Using FARMS for summarization
Using I/NI-calls for gene filtering

Djork-Arné Clevert

October 25, 2007

Contents

1 Introduction

The *farms* package provides a new summarization algorithm called FARMS - Factor Analysis for Robust Microarray Summarization and a novel unsupervised feature selection criterion called I/NI-calls.

2 FARMS

The summarization method is based on a factor analysis model for which a Bayesian Maximum a Posteriori method optimizes the model parameters under the assumption of Gaussian measurement noise Hochreiter et al. (2006). Thereafter, the RNA concentration is estimated from the model. *farms* does not use background correction and uses either quantile normalization Bolstad et al. (2003) or cyclic loess Yang et al. (2002); Dudoit et al. (2002). *farms* uses quantile normalization as default normalization procedure because it is computational efficient. It does not apply PM corrections and uses PMs only. We set **weigth** = **8**, **mu** = **0**, and **scale** = **2.0** for quantile normalization and **scale** = **1.5** for cyclic loess as default. We further set the default vaues for the maximal EM-Steps to **cyc** = **100** and the termination criteriea factor analysis to **tol** = **0.00001** if the λ-update vector has length smaller than . For the sake of convenience *farms* package provides three wrapper function for *affy*- `expresso`:

- `q.farms` is a wrapper function to `expresso` and uses no background correction and quantile normalization as default normalization procedure.
- `l.farms` performs like `q.farms`, but uses loess normalization as default normalization procedure.
- The function `exp.farms` is a transparent wrapper to `expresso` and permits further preprocessing options.

Note: If you use this package please cite Hochreiter et al. (2006) and Talloen et al. (2007). This package is only free for non-commercial users. Non-academic users **MUST** have a valid license.

2.1 Getting Started

As usual, it is necessary to load the package.

```
> library(farms)
```

In the following, we use the `affybatch.example` data set as it is provided by the *affy* package to illustrate how to compute expression measures with *farms*.

```
> data(affybatch.example)
> eset <- q.farms(affybatch.example)

background correction: none
normalization: quantiles
PM/MM correction : pmonly
expression values: farms
background correcting...done.
normalizing...done.
150 ids to be processed
|                    |
|####################|
```

This will store expression values, in the object `eset`, as an object of class `exprSet` (see the *Biobase* package).

```
> data(affybatch.example)
> eset <- exp.farms(affybatch.example, bgcorrect.method = "rma",
+     pmcorrect.method = "pmonly", normalize.method = "constant")

background correction: rma
normalization: constant
PM/MM correction : pmonly
expression values: farms
background correcting...done.
normalizing...done.
150 ids to be processed
|                    |
|####################|
```

The available preprocessing options can be queried by using `normalize.AffyBatch.methods`, `pmcorrect.methods` or `bgcorrect.methods`.

3 I/NI calls

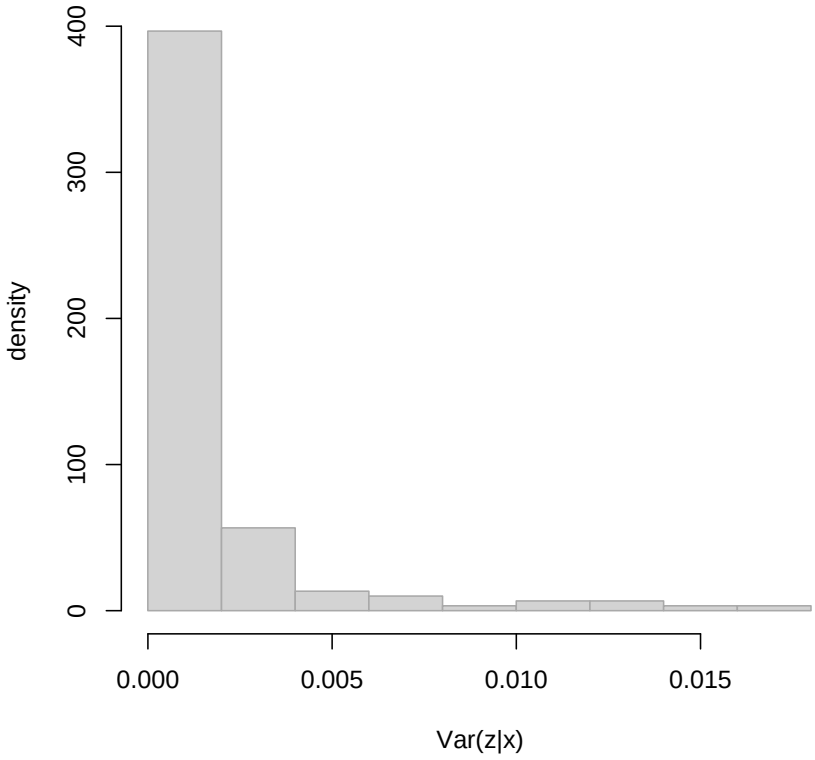
Informative/ non-informative (I/NI) calls is an objective feature filtering technique for Affymetrix GeneChips. It uses the multiple probes measuring the same target mRNA as repeated measures to quantify the signal-to-noise ratio of that specific probe set. By incorporating probe level information to assess the noisy nature of probe sets, I/NI calls provide a highly powerful and objective

