Package `flowStats`

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Type Package

Title Statistical methods for the analysis of flow cytometry data

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Description Methods and functionality to analyse flow data that is beyond the basic infrastructure provided by the flowCore package.

Depends R (>= 2.10), flowCore, fda (>= 2.2.6), cluster, flowWorkspace, ncdfFlow(>= 2.19.5)

Suggests xtable

Imports BiocGenerics, MASS, flowViz, flowCore, fda (>= 2.2.6), Biobase, methods, grDevices, graphics, stats, utils, KernSmooth, lattice,ks

Enhances RBGL,ncdfFlow,graph

License Artistic-2.0

Lazyload yes

biocViews FlowCytometry, CellBasedAssays

NeedsCompilation no

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autoGate

Description

This function tries to fit a single norm2Filter based on a rough preselection of the data. This function is considered internal. Please use the API provided by lymphGate.

Usage

autoGate(x, ..., scale = 2.5)
Arguments

- **x**: An object of class `flowSet`
- **...**: Named arguments or a list of the ranges used for the initial rough preselection. This gets passed on to `rectangleGate`, see its documentation for details.
- **scale**: The scale parameter that gets passed on to `norm2Filter`.

Details

The `flowSet` is first filtered using a `rectangleGate` and the `norm2Filter` is subsequently fitted to the remaining subset.

Value

A list with items:

- **x**: The filtered `flowSet`.
- **n2gate**: The `norm2Filter` object.
- **n2gateResults**: The `filterResult` after applying the `norm2Filter` on the `flowSet`.

Author(s)

Florian Hahne

See Also

`lymphGate`, `norm2Filter`

Examples

```r
data(GvHD)
flowStats:::autoGate(GvHD[10:15], "FSC-H"=c(100,500), "SSC-H"=c(0, 400))
```

---

**BackGating**

**Sample backgating results**

Description

A data frame containing the sub-populations of ITN dataset corresponding to the high-density areas on "FSC" and "SSC" channels. This dataset is yielded by `backGating` on channel CD3, CD8, and CD4 of the ITN sample data.

Usage

```r
data(BackGating)
```

Source

Results from executing the following code:

```r
data(ITN)
flowStats:::backGating(ITN, xy=c("FSC", "SSC"), channels=c("CD3", "CD8", "CD4"))
```
**binByRef**  
*Bin a test data set using bins previously created by probability binning a control dataset*

**Description**

The bins generated by probability binning a control data set can be applied to a test data set to perform statistical comparisons by methods such as the Chi-squared test or the probability binning statistic.

**Usage**

```r
binByRef(binRes, data)
```

**Arguments**

- `binRes`: The result generated by calling the `probBin` function on a control dataset.
- `data`: An object of class `flowFrame`

**Value**

An environment containing the matrices for each bin of the test data set

**Author(s)**

Nishant Gopalakrishnan

**See Also**

`plotBins`, `proBin`

**Examples**

```r
data(GvHD)
resCtrl<-proBin(GvHD[[1]],200)
resSample<-binByRef(resCtrl,GvHD[[2]])
ls(resSample)
```

---

**calcPBChiSquare**  
*Probability binning metric for comparing the probability binned datasets*

**Description**

This function calculates the Probability binning metric proposed by Baggerly et al. The function utilizes the data binned using the `probBin` and `binByRef` functions.

**Usage**

```r
calcPBChiSquare(ctrlRes,sampRes,ctrlCount,sampCount)
```
calcPearsonChi

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ctrlRes</td>
<td>The result generated by calling the probBin function on a control dataset.</td>
</tr>
<tr>
<td>sampRes</td>
<td>The result generated by calling the byByRef function on a test sample dataset.</td>
</tr>
<tr>
<td>ctrlCount</td>
<td>The number of events in the control sample.</td>
</tr>
<tr>
<td>sampCount</td>
<td>The number of events in the test sample being compared.</td>
</tr>
</tbody>
</table>

Value

A list containing the statistic, p.value, observed, expected counts and the residuals.

Author(s)

Nishant Gopalakrishnan

See Also

proBin, calcPBChiSquare

Examples

data(GvHD)
# flow frame 1 is treated as control dataset and used to generate bins
resCtrl<-proBin(GvHD[[1]],c("FSC-H","SSC-H","Time"),200)
plotBins(resCtrl,GvHD[[1]],channels=c("FSC-H","SSC-H","Time"),title="Binned control data")
# Same bins are applied to flowFrame 16
resSample<-binByRef(resCtrl,GvHD[[16]],c("FSC-H","SSC-H","Time"))
ctrlCount<-nrow(GvHD[[1]])
sampCount<-nrow(GvHD[[16]])
stat<-calcPBChiSquare(resCtrl,resSample,ctrlCount,sampCount)

calcPearsonChi

Pearsons chi-square statistic for comparing the probability binned datasets

Description

This function calculates the Pearson's chi-squared statistic for comparing data binned using the probBin and byByRef functions. Internally, the function utilizes the chisq.test function.

Usage

calcPearsonChi(ctrlRes,sampRes)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ctrlRes</td>
<td>The result generated by calling the probBin function on a control dataset.</td>
</tr>
<tr>
<td>sampRes</td>
<td>The result generated by calling the byByRef function on a sample dataset.</td>
</tr>
</tbody>
</table>

Value

A list containing the statistic, p.value, observed, expected counts and the residuals.
### curv1Filter-class

**Description**

Class and constructor for data-driven filter objects that selects high-density regions in one dimension.

**Usage**

```r
curv1Filter(x, bwFac=1.2, gridsize=rep(401, 2), filterId="defaultCurv1Filter")
```

**Arguments**

- `x`: Character giving the name of the measurement parameter on which the filter is supposed to work on. This can also be a list containing a single character scalar for programmatic access.
- `filterId`: An optional parameter that sets the `filterId` slot of this filter. The object can later be identified by this name.
- `bwFac`, `gridsize`: Numerics of length 1 and 2, respectively, used to set the `bwFac` and `gridsize` slots of the object.

**Details**

Areas of high local density in one dimensions are identified by detecting significant curvature regions. See Duong, T. and Cowling, A. and Koch, I. and Wand, M.P., *Computational Statistics and Data Analysis* 52/9, 2008 for details. The constructor `curv1Filter` is a convenience function for object instantiation. Evaluating a `curv1Filter` results in potentially multiple sub-populations, an hence in an object of class `multipleFilterResult`. Accordingly, `curv1Filters` can be used to split flow cytometry data sets.

### Examples

```r
data(GvHD)
# flow frame 1 is treated as control dataset and used to generate bins
resCtrl<-proBin(GvHD[[1]][,c("FSC-H","SSC-H","Time")],200)
plotBins(resCtrl,GvHD[[1]],channels=c("FSC-H","SSC-H","Time"),title="Binned control data")
# Same bins are applied to flowFrame 16
resSample<-.binByRef(resCtrl,GvHD[[16]][,c("FSC-H","SSC-H","Time")])
stat<-.calcPearsonChi(resCtrl,resSample)
```
Value

Returns a `curv1Filter` object for use in filtering `flowFrames` or other flow cytometry objects.

