Package 'openCyto'

April 5, 2014

Type Package

Title Hierarchical Gating Pipeline for flow cytometry data

Version 1.0.14

Date 2012-06-11

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Description This package is designed to facilitate the automated gating methods in sequential way to mimic the manual gating strategy.

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LazyLoad yes

Collate 'AllGenerics.R' 'AllClasses.R' 'bayes-flowClust.R'
 'csvTemplate-parser.R' 'functions.R' 'gating-methods.R'
 'gating-functions.R' 'gatingTemplate-methods.R' 'gtMethod-methods.R' 'gtPopulation-methods.R'
 'fcFilterList-methods.R' 'fcTree-methods.R' 'fcFilter-methods.R' 'wrapper-functions.R'
 'preprocessing-method.R' 'pluginFramework.R' 'unlockNamespace.R' 'zzz.R'

Depends methods,flowWorkspace

Imports

Biobase,gtools,flowCore,flowStats,flowClust,MASS,clue,plyr,RBGL,Rgraphviz,graph,data.table,ks,RColorBrewer,lattice,

Suggests flowWorkspaceData, knitr, testthat, utils, tools

biocViews FlowCytometry, DataImport, Preprocessing, DataRepresentation

Enhances flowDensity

VignetteBuilder knitr

28

Index

R topics documented:

| isPolyfunctional | 3 |
|-----------------------|----|
| prior_flowClust1d | 3 |
| prior_kmeans | 5 |
| boolMethod-class | 6 |
| fcFilter-class | 6 |
| fcFilterList | 6 |
| fcFilterList-class | 7 |
| fcPolygonGate | 7 |
| fcPolygonGate-class | 7 |
| fcRectangleGate | 8 |
| fcRectangleGate-class | 8 |
| fcTree | 8 |
| fcTree-class | 9 |
| flowClust.1d | 9 |
| flowClust.2d | 11 |
| gating | 12 |
| gatingTemplate-class | 14 |
| getNodes | 15 |
| gtMethod-class | 16 |
| gtPopulation-class | 17 |
| gtSubsets-class | 17 |
| listgtMethods | 17 |
| mindensity | 18 |
| names | 19 |
| openCyto | 20 |
| plot | 21 |
| polyFunctions-class | 22 |
| posteriors | 22 |
| ppMethod-class | 22 |
| preprocessing | 22 |
| priors | 23 |
| prior_flowClust | 23 |
| refGate-class | 24 |
| registerPlugins | 24 |
| tailgate | 25 |
| templateGen | 26 |
| | |

isPolyfunctional 3

.isPolyfunctional

A function to tell wether a gating method is polyFunctions

Description

A function to tell wether a gating method is polyFunctions

Usage

```
.isPolyfunctional(gm)
```

Arguments

gm

an object that extends gtMethod

.prior_flowClust1d

Elicits data-driven priors from a flowSet object for a specified channel

Description

We elicit data-driven prior parameters from a flowSet object for a specified channel. For each sample in the flowSet object, we apply a kernel-density estimator (KDE) and identify its local maxima (peaks). We then aggregate these peaks to elicit a prior parameters for each of K mixture components.

Usage

```
.prior_flowClust1d(flow_set, channel, K = NULL,
    hclust_height = NULL,
    clust_method = c("kmeans", "hclust"),
    hclust_method = "complete", artificial = NULL, nu0 = 4,
    w0 = 10, adjust = 2, min = NULL, max = NULL,
    vague = TRUE)
```

Arguments

flow_set a flowSet object

channel the channel in the flowSet from which we elicit the prior parameters for the

Student's t mixture

K the number of mixture components to identify. By default, this value is NULL

and determined automatically

hclust_height the height of the hclust tree of peaks, where the should be cut By default, we

use the median of the distances between adjacent peaks. If a value is specified,

we pass it directly to cutree.

4 .prior_flowClust1d

clust_method the method used to cluster peaks together when for prior elicitation. By default, kmeans is used. However, if K is not specified, hclust will be used instead.

hclust_method the agglomeration method used in the hierarchical clustering. This value is

passed directly to hclust. Default is complete linkage.

artificial a numeric vector containing prior means for artificial mixture components. The

remaining prior parameters for the artificial components are copied directly from the most informative prior component elicited. If NULL (default), no artificial

prior components are added.

nu0 prior degrees of freedom of the Student's t mixture components.

w0 the number of prior pseudocounts of the Student's t mixture components.

adjust the bandwidth to use in the kernel density estimation. See density for more

information.

min a numeric value that sets the lower bound for data filtering. If NULL (default), no

truncation is applied.

max a numeric value that sets the upper bound for data filtering. If NULL (default), no

truncation is applied.

vague logical Whether to elicit a vague prior. If TRUE, we first calculate the median of

standard deviations from all flowFrames. Then, we divide the overall standard

deviation by the number of groups to the scale the standard deviation.

