Microarray Data Analysis

Preprocessing for Affymetrix GeneChip Data

國立台灣大學資訊所

Course: 生物資訊與計算分子生物學

2007/11/06

吳漢銘
hmwu@stat.sinica.edu.tw
http://idv.sinica.edu.tw/hmwu

中央研究院 統計科學研究所
Institute of Statistical Science, Academia Sinica

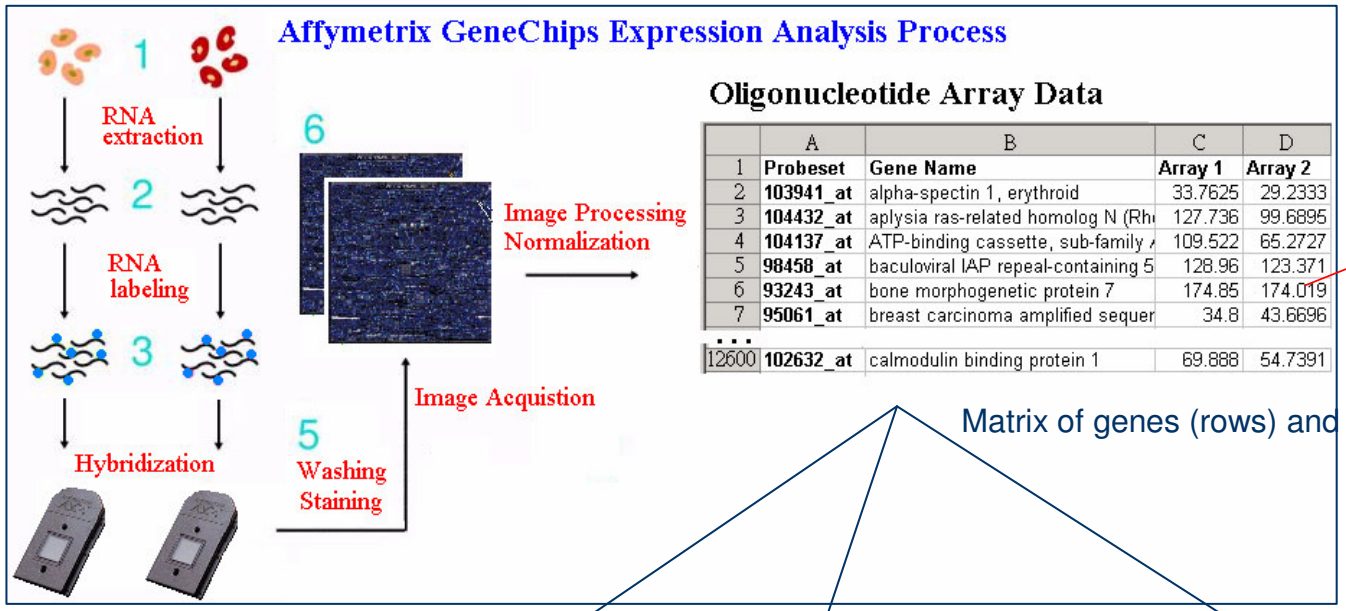
Outlines

- GeneChip Expression Array Design
- Assay and Analysis Flow Chart
- Quality Assessment
- Low Level Analysis
  (from probe level data to expression value)
- Software
- Useful Links and Reference

Affymetrix Dominates DNA Microarrays Market (75%~85%)

http://www.gene2drug.com/about/archives.asp?newsId=180

Overview of Microarray Analysis

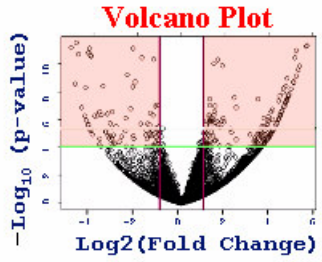


	A	B	C	D
1	Probeset	Gene Name	Array 1	Array 2
2	103941_at	alpha-spectin 1, erythroid	33.7625	29.2333
3	104432_at	aplysia ras-related homolog N (Rh	127.736	99.6895
4	104137_at	ATP-binding cassette, sub-family	109.522	65.2727
5	98458_at	baculoviral IAP repeal-containing 5	128.96	123.371
6	93243_at	bone morphogenetic protein 7	174.85	174.019
7	95061_at	breast carcinoma amplified sequer	34.8	43.6696
...				
12600	102632_at	calmodulin binding protein 1	69.888	54.7391

Discovery of differentially expressed genes

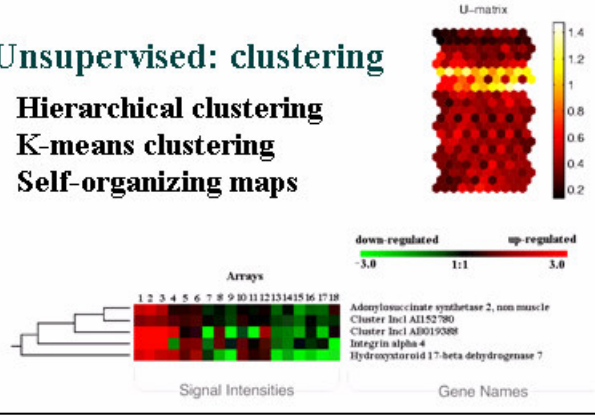
Parametric : t-test

Non-parametric : Wilcoxon, Mann-Whitney test



Unsupervised: clustering

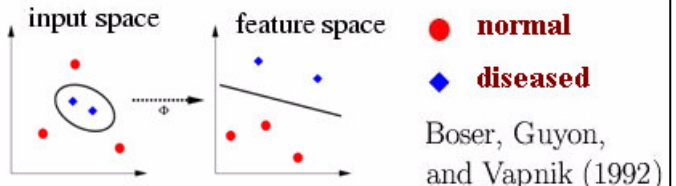
- Hierarchical clustering
- K-means clustering
- Self-organizing maps



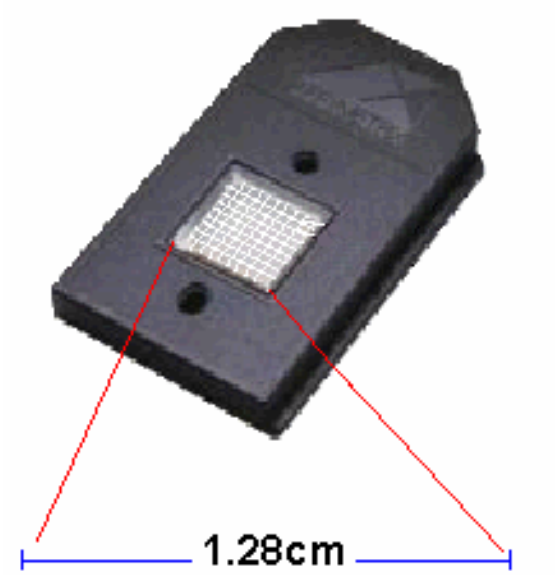
Supervised: classification

- Linear discriminants
- Decision trees
- Support vector machines

Support Vector Classifiers



GeneChip Expression Array Design



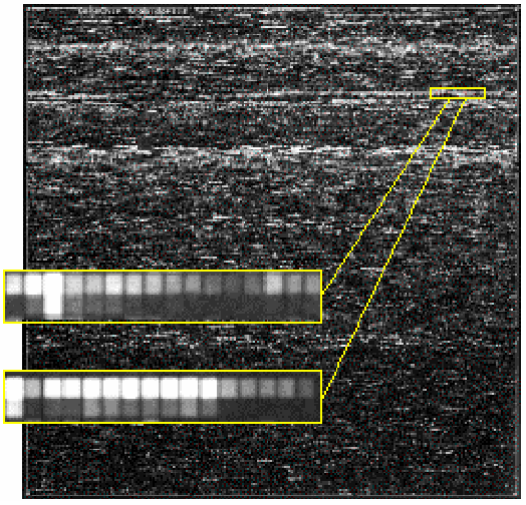
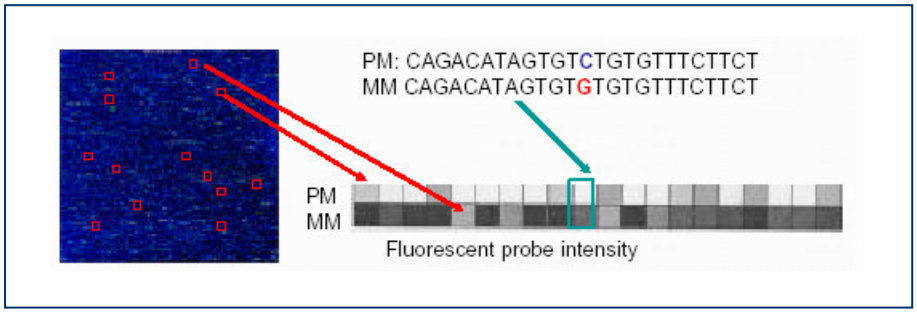
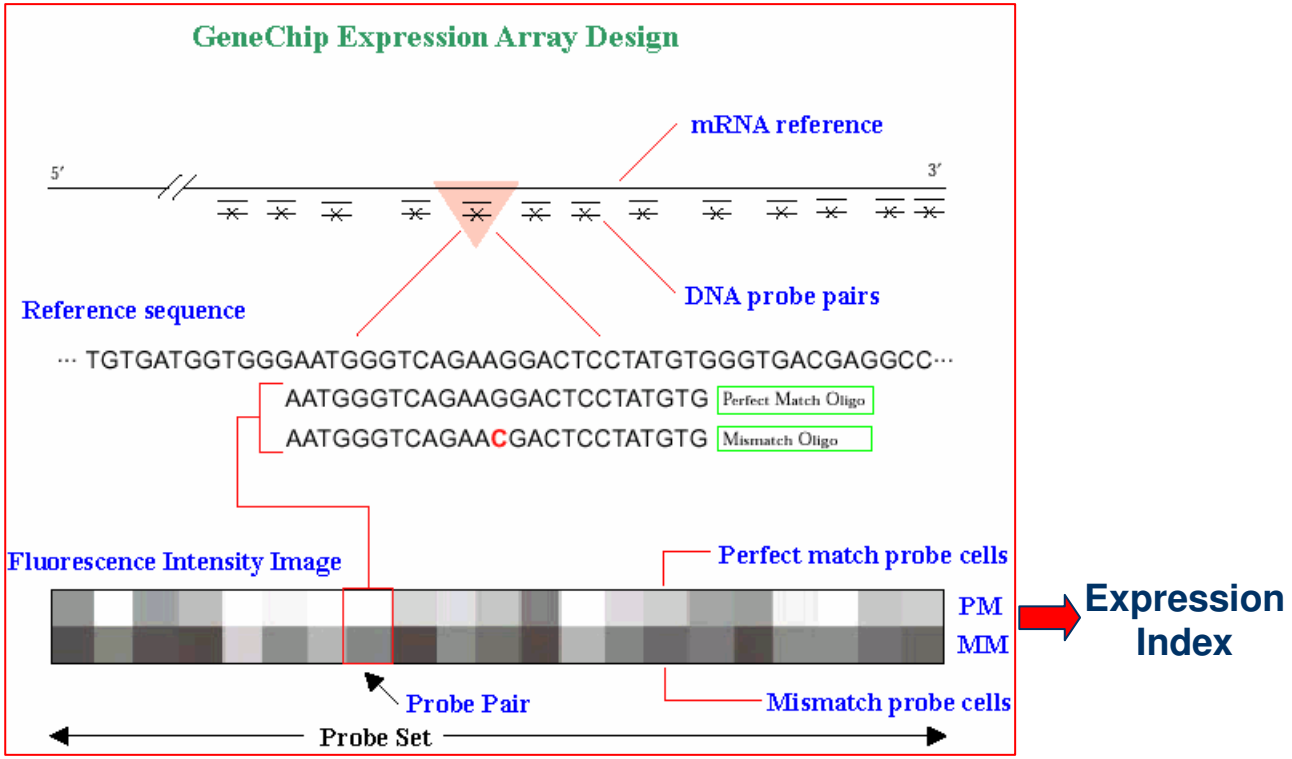


Image of hybridized probe array



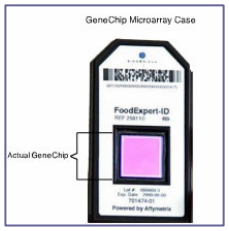


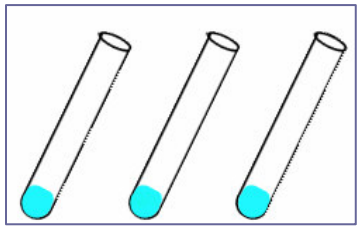
Animations

The Structure of a GeneChip® Microarray

How to Use GeneChip® Microarrays to Study Gene Expression

http://www.affymetrix.com/corporate/outreach/lesson_plan/educator_resources.affx
http://www.affymetrix.com/corporate/outreach/educator.affx





Genisphere

http://www.genisphere.com/ed_data_ref.html

HHMI (Howard Hughes Medical Institute)

http://www.hhmi.org/biointeractive/genomics/video.html
http://www.hhmi.org/biointeractive/genomics/animations.html
http://www.hhmi.org/biointeractive/genomics/click.html

DNA Interactive Site from Cold Spring Harbor Labs

http://www.dnai.org/index.htm
"Applications", => "Genes and Medicine" => "Genetic Profiling"

