GS01 0163
Analysis of Microarray Data

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Lecture 14: Comparing Microarray Analysis Methods

- Review of the last lecture
- Comparing processing methods
  - Shedden et al.
  - Wilcoxon rank-sum tests
  - Thresholds
  - Cope et al.

Review of the last lecture

We looked at the ALL-MLL-AML data from the paper by Armstrong et al. in *Nature Genetics*, 2002; 30:41-47.

We learned that the AML samples were run on the U95Av2 chip, while the ALL and MLL samples were run on the U95A.

We processed the data in dChip, including finding differentially expressed genes and clustering. We exported the data from dChip, imported it to R, and created an `exprSet`.

We also processed the CEL files in BioConductor using the `just.rma` function to produce another `exprSet`.

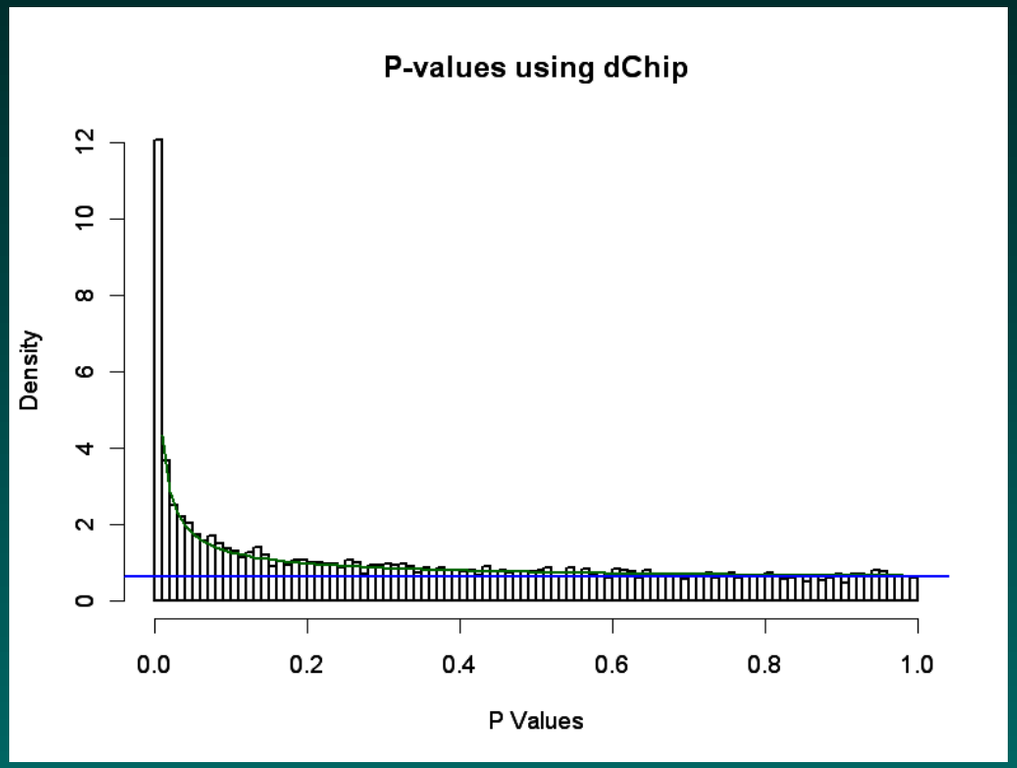
We loaded the `ClassComparison` package from `http://bioionformatics.mdanderson.org/Software/OOMPA` and had just started looking at differential expression.

Loading the ClassComparison package

```
> require(ClassComparison)
Loading required package: ClassComparison
Loading required package: splines
Loading required package: oompaBase
Loading required package: PreProcess
Creating a new generic function for ’plot’ in ’PrePro
Creating a new generic function for ’print’ in ’PreP
Creating a new generic function for ’as.data.frame’ i
[1] TRUE
```

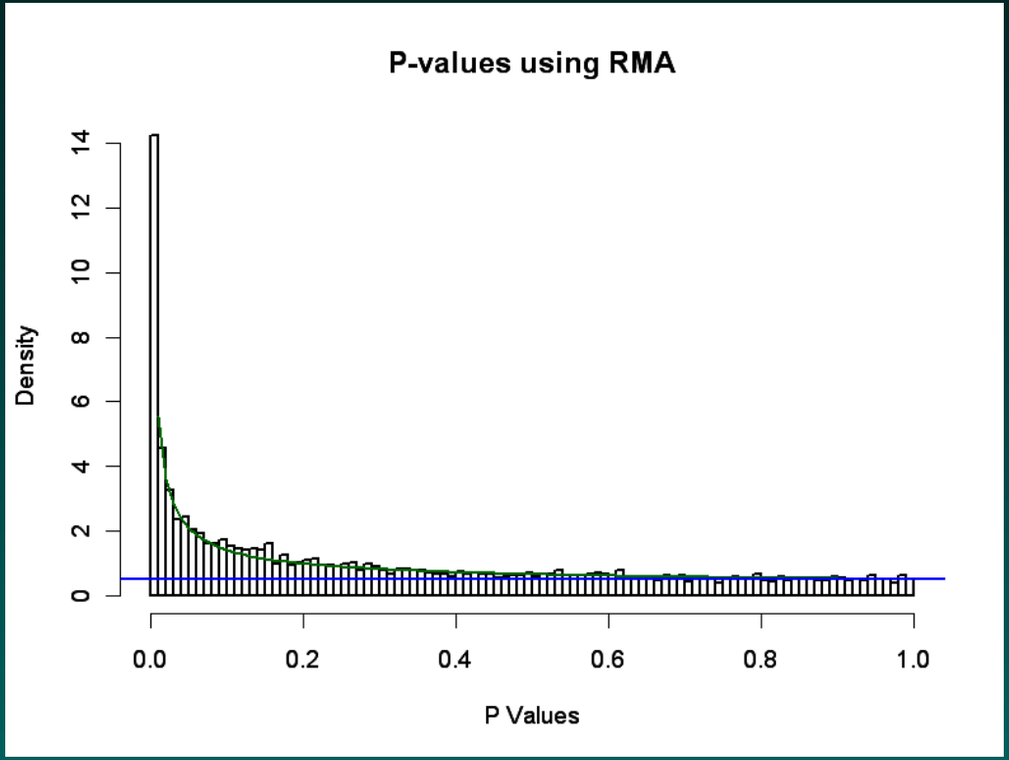
T-test, take one

```
> notAML <- pd@pData$type != 'AML'
> dchip.t <- MultiTtest(dchip[, notAML], 'ALLvMLL')
> dchip.b <- Bum(dchip.t@p.values)
> hist(dchip.b, main='P-values using dChip')
```



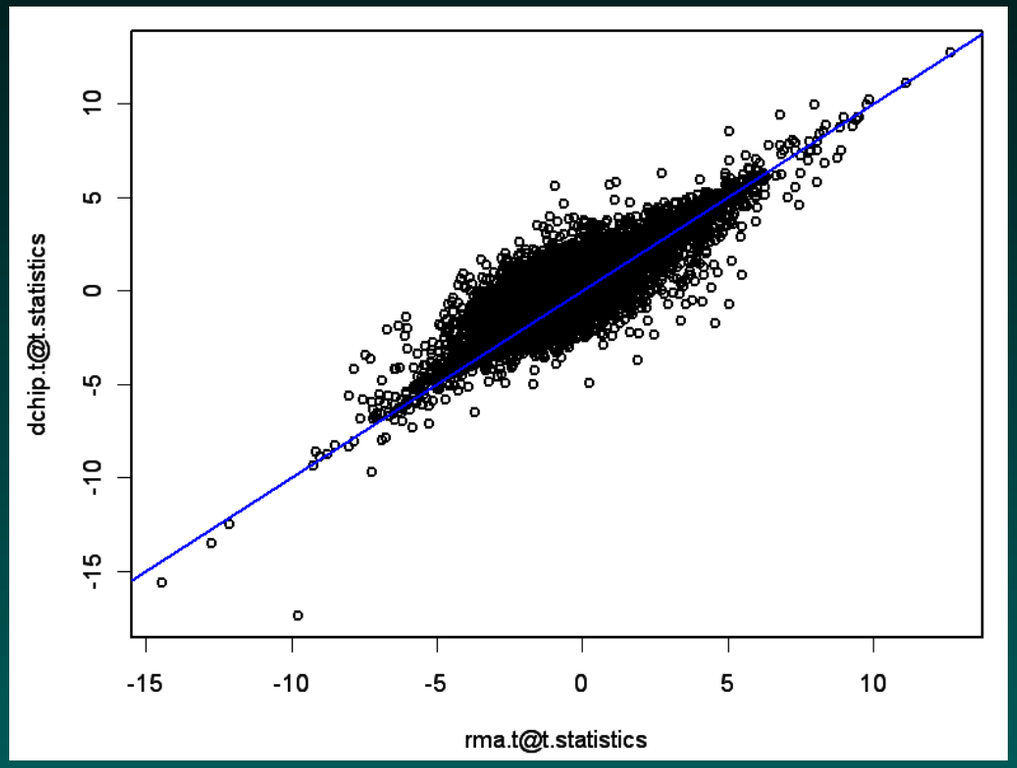
T-test, take two

```
> rma.t <- MultiTtest(rmaData[, notAML], 'ALLvMLL')
> rma.b <- Bum(rma.t@p.values)
> hist(rma.b, main='P-values using RMA')
```



Do the two methods agree?

```
> plot(rma.t@t.statistics, dchip.t@t.statistics)
> abline(0,1, col='blue')
```



How do we tell if the methods agree?

We have seen that the BUM plots for t-tests when we used different quantifications methods look similar. We have also seen that the t-statistics roughly agree, in the sense that they more or less follow the identiity line. The haze around that line is rather "fat", however, which suggests that the exact lists of genes we get with the two methods may not be quite the same. Here's one difference:

```
> alpha <- 0.05
> countSignificant(dchip.b, alpha=alpha, by='FDR')
[1] 1520
> countSignificant(rma.b, alpha=alpha, by='FDR')
[1] 2353
```

Try a smaller FDR

```
> alpha <- 0.01
> countSignificant(dchip.b, alpha=alpha, by='FDR')
[1] 681
> countSignificant(rma.b, alpha=alpha, by='FDR')
[1] 992
```

There certainly appear to be a lot of differentially expressed genes. However, RMA seems to give us more genes than dChip at the same level of the False Discovery Rate. That already tells us something about the processing methods.

