

Package ‘immunoClust’

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Type Package

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Suggests BiocStyle, utils

Description

immunoClust is a model based clustering approach for Flow Cytometry samples. The cell-events of single Flow Cytometry samples are modelled by a mixture of multinomial normal- or t-distributions. The cell-event clusters of several samples are modelled by a mixture of multinomial normal-distributions aiming stable co-clusters across these samples.

Collate generics.R class.immunoClust.R plot.immunoClust.R splom.immunoClust.R methods.immunoClust.R class.immunoMeta.R plot.immunoMeta.R methods.immunoMeta.R data.R process.R cell.clustering.R meta.clustering.R meta.annotation.R meta.export.R clust.util.R transform.R

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immunoClust-package	<i>immunoClust - Automated Pipeline for Population Detection in Flow Cytometry</i>
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Description

Model based clustering and meta-clustering routines for Flow Cytometry (FC) data.

The immunoClust-pipeline consists of two major procedures:

cell.process	Clustering of cell-events
meta.process	Meta-clustering of cell-clusters

Cell-events clustering is performed for each FC data sample separately. After this all cell-clustering results are collected in a vector and meta-clustering is performed to obtain the across samples populations.

Details

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Author(s)

Till Sörensen <till-antoni.soerensen@charited.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

bhattacharyya

Bhattacharyya Distance, Coefficient and Probability

Description

Calculates the Bhattacharyya Distance, Coefficient and Probability

Usage

```
bhattacharyya.prob(gM,gS, cM,cS, alpha=1)
```

```
bhattacharyya.dist(gM, gS, cM, cS)
```

```
bhattacharyya.coeff(gM,gS, cM,cS, alpha=1)
```

Arguments

gM, cM	P-dimensional vector of cluster means
gS, cS	PxP-dimensional matrix of clusters co-variances
alpha	A value between 0 and 1 used to balance the bhattacharyya probabilities, coefficients calculated with either the full covariance matrices or using only the diagonal elements of it.

Details

Calculates the bhattacharyya probability, distance or coefficient of the clusters, i.e. Gaussian distributions. Distance and Coefficient are symmetric for both clusters, whereas the probability is not.

Value

The Bhattacharyya probability, distance or coefficient

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

cell.ClustData

Model Based Clustering of Data for a pre-defined Number of Clusters

Description

Performs EM-iteration on cell events, where an initial event cluster membership is obtained by hierarchical clustering on a sample subset given a number of clusters.

Usage

```
cell.ClustData(data, K, parameters=NULL, expName="immunoClust Experiment",
               sample.number=1500, sample.standardize=TRUE,
               B=50, tol=1e-5, modelName="mvt")
```

Arguments

data	A numeric matrix, data frame of observations, or object of class flowFrame. Rows correspond to observations and columns correspond to measured parameters.
K	Given number of clusters for the final model.
parameters	A character vector specifying the parameters (columns) to be included in clustering. When it is left unspecified, all the parameters will be used.
expName	The name of the clustering experiment.
sample.number	The maximum number of samples used for initial hierarchical clustering.
sample.standardize	A numeric indicating whether the samples for hierarchical clustering are standardized (mean=0, SD=1).
B	The maximum number of EM-iterations.
tol	The tolerance used to assess the convergence of the EM-algorithm.
modelName	Used mixture model; either "mvt" for a t-mixture model or "mvm" for a Gaussian Mixture model.

Details

Although this function provides the possibility to cluster an arbitrary set of observed data into a fixed number of clusters, this function is used in the immunoClust-pipeline only for the calculation of the initial model with one cluster.

Value

The fitted model cluster information in an object of class `immunoClust`.

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

References

Sørensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[immunoClust-object](#), [cell.hclust](#)

Examples

```
data(dat.fcs)
res <- cell.ClustData(dat.fcs, parameters=c("FSC-A", "SSC-A"), 5)
summary(res)
```

cell.EM	<i>immunoClust EMt-iteration on Cell-events given initial Model Parameters</i>
---------	--

Description

Performs EMt-iteration on cell event observations giving initial model parameters and returns the fitted clusters information in an object of class [immunoClust](#).

Usage

```
cell.EM(data, parameters=NULL, expName="immunoClust Experiment",
        history=NULL, state=NULL,
        K, w, m, s, B=50, tol=1e-5, bias=0.5, modelName="mvt")

cell.Estimation(data, parameters=NULL, expName="immunoClust Experiment",
               history=NULL, state=NULL,
               K, w, m, s, modelName="mvt")
```

Arguments

data	A numeric matrix, data frame of observations, or object of class flowFrame.
parameters	A character vector specifying the parameters (columns) to be included in clustering. When it is left unspecified, all the parameters will be used.
expName	The name of the clustering experiment.
history	experimental; unused so far.
state	experimental: unused so far.
K	The number of clusters.
w	The K -dimensional vector of the mixture proportions.
m	The $K \times P$ -dimensional matrix of the K estimated cluster means.
s	The $K \times P \times P$ -dimensional matrix of the K estimated cluster covariance matrices.
B	The maximum number of EMt-iterations.

tol	The tolerance used to assess the convergence of the EMt-algorithms.
bias	The ICL-bias used in the EMt-algorithm.
modelName	Used mixture model; either "mvt" or "mvn" for a <i>t</i> - or Gaussian mixture model respectively.

Details

Whereas `cell.EM` performs a complete EMt-iteration, `cell.Estimate` only calculates the posterior probabilities and the Maximum-A-Posterior estimators of cluster membership for all events.

Value

The fitted clusters information in an object of class `immunoClust`.

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

References

Sørensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[cell.ME](#), [cell.FitModel](#)

Examples

```
data(dat.fcs)
data(dat.exp)
## cell.clustering result for dat.fcs
r <- dat.exp[[1]]
summary(r)
## apply model parameter to all (unfiltered) events
dat.trans <- trans.ApplyToData(r, dat.fcs)
#r2 <- cell.EM(dat.trans, parameters=r@parameters,
# K=r@K, w=r@w,m=r@mu,s=r@sigma)
r2 <- cell.EM(dat.trans, parameters(r), K=ncls(r),
w=weights(r),m=mu(r),s=sigma(r))
summary(r2)
```

cell.FitModel	<i>immunoClust EMt-iteration on Cell-events given initial Model Parameters</i>
---------------	--

Description

The function fits initial model parameters to specific observed cell event data. The function returns the cluster information of the fitted model in an object of class `immunoClust`.

Usage

```
cell.FitModel(x, data, B=50, tol=1e-5, bias=0.5, modelName="mvt" )  
  
cell.Classify(x, data, modelName="mvt" )
```

Arguments

x	An immunoClust object with the initial model parameter (<i>parameters</i> , K , w , μ , σ).
data	A numeric matrix, data frame of observations, or object of class flowFrame.
B	The maximum number of EMt-iterations.
tol	The tolerance used to assess the convergence of the EMt-algorithms.
bias	The ICL-bias used in the EMt-algorithm.
modelName	Used mixture model; either "mvt" or "mvn" for a t - or Gaussian mixture model respectively.

Details

These functions are wrapper of the functions `cell.EM` and `cell.Estimation`, when model cluster parameters are combined in an object of class `immunoClust` and are used in the iterative cell event clustering process `cell.process` of `immunoClust` and are not intended to be called directly.

Value

The fitted model cluster information in an object of class `immunoClust`.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[cell.process](#), [cell.EM](#), [cell.Estimation](#)

Examples

```
data(dat.fcs)  
data(dat.exp)  
r1 <- dat.exp[[1]]  
dat.trans <- trans.ApplyToData(r1, dat.fcs)  
r2 <- cell.FitModel(r1, dat.trans)
```

cell.hclust	<i>Hierarchical Model Based Clustering of Cell-events in the immunoClust-pipeline</i>
-------------	---

Description

Performs model based agglomerative clustering on cell event observations with weights. It is used in the iterative cell event clustering approach of *immunoClust* to obtain an initial cluster membership for the EM(t)-iteration.

Usage

```
cell.hclust(data, weights=NULL)
```

Arguments

data	The numeric $N \times P$ -dimensional data matrix to cluster. Each row contains a P -dimensional overservation vector.
weights	The N -dimensional vector of optional weights to be applied for the overservations.

Details

This function is used internally in [cell.TestSubCluster](#) procedure of **immunoClust**.

Value

A numeric $(N - 1) \times 2$ -dimensional matrix which gives the minimum index for observations in each of the two clusters merged at the i th step in each row.