Extends

Class "`parameterFilter`", directly.
Class "`concreteFilter`", by class `parameterFilter`, distance 2.
Class "`filter`", by class `parameterFilter`, distance 3.

Slots

`bwFac`: Object of class "`numeric`". The bandwidth factor used for smoothing of the density estimate.
`gridsize`: Object of class "`numeric`". The size of the bins used for density estimation.
`parameters`: Object of class "`character`", describing the parameter used to filter the `flowFrame`.
`filterId`: Object of class "`character`", referencing the filter.

Objects from the Class

Objects can be created by calls of the form `new("curvFilter", ...)` or using the constructor `curv1Filter`. Using the constructor is the recommended way of object instantiation:

Methods

%\texttt{in}\% signature(x = "flowFrame", table = "curv1Filter"): The workhorse used to evaluate the filter on data. This is usually not called directly by the user, but internally by calls to the `filter` methods.

`show` signature(object = "curv1Filter"): Print information about the filter.

Note

See the documentation in the `flowViz` package for plotting of `curv1Filters`.

Author(s)

Florian Hahne

See Also

`curv2Filter`, `flowFrame`, `flowSet`, `filter` for evaluation of `curv1Filter` objects and `split` for splitting of flow cytometry data sets based on that.

Examples

```r
library(flowStats)
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))

## Create directly. Most likely from a command line
curv1Filter("FSC-H", filterId="myCurv1Filter", bwFac=2)
```
## To facilitate programmatic construction we also have the following
c1f <- curv1Filter(filterId="myCurv1Filter", x=list("FSC-H"), bwFac=2)

## Filtering using curv1Filter
fres <- filter(dat, c1f)
fres
summary(fres)
names(fres)

## The result of curv1 filtering are multiple sub-populations
## and we can split our data set accordingly
split(dat, fres)

## We can limit the splitting to one or several sub-populations
split(dat, fres, population="rest")
split(dat, fres, population=list(keep=c("peak 2", "peak 3")))

### curv2Filter-class

Class “curv2Filter”

#### Description

Class and constructor for data-driven filter objects that selects high-density regions in two dimensions.

#### Usage

curv2Filter(x, y, filterId="defaultCurv2Filter", bwFac=1.2, gridsize=rep(151, 2))

#### Arguments

- **x, y** Characters giving the names of the measurement parameter on which the filter is supposed to work on. y can be missing in which case x is expected to be a character vector of length 2 or a list of characters.
- **filterId** An optional parameter that sets the filterId slot of this filter. The object can later be identified by this name.
- **bwFac, gridsize** Numerics of length 1 and 2, respectively, used to set the bwFac and gridsize slots of the object.

#### Details

Areas of high local density in two dimensions are identified by detecting significant curvature regions. See Duong, T. and Cowling, A. and Koch, I. and Wand, M.P., Computational Statistics and Data Analysis 52/9, 2008 for details. The constructor curv2Filter is a convenience function for object instantiation. Evaluating a curv2Filter results in potentially multiple sub-populations, hence in an object of class multipleFilterResult. Accordingly, curv2Filters can be used to split flow cytometry data sets.
Value

Returns a `curv2Filter` object for use in filtering `flowFrames` or other flow cytometry objects.

Extends

Class "parameterFilter", directly.
Class "concreteFilter", by class `parameterFilter`, distance 2.
Class "filter", by class `parameterFilter`, distance 3.

Slots

`bwFac`: Object of class "numeric". The bandwidth factor used for smoothing of the density estimate.
`gridsize`: Object of class "numeric". The size of the bins used for density estimation.
`parameters`: Object of class "character", describing the parameters used to filter the `flowFrame`.
`filterId`: Object of class "character", referencing the filter.

Objects from the Class

Objects can be created by calls of the form `new("curv2Filter", ...)` or using the constructor `curv2Filter`. The constructor is the recommended way of object instantiation:

Methods

`%in%` signature(`x = "flowFrame", table = "curv2Filter"`): The workhorse used to evaluate the filter on data. This is usually not called directly by the user, but internally by calls to the `filter` methods.
`show` signature(`object = "curv2Filter"`): Print information about the filter.

Note

See the documentation in the `flowViz` package for plotting of `curv2Filters`.

Author(s)

Florian Hahne

See Also

`curv1Filter`, `flowFrame`, `flowSet`, `filter` for evaluation of `curv2Filters` and `split` for splitting of flow cytometry data sets based on that.

Examples

```r
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))

## Create directly. Most likely from a command line
curv2Filter("FSC-H", "SSC-H", filterId="myCurv2Filter")

## To facilitate programmatic construction we also have the following
```
c2f <- curv2Filter(filterId="myCurv2Filter", x=list("FSC-H", "SSC-H"), bwFac=2)
c2f <- curv2Filter(filterId="myCurv2Filter", x=c("FSC-H", "SSC-H"), bwFac=2)

## Filtering using curv2Filter
fres <- filter(dat, c2f)
fres
summary(fres)
names(fres)

## The result of curv2 filtering are multiple sub-populations
## and we can split our data set accordingly
split(dat, fres)

## We can limit the splitting to one or several sub-populations
split(dat, fres, population="rest")
split(dat, fres, population=list(keep=c("area 2", "area 3")))

curv2Filter("FSC-H", "SSC-H", filterId="test filter")

curvPeaks

Parse curv1Filter output

Description

Parse the output of curv1Filter and find modes and midpoints of the high-density regions. This function is considered to be internal.

Usage

curvPeaks(x, dat, borderQuant = 0.01, n = 201, from, to, densities=NULL)

Arguments

x A multipleFilterResult produced by a curv1Filter operation.
dat The corresponding flowFrame.
borderQuant A numeric in [0,1] giving the extreme quantiles for which high-density regions are ignored.
n, from, to Arguments are passed on to density.
densities The optional y values of the density estimate computed for the respective data.

Value

A list with items

peaks x and y locations of the modes of the regions in the density estimates.
regions the left and right margins of the regions.
density1d

midpoints  the mean of regions.
regPoints  x and y locations of the outline of the significant density regions.
densFuns  an approximation function of the density estimate

Author(s)

Florian Hahne

See Also

landmarkMatrix

Examples

data(GvHD)
tmp <- filter(GvHD[[10]], curv1Filter("FSC-H"))
res <- flowStats:::curvPeaks(tmp, exprs(GvHD[[10]])[, "FSC-H"])

density1d  Find most likely separation between positive and negative populations  in 1D

Description

The function tries to find a reasonable split point between the two hypothetical cell populations "positive" and "negative". This function is considered internal, please use the API provided by rangeGate.

Usage

density1d(x, stain, alpha = "min", sd = 2, plot = FALSE, borderQuant = 0.1, absolute = TRUE, inBetween = FALSE, refLine=NULL,rare=FALSE,bwFac=1.2 ,sig=NULL,peakNr=NULL, ...)

