MANOR: Micro-Array NORmalization of array-CGH data

Pierre Neuvial 1,2,3, Philippe Hupé 1,2,3,4, Isabel Brito 1,2,3, Emmanuel Barillot 1,2,3

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1. Institut Curie, 26 rue d’Ulm, Paris cedex 05, F-75248 France
2. INSERM, U900, Paris, F-75248 France
3. École des Mines de Paris, ParisTech, Fontainebleau, F-77300 France
4. UMR 144 CNRS, Paris, F-75248 France
manor@curie.fr

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1 Overview

This document gives an overview of the MANOR package, which is devoted to the normalization of Array Comparative Genomic Hybridization (array-CGH) data\(^9\)\(^7\)\(^8\)\(^4\)\(^3\). Normalization is a crucial step of microarray analysis which aims at separating biologically relevant signal from experimental artifacts. Typical input data is a file generated by an image analysis software such as Genepix or SPOT\(^5\), containing several measurements for each biological variable of interest, i.e. several replicated spots for each clone; this spot-level data is filtered with various statistical criteria (including a spatial bias detection step which is described in\(^6\)), and aggregated into clean clone-level data.

Using the \texttt{arrayCGH} framework developped in the package GLAD, which is available under \texttt{Bioconductor}. We propose the formalism of flags to handle clone and spot filtering: the core of the normalization process consists in applying to an \texttt{arrayCGH} object a list of flags that successively exclude from the data all irrelevant spots or clones.

We also define quality scores (\texttt{qscores}) that quantify the quality of an array after normalization: these scores can be used directly to compare the quality of different arrays after the same normalization process, or to compare the efficiency of different normalization processes on a given array or on a given batch of arrays.

This document is organized as follows: after a short description of optional items we add to \texttt{arrayCGH} objects (section\(^2\)), we introduce the classes \texttt{flag} (section\(^3\)) and \texttt{qscore} (section\(^4\)) with their attributes and dedicated methods; then we describe two useful graphical representation functions (section\(^6\)), namely \texttt{genome.plot} and \texttt{report.plot}; Afterwards we give a short description of the array-CGH datasets we provide (section\(^5\)); finally we illustrate the usage of MANOR by a sample R script (section\(^7\)).

[1] "Have fun with GLAD"
[1] "For smoothing it is possible to use either"
[1] "the AWS algorithm (Polzehl and Spokoiny, 2002)"
[1] "or the HaarSeg algorithm (Ben-Yaacov and Eldar, 2008)"
[1] '\'
arrayCGH class

For the purpose of normalization we have added several optional items to the arrayCGH objects defined in the R package GLAD, including:

cloneValues a data frame with aggregated (clone-level) information, quite similar to profileCGH objects of GLAD

id.rep the name of a variable common to cloneValues and arrayValues, that can be used as an identifier for the replicates.

flag class

We view the process of filtering microarray data, and especially array-CGH data, as a succession of steps consisting in excluding from the data unreliable spots or clones (according to criteria such as signal to noise ratio or replicate consistency), and correcting signal values from various non-biologically relevant sources of variations (such as spotting effects, spatial effects, or intensity effects).

We introduce the formalism of flags to deal with this filtering issue: in the two following subsections, we describe the attributes and methods devoted to flag objects.

3.1 Attributes

A flag object f is a list whose most important items are a function (f$FUN) which has to be applied to an object of class arrayCGH, and a character value (f$char) which identifies flagged spots. Optionally further arguments can be passed to f$FUN via f$args, and a label can be added via f$label. The examples of this subsection use the function to.flag, which is explained in subsection 3.2.

