Bioinformatics and Genomics: A New SP Frontier?

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Outline

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

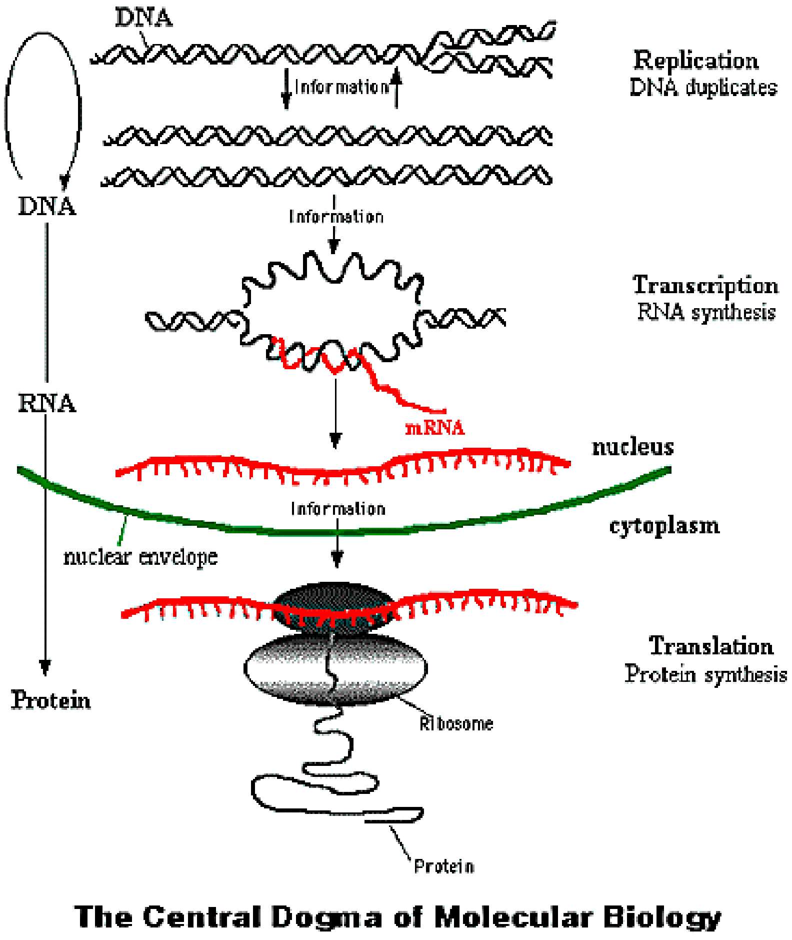


Figure 1: `http://www.accessexcellence.org`

I. Bioinformatics background

- Every human cell contains 6 feet of double stranded (ds) DNA
- This DNA has 3,000,000,000 basepairs representing 50,000-100,000 genes
- This DNA contains our complete genetic code or *genome*
- DNA regulates all cell functions including response to disease, aging and development
- Gene expression pattern: snapshot of DNA in a cell
- Gene expression profile: DNA mutation or polymorphism over time
- Genetic pathways: changes in genetic code accompanying metabolic and functional changes, e.g. disease or aging.

Genomics: study of gene expression patterns in a cell or organism

Possible Impact

- Understanding role of genetics in cell function and metabolism
- Discovering genetic markers and pathways for different diseases
- Understanding pathogen mechanisms and toxicology studies
- Development of genotype-specific drugs
- Development of genetic computing machines
- In situ genetic monitoring and drug delivery

Kellog Sensory Gene Microarray Node: Objectives

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

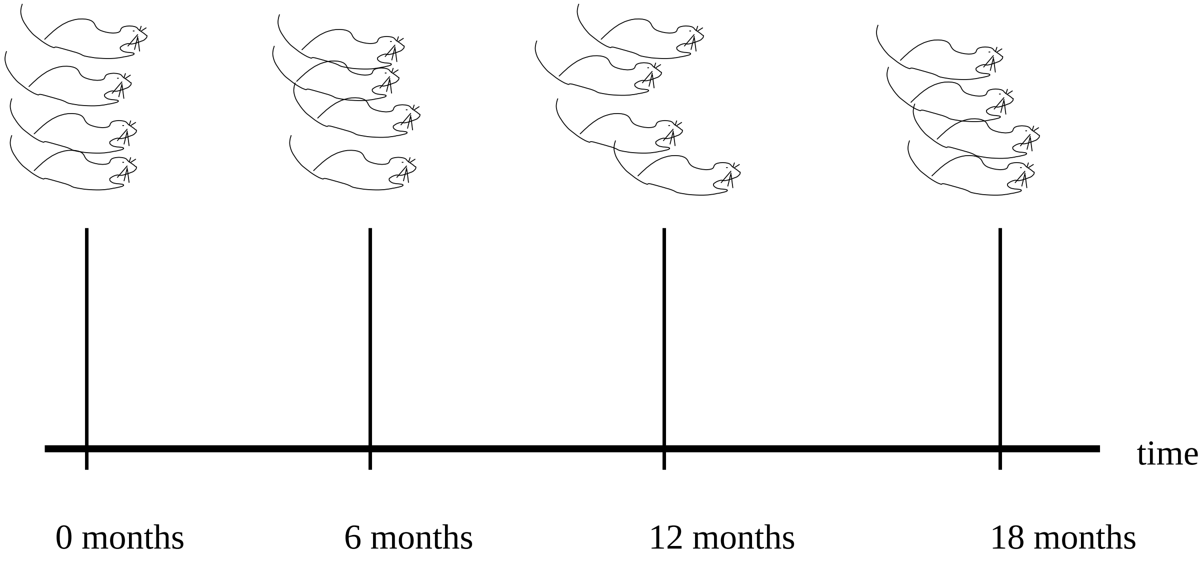
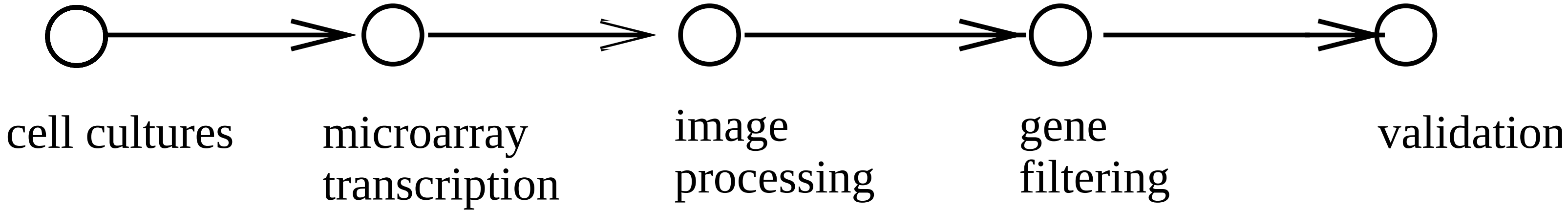


Figure 2: *Sample gene trajectories over time.*

II. Gene Microarrays

Two kinds of “Shotgun sequencing:”