Arguments

x  A flowSet or flowFrame.
stain  A character scalar giving the flow parameter for which to compute the separation.
alpha  A tuning parameter that controls the location of the split point between the two populations. This has to be a numeric in the range [0,1], where values closer to 0 will shift the split point closer to the negative population and values closer to 1 will shift towards the positive population. Additionally, the value of alpha can be "min", in which case the split point will be selected as the area of lowest local density between the two populations.
sd  For the case where there is only a single population, the algorithm falls back to estimating the mode of this population and a robust measure of the variance of it distribution. The sd tuning parameter controls how far away from the mode the split point is set.
Create a plot of the results of the computation.

Usually the instrument is set up in a way that the positive population is somewhere on the high end of the measurement range and the negative population is on the low end. This parameter allows to disregard populations with mean values in the extreme quantiles of the data range. Its value should be in the range $[0, 1]$.

Logical controlling whether to classify a population (positive or negative) relative to the theoretical measurement range of the instrument or the actual range of the data. This can be set to TRUE if the alignment of the measurement range is not optimal and the bulk of the data is on one end of the theoretical range.

Force the algorithm to put the separator in between two peaks. If there are more than two peaks, this argument is ignored.

Either NULL or a numeric of length 1. If NULL, this parameter is ignored. When it is set to a numeric, the minor sub-population (if any) below this reference line will be ignored while determining the separator between positive and negative.

Either TRUE or FALSE, assumes that there is one major peak, and that the rare positive population is to the right of it. Use a robust estimate of mean and variance to gate the positive cells.

The bandwidth for smoothing the density estimate. User-tunable

A value of c(NULL,"L","R"), when sig is not NULL, use the half (left or right) of signal to estimate the std and mean.

when peakNr is not NULL, drop the less significant peaks by their heights

Further arguments.

The algorithm first tries to identify high density regions in the data. If the input is a flowSet, density regions will be computed on the collapsed data, hence it should have been normalized before (see warpSet for one possible normalization technique). The high density regions are then classified as positive and negative populations, based on their mean value in the theoretical (or absolute if argument absolute=TRUE) measurement range. In case there are only two high-density regions the lower one is usually classified as the negative populations, however the heuristics in the algorithm will force the classification towards a positive population if the mean value is already very high. The absolute and borderQuant arguments can be used to control this behaviour. The split point between populations will be drawn at the value of minimum local density between the two populations, or, if the alpha argument is used, somewhere between the two populations where the value of alpha forces the point to be closer to the negative ($0 - 0.5$) or closer to the positive population ($0.5 - 1$).

If there is only a single high-density region, the algorithm will fall back to estimating the mode of the distribution (hubers) and a robust measure of its variance and, in combination with the sd argument, set the split point somewhere in the right or left tail, depending on the classification of the region.

For more than two populations, the algorithm will still classify each population into positive and negative and compute the split point between those clusters, similar to the two population case.

A numeric indicating the split point between positive and negative populations.


**gate_singlet**

*Creates a singlet polygon gate using the prediction bands from a robust linear model*

**Description**

The function constructs a singlet polygon gate using the prediction bands from a robust linear model trained on the area and height of one flow parameter (usually forward scatter).

**Usage**

`gate_singlet(x, area = "FSC-A", height = "FSC-H", sidescatter = NULL, prediction_level = 0.99, subsample_pct = NULL, wider_gate = FALSE, filterId = "singlet", maxit = 5, ...)`

**Arguments**

- **x**  
  A `flowFrame` object
- **area**
  Character giving the channel name that records the signal intensity as peak area
- **height**
  Character giving the channel name that records the signal intensity as peak height
- **sidescatter**
  Character giving an optional channel name for the sidescatter signal. By default, ignored.
- **prediction_level**
  A numeric value between 0 and 1 specifying the level to use for the prediction bands

**Examples**

```r
data(GvHD)
dat <- GvHD[pData(GvHD)$Patient==10]
dat <- transform(dat, "FL4-H"=asinh('FL4-H'), "FL3-H"=asinh('FL3-H'))
d <- flowStats:::density1d(dat, "FL4-H", plot=TRUE)
if(require(flowViz))
densityplot(~'FL4-H', dat, reline=d)

## tweaking the location
flowStats:::density1d(dat, "FL4-H", plot=TRUE, alpha=0.8)

## only a single population
flowStats:::density1d(dat, "FL3-H", plot=TRUE)
flowStats:::density1d(dat, "FL3-H", plot=TRUE, sd=2)
```

**Author(s)**

Florian Hahne

**See Also**

`warpSet, rangeGate`
gate_singlet

subsample_pct  a numeric value between 0 and 1 indicating the percentage of observations that should be randomly selected from x to construct the gate. By default, no subsampling is performed.

wider_gate  logical value. If TRUE, the prediction bands used to construct the singlet gate use the robust fitted weights, which increase prediction uncertainty, especially for large FSC-A. This leads to wider gates, which are sometimes desired.

filterId  the name for the filter that is returned

maxit  the limit on the number of IWLS iterations passed to rlm

...  additional arguments passed to rlm

Details

We construct a singlet gate by applying a robust linear model with rlm. By default, we model the forward-scatter height (FSC-H) as a function of forward-scatter area (FSC-A). If sidescatter is given, forward-scatter height is as a function of area + sidescatter + sidescatter / area.

Because rlm relies on iteratively reweighted least squares (IRLS), the runtime to construct a singlet gate is dependent in part on the number of observations in x. To improve the runtime, we provide an option to subsample randomly a subset of x. A percentage of observations to subsample can be given in subsample_pct. By default, no subsampling is applied.

Value

polygonGate.

Author(s)

Greg Finak, John Ramey

See Also

rangeGate, polygonGate

Examples

## Not run:
# fr is a flowframe
sg <- gate_singlet(fr, area = "FSC-A", height = "FSC-H")
sg
# plot the gate
xyplot(~FSC-H ~ FSC-A, fr, filter = sg)

## End(Not run)
gaussNorm

Per-channel normalization based on landmark registration

Description

This function normalizes a set of flow cytometry data samples by identifying and aligning the high density regions (landmarks or peaks) for each channel. The data of each channel is shifted in such a way that the identified high density regions are moved to fixed locations called base landmarks.

Usage

```r
gaussNorm(flowset, channel.names, max.lms=2, base.lms=NULL, peak.density.thr=0.05, peak.distance.thr=0.05, debug=FALSE, fname="'")
```

Arguments

- `flowset`: A `flowSet`.
- `channel.names`: A character vector of flow parameters in `flowset` to be normalized.
- `max.lms`: A numeric vector of the maximum number of base landmarks to be used for normalizing each channel. If it has only one value that will be used as the maximum number of base landmarks for all the channels.
- `base.lms`: A list of vector for each channel that contains the base landmarks for normalizing that channel. If not specified the base landmarks are computed from the set of extracted landmarks.
- `peak.density.thr`: The peaks with density value less than "peak.density.thr times maximum peak density" are discarded.
- `peak.distance.thr`: The sequences of peaks that are located closer than "peak.distance.thr times range of data" are identified. Then for each sequence only one peak (the one with the highest intensity value) is used as a landmark. In other words no two landmarks are located closer than "peak.distance.thr times range of data" to each other.
- `debug`: Logical. Forces the function to draw before and after normalization plots for each sample. The plot of the i-th sample is stored in `paste(fname, i)` file.
- `fname`: The pre- and post- normalization plots of the i-th sample is stored in `paste(fname, i)` file if `debug` is set to TRUE. If default value is used the plots are drawn on separate X11 windows for each sample. In this case, the function waits for a user input to draw the plots for the next sample.