3.1.1 Exclusion and correction flags

As stated above, we make the distinction between flags that exclude spots from further analysis and flags that correct signal values:

exclusion flags If f is an exclusion flag, f$FUN returns a list of spots to exclude and f$char is a non NULL value that quickly identifies the flag. In the following example, we define SNR.flag, a flag objects that excludes spots whose signal to noise ratio lower than the threshold snr.thr.

```r
> SNR.FUN <- function(arrayCGH, var.FG, var.BG, snr.thr) {
+     which(arrayCGH$arrayValues[[var.FG]] < arrayCGH$arrayValues[[var.BG]] * 
+         snr.thr)
```

correction flags  If f is a correction flag, f$FUN returns an object of type arrayCGH and f$char is NULL. In the following example, global.spatial.flag computes a spatial trend on the array, and corrects the signal log-ratios from this spatial trend:

```r
define spatial trend on the array, and corrects the signal log-ratios from this spatial trend:
```
3.2 Methods

3.2.1 to.flag

The function `to.flag` is used for the creation of `flag` objects, with the specificities described in subsection 3.1.

```r
> args(to.flag)
function (FUN, char = NULL, args = NULL, type = "perm.flag", label = NULL)
```

3.2.2 flag.arrayCGH

Function `flag.arrayCGH` simply applies function `flag$FUN` to a `flag` object for filtering, and returns:

- a filtered array with field `arrayCGH$arrayValues$Flag` filled with the value of `flag$char` for each spot to be excluded from further analysis in the case of an exclusion flag;
- an array with corrected signal value in the case of a correction flag.

```r
> args(flag.arrayCGH)
function (flag, arrayCGH)
```

3.2.3 flag.summary

Function `flag.summary` computes spot-level information about normalization (including the number of flagged spots and numeric normalization parameters), and displays it in a convenient way. This function can either be applied to an object of type `arrayCGH`:

```r
> args(flag.summary.arrayCGH)
function (arrayCGH, flag.list, flag.var = "Flag", nflab = "not flagged", ...)
```

or to plain spot-level information, by using the default method:

```r
> args(flag.summary.default)
function (spot.flags, flag.list, nflab = "not flagged", ...)
```
4 \textit{qscore} class

As we point out in the introduction of this document, evaluating the quality of an array-CGH after normalization is of major importance, since it helps answering the following questions:

- which is the best normalization process ?
- which array is of best quality ?
- what is the quality of a given array ?

To this purpose we define quality scores \textit{(qscores)}, which attributes and methods are explained in the two following subsections.

4.1 Attributes

A \textit{qscore} object \textit{qs} is a list which contains a function (\textit{qs$FUN}), a name (\textit{qs$name}), and optionally a label (\textit{qs$label}) and arguments to be passed to \textit{qs$FUN} (\textit{qs$args}). In the following example, the quality score \textit{pct.spot.qscore} evaluates the percentage of spots that have passed the filtering steps of normalization; it provides an evaluation of the array quality for a given normalization process. The function \textit{to.qscore} is explained in subsection 4.2.

\begin{verbatim}
> pct.spot.FUN <- function(arrayCGH, var) {
+ 100 * sum(!is.na(arrayCGH$arrayValues[[var]]))/dim(arrayCGH$arrayValues)[1]
+ }
> pct.spot.name <- "SPOT_PCT"
> pct.spot.label <- "Proportion of spots after normalization"
> pct.spot.qscore <- to.qscore(pct.spot.FUN, name = pct.spot.name,
+                        args = alist(var = "LogRatioNorm"), label = pct.spot.label)
\end{verbatim}

4.2 Methods

4.2.1 \textit{to.qscore}

The function \textit{to.qscore} is used of the creation of \textit{qscore} objects, with the specificities described in subsection 4.1.

\begin{verbatim}
> args(to.qscore)

function (FUN, name = NULL, args = NULL, label = NULL, dec = 3)
NULL
\end{verbatim}

4.2.2 \textit{qscore.arrayCGH}

Function \textit{qscore.arrayCGH} simply computes and returns the value of \textit{qscore} for \textit{arrayCGH}:

\begin{verbatim}
> args(qscore.arrayCGH)

function (qscore, arrayCGH)
NULL
\end{verbatim}
4.2.3 qscore.summary.arrayCGH

Function qscore.summary.arrayCGH computes all quality scores of a list (using function qscore.arrayCGH), and displays the results in a convenient way.

```r
> args(qscore.summary.arrayCGH)

function (arrayCGH, qscore.list)

NULL
```

5 Data

We provide examples of array-CGH data coming from two different platforms. These data illustrate the need for appropriate within-array normalization methods, and especially the need for methods that handle spatial effects.

```r
> data(spatial)
```

For each array we provide raw data (generated by Genepix or SPOT), as well as the corresponding arrayCGH object before and after normalization.

