Microarray Data Analysis

Data Preprocessing for Affymetrix GeneChip

國立台灣大學資訊所

Course: 生物資訊與計算分子生物學

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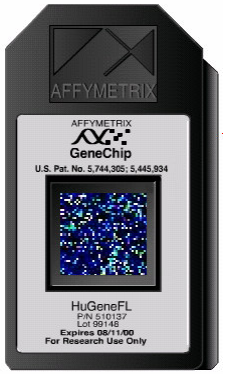
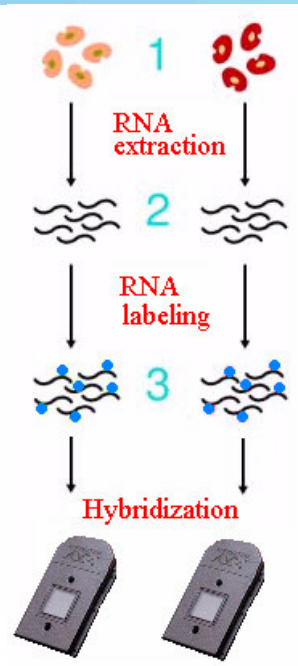
Outlines

- **GeneChip Expression Array Design**
- **Assay and Analysis Flow Chart**
  - Image Analysis, Affymetrix Data Files, from DAT to CEL.
- **Quality Assessment**
  - RNA Sample Quality Control
  - Array Hybridization Quality Control
  - Statistical Quality Control (Diagnostic Plots)
- **Low level Analysis**
  (from probe level data to expression value)
  - Background Correction, Normalization, PM Correction, Expression Index
  - Liwong Model, RMA
- **Software**
  - Freeware: BioConductor, dChip, RMAExpress
  - Commercial: GCOS, GeneSpring



