TurboNorm: A fast scatterplot smoother with applications for microarray normalization

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Contents

1 Introduction

This vignette show how piecewise constant P-splines [1] can be used for normalization of either single- or two-colour data. The `pspline()`-function can be used for two-colour data objects of type `RGList` and `MarrayRaw` from respectively from *limma* [2] and from the package *marray*. For single colour microarray data wrapper functions are writting based on the *affy* [3] functions `normalize.loess()` and `normalize.AffyBatch.loess()` namely `normalize.pspline()` and `normalize.AffyBatch.pspline()`. Also a `panel`-function, `panel.pspline()`, is available for adding the smoothed curve to *lattice* [4] graphic panels.

The P-spline smoother introduced by Eilers and Marx [1] is a combination of B-splines with a difference penalty on the regression coefficients. P-splines belong to the family of penalized splines using B-spline basis functions, where the penalization is on the curvature of the smoothed function. For the P-splines of Eilers and Marx [1], a discrete approximation to the integrated squared second derivative of the B-splines is made. This results in an easy-to-construct penalty matrix, and the resulting band-diagonal system of equations can be efficiently solved. Using piecewise constant B-splines as a basis makes the construction of the B-spline basis even easier. The resulting linear system of equations can be solved either using a QR decomposition or a Cholesky decomposition [5].

Additionally to the P-spline smoother proposed by [1] we introduce a weighted P-spline smoother. The weighted P-spline smoother leads to the following system of equations:

$$(X'WX + \lambda D'D)\hat{\boldsymbol{\beta}} = X'W\mathbf{y}, \tag{1}$$

where X is the B-spline basis matrix (with X' its transpose), W is a diagonal matrix of weights, D is a matrix operator for the second-order differences and $\mathbf{y}$ represents the vector of observations. The value of penalty parameter, λ, can be determined by cross-validation, for example. The original P-spline smoother of Eilers and Marx [1] has W, the identity matrix. When piecewise constant basis functions are used, both $X'WX$ and $X'W\mathbf{y}$ become diagonal matrices, and can be constructed very efficiently [6]. The regression coefficients of the weighted P-spline smoother are now given by:

$$\hat{\boldsymbol{\beta}} = (X'WX + \lambda D'D)^{-1}X'W\mathbf{y}. \tag{2}$$

See for a detailed description of the method and several applications van Iterson *et al.* [7].

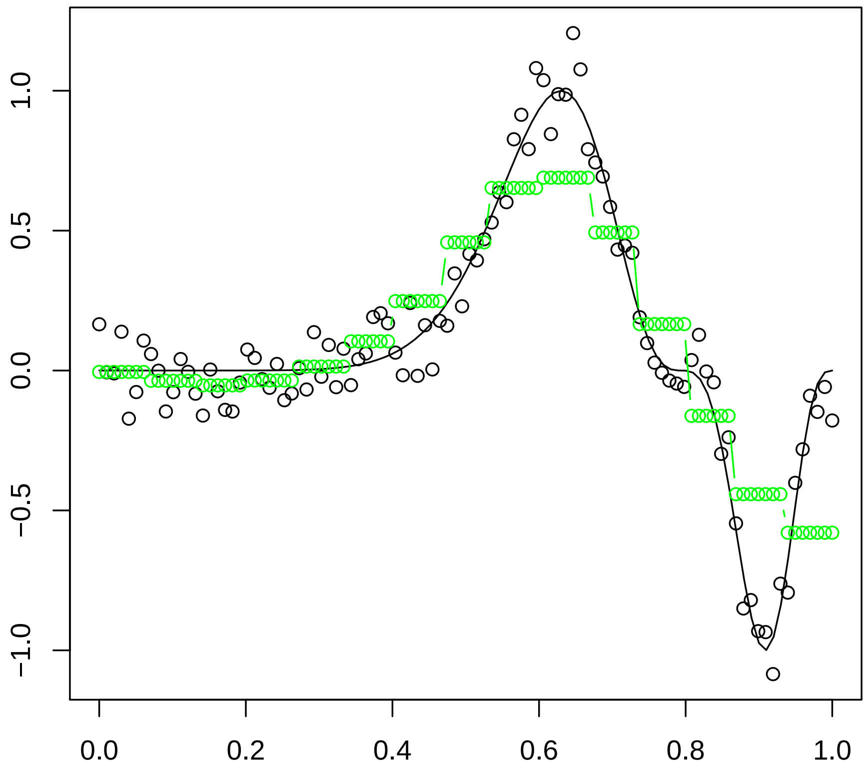
2 Smoothing using piecewise constant P-splines

The main workhorse of the package is the function `turbotrend()`. Given data the function returns an object containing the smoothed values and some additional information like, effective degrees of freedom, optimized penalty value, λ, and the generalized cross-validation error at the optimal penalty value.
The following toy example shows the use of the `turbotrend()`. First we load the library and generate some data:

```
> library(TurboNorm)
> funky <- function(x) sin(2*pi*x^3)^3
> m <- 100
> x <- seq(0, 1, length=m)
> y <- funky(x) + rnorm(m, 0, 0.1)
```

