

Bioconductor's nnNorm package

Laurentiu A. Tarca¹

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1. CRBF, Laval University, CANADA, <http://www.crbf.ulaval.ca>

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1 Overview

The **nnNorm** package contains mainly a function for intensity and spatial normalization of cDNA two color data, or paired single channel data, based on neural networks fitting. Functionality to compare the distributions of the normalized log ratios is also provided. For the simpler case when only intensity normalization is performed (univariate distortion color model), we provide functionality to plot the bias estimate against the level of intensity for every print tip group.

Introduction to intensity and spatial normalization. This document provides a tutorial for using the **nnNorm** package. For a detailed description of the principles and algorithms implemented by this package consult Tarca et al. (2005).

Spatial and intensity normalization implemented in nnNorm. The **nnNorm** package implements intensity and spatial normalization of cDNA data based on neural networks. In this approach the log average intensity **A** as well as the spatial coordinates **X** and **Y** (obtained by binning the print tip group space) are used as regressors into a neural network model. Resistance to outliers is enhanced by assigning to each spot a weight which is dependent on how extreme its log ratio, **M**, is with respect to that of the spots having about the same **A** level belonging to the same print tip group. A 4-fold crossvalidation procedure is used to obtain the bias estimate for every spot.

Changes with respect to the previous version. The current version 1.1.0 of the **nnNorm** package differs from the ancestor 1.0.1 version by implementing a 4-fold cross-validation procedure to predict the bias estimates, instead of using all data in the model training as was the case in the previous version. The 4-fold cross-validation trains neural models on three quarters of data and make the prediction on the remaining fraction. Although by doing so the computation time is increased, the results are more robust. The models parameters saving option was removed and the

seed of the random number generator is fixed to obtain result which are exactly reproducible from one trial to another on the same data.

Help files. As with any R package, detailed information on functions, their arguments and value, can be obtained in the help files. For instance, to view the help file for the function `maNormNN` in a browser, use `help.start()` followed by `? maNormNN`.

2 Case study: Apo AI dataset, Callow et al.

We demonstrate the functionality of this package using gene expression data from the Apo AI study of Callow et al. (2000). To load the Apo dataset in a object called `Apo` of type `marrayRaw`, we use the following lines:

```
> library(marray)
> dataweb <- "http://www.stat.berkeley.edu/users/terry/zarray/Data/ApoA1/rg_aiko_morph.txt"
> data <- read.table(dataweb, header = TRUE, sep = ",", dec = ".")
> ApoLayout <- new("marrayLayout", maNgr = 4, maNgc = 4, maNsr = 19,
+   maNsc = 21)
> spots <- 4 * 4 * 19 * 21
> Gb <- Rb <- array(c(0), c(spots, 16))
> Gf <- as.matrix(data[, seq(2, 33, 2)])
> Rf <- as.matrix(data[, seq(3, 33, 2)])
> labs <- c(paste(c("c"), 1:8, sep = ""), paste(c("k"), 1:8, sep = ""))
> maInfo <- new("marrayInfo", maLabels = labs, maInfo = data.frame(Names = labs))
> Apo <- new("marrayRaw", maRf = Rf, maGf = Gf, maRb = Rb, maGb = Gb,
+   maLayout = ApoLayout, maTargets = maInfo)
```

Note that the background values were set to zero as the data file contains background corected red and green intensities.

Due to several factors, the bias in the microarray experiments may depend not only on the average log intensity level but also on the spatial coordinates Yang et al. (2002). Figure 1 shows the reconstructed image of log ratios M , for slide No. 16 in the Apo AI data set. This image is obtained using the `maImage` function of `marray` package.

```
> RGcol <- maPalette(low = "green", high = "red", k = 20)
> maImage(Apo[, 16], x = "maM", col = RGcol, zlim = c(-2, 2))
```

Observe the clearly uneven distribution of M values in the print tip groups situated at the bottom of the figure.