Overview of Microarray Experiment



5 Washing Staining

6 Image Acquistion

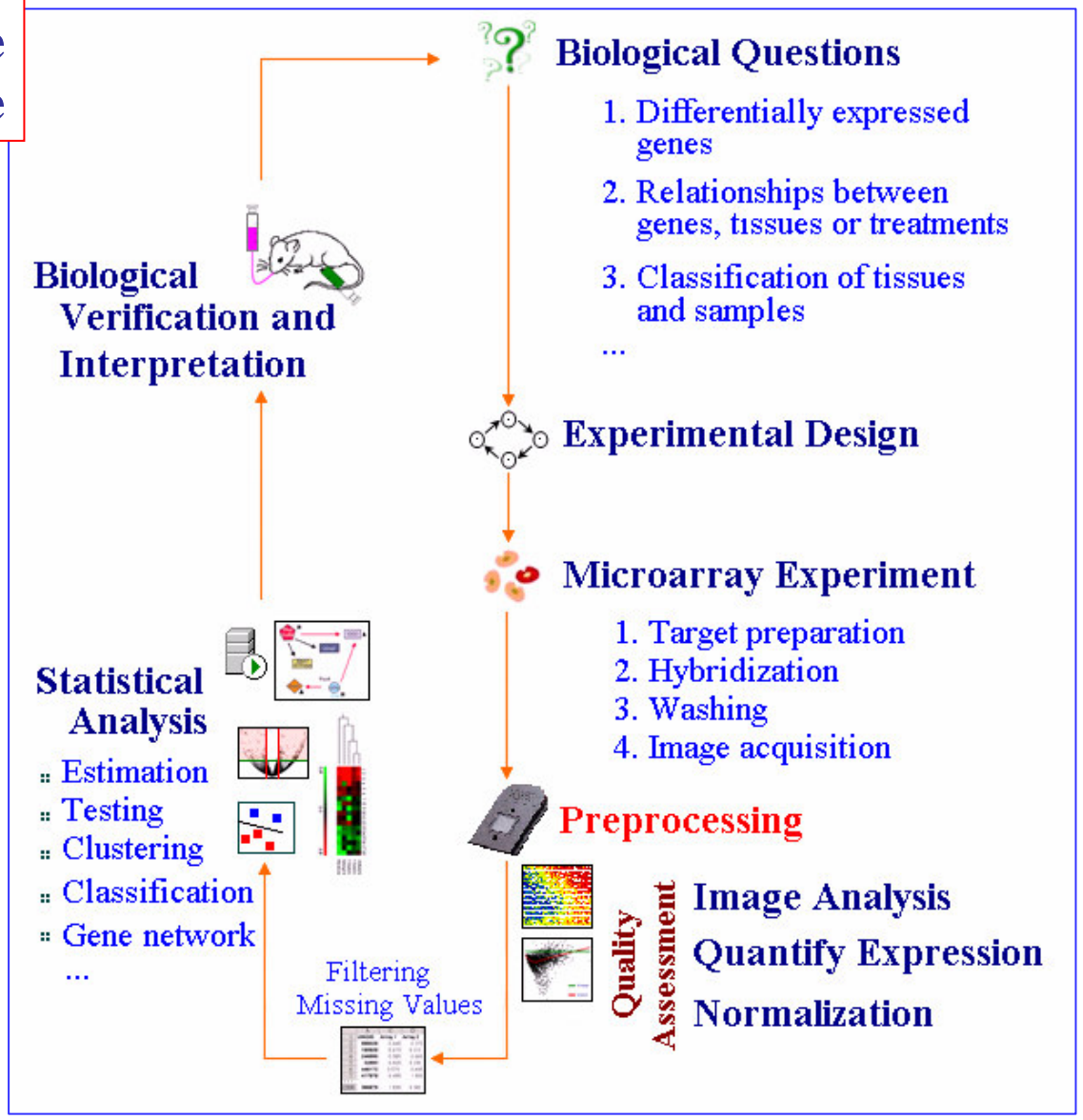
Image Processing Normalization

Use to calculate log2 ratios

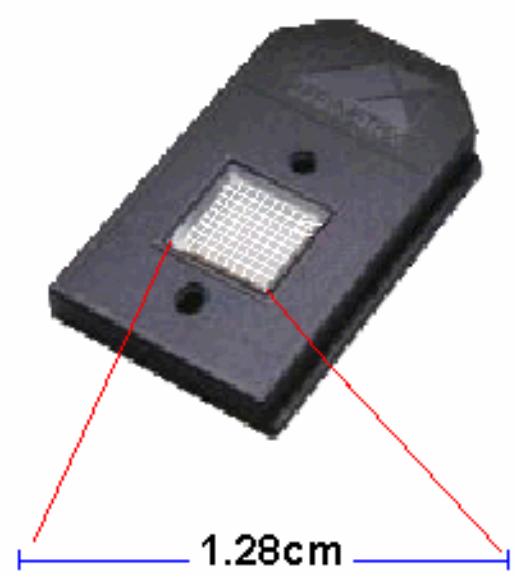
Oligonucleotide Array Data

	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

Microarray Life Cycle



GeneChip Expression Array Design



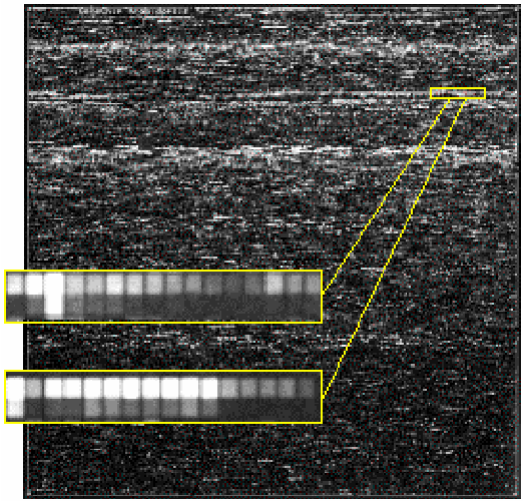
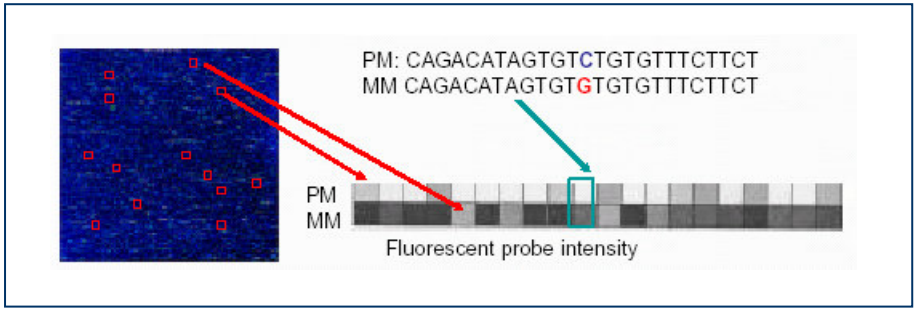
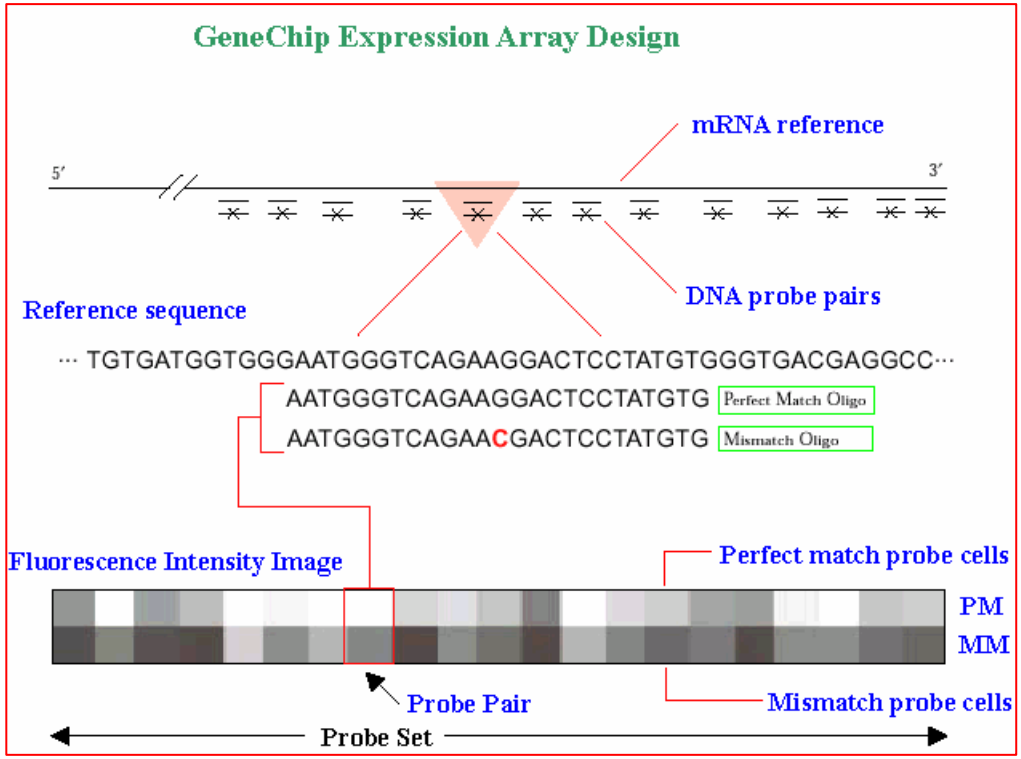
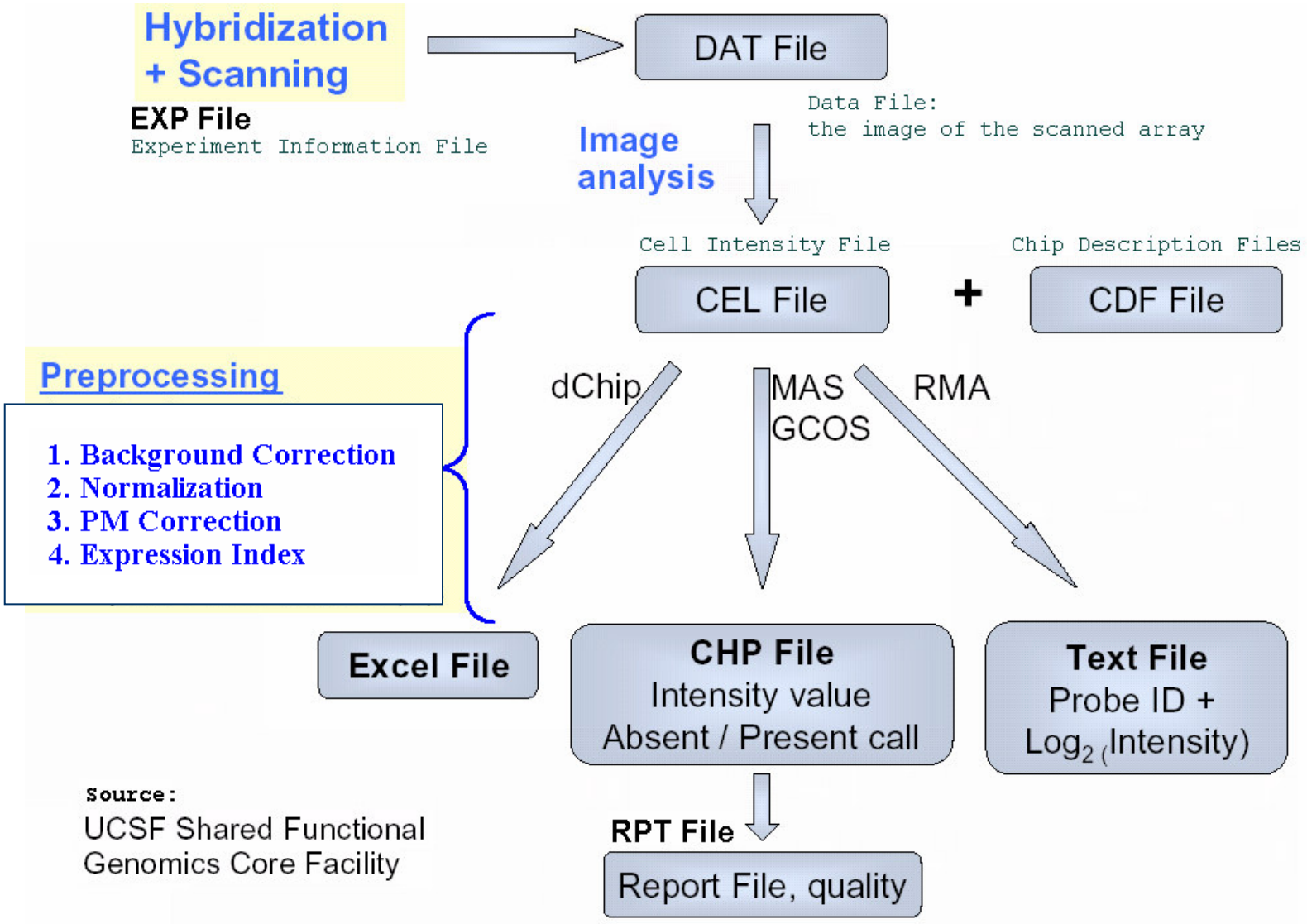


Image of hybridized probe array





Assay and Analysis Flow Chart



Affymetrix Data Files

*.EXP file

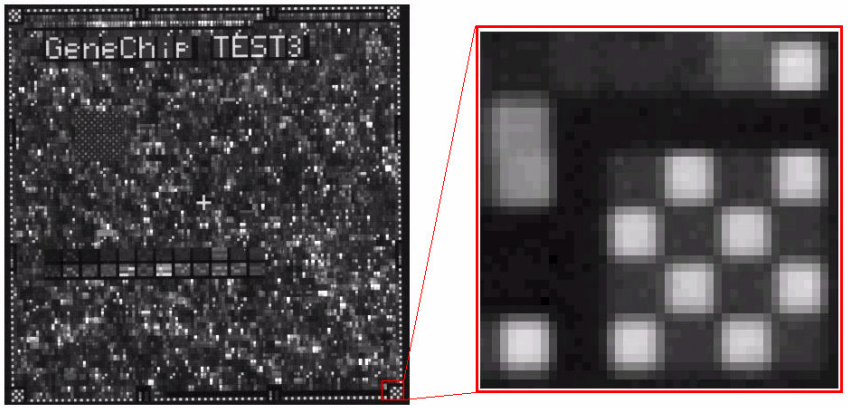
```
Affymetrix GeneChip Experiment Information
Version 1

[Sample Info]
Chip Type        HG-U133A
Chip Lot
Operator         array
Sample Type      RNA
Description
Project Dr. Mi
Comments
Solution Type
Solution Lot

[Fluidics]
Protocol         EukGE-WS2v4
Completed
Station 1
Module  2
Hybridize Date   Oct 19 2004 01:17PM

[Scanner]
Pixel Size       3
Filter  570
Scan Temperature
Scan Date        Oct 19 2004 01:41PM
Scanner ID
Number of Scans 2
Scanner Type     HP
```

*.DAT file ~50MB



*.CEL file ~12MB

(Version 4) ~5MB

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TotalY=712
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OffsetY=0
GridCornerUL=230 231
GridCornerUR=4503 235
GridCornerLR=4499 4506
GridCornerLL=226 4502
Axis-invertX=0
AxisInvertY=0
swapXY=0
DatHeader=[9..46155]  7:CLS=4733 RWS=4733 XIN=3  YIN=3  VE=17        2.0 02/24/04 13:41:05   HP
Algorithm=Percentile
AlgorithmParameters=Percentile:75;CellMargin:2;OutlierHigh:1.500;OutlierLow:1.004

[INTENSITY]
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          1    0       4711.5  721.0    16
          2    0       111.8   13.9     16
                                 ⋮
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ffsetY=0 GridCornerUL=230 231 GridCornerUR=4503 235 GridCornerLR=4499 4506
GridCornerLL=226 4502 Axis-invertX=0 AxisInvertY=0 swapXY=0 DatHeader=[9..4
6155]  7:CLS=4733 RWS=4733 XIN=3  YIN=3  VE=17        2.0 02/24/04 13:41:05
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AlgorithmParameters=Percentile:75;CellMargin:2;OutlierHigh:1.500;OutlierLow
:1.004  ■■■Percentile>■■■Percentile=75 CellMargin=2 OutlierHigh=1.500 Outli
erLow=1.004  ■■■?■■■■■■■■■■■■嘖6zkA ■■<堵zB4D ■■■窒t訟A ■■?E?AD ■■■  ?J
A ■■■嫀P9HA ■■曉EF?D ■■■劑??A ■■拎E O D ■■■澌w⓯A ■■ rE澹 D ■■■哼v_ A ■■
■EhG)D ■■■愖浭4A ■■4mE? D ■■■趨 ?A ■■ iE h D ■■■譒!覣A ■■xfE? D ■■■嫀I輯
                                 ⋮
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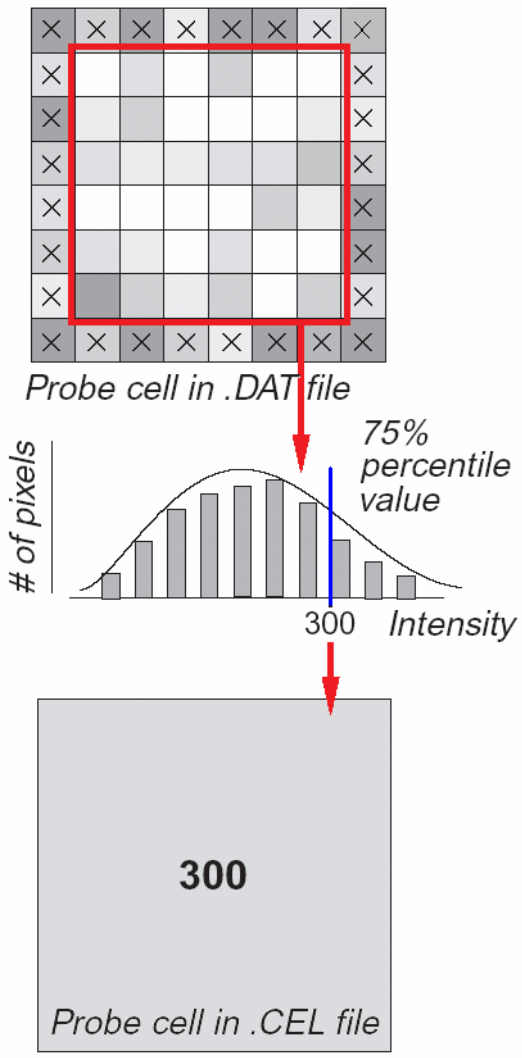
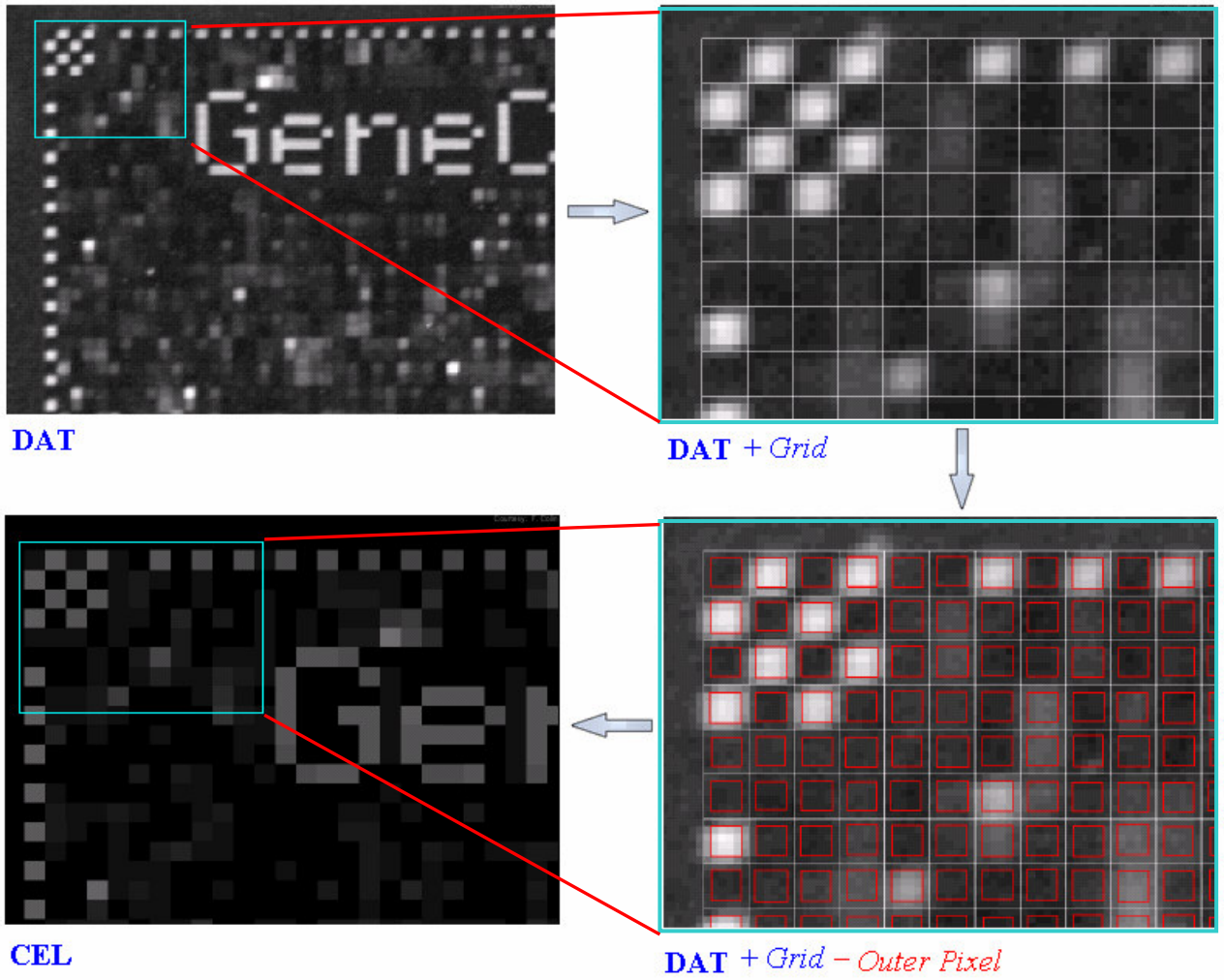
CEL File Conversion Tool

