Genomic signal processing

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Outline

1. Bioinformatics background
2. Gene microarrays
3. Normalization
4. Gene clustering and filtering for gene pattern extraction
5. Application: development and aging in retina

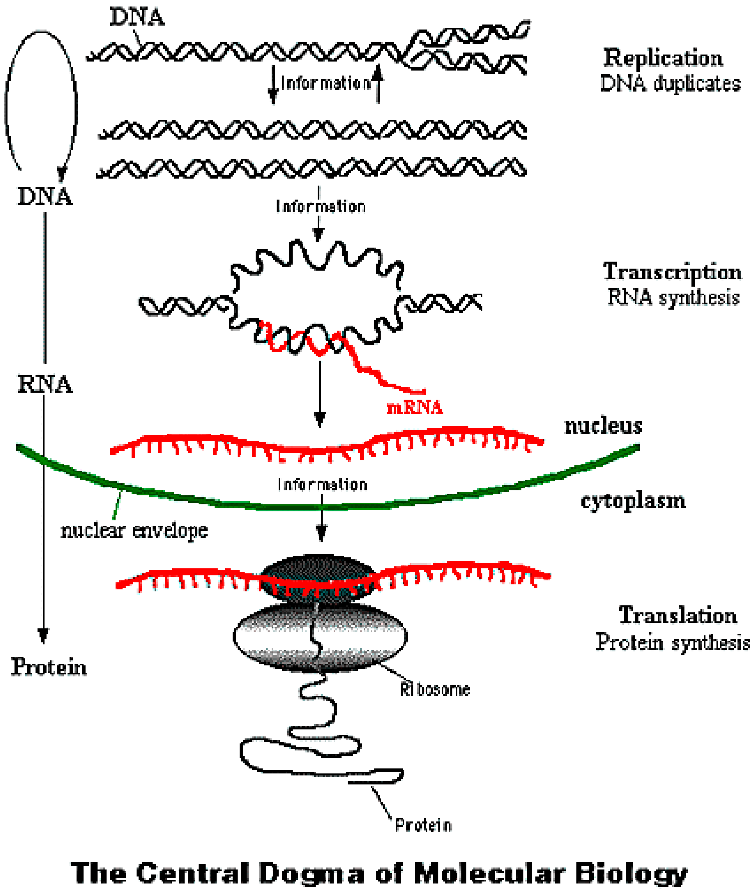


Figure 1: `http://www.accessexcellence.org`

Kellog Sensory Gene Microarray Node: Objectives

Establish genetic basis for development, aging, and disease in the retina

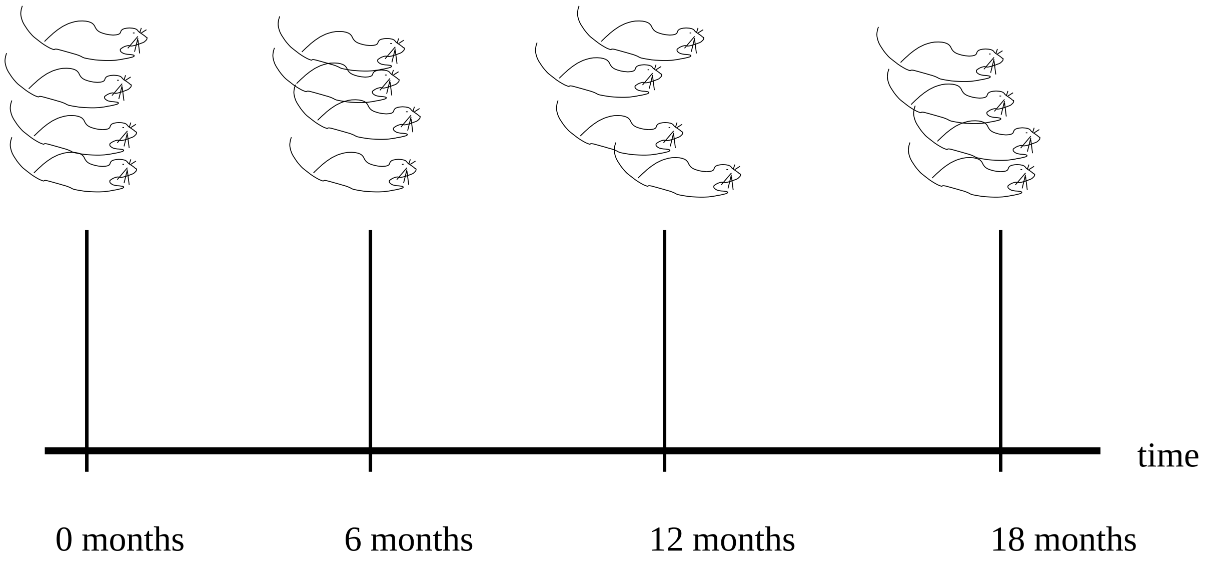
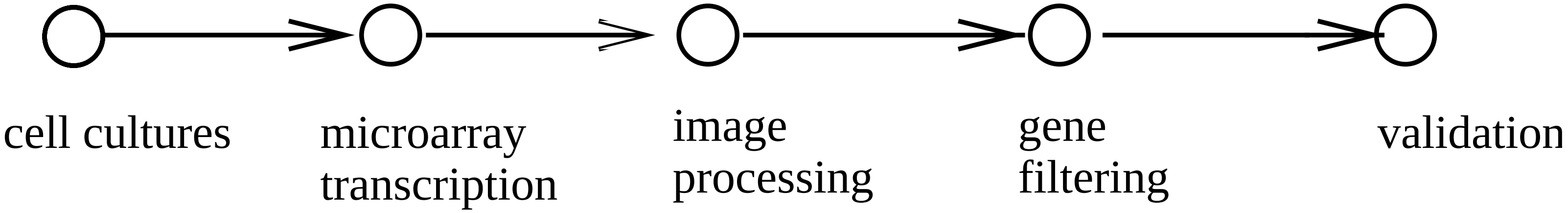


Figure 2: *Sample gene trajectories over time.*

II. Gene Microarrays

“Shotgun sequencing”



cell cultures

microarray transcription

image processing

gene filtering

validation

Figure 3: *Microarray experiment cycle.*

Microarray Technologies

Two principal microarray technologies:

1. Oligonucleotide chips (Affymetrix GeneChip)
2. cDNA spotted arrays (Synteni/Stanford chip)

These technologies share common experimental procedure...

1. Specific complementary ss DNA segments (gene probes) are deposited at locations on a slide (*arraying* or *photo-lithography*)
2. Dye-labeled DNA from sample is distributed over slide - complementary DNA binds to probes (*hybridization*)
3. Presence of bound DNA is read out by detecting spot flourescence by laser excitation (*scanning*)

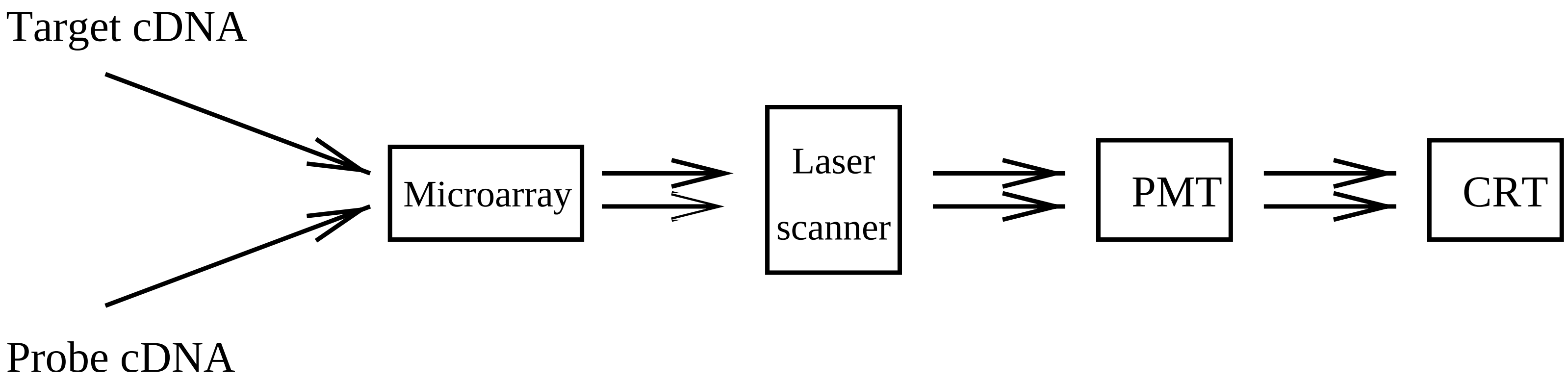


Figure 4: *Image formation process.*

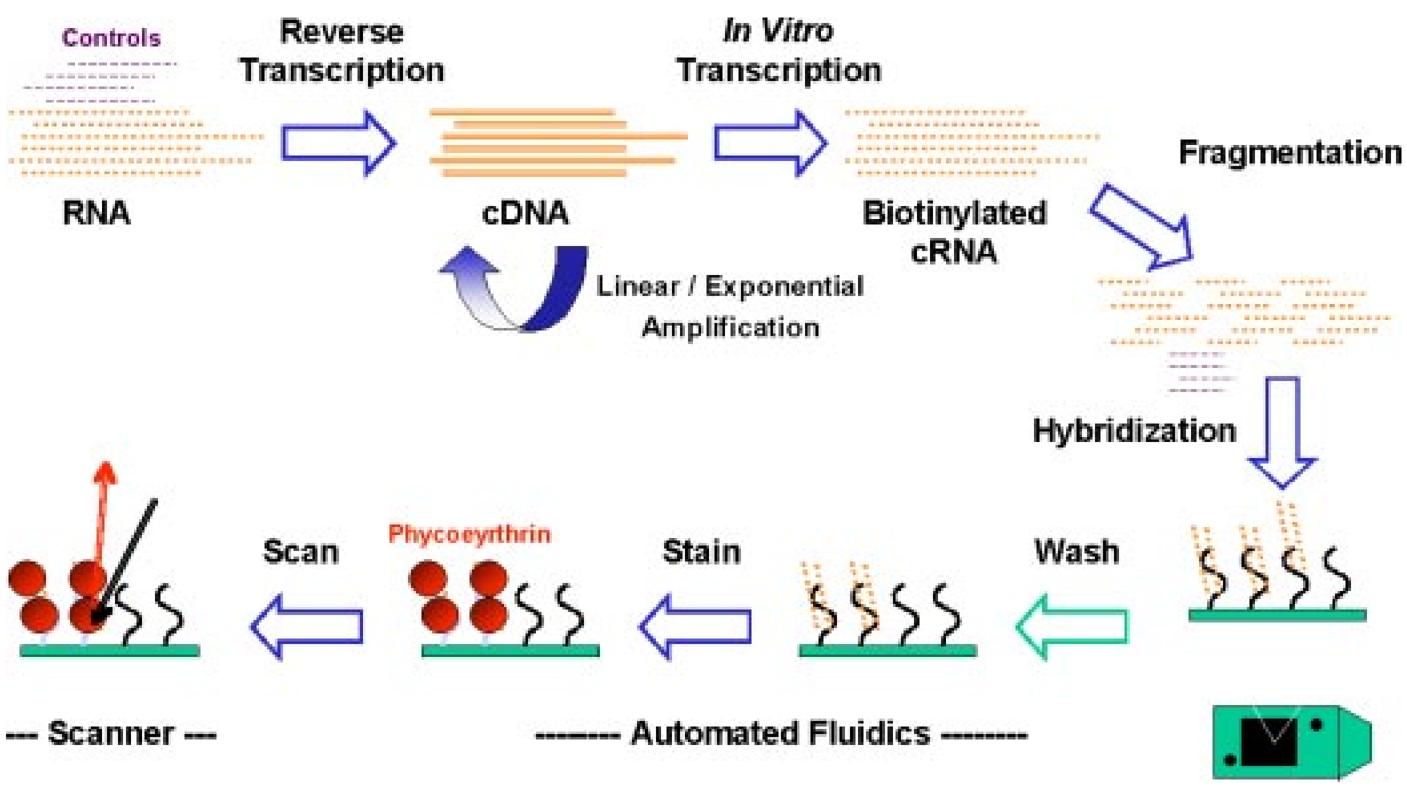


Figure 5: *Oligonucleotide (GeneChip) system* (`pathbox.wustl.edu`).

