Pre-processing in oligonucleotide microarray experiments

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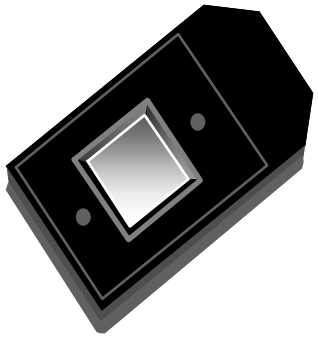
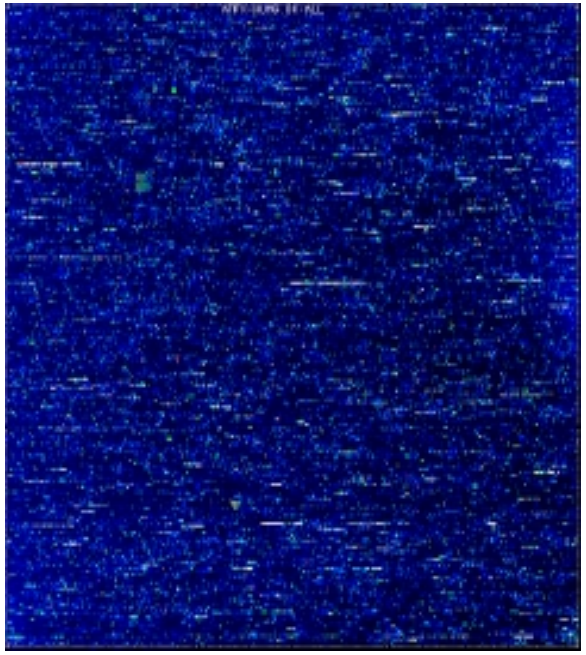
Oligonucleotide chips





Affymetrix files

- Main software from Affymetrix company MAS - MicroArray Suite, 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 (genes, gene fragments, ESTs).

Image analysis

- Raw data, **DAT image files → CEL files**
- Each probe cell: 10x10 pixels.
- **Gridding**: estimate location of probe cell centers.
- **Signal**:
  - Remove outer 36 pixels → 8x8 pixels.
  - The probe cell signal, PM or MM, is the 75th percentile of the 8x8 pixel values.
- **Background**: Average of the lowest 2% probe cell values is taken as the background value and subtracted.
- Compute also quality measures.

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 16 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., 20 PM and MM pairs, into a single **expression measure**.
- Expression measures may then be compared within or between chips for detecting differential expression.

Expression measures
MAS 4.0

- GeneChip® MAS 4.0 software uses **AvDiff**

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

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 $d_{(2)}, \ldots, d_{(J-1)}$.

- Log-ratio version is also used: average of log(PM/MM).

Expression measures
MAS 5.0

- GeneChip® MAS 5.0 software uses **Signal**

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

with MM * 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.

Expression measures
Li & Wong

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

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

  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*.
- Need at least 10 or 20 chips.
- Current version works with PMs only.

Expression measures

- Most expression measures are based on **PM-MM**, with the intention of correcting for non-specific binding and background noise.
- Problems:
  - MMs are PMs for some genes,
  - removing the middle base does not make a difference for some probes .
- Why not simply average PM or log PM? Not good enough, still need to adjust for background.
- Also need to normalize.

Expression measures
RMA

Irizarry et al. (2002).

1. Estimate **background** BG and use only background-corrected PM: $\log_2$(PM-BG).
2. Probe level **normalization** of $\log_2$(PM-BG) for suitable set of chips.
3. **Robust Multi-chip Analysis**, **RMA**, of $\log_2$(PM-BG).

RMA background, I

Simple background estimation

- Estimate $\log_2$(BG) as the mode of the $\log_2$(MM) distribution for a given chip (kernel density estimate).

- Quick fix when PM <= BG: use half of the minimum of $\log_2$(PM-BG) for PM > BG over all chips and probes.

RMA background, II

More refined background estimation

- Model observed PM as the sum of a signal intensity SG and a background intensity BG

$$PM = SG + BG,$$

  where it is assumed that SG is *Exponential* (α), BG is *Normal* (μ, σ^2), and SG and BG are independent.

- Background adjusted PM values are then **E(SG|PM)**.

Quantile normalization

- **Probe level quantile normalization** (Bolstad et al., 2002).
- Co-normalize probe level intensities, e.g. PM-BG or just PM or MM, for *n* chips by averaging each quantile across chips.
- Assumption: same probe level intensity distribution across chips.
- No need to choose a baseline or work in a pairwise manner.
- Deals with non-linearity.

Curve-fitting normalization

- Astrand (2001). Generalization of M vs. A robust local regression normalization for cDNA arrays.
- For *n* chips, regress orthonormal contrasts of probe level statistics on the average of the statistics across chips.

RMA expression measures, I

Simple measure

$$\mathrm{RMA} = \frac{1}{|\mathrm{A}|} \sum_{j \in A} \log_2 (PM_j - BG_j)$$

with A a set of "suitable" pairs.

RMA expression measures, II

- **Robust regression method** to estimate expression measure and SE from PM-BG values.
- Assume additive model

$$\log_2(PM_{ij} - BG) = a_i + b_j + \varepsilon_{ij}$$

- 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).
- Fine with *n=2* or more chips.

Conclusions

- Don’t use MM.
- “Background correct” PM. Even global background improves on probe-specific MM.
- Take logs: probe effect is additive on log scale.
- PMs need to be normalized (e.g. quantile normalization).
- RMA is arguably the best summary in terms of bias, variance, and model fit. Comparison study in Irizarry et al. (2002).

R software for pre-processing of Affymetrix data

- Bioconductor R package **`affy`**.
- Background estimation.
- Probe-level normalization: quantile, curve-fitting.
- Expression measures: AvDiff, Signal, Li & Wong (2001), RMA.
- Two main functions: **`ReadAffy`**, **`express`**.

Combining data across slides

Data on *G* genes for *n* hybridizations

→ *G x n* genes-by-arrays data matrix

Arrays

Genes	Array1	Array2	Array3	Array4	Array5	...
Gene1	0.46	0.30	0.80	1.51	0.90	...
Gene2	-0.10	0.49	0.24	0.06	0.46	...
Gene3	0.15	0.74	0.04	0.10	0.20	...
Gene4	-0.45	-1.03	-0.79	-0.56	-0.32	...
Gene5	-0.06	1.06	1.35	1.09	-1.09	...
...	...	...	...	...	...	

M = $\log_2$(Red intensity / Green intensity)

expression measure, e.g, RMA

Combining data across slides

… but columns have **structure**

How can we design experiments and combine data across slides to provide accurate estimates of the effects of interest?

Experimental design
Regression analysis