RMA+ and RMA++ using the RefPlus package

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Dec 23, 2008

Abstract
In this vignette, we introduce the ideas behind Extrapolation Strategy (RMA+) and Extrapolation Averaging (RMA++) methods, and give examples of using the functions in this package.

1 Introduction
The Extrapolation Strategy and Extrapolation Averaging are Affymetrix GeneChip microarray data pre-processing methods proposed by Goldstein (2006). These methods were independently developed by Chang, Harbron and South (2006), termed RMA+ and RMA++. Katz et al. (2006) also independently developed the RMA+ method, termed refRMA. This vignette will use the “RMA+” and “RMA++” nomenclature for these algorithms. RMA+ is an extension to the RMA algorithm by Irizarry et al. (2004), and RMA++ is a further extension based on the RMA+ method.

The RMA+ algorithm calculates the microarray intensities using a pre-stored RMA model trained on a reference microarray set (can be standard reference microarrays, microarrays from an independent study, or an incomplete set of microarrays in a study). RMA+ measurements of a microarray can be considered as an approximation to the RMA measurements of this microarray when the microarray is RMAed with the reference set microarrays in one batch.

RMA++ measurements of a microarray are the average of multiple RMA+ measurements of a microarray based on several reference sets. If the reference sets cover more information of the microarrays to be pre-processed than a single reference set does, the RMA++ measurements will provide a better approximation to the RMA measurements.

2 RMA+

RMA+ procedure:
1. Fit the RMA model on the reference set and store the normalizing quantiles and the estimated probe effects;
2. Background correct the probe intensities of the microarrays to be pre-processed;

3. Normalize the background-corrected probe intensities to the normalizing quantiles (reference quantiles);

4. Derive the probeset intensity using the estimated probe effects and normalized background-corrected probe intensity data.

Step 1 can be done using the \texttt{rma.para} function in the package. The normalizing quantiles and the estimated probe effects are returned. Step 2-4 can be done using the \texttt{rmaplus} function.

Both functions provide an option of skipping the background correction step. In this case, the microarrays can be background-corrected independently.

3 \textbf{RMA++}

\textbf{RMA++ procedure}

1. Fit multiple RMA models on several reference sets and store the normalizing quantiles and the estimated probe effects of these reference sets;

2. Calculate the RMA+ measurements of the microarrays of interest for each reference set;

3. Average multiple RMA+ measurements of the microarray based on these reference sets.

4 \textbf{Example}

4.1 \textbf{RMA+}

The Dilution dataset in the \texttt{affydata} package consists of 4 microarray samples.

\begin{verbatim}
> ##Use Dilution in affydata package
> library(RefPlus)
> library(affydata)

Package LibPath Item
[1,] "affydata" "/home/biocbuild/bbs-3.4-bioc/R/library" "Dilution"
Title
[1,] "AffyBatch instance Dilution"

> data(Dilution)
> sampleNames(Dilution)
[1] "20A" "20B" "10A" "10B"
\end{verbatim}

Firstly, we calculate the RMA measurements of the 4 microarrays Ex0:

\begin{verbatim}
> ##Calculate RMA intensities using the rma function.
> Ex0<EXPRS(rma(Dilution))
\end{verbatim}
Background correcting
Normalizing
Calculating Expression

Secondly, we form a reference set using the first 3 samples and derive the reference quantiles and the reference probe effects:

```
> ##Background correct, estimate the probe effects, and calculate the
> ##RMA intensities using rma.para function.
> Para<-rma.para(Dilution[,1:3],bg=TRUE,exp=TRUE)
> Ex1 <- Para[[3]]
```

Then, we calculate the RMA+ measurements of all microarrays $Ex_2$. Figure 1 compares the RMA measurements and the RMA+ measurements of these 4 microarrays.

```
> ##Calculate the RMA+ intensity using rmaplus function.
> Ex2 <- rmaplus(Dilution, rmapara=Para, bg = TRUE)
```

Use rmapara.

4.2 RMA++

Now, we form another reference set using the 2-4 samples and calculate a new set of RMA+ measurements $Ex_3$.

```
> Para2 <- rma.para(Dilution[,2:4],bg=TRUE,exp=TRUE)
> Ex3 <- rmaplus(Dilution, rmapara=Para2, bg = TRUE)
```

Use rmapara.

We can then obtain a set of RMA++ measurements by averaging these two sets of RMA+ measurements $Ex_4$. Figure 2 compares the RMA measurements and the RMA++ measurements of these 4 microarrays.

```
> Ex4 <- (Ex2+Ex3)/2
```
Figure 1: RMA (Ex0) vs. RMA+ (Ex2).
> par(mfrow=c(2,2))
> plot(Ex0[,1],Ex4[,1],pch=".",main=sampleNames(Dilution)[1])
> plot(Ex0[,2],Ex4[,2],pch=".",main=sampleNames(Dilution)[2])
> plot(Ex0[,3],Ex4[,3],pch=".",main=sampleNames(Dilution)[3])
> plot(Ex0[,4],Ex4[,4],pch=".",main=sampleNames(Dilution)[4])

Figure 2: RMA (Ex0) vs. RMA++ (Ex4).
The root mean squares differences (RMSD) between RMA measurements and 2 RMA+ measurements, are

$$> \sqrt{\text{mean}((Ex0-Ex2)^2)}$$

[1] 0.2138337

$$> \sqrt{\text{mean}((Ex0-Ex3)^2)}$$

[1] 0.228387

and the RMSD between RMA measurements and RMA++ measurements is

$$> \sqrt{\text{mean}((Ex0-Ex4)^2)}$$

[1] 0.06549345

We can see that the RMA++ measurements can provide a better approximation to the RMA measurements, which is consistent with the comparison between figure 1 and figure 2.

References


