# Package ‘flowVS’

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**Description** Per-channel variance stabilization from a collection of flow cytometry samples by Bertlett test for homogeneity of variances. The approach is applicable to microarrays data as well.  
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estParamFlowVS

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*flowVS-package*

flowVS: Variance stabilization in flow cytometry (and microarrays).

Description

Please see the vignette.

Author(s)

Ariful Azad <azad@lbl.gov>

References


See Also

transFlowVS, microVS

estParamFlowVS

Estimate optimum parameters for per-channel within-population variance stabilization.

Description

This function estimates the variance stabilizing cofactors, one for each channel for the entire dataset. When a fluorescence channel $z$ is transformed by asinh transformation with the optimum cofactor for $z$, the within-population variances of populations from all samples in the channel $z$ are approximately stabilized.

Usage

```
estParamFlowVS(fs, channels)
```

Arguments

- **fs**: A flowSet containing a collection of flow cytometry samples.
- **channels**: A character vector identifying the channels/dimensions to be transformed. If any entry in this vector is not present in the flowSet, the function returns with an error.
Details

Let \( z \) be a fluorescence channel (column of a flowFrame). We consider transforming \( z \) by asinh transformation such that after transformation we obtain the transformed channel \( \text{asinh}(z/c) \), where \( c \) is a normalizing cofactor.

The \text{estParamFlowVS} function estimates cofactors, one for each channel for the entire dataset such that the within-population variance is stabilized in each fluorescence channel. When a fluorescence channel \( z \) is transformed by asinh transformation with the optimum cofactor for \( z \), the within-population variances of populations from all samples in the channel \( z \) are approximately stabilized.

Value

\text{estParamFlowVS} returns a numeric vector representing the optimum cofactors for the requested channels. The optimum cofactor for the input channels[\( i \)] is stored in the \( i \)th entry of the returned vector.

Author(s)

Ariful Azad

References


See Also

\text{transFlowVS}

Examples

```r
data(HD)
## identify optimum cofactor for CD3 and CD4 channels (from five samples)
cofactors = estParamFlowVS(HD[1:5], channels=c('CD3','CD4'))
# See detail examples in the documentation of the transFlowVS function.
```

Description

A \text{flowSet} containing 12 flow cytometry samples from three healthy individuals "A", "C", and "D". From each individual, the samples were drawn on two different days and two technical replicates were created from each sample (i.e., 3 x 2 x 2 = 12 samples). Each HD sample was stained using labeled antibodies against CD45, CD3, CD4, CD8, and CD19 protein markers. Here, an HD sample "C_4_2" means that it is collected on day 4 from individual "C" and it is the second replicate on that day. We have identified lymphocytes in each sample of the HD dataset and apply the subsequent analysis on lymphocytes.
lymphs

Usage

data(HD)

Value

A flowSet containing 12 flowFrames. There are 3 subject groups with 4 samples each (2 days and 2 technical replicates per day).

References


lymphs

Identify lymphocyte cells from a flow cytometry sample.

Description

Identify and retain lymphocytes from a flow cytometry sample based on the forward and side scatters.

Usage

lymphs(ff, lymph.boundary, fsc, ssc, plot=FALSE)

Arguments

ff
A flowFrame containing a flow cytometry sample.

lymph.boundary
A list denoting an approximate rectangular boundary for lymphocytes. The first element of the list represents the lower and upper limit of forward scatter (FSC), and the 2nd element represents the lower and upper limit of side scatter (SSC). Example: list("FSC"=c(180000, 500000),"SSC"=c(0, 180000)).

fsc
name (or numeric index) of the forward scatter channel.

ssc
name (or numeric index) of the side scatter channel.

plot
true/false. If true then plots the rectangular and elliptical gates for the lymphocytes.

Details

At first a rectangular gate is created based on the lymph.boundary. Then the norm2Filter function is used to identify lymphocytes.

Value

lymphs returns a new flowFrame containing the lymphocytes.
Author(s)
Ariful Azad

References

See Also
estParamFlowVS

Examples
library(flowStats)
data(ITN)
# identify lymphocytes
ITN.lymphs = lymphs(ITN[[1]], list("FS"=c(200, 600),"SS"=c(0, 400)), "FSC", "SSC",TRUE)

microVS
Variance stabilization for microarray data.

Description
Variance-stabilizing inverse hyperbolic sine (asinh) transformation for microarray data.

Usage
microVS(data, cfLow=0, cfHigh=10, frac=1)

Arguments
data
The microarray data in a Matrix.
ciafLow
lowest possible value of cofactor (log scale).
ciafHigh
highest possible value of cofactor (log scale).
ciafrac
fraction of differentially expressed genes used in variance stabilization (0< frac <=1).