From DAT to CEL





MAS5.0 Analysis Output File

	Analysis Name	Probe Set Name	Stat Pairs	Stat Pairs Used	Signal	Detection	Detection p-value	Stat Comm
1	030606 En test3	Pae_16SrRNA_s_at	16	16	11.3	A	0.872355	
2	030606 En test3	Pae_23SrRNA_s_at	16	16	26.6	A	0.378184	
3	030606 En test3	PA1178_oprH_at	12	12	5.4	A	0.975070	
4	030606 En test3	PA1816_dnaQ_at	12	12	5.9	A	0.805907	
5	030606 En test3	PA3183_zwf_at	12	12	7.9	A	0.708540	
6	030606 En test3	PA3640_dnaE_at	12	12	10.8	A	0.964405	
7	030606 En test3	PA4407_ftsZ_at	12	12	9.5	A	0.921030	
8	030606 En test3	Pae_16SrRNA_s_st	16	16	8.9	A	0.660442	
9	030606 En test3	Pae_23SrRNA_s_st	16	16	22.0	A	0.561639	
10	030606 En test3	PA1178_oprH_st	12	12	35.1	P	0.024930	
11	030606 En test3	PA1816_dnaQ_st	12	12	34.7	A	0.240088	
12	030606 En test3	PA3183_zwf_st	12	12	6.5	A	0.985972	
13	030606 En test3	PA3640_dnaE_st	12	12	87.5	A	0.173261	
14	030606 En test3	PA4407_ftsZ_st	12	12	47.5	A	0.623158	
15	030606 En test3	AFFX-AthaI-Actin_5_r_at	16	16	89.8	P	0.013092	

Analysis Info | Metrics | Pivot

(*.CHP)

Metrics

	030606 En test3		Descriptions
	Signal	Detection	
Pae_16SrRNA_s_at	11.3	A	
Pae_23SrRNA_s_at	26.6	A	
PA1178_oprH_at	5.4	A	
PA1816_dnaQ_at	5.9	A	
PA3183_zwf_at	7.9	A	
PA3640_dnaE_at	10.8	A	
PA4407_ftsZ_at	9.5	A	
Pae_16SrRNA_s_st	8.9	A	
Pae_23SrRNA_s_st	22.0	A	
PA1178_oprH_st	35.1	P	
PA1816_dnaQ_st	34.7	A	
PA3183_zwf_st	6.5	A	
PA3640_dnaE_st	87.5	A	
PA4407_ftsZ_st	47.5	A	

Analysis Info | Metrics | Pivot

Pivot

Quality Assessment

- **RNA Sample Quality Control**
  - *Validation of total RNA*
  - *Validation of cRNA*
  - *Validation of fragmented cRNA*
- **Array Hybridization Quality Control**
  - Probe Array Image Inspection (DAT, CEL)
  - B2 Oligo Performance
  - MAS5.0 Expression Report Files (RPT)
    - Scaling and Normalization factors
    - Average Background and Noise Values
    - Percent Genes Present
    - Housekeeping Controls: Internal Control Genes
    - Spike Controls: Hybridization Controls: bioB, bioC, bioD, cre
    - Spike Controls: Poly-A Control: dap, lys, phe, thr, trp
- **Statistical Quality Control (Diagnostic Plots)**

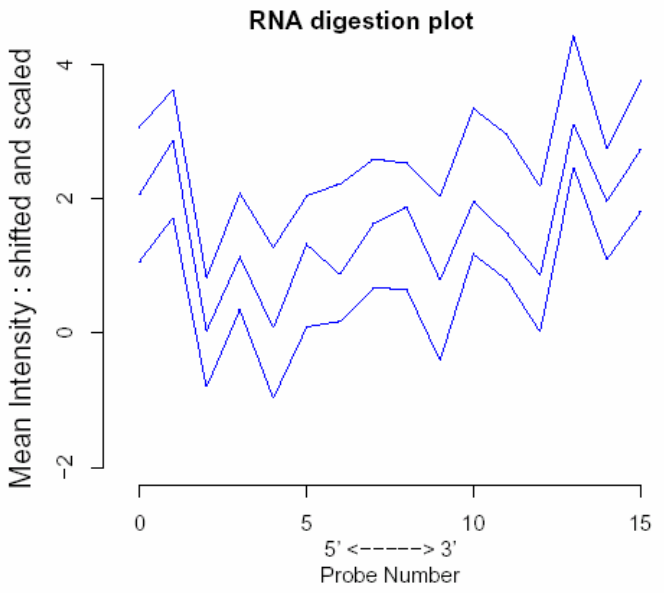
Two aspects of quality control: detecting poor hybridization and outliers

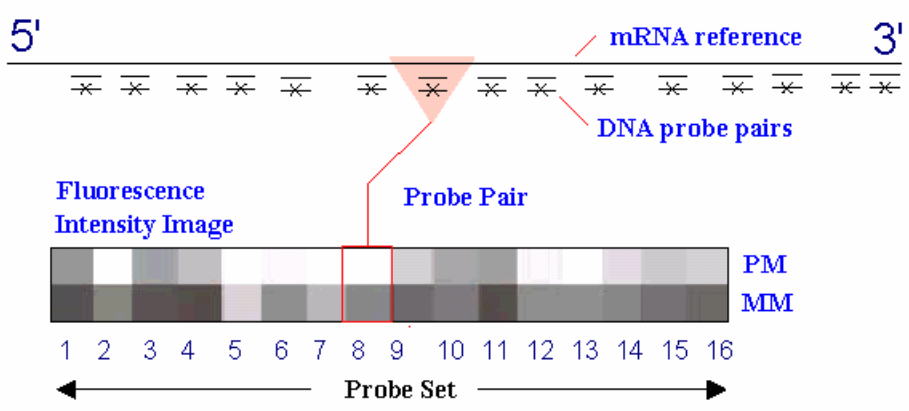
- Reasons for poor hybridizations
  - mRNA degenerated
  - one or more experimental steps failed
  - poor chip quality, …
- reasons for (biological) outliers
  - infiltration with non-tumour tissue
  - wrong label
  - contamination, …

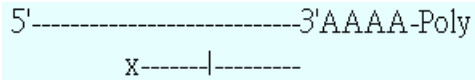
RNA Degradation Plots

Assessment of RNA Quality:

- Individual probes in a probe set are ordered by location relative to the 5' end of the targeted RNA molecule.
- Since RNA degradation typically starts from the 5' end of the molecule, we would expect probe intensities to be systematically lowered at that end of a probeset when compared to the 3' end.
- On each chip, probe intensities are averaged by location in probeset, with the average taken over probesets.
- The RNA degradation plot produces a side-by-side plots of these means, making it easy to notice any 5' to 3' trend.



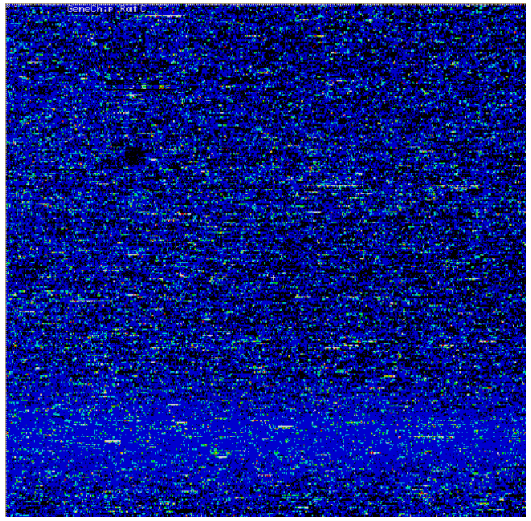




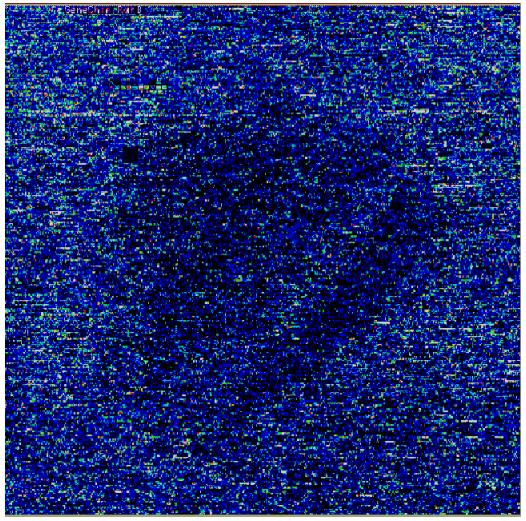
Probe Array Image Inspection

- 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.