Details

Here, we outline the approach used for prior elicitation. First, we apply a KDE to each sample and extract all of its peaks (local maxima). It is important to note that different samples may have a different number of peaks. Our goal then is to align the peaks before aggregating the information across all samples. To do this, we utilize a technique similar to the peak probability contrasts (PPC) method from Tibshirani et al (2004). Effectively, we apply hierarchical clustering to the peaks from all samples to find clusters of peaks. We compute the sample mean and variance of the peaks within each cluster to elicit the prior means and its hyperprior variance, respectively, for a flowClust mixture component. We elicit the prior variance for each mixture component by first assigning the observations within each sample to the nearest prior mean. Then, we compute the variance of the observations within each cluster. Finally, we average the variances corresponding to each mixture component across all samples in the flowSet object.

Following Tibshirani et al. (2004), we cluster the peaks from each sample using complete-linkage hierarchical clustering. The linkage type can be changed via the hclust_method argument. This argument is passed directly to hclust.

To cluster the peaks, we must cut the hierarchical tree by selecting either a value for K or by providing a height of the tree to cut. By default, we cut the tree using as the height the median of the distances between adjacent peaks within each sample. This value can be changed via the hclust_height argument and, if provided, will be passed to cutree. Also, by default, the number of mixture components K is NULL and is ignored. However, if K is provided, then it has priority over hclust_height and is passed instead directly to cutree.

To ensure that the KDEs are smooth, we recommend that the bandwidth set in the adjust argument be sufficiently large. We have defaulted this value to 2. If the bandwidth is not large enough, the KDE may contain numerous bumps, resulting in erroneous peaks.

.prior_kmeans 5

Value

list of prior parameters

References

Tibshirani, R et al. (2004), "Sample classification from protein mass spectrometry, by 'peak probability contrasts'," Bioinformatics, 20, 17, 3034-3044. http://bioinformatics.oxfordjournals.org/content/20/17/3034.

| .prior_kmeans | Elicits data-driven priors from a flowSet object for specified channels using the K-Means clustering algorithm |
|---------------|--|
| | |

Description

We elicit data-driven prior parameters from a flowSet object for specified channels. For each sample in the flowSet object, we apply kmeans to obtain K clusters. From each cluster, we determine its centroid and the sample covariance matrix. We then aggregate these two sample moments across all samples for each cluster.

Usage

```
.prior_kmeans(flow_set, channels, K, nu0 = 4, w0 = 10,
  nstart = 10, pct = 0.1, min = NULL, max = NULL, ...)
```

Arguments

| flow_set | a flowSet object |
|----------|---|
| channels | a character vector containing the channels in the flowSet from which we elicit the prior parameters for the Student's t mixture |
| K | the number of mixture components to identify |
| nu0 | prior degrees of freedom of the Student's t mixture components. |
| wO | the number of prior pseudocounts of the Student's t mixture components. |
| nstart | number of random starts used by kmeans algorithm |
| pct | percentage of randomly selected cells in each flowFrame that is used to elicit the prior parameters. The value should must be greater than 0 and less than or equal to 1. |
| min | a numeric vector that sets the lower bounds for data filtering. If NULL (default), no truncation is applied. |
| max | a numeric vector that sets the upper bounds for data filtering. If NULL (default), no truncation is applied. |
| | Additional arguments passed to kmeans |

6 fcFilterList

Details

Because the cluster labels returned from kmeans are arbitrary, we align the clusters based on the centroids that are closest to a randomly selected reference sample. We apply the Hungarian algorithm implemented using the solve_LSAP function from the clue package to assist with the alignment.

If each frame within flow_set has a large number of cells, the computational costs of kmeans can be a burden. We provide the option to randomly select pct, a percentage of the cells from each flow frame to which kmeans is applied.

Value

list of flowClust prior parameters

| | | | _ |
|------|-----|-------|------|
| hool | Met | hod-c | lass |

A class to represent a boolean gating method.

Description

It extends refGate class.

| fcF | i 1 | ter- | -clas | s |
|-----|-----|------|-------|---|
| | | | | |

a virtual class that represents the gating result generated by flowClust gating function

Description

Bascially it extends flowCore 'filter classes to have extra slot to store priors and posteriors

| _ | | ٠. | | | |
|----|----|----|----|----|----|
| fc | ۱۱ | Ιt | er | Lı | st |

 $constuctor\ for\ fcFilterList$

Description

```
constuctor for fcFilterList
```

Usage

```
fcFilterList(x)
```

Arguments

x list of fcFilter (i.e. fcPolygonGate or fcRectangleGate)

fcFilterList-class 7

| fcFilterList-class | a class that extends filter list class |
|--------------------|---|
| | a class malexienas i i i eti i si class |

Description

Each filter in the filterList must extends the fcFilter class

| fcPolygonGate d | constuctor for fcPolygonGate |
|-----------------|------------------------------|
|-----------------|------------------------------|

Description

 $constuctor\ for\ fc Polygon Gate$

Usage

```
fcPolygonGate(x, priors, posts)
```

Arguments

```
x a polygonGate objectpriors a list storing priorsposts a list storing posteriors
```

 ${\tt fcPolygonGate-class} \qquad a \ concrete \ class \ that \ reprents \ the \ polygonGate \ generated \ by \ flowClust$

Description

It stores priors and posteriors as well as the actual polygonGate.