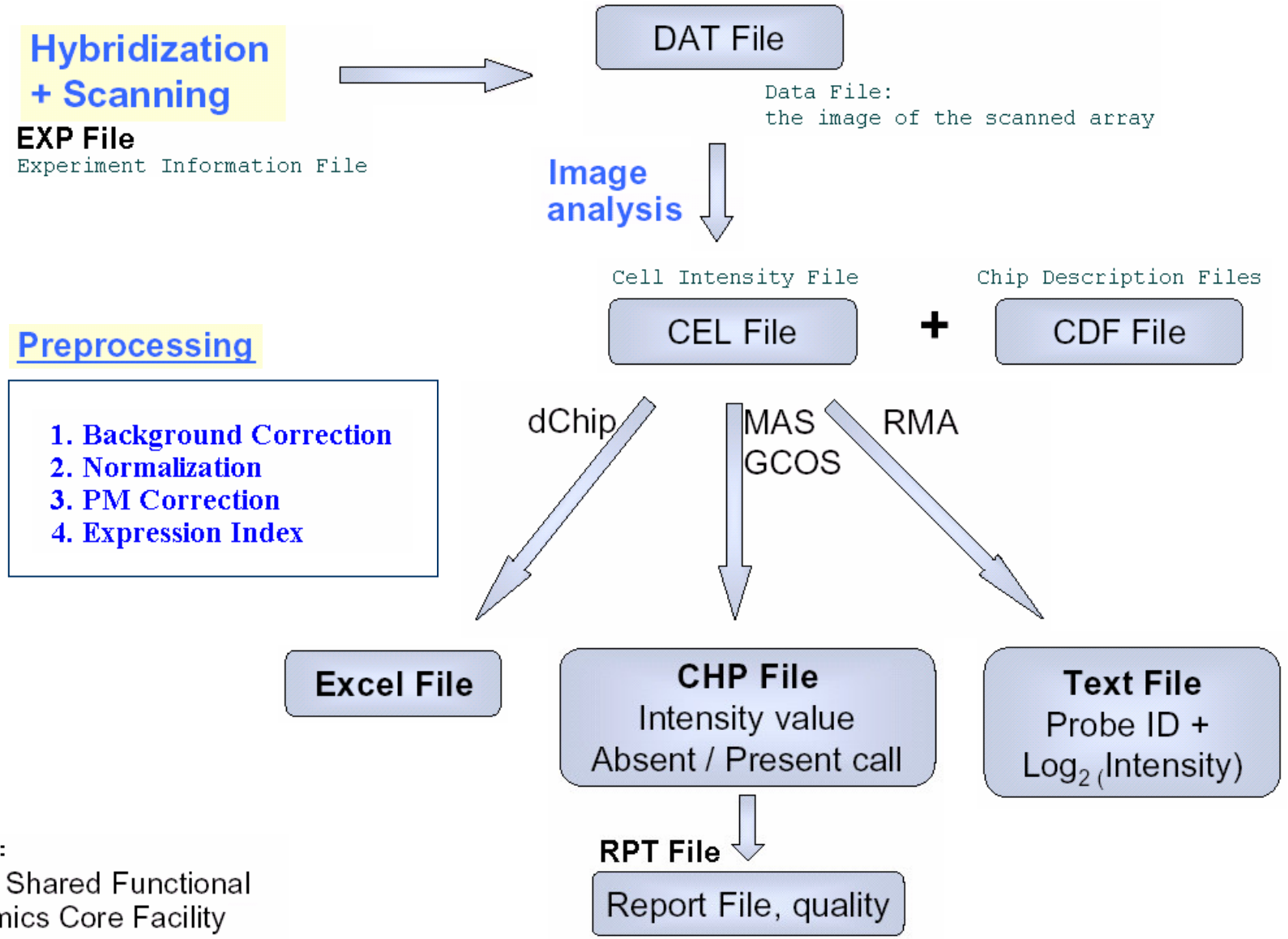
Digizyme - Web & Multimedia Design for the Sciences

http://www.digizyme.com/
http://www.digizyme.com/portfolio/microarraysfab/index.html
http://www.digizyme.com/competition/examples/genechip.swf

DNA Microarray Virtual Lab

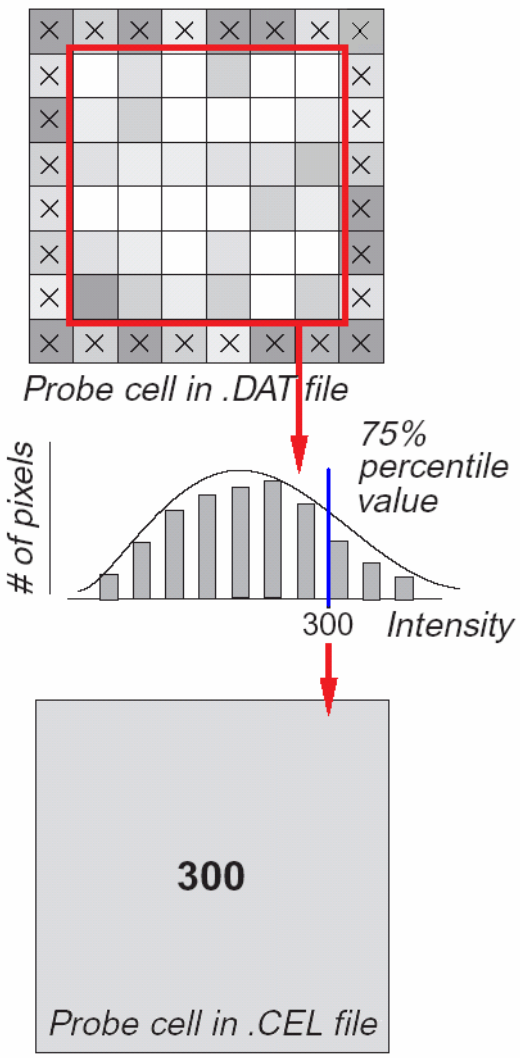
http://learn.genetics.utah.edu/units/biotech/microarray

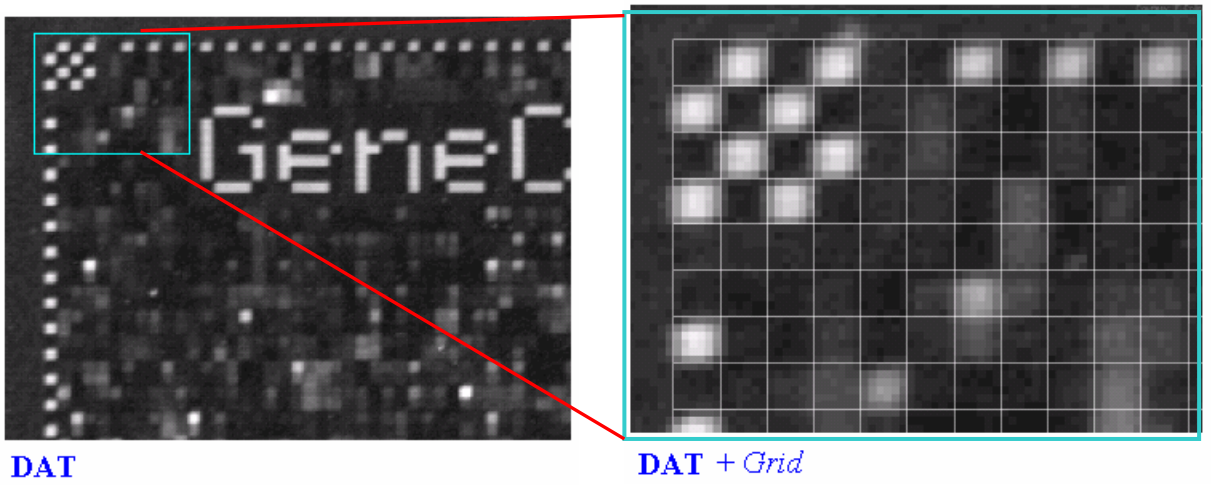
Assay and Analysis Flow Chart

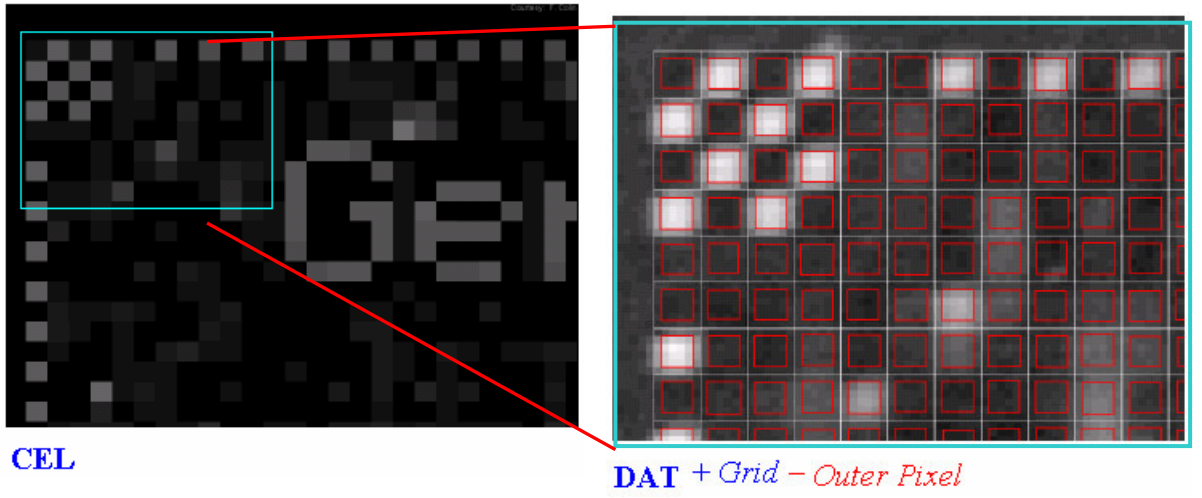


Source:
UCSF Shared Functional Genomics Core Facility

From DAT to CEL





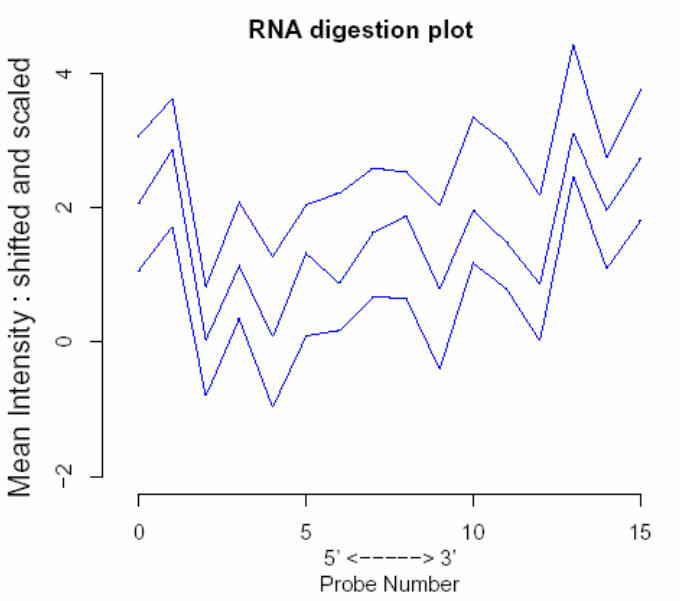


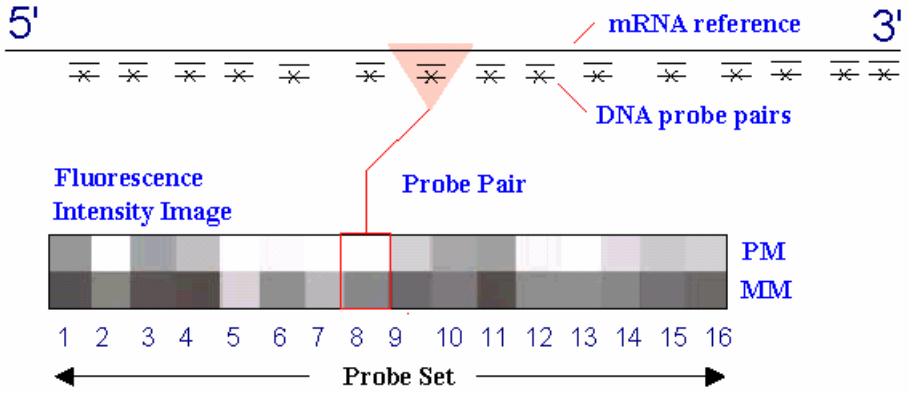
Quality Assessment

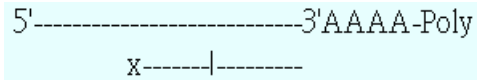
- RNA Sample Quality Control
- Array Hybridization Quality Control
- Statistical Quality Control (Diagnostic Plots)

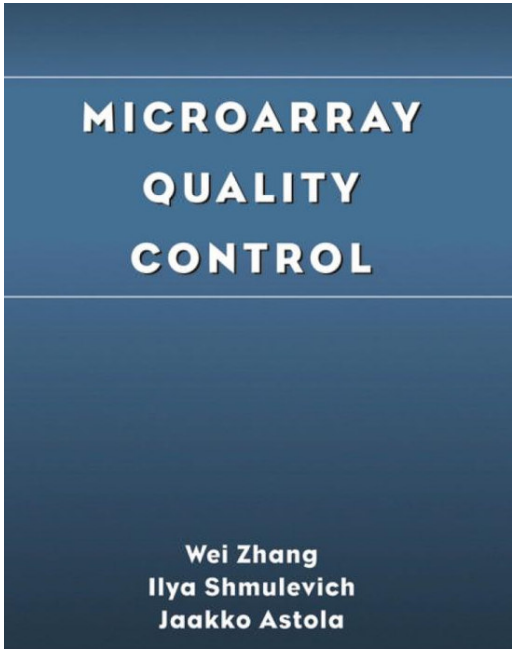
RNA Degradation Plots

Assessment of RNA Quality:





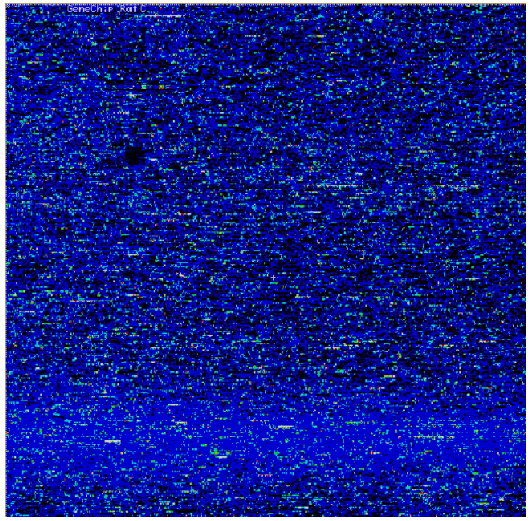




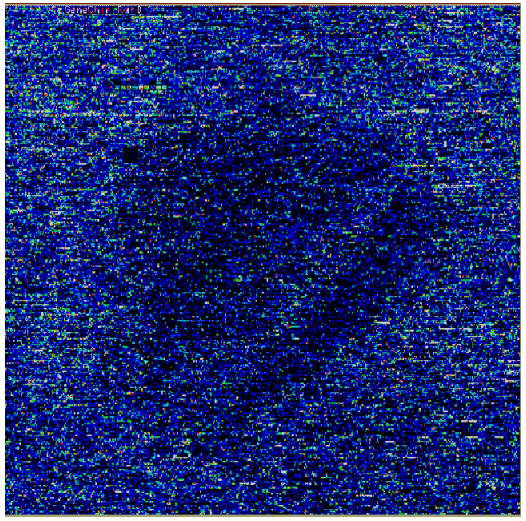
Probe Array Image Inspection

- Saturation: PM or MM cells > 46000
- Defect Classes:
  dimness/brightness, high Background, high/low intensity spots, scratches, high regional, overall background, unevenness, spots, Haze band, scratches, crop circle, cracked, cnow, grid misalignment.
- As long as these areas do not represent more than 10% of the total probes for the chip, then the area can be masked and the data points thrown out as outliers.