Now we perform normalization with the method of the `nnNorm` package called `maNormNN`. This function returns a `marrayNorm` type object (containing the normalized log ratios). The `maNormNN` function is a print tip oriented normalization method.

```
> library(nnNorm)
> ApNN2DA <- maNormNN(Apo)
```

Figure 2 shows the log ratios, for the same data as in Figure 1 after intensity and spatial normalization. Observe a rather uniform distribution of red and green colored spots.

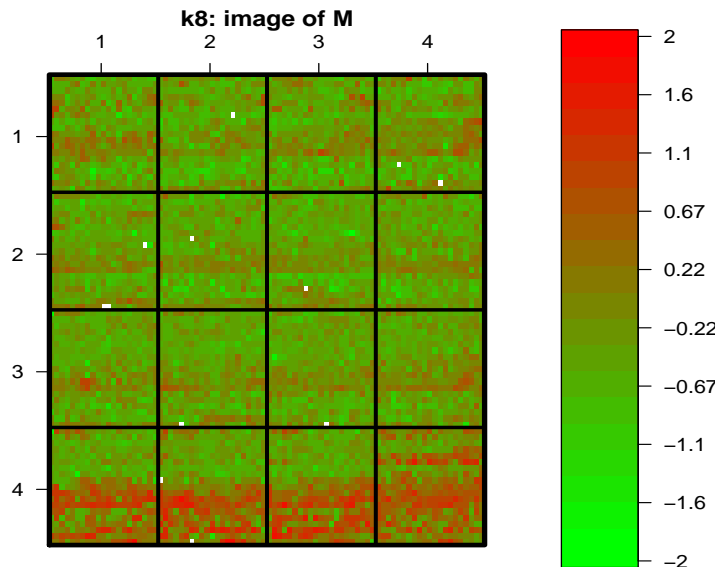


Figure 1: Image of raw M values for Apo data set, slide No. 16.

```
> maImage(ApNN2DA[, 16], x = "maM", col = RGcol, zlim = c(-2, 2))
```

We may compare the robust neural networks normalization method with other existing approaches that takes into account intensity and spatial bias fluctuations, like for e.g. a composite normalization method available in `marray` package.

```
> AcPLo2D <- maNormMain(Apo, f.loc = list(maNorm2D(), maNormLoess(x = "maA",
+   y = "maM", z = "maPrintTip")), a.loc = maCompNormEq())
```

We define a function able to compute the Westfall and Young (1993) adjusted p-values for different normalizations of the same Apo batch. For this we use functionality of the `multtest` package (Dudoit et al. (2002)).

```
> library(multtest)
```

```
Loading required package: Biobase
```

```
Welcome to Bioconductor
```

```
Vignettes contain introductory material. To view,
  simply type: openVignette()
For details on reading vignettes, see
  the openVignette help page.
```

```
Loading required package: genefilter
```

```
Loading required package: survival
```

```
Loading required package: splines
```

```
Loading required package: reposTools
```

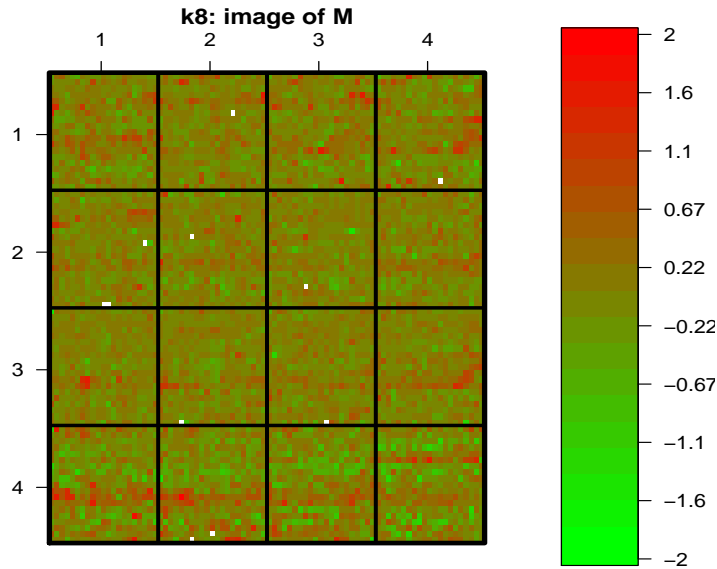


Figure 2: Image of M values after nnNorm normalization, for Apo data set, slide No. 16.

```
> getP <- function(maObj) {
+   X <- maM(maObj)
+   class <- c(rep(0, 8), rep(1, 8))
+   resT <- mt.maxT(X, class, B = 15000)
+   ord <- order(resT$index)
+   resT$adjp[ord]
+ }
```