8 fcTree

fcRectangleGate

 $constuctor \ for \ {\tt fcRectangleGate}$

Description

constuctor for fcRectangleGate

Usage

```
fcRectangleGate(x, priors, posts)
```

Arguments

x a rectangleGate objectpriors a list storing priorsposts a list storing posteriors

 $\begin{tabular}{ll} fc Rectangle Gate-class & a concrete class that reprents the rectangle Gate generated by flow-clust \\ \hline & Clust \\ \hline \end{tabular}$

Description

It stores priors and posteriors as well as the actual rectangleGate.

fcTree

 $constructor\ of\ fcTree$

Description

It adds an extra node data slot "fList" (which is a filterList object) to the gatingTemplate

Usage

```
fcTree(gt)
```

Arguments

gt

a gatingTemplate object

fcTree-class 9

| fcTree-class | A class to represent a flowClust tree. | |
|--------------|--|--|
| | | |

Description

It is a graphNEL used as a container to store priors and posteriors for each flowClust gate that can be visualized for the purpose of fine-tunning parameters for flowClust algorithm

flowClust.1d Applies flowClust to 1 feature to determine a cutpoint between the minimum cluster and all other clusters.

Description

We cluster the observations in fr into K clusters.

Usage

```
flowClust.1d(fr, params, filterId = "", K = NULL,
    trans = 0, positive = TRUE, prior = NULL,
    criterion = c("BIC", "ICL"),
cutpoint_method = c("boundary", "min_density", "quantile", "posterior_mean", "prior_density"),
    neg_cluster = 1, cutpoint_min = NULL,
    cutpoint_max = NULL, min = NULL, max = NULL,
    quantile = 0.99, quantile_interval = c(0, 10),
    plot = FALSE, ...)
```

Arguments

| fr | a flowFrame object |
|-----------|---|
| params | character channel to be gated on |
| filterId | A character string that identifies the filter created. |
| K | the number of clusters to find |
| trans | numeric indicating whether the Box-Cox transformation parameter is estimated from the data. May take 0 (no estimation), 1 (estimation) or 2 (cluster-specific estimation). NOTE: for the Bayesian version of flowClust, this value cannot be 2. |
| positive | If TRUE, then the gate consists of the entire real line to the right of the cutpoint. Otherwise, the gate is the entire real line to the left of the cutpoint. (Default: TRUE) |
| prior | list of prior parameters for the Bayesian flowClust. If NULL, no prior is used. |
| criterion | a character string stating the criterion used to choose the best model. May take either "BIC" or "ICL". This argument is only relevant when K is NULL or if $length(K) > 1$. The value selected is passed to $flowClust$. |

10 flowClust.1d

| cutpoint_method | | | |
|-------------------|---|--|--|
| | How should the cutpoint be chosen from the fitted flowClust model? See Details. | | |
| neg_cluster | integer. The index of the negative cluster. The cutpoint is computed between clusters neg_cluster and neg_cluster + 1. | | |
| cutpoint_min | numeric value that sets a minimum thresold for the cutpoint. If a value is provided, any cutpoint below this value will be set to the given minimum value. If NULL (default), there is no minimum cutpoint value. | | |
| cutpoint_max | numeric value that sets a maximum thresold for the cutpoint. If a value is provided, any cutpoint above this value will be set to the given maximum value. If NULL (default), there is no maximum cutpoint value. | | |
| min | a numeric value that sets the lower bound for data filtering. If NULL (default), no truncation is applied. | | |
| max | a numeric value that sets the upper bound for data filtering. If NULL (default), no truncation is applied. | | |
| quantile | the quantile for which we will find the cutpoint using the quantile cutpoint_method. If the cutpoint_method is not set to quantile, this argument is ignored. | | |
| quantile_interval | | | |
| | a vector of length 2 containing the end-points of the interval of values to find the quantile cutpoint. If the cutpoint_method is not set to quantile, this argument is ignored. | | |
| plot | logical value indicating that the fitted flowClust model should be plotted along with the cutpoint | | |

Details

By default, the cutpoint is chosen to be the boundary of the first two clusters. That is, between the first two cluster centroids, we find the midpoint between the largest observation from the first cluster and the smallest observations from the second cluster. Alternatively, if the cutpoint_method is min_density, then the cutpoint is the point at which the density between the first and second smallest cluster centroids is minimum.

additional arguments that are passed to flowClust

Value

a rectangleGate object consisting of all values beyond the cutpoint calculated

Examples

```
## Not run:
  gate <- flowClust.1d(fr, params = "APC-A", K =2) # fr is a flowFrame
## End(Not run)</pre>
```

flowClust.2d

| flowClust.2d | Automatic identification of a population of interest via flowClust based on two markers |
|--------------|---|
| | on two markers |

Description

We cluster the observations in fr into K clusters. We set the cutpoint to be the point at which the density between the first and second smallest cluster centroids is minimum.

