Package ‘flowDensity’

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Type  Package
Title  Sequential Flow Cytometry Data Gating
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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, car, gplots, RFOC, GEOmap, methods, grDevices
Depends  R (>= 2.10.0), methods
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CellPopulation-class

Class "CellPopulation"

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)
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Examples

`showClass("CellPopulation")`
deGate

ID density gating method

Description

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

Usage

degate(flow.frame, channel, n.sd=1.5, use.percentile = FALSE, percentile = 0.95, use.upper=FALSE, upper = NA, alpha = 0.1, sd.threshold = FALSE, graphs = FALSE, all.cuts = FALSE, tinypeak.removal=1/25, adjust.dens=1)

Arguments

flow.frame a 'FlowFrame' object.
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 value is 0.95.
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'.
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'.
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'.
graphs if TRUE, generates density distribution plot plus its corresponding threshold.
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

Value

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

Author(s)

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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)

flowDensity

Automated Sequential Gating Tool for Flow Cytometry

Description

flowDensity is an automated clustering algorithm which aims to emulate the current practice of manual sequential gating. It is designed to identify the predefined cell subsets based on the density distribution of the parent cell population by analyzing the peaks of the density curve.

When the density distribution has only one peak:

• the first argument that would be used is the percentile, the default value is 95th.
• If set to 'NA' then upper will be used when set to 'FALSE/TRUE'.
• If both upper and percentiles are set to 'NA' sd.threshold is used when set to 'TRUE'.
• If either all these arguments are 'NA', or one of them fails, the algorithm find the best cutoff based on the inflection points and by comparing the position of the peak and the mean of the population.

Usage

flowDensity(obj, channels, position, singlet.gate, ...)

Arguments

obj a 'CellPopulation' or 'flowFrame' 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.
singlet.gate if TRUE, singlet cell gate is derived and the corresponding cell population is returned.

... 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)
• 'sd.threshold' if 'TRUE', it uses 'n.sd' times standard deviation for gating.
Methods for Function flowDensity in Package flowDensity

Description

Methods for function flowDensity in package flowDensity
**Methods**

signature(obj = "CellPopulation", channels = "ANY", position = "logical", singlet.gate = "missing")

signature(obj = "CellPopulation", channels = "missing", position = "missing", singlet.gate = "logical")

signature(obj = "flowFrame", channels = "ANY", position = "logical", singlet.gate = "missing")

---

**getflowFrame**

`'CellPopulation'` class accessor.

**Description**

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

**Usage**

```r
getflowFrame(obj)
```

**Arguments**

- **obj**
  
  A 'CellPopulation' object.

**Value**

A 'FlowFrame' object.

**Author(s)**

Jafar Taghiyar <<jtaghiyar@bccrc.ca>>

**Examples**

```r
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, FALSE), upper=c(NA, TRUE), debris.gate=c(TRUE, FALSE))
f.lymph <- getflowFrame(lymph)
```
**nmRemove**  
*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, talk=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
- `talk` if TRUE, it prints the margin event in each channel

**Value**
a 'FlowFrame' object.

**Author(s)**
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**Examples**
```r
data_dir <- system.file("extdata", package = "flowDensity")
load(list.files(pattern = "Var\".FCS_2\"", data_dir, full = TRUE))
#Removing margin events of FSC-A and SSC-A channels
no.margin <- nmRemove(f2, c("FSC-A","SSC-A"), talk=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**  
*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(flow.frame, channels, position, gates, filter)
```

**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.
plotDens

Arguments

flow.frame a ‘FlowFrame’ 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)

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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(flow.frame, channels, col, main, xlab, ylab, pch=",", s=FALSE, outdir, file.name, ...)

Arguments

flow.frame a ‘FlowFrame’ object.
channels a vector of two channel names or their corresponding indices in the ‘flow.frame’.
s logical variable, if TRUE the output graph is saved.
outdir an optional string value specifying the output directory for saving the graph if ‘s’ is TRUE.
plotDens

  file.name  an optional string value used as the name for the saved file.
  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'
  pch        Either an integer specifying a symbol or a single character to be used as the
default in plotting points: see '?par'.
  ...        can be used to provide desired arguments for the plot() function used to plot the
              output results.

Value

  a scatter dot plot with density-based colors.

Author(s)

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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"))
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