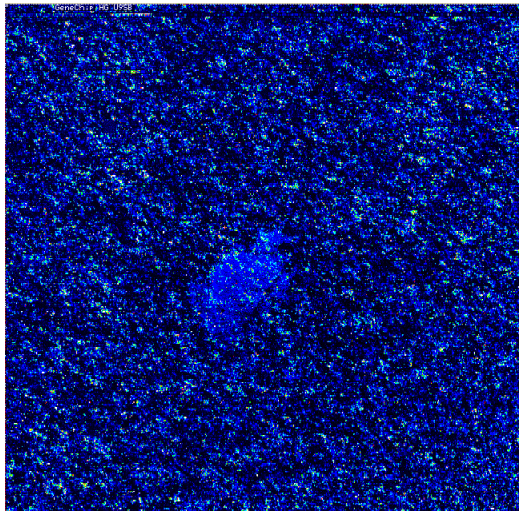
Haze Band



Crop Circles



Spots, Scratches, etc.



Source: Michael Elashoff (GLGC)

Probe Array Image Inspection (conti.)

Li, C. and Wong, W. H. (2001) Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection, Proc. Natl. Acad. Sci. Vol. 98, 31-36.

Fig. 1. A contaminated D array from the Murine 6500 Affymetrix GeneChip® set. Several particles are highlighted by arrows and are thought to be torn pieces of the chip cartridge septum, potentially resulting from repeatedly pipetting the target into the array.

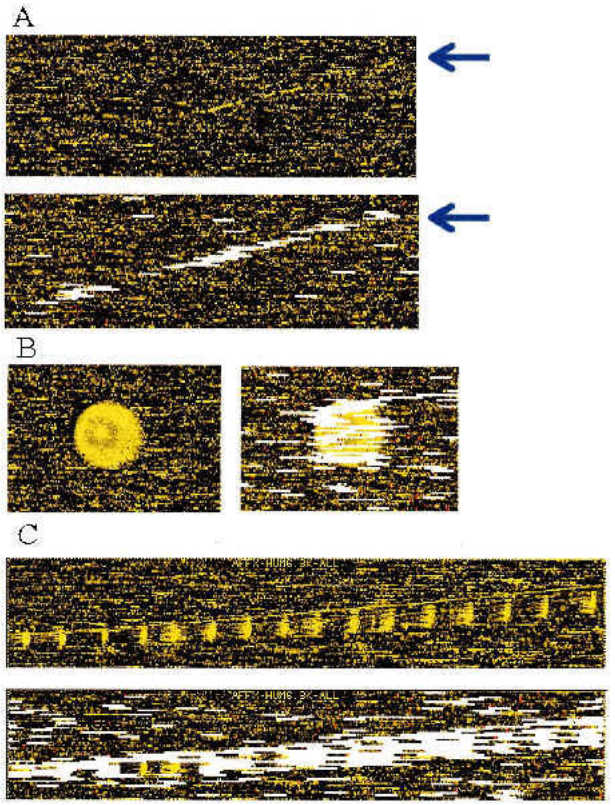
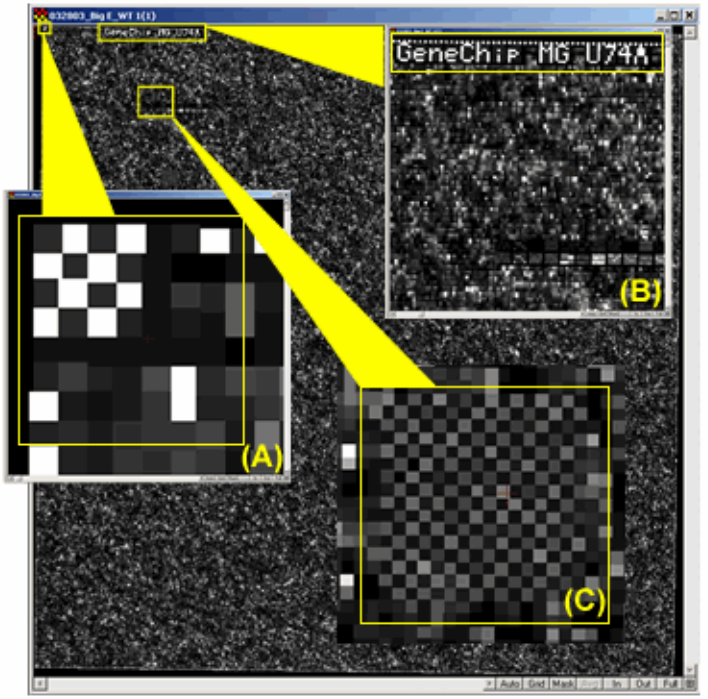


Fig. 5. (*A*) A long scratch contamination (indicated by arrow) is alleviated by automatic outlier exclusion along this scratch. (*B* and *C*) Regional clustering of array outliers (white bars) indicates contaminated regions in the original images. These outliers are automatically detected and accommodated in the analysis. Note that some probe sets in the contaminated region are not marked as array outliers, because contamination contributed additively to PM and MM in a similar magnitude and thus cancel in the PM–MM differences, preserving the correct signals and probe patterns.

B2 Oligo Performance

- Make sure the **alignment** of the grid was done appropriately.
- Look at the spiked in Oligo B2 control in order to check the **hybridization uniformity**.
- The border around the array, the corner region, the control regions in the center, are all checked to make sure the **hybridization** was successful.



Affymetrix CEL File Image- Yellow squares highlighting various Oligo B2 control regions: (A) one of the corner regions, (B) the name of the array, and (C) the "checkerboard" region.

Source: Baylor College of Medicine, Microarray Core Facility

MAS5.0 Expression Report File (*.RPT)


```
Report Type:                Expression Report
Date:                       04:42PM 02/24/2004

Filename:                   test.CHP
Probe Array Type:           HG-U133A
Algorithm:                  Statistical
Probe Pair Thr:             8
Controls:                   Antisense

Alpha1:                     0.05
Alpha2:                     0.065
Tau:                        0.015
Noise (RawQ):               2.250
Scale Factor (SF):          5.422
TGT Value:                  500
Norm Factor (NF):           1.000

Background:
        Avg: 64.23          Std: 1.75       Min: 59.50      Max: 67.70
Noise:
        Avg: 2.54           Std: 0.14       Min: 2.10       Max: 3.00
Corner+
        Avg: 49             Count: 32
Corner-
        Avg: 5377           Count: 32
Central-
        Avg: 4845           Count: 9

The following data represents probe sets that exceed the probe pair threshold
and are not called "No Call".

Total Probe Sets:           22283
Number Present:             9132    41.0%
Number Absent:              12766   57.3%
Number Marginal:            385     1.7%

Average Signal (P):         1671.0
Average Signal (A):         119.6
Average Signal (M):         350.1
Average Signal (All):       759.3
```

- The Scaling Factor- In general, the scaling factor should be around three, but as long as it is not greater than five, the chip should be okay.
- The scaling factor (SF) should remain consistent across the experiment.

- Average Background: 20-100
- Noise < 4

- The measure of Noise (RawQ), Average Background and Average Noise values should remain consistent across the experiment.

- Percent Present : 30~50%, 40~50%, 50~70%.
- Low percent present may also indicate degradation or incomplete synthesis.

MAS5.0 Expression Report File (*.RPT)

- Sig (3'/5')- This is a ratio which tells us how well the labeling reaction went. The two to really look at are your 3'/5' ratio for GAPDH and B-ACTIN. In general, they should be less than three.

- Spike-In Controls (BioB, BioC, BioD, Cre)- These spike in controls also tell how well your labelling reaction went. BioB is only Present half of the time, but BioC, BioD, & Cre should always have a present (P) call.

Housekeeping Controls:

Probe Set	Sig(5')	Det(5')	Sig(M')	Det(M')	Sig(3')	Det(3')	Sig(all)	Sig(3'/5')
AFFX-HUMISGF3A/M97935	272.8	P	856.8	P	1274.5	P	801.36	4.67
AFFX-HUMRGE/M10098	340.6	M	181.3	A	632.6	P	384.80	1.86
AFFX-HUMGAPDH/M33197	13890.6	P	15366.6	P	14060.7	P	14439.32	1.01
AFFX-HSAC07/X00351	35496.8	P	39138.0	P	31375.0	P	35336.61	0.88
AFFX-M27830	469.2	P	2206.1	A	114.3	A	929.86	0.24

Spike Controls:

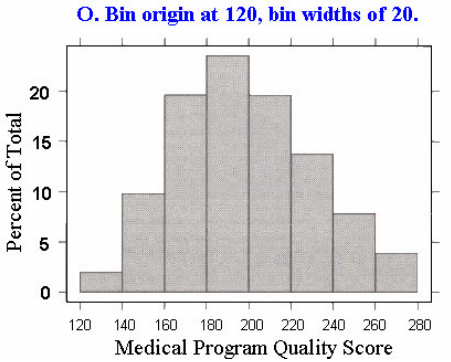
Probe Set	Sig(5')	Det(5')	Sig(M')	Det(M')	Sig(3')	Det(3')	Sig(all)	Sig(3'/5')
AFFX-BIOB	559.0	P	801.6	P	385.8	P	582.14	0.69
AFFX-BIOC	1132.9	P			818.0	P	975.47	0.72
AFFX-BIOD	874.7	P			6918.1	P	3896.42	7.91
AFFX-CRE	10070.5	P			16198.0	P	13134.27	1.61
AFFX-DAP	10.9	A	60.9	A	8.5	A	26.75	0.78
AFFX-LYS	51.5	A	86.2	A	14.1	A	50.62	0.27
AFFX-PHE	4.9	A	4.0	A	40.0	A	16.30	8.20
AFFX-THR	20.3	A	53.2	A	18.7	A	30.77	0.92
AFFX-TRP	9.8	A	11.1	A	2.7	A	7.86	0.28
AFFX-R2-EC-BIOB	497.6	P	928.0	P	479.4	P	634.98	0.96
AFFX-R2-EC-BIOC	1319.9	P			1705.0	P	1512.50	1.29
AFFX-R2-EC-BIOD	4744.0	P			4865.7	P	4804.82	1.03
AFFX-R2-P1-CRE	25429.2	P			30469.5	P	27949.37	1.20
AFFX-R2-BS-DAP	5.9	A	1.6	A	3.3	A	3.58	0.55
AFFX-R2-BS-LYS	32.2	A	43.7	M	74.7	P	50.18	2.32
AFFX-R2-BS-PHE	14.8	A	27.5	A	146.5	A	62.91	9.93
AFFX-R2-BS-THR	209.5	P	152.9	A	15.8	A	126.08	0.08

Statistical Plots: Histogram

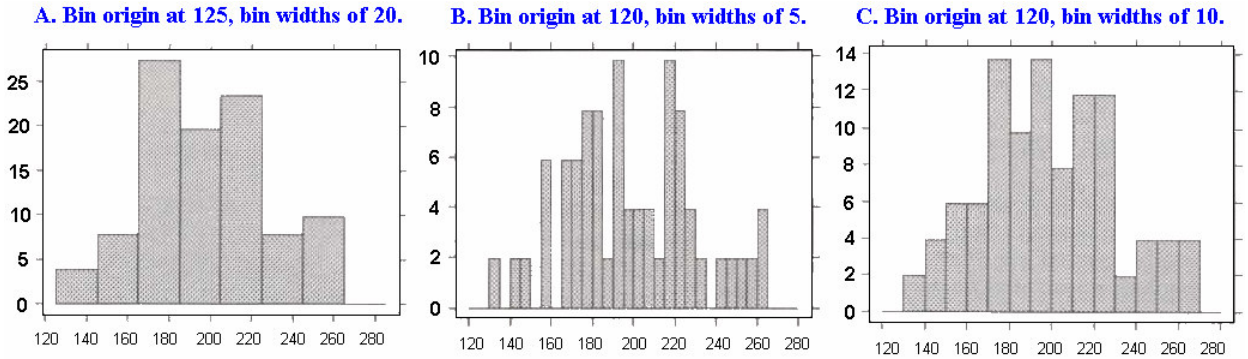
- 1/2h adjusts the height of each bar so that the total area enclosed by the entire histogram is 1.
- The area covered by each bar can be interpreted as the probability of an observation falling within that bar.

Disadvantage for displaying a variable's distribution:

- selection of origin of the bins.
- selection of bin widths.
- the very use of the bins is a distortion of information because any data variability within the bins cannot be displayed in the histogram.