Details

Normalization is archived in three phases: (i) identifying high-density regions (landmarks) for each `flowFrame` in the `flowSet` for a single channel; (ii) computing the best matching between the landmarks and a set of fixed reference landmarks for each channel called base landmarks; (iii) manipulating the data of each channel in such a way that each landmark is moved to its matching base landmark. Please note that this normalization is on a channel-by-channel basis. Multiple channels are normalized in a loop.
gpaSet

Multi-dimensional normalization of flow cytometry data

Description

This function performs a multi-dimensional normalization of flow cytometry data (flowSets) using a generalized Procrustes analysis (GPA) method.

Usage

```
gpaSet(x, params, register="backgating", bgChannels=NULL, bg=NULL, rotation.only=TRUE, downweight.missingFeatures=FALSE, thres.sigma=2.5, show.workflow=FALSE, ask=names(dev.cur())!="pdf")
```

Arguments

- **x**
  - A `flowSet`.
- **params**
  - A character vector of length 2 describing the channels of interest.
- **register**
  - A character indicating the method to be used for identifying features. Available method only includes "backgating" at the point.
- **bgChannels**
  - A character vector indicating the channels used for backgating. If NULL, backGating will find the appropriate backgating channels.
- **bg**
  - A data frame as the returning value of the backGating function. If not NULL, gpaSet will skip the backGating process and use the given data frame to extract potential features.
gpaSet

rotation.only Logical for coarsing a reflection matrix to a rotation matrix.
downweight.missingFeatures Logical. If TRUE, the missing features, labeled as bogus features, are downweighted to zero. See details.
thres.sigma A numerical value indicating the threshold of where to cut the tree, e.g., as resulting from diana, into several clusters. It is default to 2.5 sigma of the distribution of the heights of the cluster points.
show.workflow Logical. If TRUE, the workflow of gpaSet will be displayed.
ask Logical. If TRUE, the display operates in interactive mode.

Details

Normalization is achieved by first identifying features for each flowFrame in the flowSet for designated channels using backgating, subsequently labeling features, and finally aligning the features to a reference feature in the sense of minimizing the Frobenius norm of

$$||s FQ - \bar{F}||,$$

where $s$ is a scalar, $Q$ a rotational matrix, $F$ the matrix of features, and $\bar{F}$ the reference feature. Both $s$ and $Q$ are solved by using singular value decomposition (SVD).

Note that if feature $F_{ij}$ is missing, it is given a bogus value as $\bar{F}_{ij}$.

If downweight.missingFeatures is TRUE, the cost function becomes

$$||s W_0 FQ - W_0 \bar{F}||,$$

where the weighting function $W_0$ is zero if the corresponding feature is bogus.

Value

The normalized flowSet with "GPA" attribute.

Author(s)

C. J. Wong <cwon2@fhcrc.org>

References

in progress

Examples

## Example 1: calling up gpaSet directly
data(ITN)
data(BackGating)

```r
tl <- transformList(colnames(ITN)[3:7], asinh, transformationId="asinh")
dat <- transform(ITN, tl)

xy = c("FSC", "SSC")
bgChannels = c("CD8", "CD4", "CD3")

## using pre-generated backgating results: BackGating
s <- gpaSet(dat, params=xy, bgChannels=bgChannels, bg=BackGating)
```
```r
if(require(flowViz)) {
  d1 <- densityplot(~., s, channels=c("FSC", "SSC"),
                  layout=c(2,1), main="After GPA using bg")
  d2 <- xyplot(FSC ~ SSC, as(s, "flowFrame"),
               channels=c("FSC", "SSC"), main="All flowFrames")
  plot(d1)
  plot(d2)
}

## view "GPA" attribute
attr(s, "GPA")

## Not run:
## Example 2: using work flow and normalization objects
data(ITN)
ITN <- ITN[1:8,]
wf <- workFlow(ITN)
tl <- transformList(colnames(ITN)[3:7], asinh, transformationId="asinh")
add(wf, tl)
x <- Data(wf[["asinh"]])
## normalize 'FSC' and 'SSC' channels
norm <- normalization(normFun=function(x, parameters, ...)
                       gpaSet(x, parameters, ...),
               parameters = c("FSC", "SSC"),
               arguments=list(bgChannels=c("CD8", "CD3"),
                               register="backgating"),
               normalizationId="Procrustes")
add(wf, norm2, parent="asinh")
s <- Data(wf[["Procrustes"]])
if(require(flowViz)) {
  d1 <- densityplot(~., s, channels=c("FSC", "SSC"),
                   layout=c(2,1), main="After GPA using bg")
  d2 <- xyplot(FSC ~ SSC, as(s, "flowFrame"),
               channels=c("FSC", "SSC"), main="All flowFrames")
  plot(d1)
  plot(d2)
}

## End(Not run) ## end of dontrun
```

---

### idFeaturesByBackgating

*Internal use only* Identify features of flow cytometry data using backgating.

#### Description

Identify and labeling significant features using divisive clustering method such as `diana`.

#### Usage

```r
idFeaturesByBackgating(bg, nDim, thres.sigma=2.5, lambda=0.1,
```
idFeaturesByBackgating

reference.method="median",
plot.workflow=FALSE, ask=names(dev.cur())!="pdf")

Arguments

- **bg**: A data frame containing subpopulations on channels of interests. Must be a returning result from `flowStats::backGating`
- **nDim**: An integer indicating the length of channels of interest.
- **thres.sigma**: An numerical value indicating the threshold at which to cut tree, e.g., as resulting from 'diana', into several clusters.
- **lambda**: A numerical value indicating the percentage of the potential features that is used as a threshold for deciding outlier clusters. The default value is 0.1.
- **reference.method**: A character vector indicating the method for computing the reference features. If `median`, the reference feature is defined by the medain of each cluster of features. Valid methods include `median` and `mean` only.
- **plot.workflow**: Logical. If TURE, display the workflow of feature identification.
- **ask**: Logical. If TRUE, the display operates in interactive mode.

Details

Using the resulting data frame from `backGating` as potential features, the algorithm follows four major steps: (i) centering the potential features, which yields the returning value `TransMatrix`, (ii) using `diana` to compute a clustering of the potential features, (iii) cutting the tree into several clusters, and (iv) accessing outliers and rendering the final registered features with labels.

In step three, the threshold for cutting the tree is computed by

\[ sd \times thres.sigma, \]

where \( sd \) is the standard deviation of the distribution of the height between entities computed by `diana`.

A cluster is determined as an outlier if the number of its members is less than the median of the numbers of all clusters’ members times ‘lambda’.

Value

- **register**: A list containing registered features for each sample.