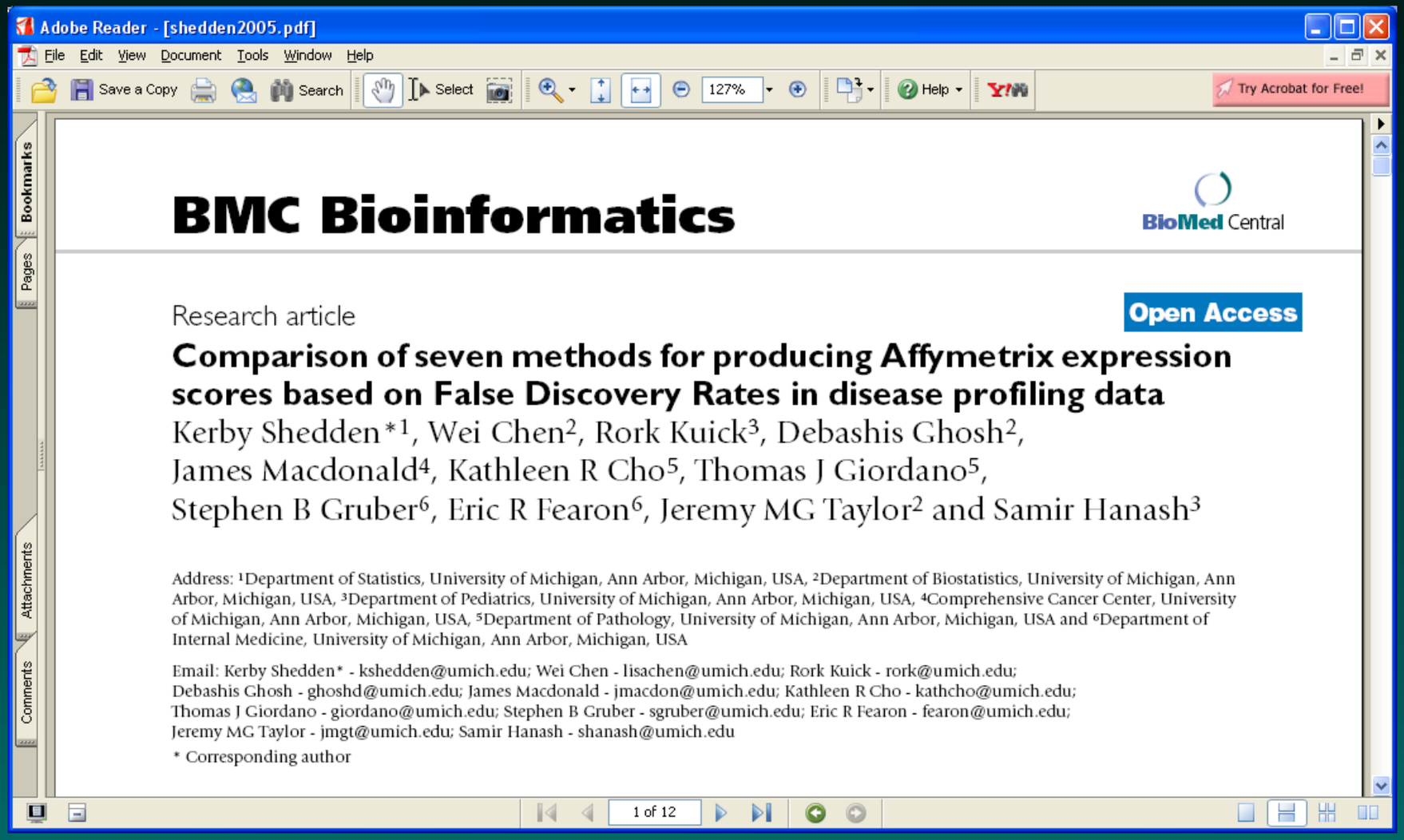
How much do the lists overlap?

```
> # logical vector: what does dChip find?
> dchip.01 <- selectSignificant(dchip.b,
+     alpha=alpha, by='FDR')
> # logical vector: what does RMA find?
> rma.01 <- selectSignificant(rma.b,
+     alpha=alpha, by='FDR')
> # Count the overlap
> sum(dchip.01 & rma.01)
[1] 563
> 563/681
[1] 0.8267254
```

Only 83% of the 681 genes found by dChip are contained in the larger list of genes found by RMA.

Comparing processing methods

So, the answers are “different”. Can we tell which is “better”?



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BMC Bioinformatics BioMed Central

Research article **Open Access**

Comparison of seven methods for producing Affymetrix expression scores based on False Discovery Rates in disease profiling data

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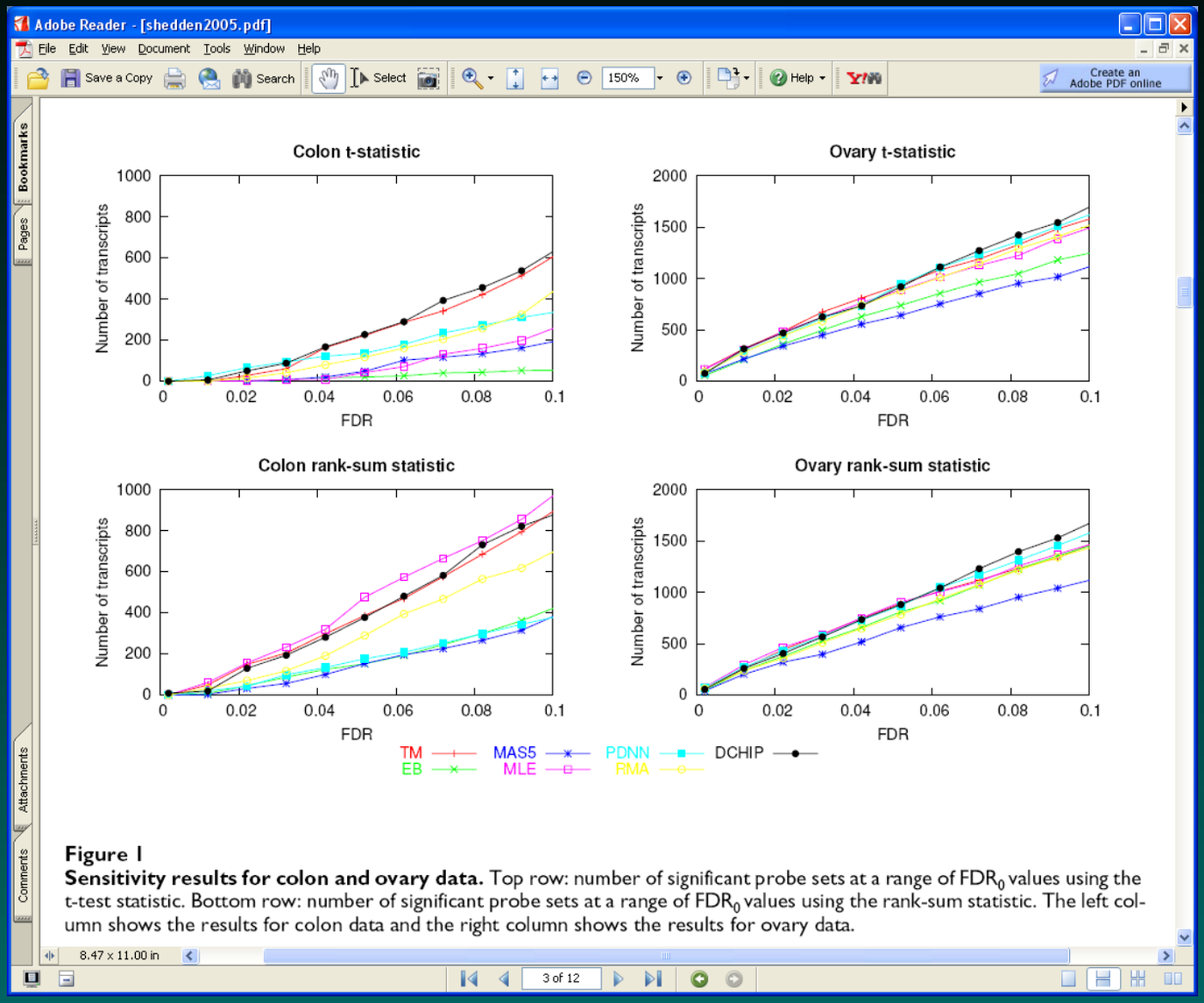
Shedden et al., BMC Bioinformatics 2005; 6:26

They looked at 7 processing methods in two different data sets.

- Methods
  - dChip
  - GCRMA-EB
  - GCRMA-MLE
  - MAS5
  - PDNN
  - RMA
  - trimmed mean (TM)
- Data Sets
  - 47 Colon cancer, U133A (40 MSS vs. 7 MSI)
  - 79 Ovarian cancer, U133A (38 endometroid vs. 41 serous)

Shedden Fig1: Number of probe sets by FDR



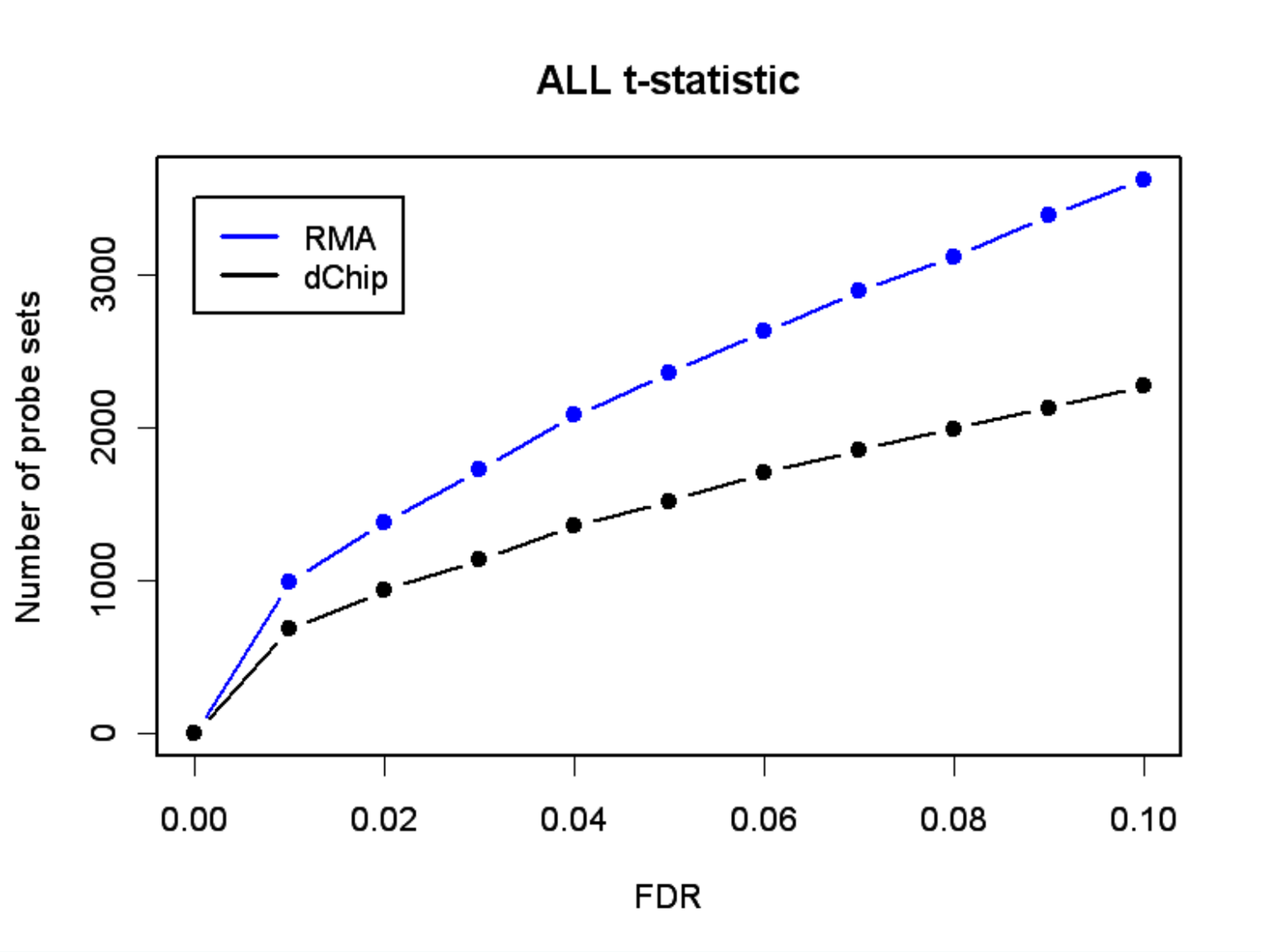
Same idea, ALL-MLL data set

```
> alpha <- seq(0, 0.1, by=0.01)
> f <- function(a, data) {
+   countSignificant(data, alpha=a, by='FDR')
+ }
>
> dchip.counts <- sapply(alpha, f, dchip.b)
> dchip.counts
 [1]    0  681  936 1139 1356 1520 1703
 [8] 1850 1990 2126 2266
> rma.counts   <- sapply(alpha, f, rma.b)
> rma.counts
 [1]    0  992 1379 1725 2078 2353 2623
 [8] 2890 3112 3383 3618
```

