multiscan: Combining multiple laser scans of microarrays

Mizanur R. Khondoker*† Chris A. Glasbey [‡] Bruce J. Worton [§]
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1 Introduction

The sensitivity level of microarray scanners is adjustable and plays a crucial role in getting reliable measurement of the fluorescence intensity. A change in scanner setting transforms the intensity measurements by a multiplicative constant. A scanner's sensitivity has to be raised to a certain level to ensure that the intensity levels of weakly expressed genes exceed the intrinsic noise level of the scanner and so become measurable. This may, however, cause another problem: signal censoring

^{*}Package maintainer, Email: Mizanur.Khondoker@ed.ac.uk

[†]Division of Pathway Medicine, The University of Edinburgh Medical School, The Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, UK.

[‡]Biomathematics & Statistics Scotland, King's Buildings, Edinburgh, EH9 3JZ, Scotland, UK. Email:chris@bioss.ac.uk.

[§]School of Mathematics, University of Edinburgh, King's Buildings, Edinburgh, EH9 3JZ, Scotland, UK. Email: Bruce.Worton@ed.ac.uk.

for highly expressed genes. Scanners cannot record pixel intensities above some software dependent threshold ($2^{16} - 1 = 65535$, for a 16-bit computer storage system), so highly expressed genes can have pixel values which are right censored at the largest possible value that the scanner software allows. It is not usually possible to find a scanner setting which is optimal for both weakly and highly expressed genes. So, it seems reasonable to consider multiple scanning of the same microarray at different scanner settings and estimate spot intensities from these combined data.

The multiscan package implements the method of Khondoker et al. (2006) for estimating gene expressions from multiple laser scans of hybridised microarrays. The method is based on a non-linear functional regression model with both additive and multiplicative error terms. Maximum likelihood estimation based on a Cauchy distribution is used to fit the model, which reduces the sampling variability in expression estimates and is able to estimate gene expressions taking account of outliers and the systematic bias caused by signal censoring of highly expressed genes.

The package contains a function multiscan, and a small data set murine for illustrating the use of the function. The function produces output of class multiscan. S3 print and plot methods are also defined for this new class.

After installation, the package can be loaded using

> library(multiscan)

2 Package documentation

2.1 Help files

After installing and loading the package, the help file with detailed information of the multiscan function, its usage, arguments and values, can be viewed by issuing the command help(multiscan) or ?multiscan at the R command prompt.

2.2 Package vignettes

To view the package vignette (this document) you need to use

> vignette("multiscan")

and a PDF file will open in your PDF reader. For Windows, the package vignette can also be accessed through the R menu bar item *Vignettes*.

3 Data

The only required argument for the multiscan function is the data argument which should be a numeric matrix or data frame containing the intensity data of a single microarray scanned at multiple (two or more) scanner settings. The data frame can be created by reading text or csv files into R, using read.table or read.csv command.

The number of rows (n) of the data matrix should be equal to the number of spots/probes on the array, and the number of columns (m) equal to the number of scans. Replicated probes on the array are treated as individual spots. Columns can be given in any order, but will be arranged in order of scanner's sensitivity before fitting the model. The data should be on the raw scale, i.e., not transformed!

An example data set, murine, has been included as part of the package, which can be loaded using

> data(murine)

and the first few rows of the data can be viewed by typing

> murine[1:10,]

```
scan1
                 scan2
                            scan3
                                      scan4
   1145.1677 1778.3423 3138.7314
                                   4516.025
1
2
    296.0000 472.0000 869.0000
                                   1374.000
3
    467.0000 692.0000 1179.8000
                                   1992.000
4
    795.4694 1247.3893 2176.6643
                                   3329.654
5
   2084.2793 3334.1765 6084.2651 10014.496
6
    525.0000
             688.6923 1169.9434
                                   1825.786
7
    266.2000
              384.1875
                        732.1111
                                   1072.600
8
    495.9074
              742.3148 1313.9796
                                   2009.833
    565.9101
             895.0319 1616.3943
                                   2495.142
10 1160.0336 1832.7114 3259.6541
                                   5295.662
```

To see a brief description of this example data use

> help(murine)

4 Example usage of multiscan

Suppose that we want to obtain estimates of gene expressions combining the 4 scans of the murine data set. This can be done using

```
> data(murine)
> fit<-multiscan(murine)</pre>
```

Estimating 1000 gene expressions using 4 scans of data

```
Log-likelihood at initial parameters: -21348.7187

End of global iteration: 1, Log-likelihood: -19542.94736
End of global iteration: 2, Log-likelihood: -19373.08458
End of global iteration: 3, Log-likelihood: -19351.64478
End of global iteration: 4, Log-likelihood: -19349.44816
End of global iteration: 5, Log-likelihood: -19348.63585
End of global iteration: 6, Log-likelihood: -19348.63585
```