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

References

Sørensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[cell.TestSubCluster](#), [cell.process](#)

Examples

```
data(dat.fcs)
inc <- sample(1:nrow(dat.fcs), 50)
result <- cell.hclust(exprs(dat.fcs)[inc,])
```

cell.ME	<i>immunoClust EM-iteration on Cell-events given initial Cluster Membership Assignment</i>
---------	--

Description

Performs an EM-iteration on cell event observations given an initial cluster membership for the cell events and returns the fitted cluster information in an object of class `immunoClust`.

Usage

```
cell.ME(data, parameters=NULL, expName="immunoClust Experiment",
        history=NULL, state=NULL, label, B=50, tol=1e-5, modelName="mvt")
```

Arguments

data	A numeric matrix, data frame of observations, or object of class <code>flowFrame</code> .
parameters	A character vector specifying the parameters (columns) to be included in clustering. When it is left unspecified, all the parameters will be used.
expName	The name of the clustering experiment.
history	experimental; unused so far.
state	experimental: unused so far.
label	The N -dimensional vector containing the initial cluster membership. A label-number of 0 for an event indicates that this event is not initially assigned to a cluster.
B	The maximum number of EMt-iterations.
tol	The tolerance used to assess the convergence of the EMt-algorithms.
modelName	Used mixture model; either "mvt" or "mvn" for a t - or Gaussian mixture model respectively.

Value

The fitted clusters information in an object of class `immunoClust`.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[cell.EM](#)

Examples

```

data(dat.fcs)
data(dat.exp)
## cell.clustering result for dat.fcs
r1 <- dat.exp[[1]]
summary(r1)
## apply model parameter to all (unfiltered) events
dat.trans <- trans.ApplyToData(r1, dat.fcs)
r2 <- cell.ME(dat.trans, parameters(r1), label=label(r1))
summary(r2)

```

cell.process

Clustering of Cell-events in the immunoClust-pipeline

Description

This function performs iterative model based clustering on cell-event data. It takes the observed cell-event data as major input and returns an object of class `immunoClust`, which contains the fitted mixture model parameter and cluster membership information. The additional arguments control the routines for data preprocessing, major loop and EMt-iteration, the model refinement routine and transformation estimation.

Usage

```

cell.process(fcs, parameters=NULL,
  apply.compensation=FALSE, classify.all=FALSE,
  N=NULL, min.count=10, max.count=10, min=NULL, max=NULL,
  I.buildup=6, I.final=4, I.trans=I.buildup,
  modelName="mvt", tol=1e-5, bias=0.3,
  sub.tol= 1e-4, sub.bias=bias, sub.thres=bias, sub.samples=1500,
  sub.extract=0.8, sub.weights=1, sub.standardize=TRUE,
  trans.estimate=TRUE, trans.minclust=10,
  trans.a=0.01, trans.b=0.0, trans.parameters=NULL)

cell.MajorIterationLoop(dat, x=NULL, parameters=NULL,
  I.buildup=6, I.final=4,
  modelName="mvt", tol=1e-5, bias=0.3,
  sub.bias=bias, sub.thres=0.0, sub.tol=1e-4, sub.samples=1500,
  sub.extract=0.8, sub.weights=1, sub.EM="MEt", sub.standardize=TRUE)

cell.MajorIterationTrans(fcs, x=NULL, parameters=NULL,
  I.buildup=6, I.final=4, I.trans=I.buildup,
  modelName="mvt", tol=1e-5, bias=0.3,
  sub.bias=bias, sub.thres=0.0, sub.tol=1e-4, sub.samples=1500,
  sub.extract=0.8, sub.weights=1, sub.EM="MEt", sub.standardize=TRUE,
  trans.minclust=5, trans.a=0.01, trans.decade=-1, trans.scale=1.0,
  trans.proc="vSHtransAw")

cell.InitialModel(dat, parameters=NULL, trans.a = 0.01, trans.b = 0.0,
  trans.decade=-1, trans.scale=1.0)

cell.classifyAll(fcs, x, apply.compensation=FALSE)

```

Arguments

<code>fcs</code>	An object of class <code>flowFrame</code> . Rows correspond to observations and columns correspond to measured parameters.
<code>dat</code>	A numeric matrix, data frame of observations, or object of class <code>flowFrame</code> . Rows correspond to observations and columns correspond to measured parameters.
<code>x</code>	An object of class <code>immunoClust</code> . Used as initial model in the major iteration loop. When left unspecified the simplest model containing 1 cluster is used as initial model.
	Arguments for data pre and post processing:
<code>parameters</code>	A character vector specifying the parameters (columns) to be included in clustering. When it is left unspecified, all the parameters will be used.
<code>apply.compensation</code>	A numeric indicator whether the compensation matrix in the <code>flowFrame</code> should be applied.
<code>classify.all</code>	A numeric indicator whether the removed over- and underexposed observations should also be classified after the clustering process.
<code>N</code>	Maximum number of observations used for clustering. When unspecified or higher than the number of observations (i.e. rows) in <code>dat</code> , all observations are used for clustering, otherwise only the first <code>N</code> observations.
<code>min.count</code>	An integer specifying the threshold count for filtering data points from below. The default is 10, meaning that if 10 or more data points are smaller than or equal to <code>min</code> , they will be excluded from the analysis. If <code>min</code> is <code>NULL</code> , then the minimum value of each parameter will be used. To suppress filtering, it is set to -1.
<code>max.count</code>	An integer specifying the threshold count for filtering data points from above. Interpretation is similar to that of <code>min.count</code> .
<code>min</code>	The lower limit set for data filtering. Note that it is a vector of length equal to the number of parameters (columns), implying that a different value can be set for each parameter.
<code>max</code>	The upper limit set for data filtering. Interpretation is similar to that of <code>min</code> .
	Arguments for the major loop and EMt-iteration:
<code>I.buildup</code>	The number of major iterations, where the number of used observations is doubled successively.
<code>I.final</code>	The number of major iterations with all observations.
<code>I.trans</code>	The number of iterations where transformation estimation is applied.
<code>modelName</code>	Used mixture model; either <code>"mvt"</code> for a <i>t</i> -mixture model or <code>"mvn"</code> for a Gaussian Mixture model. With <code>"mvt2"</code> an implementation variant for <code>"mvt"</code> is given, which is more reliable for samples with cutted values at the lower or upper edges of the parameter space (e.g. for CyTOF all values below a detection limit are set to zero which leads to wrong co-variance estimators and poor clustering results).
<code>tol</code>	The tolerance used to assess the convergence of the major EM(t)-algorithms of all observations.
<code>bias</code>	The ICL-bias used in the major EMt-algorithms of all observations.
	Arguments for model refinement (sub-clustering):

sub.tol	The tolerance used to assess the convergence of the EM-algorithms in the sub-clustering.
sub.bias	The ICL-bias used in the sub-clustering EMt-algorithms, in general the same as the ICL-bias.
sub.thres	Defines the threshold, below which an ICL-increase is meaningless. The threshold is given as the multiple (or fraction) of the costs for a single cluster.
sub.samples	The number of samples used for initial hierarchical clustering.
sub.extract	The threshold used for cluster data extraction.
sub.weights	Power of weights applied to hierarchical clustering, where the used weights are the probabilities of cluster membership.
sub.EM	Used EM-algorithm; either "MEt" for EMt-iteration or "ME" for EM-iteration without test step.
sub.standardize	A numeric indicating whether the samples for hierarchical clustering are standardized (mean=0, SD=1). Arguments for transformation optimization:
trans.estimate	A numeric indicator whether transformation estimation should be applied.
trans.minclust	The minimum number of clusters required to start transformation estimation.
trans.a	A numeric vector, giving the (initial) scaling a for the asinh-transformation $h(y) = a \cdot \sinh(a \cdot y + b)$. A scaling factor of $a = 0$ indicates that a parameter is not transformed.
trans.b	A numeric vector, giving the (initial) translation b for the asinh-transformation.
trans.parameters	A character vector, specifying the parameters (columns) to be applied for transformation. When it is left unspecified, the parameters to be transformed are obtained by the PxDISPLAY information of the flowFrame description parameters. All parameters with LOG display values are transformed.
trans.decade	A numeric scale value for the theoretical maximum of transformed observation value. If below 0, no scaling of the transformed values is applied, which is the default in the <i>immunoClust</i> -pipeline.
trans.scale	A numeric scaling factor for the linear (i.e. not transformed) parameters. By default the linear parameters (normally the scatter parameters) are not scaled.
trans.proc	An experimental switch for alternative procedures; should be "vsHtransAw".

Details

The `cell.process` function does data preprocessing and calls the major iteration loop either with or without integrated transformation optimization. When transformation optimization is applied the transformation parameters give the initial transformation otherwise they define the fixed transformation.

The major iteration loop with included transformation optimization relies on `flowFrames` structure from the `flowCore`-package for the storage of the observed data.