Author(s)

Chao-Jen Wong

See Also

diana, BackGating, gpaSet.
Examples

```r
## Not run:
data(ITN)
wf <- workFlow(ITN)
tl <- transformList(colnames(ITN)[3:7], asinh, transformationId="asinh")
dat <- transformList(ITN, tl)
bg <- backGating(dat, xy=c("FSC", "SSC"), channels="CD3")
## End(Not run)

data(BackGating)
results <- flowStats:::idFeaturesByBackgating(bg=BackGating, nDim=2,
plot.workflow=TRUE, ask=TRUE)
```

### iProcrustes

Procrustes analysis. Using singular value decomposition (SVD) to determine a linear transformation to align the points in X to the points in a reference matrix Y.

Description

Based on generalized Procrustes analysis, this function determines a linear transformation (rotation/reflection and scalling) of the points in matrix x to align them to their reference points in matrix xbar. The alignment is carried out by minimizing the distance between the points in x and xbar.

Usage

```r
iProcrustes(x, xbar, rotation.only=TRUE, scalling=TRUE, translate=FALSE)
```

Arguments

- `x` A numerical matrix to be align to points in xbar, the second argument. The columns represents the coordinates of the points. The matrices x and xbar must have the same dimensions.
- `xbar` A numerical, reference matrix to which points in matrix x are to be aligned.
- `rotation.only` Logical. When rotation.only is TRUE, it allows the function to lose reflection component of the linear transformation. Although it might not give the best-fitting alignment, when dealing with flow cytometry data alignment, a non-reflection transformation is preferred. When rotation.only is FALSE, it allows the function to retain the reflection component.
- `scalling` Logical. When scalling is FALSE, it allows the function to calculate the linear transformation without a scalling factor. That is, the returning scalling factor is set to 1.
- `translate` Logical. Set translate to FALSE when the points in matrices x and xbar are already centralized prior to applying this function. When translate is TRUE, it allows the function to translate the centroid the points in matrix x to that of points in xbar.
**Details**

Suppose the points in matrix $X$ and $\bar{X}$ are centralized (meaning their centroids are at the origin). The linear transformation of $X$ for aligning $X$ to its reference matrix $\bar{X}$, i.e., $\min ||sXQ - \bar{X}||_F$, is given by:

$$Q = VU^T,$$

and

$$s = \frac{\text{trace}(\bar{X}^TXQ)}{\text{trace}(X^TX)},$$

where $V$ and $U$ are the singular value vectors of $\bar{X}^TX$ (that is, $\bar{X}^TX = U\Sigma V^T$), and $s$ is the scaling factor.

**Value**

A list of the linear transformation with items

- **Q**: An orthogonal, rotation/reflection matrix.
- **scal**: A scaling factor
- **T**: (optional) A translation vector used to shift the centroid of the points in matrix $x$ to the origin. Returned when `translate` is `TRUE`.
- **T.xbar**: (optional) Centered $xbar$ (that is, the centroid of the points in $xbar$ is translated to the origin). Returned when `translate` is `TRUE`.

Note that the return values of this function do not include the transformed matrix $scal * x * Q$ or $scal * (x - IT) * Q$, where $T$ is the translation vector and $I$ is an $n - by - 1$ vector with elements 1.

**Author(s)**

C. J. Wong <cwon2@fhcrc.org>

**See Also**

- `gpaSet`

**Examples**

```r
## Example 1
x <- matrix(runif(20), nrow=10, ncol=2)+ 1.4
s <- matrix(c(cos(60), -sin(60), sin(60), cos(60)),
nrow=2, ncol=2, byrow=TRUE)
xbar <- 2.2 *(x %*% s) - 0.1
lt <- iProcrustes(x, xbar, translate=TRUE) ## return linear transformation
lt
## showing result
I <- matrix(1, nrow=nrow(x), ncol=1)
tx <- x - I %*% lt$T
## get the transformed matrix xnew
xnew <- lt$scal * (tx %*% lt$Q)
if (require(lattice)) {
```

```
ITN

Sample flow cytometry data

Description

A `flowSet` containing data from 15 patients.

Usage

data(ITN)

Format

A `flowSet` containing 15 `flowFrames`. There are 3 patient groups with 5 samples each.

Source

Immune Tolerance Network