Making the plot

```
> plot(alpha, rma.counts,
+      xlab='FDR', ylab='Number of probe sets',
+      main='ALL t-statistic', type='b',
+      pch=16, col='blue')
> lines(alpha, dchip.counts, type='b', pch=16)
> legend(0, 3500, c('RMA', 'dChip'), lwd=3,
+      col=c('blue', 'black'))
```

RMA gives more differences in this data set



Wilcoxon rank-sum tests

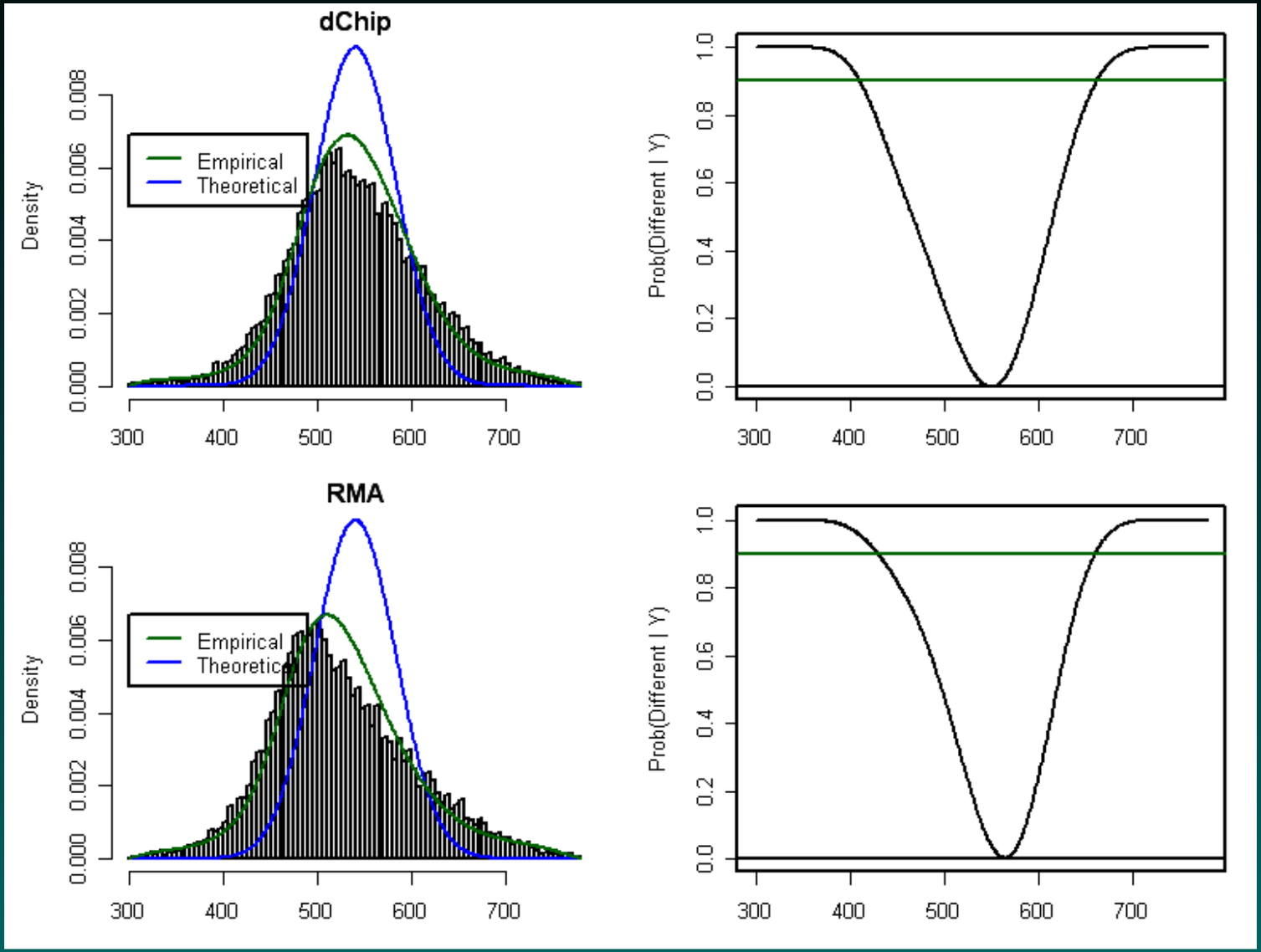
In the Shedden paper, they use a rank-sum statistic to test for differential expression, in addition to the t-statistic. To compute these statistics, we use the `MultiWilcoxonTest` function in the `ClassComparison` package.

```
> dchip.wil <- MultiWilcoxonTest(dchip[, notAML],
+     'ALLvMLL')
> rma.wil <- MultiWilcoxonTest(rmaData[, notAML],
+     'ALLvMLL')
```

Summary plots from the Wilcoxon empirical Bayes

```
> opar <- par(mai=c(0.5, 0.7, 0.2, 0.2),
+       mfrow=c(2,2))
> hist(dchip.wil, main='dChip')
> plot(dchip.wil, prior=0.725,ylim=c(0,1))
> abline(h=0)
> hist(rma.wil, main='RMA')
> plot(rma.wil, prior=0.56,ylim=c(0,1))
> abline(h=0)
> par(opar)
```

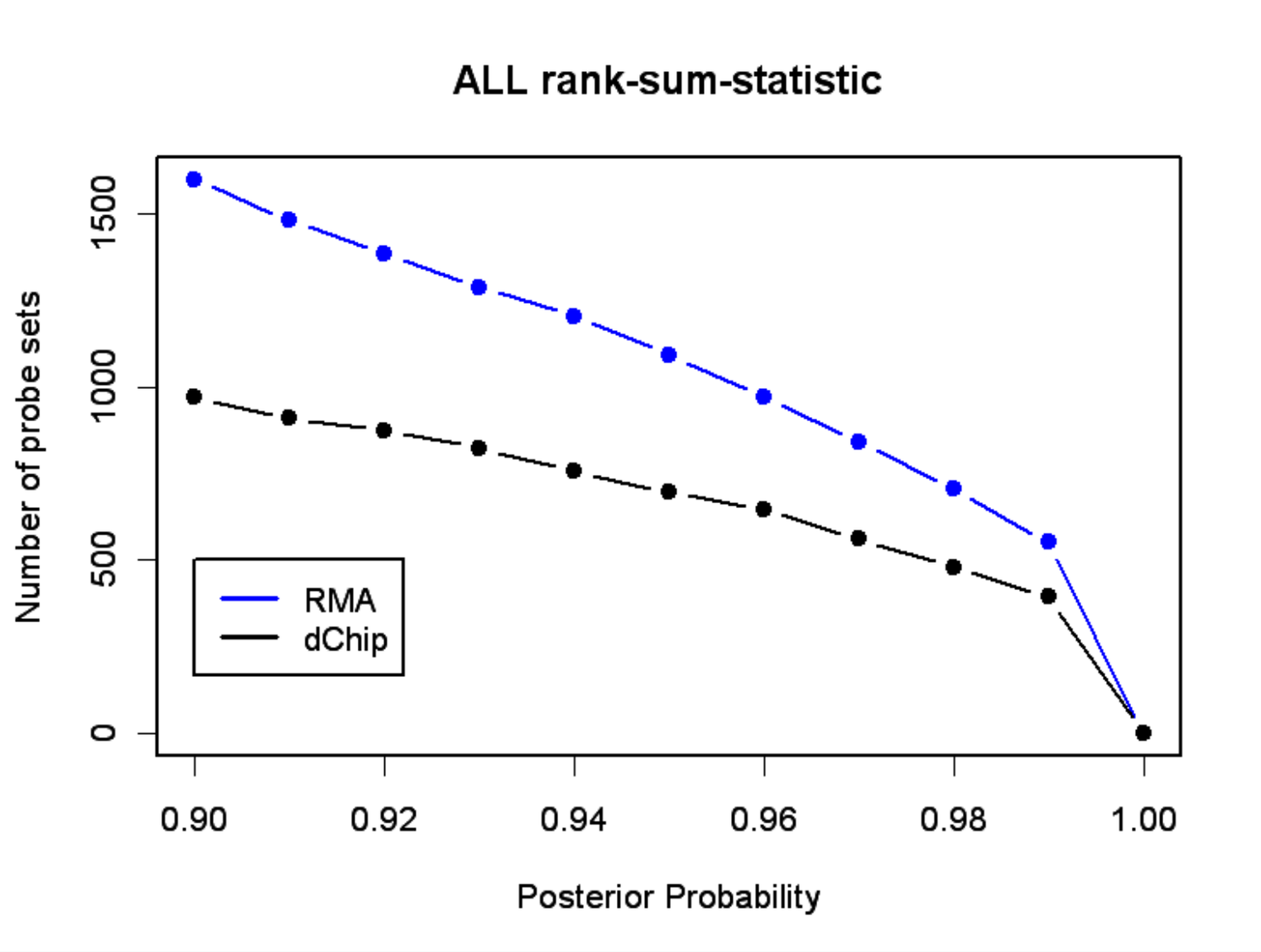
Why is the RMA version skewed?