Density Plots



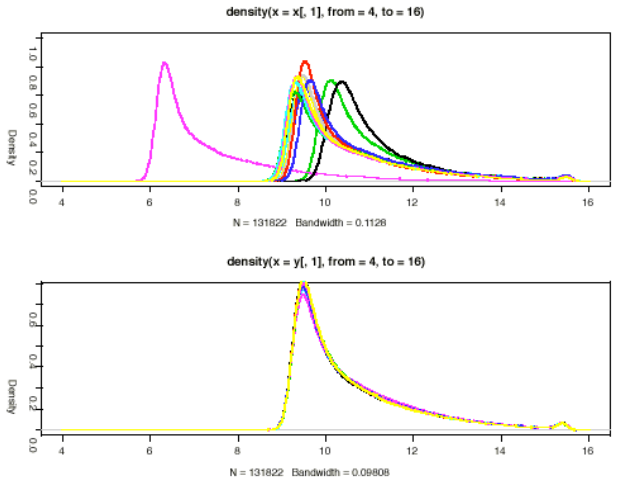
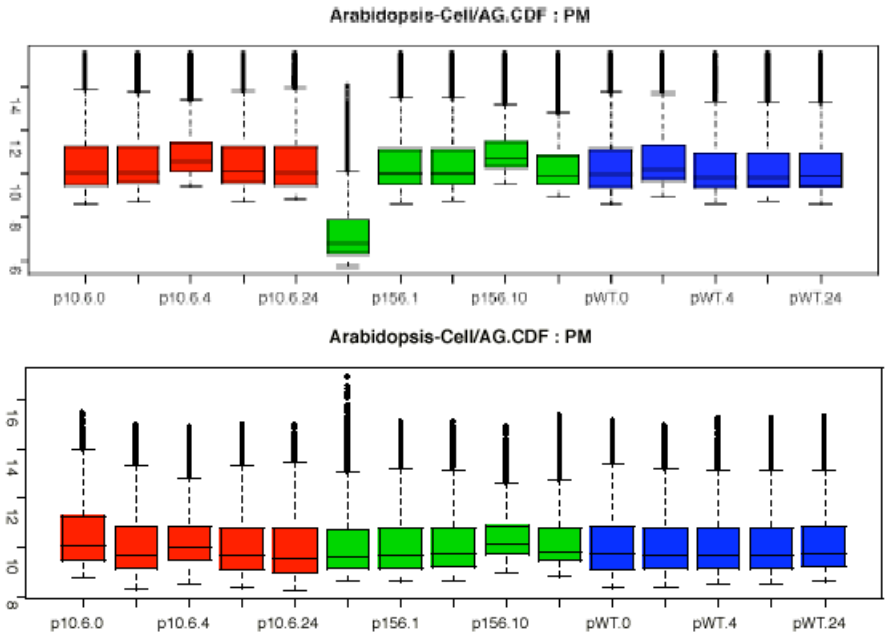
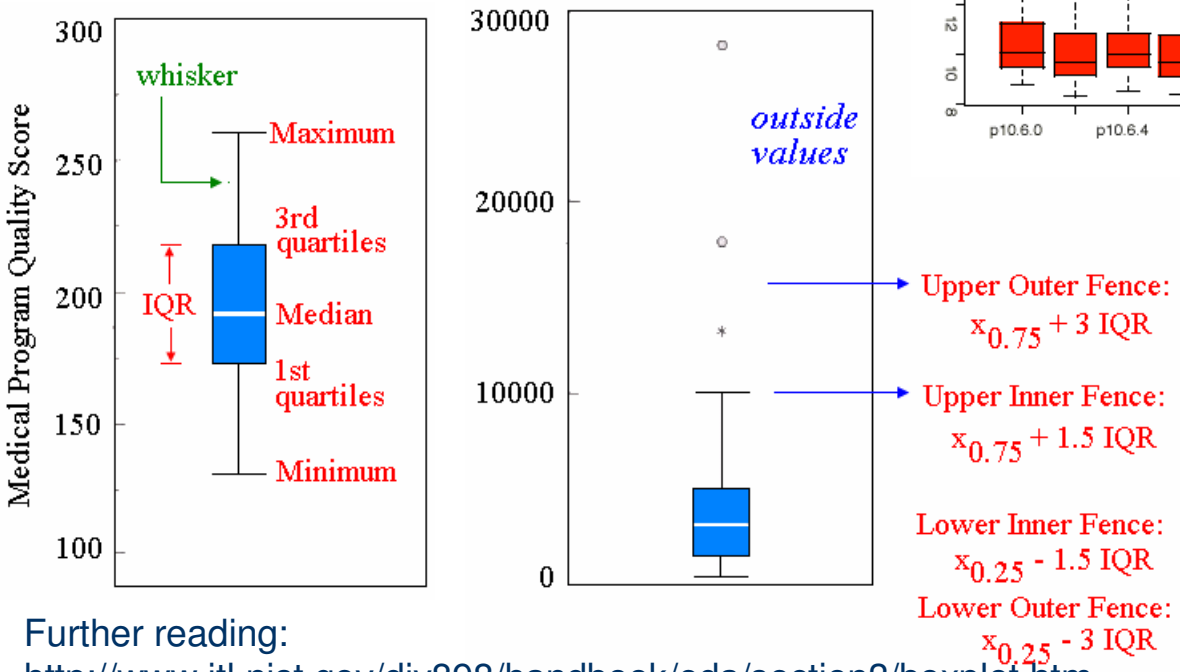


Figure Sources: Jacoby (1997).

Statistical Plots: Box Plots

- Box plots (Tukey 1977, Chambers 1983) are an excellent tool for conveying **location and variation** information in data sets.
- For detecting and illustrating location and variation changes between different groups of data.





Further reading:
http://www.itl.nist.gov/div898/handbook/eda/section3/boxplot.htm

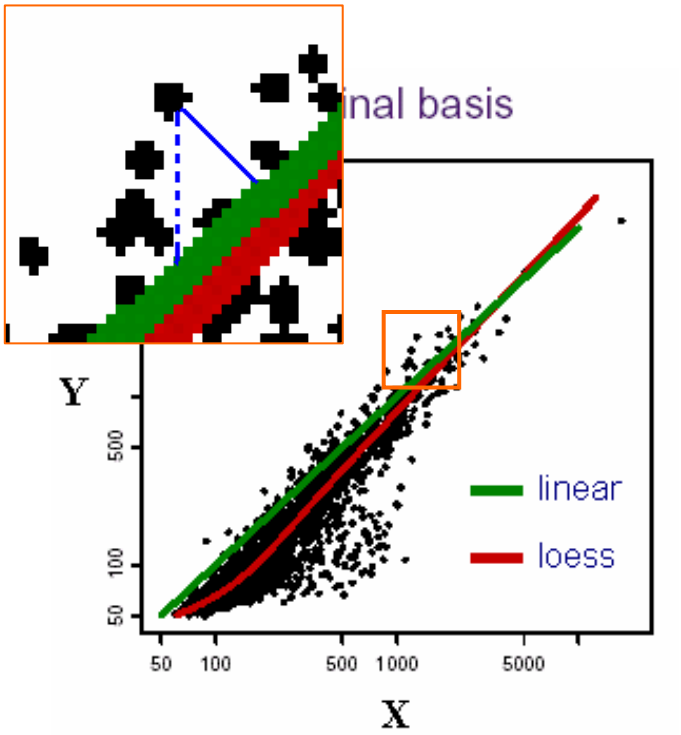
The box plot can provide answers to the following questions:

- Is a factor significant?
- Does the location differ between subgroups?
- Does the variation differ between subgroups?
- Are there any outliers?

Scatterplot and MA plot

- **Features of scatterplot.**
  - the substantial **correlation** between the expression values in the two conditions being compared.
  - the preponderance of low-intensity values.
    (the majority of genes are expressed at only a low level, and relatively few genes are expressed at a high level)

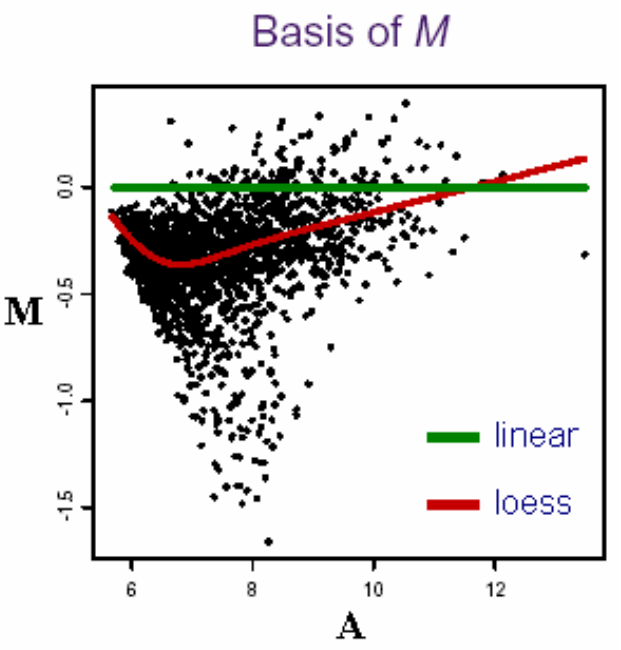
- **Goals:** to identify genes that are differentially regulated between two experimental conditions.



$$M = \log_2\left(\frac{Y}{X}\right)$$

$$A = \frac{1}{2}\log_2(X\,Y)$$

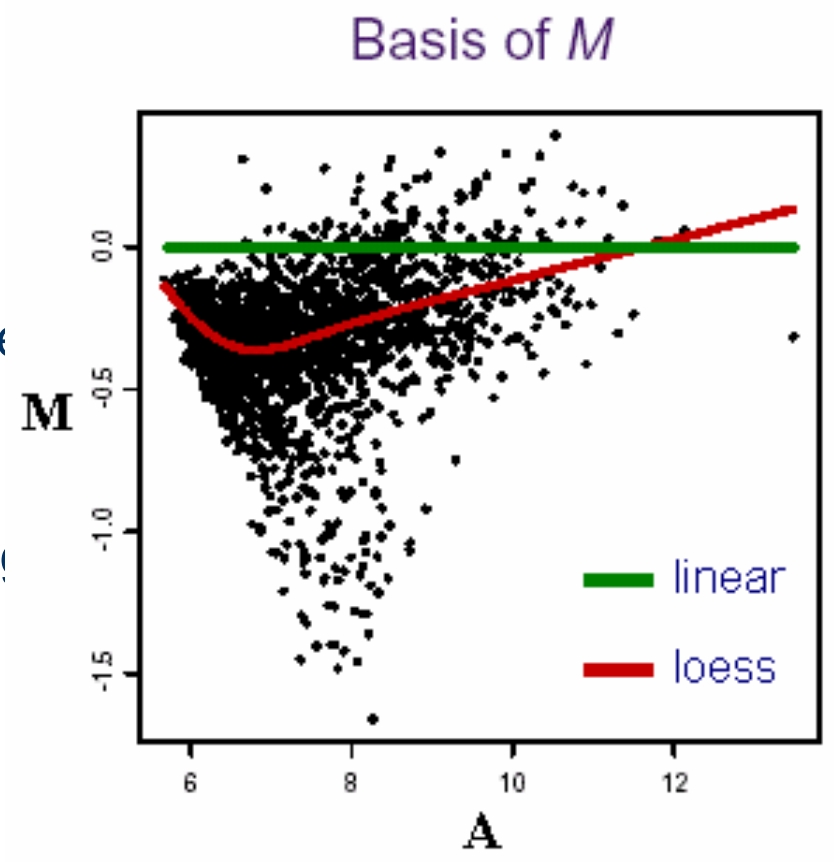
Oligo	cDNA
$X = PM_1$, $Y = PM_2$	X= Cy3, Y= Cy5
$X = PM_1\text{-}MM_1$, $Y = PM_2\text{-}MM_2$	



Scatterplot and MA plot (conti.)

- **MA plots** can show the intensity-dependant ratio of raw microarray data.
  - x-axis (mean log2 intensity): average intensity of a particular element across the control and experimental conditions.
  - y-axis (ratio): ratio of the two intensities. (fold change)

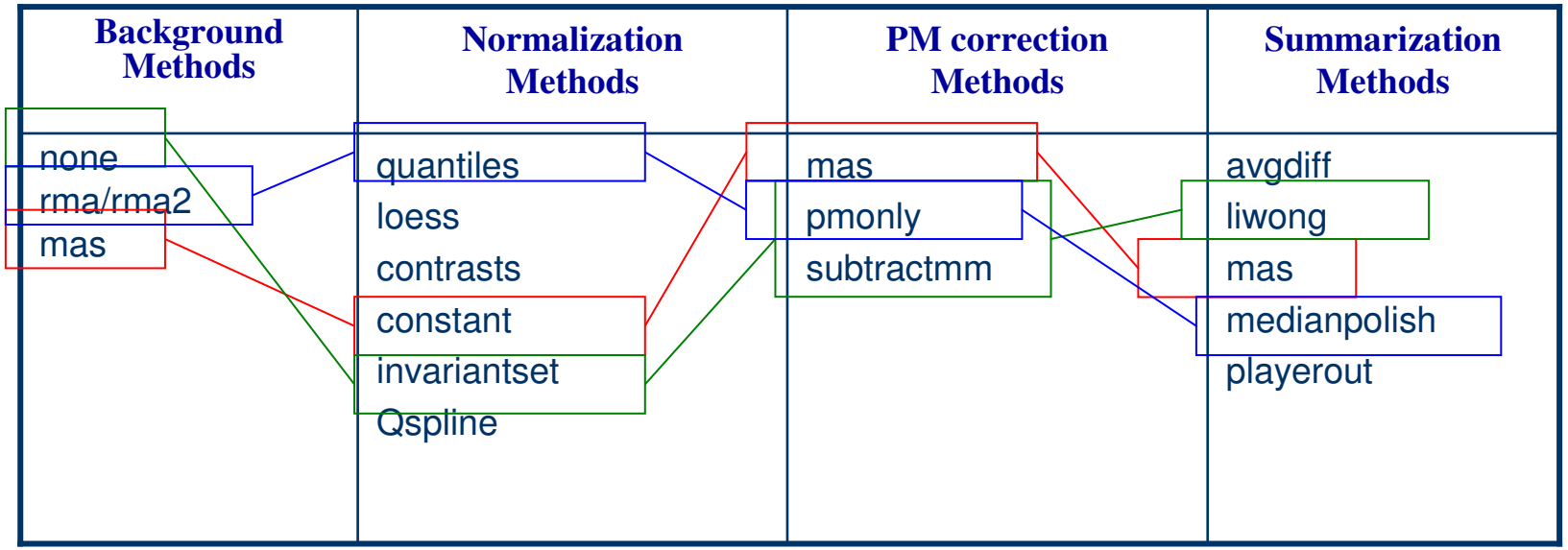
- **Outliers in logarithm scale**
  - spreads the data from the lower left corner to a more centered distribution in which the prosperities of the data are easy to analyze.
  - easier to describe the fold regulation of genes using a log scale. In log2 space, the data points are symmetric about 0.