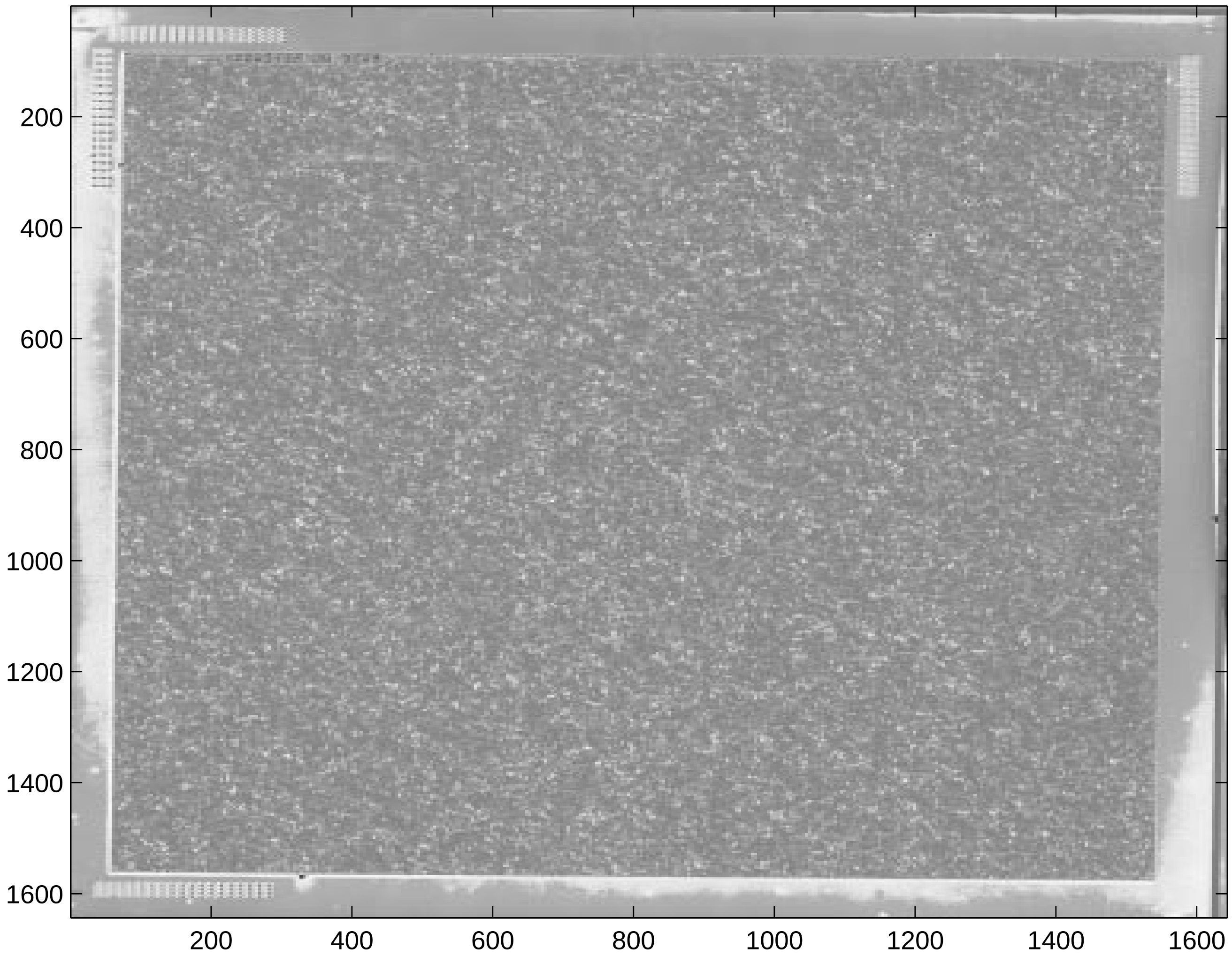


Figure 6: *Affymetrix GeneChip microarray.*

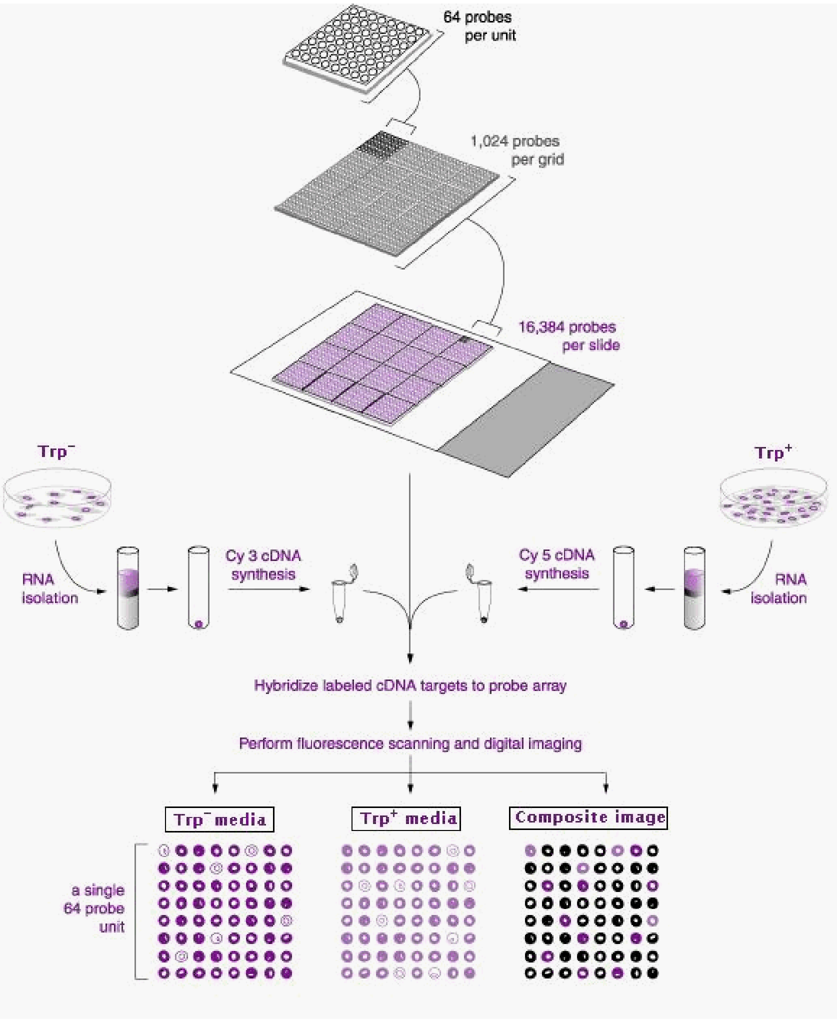


Figure 7: *cDNA (Stanford) system (*`teach.biosci.arizona.edu`*).*

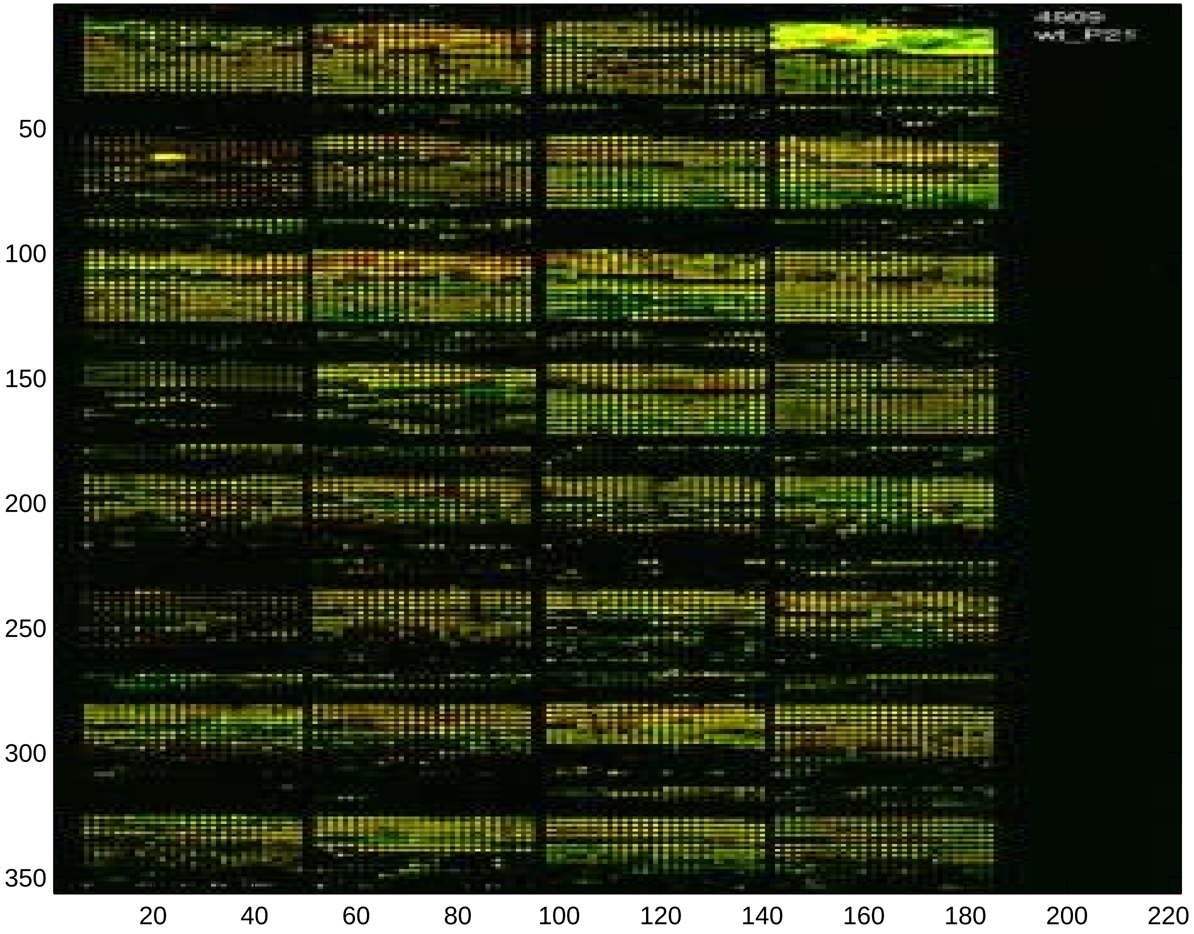


Figure 8: *cDNA spotted array.*

Signal Extraction

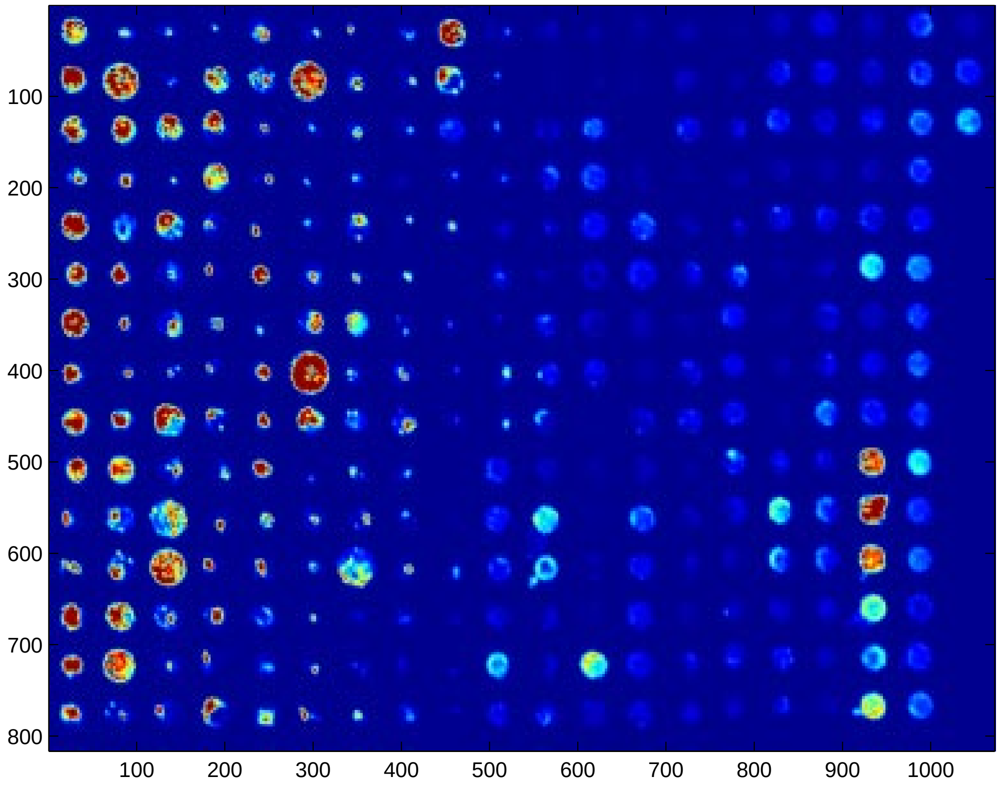


Figure 9: *Blowup of cDNA spotted array.*

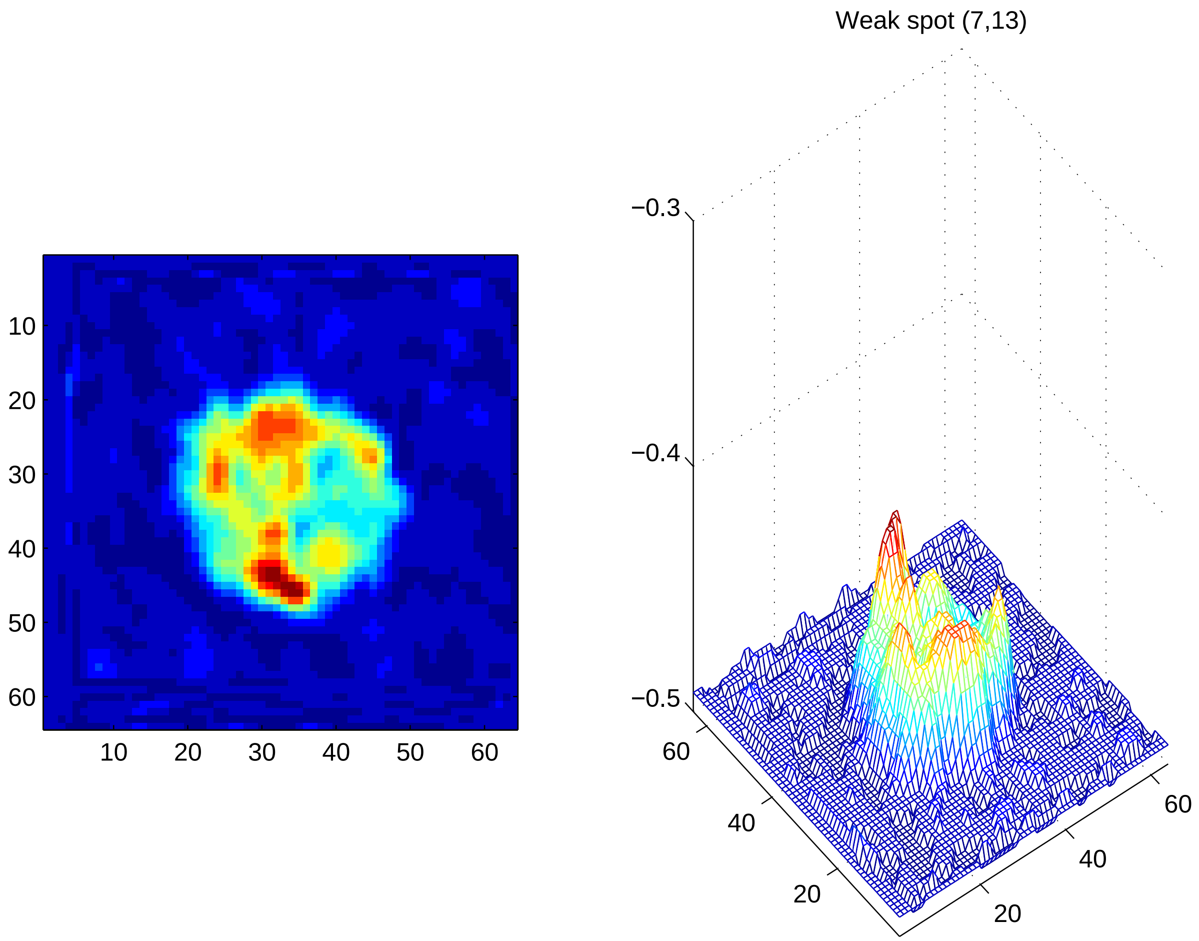


Figure 10: *Weak Spot.*

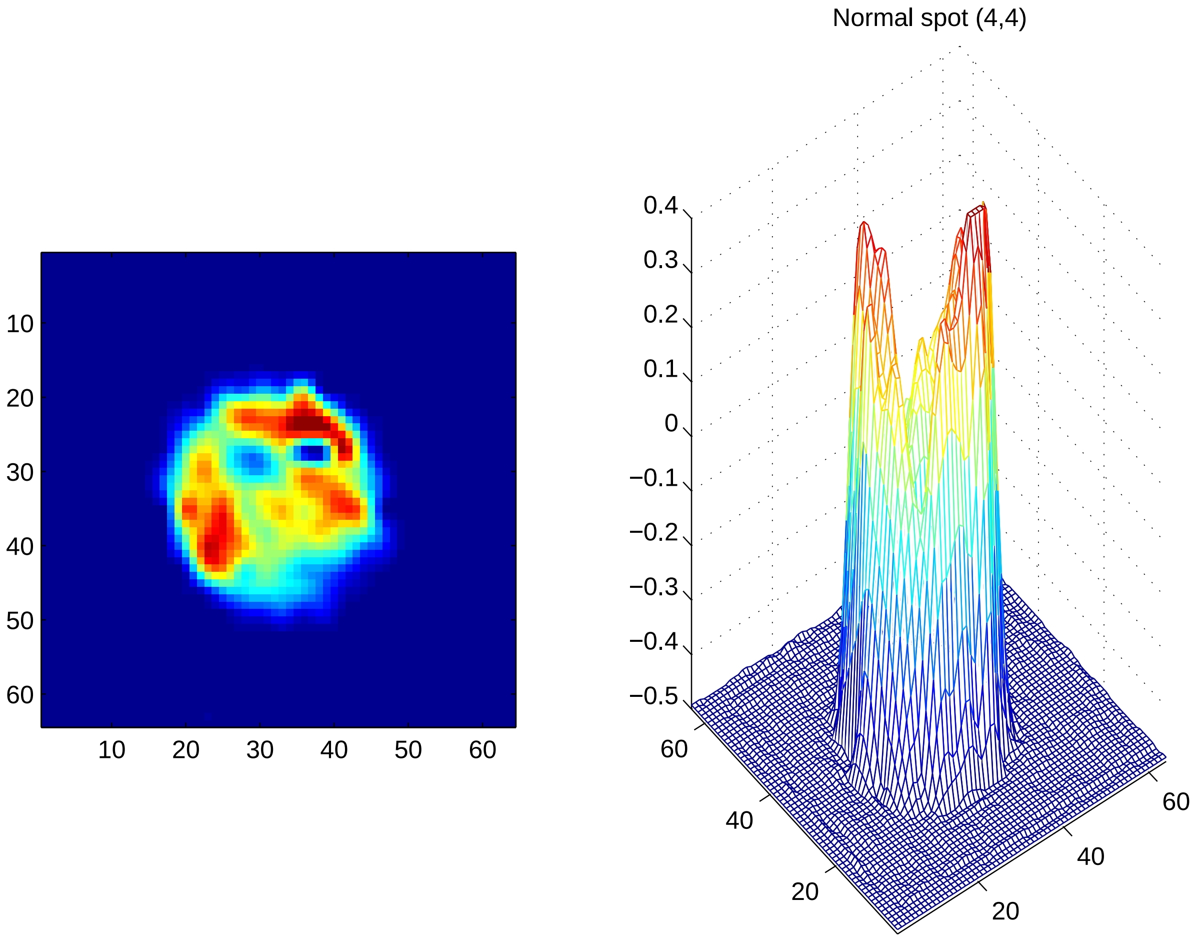


Figure 11: *Normal spot.*

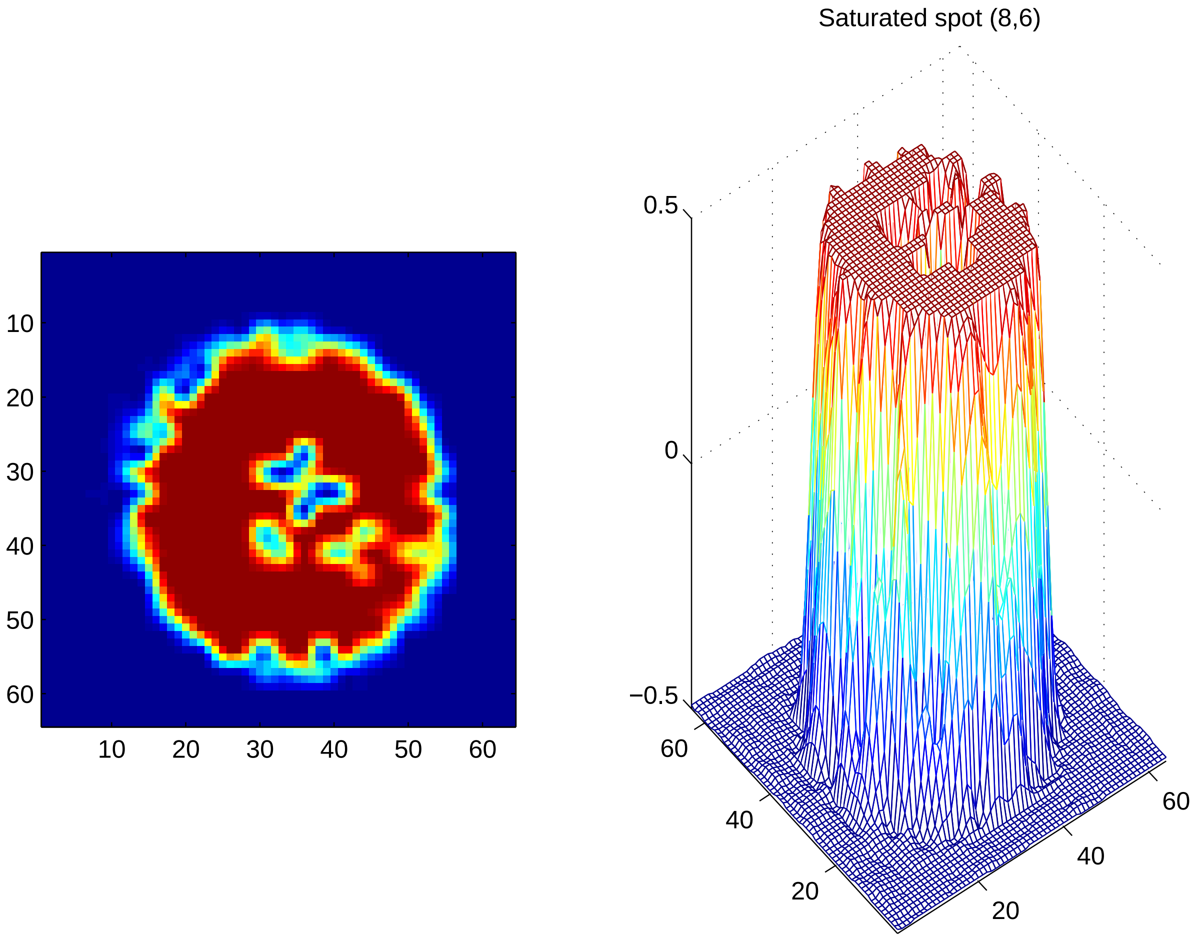


Figure 12: *Saturated spot.*

Model-based Signal Extraction (Hero:Springer02)