1. GeneChip Oligonucleotide Microarrays (Affymetrix)
2. cDNA Microarrays (Stanford)



cell cultures

microarray transcription

image processing

gene filtering

validation

Figure 3: *Microarray experiment cycle.*

Microarray Image Formation

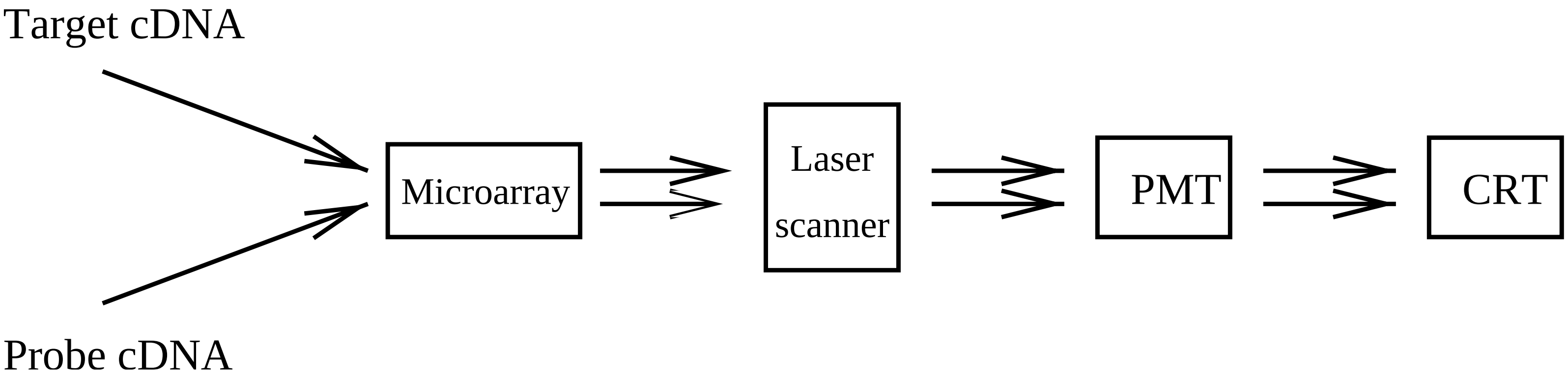


Figure 4: *Image formation process.*

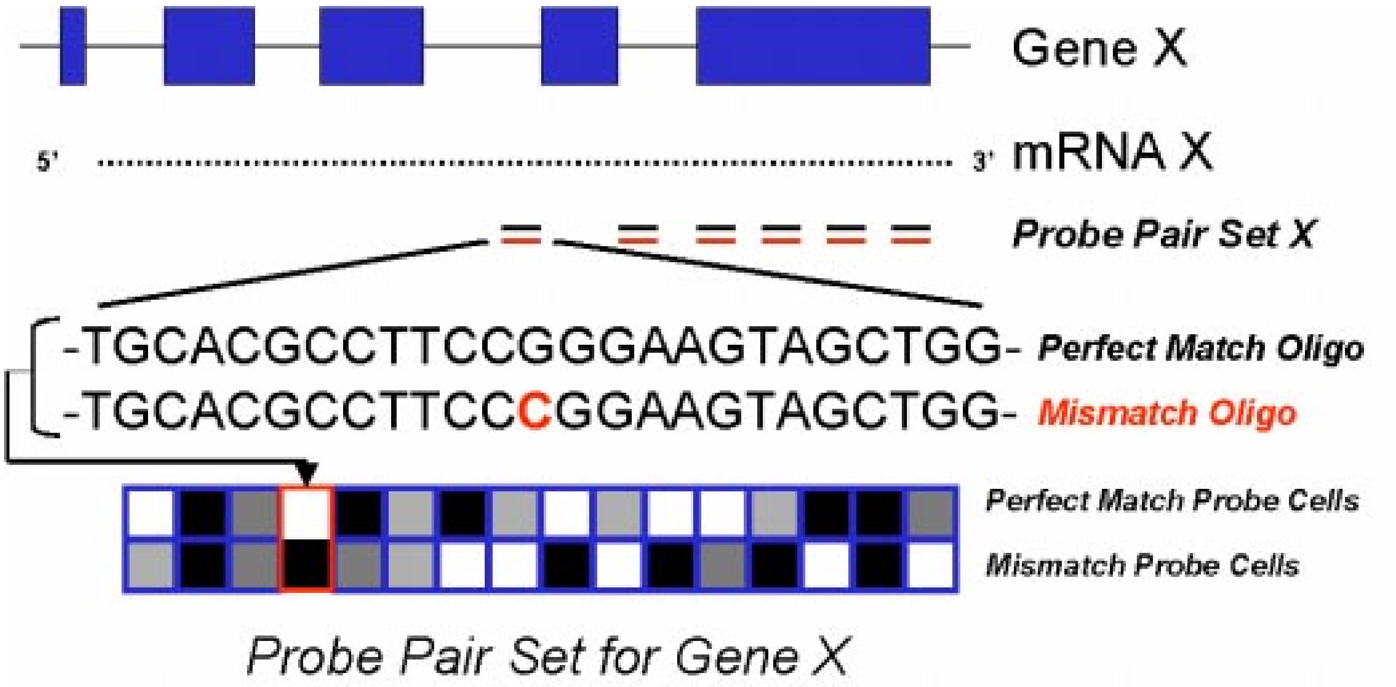

Gene X
5'
3'
mRNA X
Probe Pair Set X
-TGCACGCCTTCCGGGAAGTAGCTGG-
Perfect Match Oligo
-TGCACGCCTTCCCGGAAGTAGCTGG-
Mismatch Oligo
Perfect Match Probe Cells
Mismatch Probe Cells
Probe Pair Set for Gene X