Usage

```
flowClust.2d(fr, xChannel, yChannel, filterId = "",
   K = 2, usePrior = "no", prior = list(NA), trans = 0,
   plot = FALSE, target = NULL, transitional = FALSE,
   quantile = 0.9, translation = 0.25,
   transitional_angle = NULL, min = NULL, max = NULL, ...)
```

Arguments

| fr | a flowFrame object | | |
|--------------------|---|--|--|
| xChannel,yChan | xChannel,yChannel | | |
| | character specifying channels to be gated on | | |
| filterId | A character string that identifies the filter created. | | |
| K | the number of clusters to find | | |
| usePrior | Should we use the Bayesian version of flowClust? Answers are "yes", "no", or "vague". The answer is passed along to flowClust. | | |
| prior | list of prior parameters for the Bayesian version of flowClust. If usePrior is set to no, then the list is unused. | | |
| trans | numeric indicating whether the Box-Cox transformation parameter is estimated from the data. May take 0 (no estimation), 1 (estimation) or 2 (cluster-specific estimation). NOTE: for the Bayesian version of flowClust, this value cannot be 2. | | |
| plot | a logical value indicating if the fitted mixture model should be plotted. By default, no. | | |
| target | a numeric vector of length K containing the location of the cluster of interest. See details. | | |
| transitional | logical value indicating if a transitional gate should be constructed from the target flowClust cluster. By default, no. | | |
| quantile | the contour level of the target cluster from the flowClust fit to construct the gate | | |
| translation | a numeric value between 0 and 1 used to position a transitional gate if transitional = TRUE. This argument is ignored if transitional = FALSE. See details | | |
| transitional_angle | | | |
| | the angle (in radians) of the transitional gate. See details. Ignored if transitional = FALSE. | | |

12 gating

| min | A vector of length 2. Truncate observations less than this minimum value. The first value truncates the xChannel, and the second value truncates the yChannel. By default, this vector is NULL and is ignored. |
|-----|---|
| max | A vector of length 2. Truncate observations greater than this maximum value. The first value truncates the xChannel, and the second value truncates the yChannel. By default, this vector is NULL and is ignored. |
| | additional arguments that are passed to flowClust |

Details

The cluster for the population of interest is selected as the one with cluster centroid nearest the target in Euclidean distance. By default, the largest cluster (i.e., the cluster with the largest proportion of observations) is selected as the population of interest.

We also provide the option of constructing a transitional gate from the selected population of interest. The location of the gate can be controlled with the translation argument, which translates the gate along the major axis of the targest cluster as a function of the appropriate chi-squared coefficient. The larger translation is, the more gate is shifted in a positive direction. Furthermore, the width of the transitional gate can be controlled with the quantile argument.

The direction of the transitional gate can be controlled with the transitional_angle argument. By default, it is NULL, and we use the eigenvector of the target cluster that points towards the first quadrant (has positive slope). If transitional_angle is specified, we rotate the eigenvectors so that the angle between the x-axis (with the cluster centroid as the origin) and the major eigenvector (i.e., the eigenvector with the larger eigenvalue) is transitional_angle.

Value

a polygonGate object containing the contour (ellipse) for 2D gating.

Examples

Description

It loads the gating methods by topological order and applies them to GatingSet.

```
apply a gtMethod to the GatingSet apply a boolMethod to the GatingSet
```

It generates a batch of boolMethods based on the expression defined in polyFunctions objects. It is a convenience way to generate different boolean combinations of cytokine gates.

```
apply a refGate to the GatingSet
```

gating 13

Arguments

x a gatingTemplate object
y a GatingSet object
env_fct a environment that contains fcTree object named as 'fct'. If NULL (by default), no fcTree will be constructed. It is currently reserved for the internal debugging.

• start a character that specifies the population (correspoding to 'alias' column in csv template) where the gating process will start from. It is useful to quickly skip some gates and go directly to the target population in the testing run. Default is "root".

- stop.at a character that specifies the population (correspoding to 'alias' column in csv template) where the gating process will stop at. Default is NULL, indicating the end of gating tree.
- mc.cores passed to multicore package for parallel computing
- parallel_type character specifying the parallel type. The valid options are "none", "multicore", "cluster".
- cl cluster object passed to parallel package (when parallel_type is "cluster")

other arguments

Value

Nothing. As the side effect, gates generated by gating methods are saved in GatingSet.

Examples

```
## Not run:
    gt <- gatingTemplate(file.path(path, "data/ICStemplate.csv"), "ICS")
    gs <- GatingSet(fs) #fs is a flowSet/ncdfFlowSet
    gating(gt, gs)
    gating(gt, gs, stop.at = "v") #proceed the gating until population v
    gating(gt, gs, start = "v") # start from v
    gating(gt, gs, parallel_type = "multicore", mc.cores = 8) #parallel gating using multicore
    #parallel gating by using cluster
    cl1 <- makeCluster (8, type = "MPI")
    gating(gt, gs, parallel_type = "cluster", cl = cl1)
    stopCluster ( cl1 )

## End(Not run)</pre>
```

gatingTemplate-class a class storing the gating method and population information in a graphNEL object

Description

Each cell population is stored in graph node and is connected with its parent population or its reference node for boolGate or refGate.

It parses the csv file that specifies the gating scheme for a particular staining pannel.

Arguments

x character csv file name

name: character the label of the gating template

... other arguments passed to data.table::fread

Details

This csv must have the following columns:

'alias': a name used label the cell population, the path composed by the alias and its precedent nodes (e.g. /root/A/B/alias) has to be uniquely identifiable. So alias can not contain '/' character, which is reserved as path delimiter.

'pop': population patterns of 'A+/-' or 'A+/-B+/-', which tells the algorithm which side (postive or negative) of 1d gate or which quadrant of 2d gate to be kept. when it is in the form of 'A+/-B+/-', 'A' and 'B' should be the full name (or a substring as long as it is unquiely matched) of either channel or marker of the flow data.