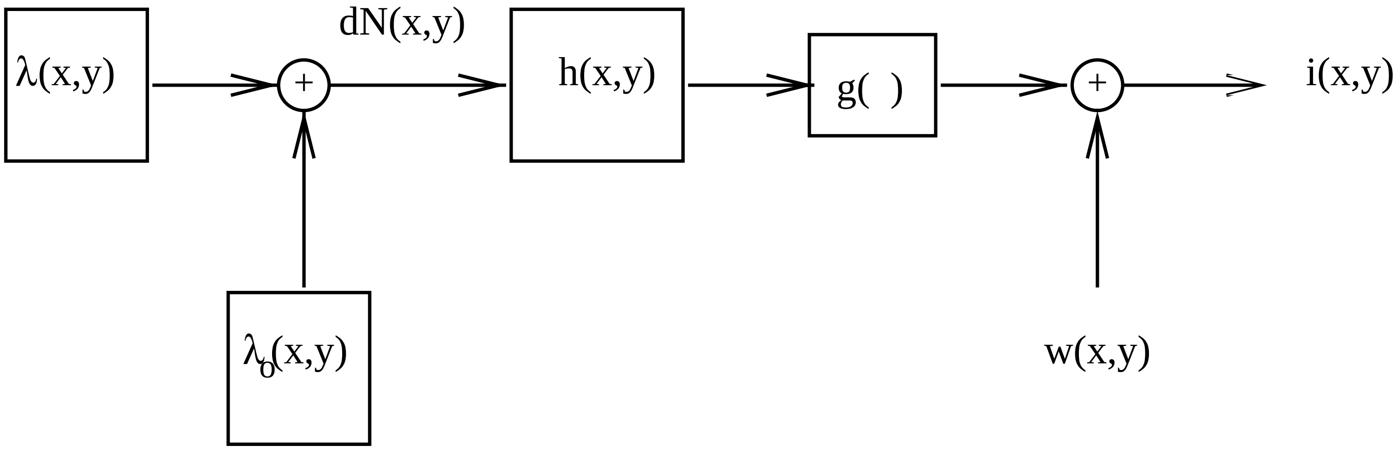


Figure 13: *Filtered Poisson model for microarray image.*

Compound Channel Representation

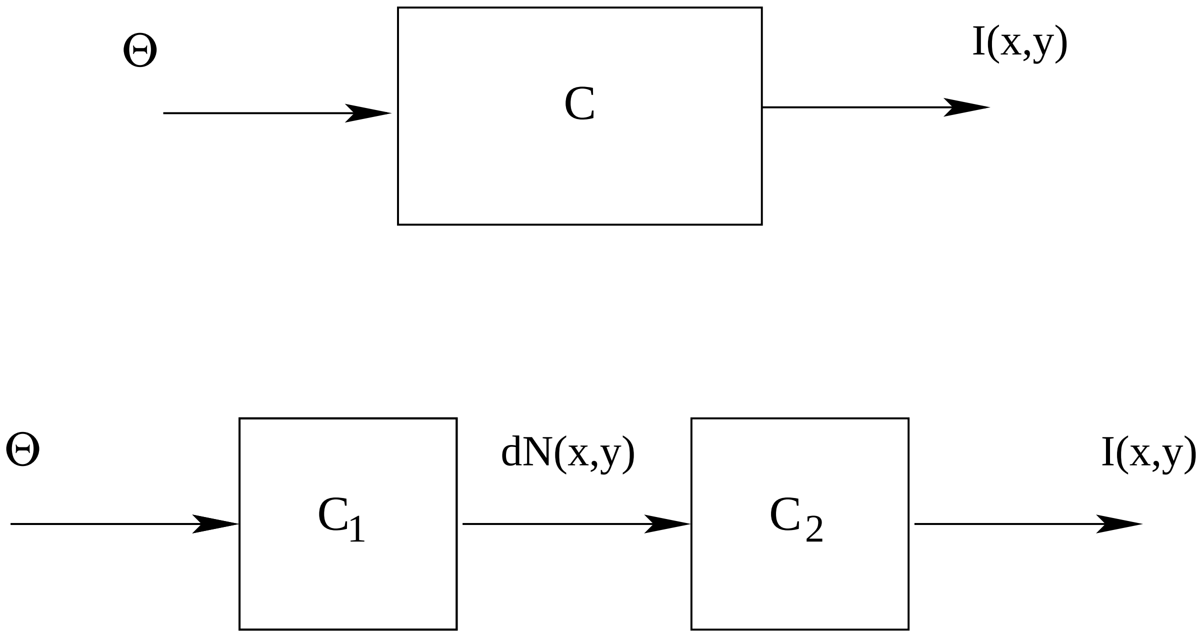


Figure 14: *Top: statistical representation of I as the output of channel C with input Θ. Bottom: decomposition of C into Poisson and Gaussian channels C_1 and C_2, respectively.*

Gabor Superposition - Width MSE

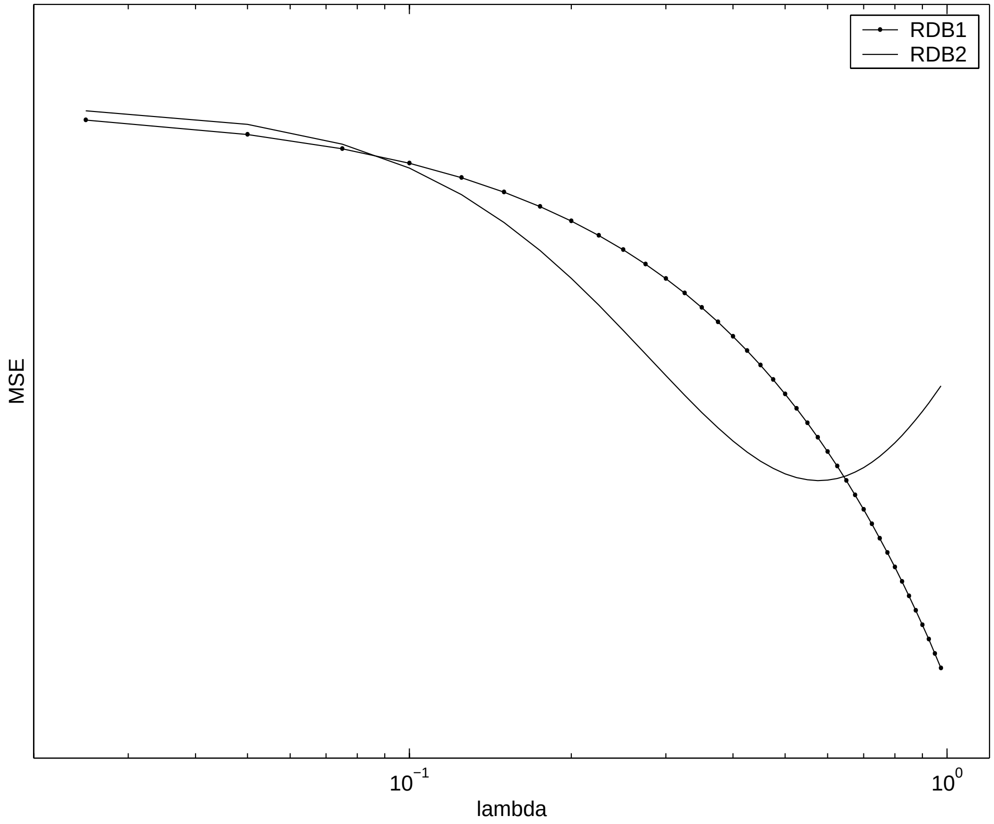


Figure 15: *Distortion-rate MSE lower bounds on Gabor widths of* $\Phi_j(x,y)$.

Morphological Spot Segmentation (Siddiqui&Hero:ICIP02)

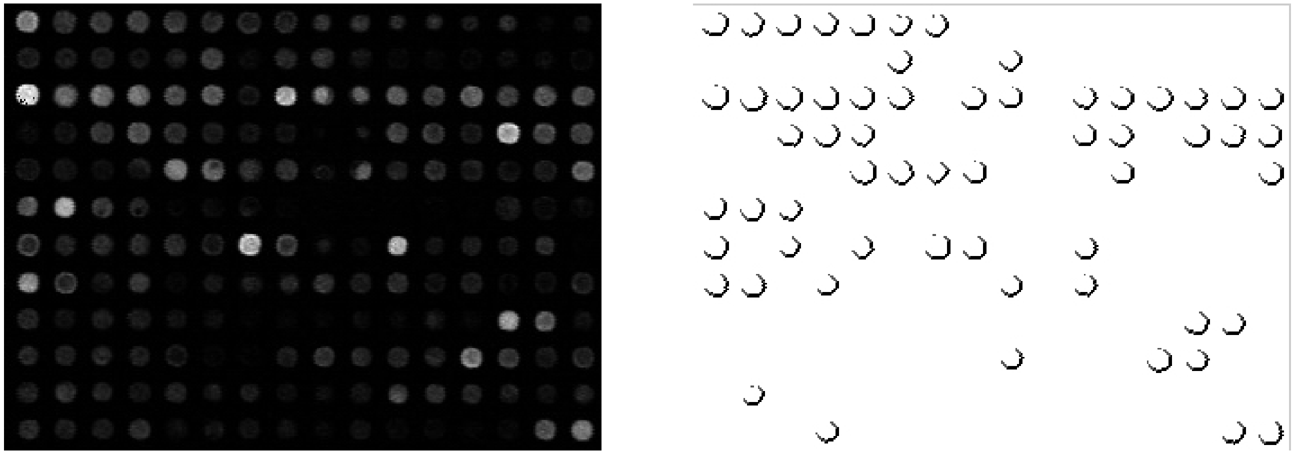


Figure 16: *(L) Original cDNA microarray image. (R) after alternating sequential filtering.*

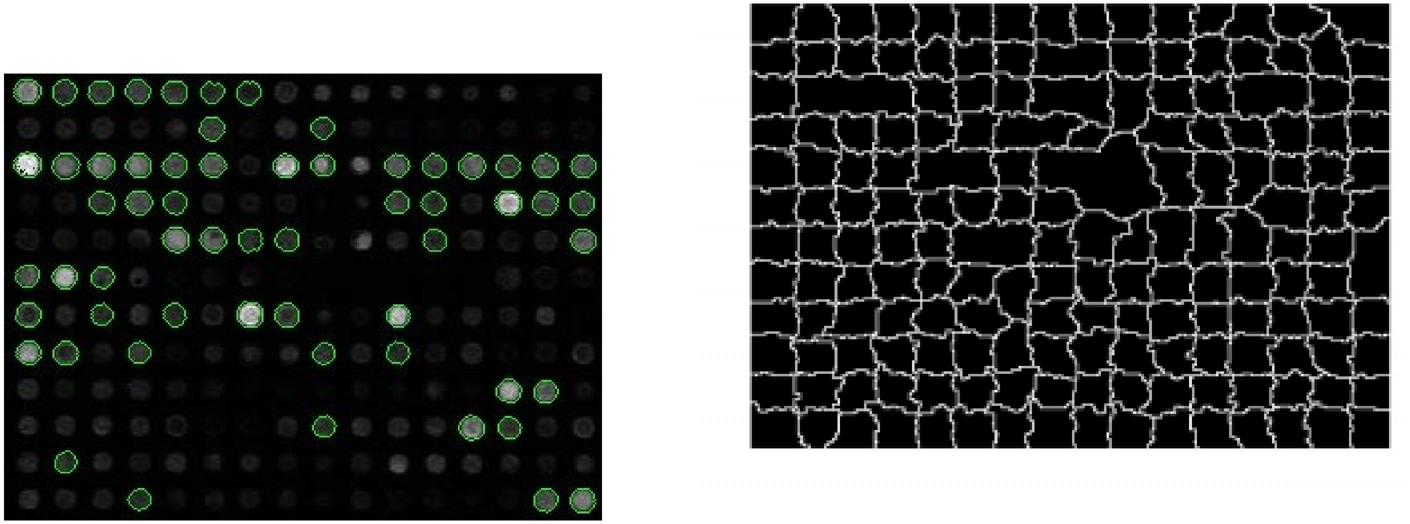


Figure 17: *(L) Final segmentation. (R) Spot watershed domains for noise averaging.*

Reference Datasets

1 (2001H) Affy human retinal aging study (Yosida, Swaroop)

- Y group: 8 individuals in age range 16-19 yrs
- O group: 8 individuals in age range 72-80 yrs

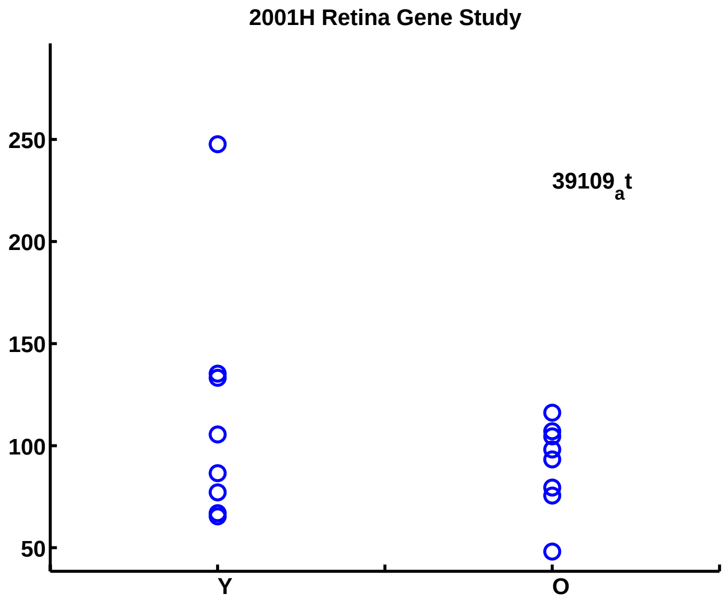


Figure 18: *Responses for a gene in human retinal aging study.*

2 (2001FW) Fred Wright’s human fibroblast mixing experiment

(`http://thinker.med.ohio-state.edu/projects/fbss/index.html`)

- 18 individuals in 3 groups of 6 subjects

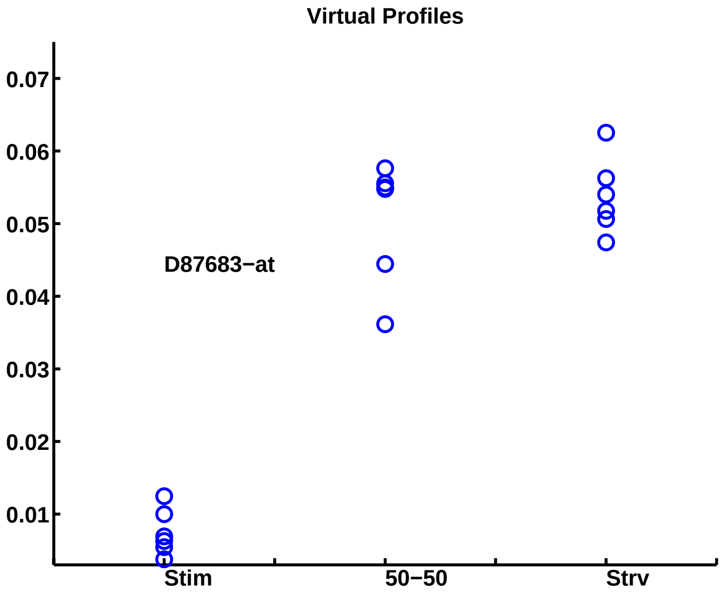


Figure 19: *Responses for a gene in FW human fibroblast mixture study.*

3 (2001M) Affy mouse retinal aging study (Yosida, Barlow, Lockhart, Swaroop)

- 24 mice in 6 groups of 4 subjects

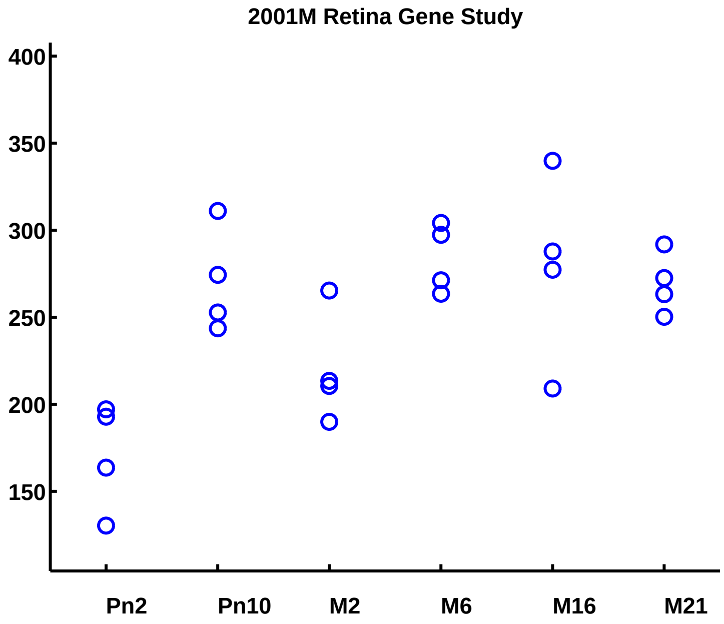


Figure 20: *Responses for a gene in mouse aging study.*

4 (2002M) Affy mouse differential study (Yosida Swaroop)

- 12 knockout mice in 3 groups of 4 subjects
- 12 wildtype mice in 3 groups of 4 subjects