The `cell.InitialModel` builds up an initial `immunoClust`-object with one cluster and the given transformation parameters.

The `cell.classifyAll` calculates the cluster membership for the removed cell events. The assignment of the cluster membership is critical for over- and underexposed observations and the interpretation is problematic.

Value

The fitted model information in an object of class `immunoClust`.

Note

- a) The data preprocessing arguments (`min.count`, `max.count`, `min` and `max`) for removing over- and underexposed observations are adopted from `flowCust`-package with the same meaning.
- b) The `sub.thres` value is given in here in relation to the single cluster costs $\frac{1}{2} \cdot P \cdot (P+1) \cdot \log(N)$. An absolute increase of the log-likelihood above is reported as reasonable from the literature. From our experience a higher value is required for this increase in FC data. For the ICL-bias and the `sub.thres` identical values were chosen. For the CyTOF dataset this value had been adjusted to 0.05 since the absolute increase of the log-likelihood became too high due to the high number of parameters.
- c) The `sub.extract` value controls the smooth data extraction for a cluster. A higher value includes more events for a cluster in the sub-clustering routine.
- d) The default value of `trans.a=0.01` for the initial transformation is optimized for Fluorescence Cytometry. For CyTOF data the initial scaling value was `trans.a=1.0`.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. `immunoClust` - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

`immunoClust-object`, `plot`, `splom`, `cell.FitModel`, `cell.SubClustering`, `trans.FitToData`

Examples

```
data(dat.fcs)
res <- cell.process(dat.fcs)
summary(res)
```

cell.removed

Brief Information of removed Cell-events by immunoClust Cell-event Clustering

Description

Gives information about the amount of overexposed cell-event observation in a FCS-file.

Usage

```
removed.above(fcs, parameters=NULL, N=NULL, max.count=10, max=NULL)
removed.below(fcs, parameters=NULL, N=NULL, min.count=10, min=NULL)
```

Arguments

<code>fcs</code>	An object of class <code>flowFrame</code> . Rows correspond to observations and columns correspond to measured parameters.
<code>parameters</code>	A character vector specifying the parameters (columns) to be included in clustering. When it is left unspecified, all the parameters will be used.
<code>N</code>	Maximum number of observations used for clustering. When unspecified or higher than the number of observations (i.e. rows) in <code>dat</code> , all observations are used for clustering, otherwise only the first <code>N</code> observations.
<code>max.count</code>	An integer specifying the threshold count for filtering data points from above. The default is 10, meaning that if 10 or more data points are larger than or equal to <code>max</code> , they will be excluded from the analysis. If <code>max</code> is <code>NULL</code> , then the maximum value of each parameter will be used. To suppress filtering, it is set to -1.
<code>max</code>	The upper limit set for data filtering. Note that it is a vector of length equal to the number of parameters (columns), implying that a different value can be set for each parameter.
<code>min.count</code>	analoguous to <code>max.count</code> .
<code>min</code>	analoguous to <code>min</code> .

Value

A table with two rows containing the number of events above `max` in each parameter and above in only this parameter. The two last columns give the sum and percentage of all events above `max` in any parameter.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

Examples

```
data(dat.fcs)
removed.above(dat.fcs)
```

<code>cell.SubClustering</code>	<i>immunoClust Model Refinement Step in iterative Cell-events Clustering</i>
---------------------------------	--

Description

These function tests each cell-cluster of a model for refining it into more sub-clusters and returns the refined model parameter in an object of class `immunoClust`.

Usage

```
cell.SubClustering(x, dat, B=50, tol=1e-5, thres=0.1, bias=0.5,
  sample.weights=1, sample.EM="EMt",
  sample.number=1500, sample.standardize=TRUE,
  extract.thres=0.8, modelName="mvt")

cell.TestSubCluster(x, y, t, cluster, J=8, B=500, tol=1e-5, bias=0.5,
  sample.EM="EMt", sample.df=5, sample.number=1500,
  sample.standardize=TRUE, modelName="mvt")
```

Arguments

x	An immunoClust object with the initial model parameter (K, w, μ, σ).
dat	A numeric matrix, data frame of observations, or object of class flowFrame.
B	The maximum number of EM(t)-iterations in Sub-Clustering.
tol	The tolerance used to assess the convergence of the EM(t)-algorithms in Sub-Clustering.
thres	Defines the threshold, below which an ICL-increase is meaningless. The threshold is given as the multiple (or fraction) of the costs for a single cluster.
bias	The ICL-bias used in the EMt-algorithm.
sample.weights	Power of weights applied to hierarchical clustering, where the used weights are the probabilities of cluster membership.
sample.EM	Used EM-algorithm; either "EMt" for EMt-iteration or "EM" for EM-iteration without test step.
sample.number	The number of samples used for initial hierarchical clustering.
sample.standardize	A numeric indicating whether the samples for hierarchical clustering are standardized (mean=0, SD=1).
extract.thres	The threshold used for cluster data extraction.
modelName	Used mixture model; either mvt for a t -mixture model or mvn for a Gaussian Mixture model.
y	A numeric matrix of the observations belonging to the particular cluster.
t	A numeric vector with the probability weights for the observations belonging to the particular cluster.
cluster	An integer index of the particular cluster
J	The number of sub-models to be builded and tested for a particular cluster.
sample.df	Degree of freedom for the t-distributions in a t-mixture model. Has to be 5 in immunoClust.

Details

These function are used internally by the cell-clustering procedures of [cell.process](#) in *immunoClust* and are not intended to be used directly.

Value

The cluster parameters of the refined model in an object of class [immunoClust](#).

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[cell.process](#), [cell.hclust](#)

Examples

```
data(dat.fcs)
data(dat.exp)
dat.trans <- trans.ApplyToData(dat.exp[[1]], dat.fcs)
#need to re-calculate the cluster membership probabilities
# not stored in dat.exp
r1 <- cell.Classify(dat.exp[[1]], dat.trans)
summary(r1)
r2 <- cell.SubClustering(r1, dat.trans)
summary(r2)
```

dat.exp

immunoClust Meta-clustering Sample

Description

A vector of immunoClust-objects with cell.process clustering results of five samples.

Usage

```
data("dat.exp")
```

Details

Cell-event clustering was performed on reduced (10.000 events) sample data of the dataset of *immunoClust*, MACS-depleted populations datasets 2010. URL <http://flowrepository.org/id/FR-FCM-ZZWB>.

Value

A vector of 5 *immnoClust-objects* for the cell clustering results of 5 FC samples.

[[1]] CD19 MACS-depleted cells

[[2]] CD15 MACS-depleted cells

[[3]] CD14 MACS-depleted cells

[[4]] CD4 MACS-depleted cells

[[4]] CD3 MACS-depleted cells

Source

<http://flowrepository.org/id/FR-FCM-ZZWB>

Examples

```
data(dat.exp)

## process meta clustering
meta <- meta.process(dat.exp, meta.bias=0.6)

## extract event counts in the 5 samples for all meta clusters
res <- meta.numEvents(meta)
```

dat.fcs

immunoClust Cell-clustering Sample

Description

flowFrame data sample with 10.000 events in 7 parameters.

Usage

```
data(dat.fcs)
```

Details

This FCS sample is a reduced (10.000 events) dataset in flowFrame format of the first sample in the dataset of immunoClust, MACS-depleted populations datasets 2010. URL <http://flowrepository.org/id/FR-FCM-ZZWB>.

Value

A flowCore flowFrame with 10.000 observations on the following 7 parameters.

FCS-A Forward scatter
SSC-A Sideward scatter
FITC-A CD14
PE-A CD19
APC-A CD15
APC-Cy7-A CD4
Pacific Blue-A CD3

Source

<http://flowrepository.org/id/FR-FCM-ZZWB>

Examples

```
data(dat.fcs)

## process cell clustering
dat.res <- cell.process(dat.fcs)

## apply asinh-transformation
dat.fcs.transformed <- trans.ApplyToData(dat.res, dat.fcs)

## plot results
splom(dat.res, dat.fcs.transformed)
```

dat.meta

immunoClust Meta-clustering Results Sample

Description

The Meta-clustering result of the [dat.exp](#) data set.

Usage

```
data("dat.meta")
```

Details

The Meta-clustering was performed with an ICL-bias of 0.4.

Value

A list-object containing the meta-clustering result. A detailed description is documented in the value section for the [meta.process](#) function.

Source

<http://flowrepository.org/id/FR-FCM-ZZWB>

Examples

```
data(dat.meta)

## extract event counts in the 5 samples for all meta clusters
res <- meta.numEvents(dat.meta)
```

generics.immunoclust *Generic function definitions in immunoClust*

Description

Collection of generic function definitions used in immunoClust either for an immunoClust or an immunoMeta object.

