

# Package ‘SamSPECTRAL’

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**Type** Package

**Title** Identifies cell population in flow cytometry data.

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**Author** Habil Zare and Parisa Shooshtari

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**Depends** R (>= 2.10)

**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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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 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**

[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.sam
```

**Arguments**

<code>full</code>	The matrix containing the coordinates of all data points.
<code>m</code>	An integer determining upper and lower bounds on the final number of sample points which will be in range $.95*m/2$ and $2.1*m$
<code>space.length</code>	An estimate for the length of a cube that is assumed to contain all data points.
<code>community.weakness.threshold</code>	The communities with number of members less than this threshold will be ignored. Normally, setting it to 1 is reasonable.
<code>talk</code>	A boolean flag with default value TRUE. Setting it to FALSE will keep running the procedure quite with no messages.
<code>do.sampling</code>	A boolean flag with default value TRUE. If set to FALSE, the sampling stage will be ignored by picking up all the data points.

**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](#)

**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)

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

## 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)
```

**Arguments**

<code>full</code>	The matrix containing the coordinates of all data points.
<code>maximum.number.of.clusters</code>	An integer used to automatically estimate the number of clusters by fitting 2 regression lines on the eigen values curve.
<code>number.of.clusters</code>	The default value is "NA" which leads to computing the number of spectral clusters automatically, otherwise this number will determine the number of spectral clusters.
<code>k.for_kmeans</code>	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.
<code>minimum.eigenvalue</code>	If not "NA", the number of spectral clusters will be determined such that corresponding eigenvalues are larger than this threshold.
<code>minimum.degree</code>	If a node in the graph has total edge sum less than this threshold, it will be considered as an isolated community.
<code>society</code>	The list of communities.
<code>conductance</code>	A matrix in which each entry is the conductance between two communities.
<code>iterations</code>	Number of iterations for the k-means algorithm used by the spectral procedure. 200 is an appropriate value.
<code>talk</code>	A boolean flag with default value TRUE. Setting it to FALSE will keep running the procedure quite with no messages.
<code>eigenvalues.num</code>	An integer with default value NA which prevents plotting the curve of eigenvalues. Otherwise, they will be plotted upto this number.
<code>stabilizer</code>	The larger this integer is, the final results will be more stable because the underlying kmeans will restart many more times.
<code>one.line</code>	If TRUE, the number of spectral clusters are estimated by fitting 1 line to the eigen values curve. Otherwise 2 lines are fitted.

**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.clusters** A list containing the desired cluster numbers.

**eigen.space** The eigen vectors and eigen values of the normalized adjacency matrix computed 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](#)

**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)

  # Compute conductance between communities
  conductance <- Conductance_Calculation(full=full, normal.sigma=ns, space.length=sl, society=society, precision=precision)

  # Use spectral clustering to cluster the data
# First example:
  clust_result <- Civilized_Spectral_Clustering(full=full, maximum.number.of.clusters=mnc, society=society, conductance=conductance,
  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)

# Second example:
number.of.clusters <- c(35,20)
# This is faster than running Civilized_Spectral_Clustering() twice because the eigen space is not needed to be computed
clust_result.not.automatic <-
Civilized_Spectral_Clustering(full=full, society=society, conductance=conductance, number.of.clusters =number.of.clusters,
  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)

## 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

```
Conductance_Calculation(full, normal.sigma, space.length, society, precision, talk=TRUE, beta=4)
```

### 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.
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 increasing 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.

### 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](#)**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, precision=precision)

## 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.factor)
```

**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.



<code>labels.for_num.of.clusters</code>	The k'th element of this list, is a vector containing the labels as result of clustering to k parts.
<code>separation.factor</code>	This threshold controls to what extend clusters should be combined or kept separate.
<code>talk</code>	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)
```

```
# Compute conductance between communities
conductance <- Conductance_Calculation(full=full, normal.sigma=ns, space.length=sl, society=society, precis

# Use spectral clustering to cluster the data
clust_result <- Civilized_Spectral_Clustering(full=full, maximum.number.of.clusters=mnc, society=society, co
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.clusters,
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)
```

**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 `l1` and `l2` 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.
```

---

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.of
  talk = TRUE, precision = 6, eigenvalues.num =NA, return_only.labels=TRUE, do.sampling=TRUE, beta=4
  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)
```

**Arguments**

<code>data.points</code>	A matrix that contains coordinates of the data points.
<code>dimensions</code>	A vector that determines which dimension of the data point matrix are chosen for investigation.
<code>normal.sigma</code>	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.
<code>separation.factor</code>	This threshold controls to what extend clusters should be combined or kept separate. Normally, an appropriate value will fall in range 0.3-2.
<code>number.of.clusters</code>	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.
<code>talk</code>	A boolean flag with default value TRUE. Setting it to FALSE will keep running the procedure quite with no messages.
<code>precision</code>	Determines the precision of computations. Setting it to 6 will work and increasing it does not improve results.
<code>eigenvalues.num</code>	An integer with default value NA which prevents plotting the curve of eigenvalues. Otherwise, they will be plotted upto this number.
<code>return_only.labels</code>	A boolean flag with default value TRUE. If the user set it to FALSE, SamSPECTRAL function will return all the intermediate objects that are computed during the sampling, similarity calculation, spectral clustering and combining stages.
<code>do.sampling</code>	A boolean flag with default value TRUE. If set to FALSE, the sampling stage will be ignored by picking up all the data points.
<code>beta</code>	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.
<code>scale</code>	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 $2.1*m$
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.

### 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` for sampling stage, `conductance` for similarity calculation, and `clustering_result`.

### 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](#)

**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='.')
```

---

`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='.')
```



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