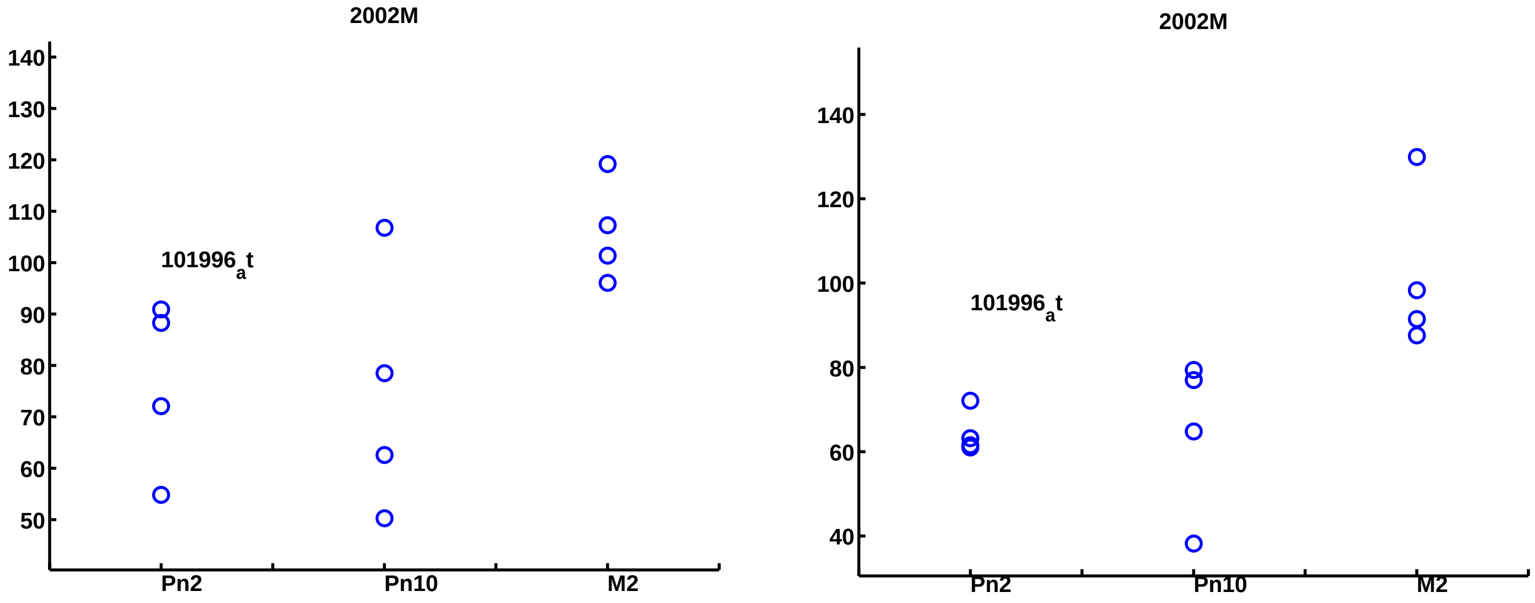


Figure 21: *Differential responses for a gene in mouse k (left) vs w (right) study.*

Microarray Normalization

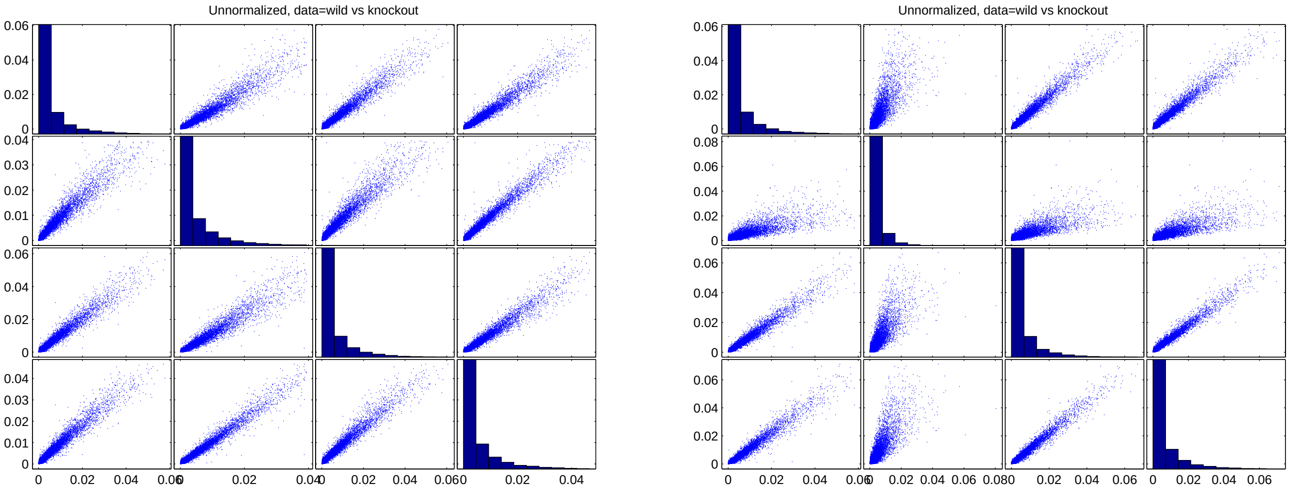


Figure 22: *Scatter of 4 microarrays from NRL knockout and wildtype at time Pn2.*

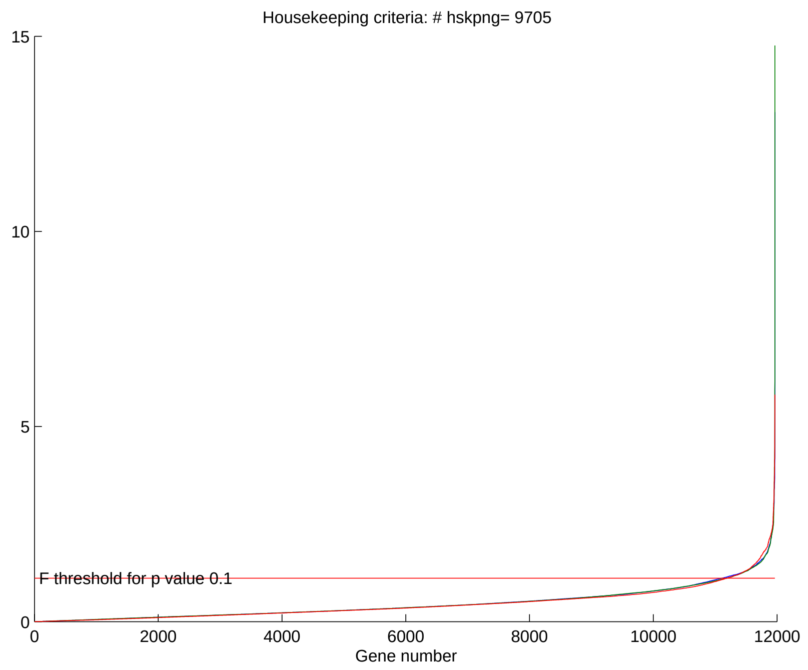


Figure 23: *Housekeeping gene rank test with threshold for Pn2 knockout and wildtype.*

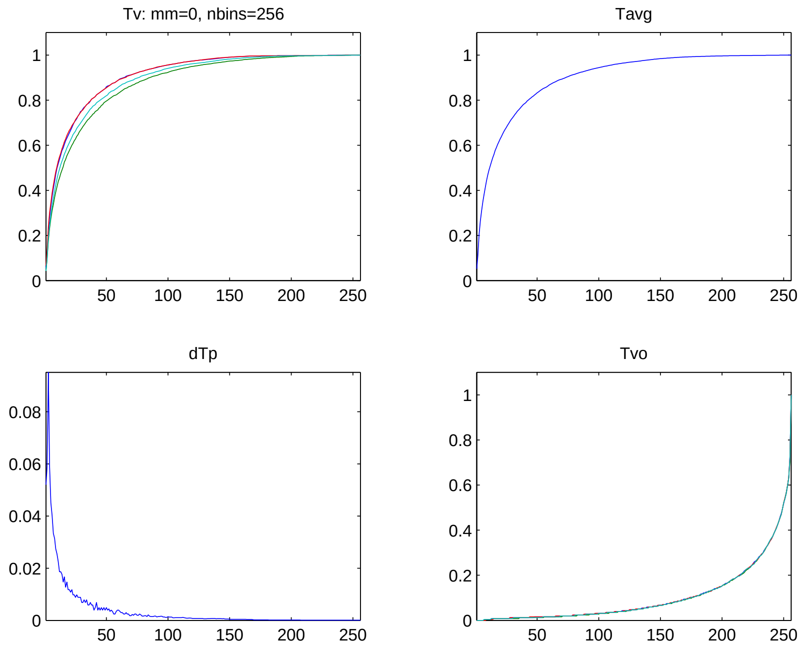


Figure 24: *Uniformization (TL), averaging (TR), differentiation (LL) and equalization (LR) mappings for knockout.*

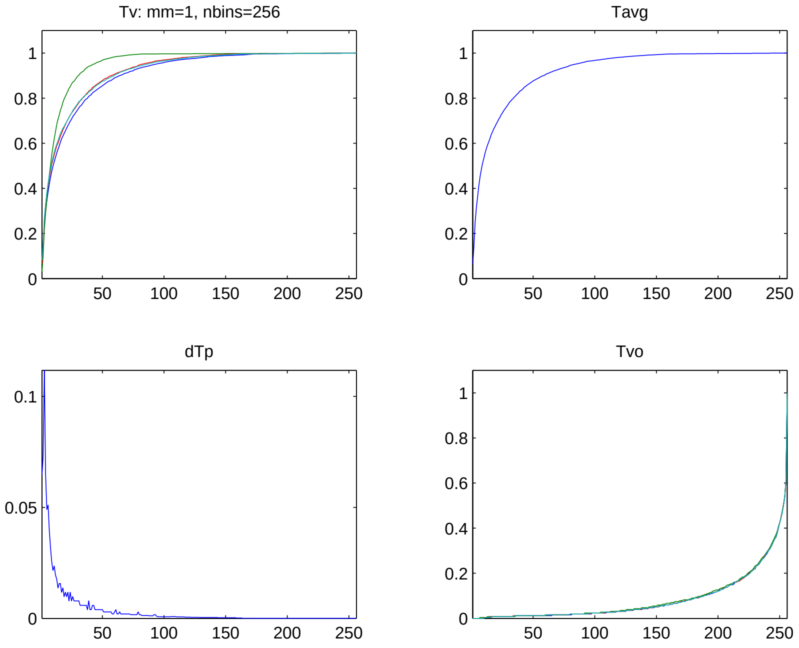


Figure 25: *Uniformization (TL), averaging (TR), differentiation (LL) and equalization (LR) mappings for wildtype.*

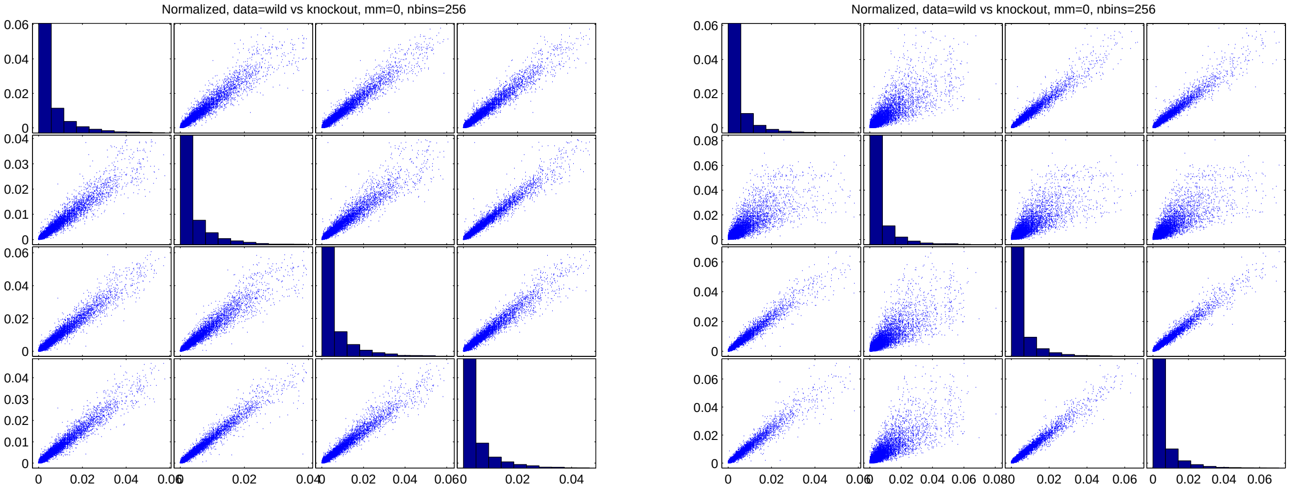


Figure 26: *Scatter of 4 normalized microarrays from NRL knockout and wildtype at time Pn2.*

Pareto Gene Filtering (Fleury&etal:ICASSP02, Hero&etal:GENSIPS02)

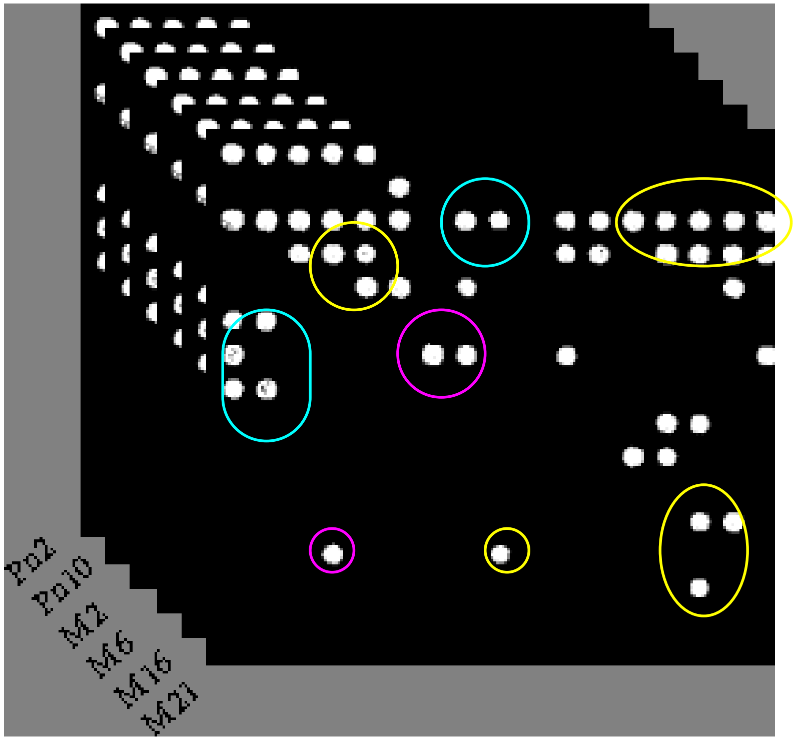


Figure 27: *Clustering on the Data Cube.*

Objective: Classify time trajectory of gene i into one of K classes

Gene Trajectory Classification

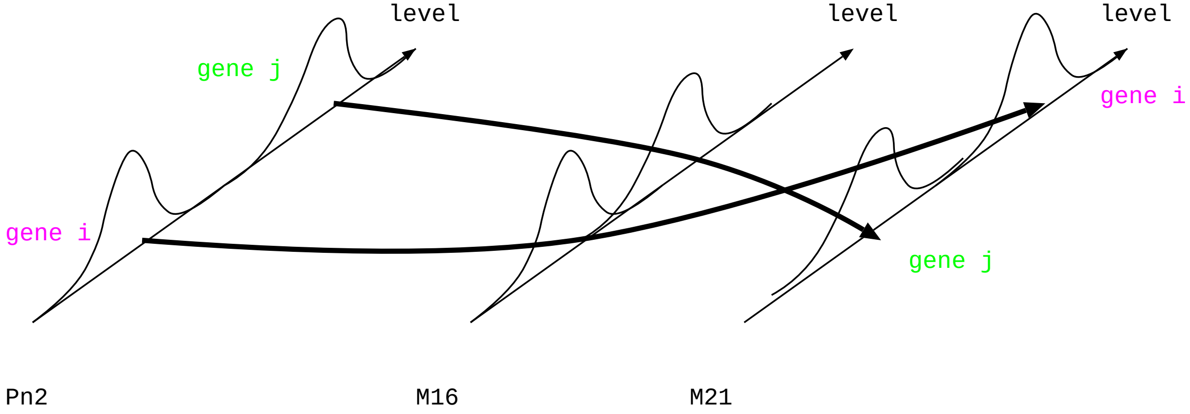


Figure 28: *Gene i is old dominant while gene j is young dominant*

Objective: extract gene trajectories (n) from sequence of repeated (m) microarray experiments over time samples (t)

$$y_{tm}(n), \quad n = 1, \ldots, N, \; t = 1, \ldots, T, \; m = 1, \ldots, M.$$

Gene Filtering via Multiobjective Optimization

Gene selection criteria: for n-th gene $\xi_1(Y(n)), \ ,\ldots, \ \xi_P(Y(n))$

Possible $\xi_p(Y(n))$'s for finding uncommon genes

- Squared mean change from $t = 1$ to $t = T$:

$$\xi_1(Y(n)) = |\bar{y}_{T*}(n) - \bar{y}_{1*}(n)|^2$$

- Standard deviation at $t = 1$:

$$\xi_2(Y(n)) = \overline{(y_{1m}(n) - \bar{y}_{1*}(n))^2}$$

- Standard deviation at $t = T$:

$$\xi_3(Y(n)) = \overline{(y_{Tm}(n) - \bar{y}_{T*}(n))^2}$$

Some possible scalar functions:

- t-test statistic (Goss etal 2000): $T(n) = \frac{\xi_1(Y(n))}{\frac{1}{2}\xi_2(Y(n))+\frac{1}{2}\xi_3(Y(n))}$
- R^2 statistic (Hastie etal 2000): $R^2(n) = \frac{T_n}{1+T_n}$
- H statistic (Sinha etal 1998): $H(n) = \frac{\xi_1(Y(n))}{\sqrt{\xi_2(Y(n))\xi_3(Y(n))}}$

Objective: find genes which maximize or minimize the selection criteria

Aggregated Criteria

Let $\{W_p\}_{p=1}^P$ be experimenter's cost "preference pattern"

$$\sum_{p=1}^P W_p = 1, \; W_i \geq 0$$

Find optimal gene via:

$$\max_n \sum_{p=1}^P W_p \xi_p(Y(n)), \quad or \quad \max_n \prod_{p=1}^P (\xi_p(Y(n)))^{W_p}$$

Q. What are the set of optimal genes for all preference patterns?

A. These are *non-dominated* genes (Pareto optimal)

Defn: Gene i is dominated if there is a $j \neq i$ s.t.