Counts as a function of posterior probability

```
> sig <- seq(1.0, 0.9, by=-0.01)
> f2 <- function(s, p, data) {
+   countSignificant(data, prior=p, signif=s)
+ }
> dchip.w.counts <- sapply(sig, f2, p =0.725,
+     data=dchip.wil)
> dchip.w.counts
 [1]    0  394  481  562  648  695  756  824
 [9]  874  908  971
> rma.w.counts <- sapply(sig, f2, p =0.56,
+     data=rma.wil)
> rma.w.counts
 [1]    0  551  707  842  971 1091 1204 1288
 [9] 1384 1479 1598
```

RMA still gives more differences



Shedden Fig3: Threshold Statistic by FDR

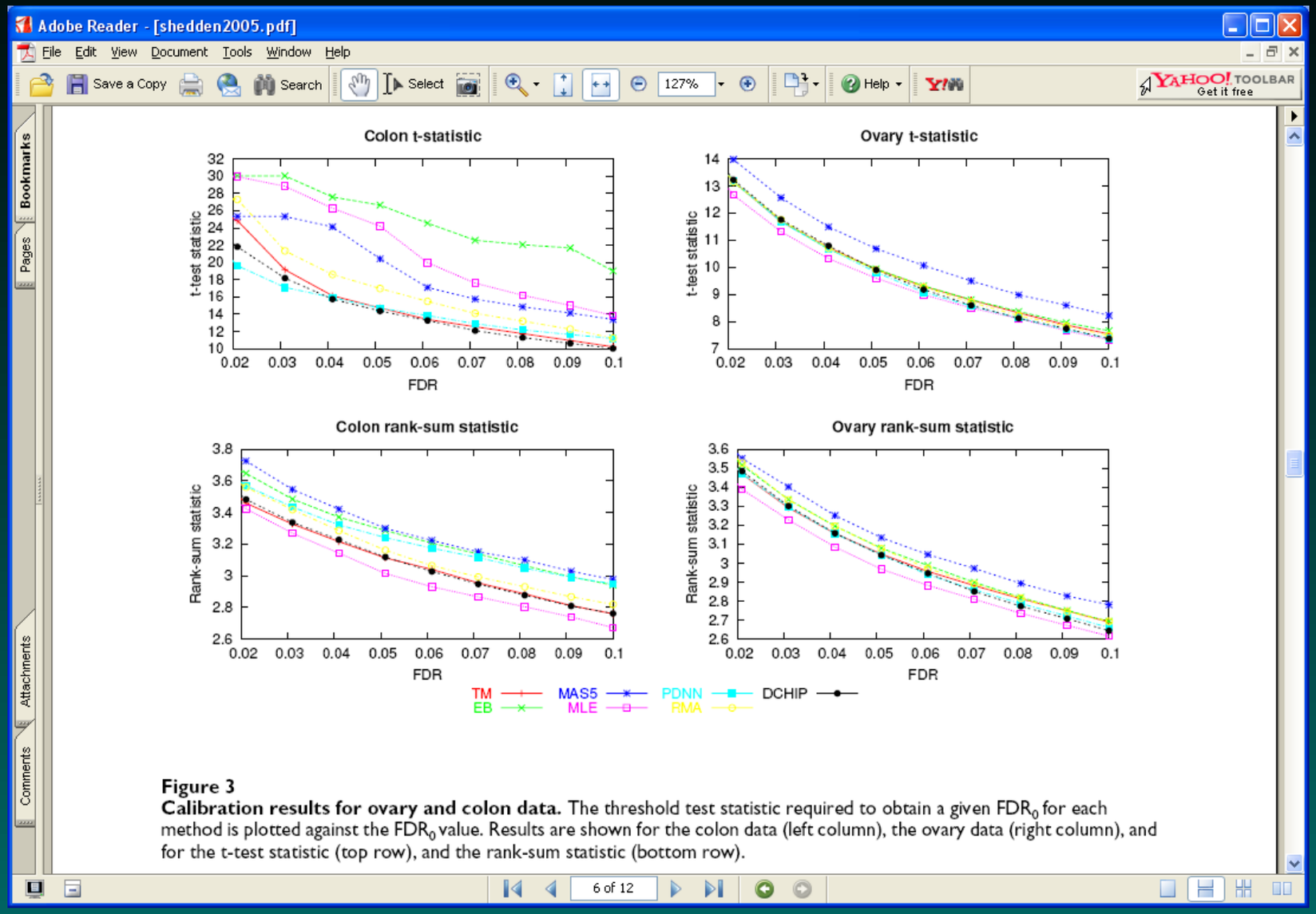
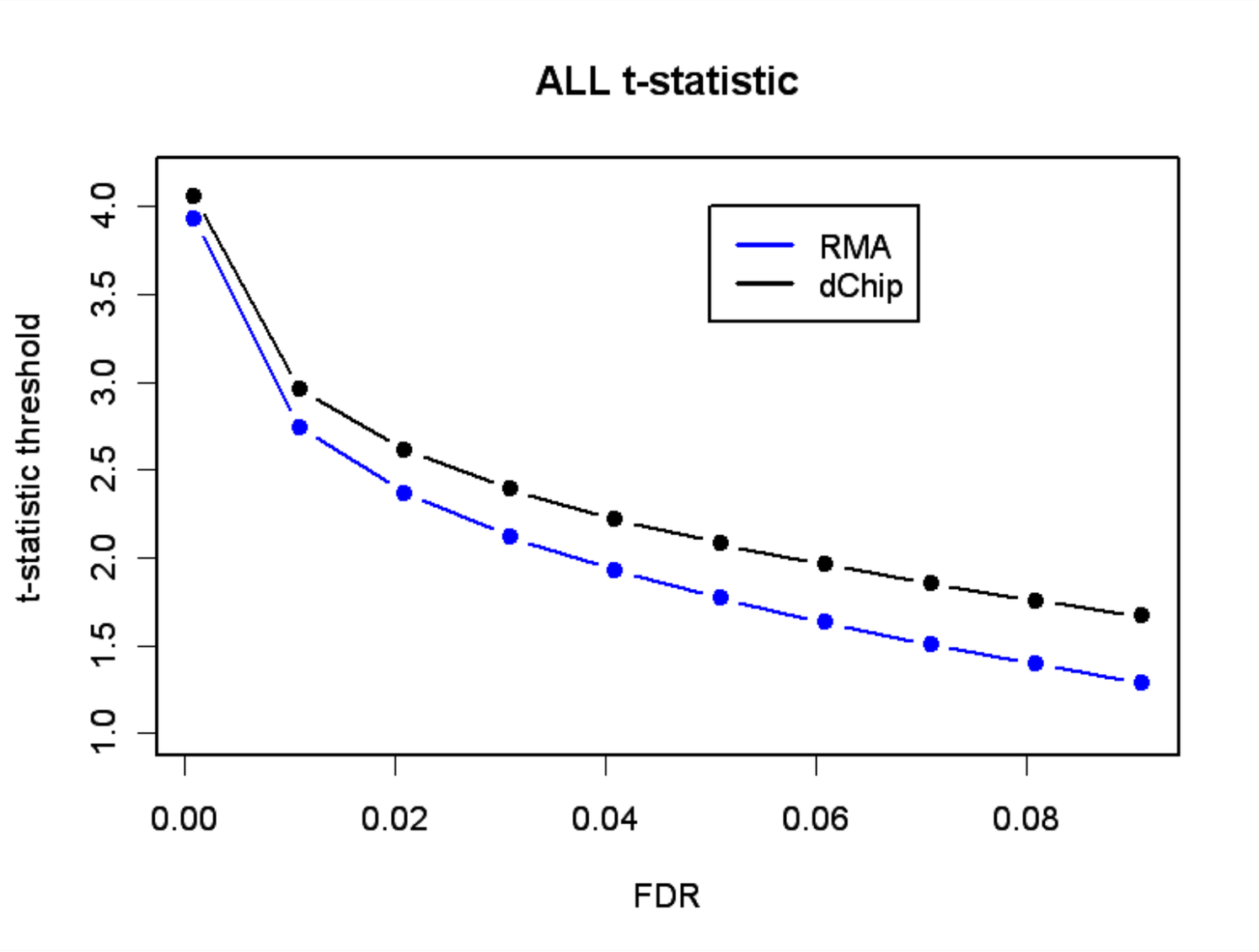


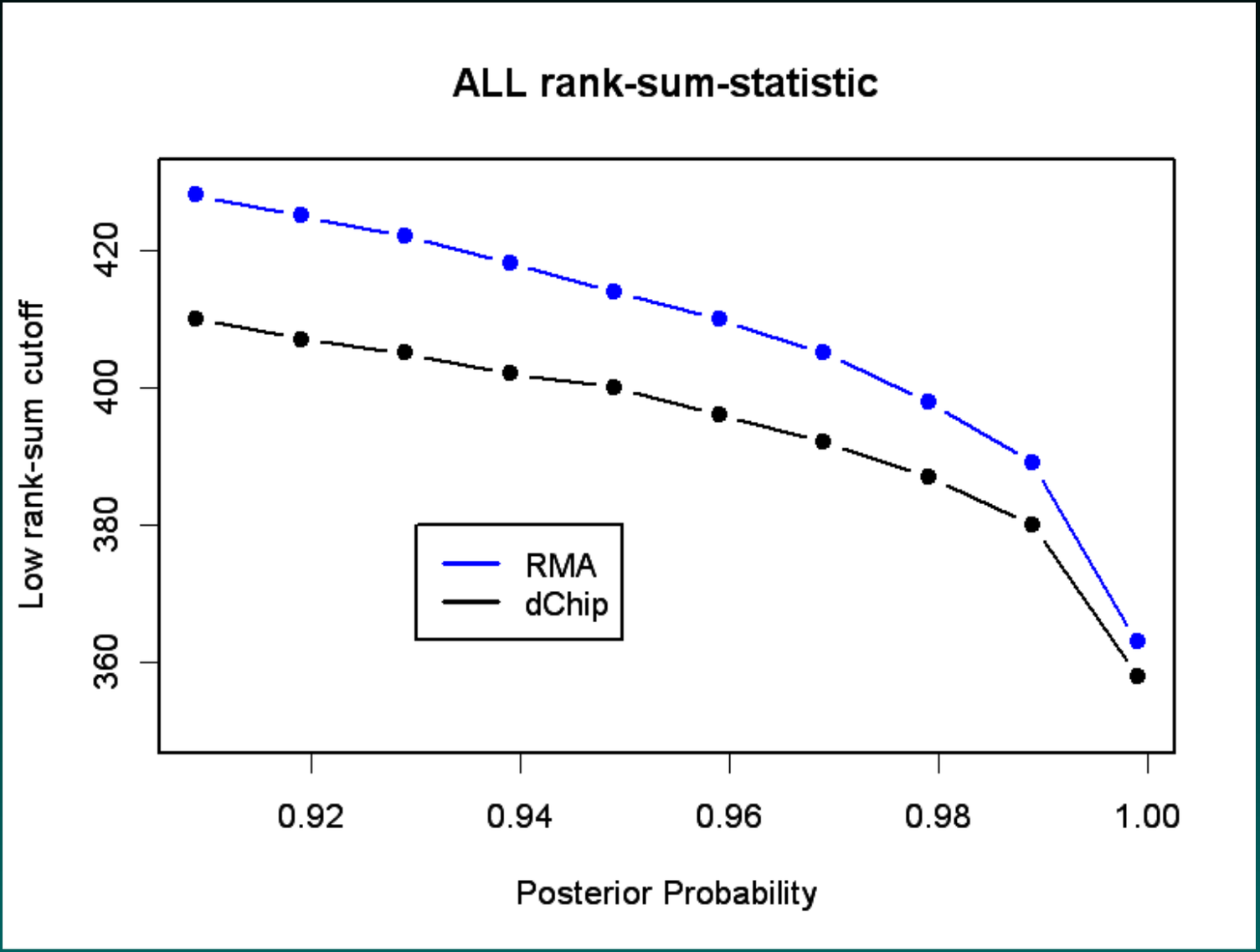
Figure 3
Calibration results for ovary and colon data. The threshold test statistic required to obtain a given FDR_0 for each method is plotted against the FDR_0 value. Results are shown for the colon data (left column), the ovary data (right column), and for the t-test statistic (top row), and the rank-sum statistic (bottom row).

```
> alpha <- seq(0.001, 0.1, by=0.01)
> g <- function(a, data) {
+   pval <- cutoffSignificant(data, alpha=a,
+             by='FDR')
+   qt(1-2*pval, 70)
+ }
> dchip.cut <- sapply(alpha, g, dchip.b)
> rma.cut   <- sapply(alpha, g, rma.b)
> plot(alpha, dchip.cut,
+      xlab='FDR', ylab='t-statistic threshold',
+      main='ALL t-statistic', type='b', pch=16,
+      ylim=c(1,4.15))
> lines(alpha, rma.cut, type='b', pch=16,
+       col='blue')
> legend(0.05, 4, c('RMA', 'dChip'), lwd=3,
+        col=c('blue', 'black'))
```