Next we plot the data and the underlying function that generated the data together with the smoothed curves based on the original piecewise constant B-spline basis.

```
> plot(x, y, type='p', xlab="", ylab="")
> curve(funky, add=TRUE)
> fitOrig <- turbotrend(x, y, n=15, method="original")
> lines(fitOrig, col="green", type='b', pch=1)
```



In order to get some more detail on the regression parameters a `show`-method is implemented.

```
> fitOrig

Call:
turbotrend(x = x, y = y, n = 15, method = "original")

Effective degrees of freedom: 5.812496
Number of bins: 15
Penalty value: 11.97688
Number of robustifying iterations: 0
GCV : 0.05451783
```

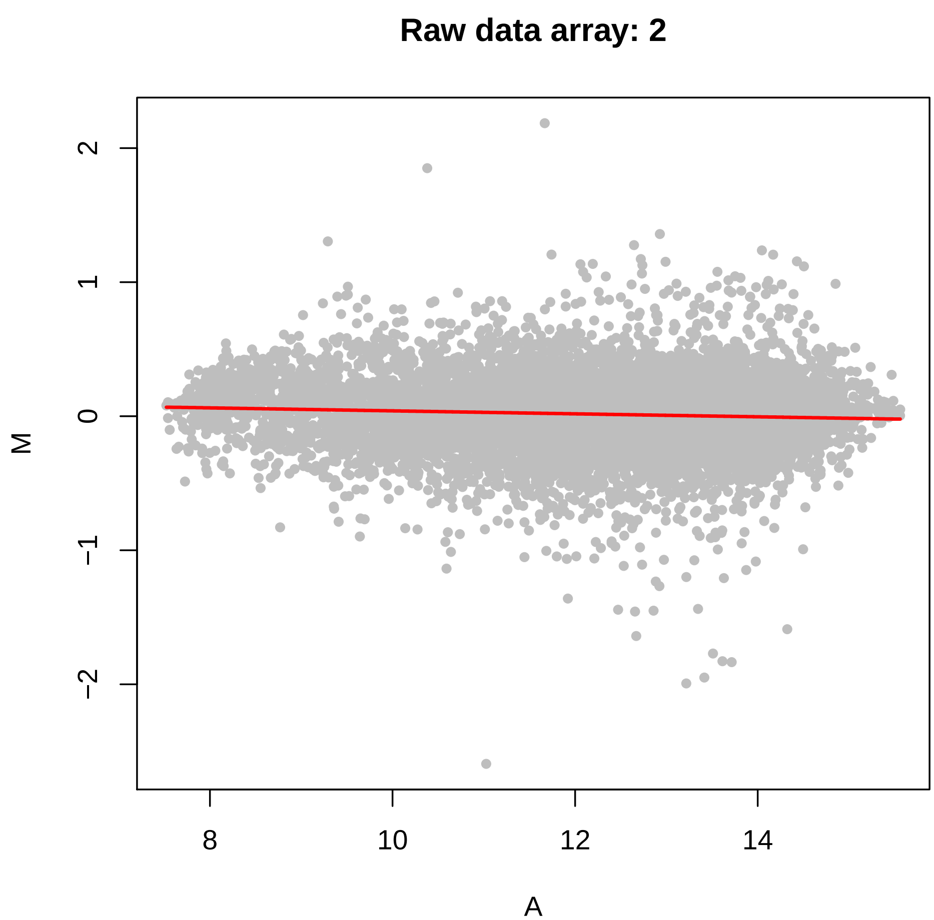
3 Normalization of single- and two-colour data

For single colour microarray data normalization the following functions are available `normalize.pspline()` and `normalize.AffyBatch.pspline()` these functions are based on functions for normalization from the *affy* package.

The `pspline()`-function can be used for normalization of two-colour microarray data. The data input is either an object of type `RGList` as defined in the package *limma* or an object of type `MarrayRaw` defined in the package *marray*. The `pspline()`-function recognizes the type of the object and returns the normalized object of the same type, i.e. `MAList` and `MarrayNorm`.

Here is an example code using the `swirl`-data from *marray*. Using the option `showArrays=2` the smoothed curve is plotted together with the data in a MA plot for array 2 (by default no plot is shown).

```
> library(marray)
> data(swirl)
> x <- pspline(swirl, showArrays=2, pch=20, col="grey")
```