Figure 5: *Oligonucleotide PM/MM layout (*`pathbox.wustl.edu`*).*

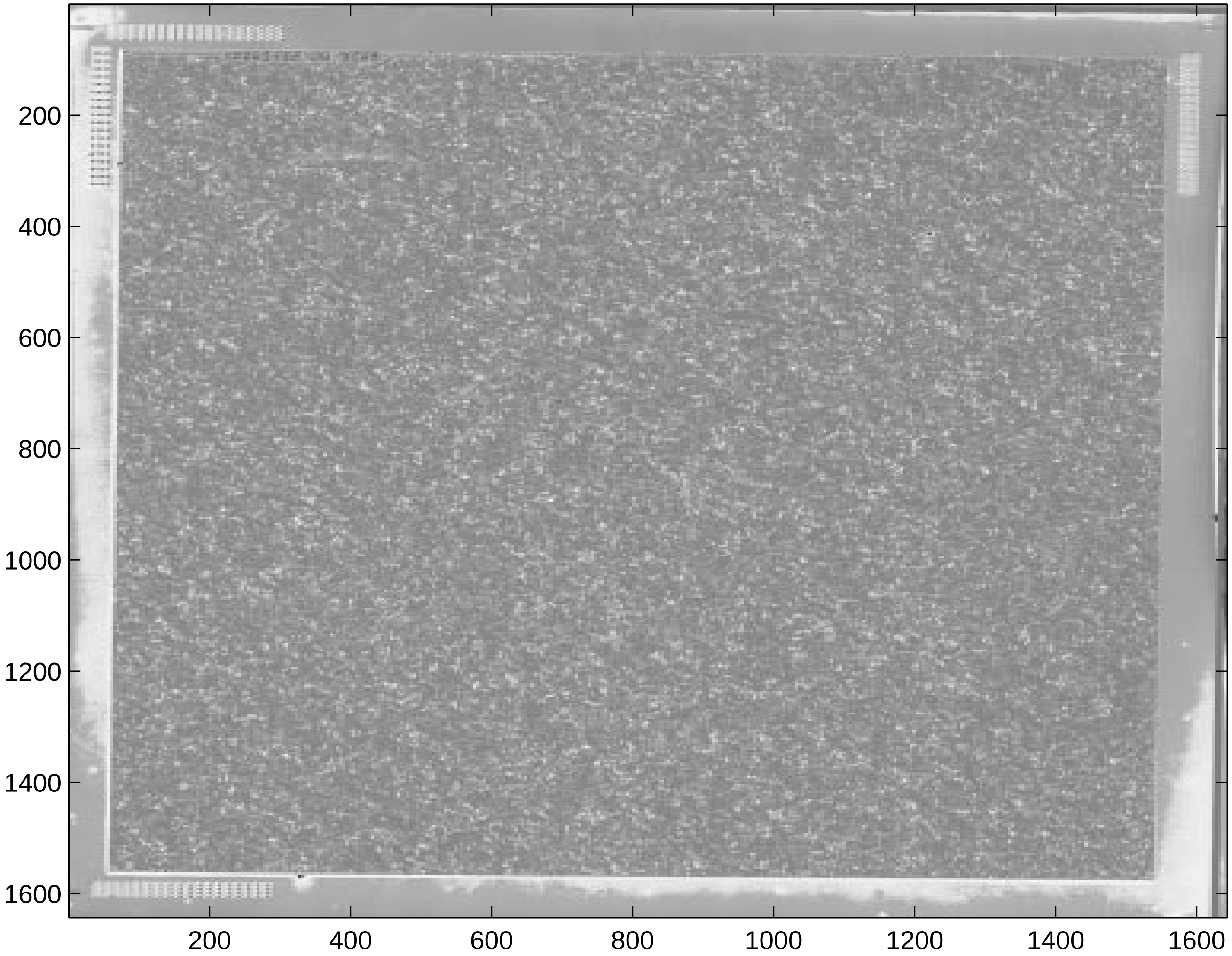


Figure 6: *Affymetrix GeneChip microarray.*

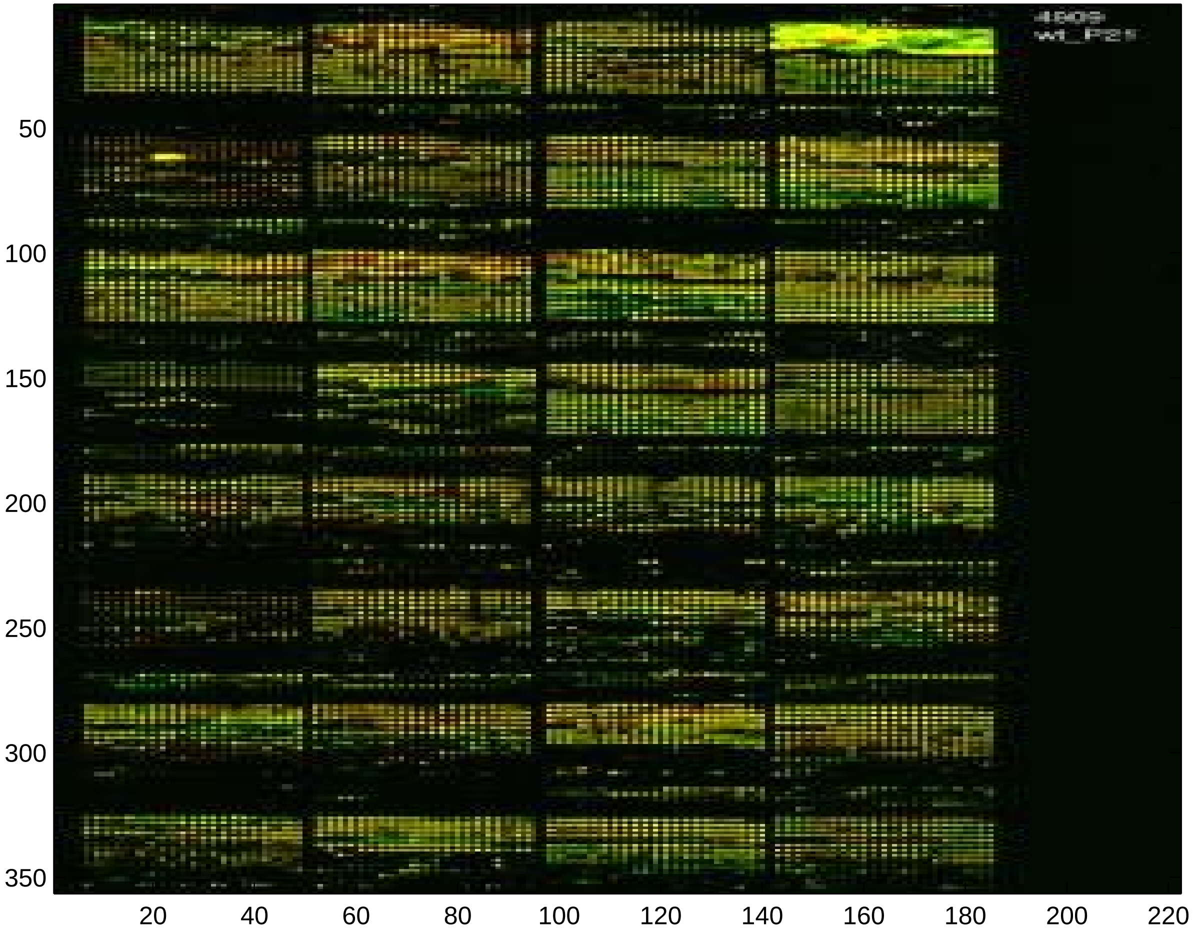


Figure 7: *cDNA spotted array.*

Control Factors Influencing Variability

- **Sample preparation**: reagent quality, temperature variations
- **Slide manufacture**: slide surface quality, dust deposition
- **Hybridization**: sample concentration, wash conditions
- **Cross hybridization**: similar but different genes bind to same probe
- **Image formation**: scanner saturation, lens aberations, gain settings
- **Imaging and Extraction**: spot misalignment, discretization, clutter

→ account for data variability

- **Scaling factors**: universal intensity amplification factor for a chip
- **Raw Q**: noise and other random variations of a chip
- **Background**: avg of lowest 2% cell intensity values
- **% P**: percentage of transcripts present