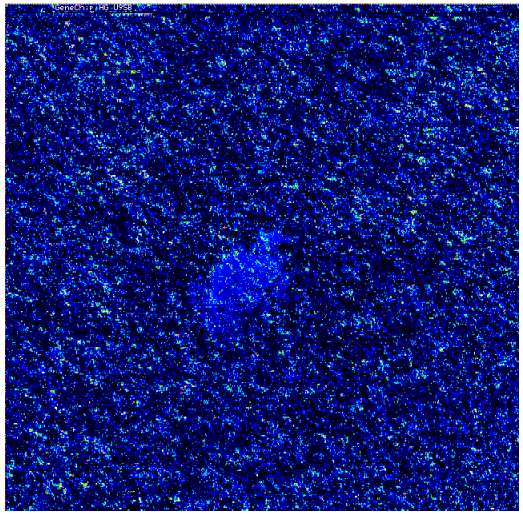
Haze Band



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.

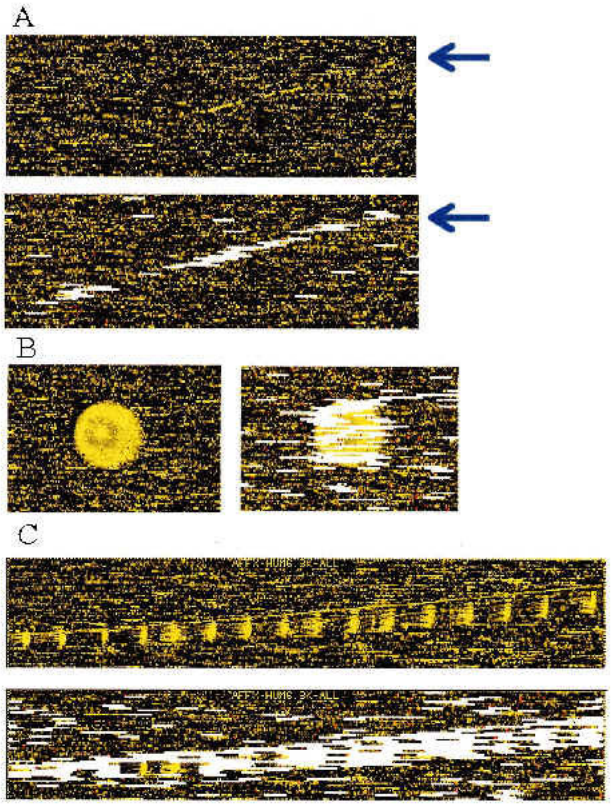
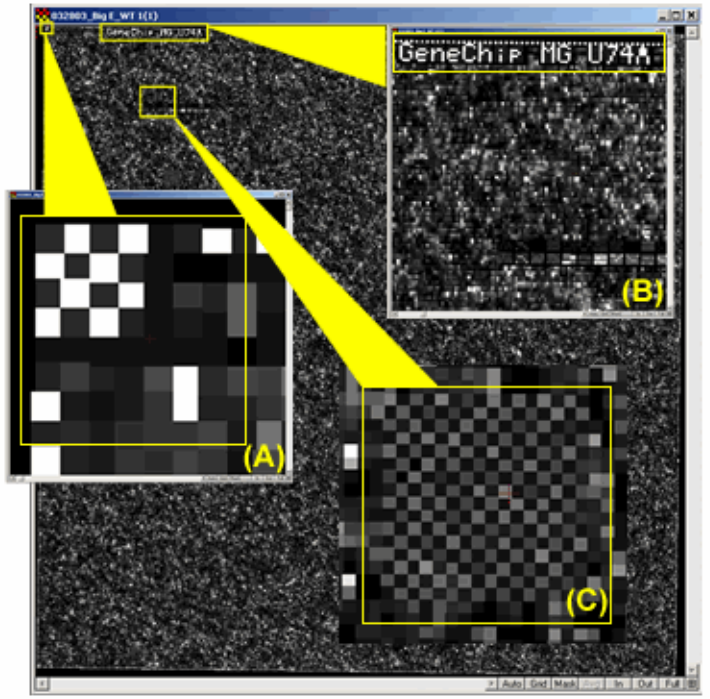


Fig. 5. (*A*) A long scratch contamination (indicated by arrow) is alleviated by automatic outlier exclusion along this scratch. (*B* and *C*) Regional clustering of array outliers (white bars) indicates contaminated regions in the original images. These outliers are automatically detected and accommodated 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:

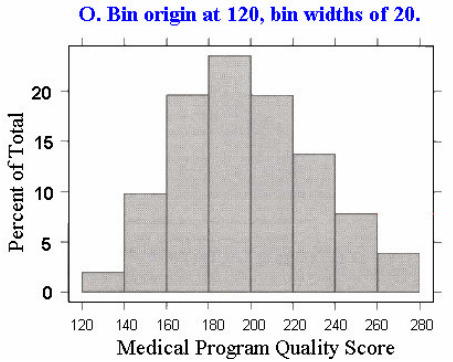
Probe Set	Sig(5')	Det(5')	Sig(M')	Det(M')	Sig(3')	Det(3')	Sig(all)	Sig(3'/5')
AFFX-HUMISGF3A/M97935	272.8	P	856.8	P	1274.5	P	801.36	4.67
AFFX-HUMRGE/M10098	340.6	M	181.3	A	632.6	P	384.80	1.86
AFFX-HUMGAPDH/M33197	13890.6	P	15366.6	P	14060.7	P	14439.32	1.01
AFFX-HSAC07/X00351	35496.8	P	39138.0	P	31375.0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

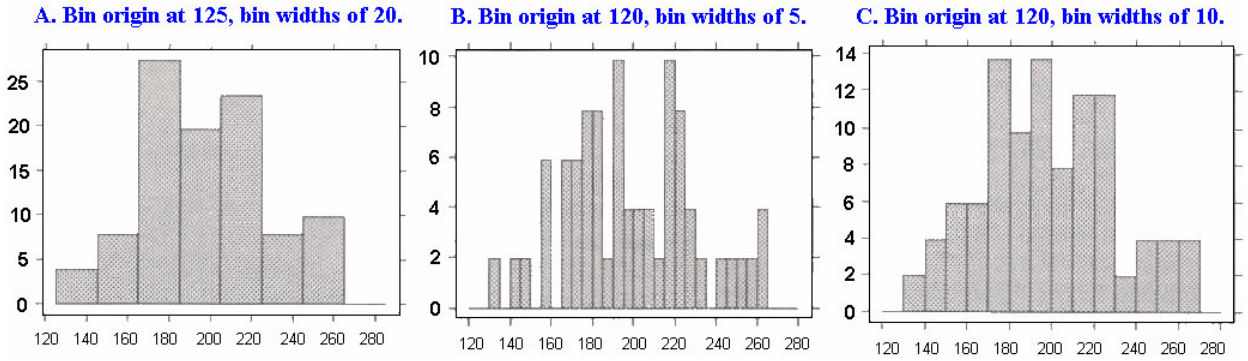
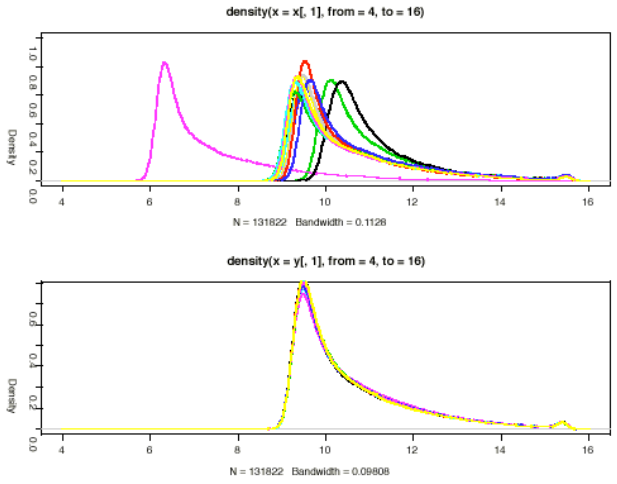


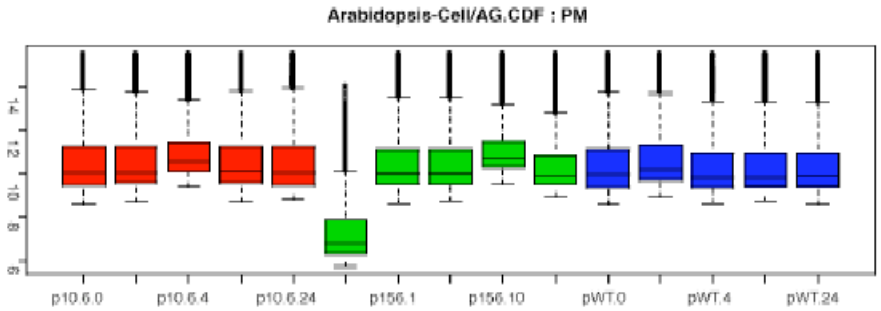
Figure Sources: Jacoby (1997).