Usage

```
nsam(object, ...)  
sam_ncls(object, ...)  
sam_clsWeights(object, ...)  
sam_clsEvents(object, ...)  
sam_clsMu(object, ...)  
sam_clsSigma(object, ...)  
nobs(object, ...)  
npar(object, ...)  
ncls(object, ...)  
weights(object, ...)  
mu(object, ...)  
sigma(object, ...)  
label(object, ...)  
posterior(object, ...)  
subset(x, ...)  
parameters(object, ...)  
transformParams(object, ...)
```

Arguments

object, x	an object to apply the function.
...	addional options to be passed to methods

Value

The appropriate value for the specific cal (see dection Details).

Details

nsam returns the number of cell-event immunoClust-objects co-clustered in the immunoMeta-object.

sam_clsWeights returns the cluster weights of all samples cell-clusters.

sam_clsEvents returns the cluster event numbers of all samples cell-clusters.

sam_clsMu returns the cluster means of all samples cell-clusters.

sam_clsSigma returns the cluster co-variance matrices of all samples cell-clusters.

nobs already generic in stats. Here, returns the number of clustered objects either cell-events or cell-clusters in cell event or meta clustering.

npar returns the number of parameters used for clustering.

ncls returns the number of cluster, either cell-event cluster or meta-cluster.

weights already generic in stats. Here, returns the weights of the mixture models for the cell-event or meta-clustering.

mu returns the cluster means.

sigma already generic in stats. Here, returns the co-variance matrices of the clusters.

label returns the cluster label, i.e. the assignment of the clustered objects to the clusters.

posterior returns the posterior probabilities of cluster membership for the clustered objects.

events returns the number of cell-events for the clusters.

subset already generic in stats. Here, returns an object with mixture model on a subset of parameters.

parameters already generic in flowCore. Here, lists the parameters used for clustering.

transformParam return an object with transformed mixture model parameters.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

See Also

[immunoClust](#), [immunoMeta](#)

immunoClust-object *immunoClust-Object*

Description

The immunoClust object contains the clustering results in the *immunoClust*-pipeline as obtained by [cell.process](#) or [meta.process](#).

Usage

```
## S4 method for signature 'immunoClust'  
summary(object)  
## S4 method for signature 'immunoClust'  
show(object)
```

Arguments

object An object of class `immunoClust` as returned by the `cell.process` or `meta.process` functions of the `immunoClust`-pipeline.

Value

An object of class `immunoClust` has the following slots:

<code>expName</code>	The name of the clustering experiment.
<code>fcsName</code>	The path of the clustered FCS-file.
<code>parameters</code>	The parameters used for clustering.
<code>removed.below</code>	Number of observations removed from below.
<code>removed.above</code>	Number of observations removed from above.
<code>trans.a</code>	The P -dimensional vector of the scaling factors for the asinh-transformation of each used parameter.
<code>trans.b</code>	The P -dimensional vector of the translations for the asinh-transformation of each used parameter.
<code>trans.decade</code>	experimental; should be -1.
<code>trans.scale</code>	experimental; should be 1.0.
<code>K</code>	The number of clusters.
<code>N</code>	The number of observations.
<code>P</code>	The number of used parameters.
<code>w</code>	The K -dimensional vector of the mixture proportions.
<code>mu</code>	The $K \times P$ -dimensional matrix of the K estimated cluster means.
<code>sigma</code>	The $K \times P \times P$ -dimensional matrix of the K estimated cluster covariance matrices.
<code>z</code>	The $K \times N$ -dimensional matrix containing the posterior probabilities of cluster membership.
<code>label</code>	The N -dimensional vector containing the maximum a posteriori estimator for cluster membership.
<code>logLike</code>	A vector of length 3 containing the BIC, ICL and the classification likelihood without penalty of the fit.
<code>BIC</code>	The Bayesian Information Criterion for the fitted mixture model.
<code>ICL</code>	The Integrate Classification Likelihood for the fitted model.
<code>history</code>	experimental; unused so far.
<code>state</code>	experimental; unused so far.

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

References

Sørensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[cell.process](#), [meta.process](#)

Examples

```
data(dat.exp)
summary(dat.exp[[1]])
```

immunoMeta-class *'immunoMeta': a class for storing meta-clustering results*

Description

The `immunoMeta` object contains the clustering results in the `immunoClust`-pipeline obtained by `meta.process`. Additionally, it offers methods to structure the meta-clusters and build up a hierarchical annotation tree.

Usage

```
immunoMeta(res, dat, gating)

## S3 method for class 'immunoMeta'
summary(object, ...)
## S3 method for class 'immunoMeta'
show(object)
```

Arguments

<code>res</code>	An <code>immunoClust</code> object as a result of the meta-clustering.
<code>dat</code>	The data on which the meta-clustering was performed.
<code>gating</code>	a hierarchical structure annotation of the meta-clusters.
<code>object</code>	An object of class <code>immunoMeta</code> as returned by the <code>meta.process</code> functions of the <code>immunoClust</code> -pipeline.
<code>...</code>	additional options for underlying methods.

Value

An object of class `immunoMeta` has the following slots:

<code>dat.clusters</code>	A dat list-object of the cell event clusters used for meta-clustering.
<code>res.clusters</code>	The <code>immunoClust-object</code> of the fitted meta-clustering mixture model.
<code>dat.scatter</code>	A dat list-object of the scatter parameters for the cell event clusters used for scatter clustering.
<code>res.scatter</code>	The <code>immunoClust-object</code> of the fitted scatter-clustering mixture model.
<code>gating</code>	A list-object containing the hierarchical annotation-tree.

The components of the `dat` list-objects are:

<code>P</code>	The number of parameters for the cell event clusters.
<code>N</code>	The number of cell-clustering experiments.
<code>K</code>	The N -dimensional vector with the numbers of cell event clusters in each experiment. The total number of clusters is $\sum_{i=1}^N K_i$.
<code>W</code>	The $totK$ -dimensional vector with the mixture proportions of all clusters.
<code>M</code>	The $totK \times P$ -dimensional matrix of all cluster means.
<code>S</code>	The $totK \times P \times P$ -dimensional matrix of all cluster covariance matrices.
<code>expNames</code>	The N -dimensional character vector with the cell-clustering experiment names.
<code>expEvents</code>	The N -dimensional vector with the numbers of events in each cell-clustering experiment.
<code>clsEvents</code>	The $totK$ -dimensional vector with the number of events in each cluster.

desc The P -dimensional character vector with the parameter description.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

See Also

[meta.process](#)

Examples

```
data(dat.meta)
summary(dat.meta)
```

meta.clustering	<i>Clustering of Cell-clusters in the immunoClust-pipeline</i>
-----------------	--

Description

This function provides a direct access to the meta-clustering procedure. The method described and discussed in this manuscript is the EMt-classification (EM-method=20) with the number of events for each cluster as weights. It returns the fitted mixture model parameter in an object of class immunoClust.

Usage

```
meta.Clustering(P, N, K, W, M, S, label=NULL, I.iter=10, B=500, tol=1e-5,
               bias=0.25, alpha=0.5, EM.method=20,
               norm.method=0, norm.blur=2, norm.minG=10)
```

Arguments

P	The number of observed parameters for the cell event clusters.
N	The number of cell-clustering experiments.
K	The N -dimensional vector with the numbers of cell event clusters in each experiment. The total number of clusters is $totK = \sum_{i=1}^K K_i$.
W	The $totK$ -dimensional vector with weights of all clusters.
M	The $totK \times P \times P$ -dimensional matrix of all cluster means.
S	The $totK \times P \times P$ -dimensional matrix of all cluster covariance matrices.
label	Optional initial cluster assignment. If label equals NULL all clusters are assigned in one cluster in the initial clustering step.
I.iter	The maximum number of major iteration steps.
B	The $totK \times P \times P$ -dimensional matrix of all cluster covariance matrices.
tol	The tolerance used to assess the convergence of the EM(t)-algorithms.
bias	The ICL-bias used in the EMt-iteration of the meta-clustering.

alpha	A value between 0 and 1 used to balance the bhattacharyya probabilities calculated with either the full covariance matrices or using only the diagonal elements of it. When working with uncompensated FC data very high correlations between parameters may be observed due to spill over. This leads to a very low bhattacharyya probability for two clusters even if they are located nearby. Using a mixture of the probabilities calculated with the complete covariance matrices and the variance information of each parameter avoids this problem. With a value of alpha=1, only the probabilities with complete covariance matrices are applied. A reasonable value for alpha is 0.5.
EM.method	0 = KL-minimization not weighted 1 = BC-maximization not weighted 10 = BC-maximization weighted 2 = EMt-classification not weighted 20 = EMt-classification weighted
norm.method	Normalization function; see meta.Normalize for details.
norm.blur	For the normalization step the A-posterior probabilities of the cell-clusters belonging to a meta-clusters a used. In order to capture narrow cell-clusters reasonable the co-variance of the cell-clusters is blurred for the A-posterior probabilities in the normalization step.
norm.minG	Minimum number of obtained meta-clusters required to process the normalization step in the major iteration loop.

