Introduction to Multivariate Analysis of Microarray Gene Expression Data using MADE4

Aedín Culhane

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1 Introduction

The package made4 facilitates multivariate analysis of microarray gene expression data. The package provides a set of functions that utilise and extend multivariate statistical and graphical functions available in ade4, [1]. made4 accepts gene expression data in a wide variety of input formats, including Bioconductor formats, AffyBatch, ExpressionSet, marrayRaw, and data.frame or matrix.

1.1 Installation

made4 requires that ade4 is installed. made4 also calls scatterplot3d. These should be installed automatically when you install made4. To install made4 from bioconductor...
if (!requireNamespace("BiocManager", quietly=TRUE))
  install.packages("BiocManager")
BiocManager::install("made4")

1.2 Further help

The package made4 is described in more detail in the RNews newsletter, December 2006.
Culhane AC and Thioulouse J. (2006) A multivariate approach to integrating datasets

We also have a tutorials on our website at [http://compbio.dfci.harvard.edu/courses/bioconductor/](http://compbio.dfci.harvard.edu/courses/bioconductor/)

Extensive tutorials, examples and documentation on multivariate statistical methods
are available from the ade4 website [http://pbil.univ-lyon1.fr/ADE-4](http://pbil.univ-lyon1.fr/ADE-4) and ade4 user
support is available through the ADE4 mailing list. The ade4 homepage is [http://pbil.univ-lyon1.fr/ADE-4](http://pbil.univ-lyon1.fr/ADE-4).

This tutorial assumes a basic knowledge of R, but we have found that Emmanuel
Paradis’s *R for Beginners* is a very good guide to those unfamiliar with R. This is

This documents assumes that data is normalised and preprocessed. Please refer
to the Bioconductor packages affy and limma, for input and initial pre-processing of

1.3 Citing

We are delighted if you use this package. Please do email us if you find a bug or have a
suggestion. We would be very grateful if you could cite:

2 Quickstart

We will very briefly demonstrate some of the functions in made4. To do this we will use
a small dataset that is available in made4. This dataset Khan contains gene expression
profiles of four types of small round blue cell tumours of childhood (SRBCT) published
by Khan et al. (2001). This is a subset of the published dataset. It contains gene
expression levels for 306 genes for 64 patient samples. Load the necessary R packages
and dataset.

> library(made4)
> library(ade4)
This experiment studied gene expression in patients with four types of SRBCT. These were neuroblastoma (NB), rhabdomyosarcoma (RMS), Burkitt lymphoma, a subset of non-Hodgkin lymphoma (BL), and the Ewing family of tumours (EWS). Gene expression profiles from both tumour biopsy and cell line samples were obtained and are contained in this dataset. In this study data were divided into a training set of 64 samples, and a blind test dataset. These 2 dataset are called khan$train and khan$test. Have a look at the data. For this example we will just example the training dataset.

> names(khan)

[1] "train"          "test"
[3] "train.classes"  "test.classes"
[5] "annotation"     "gene.labels.imagesID"
[7] "cellType"

> k.data<-khan$train
> k.class<-khan$train.classes

2.1 Overview

The made4 function overview() provides a quick way to get an overview or feel for data. overview() will draw a boxplot, histogram and dendrogram of a hierarchical analysis. Hierarchical clustering is produced using average linkage clustering with a Pearson correlation measure of similarity. This gives a quick first glance at the data.

> overview(k.data)

Often it's useful to label the samples using a class vector or covariate of interest, in this case, the tumour type (EWS, BL, NB or RMS).
> overview(k.data, labels=k.class)

Figure 1: Overview of Khan data. A) dendrogram showing results of average linkage clustering, B) boxplot and C) histogram.

