

# RMA+ and RMA++ using the RefPlus package

Kai-Ming Chang, Chris Harbron, Marie C South  
kaiming@kfsyscc.org  
Chris.Harbron@astrazeneca.com  
Marie.C.South@astrazeneca.com

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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 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 `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.

```
> ##Use Dilution in affydata package
> library(RefPlus)
> library(affydata)

Package
[1,] "affydata"
LibPath
[1,] "/Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/library"
Item      Title
[1,] "Dilution" "AffyBatch instance Dilution"

> data(Dilution)
> sampleNames(Dilution)

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

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

```
> ##Calculate RMA intensities using the rma function.
> 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:

```
> ##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 *Ex2*. 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 *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],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])

```

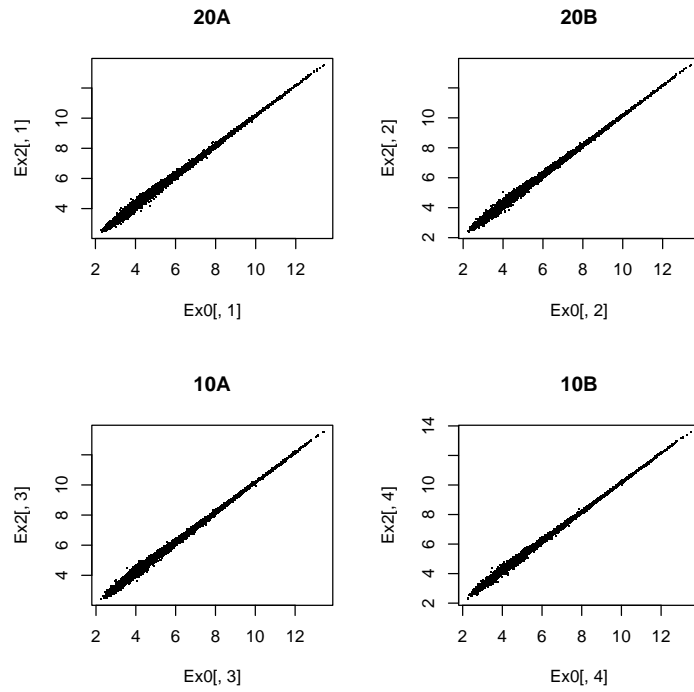


Figure 1: RMA (Ex0) vs. RMA+ (Ex2).

```

> 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])

```

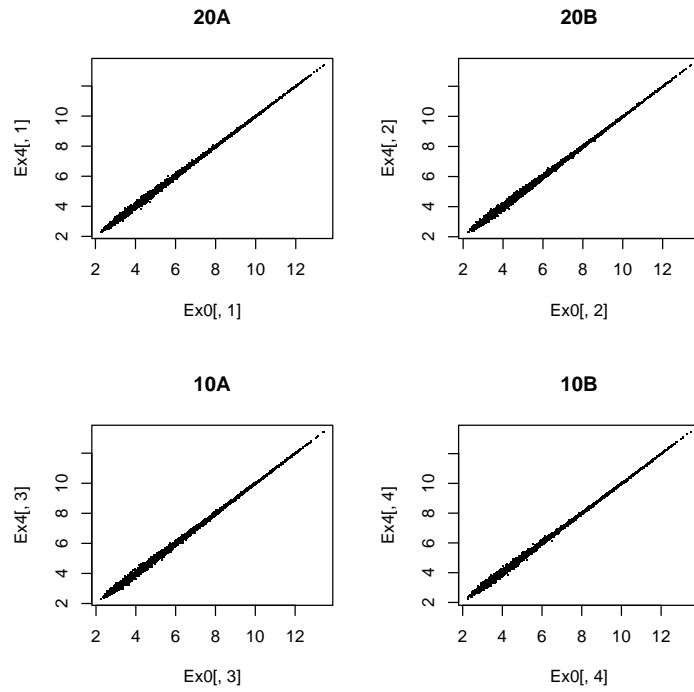


Figure 2: RMA (Ex0) vs. RMA++ (Ex4).

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] 0.228387
```

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 consistent with the comparison between figure 1 and figure 2.

## References

- Chang, K.M., Harbron, C., South, M.C. (2006) "An Exploration of Extensions to the RMA Algorithm," *Available with the RefPlus package*.
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- Harbron,C., Chang,K.M., South,M.C. (2007) "RefPlus : an R package extending the RMA Algorithm," *Bioinformatics*, 23, 2493-2494.
- 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.
- Katz,S., Irizarry,R.A., Lin,X., Tripputi,M., Porter,M. (2006) "A Summarization Approach for Affymetrix GeneChip Data Using a Reference Training Set from a Large, Biologically Diverse Database," *BMC Bioinformatics*, 7, 464.