Microarray Signal Extraction

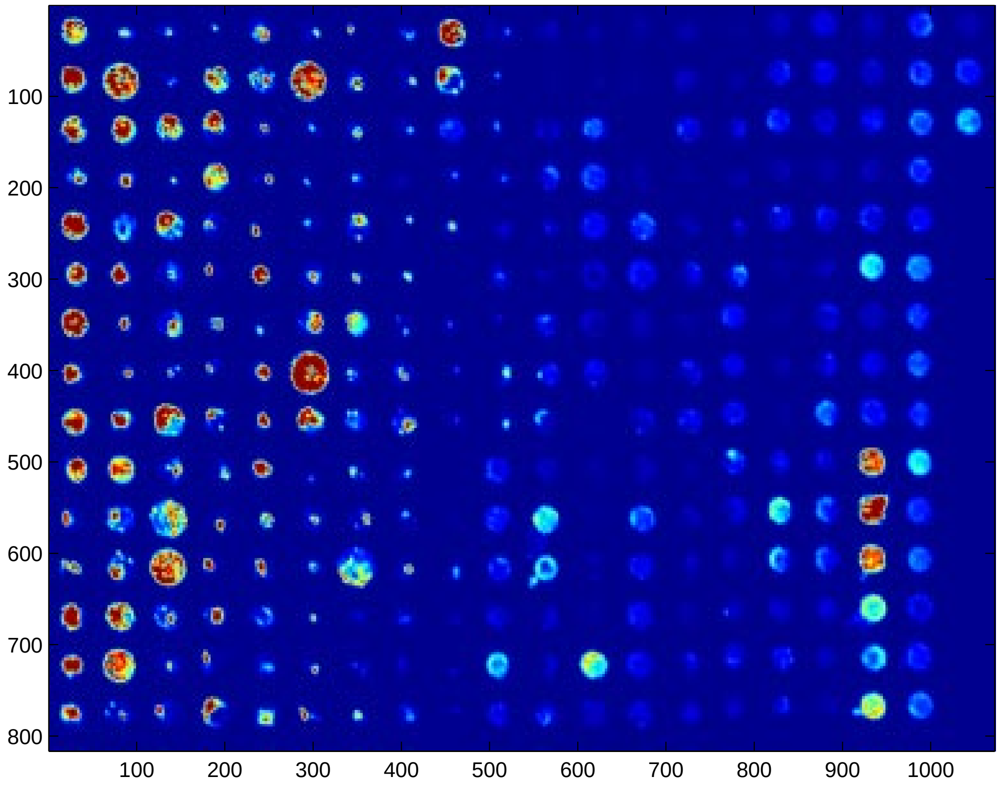


Figure 8: *Blowup of cDNA spotted array.*

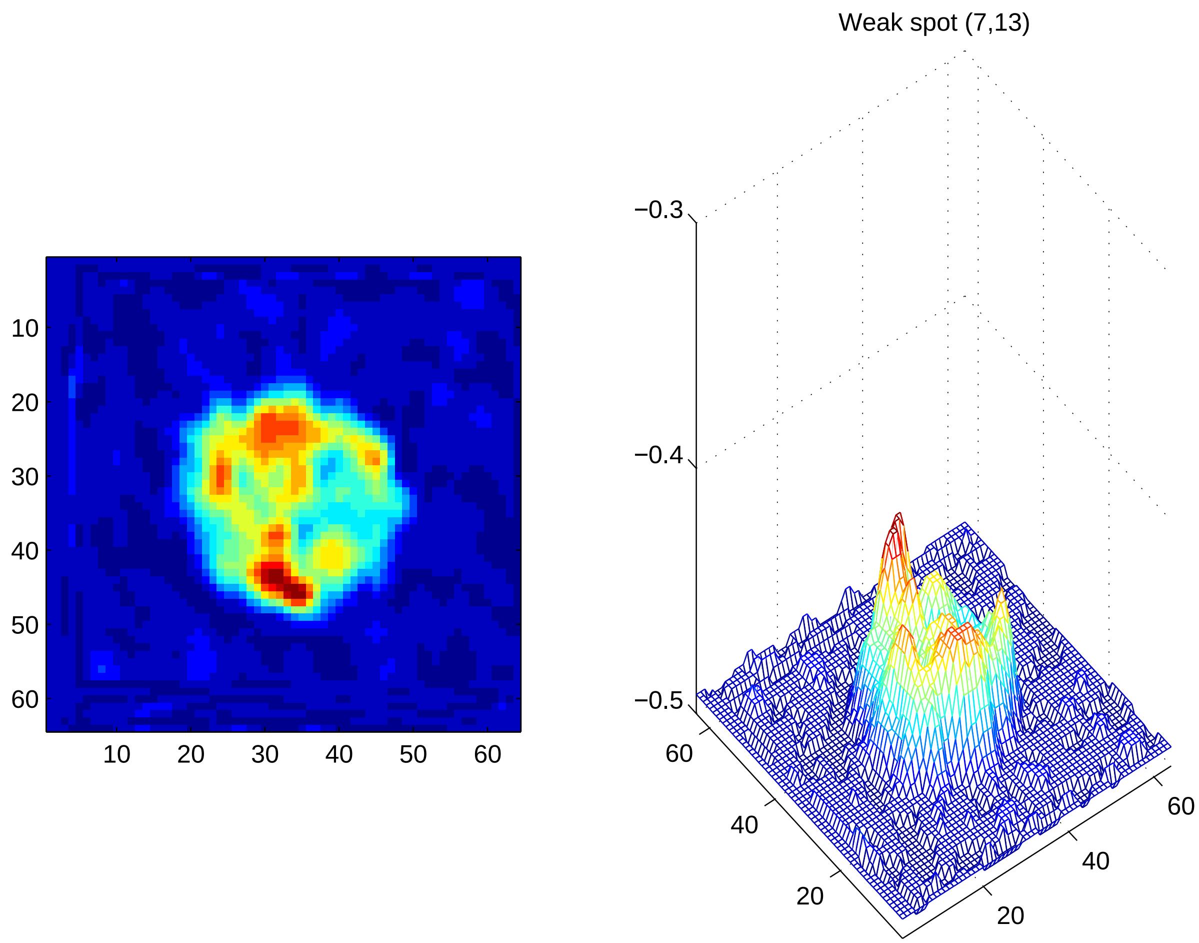


Figure 9: *Weak Spot.*

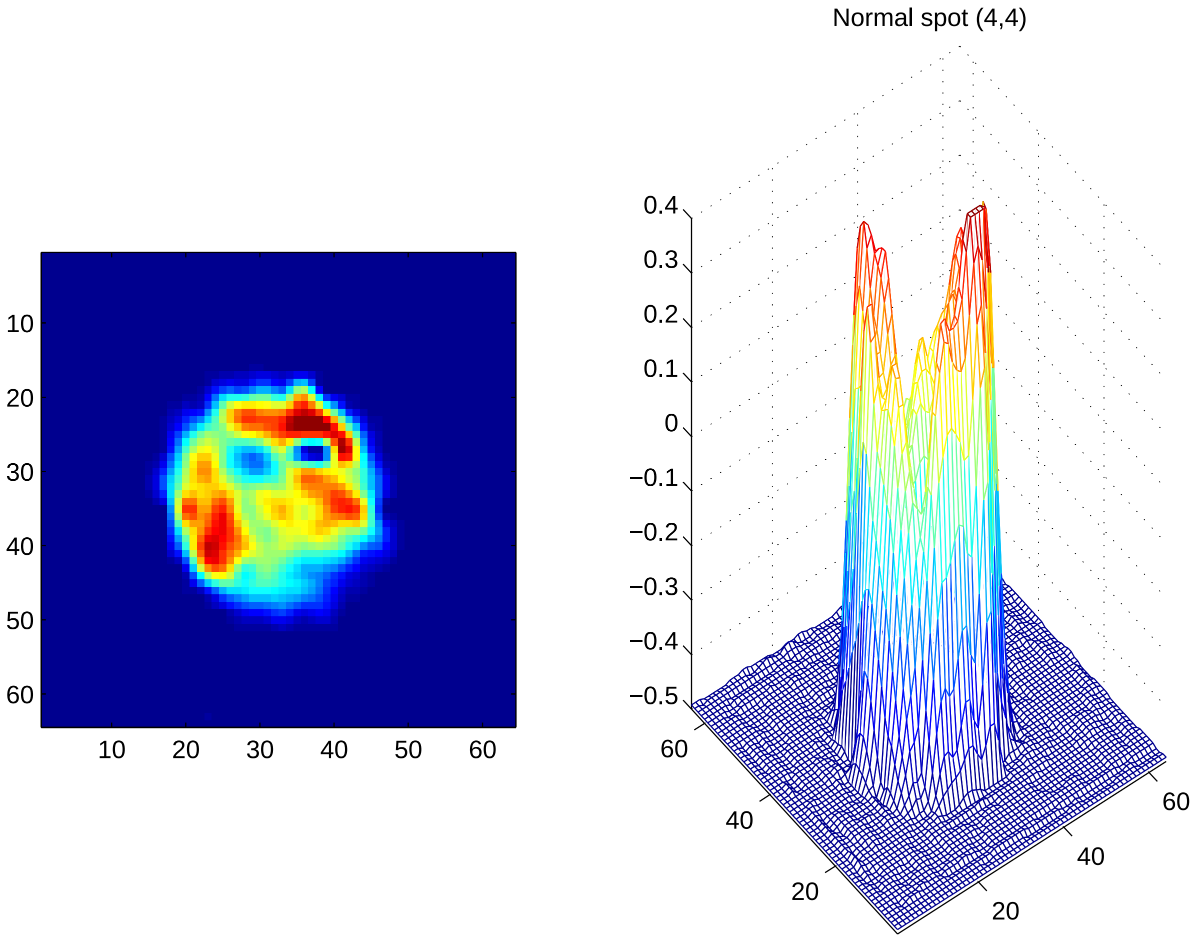


Figure 10: *Normal spot.*

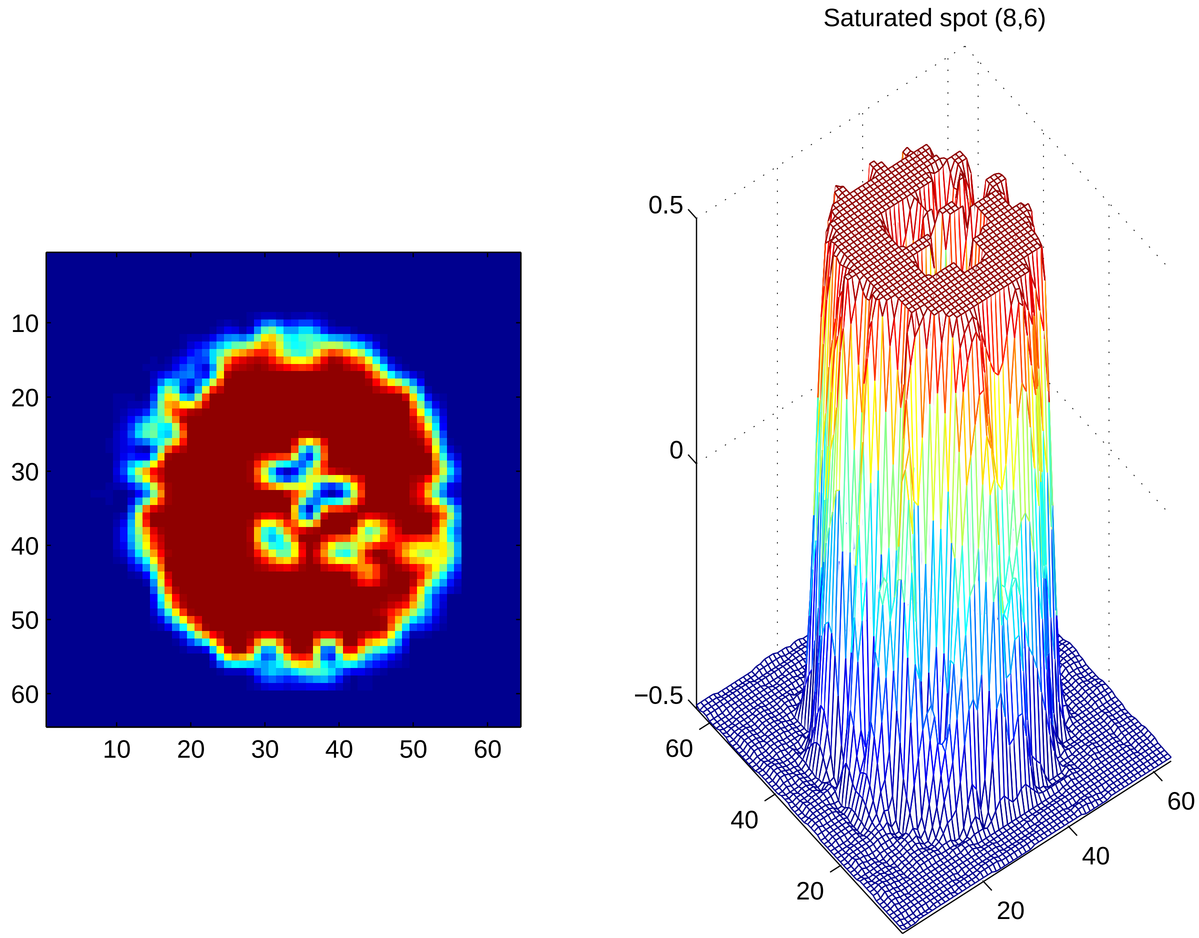


Figure 11: *Saturated spot.*

Morphological Spot Segmentation (Siddiqui&Hero:ICIP02)

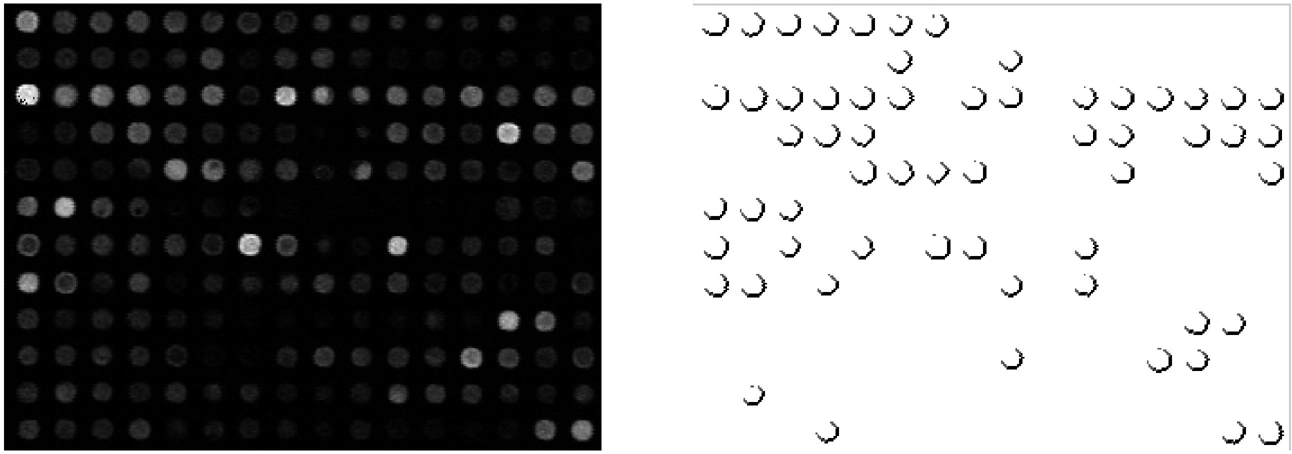


Figure 12: *(L) Original cDNA microarray image. (R) after alternating sequential filtering.*

