Package 'SamSPECTRAL'

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Type Package

Title Identifies cell population in flow cytometry data.

Version 1.30.0												
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Author Habil Zare and Parisa Shooshtari												
Maintainer Habil Zare <zare@txstate.edu></zare@txstate.edu>												
Depends R (>= 3.3.3)												
Imports methods												
Description Samples large data such that spectral clustering is possible while preserving density information in edge weights. More specifically, given a matrix of coordinates as input, SamSPECTRAL first builds the communities to sample the data points. Then, it builds a graph and after weighting the edges by conductance computation, the graph is passed to a classic spectral clustering algorithm to find the spectral clusters. The last stage of SamSPECTRAL is to combine the spectral clusters. The resulting ``connected components" estimate biological cell populations in the data sample. For instructions on manual installation, refer to the PDF file provided in the following documentation. License GPL (>= 2)												
LazyLoad yes												
biocViews FlowCytometry, CellBiology, Clustering, Cancer, FlowCytometry, StemCells, HIV												
NeedsCompilation yes												
R topics documented:												
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Description

Using a faithful sampling procedure, SamSPECTRAL reduces the size of data points such that applying spectral clustering algorithm on large data such as flow cytometry is possible. Before running the spectral clustering algorithm, it uses potential theory to define similarity between sampled points.

Details

Package: SamSPECTRAL

Type: Package Version: 1.0

Date: 2009-08-31 License: GPL-2 LazyLoad: yes

The main function is SamSPECTRAL. It can be loaded using the command library(SamSPECTRAL) in R. Some parameters should be set properly including: dimensions, normal.sigma and separation.factor. These parameters can be adjusted for a data set by running the algorithm on some samples of that data set. (Normally, 2 or 3 samples are sufficient). Then the function SamSPECTRAL() can be applied to all samples in the data set to identify cell populations in each sample data.

Author(s)

Habil Zare and Parisa Shooshtari

Maintainer: Habil Zare <hzare@bccrc.ca>

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

See Also

 ${\tt SamSPECTRAL, Building_Communities, Conductance_Calculation, Civilized_Spectral_Clustering, Connecting}$

Examples

```
## Not run:
    library(SamSPECTRAL)

# Reading data file which has been transformed using log transform
    data(small_data)

full <- small

L <- SamSPECTRAL(data.points=full,dimensions=c(1,2,3), normal.sigma = 200, separation.factor = 0.39)

plot(full, pch='.', col= L)

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

Building_Communities Builds the communities from the set of all data points.

Description

Some sample points are picked up and the points close to each sample point are considered as members of that community.

Usage

```
Building_Communities(full, m=3000, space.length=1, community.weakness.threshold=1, talk=TRUE, do.
replace.inf.with.extremum=TRUE)
```

Arguments

full The matrix containing the coordinates of all data points. An integer determining upper and lower bounds on the final number of sample m points which will be in range .95*m/2 and 21.1*m space.length An estimate for the length of a cube that is assumed to contain all data points. community.weakness.threshold The communities with number of members less than this threshold will be ignored. Normally, setting it to 1 is reasonable. talk A boolean flag with default value TRUE. Setting it to FALSE will keep running the procedure quite with no messages. A boolean flag with default value TRUE. If set to FALSE, the sampling stage do.sampling will be ignored by picking up all the data points. replace.inf.with.extremum If TRUE, the Inf and -Inf values will be replaced by maximum and minimum of

Value

Returns a society which is a list of communities.

data in each direction.

Author(s)

Habil Zare and Parisa Shooshtari

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

See Also

```
SamSPECTRAL, check. SamSPECTRAL.input
```

Examples

```
## Not run:
    library(SamSPECTRAL)

# Reading data file which has been transformed using log transform
    data(small_data)

full <- small

# Parameters:
    m <- 3000; ns <- 200; sl <- 3; cwt <-1

        # Sample the data and build the communities
        society <- Building_Communities(full=full,m=m, space.length=sl, community.weakness.threshold=cwt)

# Ploting the representatives:
    plot(full[society$representatives,])

## End(Not run)

check.SamSPECTRAL.input</pre>
```

Description

The input to SamSPECTRAL should be a numeric matrix WITHOUT any NA, NaN, and +/- Inf. This function checks the input matrix and prodeuces an error for an inappropriate input.

Checks the input to SamSPECTRAL.

Usage

```
check.SamSPECTRAL.input(data.points,dimensions=1:ncol(data.points),replace.inf.with.extremum=FAL
```

Arguments

data.points A matrix that contains coordinates of the data points.

dimensions A vector that determines which dimension of the data point matrix are chosen

for investigation.