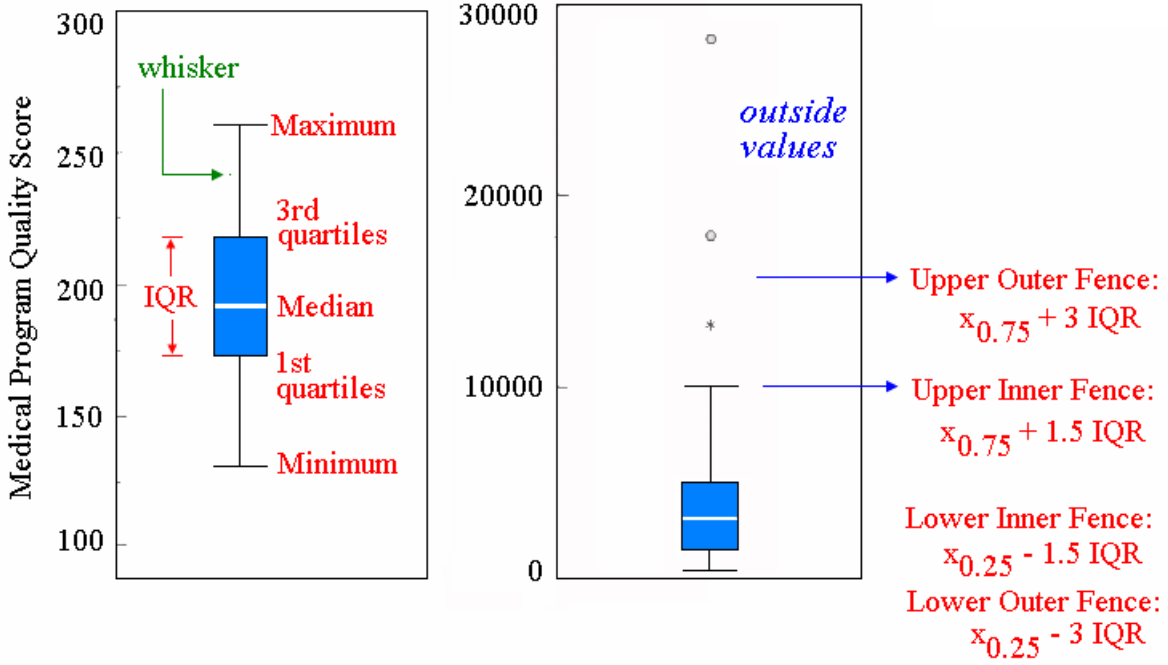
Density Plots



Statistical Plots: Box Plots

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





The box plot can provide answers to the following questions:

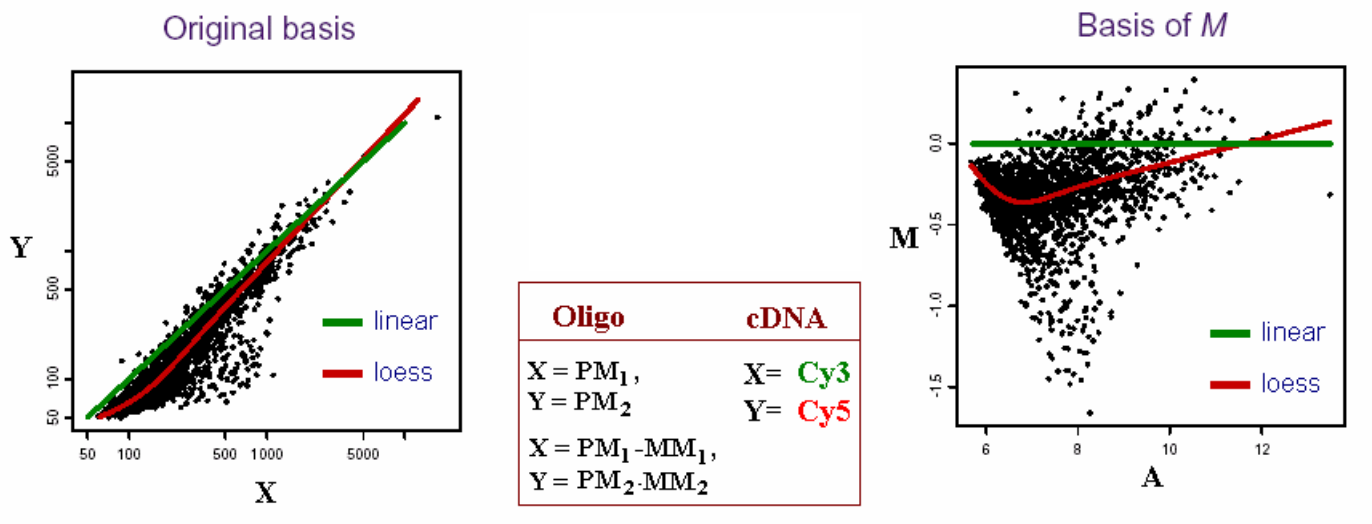
- Is a factor significant?
- Does the location differ between subgroups?
- Does the variation differ between subgroups?
- Are there any outliers?

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

Scatterplot and MA plot

- **Features of scatter plot.**
  - 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.
- **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.

- **MA plots** can show the intensity-dependant ratio of raw microarray data.

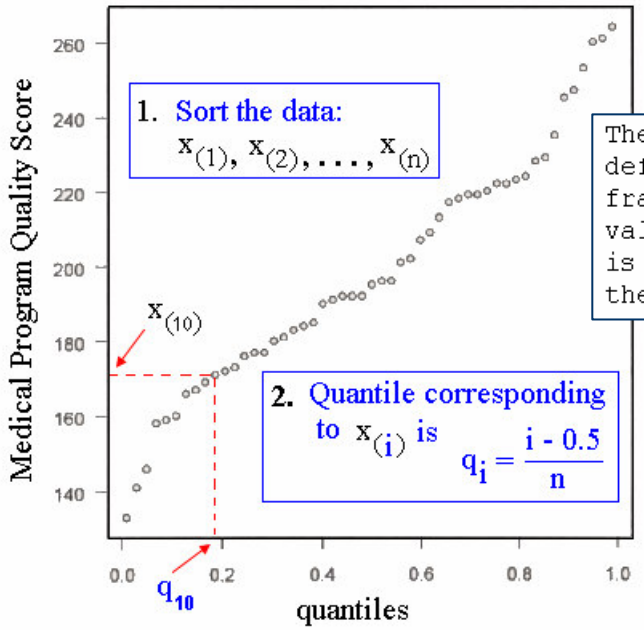


x-axis (mean log2 intensity): average intensity of a particular element across the control and experimental conditions.

y-axis (ratio): ratio of the two intensities.

Quantile Plots

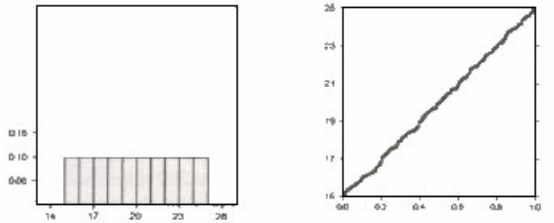
The empirical quantiles



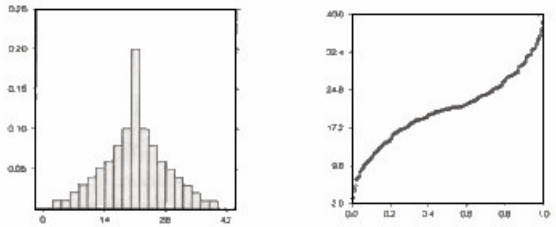
The qth quantile of a data set is defined as that value where a q fraction of the data is below that value and (1-q) fraction of the data is above that value. For example, the 0.5 quantile is the median.

Comparison of histogram and Quantile plolts for differently shaped data distribution

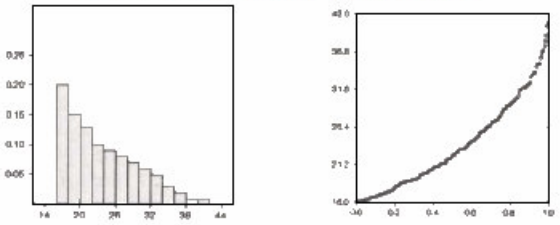
Uniform distribution



Symmetric, bell-shaped distribution



Positively skewed distribution



- 0.5 is subtracted from each i value to avoid extreme quantiles of exactly 0 or 1.
- The latter would cause problems if empirical quantiles were to be compared against quantiles derived from a theoretical. asymptotic distribution such as the normal.
- This adjustment has no effect on the shape of any graphical display.

Figures modified from Jacoby (1997)

Low level analysis

Background Methods	Normalization Methods	PM correction Methods	Summarization Methods
none rma/rma2 mas	quantiles loess contrasts constant invariantset Qspline	mas pmonly subtractmm	avgdiff liwong mas medianpolish playerout

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/Adjustment

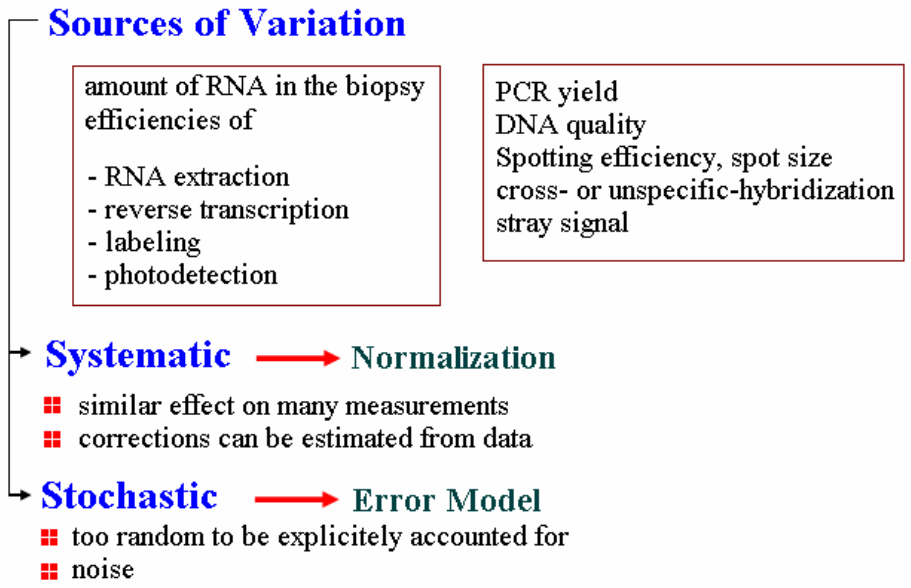
What is background?

- 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.

Normalization



What is normalization?

- Non-biological factor can contribute to the variability of data, in order to reliably compare data from multiple probe arrays, differences of non-biological origin must be minimized.
- Normalization is a process of reducing unwanted variation across chips. It may use information from multiple chips.

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.

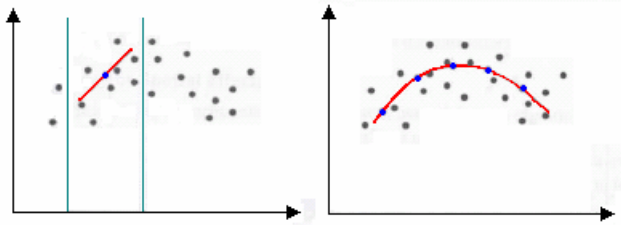
Normalization: Options

- 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

Normalization: loess Method

- 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)



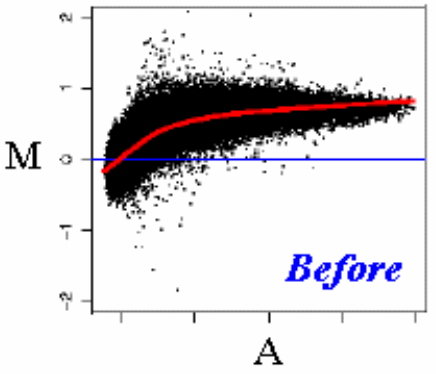
1. For any two arrays i,j with probe intensities x_{ki} and x_{kj} where $k = 1,\ldots,p$ represents the probe
2. we calculate $M_k = \log_2(x_{ki}/x_{kj})$ and $A_k = \frac{1}{2}\log_2(x_{ki}x_{kj})$.
3. A normalization curve is fitted to this M versus A plot using loess.

