

# TurboNorm: A fast scatterplot smoother with applications for microarray normalization

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## 1 Introduction

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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:

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

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,  $\lambda$ , 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:

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

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,  $\lambda$ , 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)
```

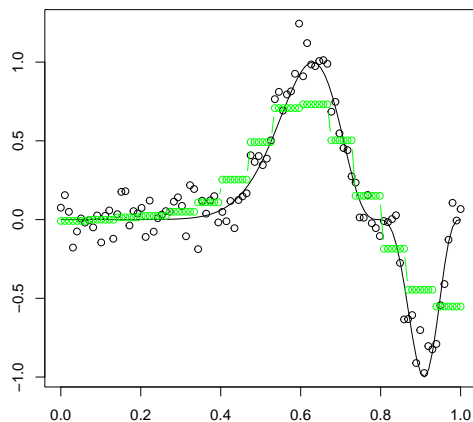
Next we plot the data and the underlying function that generated the data together with the smoothed curves based on the original piecewise constant B-spline basis.

```
> 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: 6.059443
Number of bins: 15
Penalty value: 9.973889
Number of robustifying iterations: 0
GCV : 0.05732148
```



**Figure 1:** Simple turbotrend fitting example: Piecewise constant B-splines fitted to data generated from a funky function with noise.

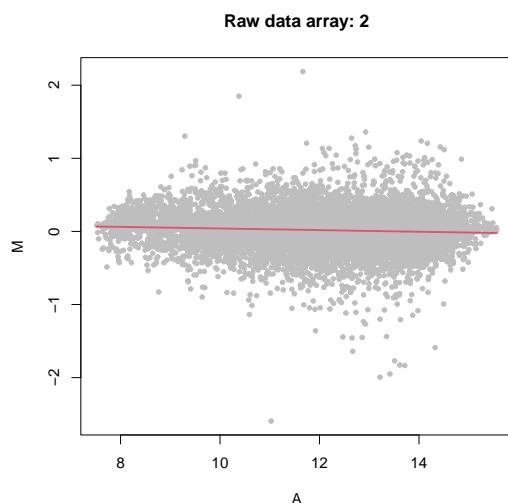
### 3 Normalization of single- and two-colour data

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")
```



**Figure 2:** Normalization of array data using pspline: Normalization of the swirl microarray data using the pspline-function, the fit to array two is shown.

## 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.

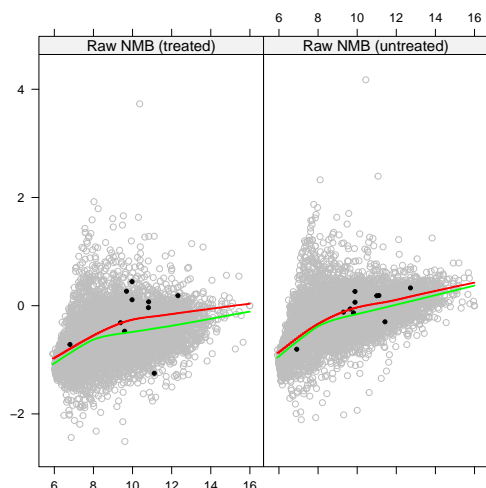
Using `data(methylation)` a random subset of the data of one of the cell lines described in the paper by van Iterson *et al.* [7] is loaded as an `RGList`. The element `weights` of the `RGList` contains the subset of invariant fragments, those without methylation-sensitive restriction sites, as a logical matrix where each column 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], pch=20, col="black")
+   panel.pspline(x, y, weights = weights, col="red", lwd=2)
+   panel.loess(x, y, col="green", lwd=2)
+ }))
```



**Figure 3:** Normalization of methylation array data using `panel.pspline`: Comparing lowess and pspline for fitting methylation array data using an invariant subset of the data. Lowess fit in green, pspline fit in red and the subset of invariant points are given as black dots.

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 4.5.1 Patched (2025-08-23 r88802), x86\_64-pc-linux-gnu
- Locale: LC\_CTYPE=en\_US.UTF-8, LC\_NUMERIC=C, LC\_TIME=en\_GB, 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
- Time zone: America/New\_York
- TZcode source: system (glibc)
- Running under: Ubuntu 24.04.3 LTS
- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.22-bioc/R/lib/libRblas.so

- LAPACK: /usr/lib/x86\_64-linux-gnu/lapack/liblapack.so.3.12.0
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: Biobase 2.70.0, BiocGenerics 0.56.0, TurboNorm 1.58.0, convert 1.86.0, generics 0.1.4, lattice 0.22-7, limma 3.66.0, marray 1.88.0
- Loaded via a namespace (and not attached): BiocManager 1.30.26, BiocStyle 2.38.0, affy 1.88.0, affyio 1.80.0, cli 3.6.5, compiler 4.5.1, digest 0.6.37, evaluate 1.0.5, fastmap 1.2.0, grid 4.5.1, htmltools 0.5.8.1, knitr 1.50, preprocessCore 1.72.0, rlang 1.1.6, rmarkdown 2.30, statmod 1.5.1, tools 4.5.1, xfun 0.53, yaml 2.3.10

## 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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