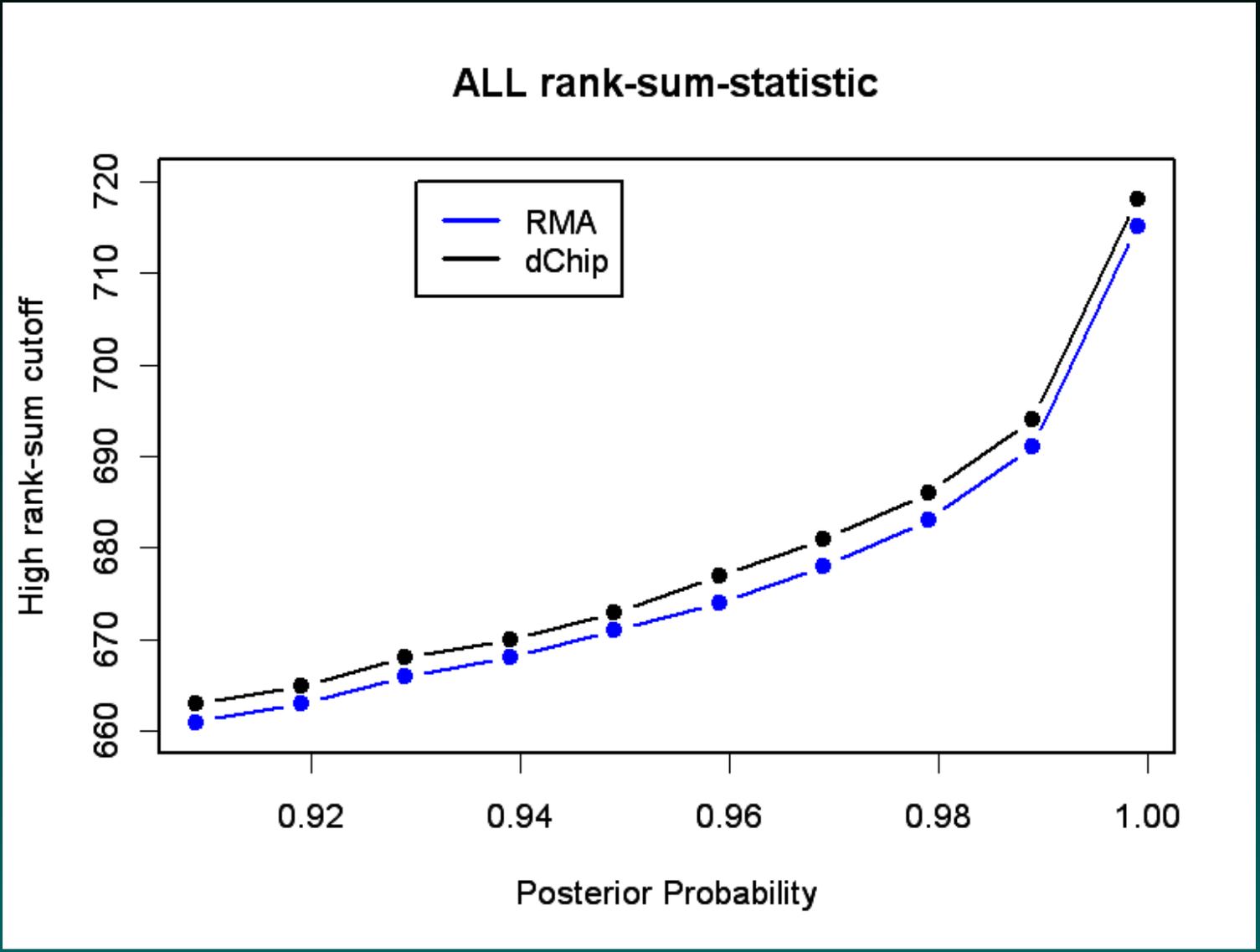
ALL T-Statistic Thresholds



ALL Rank-Sum Thresholds: Low



ALL Rank-Sum Thresholds: High



Calibration determines sensitivity

Shedden et al. found the same relation between sensitivity (the number of probe sets called different at a given FDR level) and calibration (the threshold needed to call a probe set different at a given FDR level) in their data sets that we see in our data sets.

Namely, methods that provide greater sensitivity do so by lowering the threshold required to call the statistic significant.

In contrast, they found that dChip and TM consistently performed as well or better than other methods on their two data sets. We, of course, found that RMA is “more sensitive”.

They also found (and we agree) that the choice of processing method has a bigger impact on differntial expression than the choice of using a paramteric t-statistic compared to a non-parametric rank-sum statistic.

Cope et al., Bioinformatics, 2004; 20:323-331

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A benchmark for Affymetrix GeneChip expression measures

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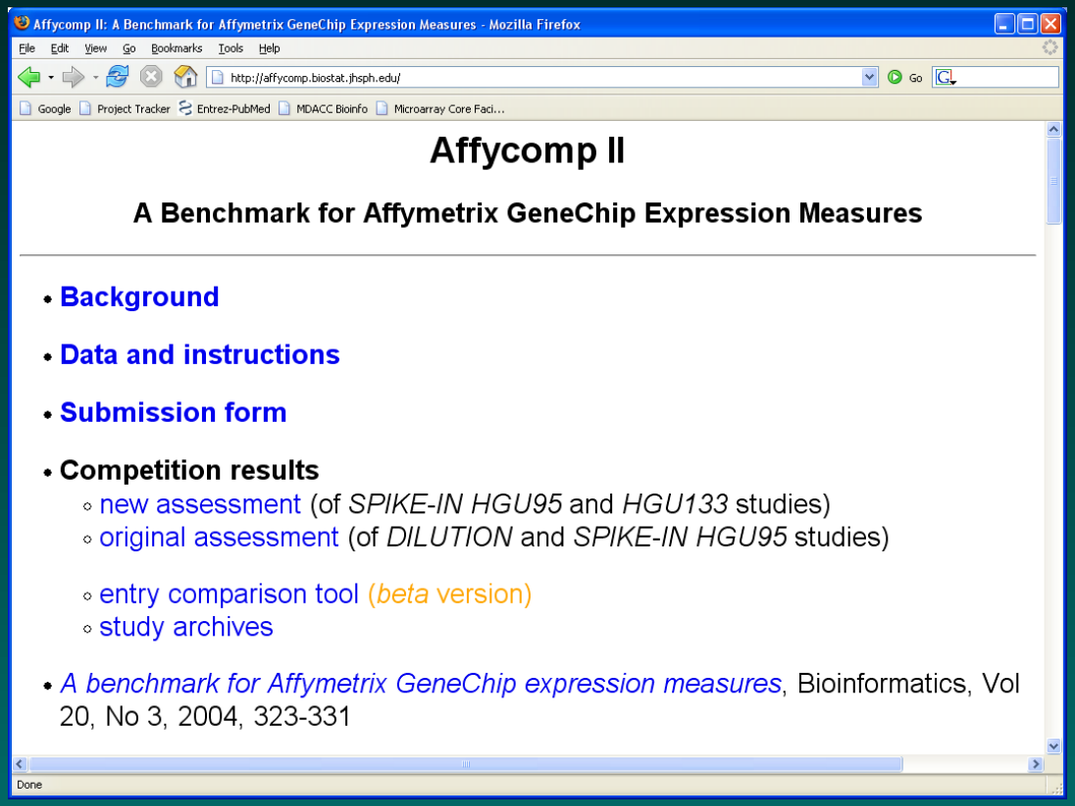
ABSTRACT

Motivation: The defining feature of oligonucleotide expression arrays is the use of several probes to assay each targeted transcript. This is a bonanza for the statistical geneticist, who can create probeset summaries with specific characteristics. There are now several methods available for summarizing probe level data from the popular Affymetrix GeneChips, but it is difficult to identify the best method for a given inquiry, offering great opportunity to create probeset summaries with specific characteristics. On the other hand, the researcher with data in hand and a particular question in mind is not necessarily able to identify the best method. Using a spike-in study prepared by Affymetrix and a dilution study by Gene Logic as benchmark data, we have developed a graphical tool for the evaluation and comparison of expression measures on the Affymetrix GeneChip platform (Lockhart *et al.*, 1996)