Now we get the adjusted p-values for the three situations: raw data (Anone), composite normalization (cpLo2DA) and robust neural networks based (pNN2DA).

```
> pAnone <- log10(getP(Apo))
> pAcPLo2D <- log10(getP(AcPLo2D))
> pApNN2DA <- log10(getP(ApNN2DA))
```

We plot now the log of adjusted p-values obtained using the normalized data. Results are shown in Figure 3.

```
> kout <- c(2149, 4139, 5356, 540, 1739, 1496, 2537, 4941)
> methods <- c("none", "cPLo2D", "pNN2DA")
> plot(rep(1, length(pAnone)), pAnone, xlim = c(1, 3), ylim = c(-4,
+   0), xlab = "Method", ylab = "log10(p)", axes = FALSE)
> points(rep(1, 8), pAnone[kout], col = "red", pch = 20)
> points(rep(2, length(pAcPLo2D)), pAcPLo2D)
```

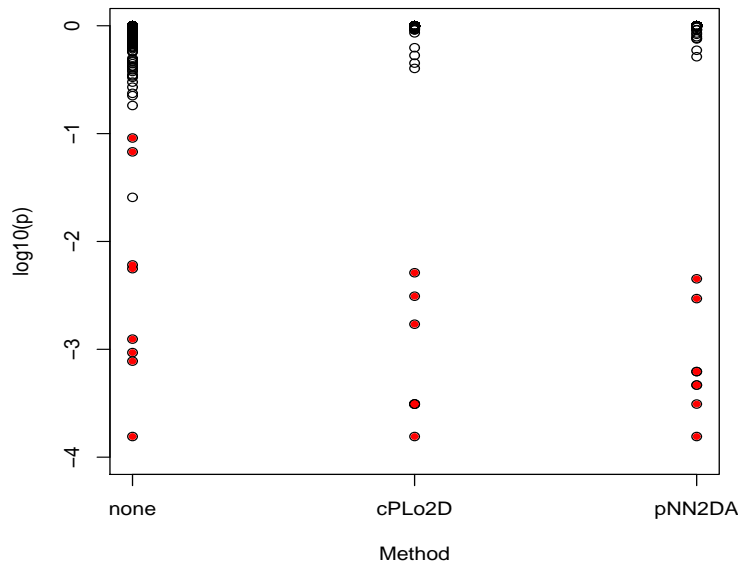


Figure 3: Adjusted p values for raw data (none), composite normalization (cpLo2D), and robust neural networks (pNN2DA)

```
> points(rep(2, 8), pAcPLo2D[kout], col = "red", pch = 20)
> points(rep(3, length(pApNN2DA)), pApNN2DA)
> points(rep(3, 8), pApNN2DA[kout], col = "red", pch = 20)
> axis(1, 1:3, methods)
> axis(2)
> box()
```

The spots with the indices `kout` above are those with smallest t-statistics after loess print tip normalization as in Yang et al. (2001).

Observe in Figure 3 that the 8 genes (represented by filled red circles) may be confidently distinguished from the bulk of non-differentially expressed genes at a threshold of $p=0.01$. As some of the genes may have received about the same adjusted p-value, less than 8 filled circles may be observed for a particular method.