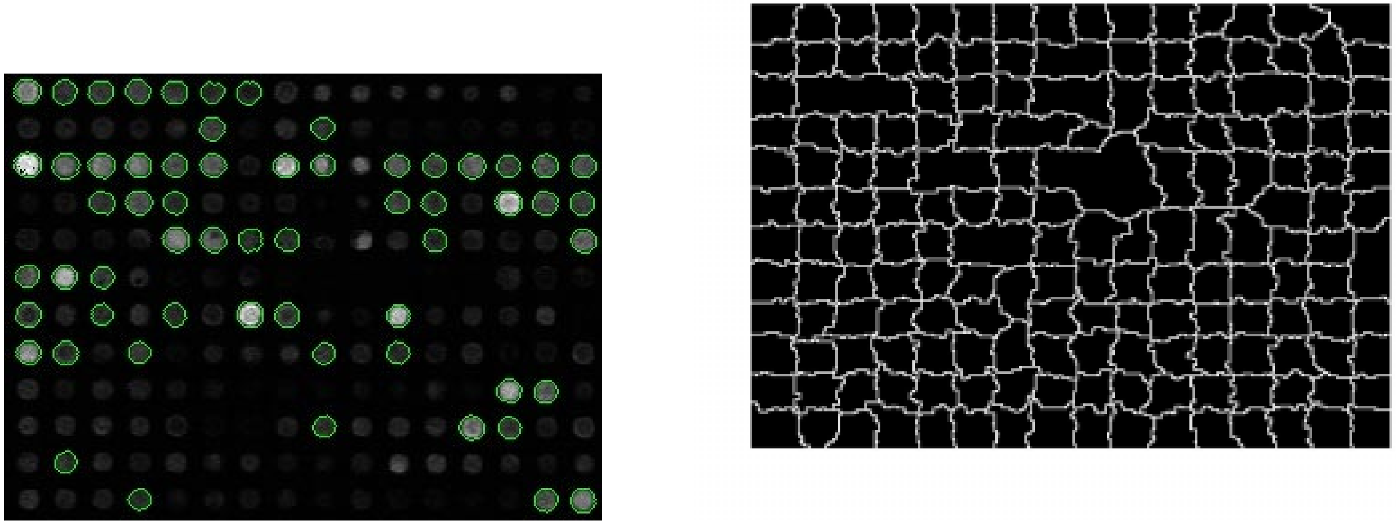


Figure 13: *(L) Final segmentation. (R) Spot watershed domains for noise averaging.*

Model-based Signal Extraction (Hero:Springer02)

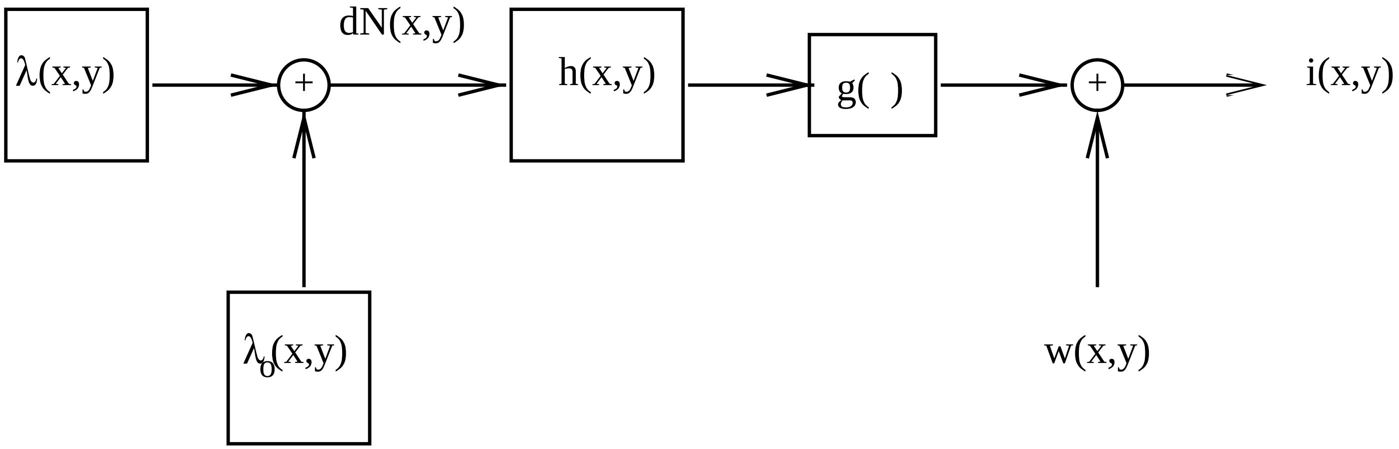


Figure 14: *Filtered Poisson model for microarray image.*

Gabor Superposition - Width MSE

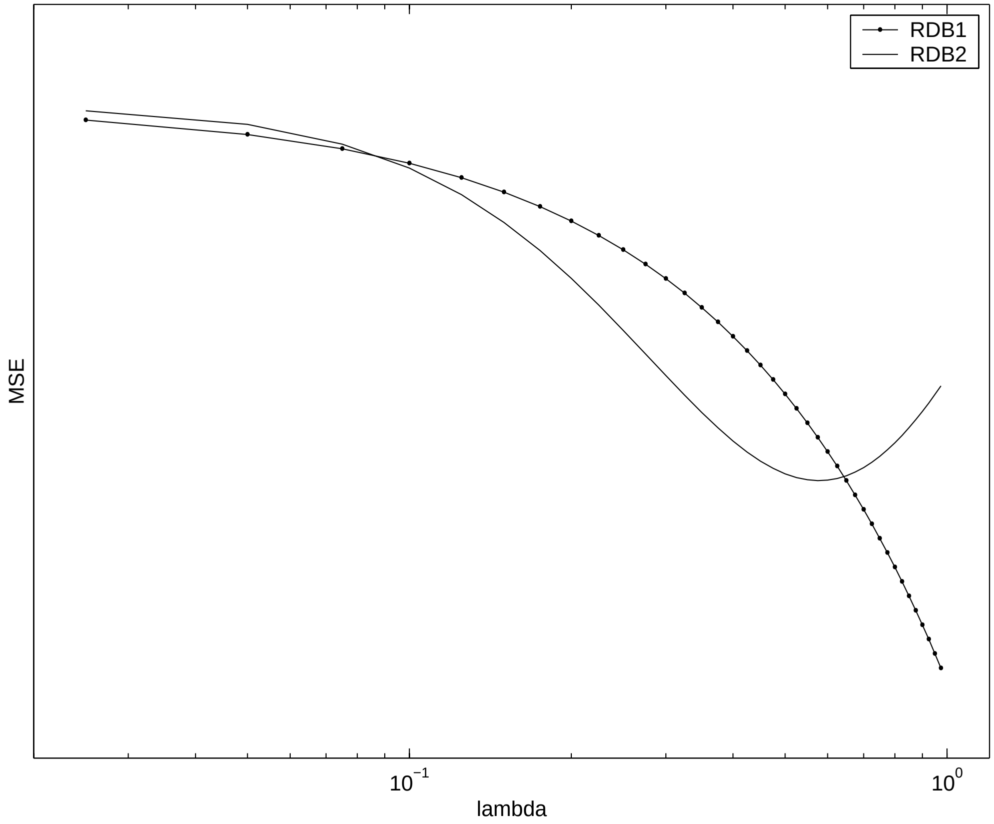


Figure 15: *Distortion-rate MSE lower bounds on Gabor widths of* $\Phi_j(x,y)$.

Gene Clustering and Filtering (Fleury&etal:ICASSP02)