Details

This function is used internally by the meta-clustering procedure [meta.process](#) in *immunoClust*.

Value

The fitted model information in an object of class [immunoClust](#).

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[immunoClust-object](#), [meta.SubClustering](#), [meta.process](#)

Examples

```
data(dat.exp)
d <- meta.exprs(dat.exp)
res <- meta.Clustering(d$P, d$N, d$K, d$clsEvents, d$M, d$S)
```

meta.export	<i>immunoClust Meta-clustering Results Export</i>
-------------	---

Description

These functions collect the output of the [meta.process](#) and extract the event numbers, relative frequencies or mean fluorescence intensities for each meta-cluster and cell-clustering experiment in a numeric table.

Usage

```
meta.numEvents(meta, out.all=TRUE, out.removed=FALSE, out.unclassified=TRUE)
meta.relEvents(meta, out.all=TRUE)
meta.relParent(meta, out.all=TRUE, out.removed=FALSE, out.unclassified=TRUE)

meta.parMFI(meta, par, out.all=TRUE)

meta.numClusters(meta, out.all=TRUE)

meta.freqTable(meta)
```

Arguments

meta	The list-object returned by the function <code>meta.process</code> .
par	An integer index to the specific parameter.
out.all	A numeric indicator whether the event numbers of all hierarchical gating levels are obtained or only the meta-clusters themselves.
out.removed	A numeric indicator whether the number of removed events, which are not used for clustering are exported.
out.unclassified	A numeric indicator whether the event numbers of the hierarchical gating levels or all meta-clusters are exported.

Value

A numeric matrix with

numEvents the number of cell events

relEvents relative frequencies, i.e. the number of cell events per total measured events

relParent relative frequencies according to parent relationship in the annotated hierarchy.

parMFI mean fluorescence intensities in one parameter, i.e. the meta-cluster centers in asinh-transformed scale

numClusters the number of cell clusters

freqTable relative frequencies with respect to all gating hierarchy levels

in each meta-cluster (and gating hierarchy level) for each cell-clustering experiment.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (submitted).

See Also

[meta.process](#)

Examples

```
data(dat.exp)
meta <- meta.process(dat.exp)
tbl <- meta.numEvents(meta)
```

meta.exprs

Collecting Data of an immunoClust vector

Description

The function takes a vector of immunoClust-object obtained by the cell.process function and extracts this information into a list object.

Usage

```
meta.exprs(exp, sub=c())
```

Arguments

exp	The vector of immunoClust object with the cell clustering results.
sub	A integer array indicating the parameter subset to be collected.

Value

A list object with the following slots:

P	The number of observed parameters for the cell event clusters.
N	The number of cell-clustering samples.
K	The N -dimensional vector with the numbers of cell event clusters in each experiment. The total number of clusters is $totK = \sum_{i=1}^N K_i$.
W	The $totK$ -dimensional vector with weights of all clusters.
M	The $totK \times P$ -dimensional matrix of all cluster means.
S	The $totK \times P \times P$ -dimensional matrix of all cluster covariance matrices.
expNames	The N -dimensional vector with the experiment names of the cell clustering samples.

expEvents	The N -dimensional vector for the total number of events of the cell clustering samples.
clsEvents	The $totK$ -dimensional vector for the event number of all clusters.
desc	The P -dimensional vector for the parameter description.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[immunoClust](#).

Examples

```
data(dat.exp)
d <- meta.exprs(dat.exp, sub=c(1,2))
```

meta.hclust	<i>Hierarchical Meta-clustering of Cell-clusters in the immunoClust-pipeline</i>
-------------	--

Description

Performs agglomerative clustering on cell-clusters. It is used in the iterative meta-clustering approach of *immunoClust* to obtain an initial meta-cluster membership for the EM(t)-iteration.

Usage

```
meta.hclust(P, N, W, M, S)
```

Arguments

P	The number of parameters.
N	The number of clusters.
W	The N -dimensional vector with cluster weights, i.e. numbers of events in a cluster.
M	The $N \times P$ -dimensional vector with cluster means.
S	The $N \times P \times P$ -dimensional vector with cluster covariance matrices.

Details

This function is used internally in [meta.TestSubCluster](#) of *immunoClust*.

Value

A numeric $(N - 1) \times 2$ -dimensional matrix which gives the minimum index for observations in each of the two clusters merged at the i th step in each row.

Note

The merging distances need not to be monotonic increasing.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[meta.TestSubCluster](#), [meta.process](#)

Examples

```
data(dat.exp)
r <- dat.exp[[1]]
#hcPairs <- meta.hclust(r@P, r@K, r@W, r@M, t(apply(r@sigma,1,c)))
hcPairs <- meta.hclust(npar(r), ncls(r), weights(r),
  mu(r), t(apply(sigma(r),1,c)))
```

meta.ME

immunoClust EM(t)-iteration on Cell-clusters

Description

Performs an EM(t)-iteration on cell-clusters given an initial meta-cluster membership for the cell-clusters and returns the fitted meta-clusters information in an object of class `immunoClust`.

Usage

```
meta.ME(P, N, K, W, M, S, label, B=100, tol=1e-5, method=20, bias=0.25,
  alpha=0.5, min.class=0)
```

Arguments

P	The number of observed parameters for the cell event clusters.
N	The number of cell-clustering experiments.
K	The N -dimensional vector with the numbers of cell event clusters in each experiment. The total number of clusters is $totK = \sum_{i=1}^K K_i$.
W	The $totK$ -dimensional vector with weights, i.e. number of events, of all clusters.
M	The $totK \times P$ -dimensional matrix of all cluster means.

S	The $totK \times P \times P$ -dimensional matrix of all cluster covariance matrices.
label	The $totK$ -dimension integer vector with the initial cell-cluster to meta-cluster membership.
B	The $totK \times P \times P$ -dimensional matrix of all cluster covariance matrices.
tol	The tolerance used to assess the convergence of the EM(t)-algorithms.
method	0 = KL-minimization not weighted 1 = BC-maximization not weighted 10 = BC-maximization weighted 2 = EMt-classification not weighted 20 = EMt-classification weighted
bias	The ICL-bias used in the EMt-iteration of the meta-clustering.
alpha	A value between 0 and 1 used to balance the bhattacharyya probabilities calculated with either the full covariance matrices or using only the diagonal elements of it.
min.class	The minimum number of clusters for the final model.

Details

This function is used internally by the meta-clustering procedures [meta.process](#) and [meta.Clustering](#) in `immunoClust`.

Value

The fitted meta-clusters information in an object of class `immunoClust`.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[meta.process](#), [meta.Clustering](#)

Examples

```
data(dat.exp)
d <- meta.exprs(dat.exp)
r <- meta.ME(d$P, d$N, d$K, d$clsEvents, d$M, d$S, label=rep(1,sum(d$K)))
```

meta.normalize	<i>immunoClust normalization step with the meta.clustering process</i>
----------------	--

Description

Performs a normalization via linear regression of the cell-cluster samples to the meta-clustering model.

Usage

```
meta.Normalize(P, N, K, W, M, S, G, Z, method=3)
```

Arguments

P	The number of observed parameters for the cell event clusters.
N	The number of cell-clustering experiments.
K	The N -dimensional vector with the numbers of cell event clusters in each experiment. The total number of clusters is $totK = \sum_{i=1}^K K_i$.
W	The $totK$ -dimensional vector with weights, i.e. number of events, of all clusters.
M	The $totK \times P$ -dimensional matrix of all cluster means.
S	The $totK \times P \times P$ -dimensional matrix of all cluster covariance matrices.
G	The number of meta-clusters.
Z	The $totK \times G$ -dimensional matrix with the A-Posterior probabilities for a cell-cluster belonging to a meta-cluster.
method	Alternative methods used for the normalization routine. Let Y denote the consensus meta-model build from all cell-event clusters of all experiments using the A-posterior Z and X the cell-event clusters in each experiment. 0 = no normalization 1 = $Y = a \times X$ 2 = $Y = a \times X + b$ 3 = $X = a \times Y$ 4 = $X = a \times Y + b$

Details

The regression used the cell-cluster and meta-cluster means weighted by the probabilities for a cell-cluster belonging to the meta-cluster. It builds a consensus meta-model from all cell-clusters using the A-posterior probabilities Z .