Now we may want to compare the ability of this new normalization method to reduce the variability of log ratios within slides. A easy way to do that is to look at the distributions of the normalized M values. The method `compNorm` of `nnNorm` package will do this (see Figure 4).

```
> compNorm(maM(Apo), maM(AcPLo2D), maM(ApNN2DA), bw = 0.9, xlim = c(-2,
+ 2), titles = methods, type = "d")
```

The same distributions may be inspected using the box plot in Figure 5.

```
> compNorm(maM(Apo), maM(AcPLo2D), maM(ApNN2DA), bw = 0.9, xlim = c(-2,
+ 2), titles = methods, type = "b")
```

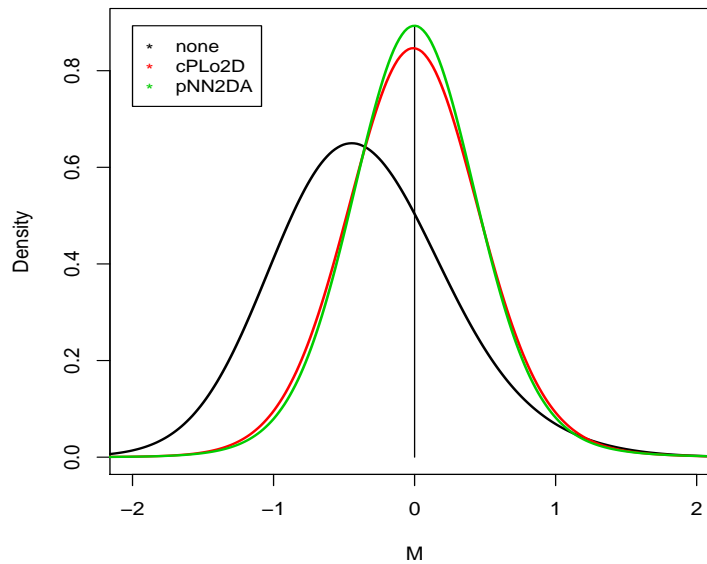


Figure 4: Density plots of normalized log ratios. The highest density curve corresponds to the robust neural networks normalization.

The `maNormNN` function in `nnNorm` package offers flexibility in choosing the type of dependence of the bias to be accounted for. For e.g. setting `binHeight` parameter as equal with the number of spot rows in a print tip group, will discard the variable `Y` in the bias model.

```
> ApoNNy <- maNormNN(Apo[, 1], binWidth = 3, binHeight = maNsr(Apo))
```

In this case only `A` and `X` will be used as regressors for the bias. Similarly by setting `binWidth=maNsc(Apo)` in the same function will suppress the contribution of the `X` variable from the bias estimation. The `M-A` plots may be shown before and after the normalization for each slide if the parameter `maplots` is set to "TRUE".

For the case when only intensity normalization is performed (`X`, and `Y` regressors discarded) we provide the facility to plot the bias estimates against the intensity level. For e.g. lets perform intensity normalization only for the 13th slide of the `Apo` batch:

```
> par(mfrow = c(2, 1))
> par(mar = c(2, 4, 2, 2))
> ApoNr <- maNormNN(Apo[, 13], binWidth = maNsc(Apo), binHeight = maNsr(Apo),
+   robust = TRUE, maplots = TRUE)
```