> fit Call: multiscan(data = murine) Scanning effects: scan1 scan2 scan3 scan4 1.000000 1.566959 2.764289 4.328168 Scale parameters: Additive Multiplicative Nu 5.241983306 0.006761393 0.390186429 Log-likelihood at convergence: Loglf -19348.64 To view the components of the fitted model use > str(fit) List of 12 \$ call : language multiscan(data = murine) : num [1:4] 1 1.57 2.76 4.33 \$ beta \$ scale : num [1:3] 5.24198 0.00676 0.39019 : num [1:1000] 1137 313 460 792 2127 ... \$ mu \$ data : num [1:1000, 1:4] 1145 296 467 795 2084 attr(*, "dimnames")=List of 2\$: chr [1:1000] "1" "2" "3" "4"\$: chr [1:4] "scan1" "scan2" "scan3" "scan4" \$ fitted : num [1:1000, 1:4] 1137 313 460 792 2127 ... \$ sdres : num [1:1000, 1:4] 0.868 -3.042 1.077 0.421 -2.813 ... \$ outerit: int 6 \$ gconv : int 0

\$ convmu : int [1:1000] 0 0 0 0 0 0 0 0 0 ...

The estimated gene expressions can be obtained by extracting the mu component of the fitted object, i.e., by doing

> gene.exprs<-fit\$mu

: int 0 \$ loglf : num -19349

- attr(*, "class")= chr "multiscan"

A plot of the fitted model can be created by using the generic plot function. For example, the plot in Figure 1 was produced using

```
> plot(fit)
```

\$ conv

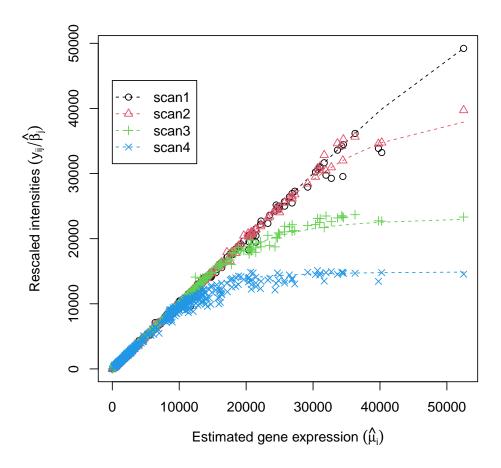


Figure 1: A plot of the rescaled intensities $(y_{ij}/\hat{\beta}_j)$ against the estimated gene expressions. The dashed lines represent the fitted model.

To view the description of the other optional arguments of the multiscan function please refer to the help file

> help(multiscan)

5 Model diagnostics

The standardised residuals of the fitted model on the input data are stored in the sdres component of the fitted multiscan object. These residuals can be plotted against the rank of the estimated expression values using

```
> op<-par(mfrow=c(2,2))
> plot(fit,residual=TRUE)
> par(op)
```

The resulting plot is shown in Figure 2.

6 Computing time and memory usage

The function is computationally slow and memory-intensive. That is due to the nested iteration loops of the numerical optimization of the likelihood function involving a large number (n+m+2) of parameters. The optimization uses an alternating algorithm with the Nelder-Mead simplex method (Nelder and Mead, 1965) in the inner loops. The function multiscan directly uses the C function nmmin, the internal code used in the general-purpose optimization tool optim, for implementing the Nelder-Mead simplex method.

For large data sets with many tens of thousands of probes, it is recommended to consider first fitting the model using a random subset (e.g. 10,000 rows) of the data matrix, and then using the estimated scanning effects and scale parameters obtained as initial values for fitting the model to the full data set.

7 Alternative software

A web interface, created by David Nutter of Biomathematics & Statistics Scotland (BioSS), based on the original Fortran code written by Khondoker et al. (2006) is available at http://www.bioss.ac.uk/ktshowcase/create.cgi. Although it uses the same algorithm, results from the web interface may not be exactly identical to that of multiscan as it uses a different (non-free IMSL routine) implementation of the Nelder-Mead simplex method.

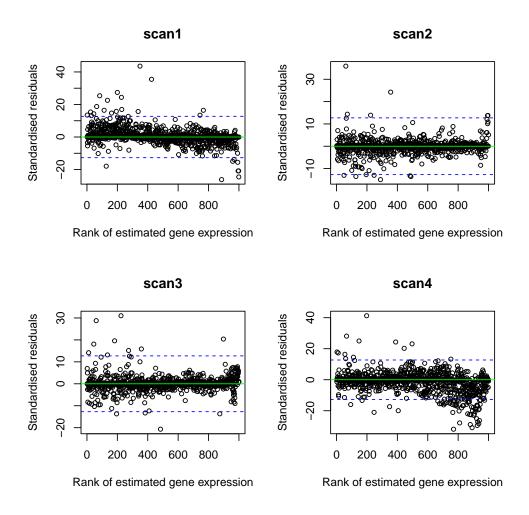


Figure 2: A plot of standardised residuals against the rank of estimated gene expressions. The dashed lines show 95% probability limits (± 12.71).

8 Acknowledgments

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9 References

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