```r
xyplot(V1 ~ V2,
do.call(make.groups, lapply(list(x=x, xbar=xbar, T.xbar=lt$T.xbar,
    xnew=xnew), as.data.frame)),
    group=which, aspect=c(0.7), pch=c(1,3,2,4), col.symbol="black",
    main="Align the points in x to xbar",
    key=list(points=list(pch=c(1,3,2,4), col="black"), space="right",
              text=list(c("x", "xbar", "T.xbar", "xnew"))))
}
## Example 2. centralized x and xbar prior to using iProcrustes
x <- matrix(runif(10), nrow=5, ncol=2)
s <- matrix(c(cos(60), -sin(60), sin(60), cos(60)),
nrow=2, ncol=2, byrow=TRUE)
xbar <- 1.2 *(x %*% s) - 2
I <- matrix(1, nrow=nrow(x), ncol=1)
x <- x-(I %*% colMeans(x))## shift the centroid of points in x to the origin
xbar <- xbar - (I %*% colMeans(xbar))## shift centroid to the origin
lt <- iProcrustes(x, xbar, translate=FALSE)## return linear transformation
## only return the rotation/reflection matrix and scalling factor
lt
xnew=lt$scal *(x %*% lt$Q)## transformed matrix aligned to centralized xbar
if (require(lattice)) {
    xyplot(V1 ~ V2,
        do.call(make.groups, lapply(list(x=x, xbar=xbar, xnew=xnew), as.data.frame)),
        group=which, auto.key=list(space="right"))
}
```
landmarkMatrix

Compute and cluster high density regions in 1D

Description

This function first identifies high-density regions for each flowFrame in a flowSet and subsequently tries to cluster these regions, yielding the landmarks matrix that needs to be supplied to landmarkreg. The function is considered to be internal.

Usage

landmarkMatrix(data, fres, parm, border=0.05, peakNr=NULL, densities = NULL, n = 201, indices=FALSE)

Arguments

data A flowSet.
fres A list of filterResultList objects generated by a filtering operation using a curv1Filter. Each list item represents the results for one of the flow parameters in parm.
parm Character scalar of flow parameter to compute landmarks for.
border A numeric in [0,1]. Ignore all high-density regions with mean values in the extreme percentiles of the data range.
peakNr Force a fixed number of peaks.
densities An optional matrix of y values of the density estimates for the flowSet. If this is not present, density estimates will be calculated by the function.
n Number of bins used for the density estimation.
indices Return matrix of population indices instead of landmark locations. These indices can be used to point into the populations identified by the curv1Filter.

Details

In order to normalize the data using the landmarkreg function in the fda, a set of landmarks has to be computed for each flowFrame in a flowSet. The number of landmarks has to be the same for each frame. This function identifies high-density regions in each frame, computes a simple clustering and returns a matrix of landmark locations. Missing landmarks of individual frames are substituted by the mean landmark location of the respective cluster.

Value

A matrix of landmark locations. Columns are landmarks and rows are flowFrames.

Author(s)

Florian Hahne

See Also

landmarkreg,warpSet
Examples

data(GvHD)
tmp <- list("FSC-H"=filter(GvHD[1:3], curv1Filter("FSC-H")))
res <- flowStats:::landmarkMatrix(GvHD[1:3], tmp, "FSC-H")

lymphFilter-class

Automated gating of elliptical cell populations in 2D.

Description

Cell populations of roughly elliptical shape in two-dimensional projections are of huge interest in many flow cytometry applications. This function identifies a single such population, potentially from a mixture of multiple populations.

Usage

lymphGate(x, channels, preselection=NULL, scale=2.5, bwFac=1.3,
          filterId="defaultLymphGate", evaluate=TRUE, plot=FALSE, ...)

Arguments

x An object of class flowSet.
channels A character vector of length 2 of valid flow parameters in x.
preselection Either NULL, in which case this boils down to fitting a regular norm2Filter, a character scalar giving one of the flow parameters in x, or a named list of numerics specifying the initial rough preselection. The latter gets passed on to rectangleGate, see it’s documentation for details.
scale The scaleFactor parameter that gets passed on to norm2Filter.
bwFac The bandwidth factor that gets passed on to curv1Filter.
filterId A character used as filterId.
evaluate A logical indicating whether the filter should be resolved (computation of the filterResult and the subset).
plot Logical. Produce plots of filter results
... Additional arguments.

Details

This algorithm does not apply real mixture modelling, however it is able to identify a single elliptical cell population from a mixture of multiple such populations. The idea is to first define a rough rectangular preselection and, in a second step, fit a bivariate normal distribution to this subset only. Depending on the value of preselection, the initial rough selection is either

**NULL:** No preselection at all

**character scalar** Preselection based on cells that are positive for a single marker only. This allows for back-gating, for instances by selecting CD4+ T-cells and using this information to back-gate lymphocytes in FSC and SSC. Positive cells are identified using a curv1Filter.

**a named list of numerics:** Preselection by a rectangular gate. The items of the list have to be numerics of length one giving the gate boundaries in the respective dimensions.
Value

A list with items

- `x` The filtered flowSet.
- `n2gate` The norm2Filter object.
- `n2gateResults` The `filterResult` after applying the norm2Filter on the flowSet.

for the `lymphGate` function. Note that `x` and `n2gateResults` are NULL when `eval=FALSE`.

Extends

Class `parameterFilter`, directly.
Class `concreteFilter`, by class "parameterFilter", distance 2.
Class `filter`, by class "parameterFilter", distance 3.

Slots

See `Arguments` section for details.

- `preselection`: Object of class `character`, the name of the flow parameter used for preselection.
- `rectDef`: Object of class `list`, the initial rectangular selection.
- `scale`: Object of class `numeric`.
- `bwFac`: Object of class `numeric`.
- `parameters`: Object of class `parameters`, the flow parameters to operate on.
- `filterId`: Object of class "character", the filter identifier.

Objects from the Class

Objects can be created by calls of the form `new("lymphFilter", parameters, ...)` or using the constructor `lymphFilter`. The constructor is the recommended way of object instantiation.

Author(s)

Florian Hahne

See Also

`norm2Filter`, `curv1Filter`

Examples

data(GvHD)
dat <- GvHD[pData(GvHD)$Patient==10]
dat <- transform(dat, "FL4-H"=asinh(\$VarFL4-H)/\$VarFL4-H))
lg <- lymphGate(dat, channels=c("FSC-H", "SSC-H"), preselection="FL4-H", scale=1.5)

if(require(flowViz))
xyplot(`SSC-H``~``FSC-H`, dat, filter=lg\$n2gate)
**normalize-methods**

normalize a GatingSet imported with flowWorkspace, using sequential normalization on the manual gates in the GatingHierarchy.

**Description**

The method will step through the gating hierarchy in a breadth first search manner and normalize each dimension and gate not explicitly excluded in skipdims, or skipgates. The normalization approach is based on warpSet, but uses sequential normalization to alternately normalize then perform gating of the cell populations. This often helps with feature registration of populations lower in the gating hierarchy. FSC and SSC, as well as time are generally excluded by default. The rule of thumb, is to only normalize a channel in a gate if it is absolutely warranted.

**Usage**

```r
normalize(data, x, ...)
```

**Arguments**

- **data**: The GatingSet to be normalized.
- **x**: Not used in here.
- **...**: Arguments passed to downstream functions.

- **target**: The target sample to normalize the other samples in the gating set to. A character vector. Must match a sample name in `x`, otherwise NULL will use the mean (average) of the peaks identified in all samples.
- **populations**: A character vector of population names that are to be normalized.
- **dims**: A character vector of parameter names to be normalized.
- **chunksize**: For a memory-efficient implementation of normalization, set the chunksize, (an integer), which will perform normalization on chunks of the data of size chunksize.
- **nPeaks**: A list of integer or an integer vector that specifies the expected number of peaks for each sample. Can be omitted to keep all peaks.
- **bwFac**: The bandwidth for density estimation, a numeric. Affects the sensitivity for smoothing and detecting distinct peaks.

**Details**

This function implements sequential normalization using a GatingSet and a set of manual gates. For each gate in the gating hierarchy, the algorithm checks if the gate should be normalized, and which dimensions in the gate should be normalized. If normalization is warranted, this is performed prior to gating. After gating, the counts for the gate in the GatingSet are updated, and the next gate is processed. This is useful in the application of template gates to data that has staining variability in one or more channels.

**Value**

Returns a GatingSet of normalized data.

**Author(s)**

Greg Finak <gfinak@fhcrc.org>
See Also

See also GatingSet-class, GatingHierarchy-class, ncdfFlowSet

Examples

```r
# Not run:
#gs is a GatingSet
gs_norm <- normalize(gs
    , target = "M+T panel_903997-25.fcs"
    , populations = "cd27gate"
    , dims = "<Violet A 610/20-A>"
    , minCountThreshold = 100
    , nPeaks = list("cd27gate" = 2)
    , chunksize = 10
    , bwFac = 2
)

#show the population statistics for before and after normalization
getPopStats(gs)
getPopStats(gs_norm)

#plot the gate to see the effects of normalization
grid.arrange(
    plotGate(gs, "cd27gate", type = "densityplot", stack = T)
    ,plotGate(gs_norm, "cd27gate", type = "densityplot", stack = T)
)
```

## Not run

---

normQA

Normalization quality assessment

Description

Create QA plots for a flow cytometry normalization process.

Usage