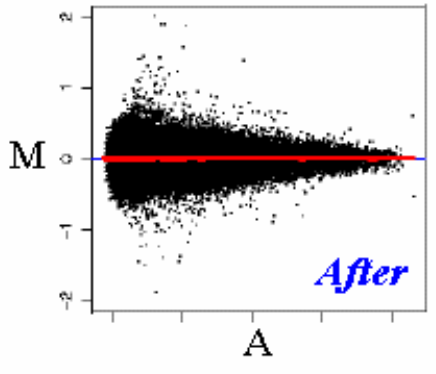
   Loess is a method of local regression (see Cleveland and Devlin (1988) for details).
4. The fits based on the normalization curve are $\hat{M}_k$
5. the normalization adjustment is $M'_k = M_k - \hat{M}_k$.
6. Adjusted probe intensites are given by $x'_{ki} = 2^{A_k + \frac{M'_K}{2}}$ and $x'_{ki} =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

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

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

Oligo	cDNA
$X = PM_1$, $Y = PM_2$	X= Cy3, Y= Cy5
$X = PM_1 - MM_1$, $Y = PM_2 - MM_2$	





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 conjunction with the liwong model.

Expression Index Estimates

Summarization

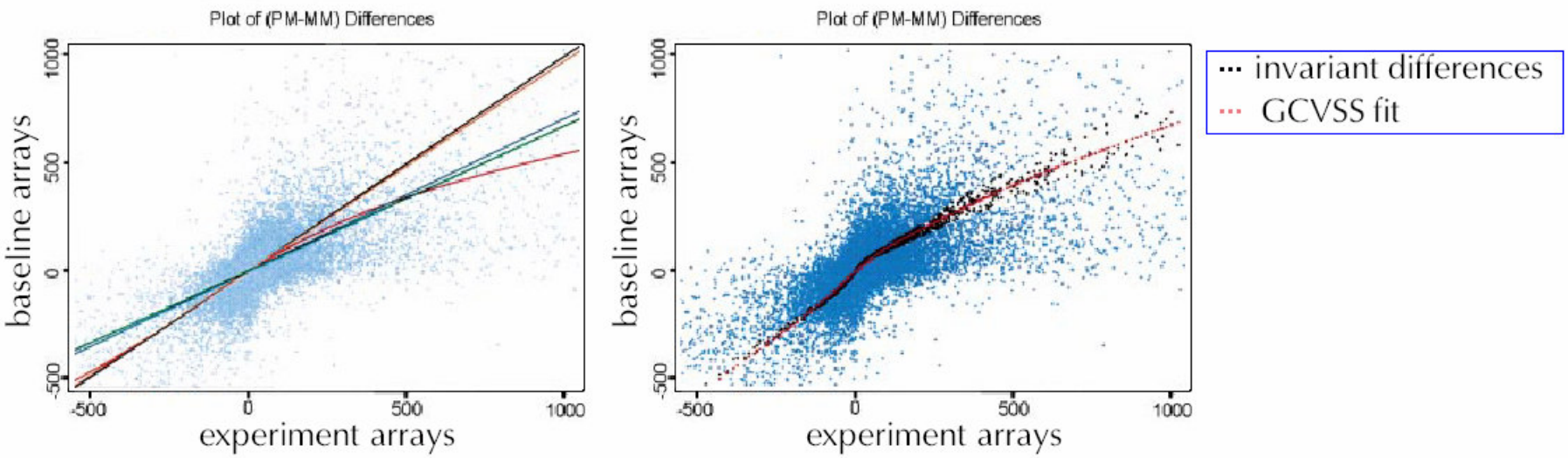
- Reduce the 11-20 probe intensities on each array to a single number for gene expression.
- The goal is to produce a measure that will serve as an indicator of the level of expression of a transcript using the PM (and possibly MM values).
- The values of the PM and MM probes for a probeset will be combined 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

Liwong: Normalization

(Li and Wong, 2001) **invariant set**

- 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.
- The non-linear relationship is used to carry out the normalization.
- 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).



Liwong: Summarization Method

(Model-Based Expression Index , MBEI)

- If there are multiple arrays from the same experiment available, this model provides an intuitive estimate of the mean and standard error of the θ s and φ s.
  - The standard error estimates of the θ s and 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. For each array, this model computes an expression level on the ith array θ i.
  - If a specific array has a large standard error relative to other arrays, possibly due to external factors like the imaging process, then this is called an **outlier array**.
  - Similarly, if the estimate of φ j for the jth probe has a large standard error, possibly due to non-specific cross-hybridization, it is called an **outlier probe**.
  - Individual PM-MM differences might also be identified by large residuals compared with the fit; these **single outliers** are regarded as missing values in the model-fitting algorithm.
- Cross-hybridization is more likely to occur at the MM probes, rather than the PM probes, and so a PM-only model exists that calculates expression values that are always positive (Li and Wong 2001). Studies suggest that the PM-only model is more robust to cross-hybridization than the PM-MM difference model.

For a gene

$$y_{ij} = \phi_i \theta_j + \epsilon_{ij}$$

y_{ij} is PM_{ij} or the difference between $PM_{ij} - MM_{ij}$.

ϕ_i is a probe response parameter

θ_j is the expression on array j.

$$\sum_j \phi_j^2 = J$$

$$\epsilon_{ij} \sim N\left(0, \sigma^2\right)$$

$i = 1, \ldots, I$ the number of chips

$j = 1, \ldots, J$ number of probe pairs

RMA: Background Correction

RMA: Robust Multichip Average (Irizarry and Speed, 2003)

- Assumes PM probes are a convolution of normal and exponentional.
- ObservedPM = Signal + Noise, (O = S + N).
- **Assume**
  - Signal is exponentional (alpha)
  - Noise (background) is Normal (mu, sigma).
- Use E[S|O=o, S>0] as the backround corrected PM.
- MM probe intensities are not corrected by RMA/RMA2.

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

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

$$b = \sigma$$

ϕ: standard normal density function

Φ: standard normal distribution function

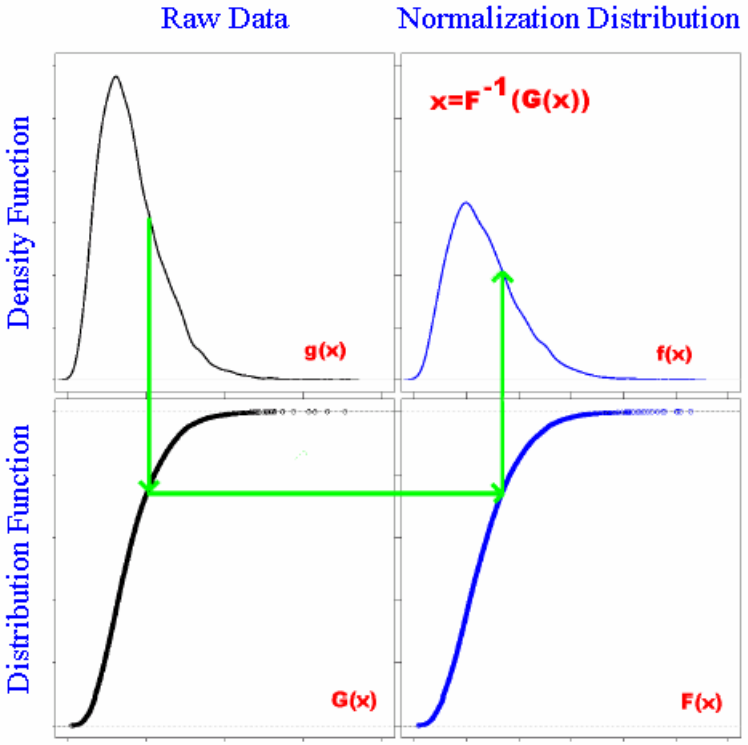
RMA: Normalization

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

1. If $q_i = (q_{i1}, \ldots, q_{iN})$ is a row in X_{sort} then the corresponding row in X'_{sort} is given by $\boldsymbol{q}'_i = \text{proj}_{\boldsymbol{d}} \boldsymbol{q}_i$
2. The projection is equivalent to talking the average of the quantile in a particular row and substituting this value for each of the individual elements in that row

$$\text{proj}_{\boldsymbol{d}} \boldsymbol{q}_i = \frac{\boldsymbol{q}_i \cdot \boldsymbol{d}}{\boldsymbol{d} \cdot \boldsymbol{d}} \boldsymbol{d} = \frac{1}{\sqrt{N}} \sum_{j=1}^{N} q_{ij} \boldsymbol{d} = \left(\frac{1}{N} \sum_{j=1}^{N} q_{ij}, \ldots, \frac{1}{N} \sum_{j=1}^{N} q_{ij} \right)$$



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.

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 with $i = 1, \ldots, I_k$ probes
and data from $j = 1, \ldots, J$ arrays

fit the following model

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

where α_i is a probe effect and
β_j is the $\log_2$ expression value.

Software

Image Analysis/Normalization

Shareware/Freeware

- Bioconductor (R, Gentleman)
- DNA-Chip Analyzer (dChip v1.3) (Li and Wong)
- RMAExpress: a simple standalone GUI program for windows for computing the RMA expression measure.