Value

Returns the normalized cell-clusters means and co-variance matrices in a list-object with the following slots:

P The number of observed parameters for the cell event clusters.

N The number of cell-clustering experiments.

K The N -dimensional vector with the numbers of cell event clusters in each experiment. The total number of clusters is $totK = \sum_{i=1}^K K_i$.

W The $totK$ -dimensional vector with weights, i.e. number of events, of all clusters.

M The $totK \times P$ -dimensional matrix of all cluster means.

S The $totK \times P \times P$ -dimensional matrix of all cluster covariance matrices.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

See Also

[meta.process](#), [meta.Clustering](#)

Examples

```
data(dat.meta)
#dat <- dat.meta$dat.clusters
res <- dat.meta$res.clusters
dat.norm <- meta.Normalize(npar(dat.meta), nsam(dat.meta),
  sam_ncls(dat.meta), sam_clsEvents(dat.meta), sam_clsMu(dat.meta),
  sam_clsSigma(dat.meta), ncls(res), posterior(res))
```

meta.process

Meta-clustering of Cell-clusters in the immunoClust-pipeline

Description

This function performs iterative model based clustering on the clusters obtained by [cell.process](#) of several samples. Its input is a vector of the immunoClust-objects of the samples.

Usage

```
meta.process(exp, dat.subset=c(), meta.iter=10, tol=1e-05, meta.bias=0.2,
  meta.alpha=.5, norm.method=0, norm.blur=2, norm.minG=10)
```

Arguments

exp	A vector of list objects, each list contains the cell-clustering result of a sample in the res field. Addition fields are name and fsc containing the cell-sample name and fcs-filename, which are used for data output and plot routines.
dat.subset	A numeric vector defining the used observed parameters for the meta-clustering. If unset, all parameters in the cell-clustering results are used.
meta.iter	The number of major iterations.
tol	The tolerance used to assess the convergence of the EM(t)-algorithms.
meta.bias	The ICL-bias used in the EMt-iteration of the meta-clustering.
meta.alpha	A value between 0 and 1 used to balance the bhattacharrya probabilities calculated with either the full covariance matrices or using only the diagonal elements of it. When working with uncompensated FC data, very high correlations between parameters may be observed due to spill over. This leads to a very low bhattacharrya probability for two clusters even if they are located nearby. Using a mixture of the probabilities calculated with the complete covariance matrices

and the variance information of each parameter avoids this problem. With a value of $\alpha=1$, only the probabilities with complete covariance matrices are applied. A reasonable value for α is 0.5.

norm.method	A numeric selector for the normalization step to be performed during the major iteration.
norm.blur	The blurring constant by which the cell-clusters co-variance matrices are increased within the normalization step.
norm.minG	Minimum number of meta-clusters required before processing the normalization step.

Value

The function returns a `immunoMeta` with the following components:

dat.clusters	A dat list-object of the cell event clusters used for meta-clustering.
res.clusters	The <code>immunoClust-object</code> of the fitted meta-clustering mixture model.
dat.scatter	A dat list-object of the scatter parameters for the cell event clusters used for scatter clustering.
res.scatter	The <code>immunoClust-object</code> of the fitted scatter-clustering mixture model.
gating	A list-object containing the hierarchical gating-tree.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

`immunoMeta-object`, `immunoClust-object`, `meta.Clustering`, `meta.export`, `cell.process`

Examples

```
data(dat.exp)
meta <- meta.process(dat.exp)
summary(meta)
tbl <- meta.numEvents(meta)
```

meta.regnorm

immunoClust normalization procedure

Description

Performs a normalization via linear regression of the sample clusters in x to the clusters in y .

Usage

```
meta.RegNorm(y, x, method=1, alpha=0.5)
```


Arguments

y	immunoClust-object with the destination clusters.
x	immunoClust-object with the cluster to normalize.
method	Alternative methods used for the normalization routine. $1 = X = a \times Y$ $2 = X = a \times Y + b$
alpha	A value between 0 and 1 used to balance the bhattacharya probabilities calculated with either the full covariance matrices or using only the diagonal elements of it.

Value

Returns the normalized cell-clusters means and co-variance matrices in a list-object with the following slots:

P The number of observed parameters for the cell event clusters.

N The number of cell-clustering experiments.

K The N -dimensional vector with the numbers of cell event clusters in each experiment. The total number of clusters is $totK = \sum_{i=1}^K K_i$.

M The $totK \times P$ -dimensional matrix of all cluster means.

S The $totK \times P \times P$ -dimensional matrix of all cluster covariance matrices.

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

Examples

```
data(dat.meta)
data(dat.exp)
dat.norm <- meta.RegNorm(dat.meta$res.clusters, dat.exp[[1]])
```

meta.SubClustering *immunoClust Model Refinement Step in iterative Meta-clustering*

Description

These function tests each meta-cluster of a model for refining it into more sub-clusters and returns the refined cluster memberships in an integer array.s

Usage

```
meta.SubClustering(P, N, W, M, S, label, tol=1e-5, bias=0.25, alpha=1.0,
  EM.method=20)
```

```
meta.TestSubCluster(P, N, W, M, S, J=8, B=500, tol=1e-5, bias=0.5, alpha=1.0,
  EM.method=2, HC.samples=2000)
```

Arguments

P	The number of parameters.
N	The number of clusters.
W	The N -dimensional vector with cluster weights, i.e. numbers of events in a cluster.
M	The $N \times P$ -dimensional vector with cluster means.
S	The $N \times P \times P$ -dimensional vector with the cluster covariance matrices.
label	The N -dimension integer vector with the cell-cluster to meta-cluster membership.
tol	The tolerance used to assess the convergence of the EM(t)-algorithms in Sub-Clustering.
bias	he ICL-bias used in the EMt-algorithm.
alpha	A value between 0 and 1 used to balance the bhattacharrya probabilities calculated with either the full covariance matrices or using only the diagonal elements of it.
J	The number of sub-models to be builded and tested for a particular cluster.
B	The maximum number of EM(t)-iterations in Sub-Clustering.
EM.method	0 = KL-minimization not weighted 1 = BC-maximization not weighted 10 = BC-maximization weighted 2 = EMt-classification not weighted 20 = EMt-classification weighted
HC.samples	The number of samples used for initial hierarchical clustering.

Details

These function are used internally by the meta-clustering procedures [meta.process](#) and [meta.Clustering](#) in *immunoClust* and are not intended to be used directly.

Value

An integer array of length N containing the cell-clusters meta-cluster memberships of the refined model.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[meta.process](#), [meta.Clustering](#), [meta.hclust](#)

Examples

```

data(dat.exp)
d <- meta.exprs(dat.exp)
label <- rep(1,sum(d$K))
label <- meta.SubClustering(d$P, sum(d$K), d$clsEvents, d$M, d$S, label=label)
## Not run:
r1 <- meta.ME(d$P, d$N, d$K, d$clsEvents, d

## End(Not run)

```

methods.immunoClust *Accessors and Methods for immunoClust Objects*

Description

Documentation of the accessors and methods for immunoClust-objects

Arguments

object, immunoClust an object of class `immunoClust` as return by `cell.process`.

cls cluster subset for retrieved slot values.

par parameter subset for retrieved slot values.

Accessors

nobs the number of cell events clustered

Usage:

nobs(immunoClust)

ncls the number of clusters.

Usage:

ncls(immunoClust)

npar the number of parameters measured, cell-clustered

Usage:

npar(immunoClust)

parameters, **parameters<-** extracts or replaces the names of measured, cell-clustered parameters

Usage:

parameters(immunoClust)

parameters(immunoClust) <-value

label the clustering label, that is the assignment of the cell-events to the clusters.

Usage:

label(immunoClust)

weights the clustering weights for the cluster selection (all cluster by default)

Usage:

weights(immunoClust,cls=seq_len(ncls(immunoClust)))

mu the cluster mean values for the cluster and parameter selection (all cluster and all parameter by default)

Usage:

```
mu(immunoClust,cls=seq_len(ncls(immunoClust)),par=seq_len(npar(immunoClust)))
```

sigma the cluster co-variance values for the cluster and parameter selection (all cluster and all parameter by default)

Usage:

```
sigma(immunoClust,cls=seq_len(ncls(immunoClust)),par=seq_len(npar(immunoClust)))
```

posterior the posterior probabilities of cluster membership for each event

Usage:

```
posterior(immunoClust)
```

events the cell-event numbers for the cluster selection (all cluster by default)

Usage:

```
events(immunoClust,ncls=seq_len(ncls(immunoClust)))
```

Methods

subset Builds the immunoClust-object for a parameter subset

Usage:

```
res <-subset(immunoClust,par)
```

transformParams Scales and translates the cluster means of the immunoClust-object in each parameter

Usage:

```
res <-transformParams(immunoClust,scale=c(),offset=c())
```

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

See Also

[immunoClust-object](#)

Examples

```
###
data(dat.exp)
## cell.clustering result for dat.fcs
res <- dat.exp[[1]]
nobs(res)
ncls(res)
```

Description

Documentation of the accessors and methods for immunoMeta-objects

Arguments

object, immunoMeta
 an object of class `immunoMeta` as return by `meta.process`.

cls
 cluster subset for retrieved slot values.

par
 parameter subset for retrieved slot values.

pos
 Gives the position in the immunoMeta-hierarchy. `pos` is an array of indices which addresses the level of interest. Each level in the immunoMeta-hierarchy consists of a name (`desc`), meta-cluster subset (array of cluster indices) and a vector of sub-levels. `pos` is the sequence of indices into these sub-levels beginning at root level.