```
replace.inf.with.extremum
```

If TRUE, the Inf and -Inf values will be replaced by maximum and minimum of data in each direction.

Value

Returns a list with the following entries:

data.matrix The data with infinite elements fixed if replace.inf.with.extremum=TRUE

dimensions All the checked dimensions.

infinite Will be TRUE if data contained infinite entries.

Author(s)

Habil Zare

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

See Also

SamSPECTRAL, Building_Communities, Conductance_Calculation, Connecting

Examples

```
## Not run:
    library(SamSPECTRAL)

# Reading data file which has been transformed using log transform
    data(small_data)

full <- small
    checked <- check.SamSPECTRAL.input(data.points=full,dimensions=c(1,2,3),replace.inf.with.extremum=TRUE)
    plot(checked$data.matrix, pch='.')

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

Civilized_Spectral_Clustering

Runs the spectral clustering algorithm on the sample points.

Description

The representatives of communities are considered as the vertices of a graph. Assuming the edges have been weighted according to the equivalent conductance between them, this function runs the classic spectral clustering on the graph.

Usage

```
Civilized_Spectral_Clustering(full, maximum.number.of.clusters, society, conductance, iterations=200, number.of.clusters="NA", k.for_kmeans="NA", minimum.eigenvalue="NA", minimum.degree=0, eigenvalues.num =NA, talk=TRUE, stabilizer=1000, one.line=FALSE, replace.inf.with.extremum=TRUE)
```

Arguments

full The matrix containing the coordinates of all data points.

maximum.number.of.clusters

An integer used to automatically estimate the number of clusters by fitting 2 regression lines on the eigen values curve.

number.of.clusters

The default value is "NA" which leads to computating the number of spectral clusters automatically, otherwise this number will determine the number of spectral clusters.

 $\hbox{k.for_kmeans} \qquad \hbox{The number of clusters for running kmeans algorithm in spectral clustering. The} \\$

default value of "NA" leads to automatic estimation based on eigen values curve.

minimum.eigenvalue

If not "NA", the number of spectral clusters will be determined such that corresponding eigenvalues are larger than this threshold.

minimum.degree If a node in the graph has total edge sum less than this threshold, it will be

considered as an isolated community.

society The list of communities.

conductance A matrix in which each entry is the conductance between two communities.

iterations Number of iterations for the k-means algorithm used by the spectral procedure.

200 is an appropriate value.

talk A boolean flag with default value TRUE. Setting it to FALSE will keep running

the procedure quite with no messages.

eigenvalues.num

An integer with default value NA which prevents ploting the curve of eigenval-

ues. Otherwise, they will be ploted upto this number.

stabilizer The larger this integer is, the final results will be more stable because the under-

lying kmeans will restart many more times.

one.line If TRUE, the number of spectral clusters are estimated by fitting 1 line to the

eigen values curve. Otherwise 2 lines are fitted.

replace.inf.with.extremum

If TRUE, the Inf and -Inf values will be replaced by maximum and minimum of data in each direction.

Value

A ClusteringResult class object with the following slots,

The k'th element of this list is a vector containing the labels as result of clustering to k parts.

labels.for_num.of.clustersnber.of.clusters A list containing the desired cluster numbers.

eigen.space The eigen vectors and eigen values of the normalized adjacency matrix computed by the eigen() function for spectral clustering.

Author(s)

Habil Zare, Nima Aghaeepour and Parisa Shooshtari

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

See Also

```
SamSPECTRAL, check. SamSPECTRAL. input
```

Examples

```
## Not run:
         library(SamSPECTRAL)
       # Reading data file which has been transformed using log transform
         data(small_data)
full <- small
# Parameters:
m <- 3000; ns <- 200; sl <- 3; cwt <-1; precision <- 6; mnc <-30
            # Sample the data and build the communities
         society <- Building_Communities(full=full,m=m, space.length=sl, community.weakness.threshold=cwt)</pre>
            # Compute conductance between communities
         conductance <- Conductance_Calculation(full=full, normal.sigma=ns, space.length=sl, society=society, pre</pre>
            # Use spectral clustering to cluster the data
# First example:
       \verb|clust_result| <- Civilized\_Spectral\_Clustering(full=full, maximum.number.of.clusters=mnc, society=society)| \\
            number.of.clusters <- clust_result@number.of.clusters</pre>
            labels.for_num.of.clusters <- clust_result@labels.for_num.of.clusters
L <- labels.for_num.of.clusters[[number.of.clusters]]</pre>
             # plot(full, pch='.', col= L)
# Second example:
number.of.clusters <- c(35,20)</pre>
# This is faster than runnig Civilized_Spectral_Clustering() twice because the eigen space is not needed to be
clust_result.not.automatic <-</pre>
{\tt Civilized\_Spectral\_Clustering(full=full, society=society, conductance=conductance, number.of. clusters = 
         labels.for_num.of.clusters <- clust_result.not.automatic@labels.for_num.of.clusters</pre>
L35 <- labels.for_num.of.clusters[[35]]
L20 <- labels.for_num.of.clusters[[20]]
          # plot(full, pch='.', col= L35)
## End(Not run)
```

Conductance_Calculation

Computes the conductance between communities.