$$\xi_p(Y(i)) \leq \xi_p(Y(j)), \; p = 1, \ldots, P$$

Pareto Optimality: increasing criteria

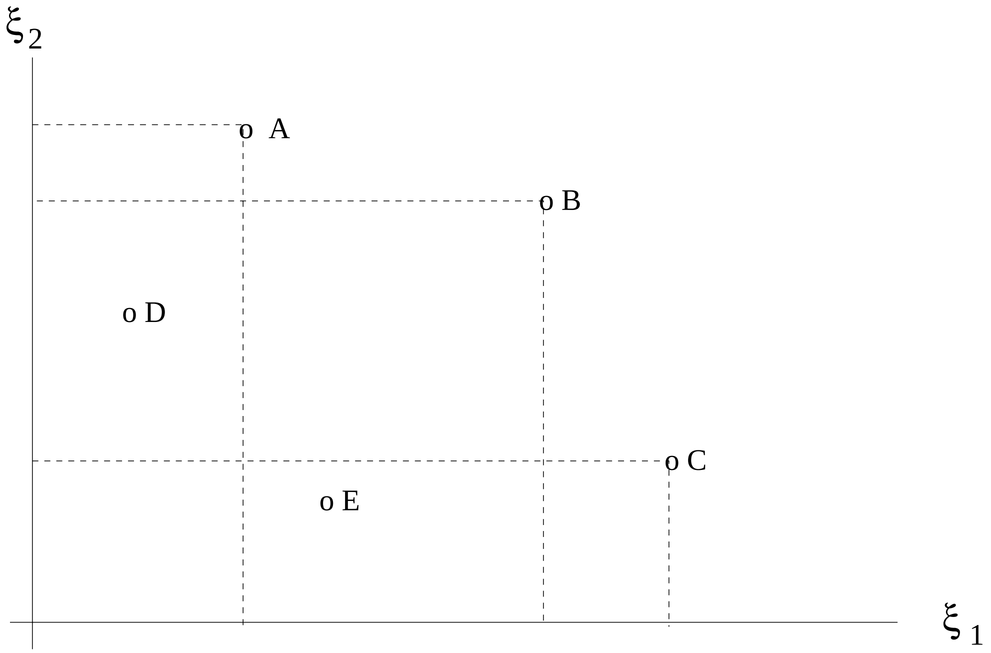


Figure 29: *For increasing criteria A, B, C are non-dominated genes and form the (first) Pareto front. A second Pareto front is formed by genes D,E.*

Pareto Optimality: inc/dec criteria

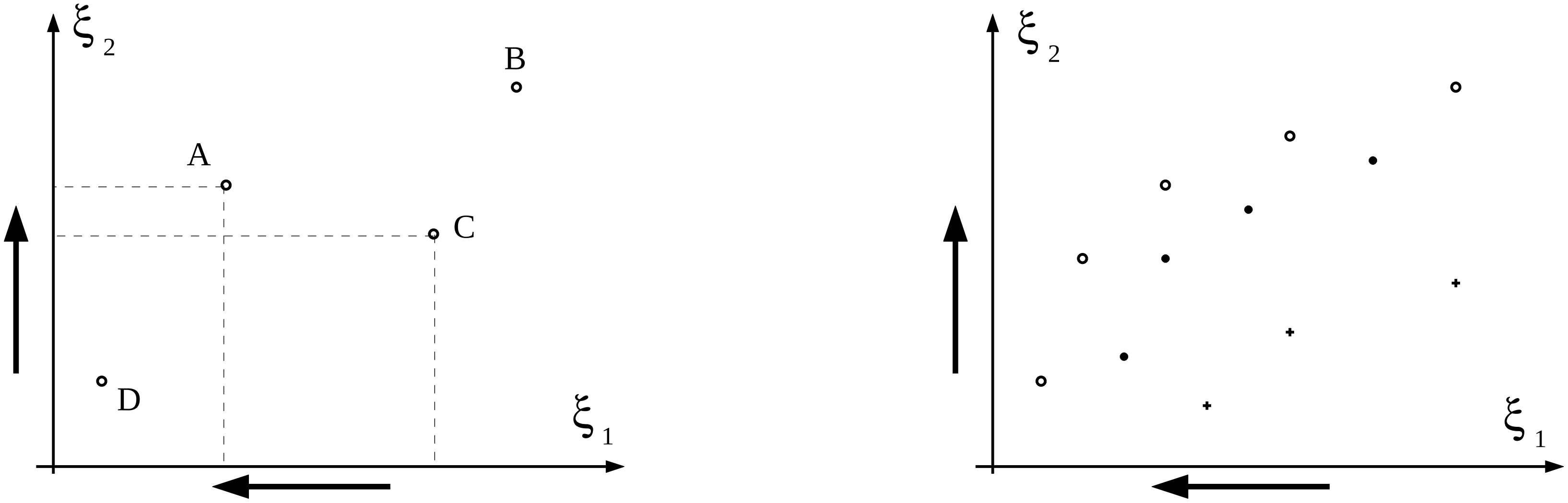


Figure 30: *a). Non-dominated property, and b). Pareto optimal fronts, in dual criteria plane.*

Pareto Gene Filtering vs. Paired T-test

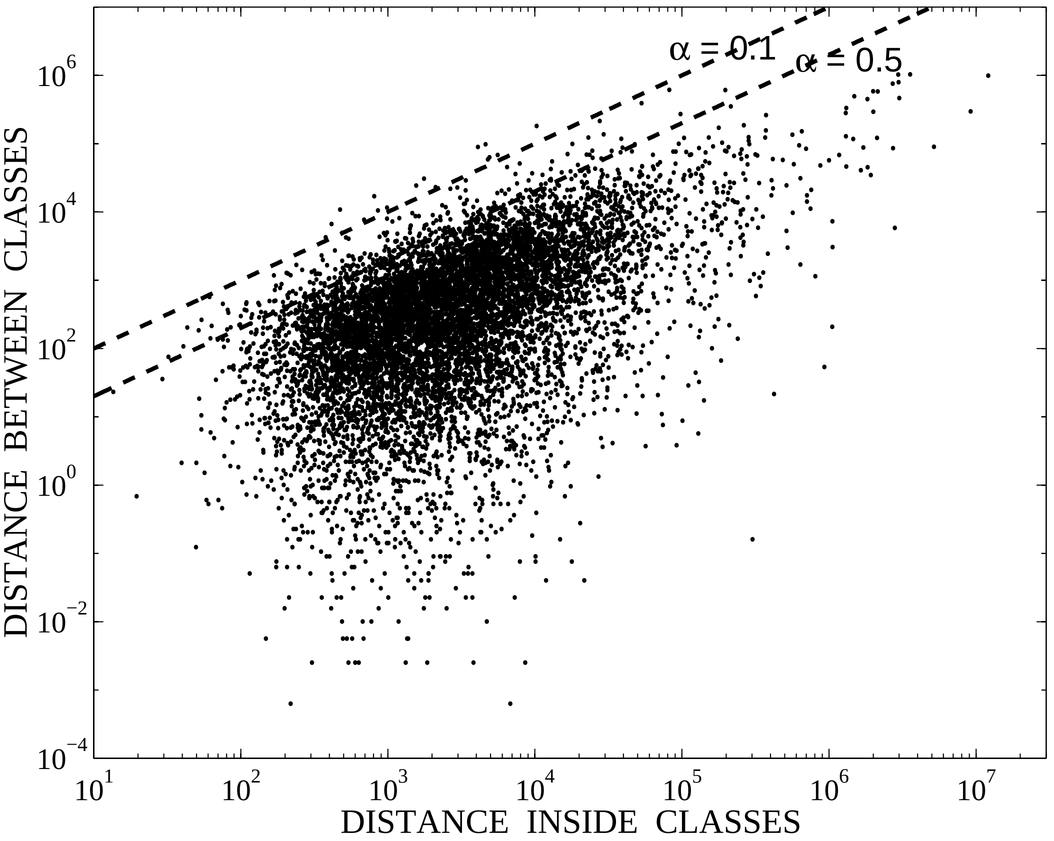


Figure 31: $\xi_1 =$ *mean change vs* $\xi_2 =$ *pooled standard deviation for 8826 human retina genes (2001H). Superimposed are T-test boundaries*

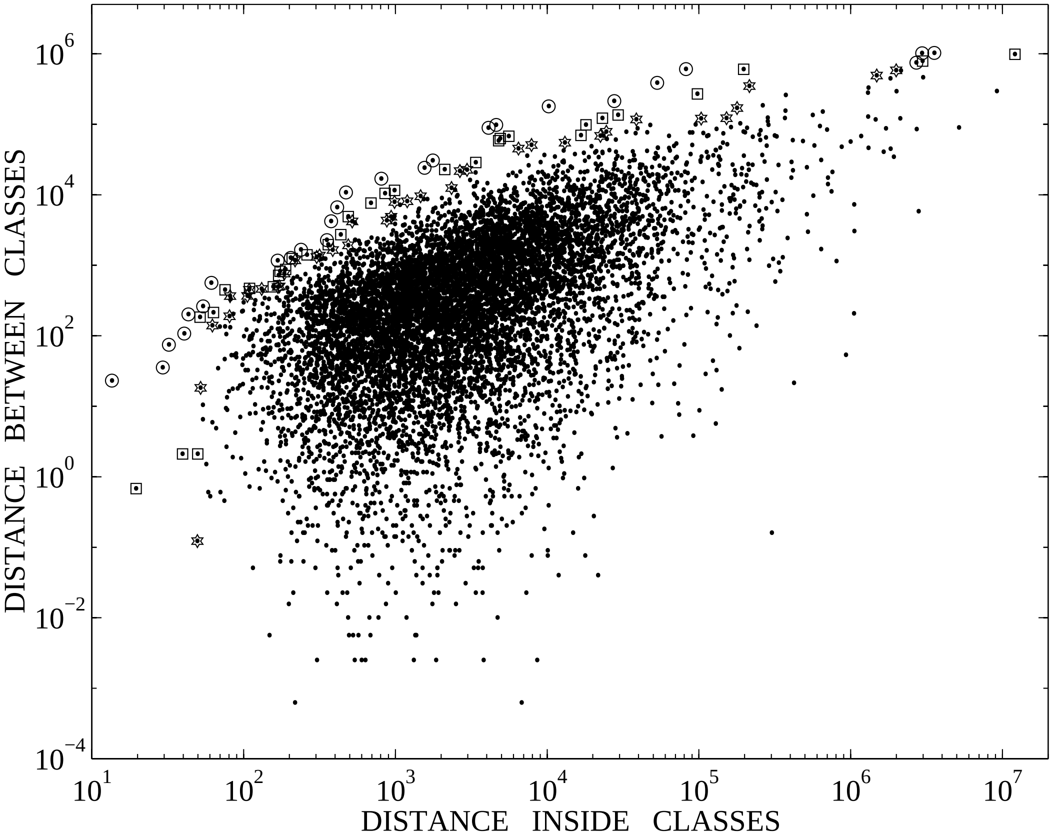


Figure 32: *First (circle) second (square) and third (hexagon) Pareto optimal fronts on (2001H) data.*

Profile Selection Criteria

1. Profile contrasts for trajectory $\{y_{mt}(n)\}_t$

$$\begin{bmatrix} \xi_1(n) \\ \vdots \\ \xi_P(n) \end{bmatrix} = \begin{bmatrix} a_{11} & \cdots & a_{1T} \\ \vdots & \ddots & \vdots \\ a_{P1} & \cdots & a_{PT} \end{bmatrix} \begin{bmatrix} \bar{y}_{1*}(n) \\ \vdots \\ \bar{y}_{T*}(n) \end{bmatrix}$$

$$\begin{aligned} A_2 &= \begin{bmatrix} -1 & 1 \\ 1 & 1 \end{bmatrix}, \; A_2^{'} = \begin{bmatrix} 1 & -1 \\ 1 & 1 \end{bmatrix}, \\ A_3 &= \begin{bmatrix} -1 & 0 & 1 \\ 1 & -2 & 1 \\ 1 & 1 & 1 \end{bmatrix}, \; A_3^{'} = \begin{bmatrix} -1 & 1 & 0 \\ -1 & -1 & 2 \\ 1 & 1 & 1 \end{bmatrix}, \end{aligned}$$

2. Profile monotonicity for trajectory $\{y_{mt}(n)\}_t$

$$\xi_2(n) = \prod_{t=2}^{T} I(\bar{y}_{*t}(n) - \bar{y}_{*(t-1)}(n))$$

3. Profile divergence of trajectories $\{w_{mt}(n)\}_t$, $\{k_{mt}(n)\}_t$

$$\xi_1(n) = \sum_{t=1}^{T} \bar{k}_{*t}(n) \log \frac{\bar{k}_{*t}(n)}{\bar{w}_{*t}(n)}$$

4. Combinations of above

Accounting for Sampling Errors: Cross-validation

- Leave-one-out cross validation

Let $Y^{-m}(n)$ denote one possible set of $T \times (M-1)$ samples

Cross-validation Algorithm:

```
Do $m = 1, \ldots, M^T$:

            Compute  $\left(\xi_1(Y^{-m}(n)), \xi_2(Y^{-m}(n))\right)$

    Find Genes in First 3 Pareto fronts: $G^{-m}$
    End
    $\mathit{Resistant}$ Genes = $\cap_{m=1}^{M^T} G^{-m}$
```

Accounting for Sampling Errors: Posterior Pareto Analysis

Given prior on mean expression levels $\overline{\xi}_p(n) = E[\xi_p(Y(n))]$ find

$$\begin{aligned}
& p(i|Y) \\
= \; & P\left(\cap_{j\neq i}\left\{\underline{\xi}(i) \leq \underline{\xi}(j)\right\}^c |Y\right) \\
= \; & \int dP(\underline{\xi}(i)|Y)\prod_{j\neq i} P\left(\left\{\underline{\xi}(i) \leq \underline{\xi}(j)\right\}^c |Y, \underline{\xi}(i)\right)
\end{aligned}$$

Case of two criteria ($P = 2$)

$$\begin{aligned}
p(i|Y) \; = \; & \int\int du_1 du_2 \, f_{\xi_1(i),\xi_2(i)|Y}(u_1, u_2) \\
& \prod_{j\neq i}\left[F_{\xi_1(j)|Y}(u_1) + F_{\xi_2(j)|Y}(u_2) - F_{\xi_1(j),\xi_2(j)|Y}(u_1, u_2)\right]
\end{aligned}$$

