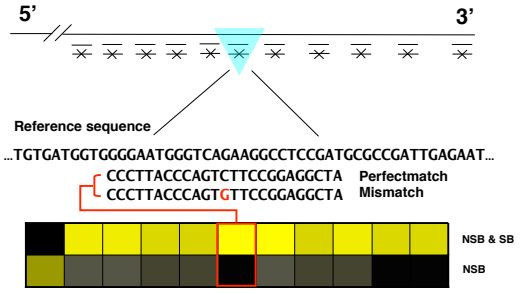
Preprocessing Affymetrix GeneChip Data

Credit for some of today's materials: Ben Bolstad, Leslie Cope, Laurent Gautier, Terry Speed and Zhijin Wu

Affymetrix GeneChip Design



Terminology

- Each gene or portion of a gene is represented by 11 to 20 oligonucleotides of 25 base-pairs.
- Reporter/Feautre/Probe: an oligonucleotide of 25 base-pairs, i.e., a 25-mer.
- Perfect match (PM): A 25-mer complementary to a reference sequence of interest (e.g., part of a gene).
- Mismatch (MM): same as PM but with a single homomeric base change for the middle (13^{th}) base (transversion purine <-> pyrimidine, G <->C, A <->T) .
- Probe-pair: a (PM,MM) pair.
- Probe-pair set: a collection of probe-pairs (11 to 20) related to a common gene or fraction of a gene.
- Affy ID: an identifier for a probe-pair set.
- The purpose of the MM probe design is to measure non-specific binding and background noise.

Affymetrix files

- Main software from Affymetrix company *MicroArray Suite* - *MAS*, now version 5.
- `DAT` file: Image file, ~10^7 pixels, ~50 MB.
- `CEL` file: Cell intensity file, probe level PM and MM values.
- `CDF` file: Chip Description File. Describes which probes go in which probe sets and the location of probe-pair sets (genes, gene fragments, ESTs).

Expression Measures

- 10-20K genes represented by 11-20 pairs of probe intensities (PM & MM)
- Obtain expression measure for each gene on each array by summarizing these pairs
- We already discussed background adjustment and normalization. We assume this has been done.
- There are many methods

Data and notation

- PM_{ijg}, MM_{ijg} = Intensity for perfect match and mismatch probe in cell *j* for gene *g* in chip *i*.
  - $i = 1,\ldots, n$ -- from one to hundreds of chips;
  - $j = 1,\ldots, J$ -- usually 11 or 20 probe pairs;
  - $g = 1,\ldots, G$ -- between 8,000 and 20,000 probe sets.
- Task: summarize for each probe set the probe level data, i.e., PM and MM pairs, into a single expression measure.
- Expression measures may then be compared within or between chips for detecting differential expression.

MAS 4.0

- **GeneChip® MAS 4.0 software used AvDiff up until 2001**

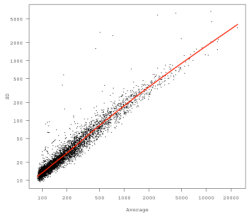
$$AvDiff = \frac{1}{|\mathrm{A}|}\sum_{j\in \mathrm{A}}(PM_j - MM_j)$$

where A is a set of "suitable" pairs, e.g., pairs with $d_j = PM_j - MM_j$ within 3 SDs of the average of $d_{(2)}, \ldots, d_{(J-1)}$

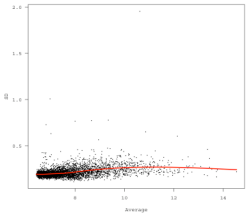
- **Obvious problems:**
  - **Negative values**
  - **No log scale**

Why use log?

Original scale



Log scale

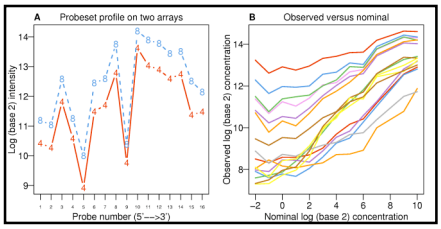


Li and Wong's observations

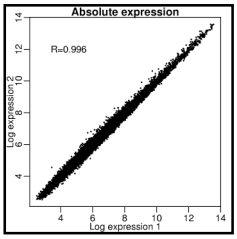
- **There is a large probe effect**
- **There are outliers that are only noticed when looking across arrays**
- **Non- linear normalization needed (discussed in previous lecture)**

