUE.
- 'upper' if 'TRUE', it finds the change in the slope after the peak with index 'peak.ind'.
- 'use.upper' if 'TRUE', forces to return the inflection point based on the first (last) peak if upper=F (upper=T)
- 'twin.factor' a value in [0,1] that is used to exclude twinpeaks.
- 'bimodal' If TRUE, it returns a cutoff that splits population closer to 50-50, when there are more than two peaks.
- 'after.peak' If TRUE, it returns a cutoff that is after the maximum peaks, when there are more than two peaks.
- 'sd.threshold' if TRUE, it uses 'n.sd' times standard deviation for gating.
- 'n.sd' an integer that is multiplied to the standard deviation to determine the place of threshold if 'sd.threshold' is 'TRUE'.
- 'tinypeak.removal' a vector of length 2, for sensitivity of peak finding for each channel. See deGate() for more information.
- 'filter' If provided it uses the given filter to gate the population.
- 'use.control' if TRUE, it finds the threshold using a matched control population and uses it for gating.
• 'control' a 'flowFrame' or 'CellPopulation' object used for calculating the gating threshold when 'use.control' is set to TRUE. If a control population is used, the other arguments ('upper', 'percentile', etc.) are applied to the control data when finding the threshold (i.e. not to 'obj').
• 'alpha' a value in [0,1) specifying the significance of change in the slope which would be detected. This is by default 0.1, and typically need not be changed.
• 'ellip.gate' if TRUE, it fits an ellipse on the data as a gate, otherwise the rectangle gating results are returned
• 'scale' a value in [0,1) that scales the size of ellipse to fit if 'ellip.gate' is TRUE

Value

a CellPopulation object.

Description

an accessor for 'CellPopulation' class to get its 'FlowFrame' object. This will remove all the NA values in the frame.

Usage

getflowFrame(obj)

Arguments

obj a 'CellPopulation' object.

Value

a 'FlowFrame' object.

Author(s)

Jafar Taghiyar «jtaghiyar@bccrc.ca»

Examples

data_dir <- system.file("extdata", package = "flowDensity")
load(list.files(pattern = 'sampleFCS_1', data_dir, full = TRUE))
lymph <- flowDensity(obj=f, channels=c('FSC-A', 'SSC-A'),
              position=c(TRUE, NA))
f.lymph <- getflowFrame(lymph)
**getPeaks**  

Finding Peaks

**Description**  
Find all peaks in density along with their indices

**Usage**  
getPeaks(obj, channel, tinypeak.removal=1/25, adjust.dens=1, verbose=F, twin.factor=1, spar = 0.4,...)

**Arguments**  
- **obj**: a 'FlowFrame', 'GatingHierarchy', 'CellPopulation' a density object or a numeric vector of density.  
- **channel**: a channel's name or its corresponding index. If the input is numeric vector, channel is NA.  
- **tinypeak.removal**: A number in \([0,1]\) to exclude/include tiny peaks in density distribution. Default is 1/25.  
- **adjust.dens**: The smoothness of density in \([0,\infty]\) to be used in density(.). The default value is 1 and should not be changed unless necessary.  
- **verbose**: If TRUE, printing warnings.  
- **twin.factor**: If smaller than 1, peaks that are of greater than height as the maximum peak*twin.factor will be removed.  
- **spar**: argument to pass to smoothSpline function, default value of spar is 0.4.  
- **...**: Other arguments that can be passed to smoothSpline function.

**Value**  
a list, including peaks, their corresponding indices and height.

**Author(s)**
Mehrnoush Malek «mmalekes@bccrc.ca»

**See Also**
deGate notSubFrame flowDensity

**Examples**
```r
data_dir <- system.file("extdata", package = "flowDensity")
load(list.files(pattern = 'sampleFCS_1', data_dir, full = TRUE))
#Find the threshold for CD20
peaks <- getPeaks(f,channel="PerCP-Cy5-5-A",tinypeak.removal=1/30)
peaks
```
Preprocessing helper function for flow cytometry data

Description

Remove the margin events on the axes. Usually, these events are considered as debris or artifacts. This is specifically useful for ‘FSC’ and ‘SSC’ channels in a ‘FlowFrame’ object. However, any channel can be input as an argument.

Usage

```
nmRemove( flow.frame, channels, neg=FALSE, verbose=FALSE, return.ind=FALSE)
```

Arguments

- `flow.frame`: a 'FlowFrame' object.
- `channels`: a vector of channel names or their corresponding indices.
- `neg`: if TRUE, negative events are also removed
- `verbose`: if TRUE, it prints the margin event in each channel
- `return.ind`: if TRUE, it return indices of margin events for each channel.

Value

- a 'FlowFrame' object, or a 'list' of indices identifying margin events for each channel.

Author(s)

Jafar Taghiyar «jtaghiyar@bccrc.ca» Mehrnoush Malek «mmalekes@bccrc.ca»

Examples

```
data_dir <- system.file("extdata", package = "flowDensity")
load(list.files(pattern = 'sampleFCS_2', data_dir, full = TRUE))
# Removing margin events of FSC-A and SSC-A channels
no.margin <- nmRemove(f2, c("FSC-A","SSC-A"),verbose=TRUE)
plotDens(f2, c("FSC-A","SSC-A"),)
# Scatter plot of FSC-A vs. SSC-A after removing margins
plotDens(no.margin, c("FSC-A","SSC-A"))
```
notSubFrame 9

Removing a subset of a FlowFrame object

Description

Remove a subset of a FlowFrame object specified by gates from the flowDensity method. It comes in handy when one needs the complement of a cell population in the input flow cytometry data.

