# Package 'SamSPECTRAL'

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Type Package

Title Identifies cell population in flow cytometry data

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**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. See the vignette for more details on how to use this package, some illustrations, and simple examples.

License GPL (>= 2)

LazyLoad yes

**biocViews** FlowCytometry, CellBiology, Clustering, Cancer, FlowCytometry, StemCells, HIV, ImmunoOncology

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SamSPECTRAL-package Identifying cell populations in flow cytometry data.

## 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 SamSPEC-TRAL() 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

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## Building\_Communities

#### 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

## 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)
```

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)
```

full	The matrix containing the coordinates of all data points.	
m	An integer determining upper and lower bounds on the final number of sample points which will be in range $.95*m/2$ and $21.1*m$	
<pre>space.length</pre>	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.	
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.	

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 society which is a list of 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

#### 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

Checks the input to SamSPECTRAL.

## 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.

#### 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.wit	h.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 <code>replace.inf.with.extremum=TRUE</code>
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)</pre>
```

plot(checked\$data.matrix, pch='.')

## End(Not run)

## 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)
```

full	The matrix containing the coordinates of all data points.	
<pre>maximum.number.</pre>	.of.clusters	
	An integer used to automatically estimate the number of clusters by fitting 2 regression lines on the eigen values curve.	
number.of.clust	ters	
	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.	
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.	
minimum.eigenva	alue	
	If not "NA", the number of spectral clusters will be determined such that corre- sponding eigenvalues are larger than this threshold.	
<pre>minimum.degree</pre>	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.clustensnber.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</pre>
# 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, prec</pre>
   # Use spectral clustering to cluster the data
# First example:
 clust_result <- Civilized_Spectral_Clustering(full=full, maximum.number.of.clusters=mnc, society=society</pre>
   number.of.clusters <- clust_result@number.of.clusters</pre>
   labels.for_num.of.clusters <- clust_result@labels.for_num.of.clusters</pre>
L <- labels.for_num.of.clusters[[number.of.clusters]]
   # plot(full, pch='.', col= L)
# Second example:
```

```
number.of.clusters <- c(35,20)</pre>
```

#### Conductance\_Calculation

```
# This is faster than runnig Civilized_Spectral_Clustering() twice because the eigen space is not needed to be
clust_result.not.automatic <-
Civilized_Spectral_Clustering(full=full, society=society, conductance=conductance, number.of.clusters =num
labels.for_num.of.clusters <- clust_result.not.automatic@labels.for_num.of.clusters
L35 <- labels.for_num.of.clusters[[35]]
L20 <- labels.for_num.of.clusters[[20]]
# plot(full, pch='.', col= L35)
```

Conductance\_Calculation

Computes the conductance between communities.

## Description

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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.
normal.sigma	The scaling parameter, the larger it is the algorithm will find smaller clusters.
<pre>space.length</pre>	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.
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.
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.

#### Connecting

#### 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; 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, preceductance)</pre>
```

## End(Not run)

```
Connecting
```

Combines the spectral clusters to build the connected components.

#### Description

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.

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 cluster-	
	ing 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.	

## 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, prec</pre>

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```
# Use spectral clustering to cluster the data
clust_result <- Civilized_Spectral_Clustering(full=full, maximum.number.of.clusters=mnc, society=society_
number.of.clusters <- clust_result@number.of.clusters
labels.for_num.of.clusters <- clust_result@labels.for_num.of.clusters
L <- labels.for_num.of.clusters[[number.of.clusters]]
# 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)
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.

## 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)
```

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.

#### SamSPECTRAL

#### 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

#### 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,number
talk = TRUE, precision = 6, eigenvalues.num =NA, return_only.labels=TRUE, do.sampling=TRUE, beta
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)
```

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.fact	or
	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.clust	
	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.
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 eigenval- ues. Otherwise, they will be ploted upto this number.
return_only.lab	
	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 under- lying 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 21.1*m
minimum.eigenva	lue
	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.wit	h.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).

#### 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

small

## 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)
```

small

Flow cytometry data to test SamSPECTRAL algorithm.

## Description

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

## 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>
```

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stmFSC

## 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])</pre>
```

plot(full, pch='.')

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