PNAS vol. 98. no. 1, **31-36**

Probe effect

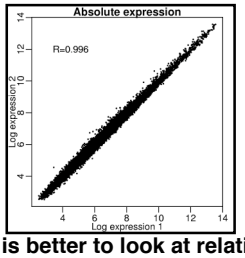


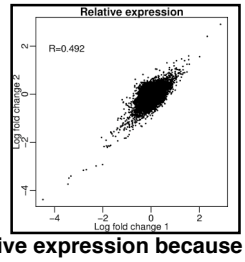
Probe effect makes correlation deceiving



Correlation for absolute expression of replicates looks great! But…

Probe effect makes correlation deceiving





- **It is better to look at relative expression because probe effect is somewhat cancelled out.**
- **Later we will see that we can take advantage of probe effect to find outlier probes.**

Li & Wong

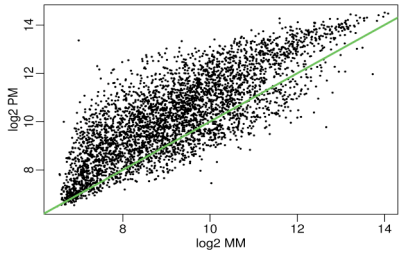
- **Li & Wong (2001) fit a model for each probe set, i.e., gene**

$$PM_{ij} - MM_{ij} = \theta_i \phi_j + \varepsilon_{ij}, \quad \varepsilon_{ij} \propto N(0, \sigma^2)$$

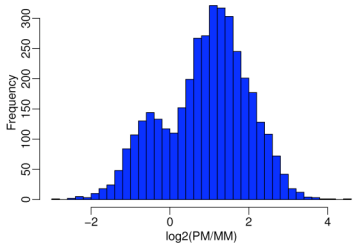
where

  - **θ_i: model based expression index (MBEI),**
  - **ϕ_j: probe sensitivity index.**
- **Maximun likelihood estimate of MBEI is used as expression measure for the gene in chip *i*.**
- **Non-linear normalization used**
- **Ad-hoc procedure used to remove outliers**
- **Need at least 10 or 20 chips**

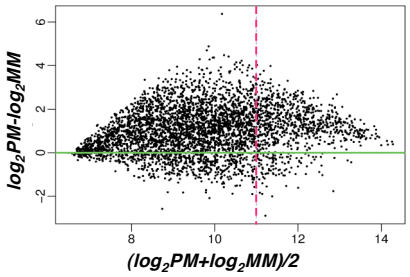
There is one more reason why PM-MM is undesirable



Especially for large PM

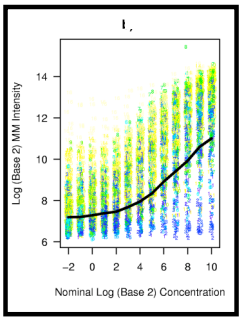


We see bimodality



Two more problems with MM

- MM detect signal
- MM cost $$$



MAS 5.0

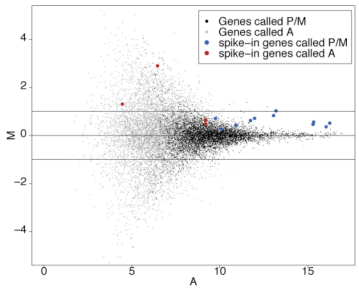
- Current version, MAS 5.0, uses Signal

$$signal = \text{Tukey Biweight}\{\log(PM_j - MM_j^*)\}$$

- Notice now log is used
- But what about negative PM-MM ?

- MM* is a new version of MM that is never larger than PM.
- If MM < PM, MM* = MM.
- If MM >= PM,
  - SB = Tukey Biweight (log(PM)-log(MM)) (log-ratio).
  - log(MM*) = log(PM)-log(max(SB, +ve)).
- Tukey Biweight: B(x) = (1 – (x/c)^2)^2 if |x|<c, 0 ow.

Can this be improved?