'parent': the parent population alias, its path has to be uniquely identifiable.

'dims': characters seperated by comma specifying the dimensions(1d or 2d) used for gating. It can be either channel name or stained marker name.

'gating_method': the name of the gating function (e.g. 'flowClust'). It is invoked by a wrapper function that has the identical function name prefixed with a dot.(e.g. '.flowClust')

'gating_args': the named arguments passed to gating function (Note that double quotes are often used as text delimiter by some csv editors. So try to use single quote instead if needed.)

'collapseDataForGating': When TRUE, data is collapsed (within groups if 'groupBy' specified) before gating and the gate is replicated across collapsed samples. When set FALSE (or blank),then 'groupBy' argument is only used by 'preprocessing' and ignored by gating.

'groupBy': If given, samples are split into groups by the unique combinations of study variable (i.e. column names of pData,e.g."PTID:VISITNO"). when split is numeric, then samples are grouped by every N samples

'preprocessing_method': the name of the preprocessing function(e.g. 'prior_flowClust'). It is invoked by a wrapper function that has the identical function name prefixed with a dot.(e.g. '.prior_flowClust') the preprocessing results are then passed to gating wrapper function through 'pps_res' argument.

'preprocessing_args': the named arguments passed to preprocessing function.

getNodes 15

Examples

```
## Not run:
    gt <- gatingTemplate(system.file("extdata/tcell.csv",package = "openCyto"))
    plot(gt)
## End(Not run)</pre>
```

getNodes

get nodes from gatingTemplate object

Description

get nodes from gatingTemplate object get children nodes get parent nodes get gating method from the node get preprocessing method from the node plot the gating scheme using Rgraphviz get nodes from fcTree get gates saved in fcTree

Arguments

| X | gatingTemplate |
|------------|--|
| у | character node index. When missing, return all the nodes |
| order | character specifying the order of nodes. options are "default", "bfs", "dfs", "tsort" |
| only.names | logical specifiying whether user wants to get the entire gtPopulation object or just the name of the population node |
| X | gatingTemplate object |
| У | either character specifying the root node which can be used to visualize only the subgraph or missing which display the entire gating scheme |
| | other arguments |
| | graphAttr, nodeAttr: graph rendering attributes passed to renderGraph showRef logical: whether to display the reference gates. Sometime it maybe helpful to hide all those reference gates which are not the cell population of interest and used primarily for generating other population nodes. |
| X | fcTree |
| у | character node name |
| obj | fcTree |
| у | character node name |

16 gtMethod-class

Examples

```
## Not run:
gt <- gatingTemplate(system.file("extdata/tcell.csv",package = "openCyto"))
getNodes(gt)[1:2]
getNodes(gt, only.names = TRUE)
getNodes(gt, "/nonDebris")
getChildren(gt, "/nonDebris")
getParent(gt, "/nonDebris/singlets")
getGate(gt, "/nonDebris", "/nonDebris/singlets")
ppMethod(gt, "/nonDebris/singlets", "/nonDebris/singlets/lymph")
plot(gt) #plot entire tree
plot(gt, "lymph") #only plot the subtree rooted from "lymph"

## End(Not run)</pre>
```

gtMethod-class

A class to represent a gating method.

Description

A gating method object contains the specifics for generating the gates.

Slots

name a character specifying the name of the gating method

dims a character vector specifying the dimensions (channels or markers) of the gate

args a list specifying the arguments passed to gating function

groupBy a character or integer specifying how to group the data if character,group the data
by the study variables (columns in pData) if integer, group the data by every N samples.

collapse a logical specifying wether to collapse the data within group before gating. it is only valid when groupBy is specified

Examples

```
## Not run:
    gt <- gatingTemplate(system.file("extdata/tcell.csv",package = "openCyto"))
    getGate(gt, 2, 3)
## End(Not run)</pre>
```

gtPopulation-class 17

| gtPopulation-class | A class to represent a cell population that will be generated by a gating method. |
|--------------------|---|
|--------------------|---|

Description

A class to represent a cell population that will be generated by a gating method.

Slots

```
id numeric unique ID that is consistent with node label of graphNEL in gating templatename character the name of populationalias character the more user friendly name of population
```

Examples

```
## Not run:
    gt <- gatingTemplate(system.file("extdata/tcell.csv",package = "openCyto"))
    getNodes(gt, 2)
## End(Not run)</pre>
```

gtSubsets-class

A class representing a group of cell populations.

Description

It extends gtPopulation class.

listgtMethods

Print a list of the registered gating methods

Description

Print a list of the registered gating methods

Usage

```
listgtMethods()
```

Value

Does not return anything. Prints a list of the available gating methods.

18 mindensity

| mindensity | Determines a cutpoint as the minimum point of a kernel density esti- mate between two peaks |
|------------|--|
| | |

Description

We fit a kernel density estimator to the cells in the flowFrame and identify the two largest peaks. We then select as the cutpoint the value at which the minimum density is attained between the two peaks of interest.