Description

For each two communities, the conductance between their members is summed up and the result is returned as the conductance between the two communities.

Usage

Arguments

full	The matrix	containing the	coordinates	of all data points.
IUII	THE IHAUIA	containing the	coordinates	or air data points.

normal.sigma The scaling parameter, the larger it is the algorithm will find smaller clusters.

space.length An estimate for the length of a cube that is assumed to contain all data points.

society The list of communities.

precision Determines the precision of computations. Setting it to 6 will work and increas-

ing it does not improve results.

talk A boolean flag with default value TRUE. Setting it to FALSE will keep running

the procedure quite with no messages.

A parameter with default value 4 which must NOT be changed except for huge

samples with more than 100,000 data points or for developmental purposes. Setting beta to zero will reduce computational time by applying the following approximation to the conductance calculation step. For each two community, the conductance will be the conductance between their representatives times their

sizes.

replace.inf.with.extremum

If TRUE, the Inf and -Inf values will be replaced by maximum and minimum of

data in each direction.

Value

Returns a matrix in which each entry is the conductance between two communities.

Author(s)

Habil Zare and Parisa Shooshtari

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

See Also

SamSPECTRAL, check. SamSPECTRAL.input

Connecting 9

Examples

```
## Not run:
    library(SamSPECTRAL)

# Reading data file which has been transformed using log transform
    data(small_data)

full <- small

# Parameters:
m <- 3000; ns <- 200; sl <- 3; cwt <-1; precision <- 6

# Sample the data and build the communities
    society <- Building_Communities(full=full,m=m, space.length=sl, community.weakness.threshold=cwt)

# Compute conductance between communities
    conductance <- Conductance_Calculation(full=full, normal.sigma=ns, space.length=sl, society=society, pred
## End(Not run)</pre>
```

Description

Connecting

Considering some biological criterion based on density, the clusters which are identified by spectral clustering are combined to estimate biological populations.

Usage

Connecting(full, society,conductance, number.of.clusters, labels.for_num.of.clusters, separation

Combines the spectral clusters to build the connected components.

Arguments

full The matrix containing the coordinates of all data points.

society The list of communities.

conductance A matrix in which each entry is the conductance between two communities.

number.of.clusters

A list containing the desired cluster numbers.

labels.for_num.of.clusters

The k'th element of this list, is a vector containing the labels as result of clustering to k parts

separation.factor

This threshold controls to what extend clusters should be combined or kept separate.

talk A boolean flag with default value TRUE. Setting it to FALSE will keep running

the procedure quite with no messages.

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Details

A hint for setting separation.factor: While separation.factor=0.7 is normally an appropriate value for many datasets, for others some value in range 0.3 to 1.2 may produce better results depending on what populations are of particular interest.

Value

Returns two objects: 1) label, a vector containing the labels that determines to which component each data point belongs. 2) clusters.graph, the max.conductance matrix that describes the original graph based on clusters.

Author(s)

Habil Zare and Parisa Shooshtari

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

See Also

SamSPECTRAL

Examples

```
## Not run:
            library(SamSPECTRAL)
         # Reading data file which has been transformed using log transform
            data(small_data)
full <- small
# Parameters:
m <- 3000; ns <- 200; sl <- 3; cwt <-1; precision <- 6; mnc <-30
               # Sample the data and build the communities
            society <- Building_Communities(full=full,m=m, space.length=sl, community.weakness.threshold=cwt)</pre>
               # Compute conductance between communities
            conductance <- Conductance_Calculation(full=full, normal.sigma=ns, space.length=sl, society=society, pre</pre>
               # Use spectral clustering to cluster the data
          \verb|clust_result| <- Civilized\_Spectral\_Clustering(full=full, maximum.number.of.clusters=mnc, society=society)| <- Civilized\_Spectral\_Clusters=mnc, society=society| <- Civilized\_Spectral\_Clusters=mnc, society=society=society| <- Civilized\_Spectral\_Clusters=mnc, society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=society=societ
               number.of.clusters <- clust_result@number.of.clusters</pre>
               labels.for_num.of.clusters <- clust_result@labels.for_num.of.clusters
L <- labels.for_num.of.clusters[[number.of.clusters]]</pre>
                # plot(full, pch='.', col= L)
               # Connect components
            L <- Connecting(full=full, society=society, conductance=conductance, number.of.clusters=number.of.cluster
```

labels.for_num.of.clusters=labels.for_num.of.clusters, separation.factor=0.39)

eigen.values.10

```
plot(full, pch='.', col= L)
## End(Not run)
```

eigen.values.10

Eigenvalues for building the SamSPECTRAL vignette.