These arrays illustrate the main source of non biological variability of these data sets, namely spatial effects. We classify these effects into two non exclusive types: local bias and global gradients. In the case of local bias, entire areas of the array show lower or higher signal values than the rest of the array, with no biological explanation (array edge); to our experience, this particular type of artifact roughly affects an array out of two. In the case of global gradients, the array shows an obvious signal gradient from one side of the slide to the other (array gradient).

5.1 edge

Bladder cancer tumors were collected at Henri Mondor Hospital (Créteil, France) and hybridized on arrays CGH composed of 2464 Bacterian Artificial Chromosomes (F. Radvanyi, D. Pinkel et al., unpublished results); each of these BAC is spotted three times on the array, and the three replicates are neighbors on the array. We give the example of an arrayCGH with local spatial effects (figure 1): high log-ratios cluster in the upper-right corner of the array.

5.2 gradient

We give the example of two arrays from a breast cancer data set from Institut Curie (O. Delattre, A. Aurias et al., unpublished results). These arrays consist of 3342 clones, organized as a 4 × 4 superblock that is replicated three times. This data set is affected by the two types of spatial effects: local bias areas (as for the previous data set), and spatial gradients from one side of the array to the other. The array gradient illustrates this second type of spatial effect.

6 Graphical representations

As for any type of data analysis, appropriate graphical representations are of major importance for data understanding. Array-CGH data are typically ratios or log-ratios, that correspond to
> data(spatial)
> arrayPlot(edge, "LogRatio", main = "Local spatial effects", zlim = c(-1, + 1), mediancenter = TRUE, bar = "h")

Figure 1: array with local spatial effects.
> data(spatial)
> arrayPlot(gradient, "LogRatio", main = "Spatial gradient", zlim = c(-2, + 2), mediancenter = TRUE, bar = "h")

Figure 2: Example of array with spatial gradient.
locations on the array (spots) and to locations on the genome (clones). Therefore in the case of
array-CGH data normalization, two complementary types of representations are necessary:

- a dotplot of the array, that takes into account the array design. This is a crucial tool in
the case of array-CGH data normalization for two reasons: first it provides an easy way

to identify spatial artifacts such as row, column, print-tip group effects, as well as spatial
bias and spatial gradients on the array; then it performs a post-normalization control, to
ensure that the normalization procedure reached its goals, i.e. significantly reduced the
observed effects.

- a plot of the signal values along the genome, which gives a visual impression of the array
quality on the edge of biological relevance; comparing the signal shape before and after
normalization provides a qualitative idea of the improvement in data quality provided by
the normalization method.

The arrayPlot method provided by the GLAD package and based on maImage [2] addresses
the first point; we add two methods to this toolbox:

- the genome.plot method displays a plot of any signal value (e.g. log-ratios) along the
genome;

- the report.plot method successively calls arrayPlot and genome.plot in order to pro-
vide a simultaneous vision of the data using the two relevant metrics (array and genome),
with appropriate color scales.

6.1 genome.plot

This method provides a convenient way to plot a given signal along the genome; the signal values
can be colored according to their level (which is the default comportment of the function) or to
the level of any other variable, in the following way:

- if the variable is numeric (e.g. signal to noise ratio), the function assumes that it is a
quantitative variable and adapts a color palette to its values (figure 3)

- if the variable is not numeric (e.g. the copy number variation as estimated by GLAD, or
a character variable making the distinction between flagged and un-flagged clones), the
function counts the number of modalities of the variable and defines an appropriate color
scale using the rainbow function (figure 4).