Usage

```
mindensity(flow_frame, channel, filter_id = "",
  positive = TRUE, pivot = FALSE, gate_range = NULL,
  min = NULL, max = NULL, ...)
```

Arguments

| flow_frame | a flowFrame object |
|------------|---|
| channel | TODO |
| filter_id | TODO |
| positive | If TRUE, then the gate consists of the entire real line to the right of the cutpoint. Otherwise, the gate is the entire real line to the left of the cutpoint. (Default: TRUE) |
| pivot | logical value. If TRUE, we choose as the two peaks the largest peak and its neighboring peak. See details. |
| gate_range | numeric vector of length 2. If given, this sets the bounds on the gate applied. If no gate is found within this range, we set the gate to the minimum value within this range if positive is TRUE and the maximum value of the range otherwise. |
| min | a numeric value that sets the lower boundary for data filtering |
| max | a numeric value that sets the upper boundary for data filtering |
| • • • | Additional arguments for peak detection. |
| | |

Details

In the default case, the two peaks of interest are the two largest peaks obtained from the link{density} function. However, if pivot is TRUE, we choose the largest peak and its neighboring peak as the two peaks of interest. In this case, the neighboring peak is the peak immediately to the left of the largest peak if positive is TRUE. Otherwise, the neighboring peak is selected as the peak to the right.

In the special case that there is only one peak, we are conservative and set the cutpoint as the min(x) if positive is TRUE, and the max(x) otherwise.

Value

a rectangleGate object based on the minimum density cutpoint

names 19

Examples

```
## Not run:
  gate <- mindensity(fr, channel = "APC-A") # fr is a flowFrame
## End(Not run)</pre>
```

names

get gating method name

Description

get gating method name

get gating method dimensions

get parameters of the gating method/function

When TRUE, the flow data(multiple flowFrames) is collapsed into one and the gating method is applied on the collapsed data. Once the gate is generated, it is then replicated and applied to the each single flowFrame.

When specified, the flow data is grouped by the grouping variable (column names in pData). Within each group, when isCollapse is set to TRUE, the gating method is applied to the collapsed data. Otherwise, it is done indepentently for each individual sample(flowFrame). Grouping variable is also used by preprocessing method.

get population name

get population alias

Arguments

| object | gtMethod |
|--------------|----------|
| object | gtMethod |
| gtPopulation | object |
| gtPopulation | object |

Value

logical

20 openCyto

Examples

```
## Not run:
gt <- gatingTemplate(system.file("extdata/tcell.csv",package = "openCyto"))
gtMthd <- getGate(gt, "/nonDebris/singlets", "/nonDebris/singlets/lymph")
names(gtMthd)
dims(gtMthd)
parameters(gtMthd)
isCollapse(gtMthd)
groupBy(gtMthd)
gtPop <- getNodes(gt, "/nonDebris/singlets/lymph/cd3/cd4+cd8-/CD38+")
names(gtPop)
alias(gtPop)
## End(Not run)</pre>
```

openCyto

Hierarchical Gating Pipeline for flow cytometry data

Description

Hierarchical Gating Pipeline for flow cytometry data.

Details

openCyto is a package designed to facilitate the automated gating methods in sequential way to mimic the manual gating strategy.

Package: openCyto
Type: Package
Version: 0.99.1
Date: 2013-08-10
License: GPL (>= 2)
LazyLoad: yes

Author(s)

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Maintainer: Mike Jiang <wjiang2@fhcrc.org>

See Also

See gating, flowClust.1d, for an overview of gating functions.

plot 21

Examples

```
## Not run: gatingTemplate(test.csv)
```

plot plot a fcFilterList

Description

It is usually called by plot method for fcTree instead of directly by users.

This provides the priors and posteriors as well as the gates for the purpose of debugging flowClust gating algorithm

Arguments

| X | fcFilterList |
|------------|--|
| У | character channel name |
| samples | character a vector of sample names to be plotted |
| posteriors | logical indicating whether posteriors should be plotted |
| xlim,ylim | scale settings for x,y axises |
| lwd | line width |
| breaks | passed to hist |
| data | GatingSet object |
| node | character population name associated with the fcFilterList |
| | other arguments passed to base plot |
| х | fcTree |
| у | character node name in the fcTree |
| channel | character specifying the channel. |
| data | GatingSet that the fcTree is associated with |
| | other arguments |

Examples

```
## Not run:
    env1<-new.env(parent=emptyenv())
    #gt is a gatingTemplate, gs is a GatingSet
    gating(gt,gs,env1) #the flowClust gating results are stored in env1
    plot(env1$fct,"nonDebris",post=T) #plot the priors as well as posteriors for the "nonDebris" gate
## End(Not run)</pre>
```

22 preprocessing

polyFunctions-class

A class to represent a polyFunctions gating method.

Description

It extends boolMethod class and will be expanded to multiple boolMethod object.

posteriors

get posteriors from a fcFilter object

Description

get posteriors from a fcFilter object

Arguments

x fcFilter

y character or missing that specifiy which channel to look for

ppMethod-class

A class to represent a preprocessing method.

Description

It extends gtMethod class.