Details
This function transforms a microarray data matrix $z$ by $\text{asinh}(z/c)$ transformation where $c$ is a normalizing cofactor. The cofactor is searched in the range $[\text{cfLow}, \text{cfHigh}]$ and an optimum cofactor is obtained for which the transformed data is variance stabilized. The optimum cofactor is obtained by minimizing Bartlett’s test statistics for homogeneity of variance. If the parameter $\text{frac}$ is less then one, a fraction of differentially expressed genes are used in estimating the cofactor.
Value

microVS returns a matrix of the variance-stabilizing microarray data.

Author(s)

Ariful Azad

References


Examples

# stabilize variance of the Kidney microarray data from the vsn package
library(vsn)
data(kidney)
kidney.t = microVS(exprs(kidney))
plotMeanSd(kidney.t)

plotMeanSd(x, ranks = TRUE,
xlab = ifelse(ranks, "Rank of means (ascending order)", "mean"),
ylab = "Standard deviation",
pch = ".",
plot = TRUE,
...)  

Arguments

x An object of class matrix
ranks Logical, indicating whether the x-axis (means) should be plotted on the original scale (FALSE) or on the rank scale (TRUE). The latter distributes the data more evenly along the x-axis and allows a better visual assessment of the standard deviation as a function of the mean.
xlab Character, label for the x-axis.
transFlowVS

ylab Character, label for the y-axis.
pch Plot symbol.
plot Logical. If TRUE (default), a plot is produced. Calling the function with plot=FALSE can be useful if only its return value is of interest.
... Further arguments that get passed to plot.default.

Details

Standard deviation and mean are calculated row-wise from the expression matrix (in) x. The scatterplot of these versus each other allows to visually verify whether there is a dependence of the standard deviation (or variance) on the mean. The red dots depict the running median estimator (window-width 10%). If there is no variance-mean dependence, then the line formed by the red dots should be approximately horizontal.

Value

A named list with four components: its elements px and py are the x- and y-coordinates of the individual data points in the plot; its first and second element are the x-coordinates and values of the running median estimator (the red dots in the plot). Depending on the value of plot, the method can also have a side effect, which is to create a plot on the active graphics device.

Examples

library(vsn)
data(kidney)
kidney.t = microVS(exprs(kidney))
plotMeanSd(kidney.t)

---

transFlowVS Transform a flowSet by asinh transformation.

Description

This function transforms a flowSet by asinh transformation with the cofactors passed on to the function. The optimum cofactors that stabilize within-population variances in different fluorescence channels are estimated beforehand and passed to this function for data transformation.

Usage

transFlowVS(fs, channels, cofactors)

Arguments

fs A flowSet containing a collection of flow cyometry samples.
channels A character vector identifying the channels/dimensions to be transformed. If any entry in this vector is not present in the flowSet, the function returns with an error.
cofactors A numeric vector. cofactors[i] is used with asinh function to transform the column with name specified by channels[i].
transFlowVS

Details

This function transforms a flowSet by asinh transformation with selected cofactors. The column with name channels[i] of every flowFrame of the input flowSet is transformed by asinh transformation with cofactors[i]. For example, let \( z_{ij} \) be the \( i \)th column of \( j \)th flowFrame in the input flowSet \( fs \). Then after transformation \( z_{ij} \) would be converted to \( \text{asinh}(z_{ij}/\text{cofactors}[i]) \).

For variance stabilization, the optimum cofactors that stabilize within-population variances in different fluorescence channels are estimated beforehand and passed to this function for data transformation. Variance stabilizing cofactors can be estimated by the \text{estParamFlowVS} function.

Value

\text{transFlowVS} returns a new flowSet with the transformed channels.

Author(s)

Ariful Azad

References


See Also

\text{estParamFlowVS}

Examples

```r
## ------------------------------------------------
## Example 1: Healthy data from flowVS package
## ------------------------------------------------
data(HD)
## identify optimum cofactor for CD3 and CD4 channels (from five samples)
cofactors = \text{estParamFlowVS}(HD[1:5], channels=c('CD3', 'CD4'))
## transform CD3 and CD4 channels in all samples
HD.VS = \text{transFlowVS}(HD, c('CD3', 'CD4'), cofactors)
densityplot(~CD3+CD4, HD.VS, main="Transformed CD3 and CD4 channels in HD data")
## ------------------------------------------------
## Example 2: ITN data from flowStats package
## ------------------------------------------------
library(flowStats)
data(ITN)
# identify lymphocytes
ITN.lymphs = \text{fsApply}(ITN.lymphs, list("FS"=c(200, 600), "SS"=c(0, 400)), "FSC", "SSC", FALSE)
## identify optimum cofactor for CD3 and CD4 channels (from five samples)
cofactors = \text{estParamFlowVS}(ITN.lymphs[1:5], channels=c('CD3', 'CD4'))
## transform CD3 and CD4 channels in all samples
ITN.VS = \text{transFlowVS}(ITN.lymphs, c('CD3', 'CD4'), cofactors)
```
densityplot(~CD3+CD4, ITN.VS, main="Transformed CD3 and CD4 channels in ITN data")
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