Low level Analysis

- Background correction (local vs. global)
- Normalization (baseline array vs. complete data)
- PM Correction
- Summarization [Expression Index] (single vs. multiple chips)

Low level analysis



The Bioconductor: affy package

- MAS5

```
eset.mas5 <- expresso(Data, bg.correct="mas", normalize.method = "constant",
                      pmcorrect.method="mas",  summary.method="mas")
```

- Liwong (PM-only Model)

```
eset.liwong <- expresso(Data, bg.correct=FALSE, normalize.method = "invariantset",
                        pmcorrect.method="pmonly", summary.method="liwong")
```

- Liwong (PM-MM Model)

```
eset.liwong <- expresso(Data, bg.correct=FALSE, normalize.method = "invariantset",
                        pmcorrect.method="subtractmm ", summary.method="liwong")
```

- RMA

```
eset.rma <- expresso(Data, bg.correct="rma", normalize.method = "quantiles",
                     pmcorrect.method="pmonly", summary.method="medianpolish")
```

- Other

```
eset <- expresso(Data, bg.correct="mas", normalize.method="qspline",
                 pmcorrect.method="subtractmm",  summary.method="playerout")
```

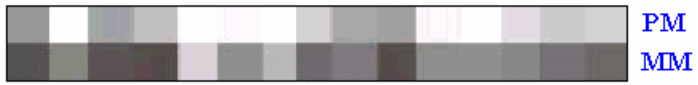
Background Correction

What is background?

- A measurement of signal intensity caused by auto fluorescence of the array surface and non-specific binding.
- Since probes are so densely packed on chip must use probes themselves rather than regions adjacent to probe as in cDNA arrays to calculate the background.
- In theory, the **MM** should serve as a biological background correction for the PM.

What is background correction?

- A method for removing background noise from signal intensities using information from only one chip.



Why Normalization?

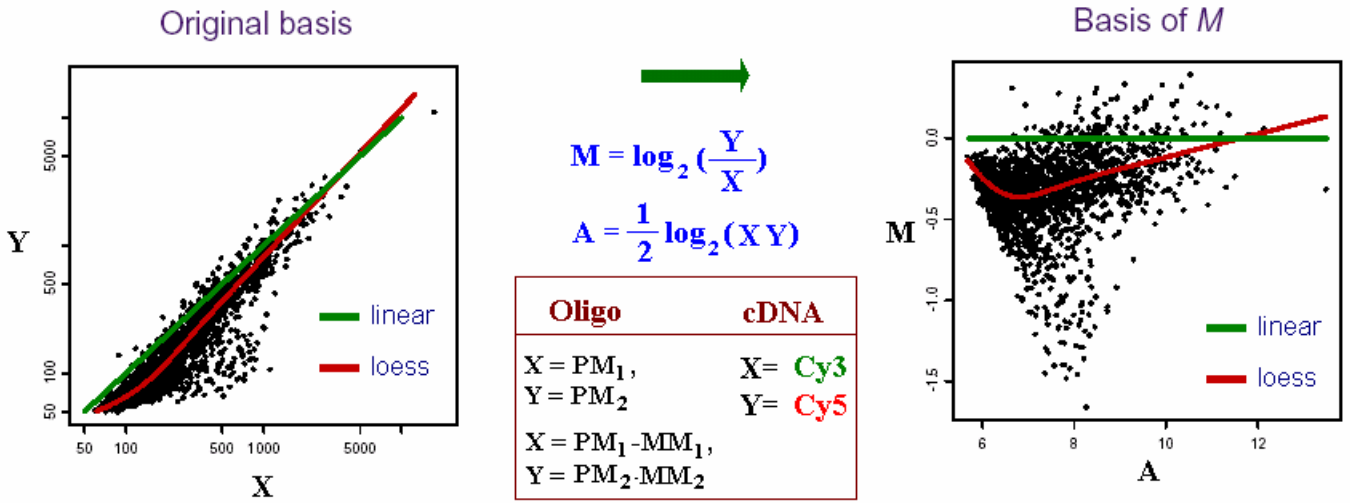
Normalization corrects for overall chip brightness and other factors that may influence the numerical value of expression intensity, enabling the user to more confidently compare gene expression estimates between samples.

Main idea

Remove the systematic bias in the data as completely possible while preserving the variation in the gene expression that occurs because of biologically relevant changes in transcription.

Assumption

- The average gene does not change in its expression level in the biological sample being tested.
- Most genes are not differentially expressed or up- and down-regulated genes roughly cancel out the expression effect.



The Options on Normalization

- Levels
  - PM&MM, PM-MM, Expression indexes
- Features
  - All, Rank invariant set, Spike-ins, housekeeping genes.
- Methods
  - Complete data: no reference chip, information from all arrays used: Quantiles Normalization, MVA Plot + Loess
  - Baseline: normalized using reference chip: MAS 4.0, MAS 5.0, Li-Wong's Model-Based, Qspline

Constant Normalization

Normalization and Scaling

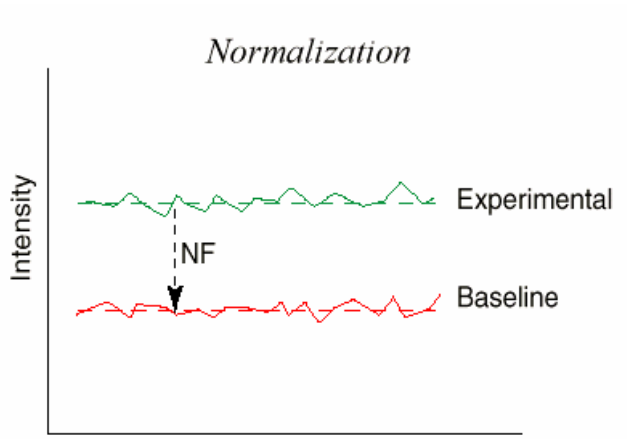
- The data can be normalized from:
  - a limited group of probe sets.
  - all probe sets.

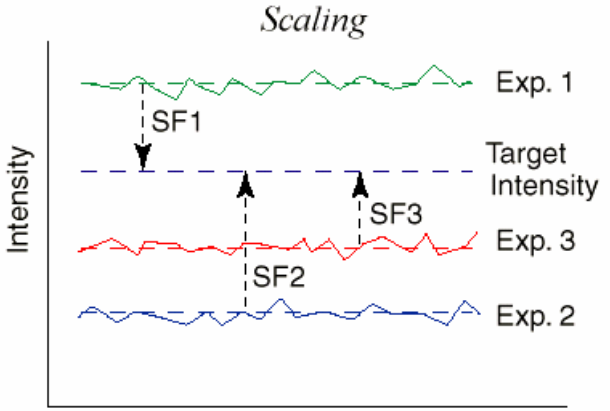
- **Global Scaling**
  the average intensities of all the arrays that are going to be compared are multiplied by scaling factors so that all average intensities are made to be numerically equivalent to a preset amount
  (termed target intensity).

$$SF = \frac{TGT}{TrimMean\left(2^{SignalLogValue_i}, 0.02, 0.98\right)}$$

$$A \times SF = TGT$$

$$\Rightarrow SF = \frac{TGT}{A}$$





- **Global Normalization**
  the normalization of the array is multiplied by a Normalization Factor (NF) to make its Average Intensity equivalent to the Average Intensity of the baseline array.

$$A_{exp} \times NF = A_{base}$$

$$\Rightarrow NF = \frac{A_{base}}{A_{exp}}$$

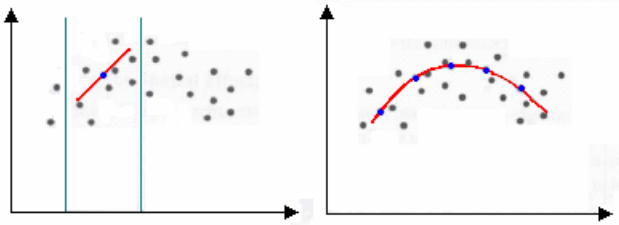
$$nf = \frac{TrimMean\left(SPVb_{i,}\ 0.02, 0.98\right)}{TrimMean\left(SPVe_{i,}\ 0.02, 0.98\right)}$$

Average intensity of an array is calculated by averaging all the Average Difference values of every probe set on the array, excluding the highest 2% and lowest 2% of the values.

LOESS Normalization

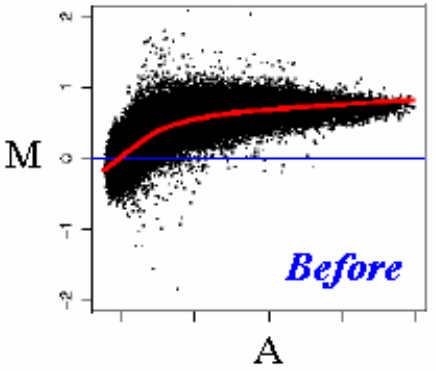
- Loess normalization (Bolstad *et al.*, 2003) is based on **MA plots**. Two arrays are normalized by using a lowess smoother.
- **Skewing** reflects experimental artifacts such as the
  - contamination of one RNA source with genomic DNA or rRNA,
  - the use of unequal amounts of radioactive or fluorescent probes on the microarray.
- Skewing can be corrected with local normalization: fitting a local regression curve to the data.

Loess regression
(locally weighted polynomial regression)



1. For any two arrays i,j with probe intensities x_{ki} and x_{kj} where $k = 1, \ldots, p$ represents the probe
2. we calculate $M_k = \log_2(x_{ki}/x_{kj})$ and $A_k = \frac{1}{2}\log_2(x_{ki}x_{kj})$.
3. A normalization curve is fitted to this M versus A plot using loess.

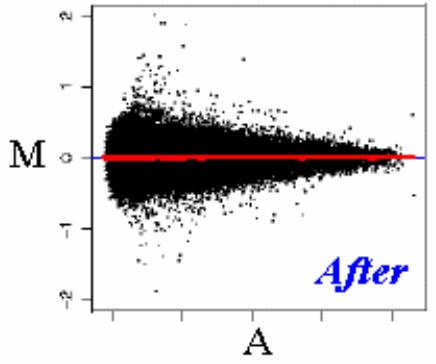
   Loess is a method of local regression (see Cleveland and Devlin (1988) for details).
4. The fits based on the normalization curve are $\hat{M}_k$
5. the normalization adjustment is $M'_k = M_k - \hat{M}_k$.
6. Adjusted probe intensites are given by $x'_{ki} = 2^{A_k + \frac{M'_K}{2}}$ and $x'_{ki} = 2^{A_K - \frac{M'_k}{2}}$.



$$M = \log_2\left(\frac{Y}{X}\right)$$

$$A = \frac{1}{2}\log_2(XY)$$

Oligo	cDNA
$X = PM_1$, $Y = PM_2$	X= Cy3, Y= Cy5
$X = PM_1\text{-}MM_1$, $Y = PM_2\text{-}MM_2$	



PM Correction Methods

- **PM only**
  make no adjustment to the PM values.
- **Subtract MM from PM**
  This would be the approach taken in MAS 4.0 Affymetrix (1999). It could also be used in conjuntion with the liwong model.

Table 1: Summary Table

Method	Assumptions	Benefits	Drawbacks
PM-MM	Background effects are large and potentially variable between features across experiments relative to effects of interest	Background effects minimized due to low bias Sensitivity to low expressors	Slightly noisier when signal is higher than background
PM-B	Features have approximately the same background	Low noise	May not represent all probe sets accurately, typically leading to underestimated differential change
PM Only	Background variation is insignificant	Low noise Approximately constant CV	All probe sets biased Compression of differential change at the low end
MM treated as additional PM	Background variation is insignificant Abundances moderate to large	Added statistical power Low noise Constant CV	All probe sets biased Compression of differential change at the low end

Affymetrix: Guide to Probe Logarithmic Intensity Error (PLIER) Estimation. Edited by: Affymetrix I. Santa Clara, CA, ; 2005.

Expression Index Estimates

Summarization

- Reduce the 11-20 probe intensities on each array to a single number for gene expression.
- The goal is to produce a measure that will serve as an indicator of the level of expression of a transcript using the PM (and possibly MM values).
- The values of the PM and MM probes for a probeset will be combined to produce this measure.
- **Single Chip**
  - avgDiff : no longer recommended for use due to many flaws.
  - **Signal** (MAS5.0): use One-Step Tukey biweight to combine the probe intensities in log scale
  - average log 2 (PM - BG)
- **Multiple Chip**
  - **MBEI** (li-wong): a multiplicative model
  - **RMA**: a robust multi-chip linear model fit on the log scale

Three Well-Known Methods

- MAS5 & PLIER
- Li-Wong Model
- RMA

MAS5 & PLIER (Affymetrix, 2005)

- Guide to Probe Logarithmic Intensity Error (PLIER) Estimation

	Previous Generation	2.0 Platform
Array Technology	• 18-µm features • Edge minimization mask strategy	• 11-µm features • Chrome setback mask design strategy • ARC
Image Analysis	Global gridding	Feature extraction (in addition to global gridding)
Data Management	MAS / LIMS	GCOS Client / Server
Analysis	MAS Statistical Algorithm	GREX including PLIER algorithm (in addition to MAS Statistical Algorithm)
Scanning Technology	GeneArray® 2500 or GeneChip® Scanner 3000	GeneChip® Scanner 3000 (high resolution)
Fluidics	Fluidics Station 400/Fluidics Station 450	Fluidics Station 450
AutoLoader	Not available on GeneArray® 2500 (optional for GeneChip® Scanner 3000)	Optional for GeneChip® Scanner 3000
Reagents	• 3rd-party cDNA reagents • Enzo labeling kits	• GeneChip® One- and Two-Cycle cDNA Kits • GeneChip® IVT Labeling Kit

Affymetrix: Guide to Probe Logarithmic Intensity Error (PLIER) Estimation. Edited by: Affymetrix I. Santa Clara, CA, ; 2005.