Examples

```
## Not run:
    gt <- gatingTemplate(system.file("extdata/tcell.csv",package = "openCyto"))
    ppMethod(gt, 3, 4)
## End(Not run)</pre>
```

preprocessing

apply a ppMethod to the GatingSet

Description

apply a ppMethod to the GatingSet

Arguments

x ppMethod

x GatingSet ... other arguments

priors 23

| priors | | |
|----------|-----------------------------------|--|
| p. 10. 5 | get priors from a fcFilter object | |

Description

get priors from a fcFilter object

Arguments

x fcFilter object y character specifying channel name. if missing then extract priors for all the

channels

prior_flowClust

Elicits data-driven priors from a flowSet object for specified channels

Description

We elicit data-driven prior parameters from a flowSet object for specified channels. For each sample in the flowSet object, we apply the given prior_method to elicit the priors parameters.

Usage

```
prior_flowClust(flow_set, channels,
  prior_method = c("kmeans"), K = 2, nu0 = 4,
  w0 = c(10, 10), shrink = 1e-06, ...)
```

Arguments

flow_set a flowSet object channels a character vector containing the channels in the flowSet from which we elicit the prior parameters for the Student's t mixture prior_method the method to elicit the prior parameters Κ the number of mixture components to identify prior degrees of freedom of the Student's t mixture components. nu0 w0 the number of prior pseudocounts of the Student's t mixture components. shrink the amount of eigenvalue shrinkage to add in the case the prior covariance matrices are singular. See details. Additional arguments passed to the prior elicitation method selected

24 registerPlugins

Details

Currently, we have implemented only two methods. In the case that one channel is given, we use the kernel-density estimator (KDE) approach for each sample to obtain K peaks from which we elicit prior parameters. Otherwise, if more than one channel is specified, we apply K-Means to each of the samples in the flowSet and aggregate the clusters to elicit the prior parameters.

In the rare case that a prior covariance matrix is singular, we shrink the eigenvalues of the matrix slightly to ensure that it is positive definite. For instance, if the flow_set has two samples, this case can occur. The amount of shrinkage is controlled in shrink.

Value

list of the necessary prior parameters

refGate-class

A class to represent a reference gating method.

Description

It extends gtMethod class.

Slots

refNodes character specifying the reference nodes

registerPlugins

Register a gating or preprocessing function with OpenCyto

Description

Function registers a new gating or preprocessing method with openCyto so that it may be used in the csv template.

Usage

```
registerPlugins(fun = NA, methodName, dep = NA, ...)
```

Arguments

fun function to be registered

methodName character name of the gating or preprocessing method

dep character name of the library dependency required for the plugin method to

work.

... other arguments type character specifying the type of registering method.

Should be either "gating" or "preprocessing".

tailgate 25

Details

The fun argument should be a wrapper function definition for the gating or preprocessing method. Gating method must have formal arguments:

```
fraflowFrame
```

pp res a pre-processing result

xChannel character (optional)

yChannel character (required)

filter_id character

... ellipses for the additional parameters.

Preprocessing method must have formal arguments:

fs a flowSet that stores the flow data (could be subgrouped data if groupBy column is defined in the csv template

```
gs a GatingSet
```

gm a gtMethod object that stores the information from gating method

xChannel character (required)

yChannel character (required)

... ellipses for the additional parameters.

The gating function must return a filter (i.e. polygonGate or other instance) from flowCore. The preprocessing can return anything and it will be passed on to the gating function. So it is up to gating function to use and interpret the results of preprocessing. Not all formal parameters need to be used. Additional arguments are passed via the ... and can be processed in the wrapper

Value

logical TRUE if successful and prints a message. FALSE otherwise.

tailgate

Gates the tail of a density using the derivative of a kernel density estimate after standardizing and collapsing flowFrames

Description

Gates the tail of a density using the derivative of a kernel density estimate after standardizing and collapsing flowFrames

Usage

```
tailgate(fr, channel, filter_id = "", num_peaks = 1,
  ref_peak = 1, tol = 0.01, positive = TRUE,
  side = "right", ...)

cytokine(fr, channel, filter_id = "", num_peaks = 1,
  ref_peak = 1, tol = 0.01, positive = TRUE,
  side = "right", ...)
```

26 templateGen

Arguments

| fr | a flowFrame object |
|-----------|--|
| channel | the channel from which the cytokine gate is constructed |
| filter_id | the name of the filter |
| num_peaks | the number of peaks expected to see. This effectively removes any peaks that are artifacts of smoothing |
| ref_peak | After num_peaks are found, this argument provides the index of the reference population from which a gate will be obtained. |
| tol | the tolerance value used to construct the cytokine gate from the derivative of the kernel density estimate |
| positive | If TRUE, then the gate consists of the entire real line to the right of the cutpoint. Otherwise, the gate is the entire real line to the left of the cutpoint. (Default: TRUE) |
| side | On which side of the density do we want to gate the tail, the right (default) or left? |
| • • • | additional arguments. |
| | |

Value

a filterList containing the gates (cutpoints) for each sample

hierarchy

Examples

Description

To ease the process of replicating the existing (usually a manual one) gating schemes, this function populate an empty gating template with the 'alias', 'pop', 'parent' and 'dims' columns that exacted from an GatingHierarchy, and leave the other columns (e.g. 'gating_method') blank. So users can make changes to that template instead of writing from scratch.

