RMA+ and RMA++ using the `RefPlus` package

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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 `RMA`ed 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 `rma.para` function in the package. The normalizing quantiles and the estimated probe effects are returned. Step 2-4 can be done using the `rmaplus` function.

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

3 `RMA++`

`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 Example

4.1 `RMA+`

The Dilution dataset in the `affydata` package consists of 4 microarray samples.

```
> library(RefPlus)
> library(affydata)
> data(Dilution)
> sampleNames(Dilution)

[1] "20A" "20B" "10A" "10B"
```

Firstly, we calculate the `RMA` measurements of the 4 microarays $Ex0$:

```
> Ex0 <- exprs(rma(Dilution))

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:

```
> Para <- rma.para(Dilution[, 1:3], bg = TRUE, exp = TRUE)
> Ex1 <- Para[[3]]
```

Then, we calculate the RMA+ measurements of all microarrays $Ex2$. Figure 1 compares the RMA measurements and the RMA+ measurements of these 4 microarrays.

```
> Ex2 <- rmaplus(Dilution, rmapara = Para, bg = TRUE)
```

Use rmapara.

```
> par(mfrow = c(2, 2))
> plot(Ex0[, 1], Ex2[, 1], pch = ".", main = sampleNames(Dilution)[1])
> plot(Ex0[, 2], Ex2[, 2], pch = ".", main = sampleNames(Dilution)[2])
> plot(Ex0[, 3], Ex2[, 3], pch = ".", main = sampleNames(Dilution)[3])
> plot(Ex0[, 4], Ex2[, 4], pch = ".", main = sampleNames(Dilution)[4])
```
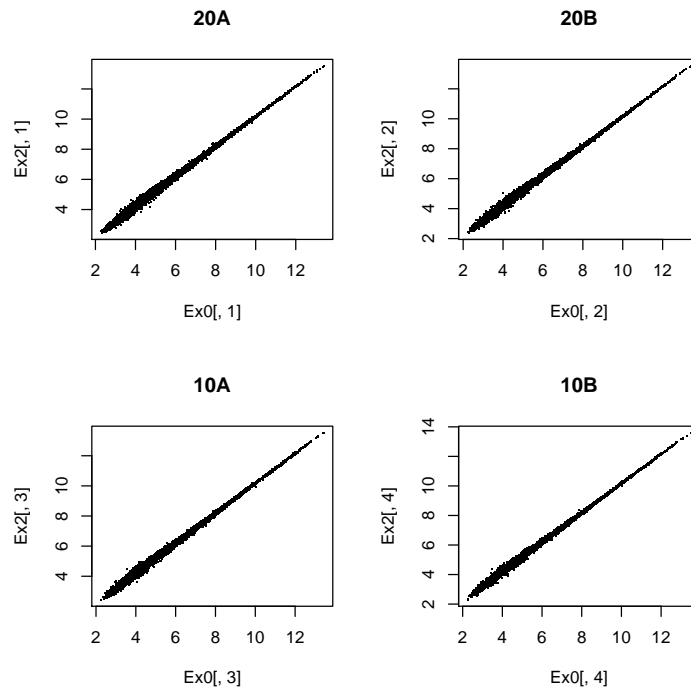

Figure 1: RMA (Ex0) vs. RMA+ (Ex2).

4.2   RMA++

Now, we form another reference set using the 2-4 samples and calculate a new set of RMA+ measurements $Ex3$.

```
> 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 $Ex4$. Figure 2 compares the `RMA` measurements and the `RMA++` measurements of these 4 microarrays.

```
> Ex4 <- (Ex2 + Ex3)/2
```

```
> 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])
```
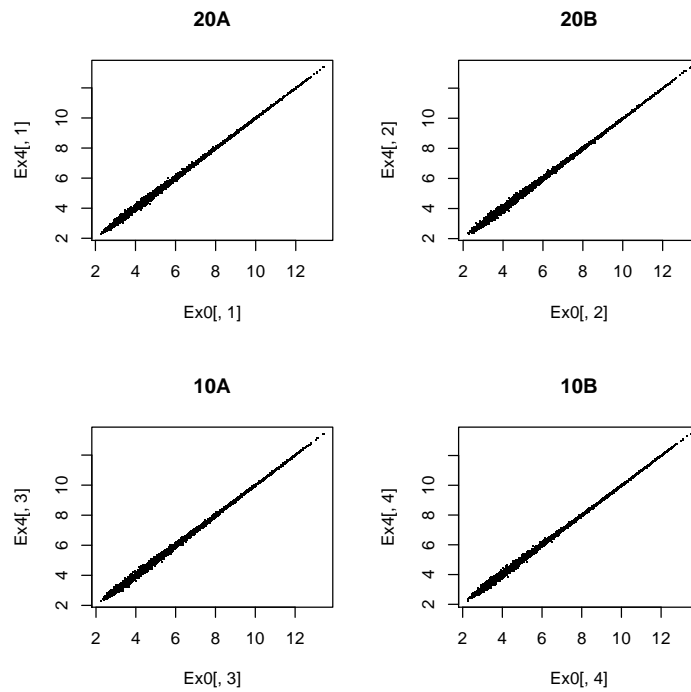

Figure 2: `RMA` (Ex0) vs. `RMA++` (Ex4).

The root mean squares differences(RMSD) between `RMA` measurements and 2 `RMA+` measurements, are

```
> sqrt(mean((Ex0 - Ex2)^2))
```

```
[1] 0.2138337
```

```
> sqrt(mean((Ex0 - Ex3)^2))
```

```
[1] 0.2283870
```

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

```
> sqrt(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 consistant with the comparison between figure 1 and figure 2.

References

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Irizarry,R.A., Hobbs,B., Collin,F., Beazer-Barclay,Y.D., Antonellis,K.J., Scherf,U. and Speed,T.P. (2003) "Exploration, Normalization, and Summaries of High density Oligonucleotide Array Probe Level Data," *Biostatistics,* 4, 249-264.

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