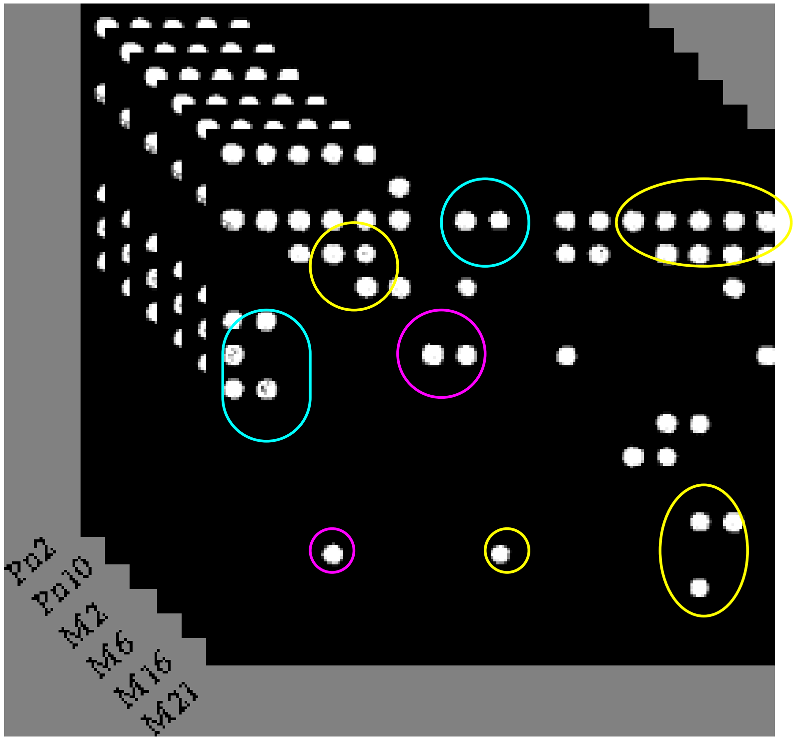


Figure 16: *Clustering on the Data Cube.*

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

Gene Trajectory Classification

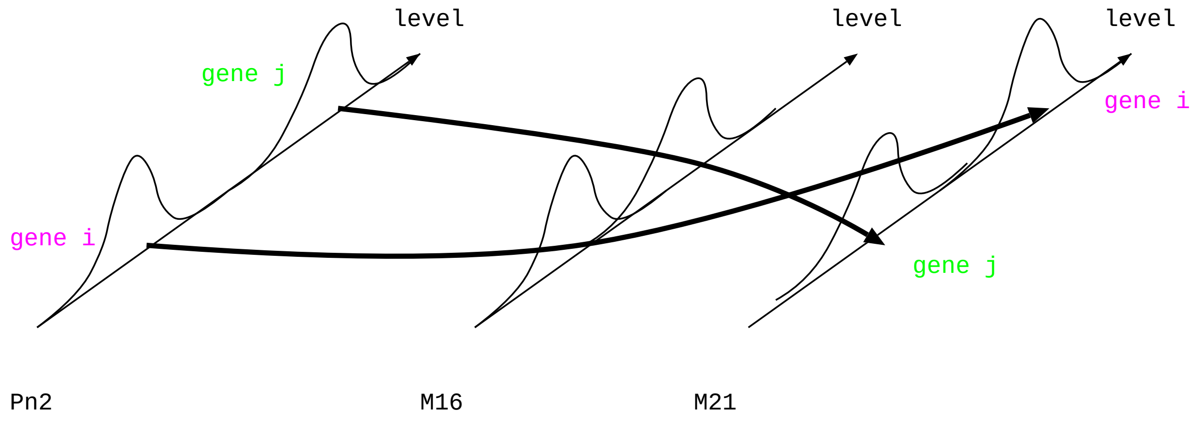


Figure 17: *Gene i is old dominant while gene j is young dominant*

Objective: classify gene trajectories from sequence of microarray experiments over time (t) and population (m)

$$\theta_i(m,t), \quad m = 1, \ldots, M, \; t = 1, \ldots, T$$

Clustering and filtering Methods

Principal approaches:

- Hierarchical clustering (kdb trees, CART, gene shaving)
- K-means clustering
- Self organizing (Kohonen) maps
- Vector support machines

Validation approaches:

- Significance analysis of microarrays (SAM)
- Bootstrapping cluster analysis
- Leave-one-out cross-validation
- Replication (additional gene chip experiments, quantitative PCR)

Gene Filtering via Multiobjective Optimization

Gene selection criteria for *i*-th gene $\xi_1(\theta_i), \;\; ,\ldots, \;\; \xi_P(\theta_i)$

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

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

$$\xi_1(\theta_i) = |\overline{\theta}_i(*,1) - \overline{\theta}_i(*,T)|^2$$

- Standard deviation at $t = 1$:

$$\xi_2(\theta_i) = \overline{\left(\theta_i(*,1) - \overline{\theta}_i(*,1)\right)^2}$$

- Standard deviation at $t = T$:

$$\xi_3(\theta_i) = \overline{\left(\theta_i(*,T) - \overline{\theta}_i(*,T)\right)^2}$$

Some possible scalar functions:

- t-test statistic (Goss etal 2000): $T_i = \frac{\xi_1(\theta_i)}{\frac{1}{2}\xi_2(\theta_i)+\frac{1}{2}\xi_3(\theta_i)}$
- R^2 statistic (Hastie etal 2000): $R_i^2 = \frac{T_i}{1+T_i}$
- H statistic (Sinha etal 1998): $H_i = \frac{\xi_1(\theta_i)}{\sqrt{\xi_2(\theta_i)\xi_3(\theta_i)}}$

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:

$$\max_i \sum_{p=1}^{P} W_p \xi_p(\theta_i), \quad or \quad \max_i \prod_{p=1}^{P} (\xi_p(\theta_i))^{W_p}$$

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.

$$\xi_p(\theta_i) \leq \xi_p(\theta_j),\ p = 1, \ldots, P$$

Pareto Optimal Fronts

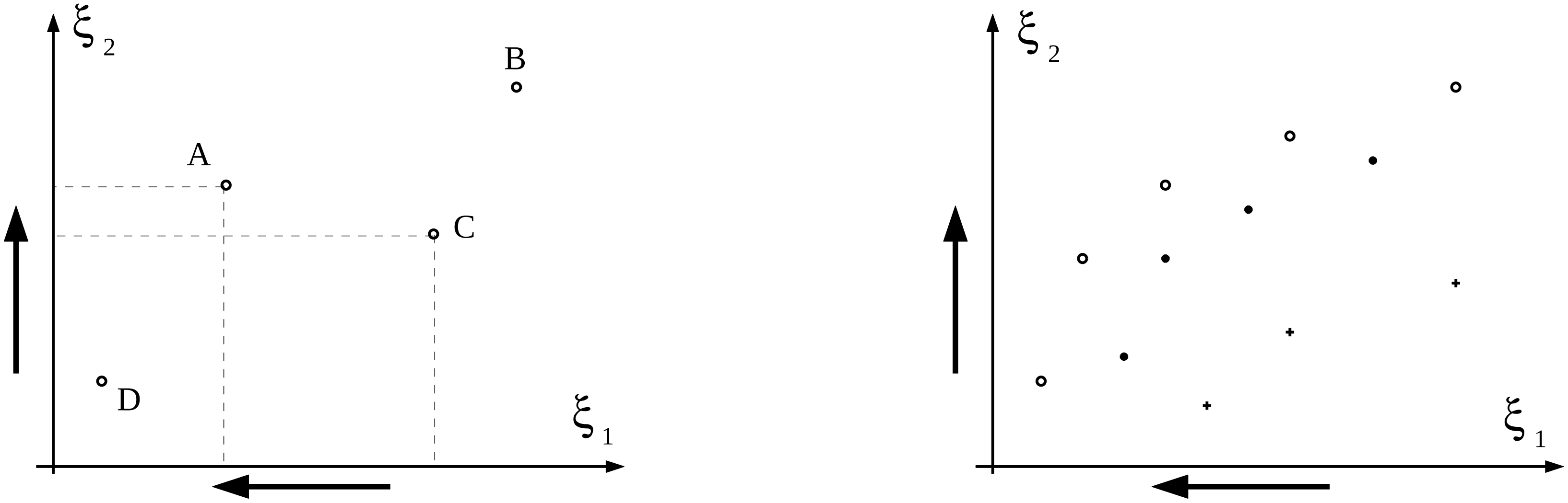


Figure 18: *a). Non-dominated property, and b). Pareto optimal fronts, in dual criteria plane.*

Pareto Gene Filtering vs. Paired T-test

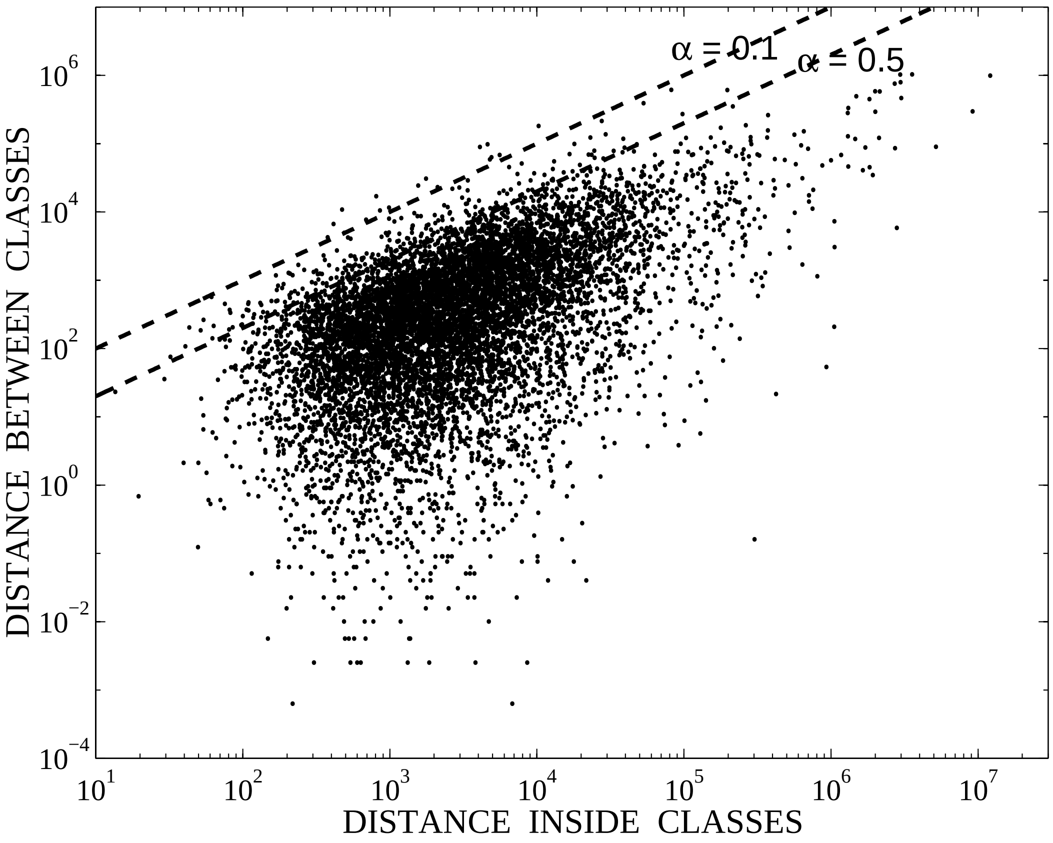


Figure 19: $\xi_1 =$ *mean change vs* $\xi_2 =$ *pooled standard deviation for 8826 mouse retina genes. Superimposed are T-test boundaries*