```r
normQA(data, morph = c("^fsc", "^ssc"), channels, odat = NULL, ask = names(dev.cur()) != "pdf", grouping)
```

Arguments

- **data**: a normalized `flowSet`.
- **morph**: A character vector of channel names to use for the backgating into the morphological channels.
- **channels**: The channels for which to create plots. Defaults to all normalized channels.
- **odat**: The original data set, always needed if there are no warping functions available.
- **ask**: Ask before creating a new plot.
- **grouping**: A grouping variable in data's `phenoData` slot.
tag.outliers Logical. Add sample name to outliers in the plots.
peaksOnly Logical. Only use data when a peak was detected in a particular sample. If set to FALSE, a average peak location is estimated.

Details
This function assumes that the necessary information has been added as attributes to data during the normalization procedure. Depending on the available information, a set of QA plots is generated. Available plots are:
- Amount of peak adjustment
- Warping functions
- Landmark classification confidence
- Backgating of peak events in morphological channels

Value
This function is called for its side effect of generating plots.

Author(s)
Florian Hahne

---

plotBins

Plots the probability bins overlaid with flowFrame data

Description
This function is useful in visualizing the differences between the binned control and sample datasets. The bins generated from the control dataset are overlaid with the sample dataset. An optional argument residuals can be used to shade each bin based on a calculated statistical measure of difference between the number of events in each bin.

Usage
plotBins(binRes, data, channels, title, residuals, shadeFactor)

Arguments
- binRes The result generated by calling the probBin function on a control dataset.
- data An object of class flowFrame sample(dataset)
- channels The flow parameters to be plotted. In cases where more than two parameters are binned from the control set, the plotBins function plots the projections of the hyperplanes in 2 dimensions.
- title Optional title for the plot generated
- residuals A vector of length equal to the number of bins generated that can be used to shade each bin. The residuals from the calcPearsonChi function or the calcPBChiSquare function can be used to highlight the bins that are different between control and sample datasets
- shadeFactor Optional argument between 0 and 1 that controls the intensity of the shading of bins
Author(s)
Nishant Gopalakrishnan

See Also
`proBin`, `calcPearsonChi`, `calcPBChiSquare`

Examples

```r
data(GvHD)
# flow frame 1 is treated as control dataset and used to generate bins
resCtrl<-proBin(GvHD[[1]],200,channels=c("FSC-H","SSC-H"))
plotBins(resCtrl,GvHD[[1]],channels=c("FSC-H","SSC-H"),title="Binned control data")
# Same bins are applied to flowFrame 16
resSample<-binByRef(resCtrl,GvHD[[16]])
stat<-calcPearsonChi(resCtrl,resSample)
dev.new()
plotBins(resCtrl,data=GvHD[[16]],channels=c("FSC-H","SSC-H","Time"),title="Comparison 1 & 16",
residuals=stat$residuals[2,],shadeFactor=0.7)
```

---

**Description**

This function divides the flowframe events into bins such that each bin contains the same number of events. The number of events falling into each bin can then be compared across the control and test samples using statistical methods such as the Chi-squared test.

**Usage**