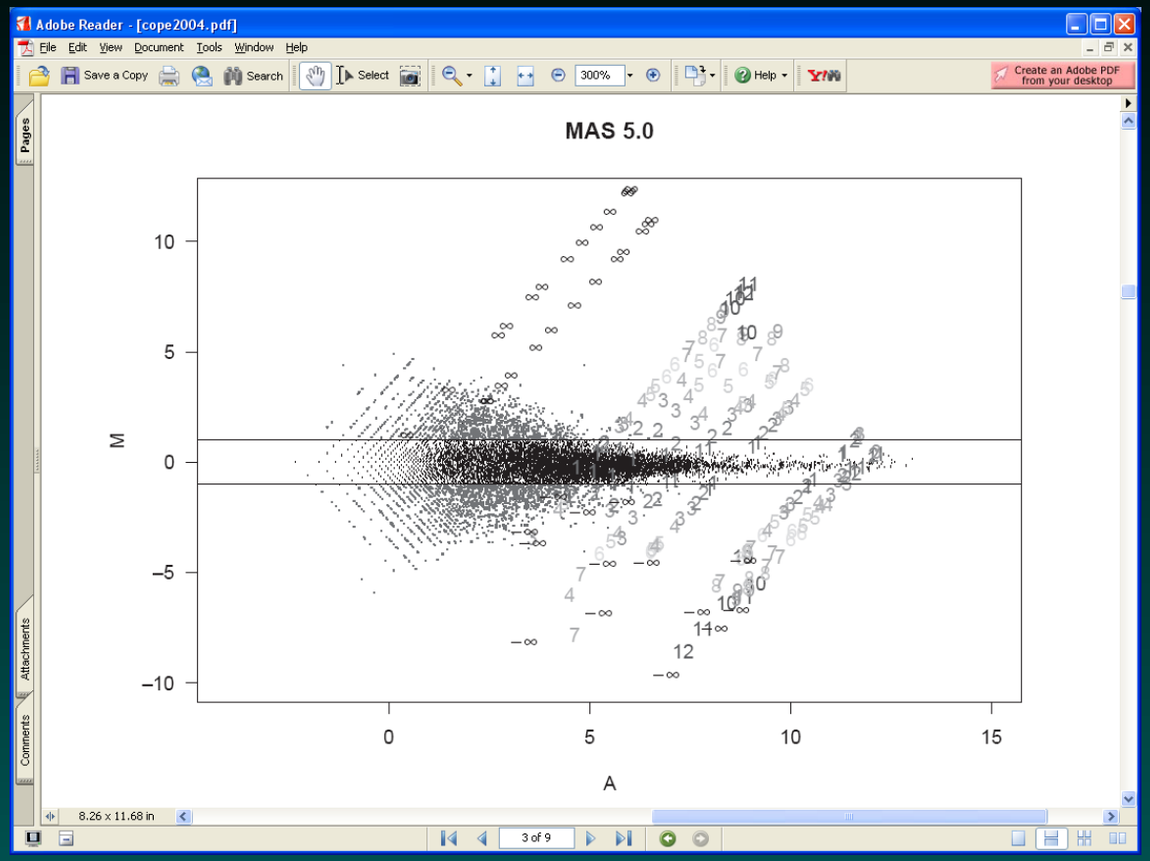
Benchmarking methods using “calibration” data

An alternative approach to comparing the results of different processing methods relies on standard sets of spike-in experiments. The performance measures described by Cope et al, are available on a web site:

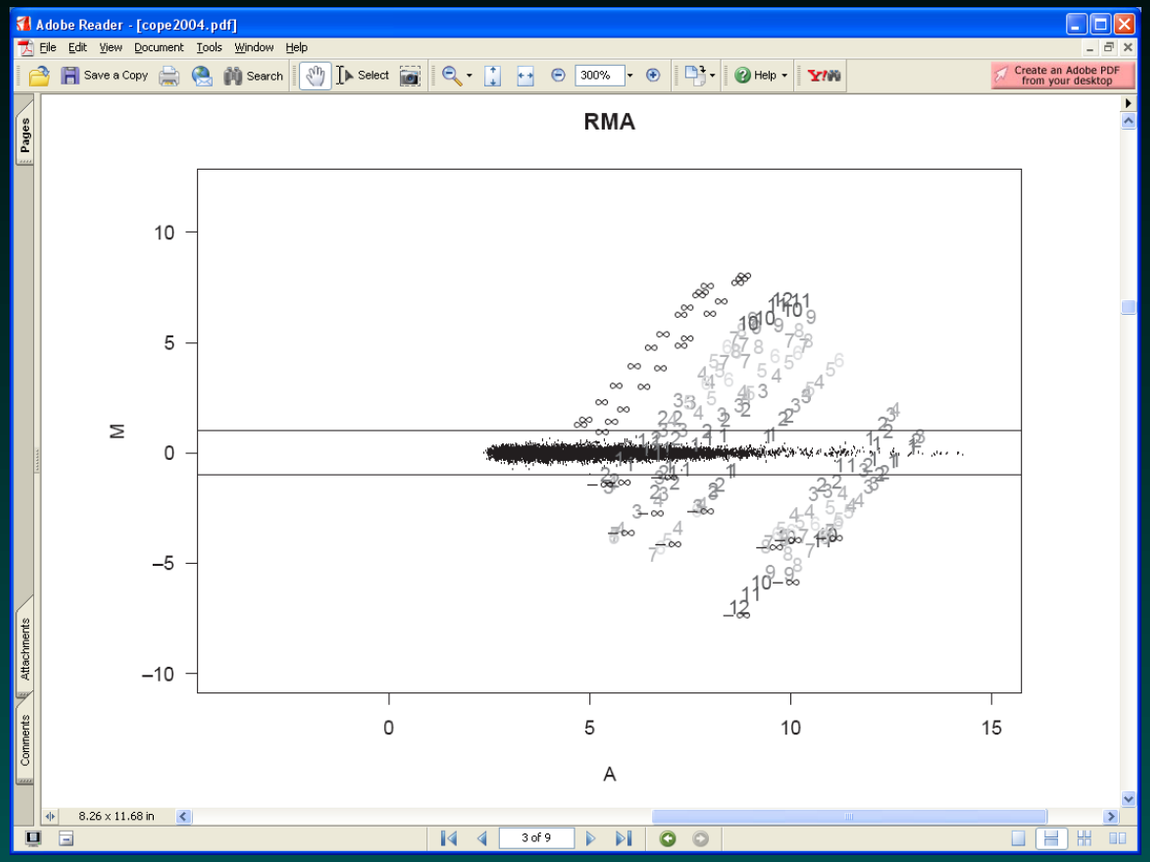


- They use
  - the GeneLogic dilution study that mixed RNA from liver and CNS tissue in different dilutions and proportions
  - the Affymetrix latin-square spike-in study on U95A arrays
  - the Affymetrix latin-square spike-in study on U133A arrays

MAplot of Latin-square data: MAS5

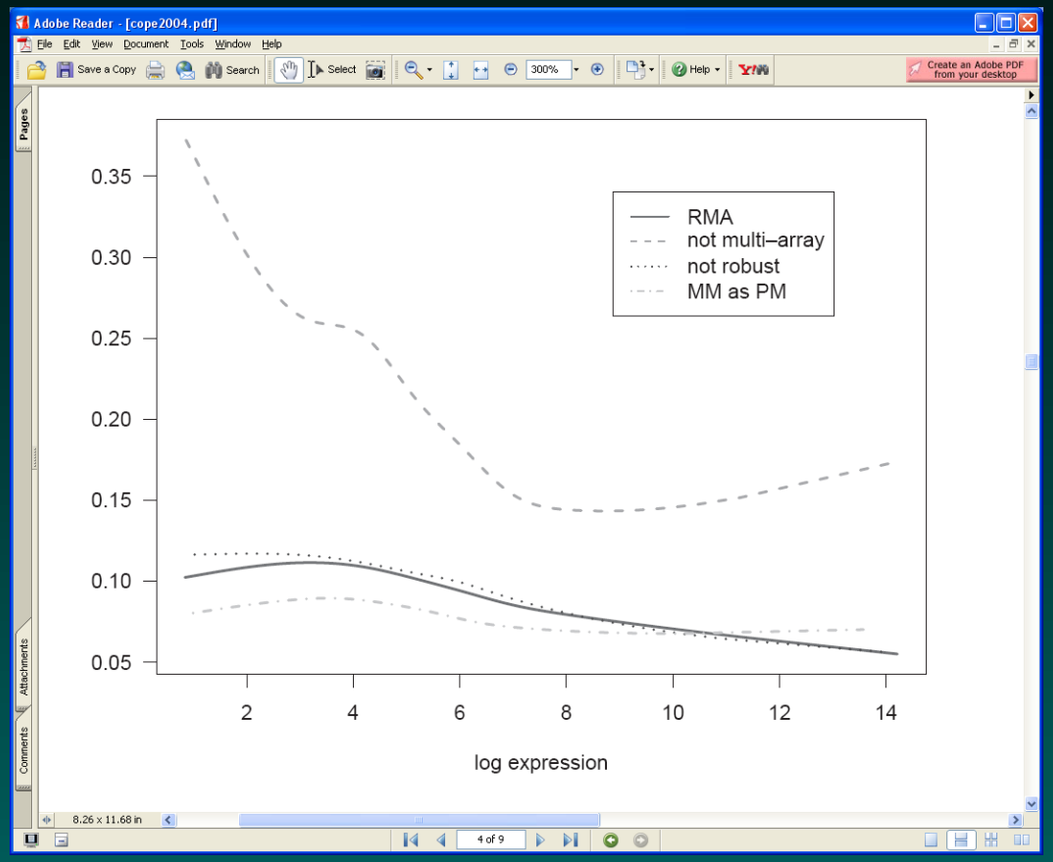


MAplot of Latin-square data: RMA



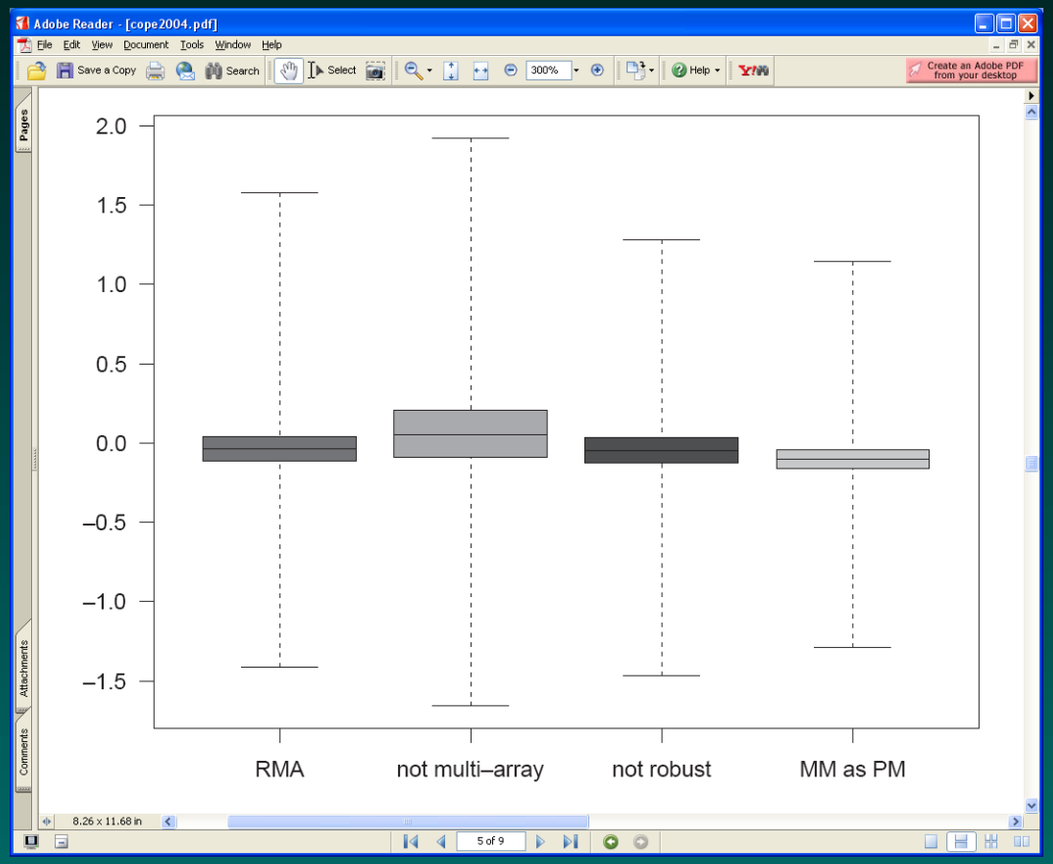
Standard deviation across replicate dilution arrays

This figure shows the results for four different methods.