Liwong: Normalization

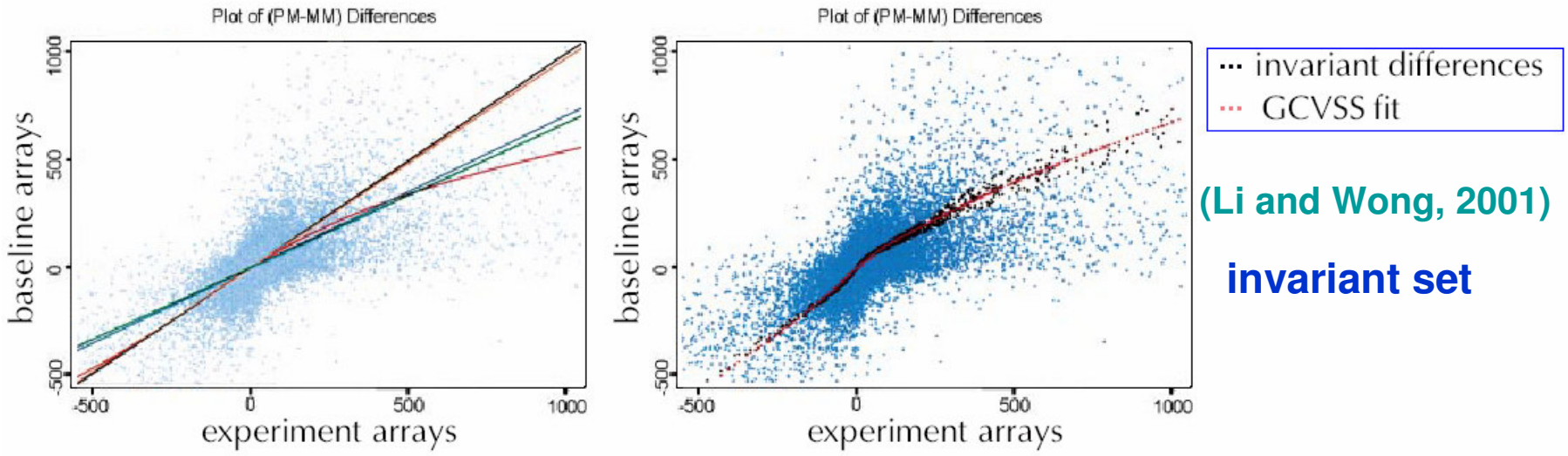
Liwong (PM-only Model)

```
eset.liwong <- expresso(Data, bg.correct=FALSE, normalize.method = "invariantset",
                        pmcorrect.method="pmonly", summary.method="liwong")
```

Liwong (PM-MM Model)

```
eset.liwong <- expresso(Data, bg.correct=FALSE, normalize.method = "invariantset",
                        pmcorrect.method="subtractmm ", summary.method="liwong")
```

- Using a baseline array, arrays are normalized by selecting invariant sets of genes (or probes) then using them to fit a *non-linear relationship* between the "treatment" and "baseline" arrays.
- A set of probe is said to be invariant if ordering of probe in one chip is same in other set.
- Fit the non-linear relation using cross validated smoothing splines (GCVSS).

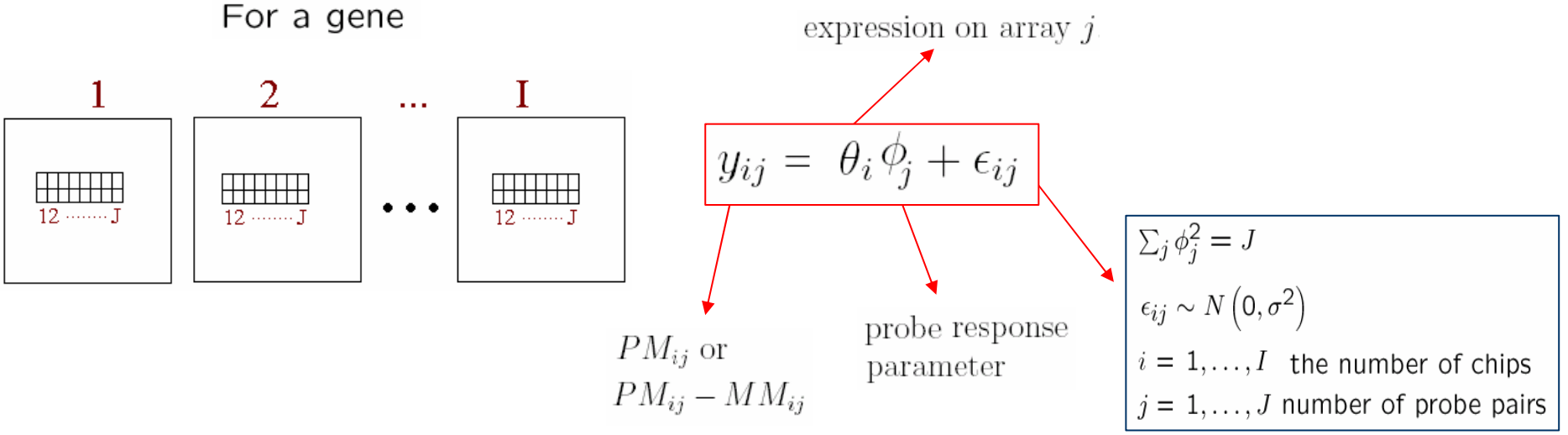


(Li and Wong, 2001)

invariant set

Liwong: Summarization Method

(Model-Based Expression Index , MBEI)



- θ i: this model computes an expression level on the ith array.
- SE(θ) 's and SE(φ) 's: can be used to identify outlier arrays and probes that will consequently be excluded from the final estimation of the probe response pattern.

- **Outlier array:** large SE(θ i), possibly due to external factors like the imaging process.
- **Outlier probe:** large SE(φ j), possibly due to non-specific cross-hybridization.
- **Single outliers:** individual PM-MM differences might also be identified by large residuals compared with the fit. (these are regarded as missing values in the model-fitting algorithm).

RMA: Background Correction


```
RMA
eset.rma <- expresso(Data, bg.correct="rma", normalize.method = "quantiles",
                           pmcorrect.method="pmonly", summary.method="medianpolish")
```

RMA: Robust Multichip Average (Irizarry and Speed, 2003):
assumes PM probes are a convolution of Normal and Exponential.

Observed PM = Signal + Noise $O = S + N$

Exponential (alpha) Normal (mu, sigma)

Use $E[S|O=o, S>0]$ as the background corrected PM.

$$E(s|O=o) = a + b\frac{\phi\left(\frac{a}{b}\right) - \phi\left(\frac{o-a}{b}\right)}{\Phi\left(\frac{a}{b}\right) + \Phi\left(\frac{o-a}{b}\right) - 1}$$

$a = s - \mu - \sigma^2\alpha$

$b = \sigma$

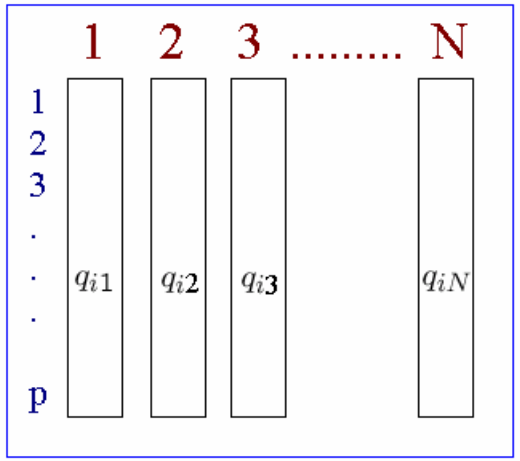
ϕ : standard normal density function

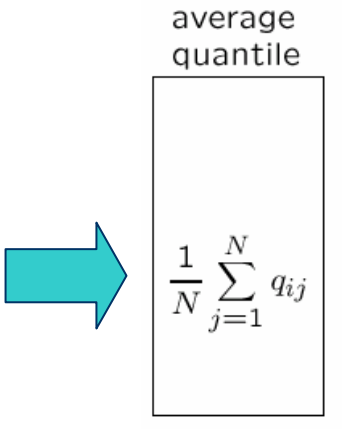
Φ : standard normal distribution function

Ps. MM probe intensities are not corrected by RMA/RMA2.

RMA: Normalization

- **Quantiles Normalization** (Bolstad *et al*, 2003) is a method to make the distribution of probe intensities the same for every chip.
- Each chip is really the transformation of an underlying common distribution.





The qth quantile of a data set is defined as that value where a q fraction of the data is below that value and (1-q) fraction of the data is above that value. For example, the 0.5 quantile is the median.

- The two distribution functions are effectively estimated by the sample quantiles.
- The normalization distribution is chosen by averaging each quantile across chips.

1. Given N datasets of length p form X of dimension $p \times N$ where each dataset is a column
2. Set $d = \left(\frac{1}{\sqrt{N}}, \ldots, \frac{1}{\sqrt{N}}\right)$
3. Sort each column of X to give X_{sort}
4. Project each row of X_{sort} onto d to get X'_{sort}
5. Get X_{norm} by rearranging each column of X'_{sort} to have the same ordering as original X

RMA: Summarization Method

MedianPolish

- This is the summarization used in the RMA expression summary Irizarry et al. (2003).
- A **multichip linear model** is fit to data from each probeset.
- The medianpolish is an algorithm (see Tukey (1977)) for fitting this model robustly.
- Please note that expression values you get using this summary measure will be in log2 scale.

for a probeset k

$$\log_2\left(PM_{ij}^{(k)}\right) = \alpha_i^{(k)} + \beta_j^{(k)} + \epsilon_{ij}^{(k)}$$

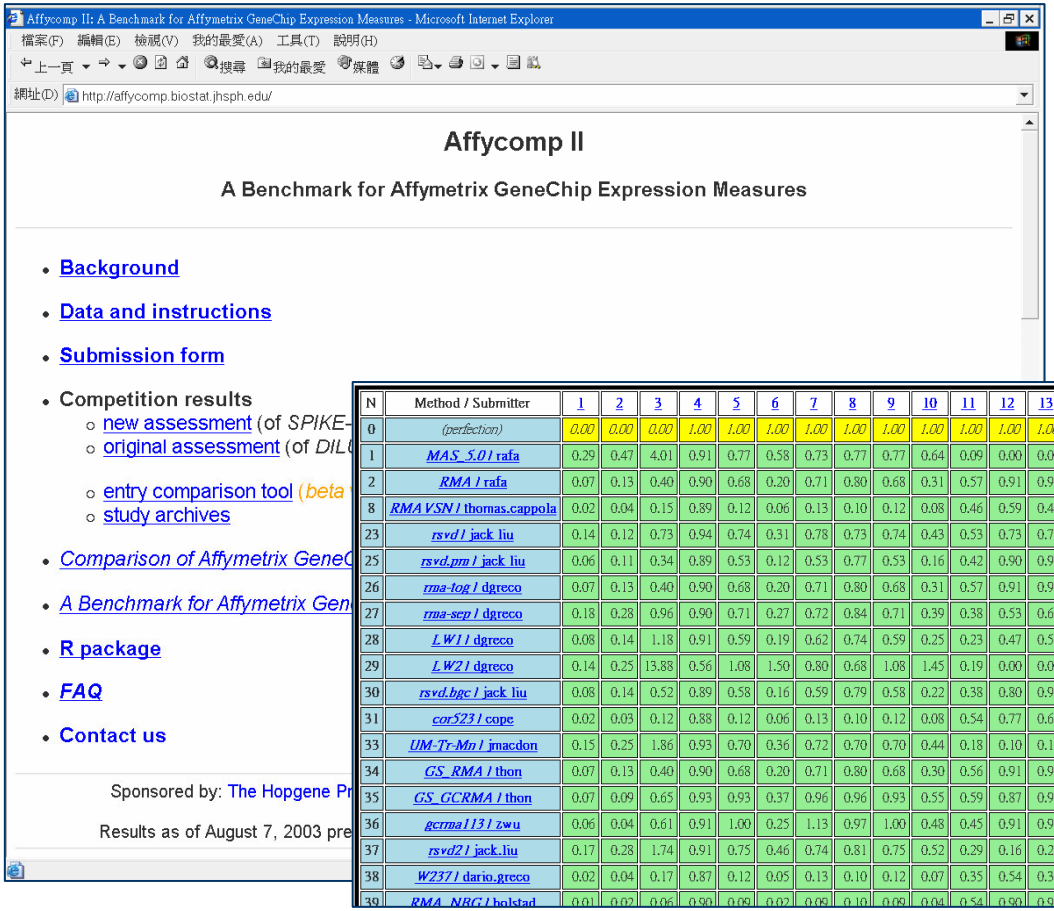
$i = 1, \ldots, I_k$ probes

$j = 1, \ldots, J$ arrays

probe effect

$\log_2$ expression value

Comparison of Affymetrix GeneChip Expression Measures



N	Method / Submitter	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	*(perfection)*	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.0
1	*MAS_5.0* / rafa	0.29	0.47	4.01	0.91	0.77	0.58	0.73	0.77	0.77	0.64	0.09	0.00	0.00	0.0
2	*RMA* / rafa	0.07	0.13	0.40	0.90	0.68	0.20	0.71	0.80	0.68	0.31	0.57	0.91	0.96	0.6
8	*RMA VSN* / thomas.cappola	0.02	0.04	0.15	0.89	0.12	0.06	0.13	0.10	0.12	0.08	0.46	0.59	0.43	0.4
23	*rsvd* / jack liu	0.14	0.12	0.73	0.94	0.74	0.31	0.78	0.73	0.74	0.43	0.53	0.73	0.71	0.5
25	*rsvd.pm* / jack liu	0.06	0.11	0.34	0.89	0.53	0.12	0.53	0.77	0.53	0.16	0.42	0.90	0.96	0.5
26	*rma-tog* / dgreco	0.07	0.13	0.40	0.90	0.68	0.20	0.71	0.80	0.68	0.31	0.57	0.91	0.96	0.65
27	*rma-sep* / dgreco	0.18	0.28	0.96	0.90	0.71	0.27	0.72	0.84	0.71	0.39	0.38	0.53	0.63	0.42
28	*LW1* / dgreco	0.08	0.14	1.18	0.91	0.59	0.19	0.62	0.74	0.59	0.25	0.23	0.47	0.55	0.29
29	*LW2* / dgreco	0.14	0.25	13.88	0.56	1.08	1.50	0.80	0.68	1.08	1.45	0.19	0.00	0.00	0.14
30	*rsvd.bgc* / jack liu	0.08	0.14	0.52	0.89	0.58	0.16	0.59	0.79	0.58	0.22	0.38	0.80	0.90	0.49
31	*cor523* / cope	0.02	0.03	0.12	0.88	0.12	0.06	0.13	0.10	0.12	0.08	0.54	0.77	0.61	0.60
33	*UM-Tr-Mn* / jmacdon	0.15	0.25	1.86	0.93	0.70	0.36	0.72	0.70	0.70	0.44	0.18	0.10	0.10	0.16
34	*GS_RMA* / thon	0.07	0.13	0.40	0.90	0.68	0.20	0.71	0.80	0.68	0.30	0.56	0.91	0.96	0.65
35	*GS_GCRMA* / thon	0.07	0.09	0.65	0.93	0.93	0.37	0.96	0.96	0.93	0.55	0.59	0.87	0.90	0.66
36	*gcrma113* / zwu	0.06	0.04	0.61	0.91	1.00	0.25	1.13	0.97	1.00	0.48	0.45	0.91	0.92	0.57
37	*rsvd2* / jack.liu	0.17	0.28	1.74	0.91	0.75	0.46	0.74	0.81	0.75	0.52	0.29	0.16	0.21	0.26
38	*W237* / dario.greco	0.02	0.04	0.17	0.87	0.12	0.05	0.13	0.10	0.12	0.07	0.35	0.54	0.39	0.39
39	*RMA_NBG* / bolstad	0.01	0.02	0.06	0.90	0.09	0.02	0.09	0.10	0.09	0.04	0.54	0.90	0.93	0.63

