

# 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, 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

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|                      |                        |
|----------------------|------------------------|
| CellPopulation-class | Class "CellPopulation" |
|----------------------|------------------------|

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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 <email: <jtaghiyar@bccrc.ca>

## Examples

```
showClass("CellPopulation")
```

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|        |                                 |
|--------|---------------------------------|
| deGate | <i>1D density gating method</i> |
|--------|---------------------------------|

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## 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,ver
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, spa
```

## 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'.                                 |

|                               |                                                                                                                                                |
|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>verbose</code>          | Logical. If TRUE, Prints a message if only one peak is found, or when inflection point is used to set the gates.                               |
| <code>twin.factor</code>      | a value in [0,1] that is used to exclude twinpeaks                                                                                             |
| <code>bimodal</code>          | Logical. If TRUE, it returns a cutoff that splits population closer to 50-50, when there are more than two peaks.                              |
| <code>after.peak</code>       | Logical. If TRUE, it returns a cutoff that is after the maximum peaks, when there are more than two peaks.                                     |
| <code>alpha</code>            | 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. |
| <code>sd.threshold</code>     | if TRUE, uses 'n.sd' times standard deviation as the threshold. Default value is set to 'FALSE'.                                               |
| <code>all.cuts</code>         | if TRUE, returns all the identified cutoff points, i.e. potential thresholds for that channel. Default value is set to 'FALSE'.                |
| <code>tinypeak.removal</code> | A number in [0,1] to exclude/include tiny peaks in density distribution.                                                                       |
| <code>adjust.dens</code>      | The smoothness of density in [0,Inf] to be used in density(.). The default value is 1 and should not be changed unless necessary               |
| <code>count.lim</code>        | minimum limit for events count in order to calculate the threshold. Default is 20, returning NA as threshold.                                  |
| <code>magnitude</code>        | A value between 0 and 1, for tracking a slope and reporting changes that are smaller than $magnitude * peak\_height$                           |
| <code>slope.w</code>          | window.width for tracking slope. Default is 4, calculating a slope based on 4 points before and after the current point.                       |
| <code>seq.w</code>            | value used for making the sequence of density points, used in trackSlope.                                                                      |
| <code>spar</code>             | value used in smooth.spline function, used in generating the density, default is 0.4.                                                          |
| <code>...</code>              | 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 be 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)
```

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flowDensity-methods      *Methods for Function flowDensity in Package **flowDensity***

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## Description

Methods for function flowDensity in package **flowDensity**

## Arguments

|          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| obj      | GatingHierarchy or <a href="#">CellPopulation</a> 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.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| ...      | <p>This can be used to pass one of the following arguments:</p> <ul style="list-style-type: none"> <li>• 'use.percentile' if TRUE, returns the 'percentile'th threshold.</li> <li>• 'percentile' a value in [0,1] that is used as the percentile if 'use.percentile' is TRUE.</li> <li>• 'upper' if 'TRUE', it finds the change in the slope after the peak with index 'peak.ind'.</li> <li>• 'use.upper' if 'TRUE', forces to return the inflection point based on the first (last) peak if upper=F (upper=T)</li> <li>• 'twin.factor' a value in [0,1] that is used to exclude twinpeaks.</li> <li>• 'bimodal' If TRUE, it returns a cutoff that splits population closer to 50-50, when there are more than two peaks.</li> <li>• 'after.peak' If TRUE, it returns a cutoff that is after the maximum peaks, when there are more than two peaks.</li> <li>• 'sd.threshold' if TRUE, it uses 'n.sd' times standard deviation for gating.</li> <li>• 'n.sd' an integer that is multiplied to the standard deviation to determine the place of threshold if 'sd.threshold' is 'TRUE'.</li> <li>• 'tinypeak.removal' a vector of length 2, for sensitivity of peak finding for each channel. See deGate() for more information.</li> <li>• 'filter' If provided it uses the given filter to gate the population.</li> <li>• 'use.control' if TRUE, it finds the threshold using a matched control population and uses it for gating.</li> </ul> |

- '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.

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|              |                                         |
|--------------|-----------------------------------------|
| getflowFrame | <i>'CellPopulation' class accessor.</i> |
|--------------|-----------------------------------------|

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### 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,Inf] 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**

```
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
```

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, 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 | <i>Removing a subset of a FlowFrame object</i> |
|-------------|------------------------------------------------|

---

## 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.type=c("l","l"), transparency=1, adjust.dens=1, show.contour=F, contour.col="darkgrey", verbose=T)
```

**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.                                    |

**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](mailto:mmalekes@bccrc.ca)» Jafar Taghiyar «[jtaghiyar@bccrc.ca](mailto: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"))
```

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