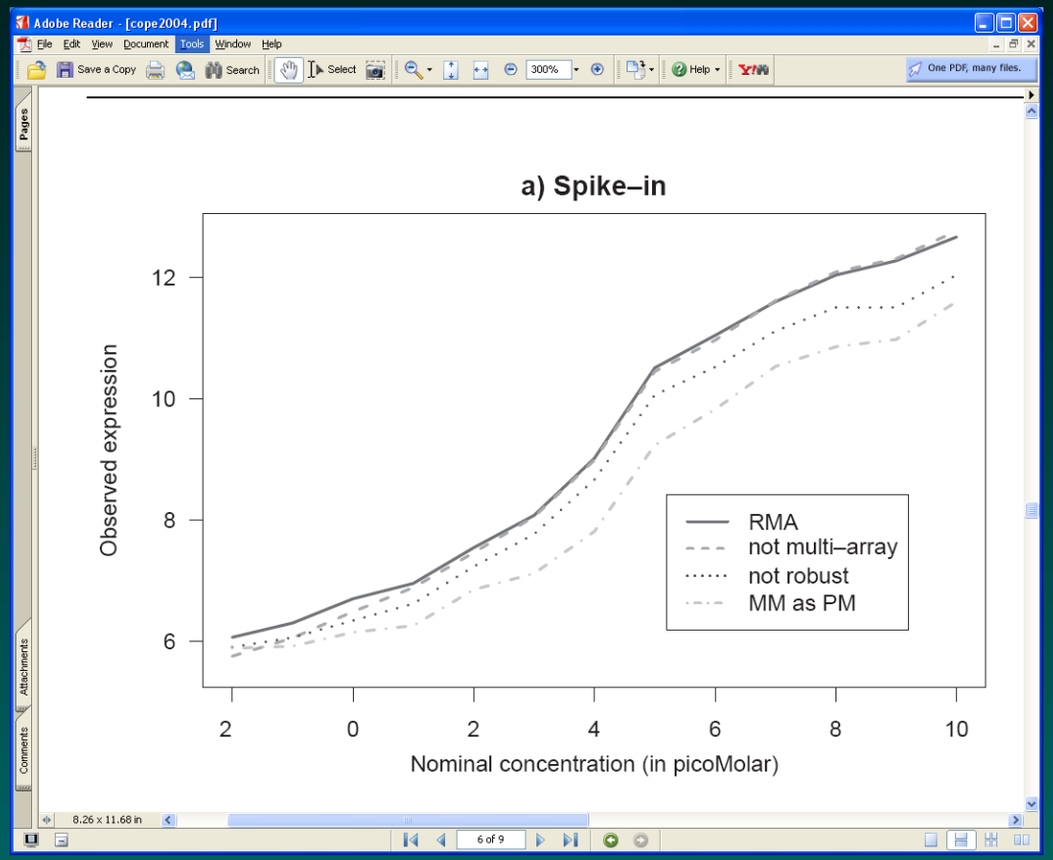
Sensitivity to total amount of RNA

For each method, compute the log ratios (fold changes) between lowest (1.25 μg) and highest (20 μg) concentrations in the dilution experiment.



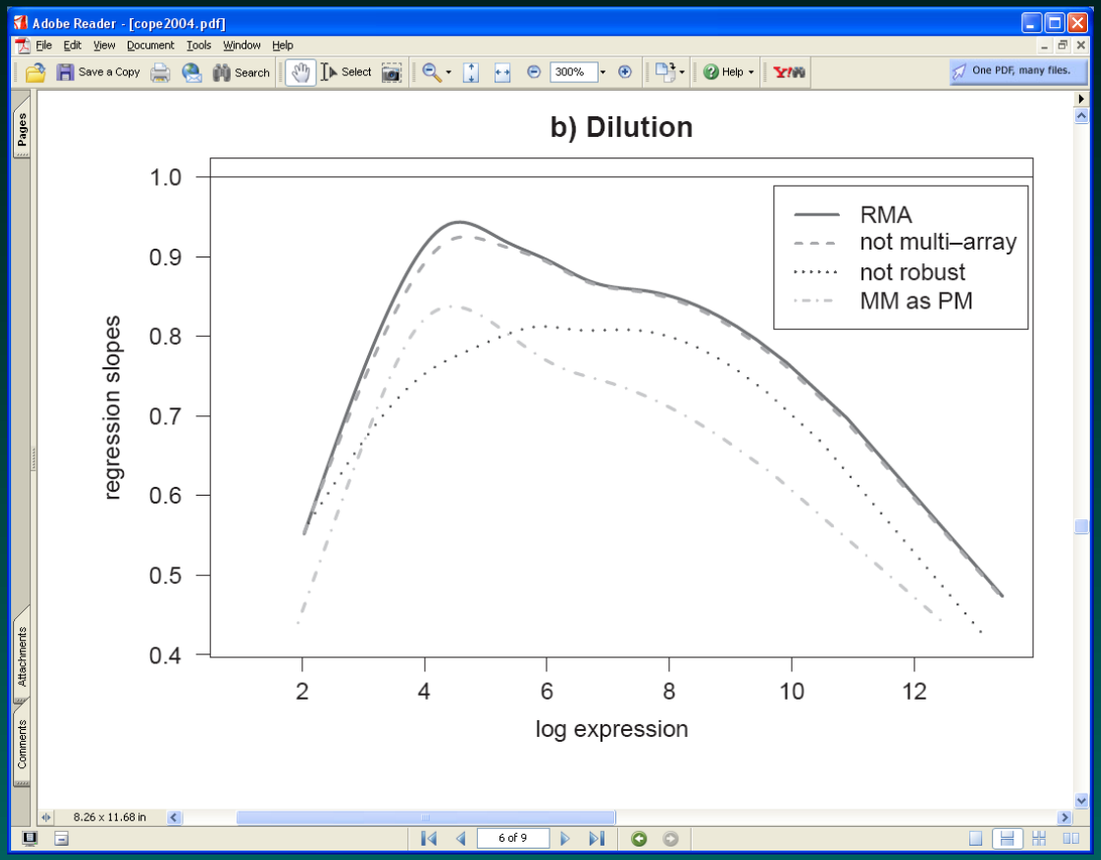
Observed expression vs. nominal concentration in Latin-square

This figure shows the results for four different methods.



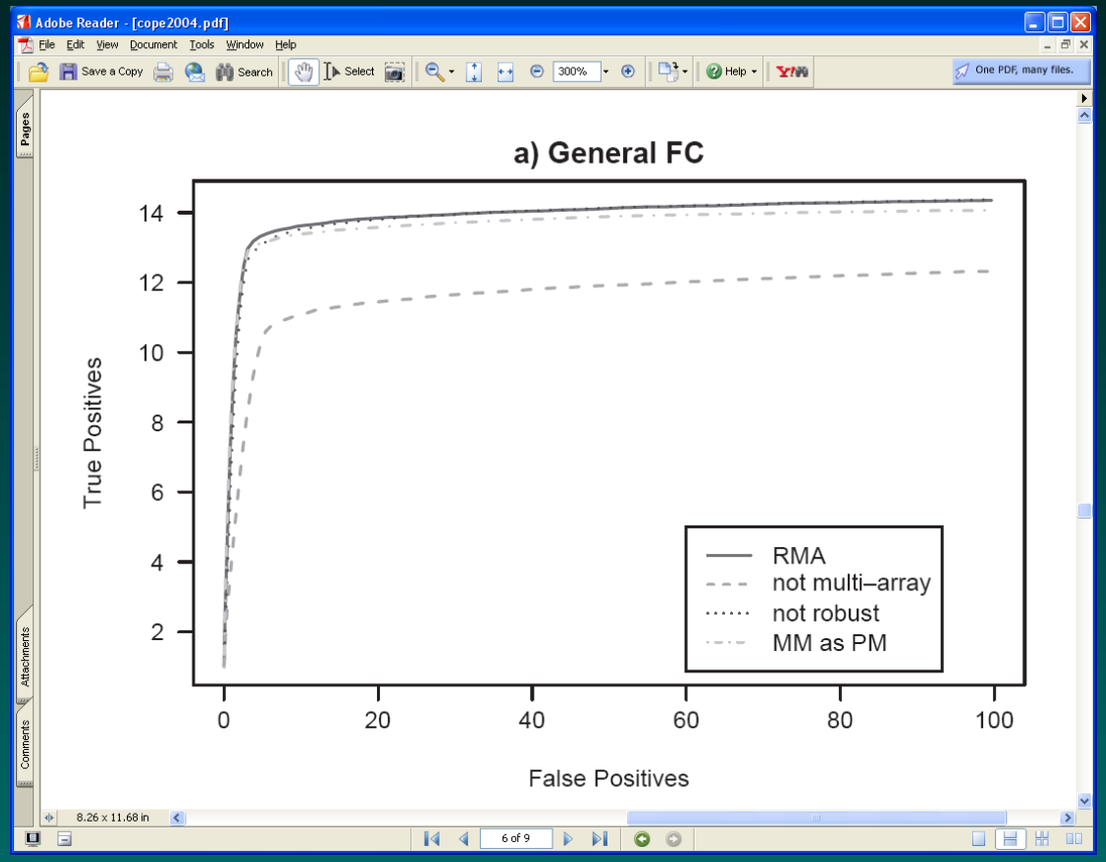
Observed vs. Nominal in Dilution

This figure shows the results for four different methods; they fit regressions to intensity as a function of dilution.