tool for gene filtering. I/NI calls consequently offers a key solution to the main problem in the analysis of high-dimensional microarray data, being multiple testing and overfitting. I/NI calls can be used in combination with summarization techniques like FARMS, but also with any other summarization technique like MAS5 or (GC)RMA.

The following example shows how this summarization method can be used as a filtering tool, based on informative / non-informative calls.

```
> data(affybatch.example)
> eset <- exp.farms(affybatch.example, bgcorrect.method = "rma",
+     pmcorrect.method = "pmonly", normalize.method = "constant")

background correction: rma
normalization: constant
PM/MM correction : pmonly
expression values: farms
background correcting...done.
normalizing...done.
150 ids to be processed
|                    |
|####################|

> IN_genes <- propINIcalls(eset)
> cat(paste(IN_genes, " percentage of the genes are informative",
+     sep = " "))

0  percentage of the genes are informative

> data(affybatch.example)
> eset <- exp.farms(affybatch.example, bgcorrect.method = "rma",
+     pmcorrect.method = "pmonly", normalize.method = "constant")

background correction: rma
normalization: constant
PM/MM correction : pmonly
expression values: farms
background correcting...done.
normalizing...done.
150 ids to be processed
|                    |
|####################|

> eset

ExpressionSet (storageMode: lockedEnvironment)
assayData: 150 features, 3 samples
  element names: exprs, se.exprs
phenoData
  sampleNames: 20A, 20B, 10A
  varLabels and varMetadata:
    sample: arbitrary numbering
featureData
  featureNames: A28102_at, AB000114_at, ..., HG2188-HT2258_at (150 total)
  varLabels and varMetadata: none
experimentData: use 'experimentData(object)'
Annotation [1] ""
```

```
> eset_INI <- INIcalls(eset)
> eset_INI

ExpressionSet (storageMode: lockedEnvironment)
assayData: 150 features, 3 samples
  element names: exprs, se.exprs
phenoData
  sampleNames: 20A, 20B, 10A
  varLabels and varMetadata:
    sample: arbitrary numbering
featureData
  rowNames: A28102_at, AB000114_at, ..., HG2188-HT2258_at (150 total)
  varLabels and varMetadata: none
experimentData: use 'experimentData(object)'
Annotation [1] ""
```



Enjoy!

References

B. M. Bolstad, R. A. Irizarry, M. Astrand, and T. P. Speed. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*, 19(2):185–193, 2003.

S. Dudoit, Y. H. Yang, M. J. Callow, and T. P. Speed. Statistical methods for identifying genes with differential expression in replicate cDNA microarray experiments. *Stat. Sin*, 12(1):111–139, 2002.

Sepp Hochreiter, Djork-Arne Clevert, and Klaus Obermayer. A new summarization method for affymetrix probe level data. *Bioinformatics*, page btl033, 2006. doi: 10.1093/bioinformatics/btl033. URL `http://bioinformatics.oxfordjournals.org/cgi/content/abstract/btl033v1`.

Willem Talloen, Djork-Arne Clevert, Sepp Hochreiter, Dhammika Amaratunga, Luc Bijnens, Stefan Kass, and Hinrich W.H. Goehlmann. I/ni-calls for the exclusion of non-informative genes: a highly effective filtering tool for microarray data. *Bioinformatics*, page btm478, 2007. doi: doi:10.1093/bioinformatics/btm478. URL `http://bioinformatics.oxfordjournals.org/cgi/content/short/btm478v1`.

Y. H. 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(4):e15, 2002.