Application to Fred Wright's Mixture Study

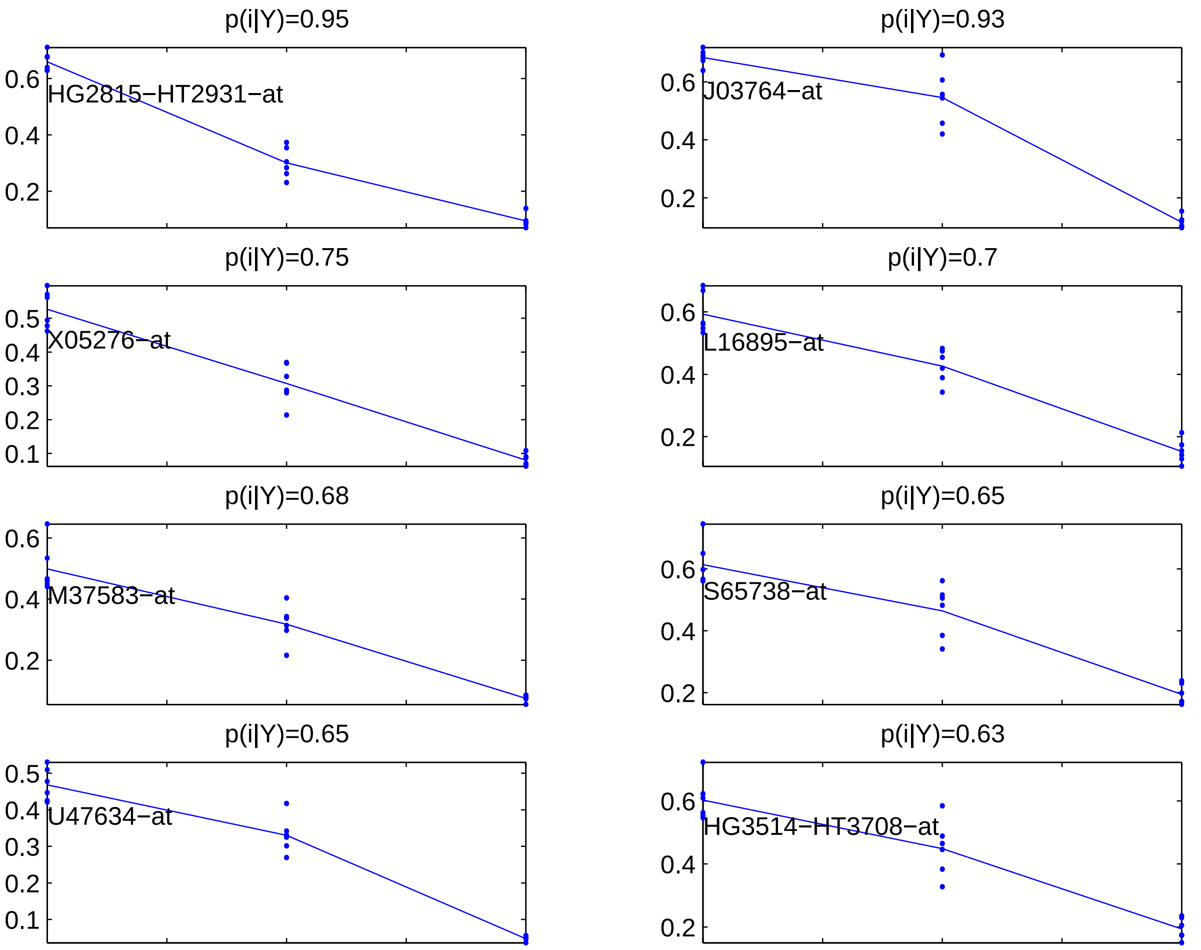


Figure 33: *8 ranked monotone decreasing gene profiles.*

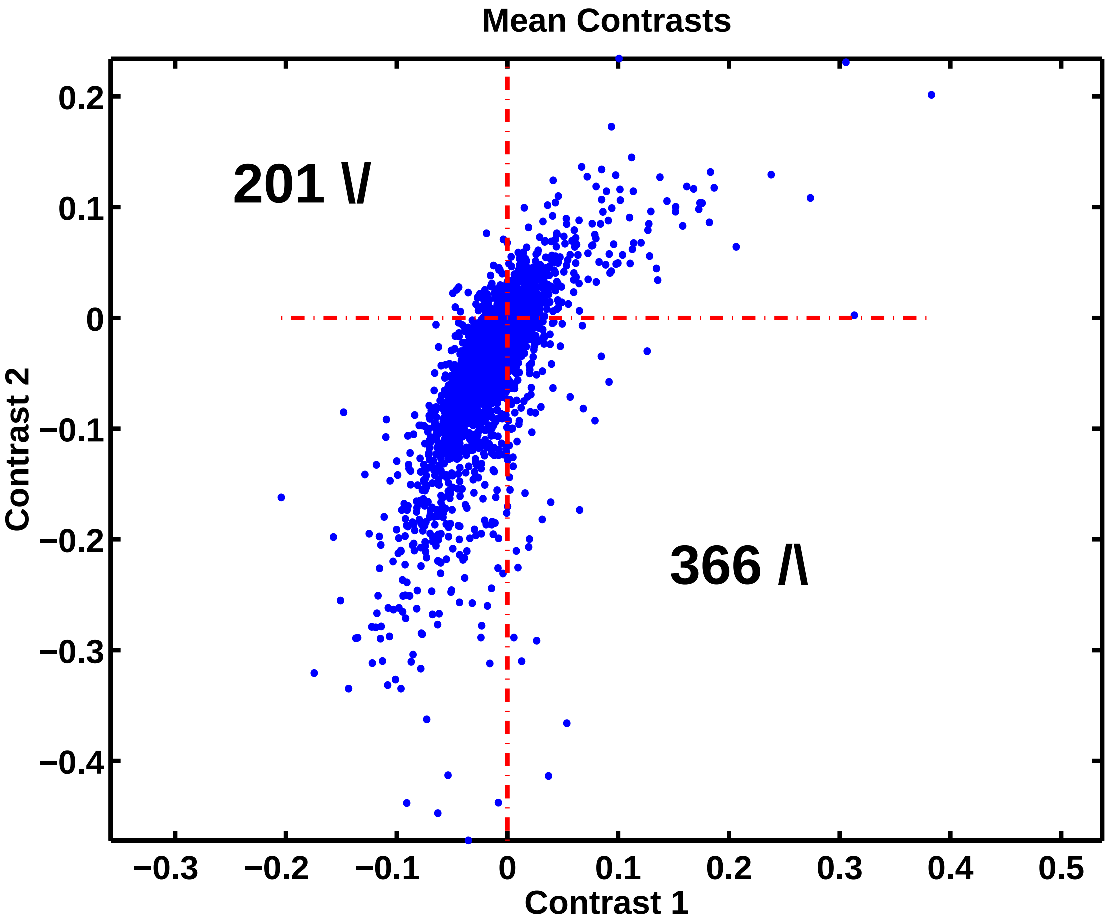


Figure 34: *Multicriterion scattergram for first two rows of* A'_3.

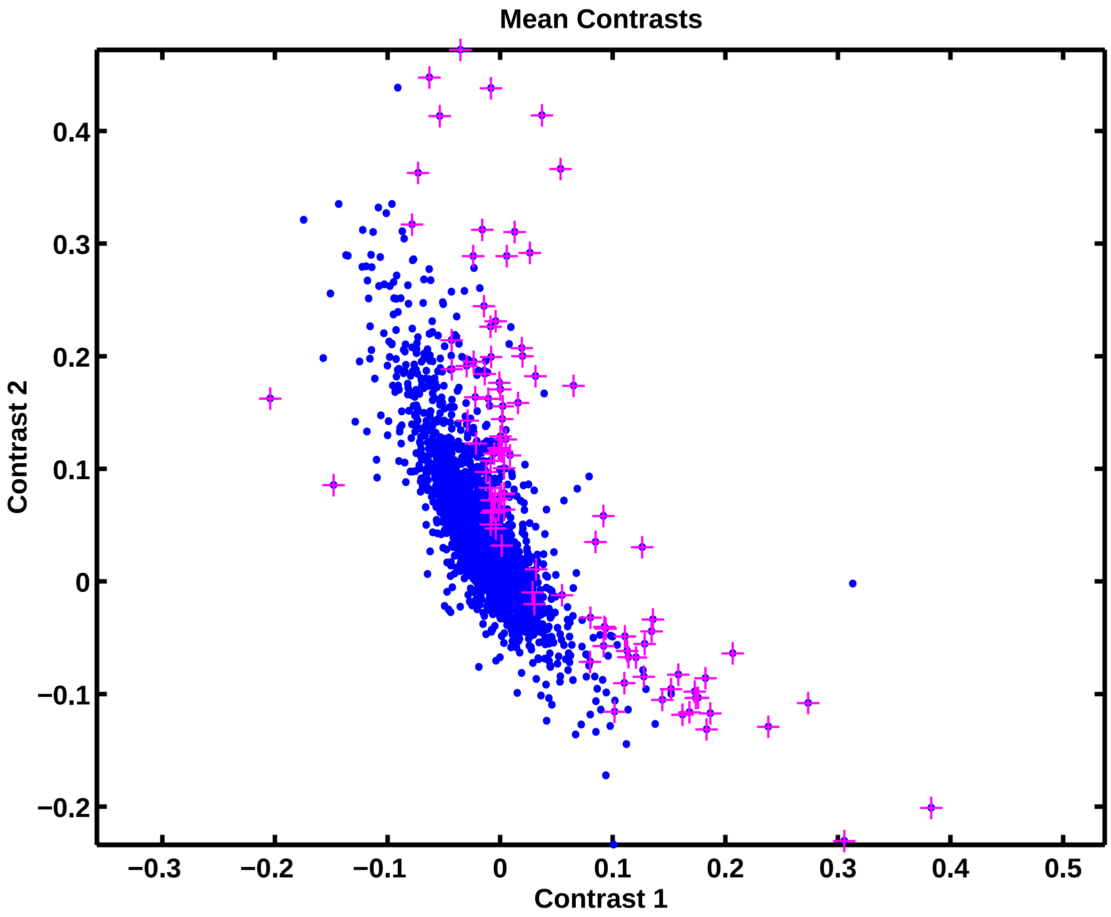


Figure 35: *Multicriterion scattergram for* $A = [-1, 1, 0; -1, -1, 2]$. *98 genes are non-linear profiles (p-value of 0.1).*

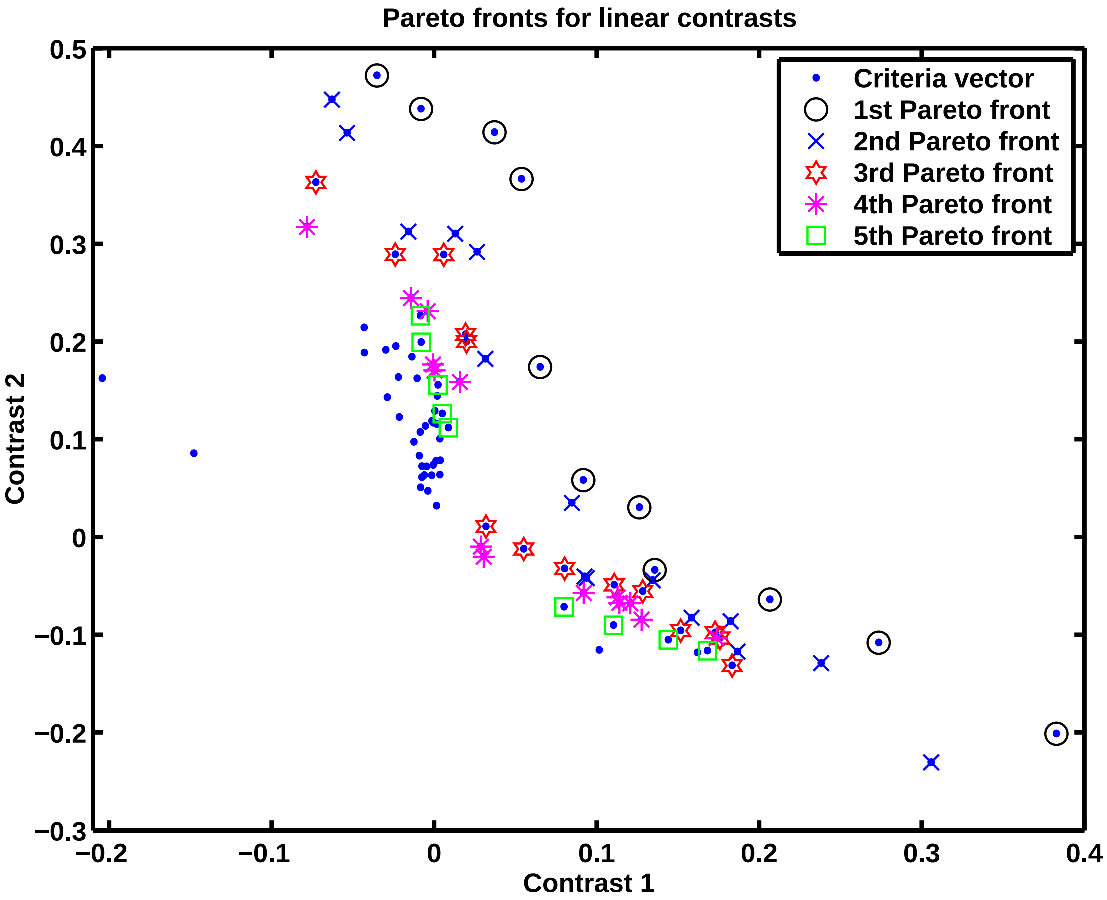


Figure 36: *The first five Pareto fronts for the genes with non-linear profiles shown in Fig. 35.*

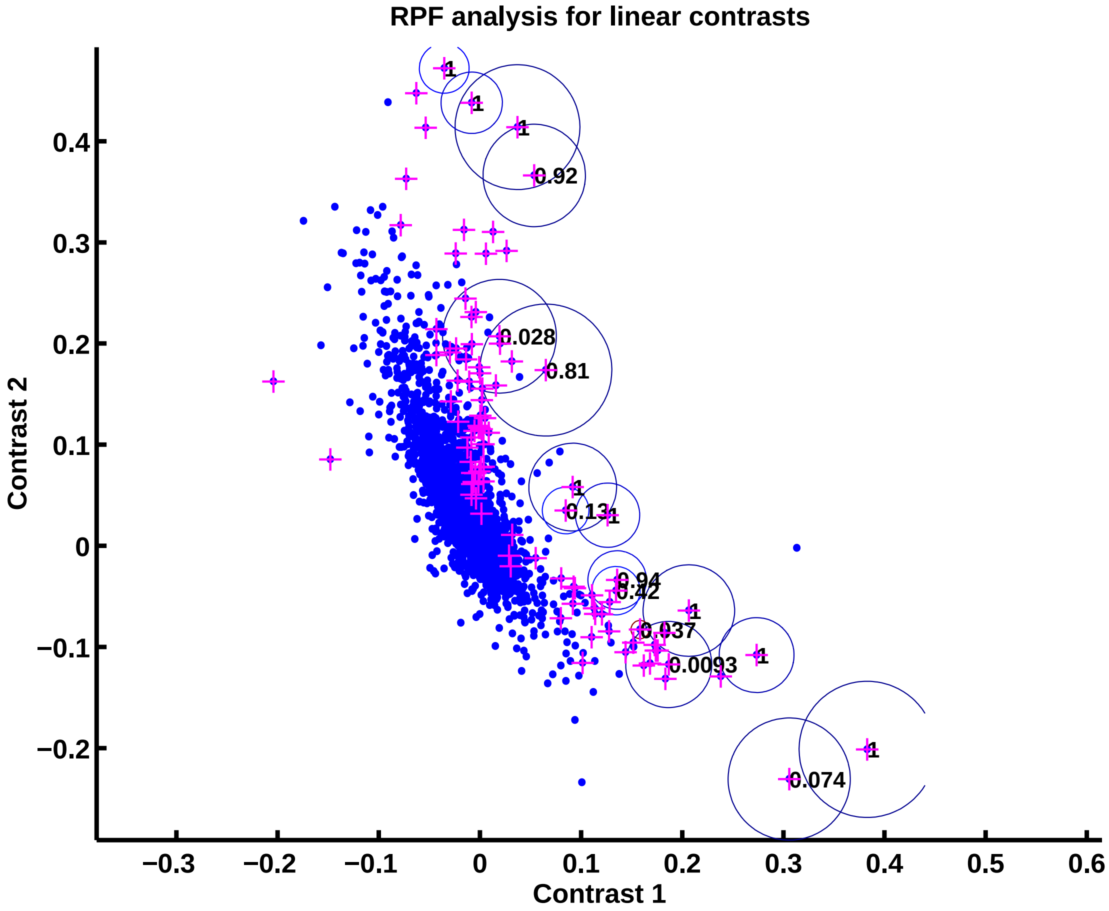


Figure 37: *17 genes in first Pareto front with non-zero probability by cross-validation.*

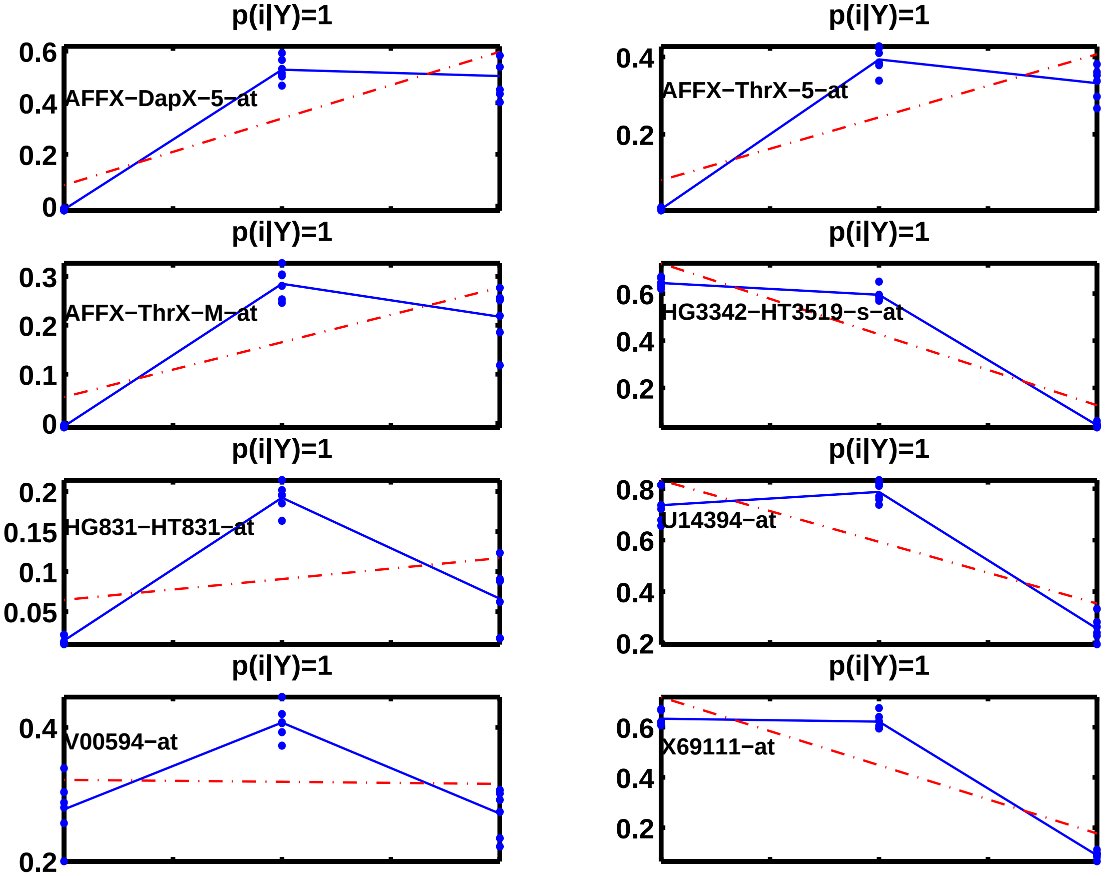


Figure 38: *The 8 top cross-validation ranked gene profiles remaining on the first Pareto front.*

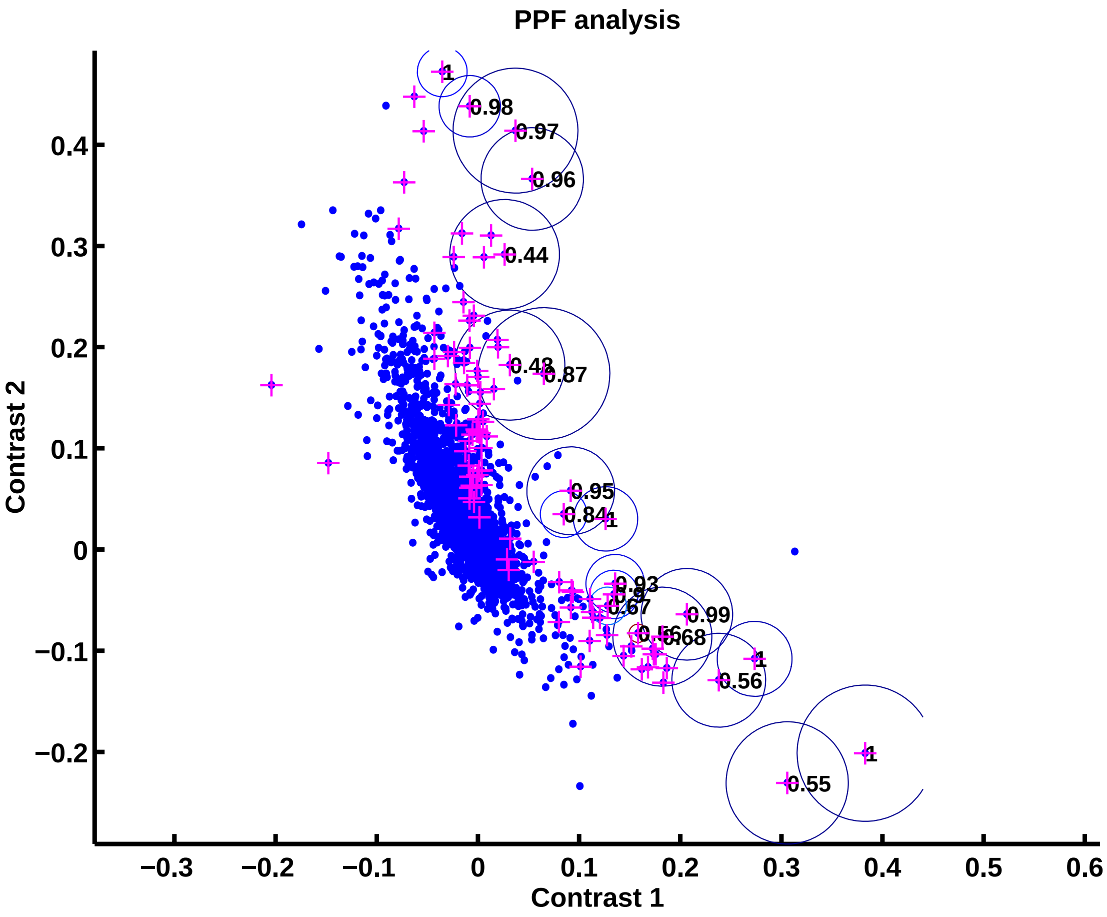


Figure 39: *PPF and posterior probabilities of belonging to the first Pareto front.*