Accessors

nsam the number of immunoClust-objects (samples) which are co-clustered.

Usage:

```
nsam(immunoMeta)
```

sam_ncls the number of cell event clusters in the immunoClust-objects (samples) which are co-clustered.

Usage:

```
sam_ncls(immunoMeta, for.samples=seq_len(nsam(meta)))
```

sam_clsWeights the weights of all cell event clusters which are collected for co-clustering.

Usage:

```
sam_clsWeights(immunoMeta)
```

sam_clsMu the means of all cell event clusters which are collected for co-clustering.

Usage:

```
sam_clsMu(immunoMeta)
```

sam_clsSigma the co-variance matrices of all cell event clusters which are collected for co-clustering.

Usage:

```
sam_clsSigma(immunoMeta)
```

sam_clsEvents the event numbers of all cell event clusters which are collected for co-clustering.

Usage:

```
sam_clsEvents(immunoMeta)
```

nobj the number of cell events clusters from sample cell-clustering which are co-clustered.

Usage:

```
nobj(immunoMeta)
```

ncls the number of meta-clusters.

Usage:

```
ncls(immunoMeta)
```

npar the number of parameters measured, cell-clustered and meta-clustered

Usage:

```
npar(immunoMeta)
```

parameters, parameters<- extracts or replaces the names of measured, cell-clustered and meta-clustered parameters

Usage:

```
parameters(immunoMeta)
parameters(immunoMeta) <-value
```

label the meta-clustering label, that is the assignment of the cell-clusters to the meta-clusters.

Usage:

```
label(immunoMeta, for.sample=NA)
```

If for.sample is specified, the label part for this sample only.

weights the meta-clustering weights for the cluster selection (all meta-cluster by default)

Usage:

```
weights(immunoMets, cls=seq_len(ncls(immunoMeta)))
```

mu the meta-cluster mean values for the cluster and parameter selection (all meta-cluster and all parameter by default)

Usage:

```
mu(immunoMeta, cls=seq_len(ncls(immunoMeta)), par=seq_len(npar(immunoMeta)))
```

sigma the meta-cluster co-variance values for the cluster and parameter selection (all meta-cluster and all parameter by default)

Usage:

```
sigma(immunoMeta, cls=seq_len(ncls(immunoMeta)), par=seq_len(npar(immunoMeta)))
```

posterior the posterior probabilities of cluster membership for each cell-cluster

Usage:

```
posterior(immunoMeta)
```

events the cell-event numbers for each sample for the cluster selection (all meta-cluster by default)

Usage:

```
events(immunoMeta, ncls=seq_len(ncls(immunoMeta)))
```

prop, prop<- get or a property value in the hierarchy level given by pos and named name

Usage:

```
prop(immunoMeta, name, pos=c())
```

```
prop(immunoMeta, name, pos, for.level=TRUE, for.sublevels=FALSE) <-value
```

If the option for.sublevels is set, the property value will be setted deep for all sub-levels of the by pos specified level.

The prop interface is very basic and no checks for meaningful properties and values are performed. It could be used for everything at any time. Nevertheless, there are some property keys which are used internally mainly to control the plot routine for the levels.

desc the name of this level.

M the mean of all clusters in this level

S the co-variance matrix of all clusters in this level

pscales a list of npar entries for the limits and ticks information. Normally, only set on root-level and then used for all sub-levels. But could set and altered at any level.

plot.subset an array of parameter indices used as default for the plot this level.

plot.color an index in the palette or other specified color used for plots of this level in its parent level.

plot.childs to be renamed in plot.levels.

plot.parent when set, additionally all cluster of the parent level are plotted in light gray.

desc, desc<- Get or set the desc property in the by pos specified level.

Usage:

```
desc(immunoMeta, pos)
desc(immunoMeta, pos) <-value
```

descFull Gives the full description path for the level given by pos, i.e. the concatenate desc values of this all parent levels.

Usage:

```
descFull(immunoMeta, pos)
```

level, level<- Get or replace the level object at specified pos,

Usage:

```
value <-level(immunoMeta, pos)
level(immunoMeta, pos) <-value
```

findLevel Find the level pos value for a specific cluster cls

Usage:

```
pos <-findLevel(immunoMeta, cls)
```

clusters Retrieves the cluster subset for the level at pos.

Usage:

```
cls <-clusters(immunoMeta, pos)
```

classified Retrieves the cluster subset for the level at pos which are classified in sub-levels.

Usage:

```
cls <-classified(immunoMeta, pos)
```

unclassified Retrieves the cluster subset for the level at pos which are not classified in sub-levels.

Usage:

```
cls <-unclassified(immunoMeta, pos)
```

Manipulators

addLevel<- Adds a level at a specified hierarchy position pos. A level consists of a name (desc) and a cluster subset cls.

Usage:

```
addLevel(immunoMeta, pos, desc="new level") <-cls
```

move<- Moves a cluster subset to a specific immunoMeta level. Clusters in cls are added to parent levels if necessary and removed from other levels.

Usage:

```
move(immunoMeta, pos) <-cls
```

remove<- removes a cluster subset from a specific immunoMeta level.

Usage:

```
remove(immunoMeta, pos) <-cls
```

parent<- sets the parent for this level, or this level as parent for all its sub-levels

Usage:

```
parent(immunoMeta, pos) <-c()
parent(immunoMeta, pos) <-level
```

transfer<- Overtakes the annotation of an immunoMeta-object to this immunoMeta-object

Usage:

```
transfer(immunoMeta) <-annotatedMeta
```

Methods

finalize After manipulations of a immunoMeta-object finalize restructure all levels and returns the finalized object, where the parent relations are solved and the mean and co-variances of all levels are build.

Usage:

```
immunoMeta <-finalize(immunoMeta)
```

subset Builds the immunoMeta-object for a cluster and/or parameter subset

Usage:

```
subsetMeta <-subset(immunoMeta,cls=seq_len(ncls(meta)),par=seq_len(npar(meta)))
```

transformParams Scales and translates the cluster means of the immunoMeta-object in each parameter

Usage:

```
transformedMeta <-transformParams(immunoMeta,scale=c(),offset=c())
```

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

See Also

[immunoMeta-object](#)

Examples

```
###
data(dat.meta)
npar(dat.meta)
ncls(dat.meta)
cls <- clusters(dat.meta,c(1))
move(dat.meta,c(2)) <- cls
```

plot.immunoClust

Scatterplot of immunoClust Clustering Results

Description

This method generates scatterplot revealing the cluster assignment.

Usage

```
## S4 method for signature 'immunoClust'
plot(x, data, subset=c(1,2), ellipse=T,
show.rm=F, include=1:(x@K), main=NULL,
col=include+1, pch=".", cex=0.6,
col.rm=1, pch.rm=1, cex.rm=0.6, ecol=col, elty=1,
npoints=501, add=F, ...)
```


Arguments

x	An object of class <code>immunoClust</code> as return by <code>cell.process</code> .
data	A matrix, data frame of observations, or object of class <code>flowFrame</code> . This is the object of observations on which <code>cell.process</code> was performed or the matrix of cell-cluster centers for the <code>meta.process</code> .
subset	A numeric vector of length two indicating which two parameters are selected for the scatterplot. Alternatively, a character vector containing the names of the two parameters is allowed if <code>x@parameters</code> is not <code>NULL</code> .
ellipse	A logical value indicating whether the cluster 90% percentil boundary is to be drawn or not.
show.rm	A logical value indicating whether filtered observations will be shown or not.
include	A numeric vector specifying which clusters will be shown on the plot. By default, all clusters are included.
main	Title of the plot.
col	Color(s) of the plotting points. May specify a different color for each cluster.
pch	Plotting character(s) of the plotting points. May specify a different character for each cluster.
cex	Size of the plotting characters. May specify a different size for each cluster.
col.rm	Color of the plotting characters denoting filtered observations.
pch.rm	Plotting character used to denote filtered observations.
cex.rm	Size of the plotting character used to denote filtered observations.
ecol	Color(s) of the lines representing the cluster boundaries. May specify a different color for each cluster.
elty	Line type(s) drawing the cluster boundaries. May specify a different line type for each cluster.
npoints	The number of points used to draw each cluster boundary.
add	A logical value. If <code>TRUE</code> , add to the current plot.
...	Further graphical parameters passed to the generic function <code>plot</code> .