Often one will known classes in the data (eg Normal v Treatment, or different tumor types). We can insert a class colourbar under the dendrogram, and colour the boxplot.
> overview(k.data, classvec=k.class, labels=k.class)

Figure 2: Overview of Khan data. A) dendrogram showing results of average linkage clustering, B) boxplot and C) histogram. In this case we have added a vector of class (classvec) to color the overview by class membership

### 2.2 Correspondence Analysis

The function `ord` simplifies the running of ordination methods such as principal component, correspondence or non-symmetric correspondence analysis. It provides a wrapper which can call each of these methods in `ade4`. To run a correspondence analysis on this dataset:

> k.coa<- ord(k.data, type="coa")
Output from `ord` is a list of length 2, containing the ordination results ($ord) and a factor ($fac) if input. The ordination results (k.coa$ord) contain a list of results (of length 12) which includes the eigenvalues ($eig) and the new column coordinates ($co) and the row (line) coordinates in $li. Hence we can visualise the projected coordinations of the genes ($li, 306 genes) and array samples ($co, 64 microarray samples).

```r
> names(k.coa)
[1] "ord" "fac"
```

```r
> summary(k.coa$ord)

Class: coa dudi
Call: dudi.coa(df = data.tr, scannf = FALSE, nf = ord.nf)

Total inertia: 1.011

Eigenvalues:
   Ax1  Ax2  Ax3  Ax4  Ax5
0.17130 0.13831 0.10317 0.05995 0.04965

Projected inertia (%):
   Ax1  Ax2  Ax3  Ax4  Ax5

Cumulative projected inertia (%):
   Ax1:2  Ax1:3  Ax1:4  Ax1:5
 16.95  30.63  40.84  46.77  51.68

(Only 5 dimensions (out of 63) are shown)
```

### 2.3 Visualising Results

There are many functions in `ade4` and `made4` for visualising results from ordination analysis. The simplest way to view the results produced by `ord` is to use `plot`. `plot(k.ord)` will draw a plot of the eigenvalues, along with plots of the variables (genes) and a plot of the cases (microarray samples). In this example Microarray samples are colour-coded using the `classvec khan$train.classes` which is saved as `k.class`.

```r
> k.class

[1] EWS  EWS  EWS  EWS  EWS  EWS  EWS  EWS  EWS
[9] EWS  EWS  EWS  EWS  EWS  EWS  EWS  EWS  EWS
```
Levels: EWS BL-NHL NB RMS
> plot(k.coa, classvec=k.class, arraycol=c("red", "blue", "yellow", "green"), genecol=c("green", "red", "blue", "black"))

Figure 3: Correspondence analysis of Khan dataset. A. plot of the eigenvalues, B. projection of microarray samples from patient with tumour types EWS (red), BL (blue), NB (yellow) or RMS (green), C. projection of genes (gray filled circles) and D. biplot showing both genes and samples. Samples and genes with a strong association are projected in the same direction from the origin. The greater the distance from the origin the stronger the association.

Genes and array projections can also be plotted using `plotgenes` and `plotarrays`. The function `s.groups` required a class vector (classvec), and allowed groups to be coloured in different colours. For example, to plot microarray samples (cases),

> plotgenes(k.coa)

To plot microarray samples, colour by group (tumour type) as specified by `khan$train.classes`
> plotarrays(k.coa, arraylabels=k.class)

Alternative you can run these analysis and give a class vector to \( \text{ord} \) and it will automatically colour samples by this class vector

> k.coa2<-ord(k.data, classvec=k.class)
> plot(k.coa2)

Plot gene projections without labels (clab=0). Typically there are a large number of genes, thus it is not feasible to label all of these. The function plotgenes is more useful to use if you wish to add labels when there are lots of variables (genes)

The gene projections can be also visualised with plotgenes. The number of genes that are labelled at the end of the axis can be defined. The default is 10.

> plotgenes(k.coa, n=5, col="red")

By default the variables (genes) are labelled with the rownames of the matrix. Typically these are spot IDs or Affymetrix accession numbers which are not very easy to interpret. But these can be easily labeled by your own labels. For example its often useful to labels using HUGO gene symbols. We find the Bioconductor annotate and annaffy annotation packages are very useful for this. Alternatively we also use biomaRt or Resourcerer or the Stanford Source database.