6.2 report.plot

This method successively calls arrayPlot and genome.plot; it checks for color scale consistency
between plots, and can automatically set the plot layout (figure 5).

7 Sample MANOR sessions

In this section we illustrate the use of MANOR on two CGH arrays. Our examples contain
several steps, including data preparation, flag definition, array normalization, quality criteria
definition, and quality assessment of the array, and highlights of the normalization process.
> data(spatial)
> genome.plot(edge.norm, chrLim = "LimitChr", cex = 1)

Figure 3: Pan-genomic profile of the array. Colors are proportional to log-ratio values.

> data(spatial)
> edge.norm$cloneValues$ZoneGNL <- as.factor(edge.norm$cloneValues$ZoneGNL)
> genome.plot(edge.norm, col.var = "ZoneGNL", chrLim = "LimitChr",
+ cex = 1)

Figure 4: Pan-genomic profile of the array. Colors correspond to the values of the variable "ZoneGNL".
7.1 array edge

7.1.1 Data preparation: import

```r
> dir.in <- system.file("data", package = "MANOR")
> spot.names <- c("LogRatio", "RefFore", "RefBack", "DapiFore",
+ "DapiBack", "SpotFlag", "ScaledLogRatio")
> clone.names <- c("PosOrder", "Chromosome")
> edge <- import(paste(dir.in, "/edge.txt", sep = ""), type = "spot",
+ spot.names = spot.names, clone.names = clone.names, add.lines = TRUE)
```

[1] "number of lines does not match array design: adding empty lines..."

7.1.2 Normalization: norm

Figure 5 shows the results of the normalization process.

```r
> data(flags)
> data(spatial)
> local.spatial.flag$args <- alist(var = "ScaledLogRatio", by.var = NULL,
+ nk = 5, prop = 0.25, thr = 0.15, beta = 1, family = "gaussian")
> flag.list <- list(spatial = local.spatial.flag, spot = spot.corr.flag,
+ ref.snr = ref.snr.flag, dapi.snr = dapi.snr.flag, rep = rep.flag,
+ unique = unique.flag)
> edge.norm <- norm(edge, flag.list = flag.list, FUN = median,
+ na.rm = TRUE)
```

[1] "spatial"
[1] "mean of unbiased zone : -0.0233232743043007"
[1] "Spatial bias has been detected"
zone.number  mu  effectif  effectif.cumul  frequency.cumul  biased.zone
4          5   0.467833333   66      66  0.00918964 1
3          4   0.046085967  1582  1648  0.22946255 0
5          3   0.005084592   2648  4296  0.59816207 0
1          2  -0.032626474  1866  6162  0.85797828 0
2          1  -0.080052941  1020  7182  1.00000000 0

[1] "spot"
[1] "ref.snr"
[1] "dapi.snr"
[1] "rep"
[1] "unique"

> edge.norm <- sort(edge.norm, position.var = "PosOrder")

> report.plot(edge.norm, chrLim = "LimitChr", zlim = c(-1, 1),
+            cex = 1)

![Pan-genomic representation](image)

Figure 6: array 'edge' after normalization.

7.1.3 Quality assessment: qscore.summary.arrayCGH

> profileCGH <- as.profileCGH(edge.norm$cloneValues)
> profileCGH <- daglad(profileCGH, smoothfunc = "lawsglad", l kern = "Exponential",
+                     model = "Gaussian", qlambda = 0.999, bandwidth = 10, base = FALSE,
+                     round = 2, lambdabreak = 6, lambdaclusterGen = 20, param = c(d = 6),
+                     alpha = 0.001, msize = 5, method = "centroid", nmin = 1,
+                     mmax = 8, amplicon = 1, deletion = -5, deltaN = 0.1, forceGL = c(-0.15,
+                     0.15), nbsigma = 3, MinBkpWeight = 0.35, verbose = FALSE)