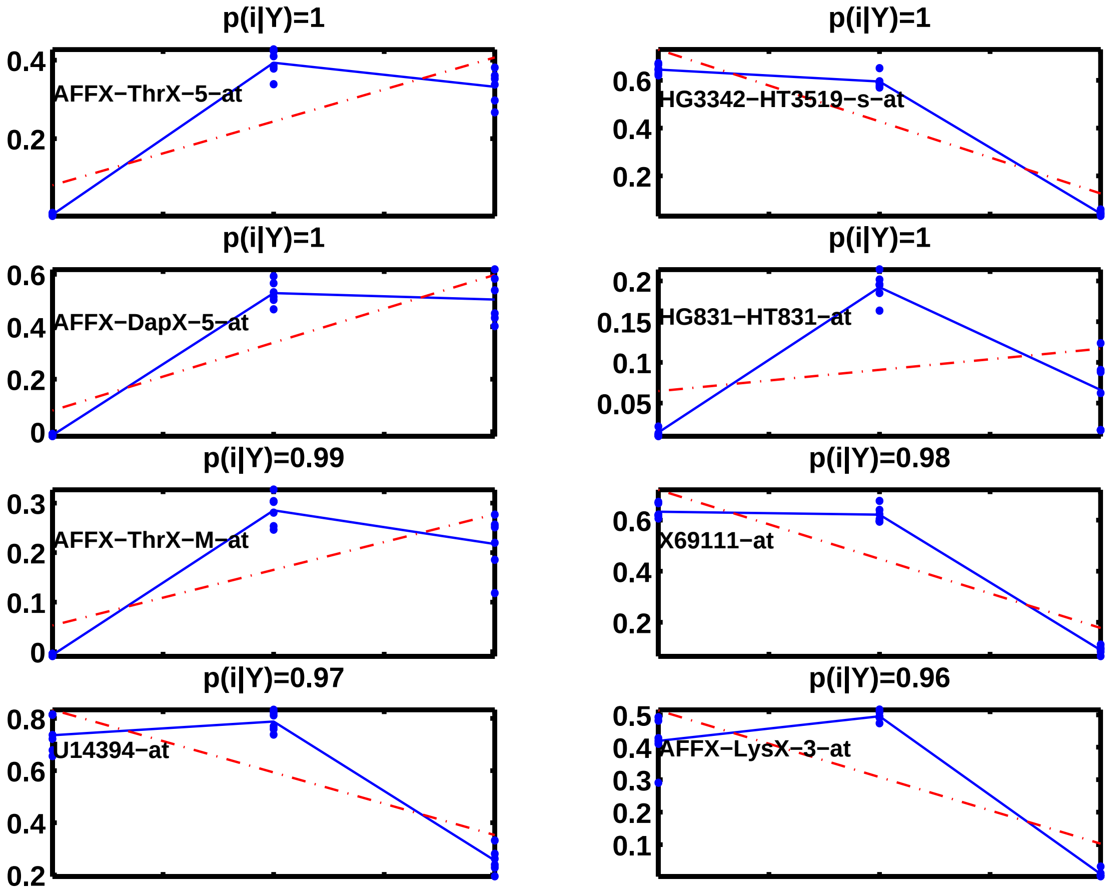


Figure 40: *The 8 top posterior ranked gene profiles remaining on the first Pareto front.*

Non-parametric Pareto filter criterion: Virtual Profiles

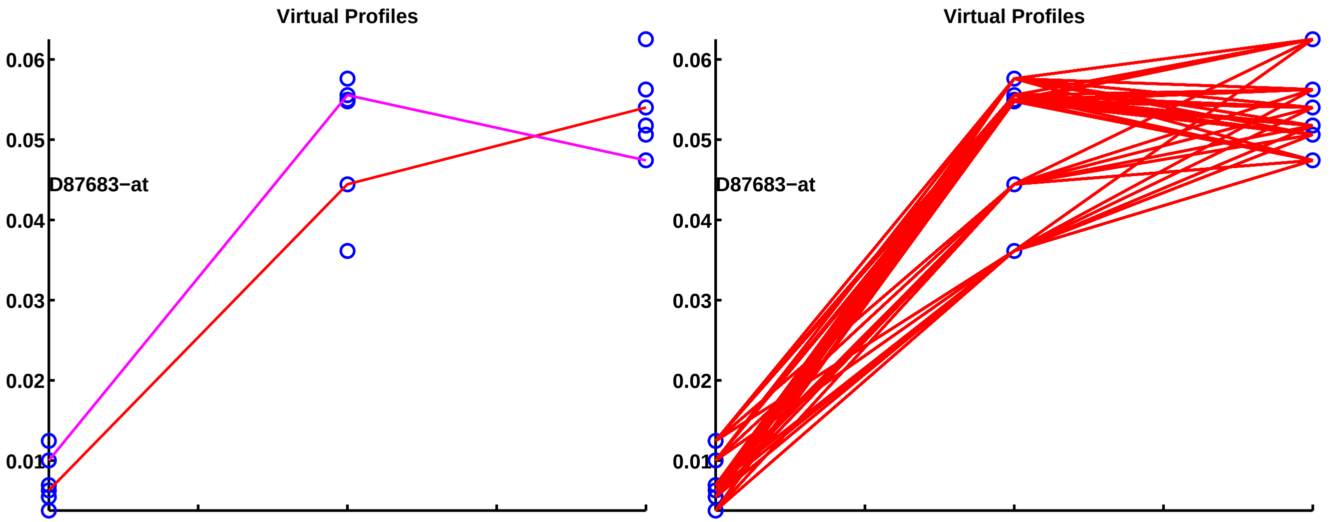


Figure 41: *Left: two virtual profiles in the data set. Right: the set of all* $3^6 = 729$ *virtual profiles for a gene in Fred Wright's dataset.*

Pareto Filtering using Virtual Sign-Profiles

Define *trend vector*: $\psi(n) = [b_1, \ldots, b_{T-1}]$, $b_i \in \{0,1\}$

- Old dominant filtering criteria:
  - Maximum end-to-end increase

$$\xi_1(Y(n)) = \bar{y}_{T*}(n) - \bar{y}_{1*}(n) = \max$$

  - Maximum number of monotone increasing T^M virtual time profiles

$$\xi_2(Y(n)) = \frac{\#\text{ virtual profiles having } \psi(n) = [1,\ldots,1]}{T^M}$$

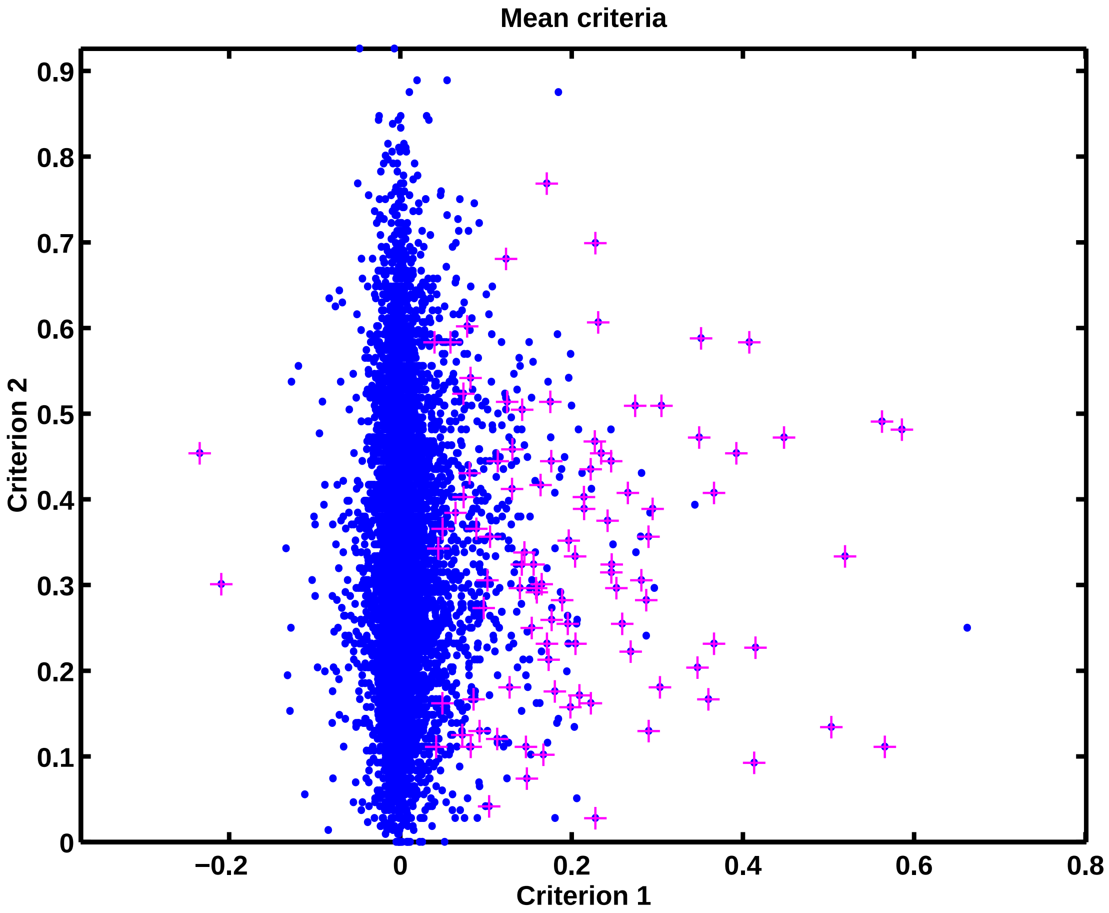


Figure 42: *Multicriterion mean scattergram for the virtual profile ranking and mean ene-to-end increase criteria.*

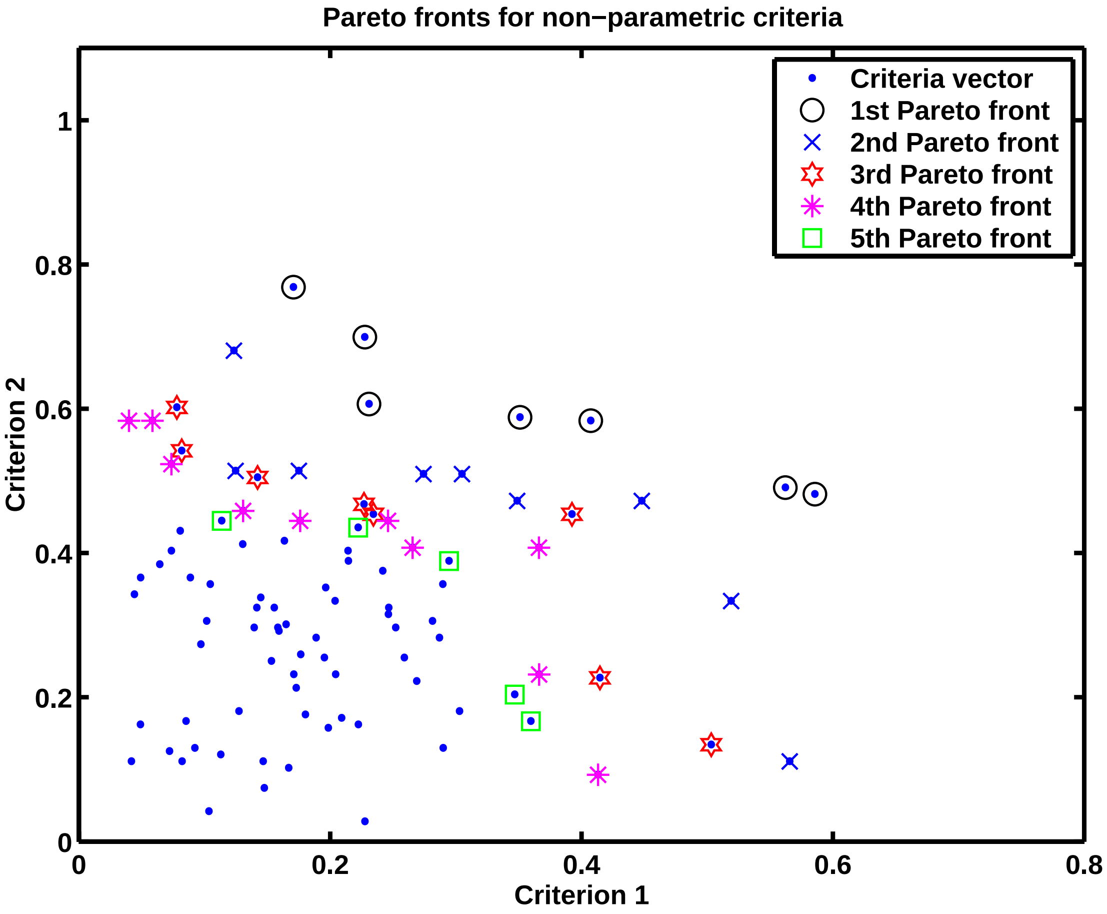


Figure 43: *The first five Pareto fronts.*

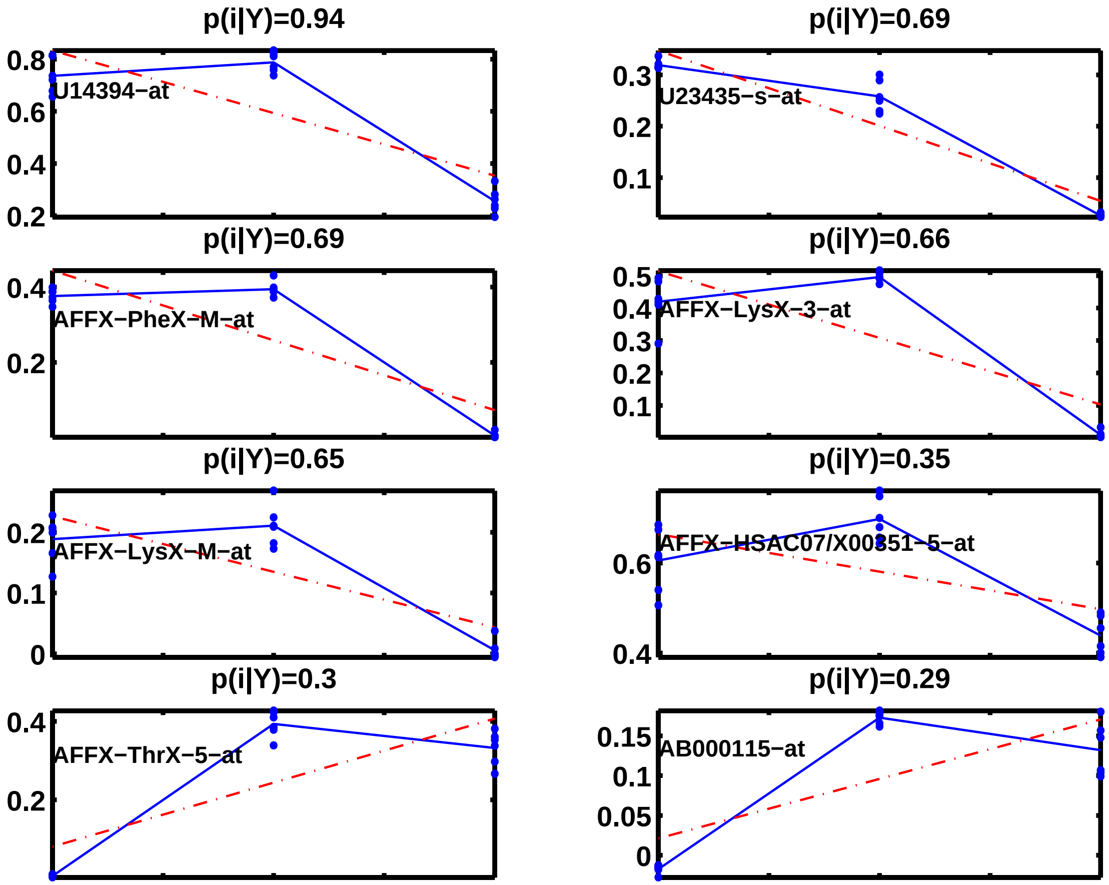
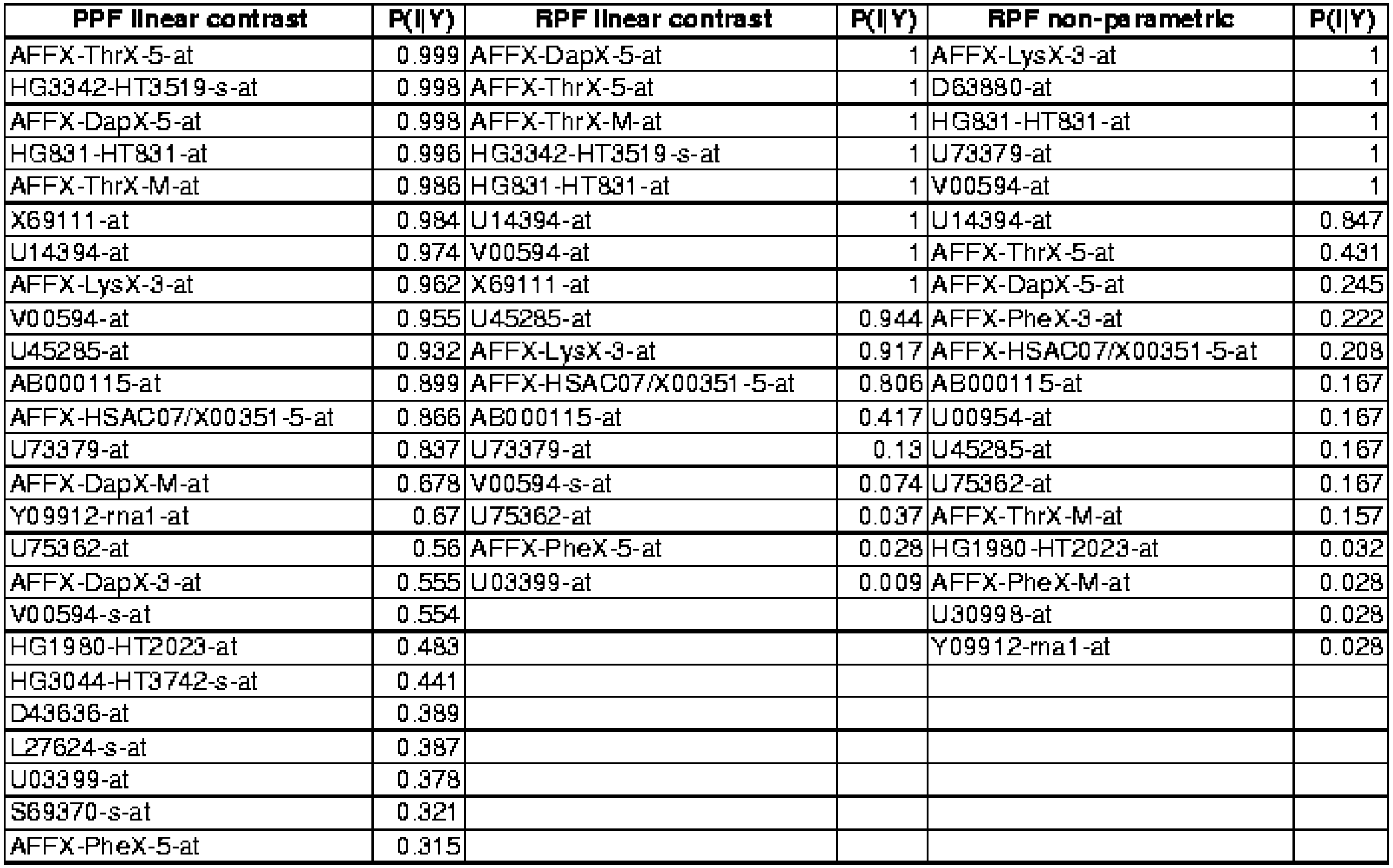