In this example we provide annotation from the Source database in khan$annotation. The gene symbol are in the column khan$annotation$Symbol

> gene.symbs<- khan$annotation$Symbol
> plotgenes(k.coa, n=10, col="red", genelabels=gene.symbs)

To get a list of variables at the end of an axes, use topgenes. For example, to get a list of the 5 genes at the negative and positive end of axes 1.

> topgenes(k.coa, axis = 1, n=5)

To only the a list of the genes (default 10 genes) at the negative end of the first axes

> topgenes(k.coa, labels=gene.symbs, end="neg")

[1] "PTPN13"  "OLFM1"  "TNFAIP6"  "GYG2"   "CAV1"
[6] "MYC"     "FVT1"    "FCGRT"   "TUBB5"   "MYC"

Two lists can be compares using comparelists.
> plotgenes(k.coa, n=10, col="red", genelabels=gene.symbs)

Figure 4: Projection of genes (filled circles) in Correspondence analysis of Khan dataset. The genes at the ends of each of the axes are labelled with HUGO gene symbols.
To visualise the arrays (or genes) in 3D either use do3d or html3d. do3d is a wrapper for scatterplot3d, but is modified so that groups can be coloured. html3d produces a "pdb" output which can be visualised using rasmol or chime. Rasmol provides a free and very useful interface for colour, rotating, zooming 3D graphs.

```r
> do3d(k.coa$ord$co, classvec=k.class, cex.symbols=3)
> html3D(k.coa$ord$co, k.class, writehtml=TRUE)
```

It is also worth exploring the package rgl which enables dynamic 3d plot (can be rotated).

```r
library(rgl)
plot3d(k.coa$co[,1], k.coa$co[,2],k.coa$co[,3], size=4, col=khan$train.classes)
rgl.snapshot(file="test.png", top=TRUE)
rgl.close()
```

### 2.4 Classification and Class Prediction using Between Group Analysis

Between Group Analysis (BGA) is a supervised classification method [3]. The basis of BGA is to ordinate the groups rather than the individual samples. In tests on two microarray gene expression datasets, BGA performed comparably to supervised classification methods, including support vector machines and artifical neural networks [2]. To train a dataset, use `bga`, the projection of test data can be assessed using `suppl`. One leave out cross validation can be performed using `bga.jackknife`. See the BGA vignette for more details on this method.

```r
> k.bga<-bga(k.data, type="coa", classvec=k.class)
```

Sometimes its useful to visualise 1 axes of an analysis. To do this use `graph1D` or `between.graph`. The latter function is specifically for visualising results from a bga as it shows the separation of classes achieved.

```r
> between.graph(k.bga, ax=1) # Show the separation on the first axes(ax)
```

### 2.5 Meta-analysis of microarray gene expression

Coinertia analysis `cia` [4] has been successfully applied to the cross-platform comparison (meta-analysis) of microarray gene expression datasets [8]. CIA is a multivariate method that identifies trends or co-relationships in multiple datasets which contain the same samples. That is either the rows or the columns of a matrix must be "matchable". CIA can be applied to datasets where the number of variables (genes) far exceeds the number of samples (arrays) such is the case with microarray analyses. `cia` calls `coinertia` in the `ade4` package. See the CIA vignette for more details on this method.
Figure 5: Output from html3D, which can be rotated and visualised on web browsers that can support chime (IE or Netscape on MS Windows or Mac).
> plot(k.bga, genelabels=gene.symbs) # Use the gene symbols earlier

Figure 6: Between group analysis of Khan dataset. A. Between graph of the microarray samples, showing their separation on the discriminating BGA axes, B. Scatterplot of the first 2 axes of microarray samples, coloured by their class, C. graph of positions of genes on the same axis. Genes at the ends of the axis are most discriminating for that group.
> # Example data are "G1_Ross_1375.txt" and "G5_Affy_1517.txt"
> data(NCI60)
> coin <- cia(NCI60$Ross, NCI60$Affy)
> names(coin)