[1] "Smoothing for each Chromosome"
[1] "Optimization of the Breakpoints"
[1] "Check Breakpoints Position"
> edge.norm$cloneValues <- as.data.frame(profileCGH)
> edge.norm$cloneValues$ZoneGNL <- as.factor(edge.norm$cloneValues$ZoneGNL)
> data(qscores)
> qscore.list <- list(smoothness = smoothness.qscore, var.replicate = var.replicate.qscore, +
  dynamics = dynamics.qscore)
> edge.norm$quality <- qscore.summary.arrayCGH(edge.norm, qscore.list)
> edge.norm$quality

<table>
<thead>
<tr>
<th>name</th>
<th>label</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOCAL_SMOOTHNESS</td>
<td>Local signal variability along the genome</td>
<td>0.021</td>
</tr>
<tr>
<td>VAR_REPLICATE</td>
<td>Average variability among replicates</td>
<td>0.011</td>
</tr>
<tr>
<td>SIGNAL_DYNAMICS</td>
<td>Dynamics of the DNA copy number variation</td>
<td>0.399</td>
</tr>
</tbody>
</table>

7.1.4 Highlights of the normalization process: html.report

Function html.report generates an HTML file with key features of the normalization process: array image and genomic profile before and after normalization, spot-level flag report, and value of the quality criteria.

> html.report(edge.norm, dir.out = ".", array.name = "an array with local bias", +
  chrLim = "LimitChr", light = FALSE, pch = 20, zlim = c(-2, +
  2), file.name = "edge")

The results of the previous command can be viewed in the file `edge.html`.

7.2 array gradient

Here we give the example of the normalization of an array with spatial gradient.

7.2.1 Data preparation: import

> spot.names <- c("Clone", "FLAG", "TEST_B_MEAN", "REF_B_MEAN", +
  "TEST_F_MEAN", "REF_F_MEAN", "ChromosomeArm")
> clone.names <- c("Clone", "Chromosome", "Position", "Validation")
> ac <- import(paste(dir.in, "/gradient.gpr", sep = ""), type = "gpr", +
  spot.names = spot.names, clone.names = clone.names, sep = "\t", +
  comment.char = "@", add.lines = TRUE)

[1] "number of lines does not match array design: adding empty lines..."
[1] "calculating array design..."

> ac$arrayValues$F1 <- log(ac$arrayValues["TEST_F_MEAN"], 2)
> ac$arrayValues$F2 <- log(ac$arrayValues["REF_F_MEAN"], 2)
> ac$arrayValues$B1 <- log(ac$arrayValues["TEST_B_MEAN"], 2)
> ac$arrayValues$B2 <- log(ac$arrayValues["REF_B_MEAN"], 2)
> Ratio <- (ac$arrayValues["TEST_F_MEAN"] - ac$arrayValues["TEST_B_MEAN"])/(ac$arrayValues +
  ac$arrayValues["REF_B_MEAN"])
> Ratio[(Ratio <= 0) | (abs(Ratio) == Inf)] <- NA
> ac$arrayValues$LogRatio <- log(Ratio, 2)
> gradient <- ac
7.2.2 Normalization: `norm`

Figure[7] shows the results of the normalization process.

```r
> data(spatial)
> data(flags)
> flag.list <- list(local.spatial = local.spatial.flag, spot = spot.flag,
+                 SNR = SNR.flag, global.spatial = global.spatial.flag, val.mark = val.mark.flag,
+                 position = position.flag, unique = unique.flag, amplicon = amplicon.flag,
+                 chromosome = chromosome.flag, replicate = replicate.flag)
> gradient.norm <- norm(gradient, flag.list = flag.list, FUN = median,
+             na.rm = TRUE)

[1] "local.spatial"
[1] "mean of unbiased zone : 8.4367711745437"
[1] "There is no spatial bias"
```

<table>
<thead>
<tr>
<th>zone.number</th>
<th>mu effectif</th>
<th>effectif.cumul</th>
<th>frequency.cumul</th>
<th>biased.zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>6</td>
<td>8.438691</td>
<td>1484</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>8.437202</td>
<td>1408</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>8.434943</td>
<td>1455</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>8.434869</td>
<td>1399</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>8.434634</td>
<td>1410</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>8.431504</td>
<td>1394</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>8.429094</td>
<td>1482</td>
<td>0</td>
</tr>
</tbody>
</table>