Usage

```
templateGen(gh)
```

Arguments

gh a GatingHierarchy likely parsed from a xml workspace

templateGen 27

Value

a gating template in data. frame format that requires further edition after output to csv

Index

| *Topic package openCyto, 20 | <pre>gating,gtMethod,GatingSet-method (gating), 12</pre> |
|---|---|
| .isPolyfunctional, 3 | gating,gtMethod,GatingSetList-method |
| .prior_flowClust1d, 3 | (gating), 12 |
| .prior_kmeans, 5 | gating, polyFunctions, GatingSet-method |
| . pr 101_kiilearis, 3 | (gating), 12 |
| alias (names), 19 | |
| alias,gtPopulation-method(names), 19 | gating, polyFunctions, GatingSetList-method |
| alias, gti opalation method (names), 17 | (gating), 12 |
| boolMethod-class, 6 | gating, refGate, GatingSet-method |
| boothethod class, v | (gating), 12 |
| cutree, <i>3</i> , <i>4</i> | gating, refGate, GatingSetList-method |
| cytokine (tailgate), 25 | (gating), 12 |
| cy tokine (taligate), 25 | gatingTemplate, 15 |
| density, 4 | <pre>gatingTemplate (gatingTemplate-class),</pre> |
| dims (names), 19 | 14 |
| dims, gtMethod-method (names), 19 | gatingTemplate,character-method |
| utilis, generiou liletilou (fialiles), 19 | (gatingTemplate-class), 14 |
| fcFilter-class, 6 | gatingTemplate-class, 14 |
| fcFilterList, 6 | <pre>getChildren(getNodes), 15</pre> |
| fcFilterList-class, 7 | <pre>getChildren,gatingTemplate,character-method</pre> |
| fcPolygonGate, 7 | (getNodes), 15 |
| fcPolygonGate-class, 7 | getGate (getNodes), 15 |
| | <pre>getGate,fcTree,character-method</pre> |
| <pre>fcRectangleGate, 8 fcRectangleGate-class, 8</pre> | (getNodes), 15 |
| | <pre>getGate,gatingTemplate,character-method</pre> |
| fcTree, 8 | (getNodes), 15 |
| fcTree-class, 9 | getNodes, 15 |
| flowClust, 4, 9–12 | getNodes, fcTree-method (getNodes), 15 |
| flowClust .1d, 9, 20 | getNodes,gatingTemplate-method |
| flowClust.2d, 11 | (getNodes), 15 |
| gating, 12, 20 | getParent (getNodes), 15 |
| | <pre>getParent,gatingTemplate,character-method</pre> |
| gating, boolMethod, GatingSet-method | (getNodes), 15 |
| (gating), 12 | groupBy (names), 19 |
| gating, boolMethod, GatingSetList-method | groupBy,gtMethod-method(names), 19 |
| (gating), 12 | gtMethod, 12 |
| gating, gatingTemplate, GatingSet-method | |
| (gating), 12 | gtMethod-class, 16 |
| <pre>gating, gatingTemplate, GatingSetList-method</pre> | gtPopulation-class, 17 |
| (gating), 12 | gtSubsets-class, 17 |

INDEX 29

| hclust, 3, 4 hist, 21 | <pre>priors,fcFilter,ANY-method(priors), 23 priors,fcFilter,character-method</pre> |
|---|--|
| <pre>isCollapse (names), 19 isCollapse, gtMethod-method (names), 19</pre> | refGate-class, 24 |
| listgtMethods, 17 | registerGatingFunction (registerPlugins), 24 |
| mindensity, 18 | registerPlugins, 24 renderGraph, <i>15</i> |
| names, 19 | chow (gatingTomplato-class) 14 |
| names, gtMethod-method (names), 19 | show (gatingTemplate-class), 14 |
| names,gtPopulation-method(names),19 | <pre>show,boolMethod-method (gatingTemplate-class), 14</pre> |
| openCyto, 20 | show,fcFilter-method |
| openCyto-package (openCyto), 20 | <pre>(gatingTemplate-class), 14 show,gatingTemplate-method</pre> |
| parameters (names), 19 | (gatingTemplate-class), 14 |
| parameters, gtMethod-method (names), 19 | <pre>show,gtMethod-method(gtMethod-class),</pre> |
| plot, 21 | 16 |
| plot,fcFilterList,ANY-method | |
| (getNodes), 15 | tailgate, 25 |
| plot,fcTree,character-method | templateGen, 26 |
| (getNodes), 15 | |
| plot, filterList, ANY-method (getNodes), | |
| 15 | |
| plot,gatingTemplate,ANY-method | |
| (getNodes), 15 | |
| plot,gatingTemplate,character-method | |
| (getNodes), 15 | |
| plot,gatingTemplate,missing-method | |
| (getNodes), 15 | |
| plot,gatingTemplate-method(getNodes), | |
| 15 | |
| polyFunctions-class, 22 | |
| posteriors, 22 | |
| posteriors, fcFilter, ANY-method | |
| (posteriors), 22 | |
| posteriors, fcFilter, character-method | |
| (posteriors), 22 | |
| ppMethod, 22 | |
| ppMethod (getNodes), 15 | |
| ppMethod,gatingTemplate,character-method | |
| (getNodes), 15 | |
| ppMethod-class, 22 | |
| preprocessing, 22 | |
| preprocessing,ppMethod,GatingSet-method | |
| (preprocessing), 22 | |
| prior_flowClust, 23 | |
| priors, 23 | |