[1] "call" "coinertia" "coa1" "coa2"

> coin$coinertia$RV

[1] 0.7859656

The RV coefficient $RV$ which is 0.786 in this instance, is a measure of global similarity between the datasets. The greater (scale 0-1) the better.
Figure 7: Coinertia analysis of NCI 60 cell line Spotted and Affymetrix gene expression dataset. The same 60 cell lines were analysed by two different labs on a spotted cDNA array (Ross) and an affymetrix array (Affy). The Ross dataset contains 1375 genes, and the affy dataset contains 1517. There is little overlap between the genes represented on these platforms. CIA allows visualisation of genes with similar expression patterns across platforms. A) shows a plot of the 60 microarray samples projected onto the one space. The 60 circles represent dataset 1 (Ross) and the 60 arrows represent dataset 2 (affy). Each circle and arrow are joined by a line, the length of which is proportional to the divergence between that samples in the two datasets. The samples are coloured by cell type. B) The gene projections from datasets 1 (Ross), C) the gene projections from dataset 2 (Affy). Genes and samples projected in the same direction from the origin show genes that are expressed in those samples. See vignette for more help on interpreting these plots.
3 Functions in made4

Data Input

array2ade4 Converts matrix, data.frame, ExpressionSet, marrayRaw microarray gene expression data input data into a data frame suitable for analysis in ADE4. The rows and columns are expected to contain the variables (genes) and cases (array samples).

overview Draw boxplot, histogram and hierarchical tree of gene expression data. This is useful only for a brief first glance at data.

Example Datasets provides with made4

khan Microarray gene expression dataset from Khan et al., 2001
NCI60 Microarray gene expression profiles of the NCI 60 cell lines

Classification and class prediction using Between Group Analysis

bga Between group analysis
bga.jackknife Jackknife between group analysis
randomiser Randomly reassign training and test samples
bga.suppl Between group analysis with supplementary data projection
suppl Projection of supplementary data onto axes from a between group analysis
plot.bga Plot results of between group analysis
between.graph Plot 1D graph of results from between group analysis

Meta analysis of two or more datasets using Coinertia Analysis

cia Coinertia analysis: Explore the covariance between two datasets
plot.cia Plot results of coinertia analysis

Graphical Visualisation of results: 1D Visualisation

graph1D Plot 1D graph of axis from multivariate analysis
between.graph Plot 1D graph of results from between group analysis
commonMap Highlight common points between two 1D plots
heatplot Draws heatmap with dendrograms (of eigenvalues)
Graphical Visualisation of results: 2D Visualisation

plotgenes  
Graph xy plot of variable (gene) projections from PCA or COA. Only label variables at ends of axes

plotarrays  
Graph xy plot of case (samples) projections from PCA or COA, and colour by group

plot.bga  
Plot results of between group analysis using plotgenes, s.groups and s.var

plot.cia  
Plot results of coinertia analysis showing s.match.col, and plotgenes

s.var  
Use plotarrays instead. Graph xy plot of variables (genes or arrays). Derived from ADE4 graphics module s.label.

s.groups  
Use plotarrays instead. Graph xy plot of groups of variables (genes or arrays) and colour by group. Derived from ADE4 graphics module s.class

s.match.col  
Use plotarrays instead. Graph xy plot of 2 sets of variables (normally genes) from CIA. Derived from ADE4 graphics module s.match

Graphical Visualisation of results: 3D Visualisation

do3d  
Generate a 3D xyz graph using scatterplot3d

rotate3d  
Generate multiple 3D graphs using do3d in which each graph is rotated

html3D  
Produce web page with a 3D graph that can be viewed using Chime web browser plug-in, and/or a pdb file that can be viewed using Rasmol

Interpretation of results

topgenes  
Returns a list of variables at the ends (positive, negative or both) of an axis

sumstats  
Summary statistics on xy co-ordinates, returns the slopes and distance from origin of each co-ordinate

comparelists  
Return the intersect, difference and union between 2 vectors

print.comparelists  
Prints the results of comparelists

References