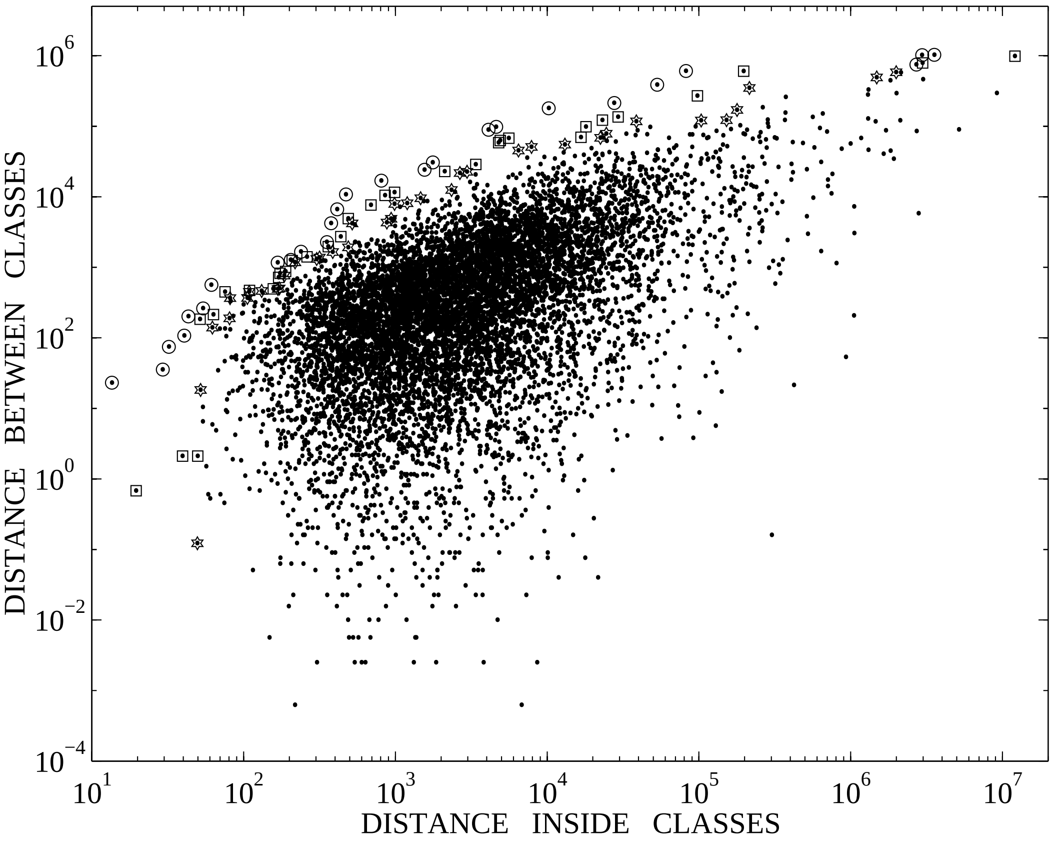


Figure 20: *First (circle) second (square) and third (hexagon) Pareto optimal fronts.*

Application: Development and Aging in Mouse Retina

Mouse Retina Experiment:

- Retinas of 24 mice sampled and hybridized
- 6 time points: Pn2, Pn10, M2, M6, M16, M21
- 4 mice per time sample
- Affymetrix GeneChip layout with 12422 poly-nucleotides
- Affymetrix attribute analyzed: “AvgDiff”
- Used Affymetrix filter to eliminate all genes labeled “A”

Objective: Find interesting gene trajectories within the set of remaining 8826 genes

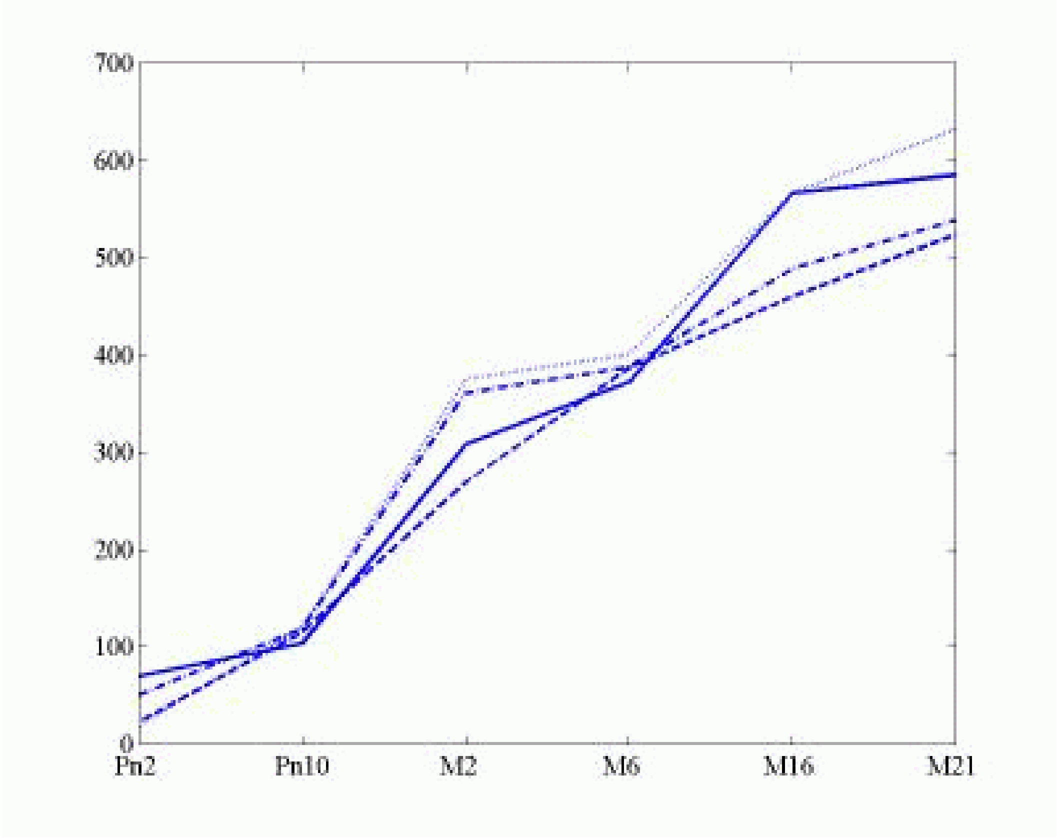


Figure 21: *4 candidate gene profiles from Mus musculus* $5'$ *end cDNA (Unigene 86632)*

Multi-objective Non-parametric Pareto Filtering

Define *trend vector*: $\psi_i = [b_1, \ldots, b_6]$, $b_i \in \{0, 1\}$

- Old dominant filtering criteria:
  - high mean slope from $t = Pn1$ to $t = M21$

$$\xi_1(\psi_i) = \overline{b_i(*,*)}$$

  - high consistency over $6^4 = 4096$ possible combinations of trajectories

$$\xi_2(\psi_i) = \frac{\text{\# trajectories having } \psi_i = [1, \ldots, 1]}{4096}$$