Description

This file contains a vector that represents the eigenvalues of the small example if normal.sigma=10.

Usage

```
data(eigen.values.10)
```

Format

This RData contains a vector.

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

Examples

```
data(eigen.values.10)
    plot(eigen.values.10)
```

eigen.values.1000

Eigenvalues for building the SamSPECTRAL vignette.

Description

This file contains a vector that represents the eigenvalues of the small example if normal.sigma=1000.

Usage

```
data(eigen.values.1000)
```

Format

This RData contains a vector.

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

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Examples

```
data(eigen.values.1000)
    plot(eigen.values.1000)
```

kneepointDetection

Fits 2 regression lines to data to estimate the knee (or elbow) point.

Description

With an appropriate sigma value, the curve of eigenvalues has a knee point shape. The bending point is a good estimate for the number of informative spectral clusters because the eigenvalues above the corresponding threshold can reasonably be assumed to be close to 1. This function estimate the knee point by fitting 2 lines using linear regression.

Usage

kneepointDetection(vect, PlotFlag=FALSE)

Arguments

vect The vector of values on which the 2 regression lines will be fitted.

PlotFlag If TRUE and in unix, an animation will be produced in tmpfigs folder that shows

how the best selected model in gif format.

Details

The running time is in order of minutes for 100 points. This function was borrowed from flowMeans package and for application in SamSPECTRAL package, it was customized such that the first line is always horizontal.

Value

Returns a list where MinIndex is the index of the knee point and 11 and 12 the fitted lines.

Author(s)

Nima Aghaeepour

References

Aghaeepour N., Nikolic R., Hoos HH., Brinkman RR.: Rapid cell population identification in flow cytometry data. Cytometry A, 2011, 79:6.

See Also

changepointDetection

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Examples

```
## Data
    values <- rep(1,times=10)
    values <- c(values,(10:0)/10)

## Looks like knee point:
plot(values)

## Find the knee point:
detected <- kneepointDetection(vect=values, PlotFlag=FALSE)
print(detected$MinIndex)
## Also, under unix, set PlotFlag=TRUE and look at animation.gif.</pre>
```

SamSPECTRAL

Identifies the cell populations in flow cytometry data.

Description

Given an FCS file as input, SamSPECTRAL first builds the communities to sample the data points. Then, it builds a graph and after weighting the edges of the graph by conductance computation, it is passed to a classic spectral clustering algorithm to find the spectral clusters. The last stage of SamSPECTRAL is to combine the spectral clusters. The resulting "connected components" estimate biological cell populations in the data sample.

Usage

```
SamSPECTRAL(data.points, dimensions=1:dim(data.points)[2], normal.sigma, separation.factor,numbe
  talk = TRUE, precision = 6, eigenvalues.num =NA, return_only.labels=TRUE, do.sampling=TRUE, bet
  k.for_kmeans = "NA", maximum.number.of.clusters=30, m=3000,
  minimum.eigenvalue = "NA", previous.result = NULL,
  replace.inf.with.extremum=TRUE, minimum.degree=0, one.line=FALSE, doOrderLabels=TRUE)
```

Arguments

data.points A matrix that contains coordinates of the data points.

dimensions A vector that determines which dimension of the data point matrix are chosen

for investigation.

normal.sigma A scaling parameter that determines the "resolution" in the spectral clustering

stage. By increasing it, more spectral clusters are identified. This can be useful when "small" population are aimed. See the user manual for a suggestion on

how to set this parameter using the eigenvalue curve.

separation.factor

This threshold controls to what extend clusters should be combined or kept separate. Normally, an appropriate value will fall in range 0.3-2.

number.of.clusters

The default value is "NA" which leads to computing the number of spectral clusters automatically, otherwise it can be a vector of integers each of which determines the number of spectral clusters. The output will contain a clustering

resulting from each value.

talk A boolean flag with default value TRUE. Setting it to FALSE will keep running

the procedure quite with no messages.