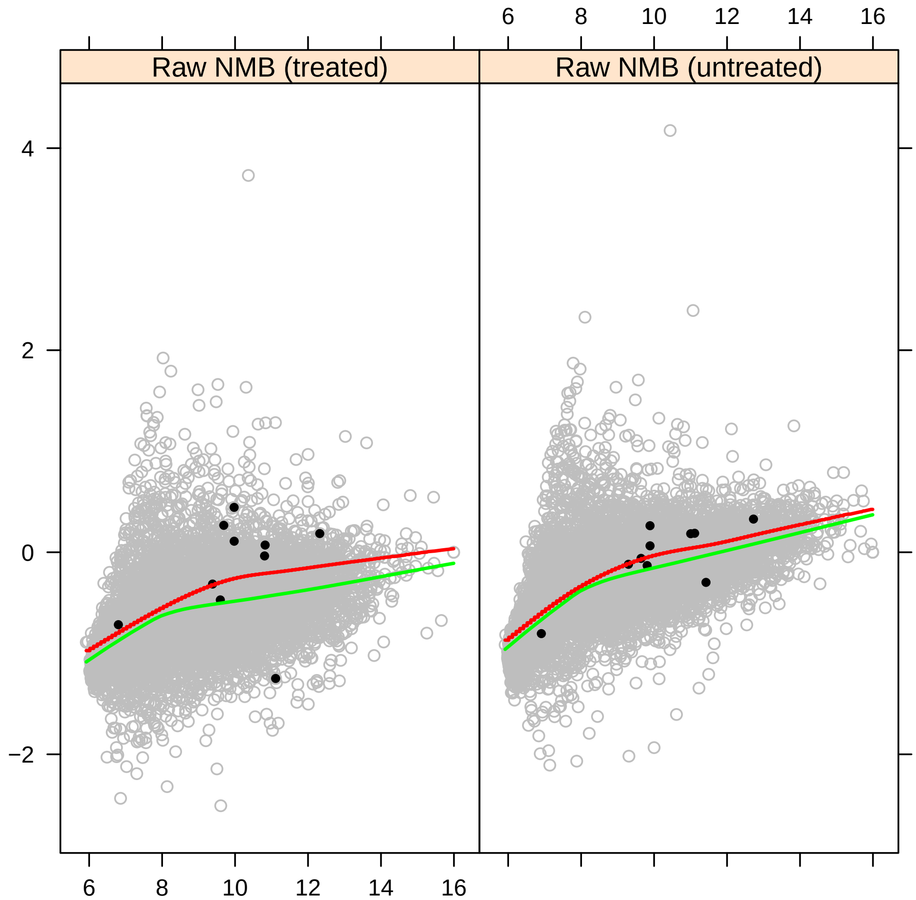
4 Normalization of array-based DNA methylation data

Here we show how a weighted normalization can be performed. This is especially useful for array-based DNA methylation data, where there is large number of differential methylation expected. Using `data(methylation)` a random subset of the data of one of the cell lines described in the paper by van Iterson *et al.* [?] is loaded as an `RGList`. The element `weights` of the `RGList` contains the subset of invariant fragments, those without methylation-sensitive restriction sites, as a logical matrix where each colunm represents an array those fragments that are part of the subset are `TRUE` and those that are not `FALSE`. The data dependent weight is in this example approximately 250.

```
> library(TurboNorm)
> data(methylation)
> indices <- methylation$weights[,1]
> weights <- rep(1, length(indices))
> weights[indices] <- length(indices)/sum(indices)
> MA <- normalizeWithinArrays(methylation, method="none", bc.method="none")
> labels <- paste("NMB", c("(untreated)", "(treated)"))
> labels <- paste(rep(c("Raw"), each=2), labels)
>
```

First we transform the intensities to M- and A-values without background correction and then the normalization is performed both weighted P-spline and ordinary lowess using *limma*. Now we use the *lattice* in order to illustrate the difference. We highlight the invariant subset in black.

```
> data <- data.frame(M=as.vector(MA$M),
+                    A=as.vector(MA$A),
+                    Array=factor(rep(labels, each=nrow(MA$A)), levels=rev(labels)))
> library(lattice)
> print(xyplot(M~A|Array, xlab="", ylab="", data=data, type='g',
+        panel = function(x, y) {
+                panel.xyplot(x, y, col="grey")
+                                                        lpoints(x[indices], y[indices
+                                                        panel.pspline(x, y, weights =
+                                                        panel.loess(x, y, col="green"
+              }))
```



This example also shows how the `panel.pspline()`-function can be used. The smoothed curve obtained by the P-spline smoother can be added to *lattice* graphics.

5 Details

This document was written using:

- R version 3.0.2 (2013-09-25), `x86_64-unknown-linux-gnu`
- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_US.UTF-8`, `LC_COLLATE=C`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: Biobase 2.22.0, BiocGenerics 0.8.0, TurboNorm 1.10.0, convert 1.38.0, lattice 0.20-24, limma 3.18.0, marray 1.40.0

- Loaded via a namespace (and not attached): BiocInstaller 1.12.0, affy 1.40.0, affyio 1.30.0, grid 3.0.2, preprocessCore 1.24.0, tools 3.0.2, zlibbioc 1.8.0

References

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[2] Gordon K. Smyth. Limma: linear models for microarray data. In R. Gentleman, V. Carey, S. Dudoit, and W. Huber R. Irizarry, editors, *Bioinformatics and Computational Biology Solutions using R and Bioconductor*, pages 397–420. Springer, New York, 2005.

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[4] D. Sarkar. *lattice: Lattice Graphics*, 2009. R package version 0.17-26.

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[7] M. van Iterson, F. A. Duijkers, J. P. Meijerink, P. Admiraal, G. J. van Ommen, J. M. Boer, M. M. van Noesel, and R. X. Menezes. A novel and fast normalization method for high-density arrays. *Statistical Applications in Genetics and Molecular Biology*, 11(4), 2012.