Occurence Histogram

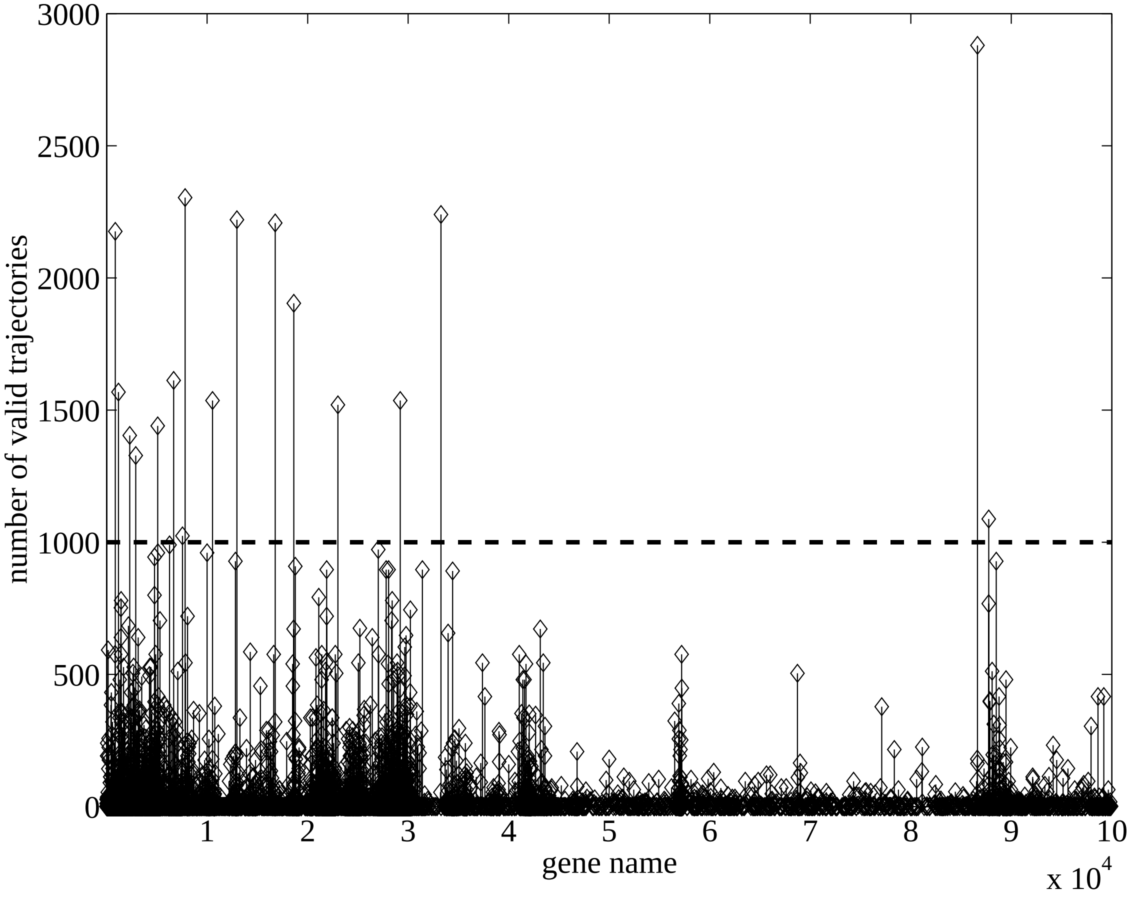
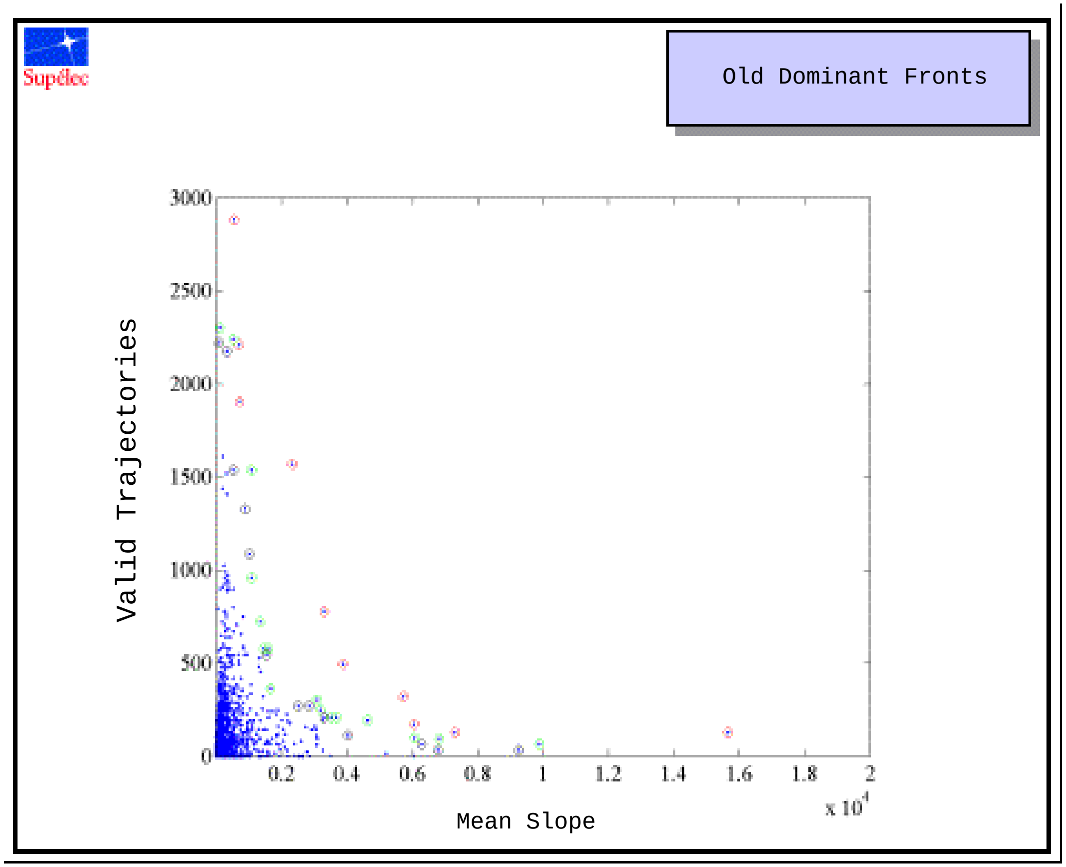


Figure 22: *Monotonicity occurrence histogram with threshold.*

Old Dominant Pareto Fronts



Old Dominant Fronts

Figure 23: *Pareto fronts for old dominant genes.*

Resistant Old Dominant Genes in first Three Fronts

- Leave-one-out cross validation

Let ψ_i^{-m} denote one possible set of $T \times (M - 1) = 6 \times 3$ samples

Cross-validation Algorithm:

```
Do $m = 1, \ldots, 4^6$:

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

   Find Genes in First 3 Pareto fronts: $G^{-m}$
   End
```

Three-objective Pareto Filtering

Objective Extract “aging genes”

- Strictly increasing filtering criteria:
  - persistent positive trend

$$\xi_1(\psi_i) = \overline{\min_t b_i(*,t)} = \max$$

  - high consistency over $6^4 = 4096$ possible combinations of trajectories

$$\xi_2(\psi_i) == \frac{\#\text{ trajectories having } \psi_i = [1,\ldots,1]}{4096} = \max$$

  - no plateau

$$\xi_3(\theta_i) = \overline{[\theta_i(*,t+1) - 2\theta_i(*,t) + \theta_i(*,t-1)]^2} = \min$$

Pareto Fronts

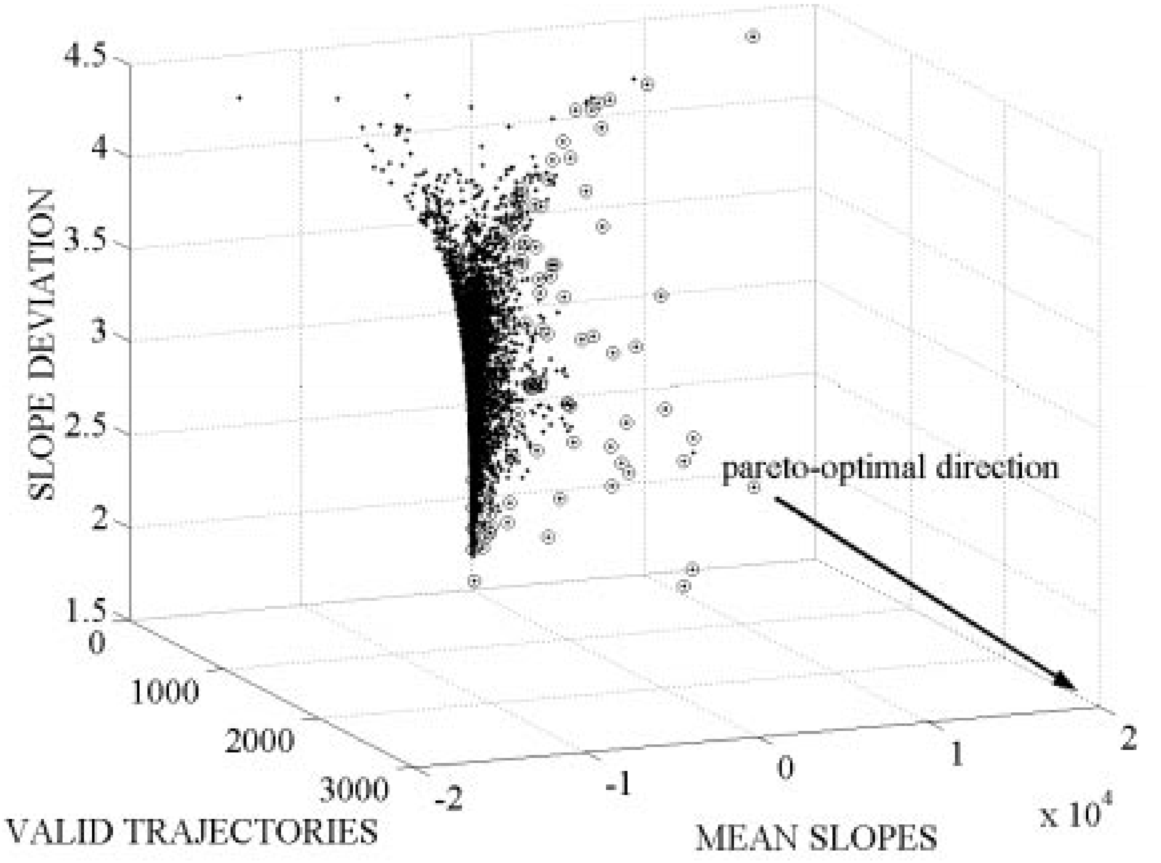


Figure 24: *First global Pareto front (o) for the three criteria (ξ_1, ξ_2 and ξ_3).*

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 is needed to account for statistical sampling uncertainty
5. Joint intensity extraction and gene filtering?
6. Optimization algorithms for large data sets?
7. Genetic priors: phylogenetic trees, BLAST database, etc?