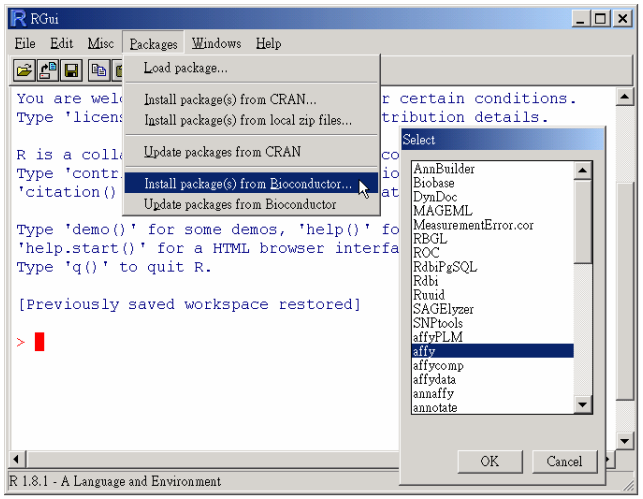
Commercial

- Affymetrix GeneChip Operating Software (GCOS v1.0)
- GeneSpring GX v7.3

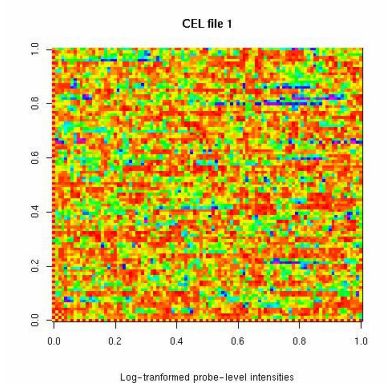
The Bioconductor: affy

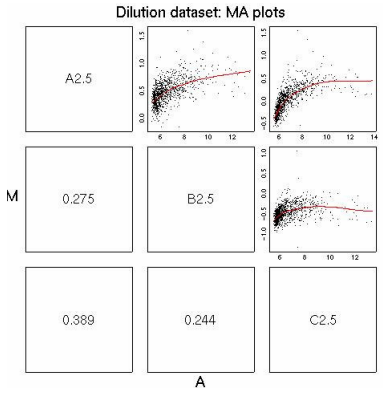
The Bioconductor Project
Release 1.7
http://www.bioconductor.org/