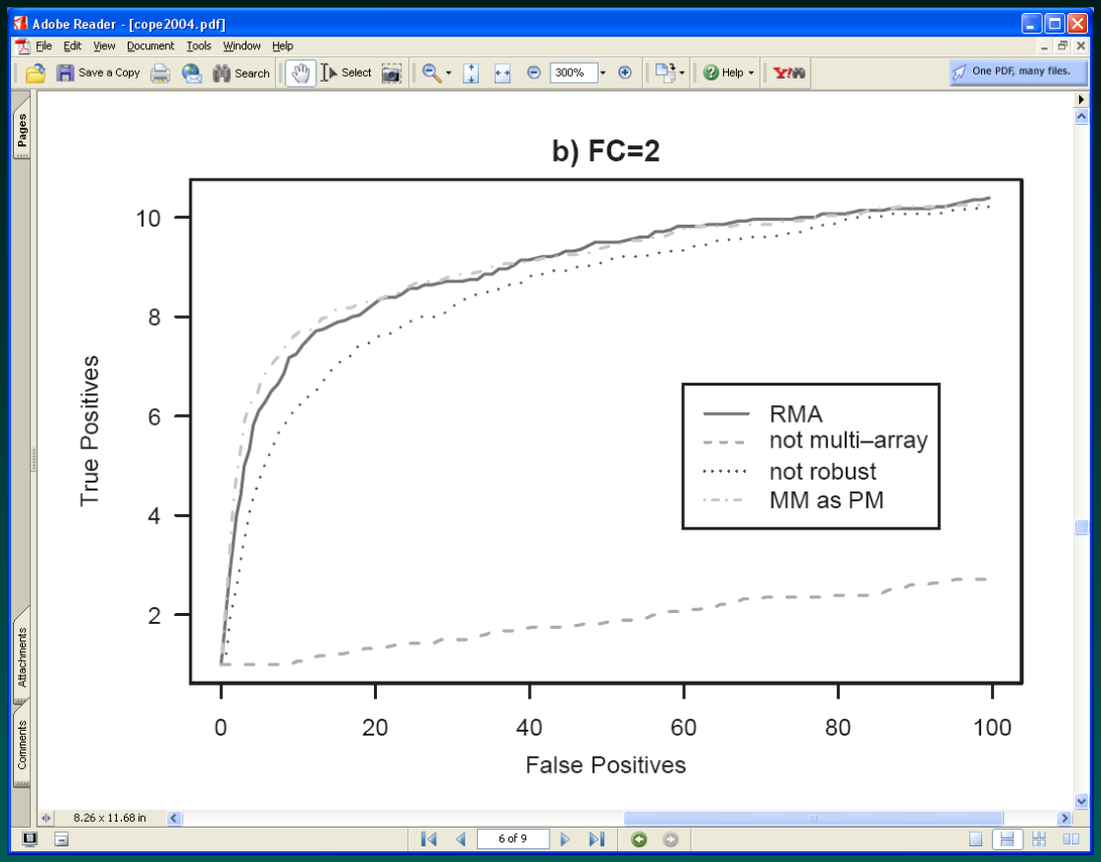
ROC curves: general FC

This figure shows the results for four different methods. In each case, they average the ROC curves for different pairwise comparisons in the Latin-square data.



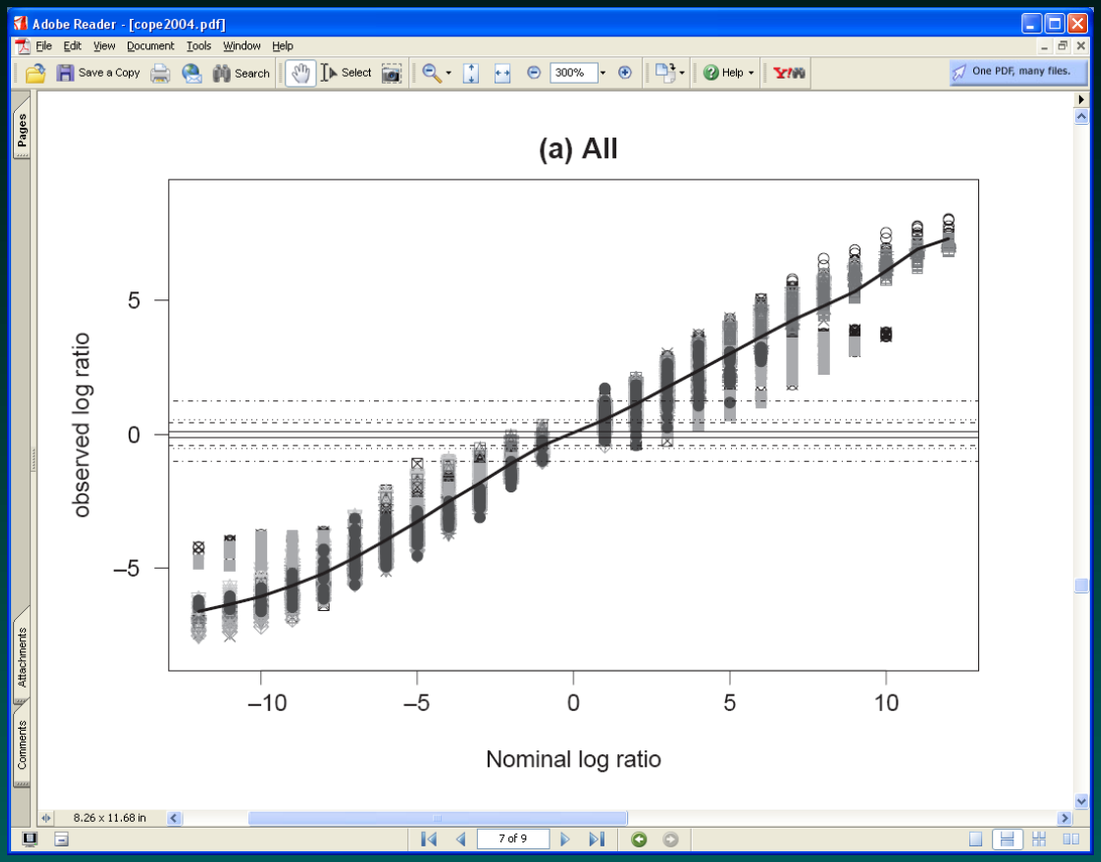
ROC curves: FC=2

This figure shows the results for four different methods



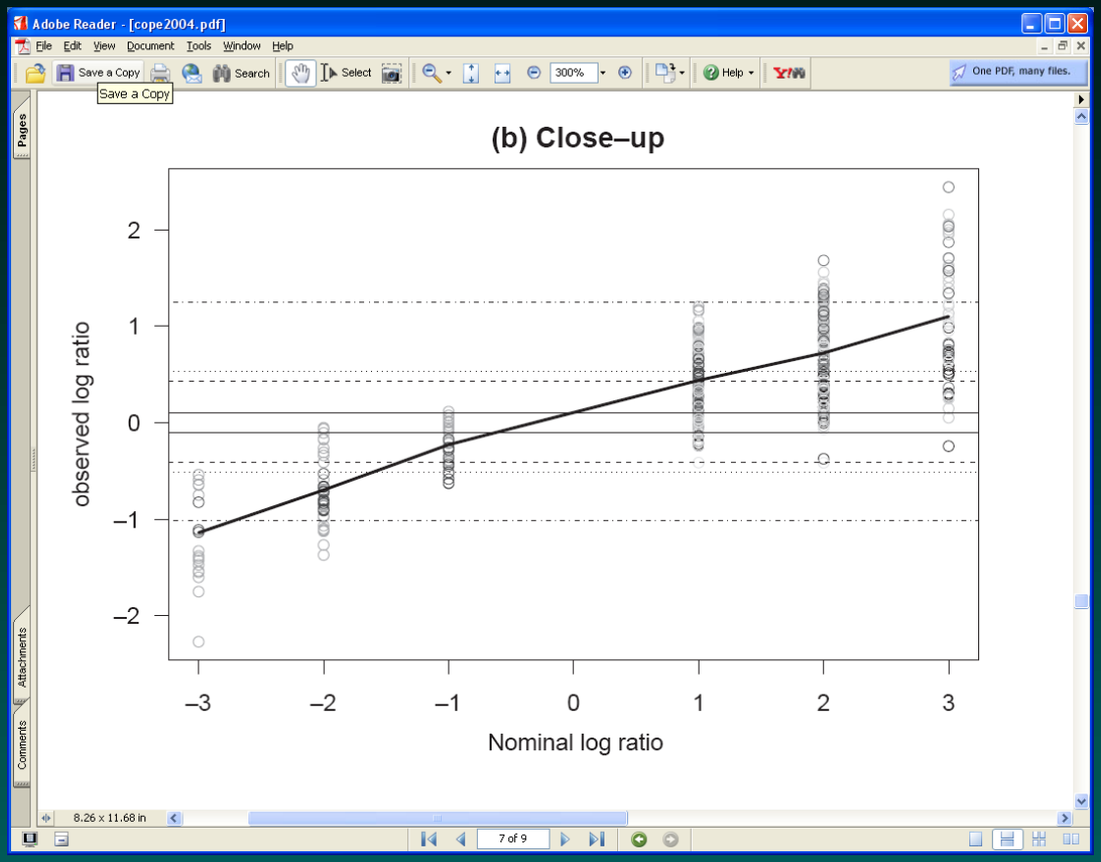
Observed vs. nominal fold change: RMA

This figure shows the results for four different methods



Observed vs. nominal fold change: RMA, close-up

This figure shows the results for four different methods



Measures of performance

Adobe Reader - [cope2004.pdf]

Table 1. Assessment summary statistics table

Assessment	Figure	MAS 5.0	dChip	RMA	Not multi-array	Not robust	MM as PM
(1) Median SD	2	0.29	0.089	**0.088**	0.19	0.092	**0.074**
(2) R2	2	0.89	0.99	**0.99**	0.98	0.99	**0.99**
(3) 1.25v20 corr	3	0.73	0.91	**0.94**	0.87	**0.94**	0.93
(4) 2-fold discrepancy	3	1200	40	**21**	99	12	**6**
(5) 3-fold discrepancy	3	330	8	**0**	12	**0**	**0**
(6) Signal detect slope	4a	**0.71**	0.53	0.63	**0.65**	0.59	0.55
(7) Signal detect R2	4a	**0.86**	0.85	0.8	**0.81**	0.76	0.72
(8) Median slope	4b	0.85	0.77	**0.87**	0.86	0.79	0.76
(9) AUC (FP < 100)	5a	0.36	0.67	**0.82**	0.69	0.82	0.81
(10) AFP, call if fc > 2	5a	3100	37	**16**	220	19	**15**
(11) ATP, call if fc > 2	5a	**13**	11	12	**12**	12	11
(12) FC=2, AUC (FP < 100)	5b	0.065	0.17	**0.54**	0.12	0.52	**0.55**
(13) FC=2, AFP, call if fc > 2	5b	1400	12	**0.5**	18	**0.5**	**0.5**
(14) FC=2, ATP, call if fc > 2	5b	**3.7**	1.3	1.7	**2.3**	1.4	1.4
(15) IQR	6	2.7	0.45	**0.31**	0.67	0.31	**0.25**
(16) Obs-intended-fc slope	6a	**0.69**	0.52	0.61	**0.64**	0.58	0.54
(17) Obs-(low)int-fc slope	6b	**0.65**	0.32	0.36	**0.45**	0.34	0.21

The second column denotes the Figure to which the summary statistic relates. Columns 3, 4 and 5 compare MAS 5.0, dChip and RMA. The statistics are described in the text. For each row, the best performing expression measure is denoted with a bold number. Columns 6, 7 and 8 compare RMA to alternatives based on RMA. For each row, if the best performing expression measure is not RMA it is denoted with a bold number.