Data and instructions

1. Download the spike-in and dilution data sets.
   - **Spike-in hgu95a Data**

Method	SD	99.9%	slope low	slope med	slope high	AUC
GCRMA	0.08	0.74	0.66	1.06	0.56	0.70
GS_GCRMA	0.10	0.79	0.62	1.03	0.55	0.66
MMEI	0.04	0.23	0.16	0.54	0.46	0.62
GL	0.05	0.25	0.16	0.55	0.46	0.62
RMA_NBG	0.04	0.24	0.16	0.56	0.46	0.61
RSVD	0.00	0.58	0.42	0.85	0.40	0.61
ZL	0.22	0.52	0.35	0.71	0.45	0.61
VSN_scale	0.09	0.43	0.28	0.91	0.70	0.59
VSN	0.06	0.28	0.18	0.6	0.46	0.59
RMA_VSN	0.09	0.48	0.31	0.74	0.46	0.57
GLTRAN	0.07	0.42	0.23	0.61	0.45	0.55
ZAM	0.09	0.50	0.30	0.70	0.47	0.54
RMA_GNV	0.11	0.58	0.35	0.76	0.47	0.52
RMA	0.11	0.57	0.35	0.76	0.47	0.52
GSrma	0.11	0.57	0.35	0.76	0.47	0.52
GSVDmod	0.07	0.44	0.22	0.64	0.42	0.51
PerfectMatch	0.05	0.40	0.18	0.56	0.43	0.50
PLIER+16	0.13	0.83	0.49	0.80	0.46	0.48
GSVDmin	0.08	0.60	0.22	0.62	0.41	0.41
MAS 5.0+32	0.14	1.07	0.35	0.71	0.44	0.12
ChipMan	0.27	2.26	0.44	1.11	0.68	0.12
qn.p5	0.12	1.09	0.13	0.50	0.52	0.11
dChip	0.13	1.44	0.31	0.67	0.39	0.09
mmgMOSgs	0.40	3.27	1.34	1.13	0.45	0.07
gMOSv.1	0.29	3.35	0.98	1.12	0.42	0.06
ProbeProfi ler	0.31	18.75	1.61	1.57	0.39	0.03
dChip PM-MM	0.23	14.83	1.40	0.86	0.35	0.02
mgMOS_gs	0.36	2.86	0.83	0.86	0.43	0.01
MAS 5.0	0.63	4.48	0.69	0.81	0.45	0.00
PLIER	0.19	123.27	0.75	0.85	0.46	0.00
UM-Tr-Mn	0.32	2.92	0.58	0.83	0.42	0.00

http://affycomp.biostat.jhsph.edu/

- Cope LM, Irizarry RA, Jaffee HA, Wu Z, Speed TP. A benchmark for Affymetrix GeneChip expression measures, Bioinformatics. 2004 Feb 12;20(3):323-31.
- Irizarry RA, Wu Z, Jaffee HA. Comparison of Affymetrix GeneChip expression measures. Bioinformatics. 2006 Apr 1;22(7):789-94.

Software

Image Analysis/Normalization

Shareware/Freeware

- Bioconductor (R, Gentleman)
- DNA-Chip Analyzer (dChip) (Li and Wong)
- RMAExpress: a simple standalone GUI program for windows for computing the RMA expression measure.

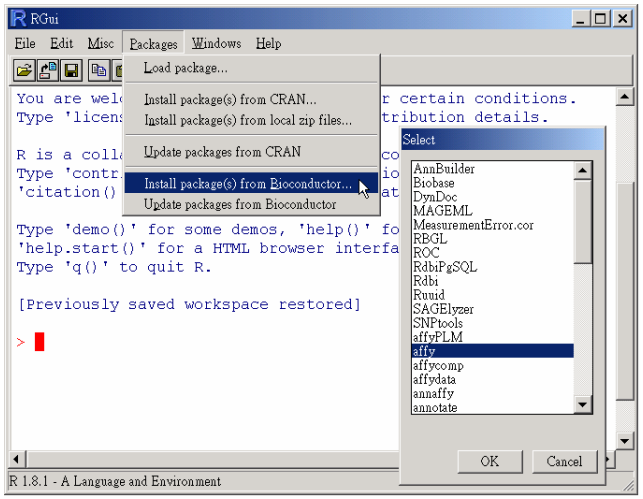
Commercial

- Affymetrix GeneChip Operating Software (GCOS v1.4)
- GeneSpring GX v7.3

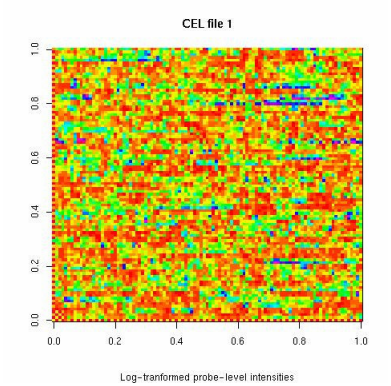
The Bioconductor: affy

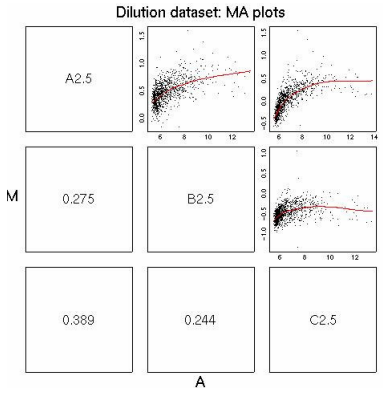
The Bioconductor Project
Release 2.1
http://www.bioconductor.org/

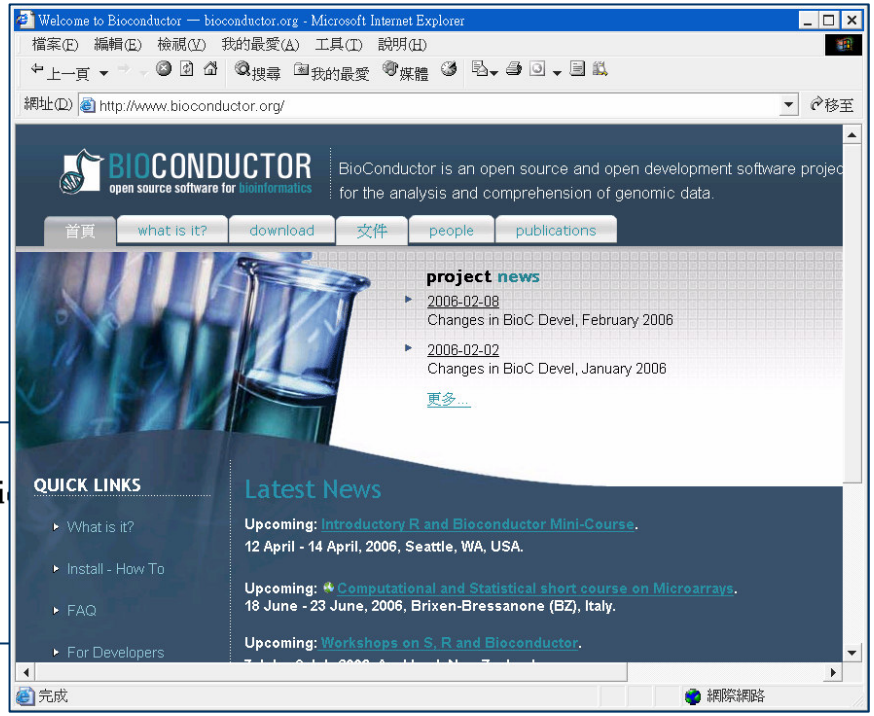
The R Project for Statistical Computing



affypdnn
affyPLM
gcrma
makecdfenv







affy	Methods for Affymetrix Oligonucleotide Arrays
affycomp	Graphics Toolbox for Assessment of Affymetrix Expressi
affydata	Affymetrix Data for Demonstration Purpose
annaffy	Annotation tools for Affymetrix biological metadata
AffyExtensions	For fitting more general probe level models

The Bioconductor: affy

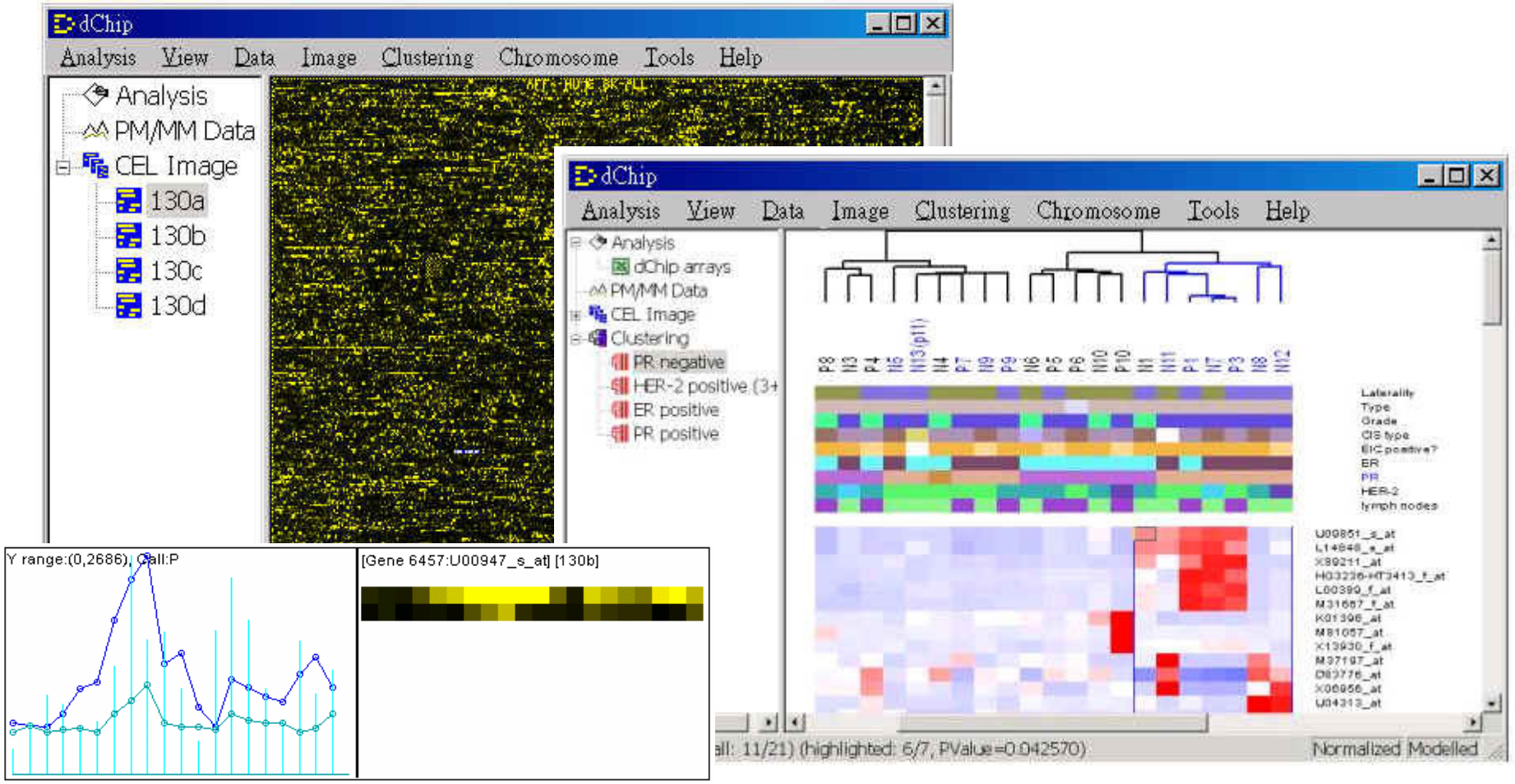
Quick Start: probe level data (*.cel) to expression measure.

```
> library(affy)
> getwd()
> list.celfiles()
> setwd("myaffy")
> getwd()
> list.celfiles()
> Data <- ReadAffy()

> eset.rma <- rma(Data)
> eset.mas <- expresso(Data,
                       normalize= FALSE,
                       bgcorrect.method="mas",
                       pmcorrect.method="mas",
                       summary.method="mas")
> eset.liwong <- expresso(Data,
                      normalize.method="invariantset",
                      bg.correct=FALSE,
                      pmcorrect.method="pmonly",
                      summary.method="liwong")
> eset.myfun <- express(Data,
                        summary.method=function(x)
                                apply(x, 2, median))

> write(eset.rma, file="mydata_rma.txt")
> write(eset.mas, file="mydata_mas.txt")
> write.exprs(eset.liwong, file="mydata_liwong.txt")
> write(eset.myfun, file="mydata_myfun.txt")
```

```
expresso(
    afbatch,

  # background correction
    bg.correct = TRUE,
    bgcorrect.method = NULL,        | none, mas, rma
    bgcorrect.param = list(),

  # normalize
    normalize = TRUE,
    normalize.method = NULL,        | constant, contrasts. invariantset, loess, qspline, quantiles, quantiles.robust
    normalize.param = list(),

  # pm correction
    pmcorrect.method = NULL,        | mas, pmonly, subtractmm
    pmcorrect.param = list(),

  # expression values
    summary.method = NULL,          | avgdiff, liwong, mas, medianpolish, playerout
    summary.param = list(),
    summary.subset = NULL,

  # misc.
    verbose = TRUE,
    warnings = TRUE,
    widget = FALSE)
```