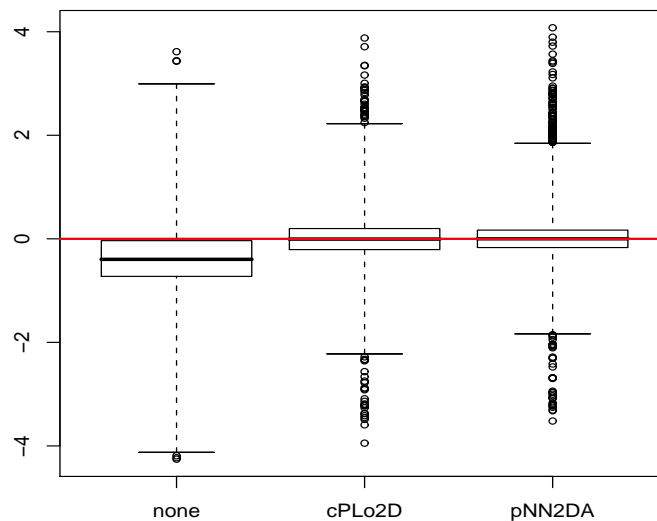


Figure 5: Box plot of normalized log ratios

References

- M. J. Callow, S. Dudoit, E. L. Gong, T. P. Speed, and E. M. Rubin. Microarray expression profiling identifies genes with altered expression in hdl-deficient mice. *Genome Research*, 10:2022–2029, 2000.
- S. Dudoit, J. P. Shaffer, and J. C. Boldrick. Multiple hypothesis testing in microarray experiments. *Statistical Science*, to appear, preprint available at UC Berkeley, Division Biostatistics working paper series: 2002-110, <http://www.bepress.com/ucbbiostat/paper110>, 2002.
- A. L. Tarca, J. E. K. Cooke, and J. Mackay. Robust neural networks approach for spatial and intensity dependent normalization of cdna data. *Bioinformatics*. (*accepted*), 2005.
- P. H. Westfall and S. S. Young. *Resampling-based multiple testing: Examples and methods for p-value adjustment*. John Wiley & Sons, 1993.
- Y. Yang, S. Dudoit, P. Luu, D. Lin, V. Peng, J. Ngai, and T. Speed. Normalization for cdna microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res.*, 30:e15, 2002.
- Y. Yang, S. Dudoit, P. Luu, and T. Speed. Normalization for cdna microarray data. *SPIE BiOS*, 2001.

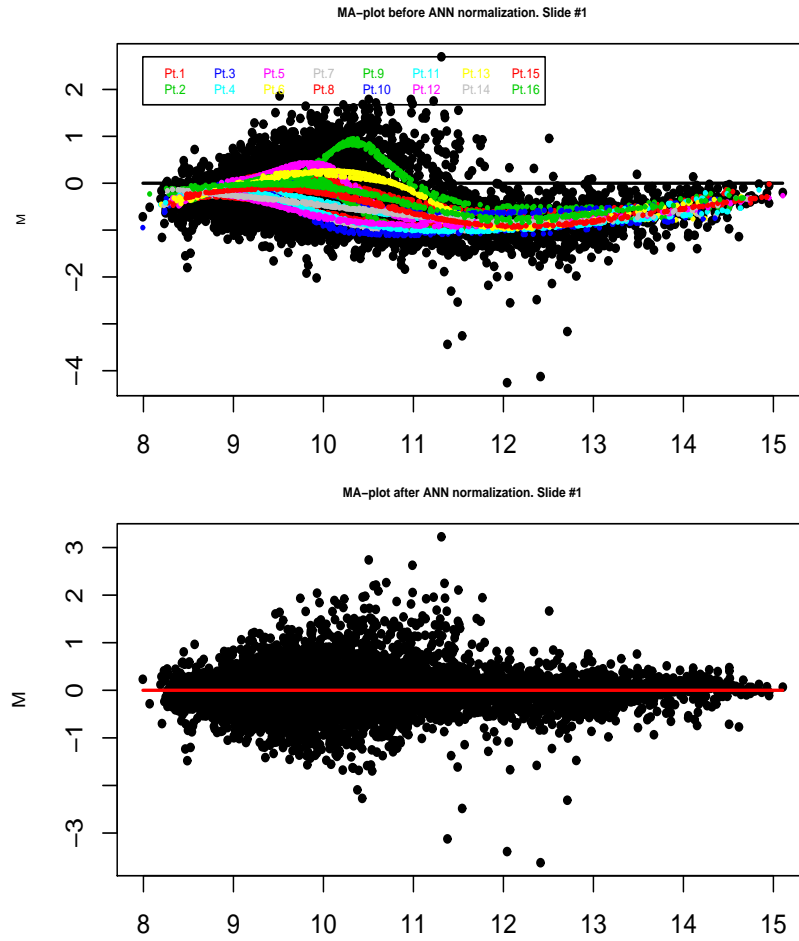


Figure 6: M-A plots before and after normalization for the whole slide.