Value

Plots the clustering assignment on an appropriate plotting device.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[immunoClust-object](#)

Examples

```

data(dat.fcs)
data(dat.exp)
dat.res <- dat.exp[[1]]
dat.trans <- trans.ApplyToData(dat.res, dat.fcs)
plot(dat.res, dat=dat.trans)

```

plot.immunoMeta *Scatterplot of immunoMeta Structured Clustering Results*

Description

This method generates scatterplot revealing the cluster assignment.

Usage

```

## S3 method for class 'immunoMeta'
plot(x, pos=c(), main="", plot.childs=TRUE,
plot.unclassified=FALSE, plot.subset=c(), inc.childs=c(), plot.ellipse=TRUE,
plot.all=FALSE, ...)

```

Arguments

x	An object of class <code>immunoMeta</code> as return by <code>meta.process</code> .
pos	gives the position in the immunoMeta-hierarchy to plot (default=c() plots the root level). pos is an array of indices, which addresses the level of interest. Each level in the immunoMeta-hierarchy has an array of sub-levels and pos is the sequences of indices into these sub-levels.
main	additional title which is concatenated with the position and description path of the plotted level.
plot.subset	an array of indices for the parameter selection to be plotted.
plot.unclassified	if set, the unclassified clusters, i.e. clusters not assigned into a sub-level, are plotted rather than the classified clusters.
plot.childs	colours the clusters by the sub-level rather than the clusters themselves. By default colours are assigned by sub-level index repeated in red, green, blue, cyan, magenta, yellow, gray, black.
inc.childs	optionally, to restrict to a particular selection of sub-levels to plot.
plot.ellipse	surrounds the cell-cluster center by an ellipse reflecting the meta-cluster deviation
plot.all	plots all sub-levels. Usefull for a full annotation documentation with a pdf file.
...	Further graphical parameters passed to the generic function plot.

Value

Plots the clustering assignment on an appropriated plotting device.

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

See Also

[immunoMeta-object](#)

Examples

```
data(dat.meta)
plot(dat.meta)
```

splom.immunoClust	<i>Scatterplot Matrix of immunoClust Clustering Results</i>
-------------------	---

Description

This method generates scatterplot matrix revealing the cluster assignment.

Usage

```
## S4 method for signature 'immunoClust,missing'
splom(x, data, include=seq_len(x@K), ...)

## S4 method for signature 'immunoClust,flowFrame'
splom(x, data, include=seq_len(x@K),
      subset=seq_len(length(attributes(x)$param)), N=NULL, label=NULL, desc=NULL,
      add.param=c(), ...)

## S4 method for signature 'immunoClust,matrix'
splom(x, data, include=seq_len(x@K),
      subset=seq_len(length(attributes(x)$param)), N=NULL, label=NULL,
      desc=NULL, ...)

datSplom(label, data, subset=seq_len(ncol(data)),
          include=seq_len(nrow(data)), ...)
```

Arguments

x	An object of class <code>immunoClust</code> as return by <code>cell.process</code> or <code>meta.process</code> .
data	Missing, a matrix, or object of class <code>flowFrame</code> . This is the object of observations on which <code>cell.process</code> was performed.
include	A numeric vector specifying which clusters will be shown on the plot. By default, all clusters are included.
subset	A numeric vector indicating which parameters are selected for the scatterplot matrix.
N	An integer for the maximum number of observations to be plotted. By default all observations are plotted.
label	A integer vector for the cluster mebership of the observations. By default this is <code>x@label</code> .

desc	A character vector for the parameter description.
add.param	A list of additional parameters to plot, which are not used for clustering.
...	Further graphical parameters passed to the generic function splom.

Value

An object of class `trellis` as returned by the generic `splom` function of the `lattice`-package. The `print` method (called by default) will plot it on an appropriate plotting device.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. `immunoClust` - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[immunoClust-object](#)

Examples

```
data(dat.fcs)
data(dat.exp)
# cell clustering results of dat.fcs
dat.res <- dat.exp[[1]]
dat.trans <- trans.ApplyToData(dat.res, dat.fcs)
splom(dat.res, data=dat.trans, N=1000)
```

trans.ApplyToData *immunoClust asinh-Transformation*

Description

Applies the transformation information of the `immunoClust` object to the raw observed FC dataset.

Usage

```
trans.ApplyToData(x, data, add.param=c(), max.decade=attr(x,"trans.decade"),
  lin.scale=attr(x,"trans.scale") )
```

Arguments

x	The <code>immunoClust</code> object containing the estimators for the transformation <code>trans.a</code> and <code>trans.b</code> .
data	The numeric matrix, data frame of observations, or object of class <code>flowFrame</code> .
add.param	A list of additional parameters in the <code>flowFrame</code> , which are not used for clustering but should be included in the final transformed resulting <code>flowFrame</code> .

max.decade	A numeric scale for the maximum transformed observation value; if missing or below 0, no scaling of the transformed values is applied, which is the default in <i>immunoClust</i> .
lin.scale	A numeric scaling factor for the linear, i.e. not transformed, parameters; if missing no scaling, i.e. <i>lin.scale</i> = 1, is applied, which is the default in <i>immunoClust</i> .

Details

In *immunoClust* an *asinh*-transformation $h(y) = \text{asinh}(a \cdot y + b)$ is applied to the fluorescence parameter in the observed data. The scatter parameter are assumed to be linear.

Value

A matrix or flowFrame with replaced transformed observation values.

Author(s)

Till Sörensen <till-antoni.soerensen@charite.de>

References

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. *immunoClust* - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[immunoClust](#), [trans.FitToData](#), [cell.process](#)

Examples

```
data(dat.fcs)
data(dat.exp)
dat.trans <- trans.ApplyToData(dat.exp[[1]], dat.fcs)
#
#plot(dat.exp[[1]], data=dat.trans)
#
```

trans.FitToData

immunoClust asinh-Transformation Optimization

Description

Performs variance stabilization transformation estimation on the fluorescence parameters of the observed cell events. It is integrated in the iterative cell event clustering approach of *immunoClust* when transformation estimation should be applied.

Usage

```
trans.FitToData(x, data, B=10, tol=1e-5, certainty=0.3, proc="vsHtransAw")
```

Arguments

x	The immunoClust object of the fitted mixture model and initial estimators for the transformation.
data	The numeric matrix, data frame of observations, or object of class flowFrame.
B	The maximum number of BFG2 minimizer iterations.
tol	The tolerance used to assess the convergence for the BFG2 minimizer.
certainty	Minimum probability for cluster membership of an observation to be taken into account.
proc	An experimental switch for alternative procedures; should be "vsHtransAw".

Details

In *immunoClust* an *asinh*-transformation $h(y) = \text{asinh}(a \cdot y + b)$ is applied for all fluorescence parameter in the observed data.

The transformation optimization `trans.FitToData` requires a fitted model of cluster information together with suitable initial transformation estimation in an `immunoClust` object. It fits the transformation based on the initial scaling values `trans.a` and translation values `trans.b` to the observed data. It returns the optimized transformation parameter in a $2 \times P$ -dimensional matrix, first row for the scaling and second row for the translation values. A scaling value of $a = 0$ on input and output indicates, that a parameter should not be transformed.

The presented transformation optimization ("vsHtransAw") fits only the scaling value. An alternative procedure ("vsHtrans_w") fits both, the scaling and the translation value, but turns out to be less robust.

Value

Optimized transformation scaling and translation values in a $2 \times P$ -dimensional matrix, first row for the scaling and second row for the translation values.

Author(s)

Till Sørensen <till-antoni.soerensen@charite.de>

References

Sørensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T. immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets. *Cytometry A* (accepted).

See Also

[trans.ApplyToData](#), [cell.process](#)

Examples

```
data(dat.fcs)
data(dat.exp)
## in dat.exp the z-matrices of the immunoClust-object are removed
## so we have to re-calculate it first ...
dat.trans <- trans.ApplyToData(dat.exp[[1]], dat.fcs)
res <- cell.Classify(dat.exp[[1]], dat.trans)
## ... now the transformation parameter can be optimized
trans.FitToData(res, dat.fcs)
```

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