DNA-Chip Analyzer (dChip)

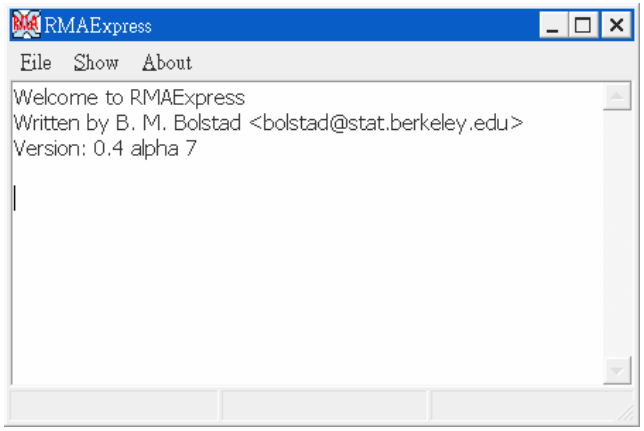
dChip Software: Analysis and visualization of gene expression and SNP microarrays

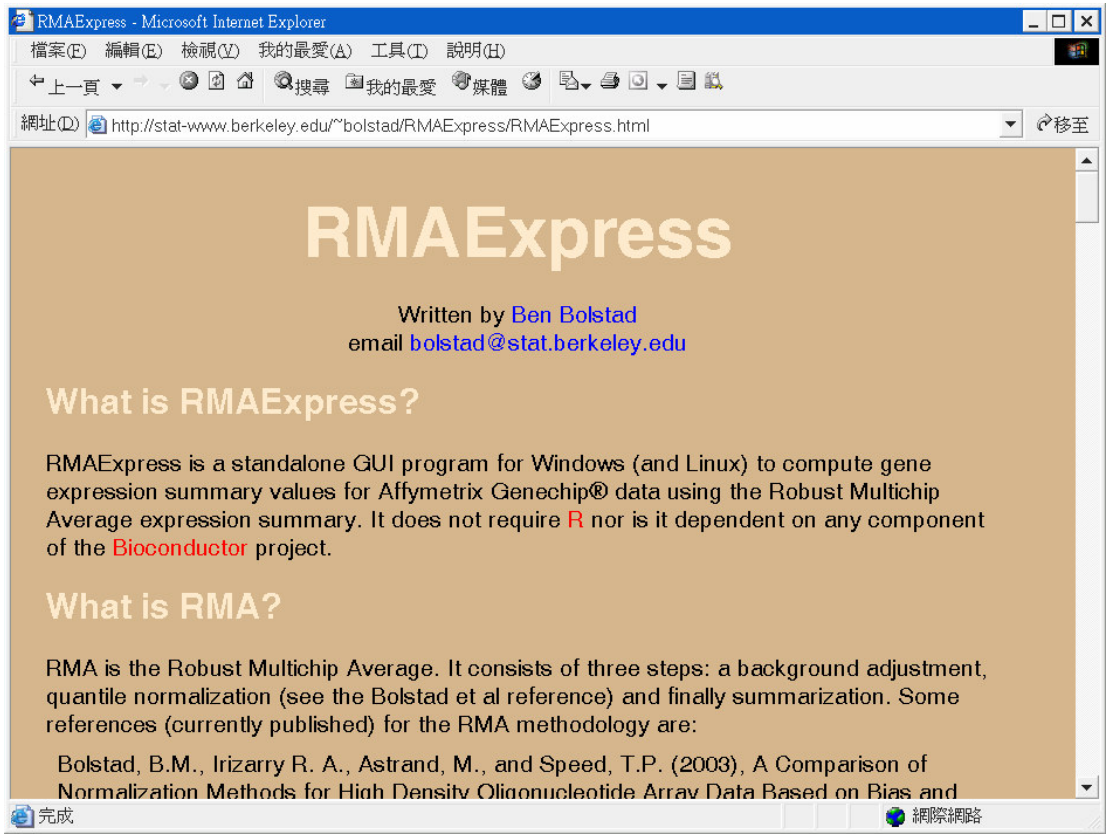


http://www.biostat.harvard.edu/complab/dchip/

RMAExpress

Ben Bolstad
Biostatistics,
University Of California, Berkeley
http://stat-www.berkeley.edu/~bolstad/
Talks Slides





http://stat-www.berkeley.edu/~bolstad/RMAExpress/RMAExpress.html

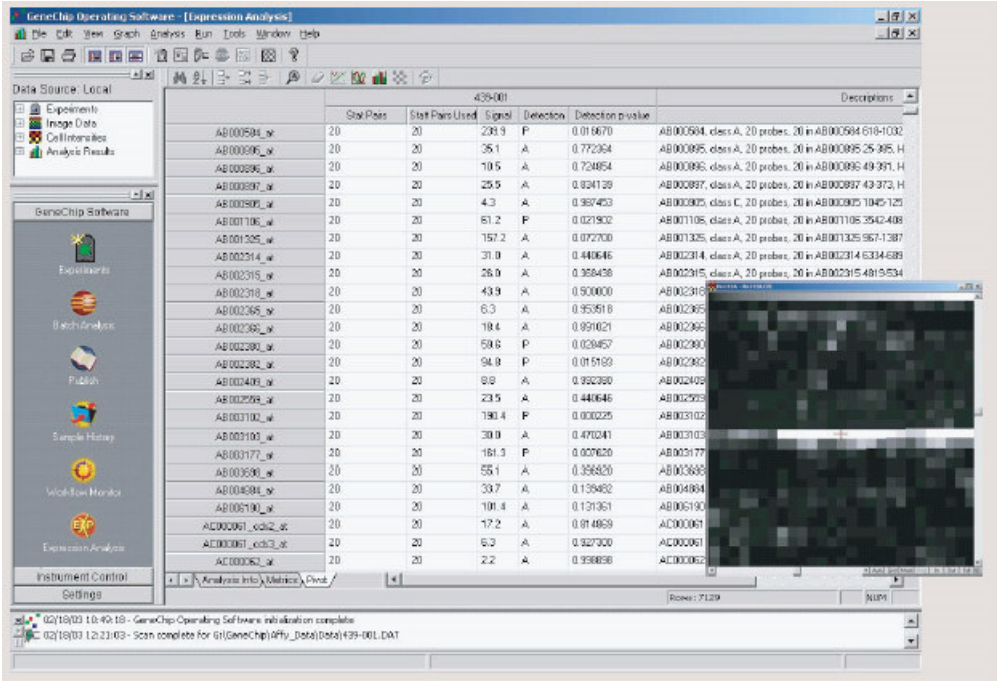
Affymetrix GeneChip Operating Software

http://www.affymetrix.com/support/technical/software_downloads.affx

AFFYMETRIX

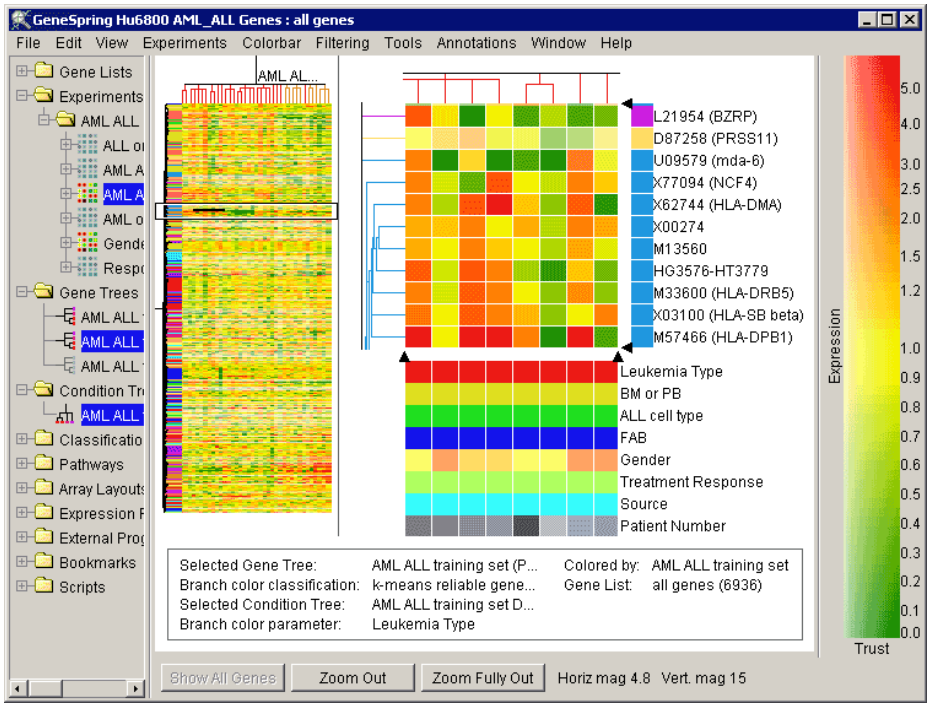
http://www.affymetrix.com

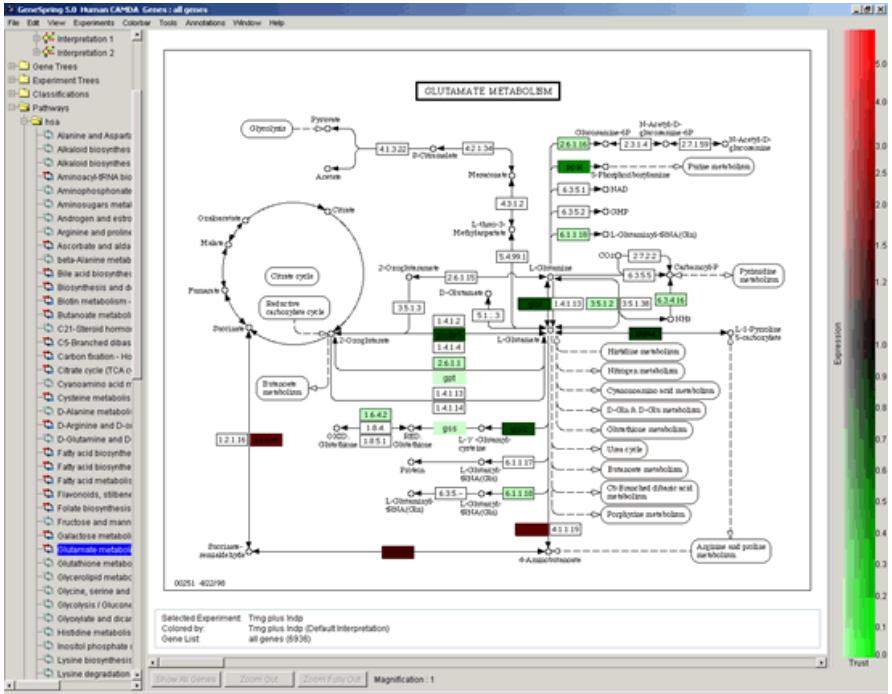
Specifications	
Instrument Support	• Affymetrix GeneChip® Fluidics Station 400 & 450 • GeneChip Scanner 3000 • GeneArray 2500 Scanner
Affymetrix Software Compatibility	• Support GeneChip DNA Analysis Software (GDAS) for mapping and resequencing data analysis • Support Affymetrix® Data Mining Tool software for statistical and clus analysis
Database Engine	• Microsoft Data Engine
GCOS Database	• Process Database • Publish Database • Gene Information Database
Database Management	• GCOS Manager • GCOS Administrator
Algorithm	• Affymetrix Statistical Expression Algorithm



GeneSpring GX v7.3.1

- RMA or GC-RMA probe level analysis
- Advanced Statistical Tools
- Data Clustering
- Visual Filtering
- 3D Data Visualization
- Data Normalization (Sixteen)
- Pathway Views
- Search for Similar Samples
- Support for MIAME Compliance
- Scripting
- MAGE-ML Export





Images from
http://www.silicongenetics.com

Agilent Technologies

2004 Articles Citing GeneSpring®

2004 : 2003 : 2002 : 2001 : pre-2001 : Reviews

More than 700 papers

Useful Links and Reference

Y.F.Leung's FUNCTIONAL GENOMICS

Home | Functional Genomics | Microarray | Bioinformatics | Proteomics | Genome mapping Complex disease mapping Linkage analysis | About Y.F.Leung

http://ihome.cuhk.edu.hk/~b400559/

AFFYMETRIX

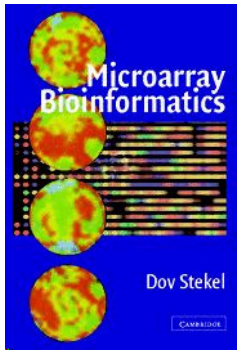
http://www.affymetrix.com

Bibliography on Microarray Data Analysis

http://www.nslij-genetics.org/microarray/

BIOINFORMATICS

http://bioinformatics.oupjournals.org



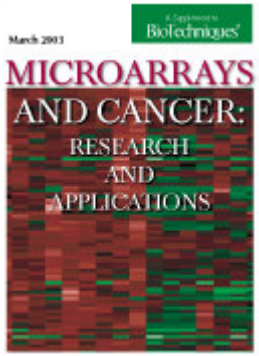
Stekel, D. (2003). Microarray bioinformatics, New York : Cambridge University Press.

- Speed Group Microarray Page: Affymetrix data analysis
http://www.stat.berkeley.edu/users/terry/zarray/Affy/affy_index.html

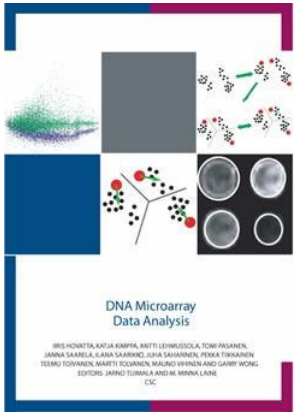
- Statistics and Genomics Short Course, Department of Biostatistics Harvard School of Public Health.
http://www.biostat.harvard.edu/~rgentlem/Wshop/harvard02.html

- Statistics for Gene Expression
http://www.biostat.jhsph.edu/~ririzarr/Teaching/688/

- Bioconductor Short Courses
http://www.bioconductor.org/workshop.htm



DNA Microarray Data Analysis
http://www.csc.fi/csc/julkaisut/oppaat/arraybook_overview



Microarrays and Cancer: Research and Applications
http://www.biotechniques.com/microarrays/