```r
proBin(m, minEvents=500, channels=NULL)
```

**Arguments**

- `m`: An object of class `flowFrame`
- `minEvents`: The minimum number of events in each bin. (i.e. the termination criterion for the probability binning algorithm)
- `channels`: A character vector for the fluorescence channels on which probability binning is to be performed. Defaults is NULL, in which case, all fluorescence channels are used for probability binning. (Time information, if provided in the flowFrame is discarded)

**Details**

The flowSet is first filtered using a rectangleGate and the norm2Filter is subsequently fitted to the remaining subset.
Value

A list with items:

table

A data.frame that stores information regarding each node of the tree generated during each stage of the probability binning algorithm. Each row in the table represents a node, the first row representing the original flowFrame matrix.

The dataIndx column provides indexes for retrieving the matrices during each stage of the binning process from the envirionment data.

The parent field indicates the row number in the table that holds the parent information for the corresponding node.

The left and right columns indicates the row numbers in the table that stores information regarding the children of that particular node. The leaf nodes that hold the binned data can be identified by the nodes with the left of right values of zero (ie. no children nodes)

The visited column is used internally by the algorithm to check if a particular node has been visited during the computation process.

data

An environment in which the matrices generated during each stage of the probability binning process is stored. The matrices stored at the leaf nodes represent the binned events obtained after the stop criterion of minEvents has been achieved. These can be identified by the corresponding dataIndx fields provided by the rows in the table with the left or right column values of zero.

limits

A list containing the the boundaries of each hyperplane generated during probability binning

splitPars

A data.frame containing two columns splitCol - indicates the column number of the flowFrame, the split was performed.

splitMed - The median value which was used as the threshold for splitting the flowFrame

The splitCol and splitMed parameters are utilized by the plotBins and shadeBins functions in visualizing the differences between control and test sample cases.

Author(s)

Nishant Gopalakrishnan

See Also

plotBins, binByRef

Examples

data(GvHD)
res<-proBin(GvHD[[1]],200,channels=c("FSC-H","SSC-H","FL1-H","FL4-H"))
quadrantGate

Description
This function tries to find the most likely separation of two-dimensional flow cytometry in four quadrants.

Usage
quadrantGate(x, stains, alpha=c("min", "min"), sd=c(2, 2), plot=FALSE, filterId="defaultQuadGate", refLine.1=NULL, refLine.2=NULL, rare=c(FALSE,FALSE), sig=c(NULL,NULL), ...) 

Arguments
x A flowSet or flowFrame.
stains A character vector of length two giving the two flow parameters for which the quad gate is to be computed.
alpha, sd Tuning factors to control the computation of the gate boundaries. See rangeGate for details.
plot Logical. Produce plots of intermediate results.
filterId Character, the name assigned to the resulting filter.
refLine.1 Either NULL or a numeric of length 1. If NULL, this parameter is ignored. When it is set to a numeric, the minor sub-population (if any) below this reference line in the first stain channel will be ignored while determining the separator between positive and negative.
refLine.2 Either NULL or a numeric of length 1. If NULL, this parameter is ignored. When it is set to a numeric, the minor sub-population (if any) below this reference line in the second stain channel will be ignored while determining the separator between positive and negative.
rare logical flags for two channels, Refer to density1d for more details.
sig parameters for two channels. Refer to density1d for more details.
... Additional arguments

Details
The most likely separation between positive and negative stains for two-dimensional data is computed based on density estimates. Essentially, the gate parameters are first fitted separately for the two parameters and later combined. See the documentation for rangeGate for details. There is a certain amount of heuristics involved in this process. The algorithm can be slightly tweaked using the alpha and sd arguments. Their values will be recycled for the two dimensions unless explicitly given as vectors of length 2.

Value
An object of class quadGate.
rangeGate

Find most likely separation between positive and negative populations in 1D

Description

The function tries to find a reasonable split point between the two hypothetical cell populations "positive" and "negative".

Usage

rangeGate(x, stain, alpha="min", sd=2, plot=FALSE, borderQuant=0.1, absolute=TRUE, filterId="defaultRectangleGate", positive=TRUE, refLine=NULL, simple = FALSE,...)

rangeFilter(stain, alpha="min", sd=2, borderQuant=0.1, filterId="defaultRangeFilter")

Arguments

x A FlowSet or flowFrame.

stain A character scalar giving the flow parameter for which to compute the separation.

alpha A tuning parameter that controls the location of the split point between the two populations. This has to be a numeric in the range [0,1], where values closer to 0 will shift the split point closer to the negative population and values closer to 1 will shift towards the positive population. Additionally, the value of alpha

Examples

## Not run:
data(GvHD)
dat <- GvHD[pData(GvHD)$Patient==10]
dat <- transform(dat, "FL4-H"=asinh('FL4-H'), "FL2-H"=asinh('FL2-H'))
qg <- quadrantGate(dat, c("FL2-H", "FL4-H"))
qg

if(require(flowViz))
  xyplot('FL2-H'~'FL4-H', dat, filter=qg)

qg <- quadrantGate(dat, c("FL2-H", "FL4-H"), alpha=c(0.1, 0.9), plot=TRUE)
qg

split(dat, qg)

## End(Not run)
can be "min", in which case the split point will be selected as the area of lowest local density between the two populations.

sd  For the case where there is only a single population, the algorithm falls back to estimating the mode of this population and a robust measure of the variance of it distribution. The sd tuning parameter controls how far away from the mode the split point is set.

plot  Create a plot of the results of the computation.

borderQuant  Usually the instrument is set up in a way that the positive population is somewhere on the high end of the measurement range and the negative population is on the low end. This parameter allows to disregard populations with mean values in the extreme quantiles of the data range. It’s value should be in the range \([0, 1]\).

absolute  Logical controlling whether to classify a population (positive or negative) relative to the theoretical measurement range of the instrument or the actual range of the data. This can be set to TRUE if the alignment of the measurement range is not optimal and the bulk of the data is on one end of the theoretical range.

filterId  Character, the name assigned to the resulting filter.

positive  Create a range gate that includes the positive (TRUE) or the negative (FALSE) population.

refline  Either NULL or a numeric of length 1. If NULL, this parameter is ignored. When it is set to a numeric, the minor sub-population (if any) below this reference line will be ignored while determining the separator between positive and negative.

simple  Logical scalar indicating whether to use a simple peak finding version of density1d algorithm.

...  Further arguments.

Details

The algorithm first tries to identify high density regions in the data. If the input is a flowSet, density regions will be computed on the collapsed data, hence it should have been normalized before (see warpSet for one possible normalization technique). The high density regions are then classified as positive and negative populations, based on their mean value in the theoretical (or absolute if argument absolute=TRUE) measurement range. In case there are only two high-density regions the lower one is usually classified as the negative populations, however the heuristics in the algorithm will force the classification towards a positive population if the mean value is already very high. The absolute and borderQuant arguments can be used to control this behaviour. The split point between populations will be drawn at the value of minimum local density between the two populations, or, if the alpha argument is used, somewhere between the two populations where the value of alpha forces the point to be closer to the negative (0 - 0.5) or closer to the positive population (0.5 - 1).

If there is only a single high-density region, the algorithm will fall back to estimating the mode of the distribution (hubers) and a robust measure of it’s variance and, in combination with the sd argument, set the split point somewhere in the right or left tail, depending on the classification of the region.

For more than two populations, the algorithm will still classify each population into positive and negative and compute the split point between those clusters, similar to the two population case.

The rangeFilter class and constructor provide the means to treat rangeGate as regular flowCore filters.
Value

A range gate, more explicitly an object of class rectangleGate.

Methods

%in% signature(x = "flowFrame", table = "rangeFilter"): the work horse for doing the actual filtering. Internally, this simply calls the rangeGate function.

Author(s)

Florian Hahne, Kyongryun Lee

See Also

warpSet, rangeGate, rectangleGate

Examples

data(GvHD)
  dat <- GvHD[pData(GvHD)$Patient==10]
  dat <- transform(dat, "FL4-H"=asinh(FL4-H), "FL3-H"=asinh(FL3-H))
  rg <- rangeGate(dat, "FL4-H", plot=TRUE)
  split(dat, rg)

  ## Test rangeGate when setting refLine=0; it does not do anything since there is no sub-population below zero.
  rangeGate(dat, "FL4-H", plot=FALSE, refLine=0)

  rf <- rangeFilter("FL4-H")
  filter(dat, rf)

warpSet

Normalization based on landmark registration

Description

This function will perform a normalization of flow cytometry data based on warping functions computed on high-density region landmarks for individual flow channels.

Usage

warpSet(x, stains, grouping = NULL, monwrd = TRUE, subsample=NULL, peakNr=NULL, clipRange=0.01, nbreaks=11, fres, bwFac=2, warpFuns=FALSE,target=NULL, ...)

warpSet
Arguments

- **x**: A `flowSet`
- **stains**: A character vector of flow parameters in `x` to be normalized.
- **grouping**: A character indicating one of the phenotypic variables in the `phenoData` slot of `x` used as a grouping factor. The within-group and between-group variance is computed and a warning is issued in case the latter is bigger than the former, indicating the likely removal of signal by the normalization procedure.
- **monwrd**: Logical. Compute strictly monotone warping functions. This gets directly passed on to `landmarkreg`.
- **subsample**: Numeric. Reduce the number of events in each `flowSet` by sub sampling for all density estimation steps and the calculation of the warping functions. This can increase computation time for large data sets, however it might reduce the accuracy of the density estimates. To be used with care.
- **peakNr**: Numeric scalar. Force a fixed number of peaks to use for the normalization.
- **clipRange**: Only use peaks within a clipped data range. Essentially, the number indicates the percent of clipping on both sides of the data range, e.g. \( \min(x) - 0.01 \times \text{diff}(\text{range}(x)) \).
- **nbreaks**: The number of spline sections used to approximate the data. Higher values produce more accurate results, however this comes with the cost of increased computing times. For most data, the default setting is good enough.
- **fres**: A named list of `filterResultList` objects. This can be used to speed up the process since the `curv1Filter` step can take quite some time.
- **bwFac**: Numeric of length 1 used to set the bandwidth factor by `curv1Filter` for smoothing of the density estimate.
- **warpFuns**: Logical indicating whether to return the normalized `flowSet` or a list of warping functions.
- **target**: Character vector specifying the target sample to which other samples in the `flowSet` should be normalized. If `NULL`, then the mean of the peaks is used.
- **...**: Further arguments that are passed on to `landmarkreg`.

Details

Normalization is achieved by first identifying high-density regions (landmarks) for each `flowFrame` in the `flowSet` for a single channel and subsequently by computing warping functions for each `flowFrame` that best align these landmarks. This is based on the algorithm implemented in the `landmarkreg` function in the `fda` package. An intermediate step classifies the high-density regions, see `landmarkMatrix` for details.

Please note that this normalization is on a channel-by-channel basis. Multiple channels are normalized in a loop.

Value

The normalized `flowSet` if `warpFuns` is `FALSE`, otherwise a list of warping functions. Additional information is attached as the `warping` attribute to the `flowSet` in form of a list.

Note

We currently use a patched `fda` version.
Author(s)
Florian Hahne

References

See Also
curv1Filter, landmarkMatrix

Examples

```r
data(ITN)
dat <- transform(ITN, "CD4"=asinh(CD4), "CD3"=asinh(CD3), "CD8"=asinh(CD8))
lg <- lymphGate(dat, channels=c("CD3", "SSC"), preselection="CD4", scale=1.5)
dat <- Subset(dat, lg$n2gate)
datr <- warpSet(dat, "CD8", grouping="GroupID", monwrd=TRUE)
if(require(flowViz)){
d1 <- densityplot(~CD8, dat, main="original", filter=curv1Filter("CD8"))
d2 <- densityplot(~CD8, datr, main="normalized", filter=curv1Filter("CD8"))
plot(d1, split=c(1,1,2,1))
plot(d2, split=c(2,1,2,1), newpage=FALSE)
}
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