[1] "spot"
[1] "SNR"
[1] "global.spatial"
[1] "val.mark"
[1] "position"
[1] "unique"
[1] "amplicon"
[1] "chromosome"
[1] "replicate"

```r
> gradient.norm <- sort(gradient.norm)
```

7.2.3 Quality assessment: `qscore.summary.arrayCGH`

```r
> profileCGH <- as.profileCGH(gradient.norm$cloneValues)
> profileCGH <- daglad(profileCGH, smoothfunc = "lawsglad", lkern = "Exponential",
+              model = "Gaussian", qlambda = 0.999, bandwidth = 10, base = FALSE,
+              round = 2, lambdabreak = 6, lambdaclusterGen = 20, param = c(d = 6),
+              alpha = 0.001, msize = 5, method = "centroid", nmin = 1,
+              nmax = 8, amplicon = 1, deletion = -5, deltaN = 0.1, forceGL = c(-0.15,
+              + 0.15), nbsigma = 3, MinBkpWeight = 0.35, verbose = FALSE)
```
> genome.plot(gradient.norm, chrLim = "LimitChr", cex = 1)

Figure 7: array gradient after normalization.

[1] "Smoothing for each Chromosome"
[1] "Optimization of the Breakpoints"
[1] "Check Breakpoints Position"

> gradient.norm$cloneValues <- as.data.frame(profileCGH)
> gradient.norm$cloneValues$ZoneGNL <- as.factor(gradient.norm$cloneValues$ZoneGNL)
> data(qscores)
> qscore.list <- list(smoothness = smoothness.qscore, var.replicate = var.replicate.qscore,
+   dynamics = dynamics.qscore)
> gradient.norm$quality <- qscore.summary.arrayCGH(gradient.norm,
+   qscore.list)
> gradient.norm$quality

<table>
<thead>
<tr>
<th>name</th>
<th>label</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOCAL_SMOOTHNESS</td>
<td>Local signal variability</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>along the genome</td>
<td></td>
</tr>
<tr>
<td>VAR_REPLICATE</td>
<td>Average variability</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>among replicates</td>
<td></td>
</tr>
<tr>
<td>SIGNAL_DYNAMICS</td>
<td>Dynamics of the DNA copy</td>
<td>0.295</td>
</tr>
<tr>
<td></td>
<td>number variation</td>
<td></td>
</tr>
</tbody>
</table>

7.2.4 Highlights of the normalization process: html.report

Function html.report generates an HTML file with key features of the normalization process:
array image and genomic profile before and after normalization, spot-level flag report, and value
of the quality criteria.

> html.report(gradient.norm, dir.out = ".", array.name = "an array with spatial gradient",
+   chrLim = "LimitChr", light = FALSE, pch = 20, zlim = c(-2,
+   2), file.name = "gradient")
The results of the previous command can be viewed in the file gradient.html.

8 Session information

The version number of R and packages loaded for generating this document are:

```r
> sessionInfo()
```

R version 2.9.0 (2009-04-17)
x86_64-unknown-linux-gnu

locale:
LC_CTYPE=en_US;LC_NUMERIC=C;LC_TIME=en_US;LC_COLLATE=en_US;LC_MONETARY=C;LC_MESSAGES=en_US;LC_PAPER=en_US;LC_NAME=C;LC_ADDRESS=C;LC_TELEPHONE=C;LC_MEASUREMENT=en_US;LC_IDENTIFICATION=C

attached base packages:
[1] tools stats graphics grDevices utils datasets methods
[8] base

other attached packages:
[1] MANOR_1.16.0 GLAD_2.0.0

9 Supplementary data

The package MANOR provides sample gpr and spot files, as examples to the import function. However, due to space limitations, only the first 100 lines these file are provided in the current distribution of MANOR. The full files can be downloaded from here:

- 'gpr' file: gradient.gpr
- 'spot' file: edge.txt

References