Figure 44: *The 8 top cross-validation ranked gene profiles.*



PPF linear contrast	P(I|Y)	RPF linear contrast	P(I|Y)	RPF non-parametric	P(I|Y)
AFFX-ThrX-5-at	0.999	AFFX-DapX-5-at	1	AFFX-LysX-3-at	1
HG3342-HT3519-s-at	0.998	AFFX-ThrX-5-at	1	D63880-at	1
AFFX-DapX-5-at	0.998	AFFX-ThrX-M-at	1	HG831-HT831-at	1
HG831-HT831-at	0.996	HG3342-HT3519-s-at	1	U73379-at	1
AFFX-ThrX-M-at	0.986	HG831-HT831-at	1	V00594-at	1
X69111-at	0.984	U14394-at	1	U14394-at	0.847
U14394-at	0.974	V00594-at	1	AFFX-ThrX-5-at	0.431
AFFX-LysX-3-at	0.962	X69111-at	1	AFFX-DapX-5-at	0.245
V00594-at	0.955	U45285-at	0.944	AFFX-PheX-3-at	0.222
U45285-at	0.932	AFFX-LysX-3-at	0.917	AFFX-HSAC07/X00351-5-at	0.208
AB000115-at	0.899	AFFX-HSAC07/X00351-5-at	0.806	AB000115-at	0.167
AFFX-HSAC07/X00351-5-at	0.866	AB000115-at	0.417	U00954-at	0.167
U73379-at	0.837	U73379-at	0.13	U45285-at	0.167
AFFX-DapX-M-at	0.678	V00594-s-at	0.074	U75362-at	0.167
Y09912-rna1-at	0.67	U75362-at	0.037	AFFX-ThrX-M-at	0.157
U75362-at	0.56	AFFX-PheX-5-at	0.028	HG1980-HT2023-at	0.032
AFFX-DapX-3-at	0.555	U03399-at	0.009	AFFX-PheX-M-at	0.028
V00594-s-at	0.554			U30998-at	0.028
HG1980-HT2023-at	0.483			Y09912-rna1-at	0.028
HG3044-HT3742-s-at	0.441				
D43636-at	0.389				
L27624-s-at	0.387				
U03399-at	0.378				
S69370-s-at	0.321				
AFFX-PheX-5-at	0.315				

Figure 45: *The top scoring genes (Affymetrix nomenclature).*

Mouse Retina Aging Study (2001M)

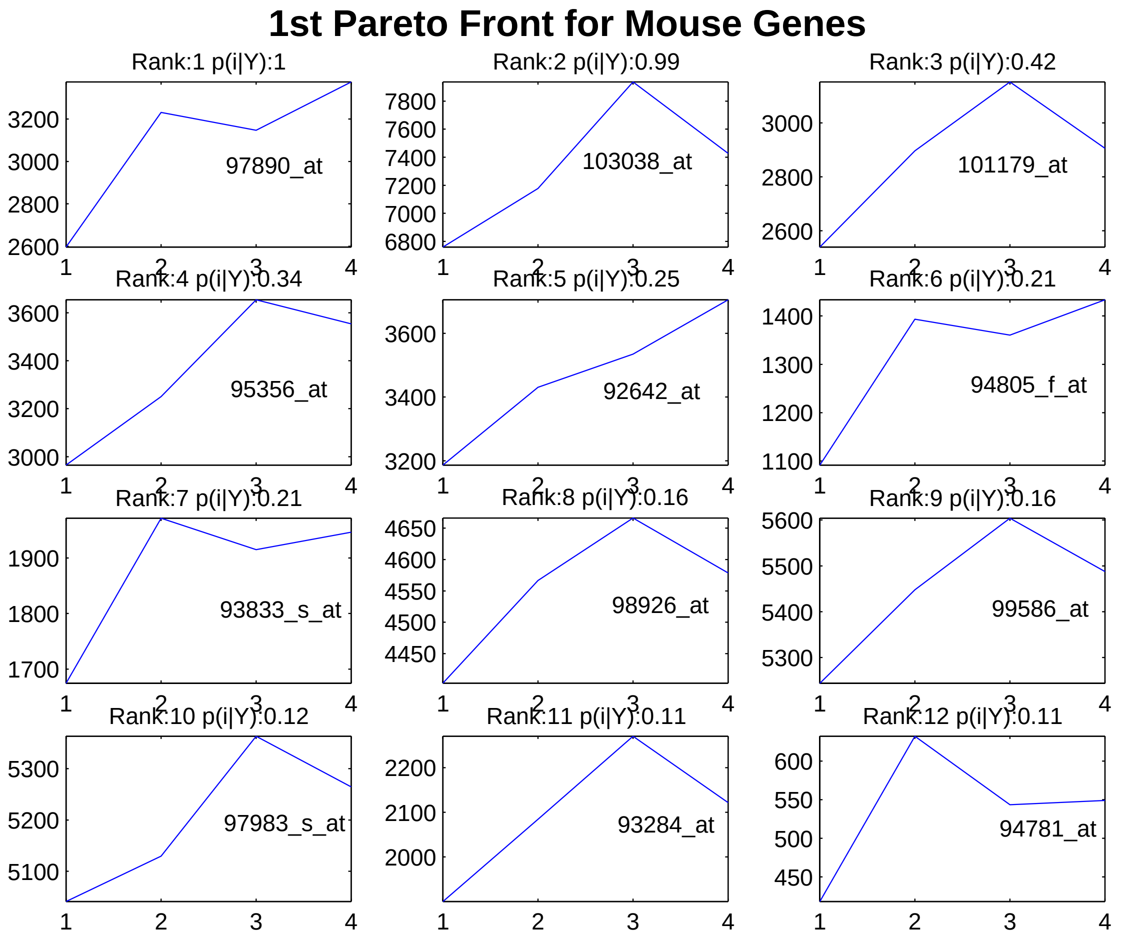


Figure 46: *Ranked first posterior Pareto front gene trajectories (Affy mouse study).*

Three-objective Pareto Filtering

Objective Extract “aging genes” in (2001M) study

- Strictly increasing filtering criteria:
  - Maximum end-to-end increase

$$\xi_1(Y(n)) = \bar{y}_{T*}(n) - \bar{y}_{1*}(n) = \max$$

  - Maximum number of monotone increasing T^M virtual time profiles

$$\xi_2(Y(n)) = \frac{\#\text{ virtual profiles having } \psi(n) = [1,\ldots,1]}{T^M}$$

  - no plateau

$$\xi_3(Y(n)) = [\bar{y}_{t+1,*}(n) - 2\bar{y}_{t*}(n) + \bar{y}_{t-1,*}(n)]^2 = \min$$

Pairwise Pareto Fronts

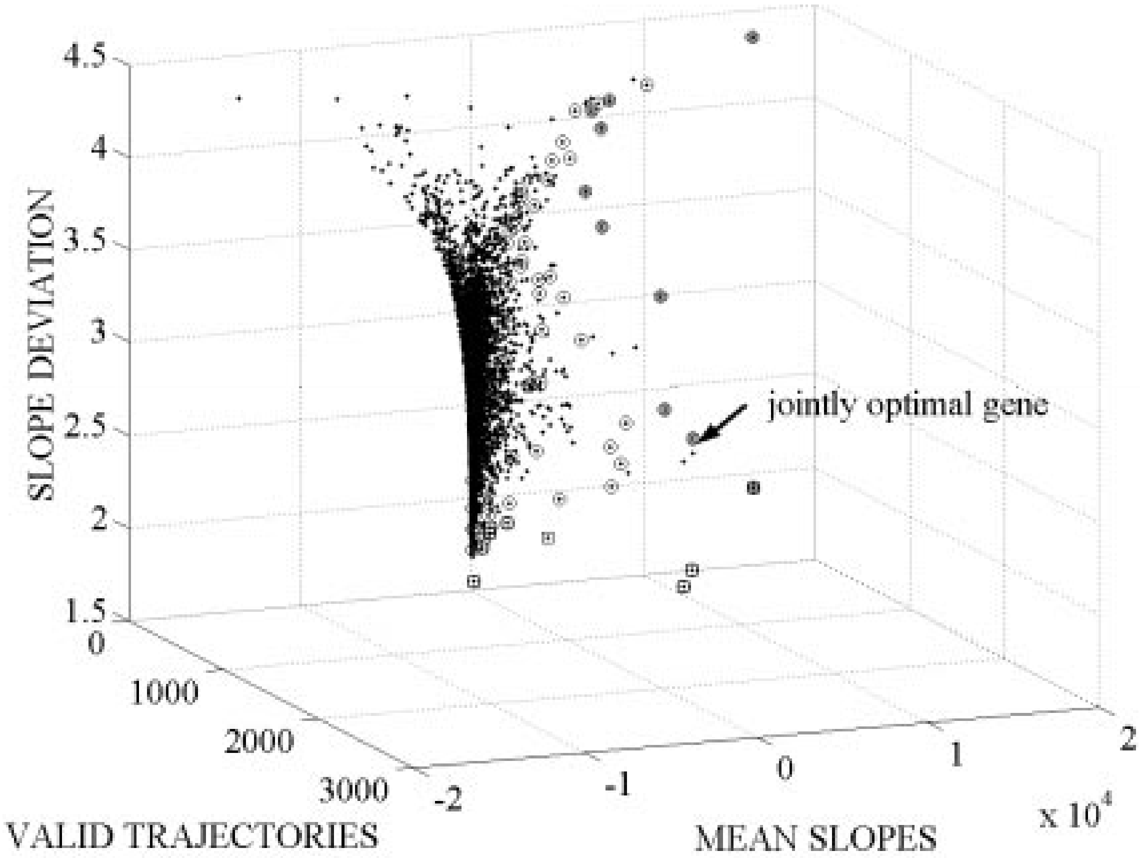


Figure 47: *First Pareto fronts for each pair of criteria taken from the set (ξ_1, ξ_2 and ξ_3).*

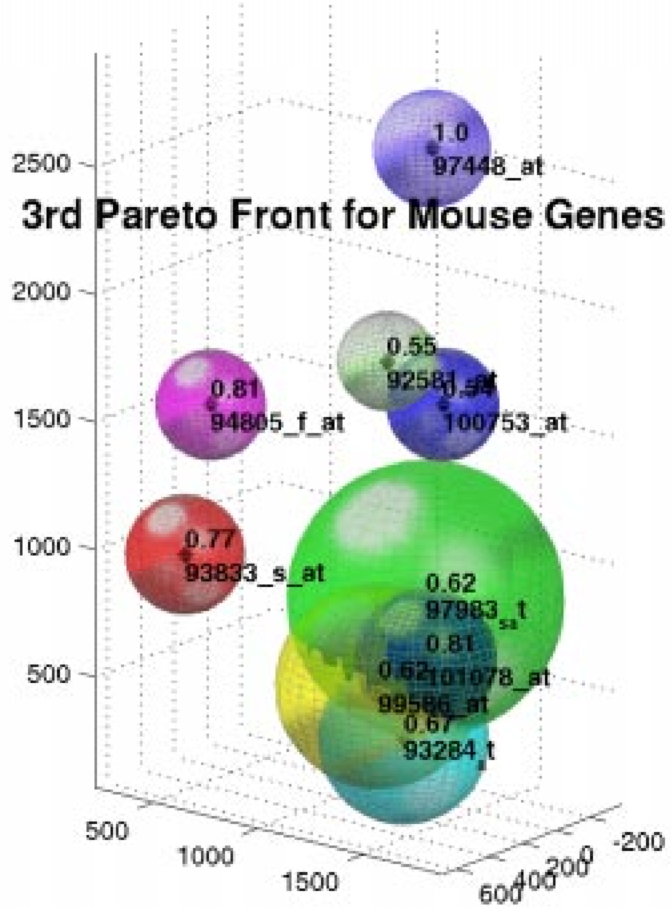


Figure 48: *Third posterior Pareto front for (affy mouse study).*

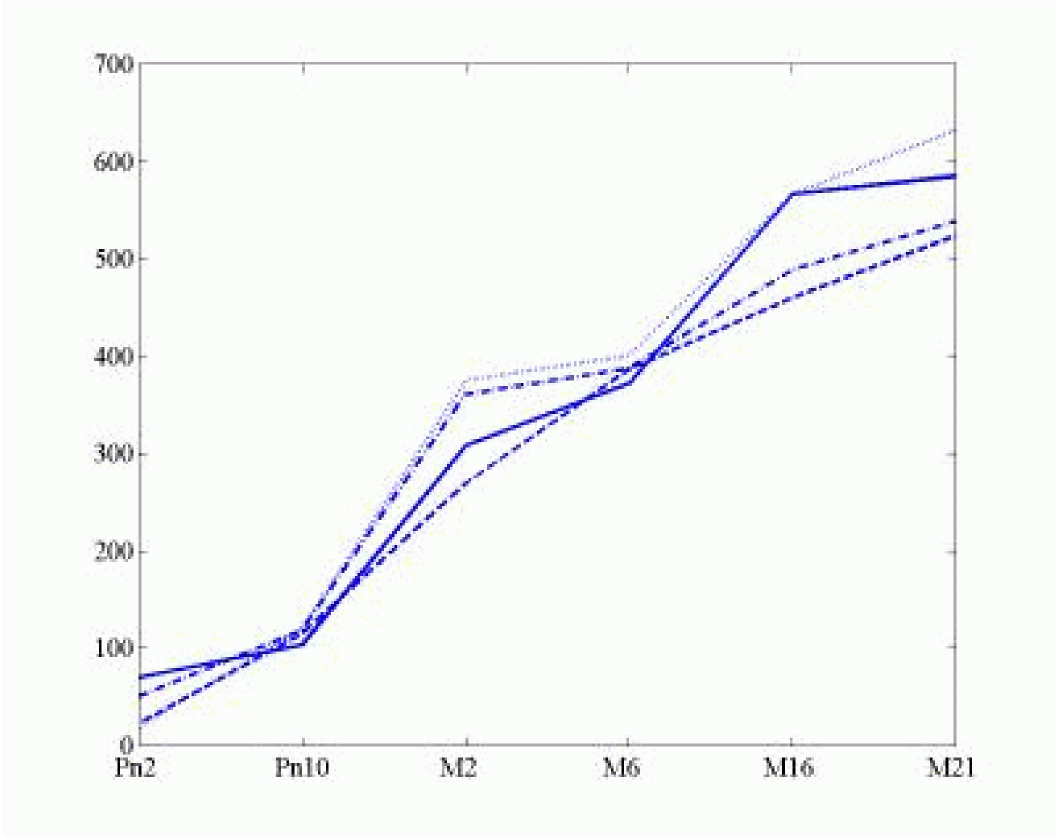


Figure 49: *Top ranked gene profile is Mus musculus* $5'$ *end cDNA (Unigene 86632)*

Conclusions

1. Signal processing has a role to play in many aspects of genomics
2. Careful physical modeling of image formation process can yield performance gains
3. New methods of data mining are needed to perform robust and flexible gene filtering
4. Cross-validation or posterior analysis can account for statistical sampling uncertainty
5. Joint intensity extraction and gene filtering is desirable