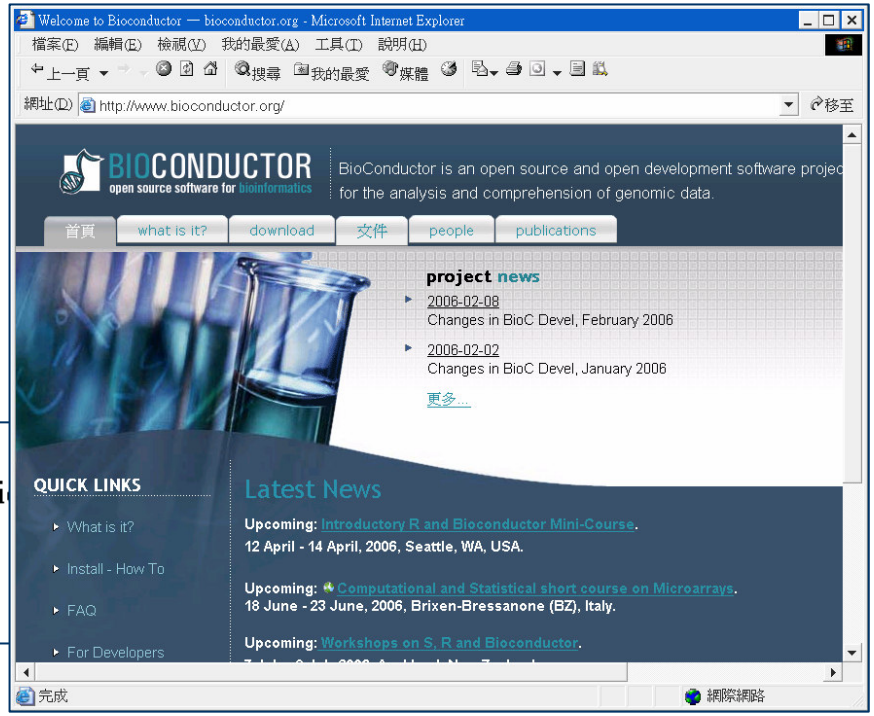
The R Project for Statistical Computing



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

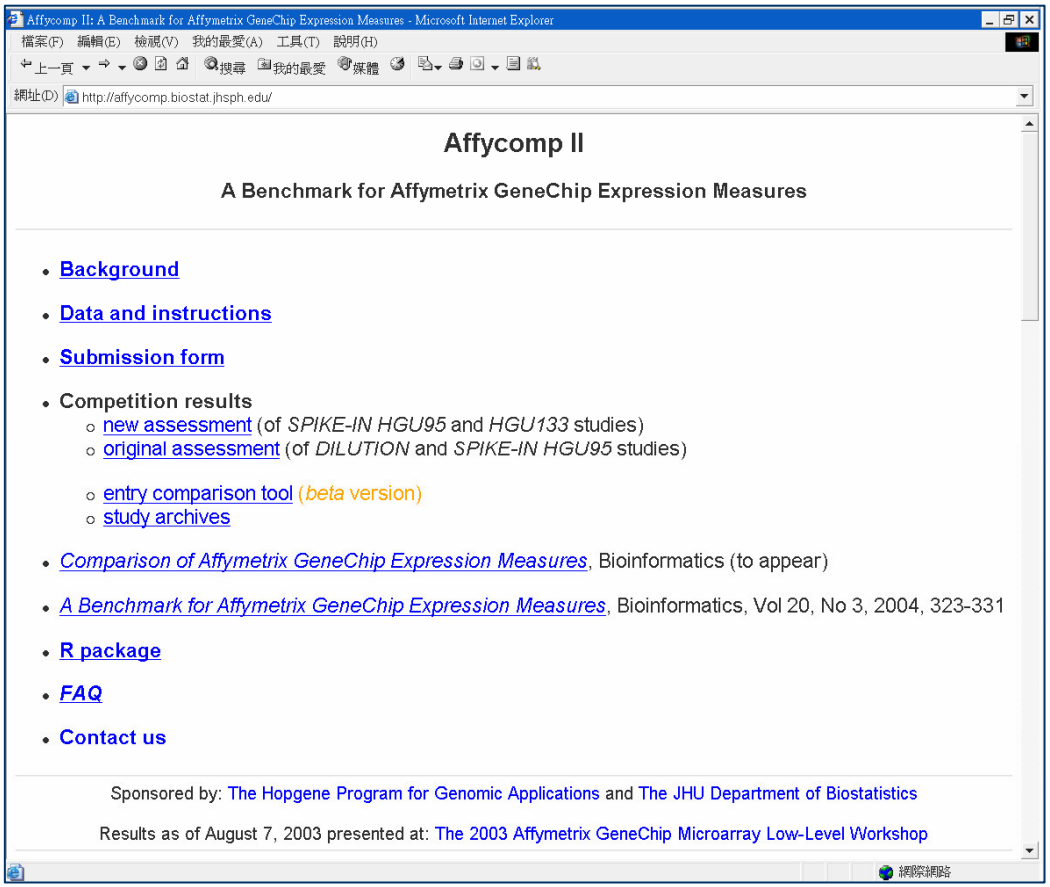
Quick Start: probe level data (*.cel) to expression measure.

```
> 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,
    bgcorrect.param = list(),       | mas,
                                    | rma

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

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

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

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

Comparison of Affymetrix GeneChip Expression Measures

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



Data and instructions

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

     Affymetrix's Spike-in hgu95a Experiment CEL files [*gzip-*

     Description file for this data [text]
   - **Spike-in hgu133a Data**

     Affymetrix's Spike-in hgu133a Experiment CEL files [*gzip*

     Description file for this data [text]
   - **Dilution Data** (optional -- see below)

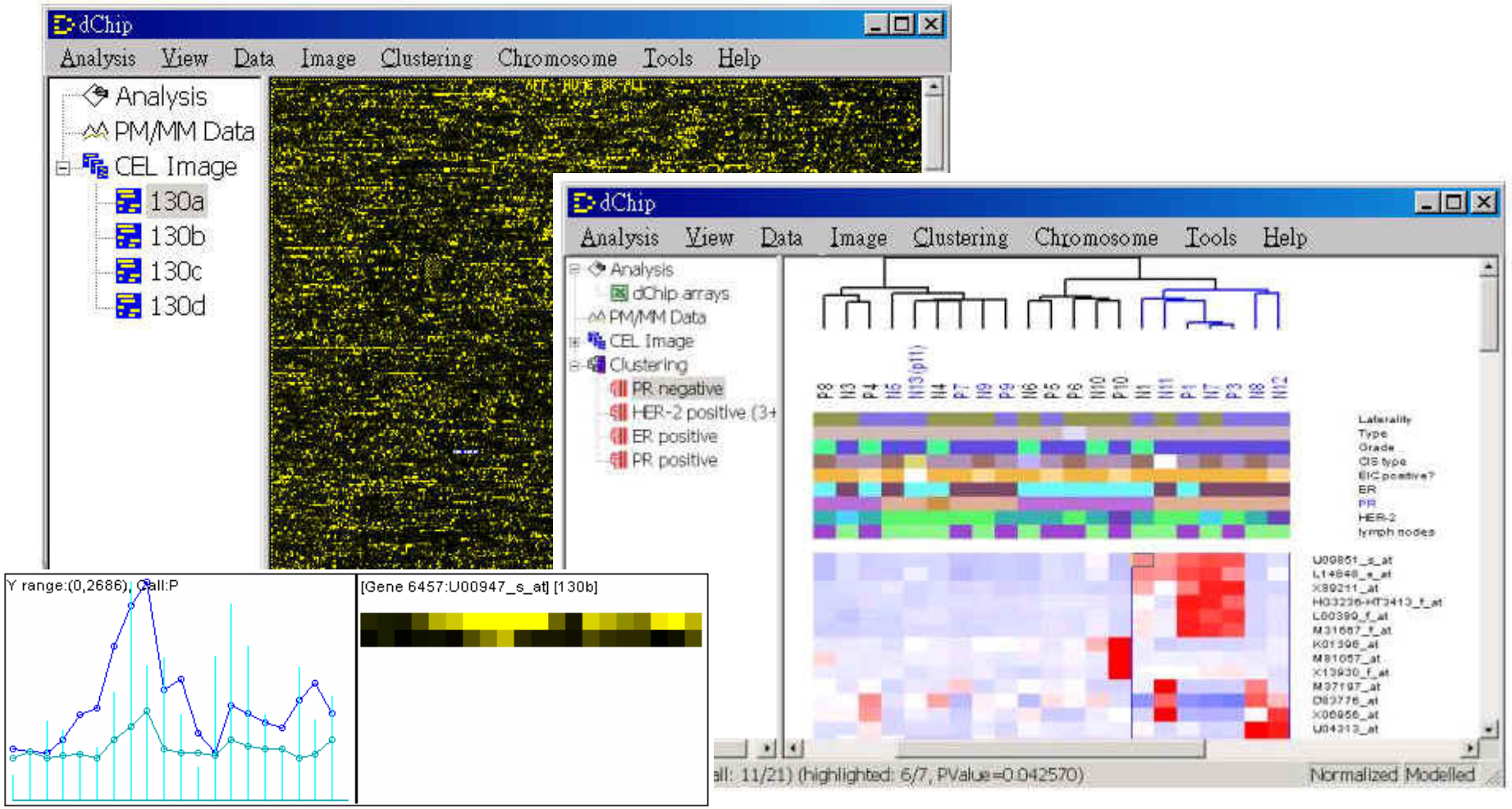
     Gene Logic's Dilution Experiment CEL files. If you have unresolvable, so note that submitting a dilution study has

     Description file for dilution data [text]

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	1.00
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	0.06
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	0.65
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	0.49
23	*rsvd1* / 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	0.58
25	*rsvd.prm* / 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	0.54
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	*W2371* / 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_NRG* / 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

- 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.

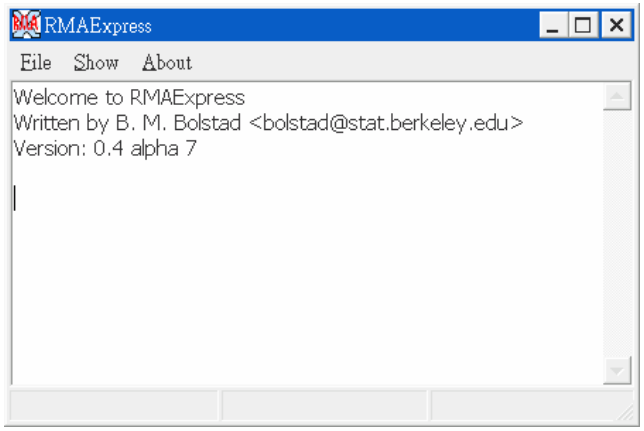
DNA-Chip Analyzer (dChip v1.3)

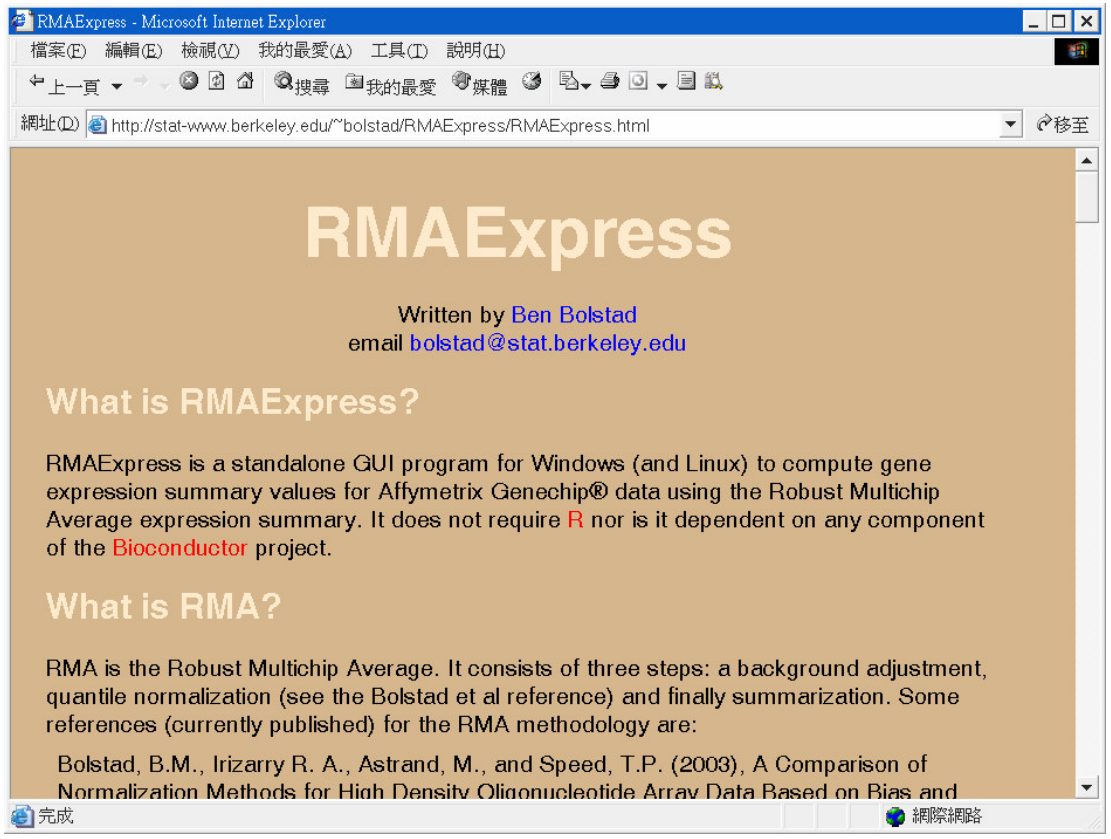


http://www.biostat.harvard.edu/complab/dchip/

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

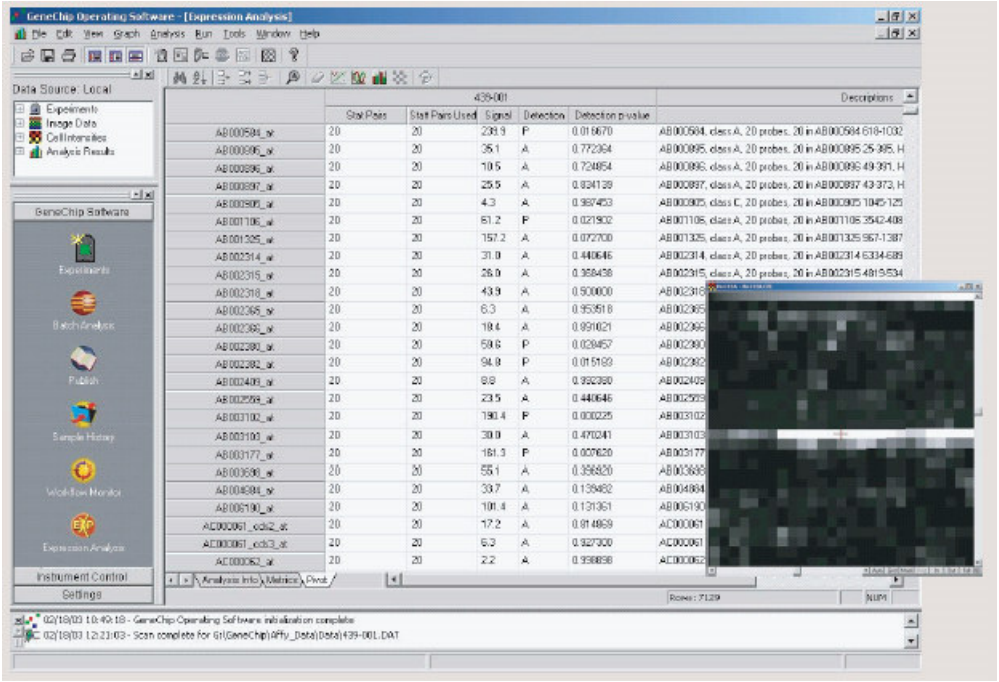
Affymetrix GeneChip Operating Software

AFFYMETRIX

http://www.affymetrix.com

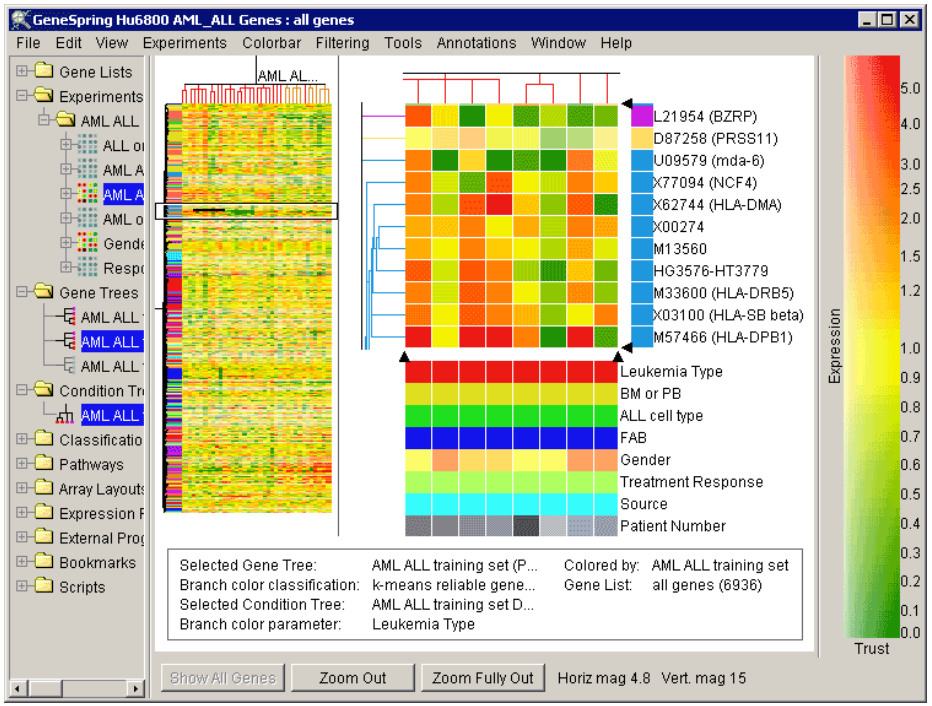
Specifications

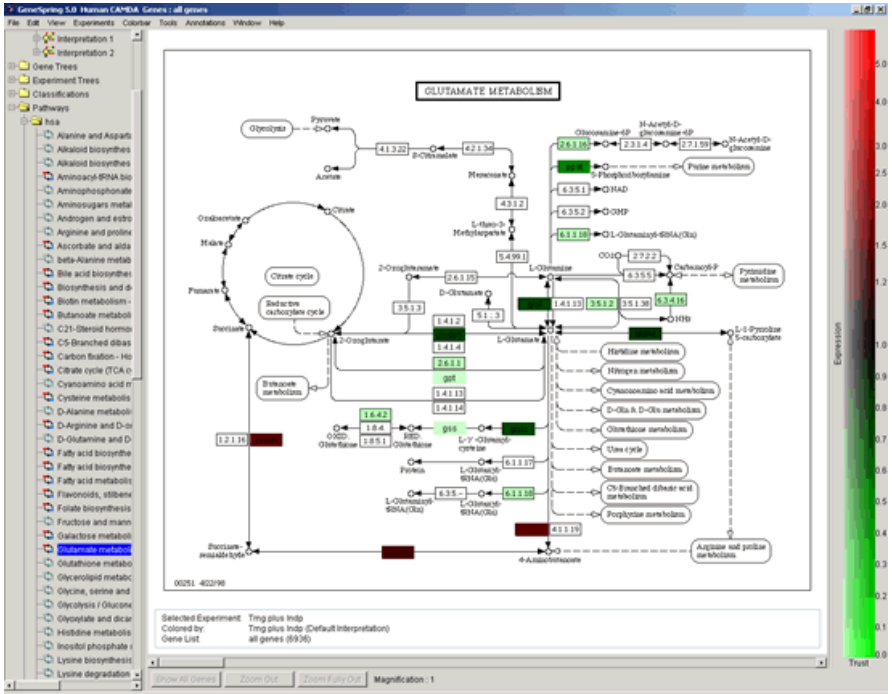
Instrument Support	• Affymetrix GeneChip® Fluidics Station 400 & 450 • GeneChip Scanner 3000 • GeneArray 2500 Scanner
Affymetrix Software Compatibility	• Support GeneChip DNA Analysis Software (GDAS) for mapping and resequencing data analysis • Support Affymetrix® Data Mining Tool software for statistical 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





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