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precision

Determines the precision of computations. Setting it to 6 will work and increasing it does not improve results.

eigenvalues.num

An integer with default value NA which prevents ploting the curve of eigenvalues. Otherwise, they will be ploted upto this number.

return_only.labels

A boolean flag with default value TRUE. If the user set it to FALSE, SamSPEC-TRAL function will return all the intermediate objects that are computed during the sampling, similarity calculation, spectral clustering and combining stages.

do.sampling

A boolean flag with default value TRUE. If set to FALSE, the sampling stage will be ignored by picking up all the data points.

beta

A parameter with default value 4 which must NOT be changed except for huge samples with more than 100,000 data points or for developmental purposes. Setting beta to zero will reduce computational time by applying the following approximation to the conductance calculation step. For each two community, the conductance will be the conductance between their representatives times their sizes.

scale

A vector the length of which is equal to the number of dimensions. The coordinates in each dimension are multiplied by the corresponding scaling factor. So, the bigger this factor is for a dimension, SamSPECTRAL will consider that dimension to be "more significant" and consequently, that dimension will be more effective in clustering.

stabilizer

The larger this integer is, the final results will be more stable because the underlying kmeans will restart many more times.

k.for_kmeans

The number of clusters for running kmeans algorithm in spectral clustering. The default value of "NA" leads to automatic estimation based on eigen values curve.

maximum.number.of.clusters

An integer used to automatically estimate the number of clusters by fitting 2 regression lines on the eigen values curve.

m

An integer determining upper and lower bounds on the final number of sample points which will be in range .95*m/2 and 2 1.1*m

minimum.eigenvalue

If not "NA", the number of spectral clusters will be determined such that corresponding eigenvalues are larger than this threshold.

previous.result

If provided, the intermediate results from previous run can be passed to save on computing time while setting the parameters.

replace.inf.with.extremum

If TRUE, the Inf and -Inf values will be replaced by maximum and minimum of data in each direction.

minimum.degree If a node in the graph has total edge sum less than this threshold, it will be considered as an isolated community.

one.line

If TRUE, the number of spectral clusters are estimated by fitting 1 line to the eigen values curve. Otherwise 2 lines are fitted.

doOrderLabels

Used for debugging. If TRUE, after connecting components, relabeling will be done such that the largest component gets label 1. If FALSE, the label of each data point will be the index of the component it belongs to (after connecting components).

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Details

Hints for setting separation.factor and normal.sigma: While separation.factor=0.7 is normally an appropriate value for many datasets, for others some value in range 0.3 to 1.2 may produce better results depending on what populations are of particular interest. The larger normal.sigma is the algorithm will find smaller clusters. It can be adjusted best by considering the plot of eigenvalues as explained in the vignette.

Value

Returns a vector of labels for data points. If the input parameter return_only.labels is set to FALSE, all the objects that are computed during the intermediate will be returned including: society from sampling stage, conductance from similarity calculation, clustering_result, component.of from connecting step (the same as labels if doOrderLabels=FALSE, used for debugging), timeTaken, and sizes which is a table of size of each component.

Author(s)

Habil Zare and Parisa Shooshtari

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

See Also

SamSPECTRAL, Building_Communities, Conductance_Calculation, Civilized_Spectral_Clustering, Connecting, check.SamSPECTRAL.input

Examples

```
## Not run:
    library(SamSPECTRAL)

# Reading data file which has been transformed using log transform
    data(small_data)

full <- small

L <- SamSPECTRAL(data.points=full,dimensions=c(1,2,3), normal.sigma = 200, separation.factor = 0.39)

plot(full, pch='.', col= L)

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

small

Flow cytometry data to test SamSPECTRAL algorithm.

Description

This FCS file is a small one used to show how to set SamSPECTRAL parameters.

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Usage

```
data(small_data)
```

Format

This is an FCS file.

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

Examples

```
data(small_data)
full <- small
    plot(full, pch='.')</pre>
```

stmFSC

Flow cytometry data to test SamSPECTRAL algorithm.

Description

This FCS file is used as demo data to illustrate SamSPECTRAL capabilities in identifying cell populations.

Usage

```
data(stm)
```

Format

The is an FCS file.

References

Zare, H. and Shooshtari, P. and Gupta, A. and Brinkman R.B: Data Reduction for Spectral Clustering to Analyse High Throughput Flow Cytometry Data. BMC Bioinformatics, 2010, 11:403.

Examples

```
data(stm)
    # Read data files and transform them using log transform
data.points <- stmFSC@exprs
dimensions <- c(3,4,7)
full <- log10(data.points[,dimensions])
    plot(full, pch='.')</pre>
```

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