Usage

notSubFrame(obj, channels, position = NA, gates, filter)

Arguments

- obj: a `FlowFrame` or `cellPopulation` object.
- channels: a vector of two channel names or their corresponding indices in the `flow.frame`.
- position: a vector of two logical values specifying the position of the cell subset of interest on the 2D plot.
- gates: the gates slot in the CellPopulation object which is output by flowDensity function. It can also be a vector of two integer values each of which specifies a threshold for the corresponding channel in `channels` argument.
- filter: boundary of the subset to be removed. This value is stored in the `filter` slot of a `CellPopulation` object.

Value

a CellPopulation object.

Author(s)

Mehrnoush Malek «mmalekes@bccrc.ca»

Examples

data_dir <- system.file("extdata", package = "flowDensity")
load(list.files(pattern = 'sampleFCS_1', data_dir, full = TRUE))
#Find the threshold for CD20
cd20.gate <- deGate(f,channel="APC-H7-A")
# Gate out the CD20- populations using the notSubFrame
CD20.pos <- notSubFrame(f,channels=c("APC-H7-A","PerCP-Cy5-5-A"),position=c(FALSE,NA),gates=c(cd20.gate,NA))
#Plot the CD20+ cells on same channels
plotDens(CD20.pos@flow.frame,c("APC-H7-A","PerCP-Cy5-5-A"))
**plotDens**

*Plot flow cytometry data with density-based colors*

**Description**

Generate a scatter dot plot with colors based on the distribution of the density of the provided channels.

**Usage**

```
plotDens(obj, channels, col, main, xlab, ylab, xlim, ylim, pch=".", density.overlay=c(FALSE,FALSE), count.lim=20, dens.col=c("grey48","grey48"), cex=1, dens.type=c("l","l"), transparency=1, adjust.dens=1, show.contour=F, contour.col="darkgrey", verbose=TRUE, ...)
```

**Arguments**

- **obj**: a `FlowFrame`, or `cellPopulation` object.
- **channels**: a vector of two channel names or their corresponding indices in the `flow.frame`.
- **col**: A specification for the default plotting color: see `?par`.
- **main**: an overall title for the plot: see `?plot`.
- **xlab**: a title for the x axis: see `?plot`.
- **ylab**: a title for the y axis: see `?plot`.
- **xlim**: a range for the x axis: see `?plot`.
- **ylim**: a range for the y axis: see `?plot`.
- **pch**: Either an integer specifying a symbol or a single character to be used as the default in plotting points: see `?par`.
- **density.overlay**: Logical vector of length 2, to plot density overlays on the x and y axes. Default is c(FALSE,FALSE).
- **count.lim**: Cutoff for number of events to set color. Default is 20. Samples with less than 20 cells will be plotted in black.
- **dens.col**: 2-character string giving the color of plot desired for density curves.
- **cex**: Size of the points for the plot. For more information look at `?plot` in graphics.
- **dens.type**: 2-character string giving the type of plot desired.
- **transparency**: Transparency of the bi-variate plot, to see the density curves in the background. The lower it is, the more transparent the plot is.
- **adjust.dens**: The smoothness of density in [0,Inf] to be used in density(). The default value is 1 and should not be changed unless necessary.
- **show.contour**: Default is FALSE. It add the contourLines to plot.
- **contour.col**: Color for contourLines. Default is darkgrey.
- **verbose**: Default is True. It will add that the sample has 0 cells in the plot title.
- **...**: can be used to provide desired arguments for the plot() function used to plot the output results.
plotDens

Value

A scatter dot plot with density-based colors, along with density overlays if desired. Set xlim and ylim when plotting if you would like to have all your plots to have same range on the axes (specially when density.overlay=TRUE)

Author(s)

Mehrnoush Malek «mmalekes@bccrc.ca» Jafar Taghiyar «jtaghiyar@bccrc.ca»

Examples

data_dir <- system.file("extdata", package = "flowDensity")
load(list.files(pattern = 'sampleFCS_1', data_dir, full = TRUE))
#Plot CD3 vs. CD19 to see the distribution of cell populations and their density
plotDens(f,c("V450-A","PerCP-Cy5-5-A"))
Index

* **Automated gating**
  plotDens, 10

* **FlowCytData**
  plotDens, 10

* **classes**
  CellPopulation-class, 2

CellPopulation, 5
CellPopulation-class, 2

degate, 3, 7

flowDensity, 4, 7
flowDensity, CellPopulation, ANY, logical
  (flowDensity-methods), 5
flowDensity, flowFrame, ANY, logical
  (flowDensity-methods), 5
flowDensity-methods, 5

gflowFrame, 4, 6
getflowFrame, CellPopulation-method
  (getflowFrame), 6
getflowFrame, CellPopulation-method
  (CellPopulation-class), 2
getPeaks, 7

nmRemove, 8
notSubFrame, 4, 7, 9

plot, flowFrame, CellPopulation-method
  (plotDens), 10
plot, flowFrame, CellPopulation-method
  (CellPopulation-class), 2
plotDens, 10