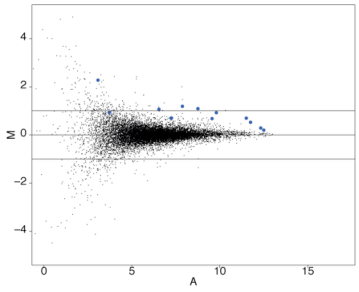


Rank of Spikeins (out of 12626)

141
250
364
368
480
586
686
838
945
1153
1567
NA
NA
NA
NA

We will discuss P/M/A calls later

Original MBEI



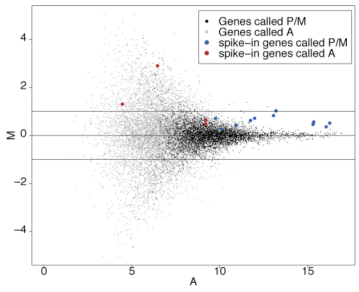
RMA

- **Robust regression method to estimate expression measure and SE from PM* (background adjusted normalized PM)**
- **Use quantile normalization**
- **Assume additive model**

$$\log_2(PM^*_{ij}) = a_i + b_j + \varepsilon_{ij}$$

- **Estimate RMA = a_i for chip *i* using robust method, such as median polish (fit iteratively, successively removing row and column medians, and accumulating the terms, until the process stabilizes).**
- **Works with *n=2* or more chips**
- **This is a robust multi-array analysis (RMA)**

Can this be improved?



Rank of Spikeins (out of 12626)

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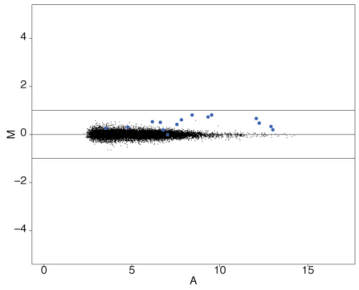
NA

RMA



Rank of Spikeins (out of 12626)

1
2
3
4
7
11
15
21
35
122
1182
230
450
1380
11700

Irizarry et al. (2003) *NAR* 31:e15

Detection

Detection

- The detection problem:
  "Given the probe-level data, which mRNA transcripts are present in the sample?"
- Biologists are mostly interested in expression levels, and so detection has received less attention
- To date only Affymetrix has tackled this, with
  - Rank-based tests
  - Implemented in MAS5.0

MAS Rank-based Detection

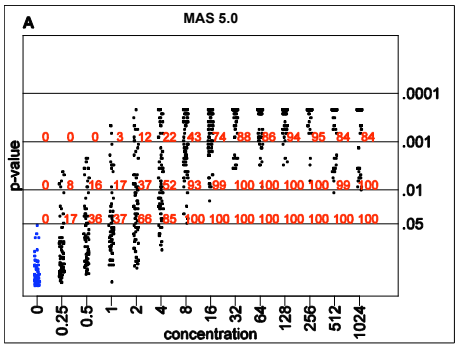
The test used in MAS 5.0 compares the following two hypotheses

H_0: median $(PM_j - MM_j)/(PM_j + MM_j) = \tau$;
H_1: median $(PM_j - MM_j)/(PM_j + MM_j) > \tau$.

Significance levels: $0 < \alpha_1 < \alpha_2 < 0.5$. If p is the p-value for the (rank) test, MAS 5.0 calls a transcript
absent: if $p > \alpha_2$,
marginal: if $\alpha_1 \leq p \leq \alpha_2$, and
present: if $p < \alpha_1$.

Typically tests are carried out with $\tau = 0.15$, $\alpha_1 = .04$ and $\alpha_2 = .06$.

Expression Detection



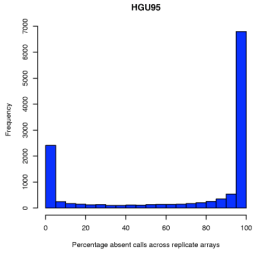
Remember uncertainty

- Some data analysts remove probesets called absent from further analysis
- This creates false negatives:

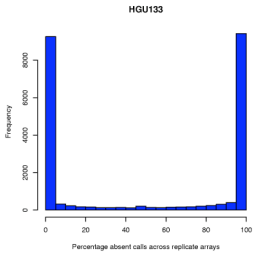
HG95	P	M	A
Present	82%	1%	17%
Absent	0%	0%	100%
HGU133	**P**	**M**	**A**
Present	77%	3%	20%
Absent	0%	0%	100%

From spike-in experiments

Consistency across reps



Consistency across reps



Current work

- **We need better estimates of means and variances of bivariate normal background noise**
- **Use observed MM intensities along with sequence information**
- **We also have a solution that does not use the MM**