

醫工導論

生物晶片與生物資訊

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10/13/2005

Two Major Biochip Technologies

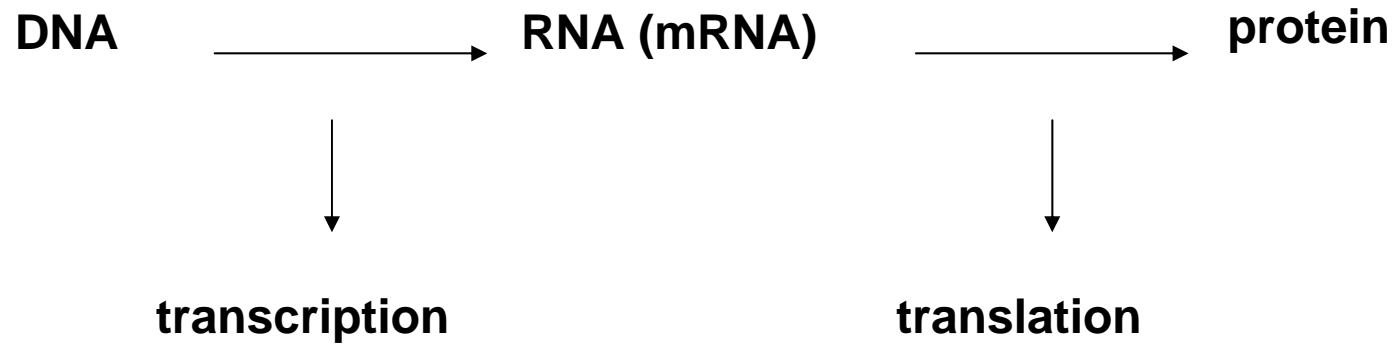
- Microarray Technologies
 - DNA
 - Protein
- Lab-on-a-chip (LOAC)
 - Capillary Electrophoresis
 - PCR

LOAC vs. Array

	Pros	Cons
LOAC	<ul style="list-style-type: none">•Easier acceptance•Potentially larger market size	<ul style="list-style-type: none">•Difficulty in technology integration•No therapeutics applications•Accuracy still not comparable to gene-based tests
Array	<ul style="list-style-type: none">•Broader applications, including diagnostics and therapeutics•Higher accuracy for gene-based testing•Multiple screening and simultaneous testing capability•Possibility to complement LOAC tests	<ul style="list-style-type: none">•Might need approvals from FDA or similar government agencies•Complex data management and analysis•Cost effectiveness

Microarray Analysis Involves Different Disciplines

- Biology
- Chemistry
- Physics
- Engineering
- Computer science
- Mathematics



DNA Chips

- Gene expression
- Genotyping-mutations, polymorphisms and gene copy number
- Gene discovery
- Functional analysis
- Molecular targets

Methods for Gene Expression Study

- Northern blot
- RT-PCR
- Differential display
- Serial analysis of gene expression (SAGE)
- Microarray

Arrays

- An array is an orderly arrangement of samples. It provides a medium for matching known and unknown DNA samples based on base-pairing rules and automating the process of identifying the unknowns.
- Standard blotting membranes or glass.
- created by hand or robotics to deposit the sample.

Microarray Technologies

- Favor small sample size and high throughput
- Gather data on thousands of genes in a single experiment
- Exploit genome data

DNA Microarray Elements

- **cDNA microarrays**

 - spotting**

 - contact – printing pins**

 - non-contact - piezoelectric dispensing**

 - (Packard BioScience/Perkin Elmer)**

- **Oligonucleotides microarrays**

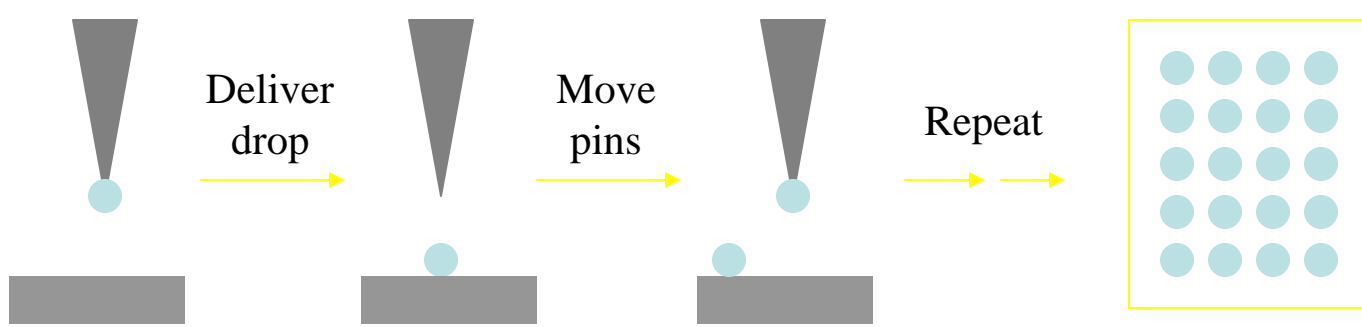
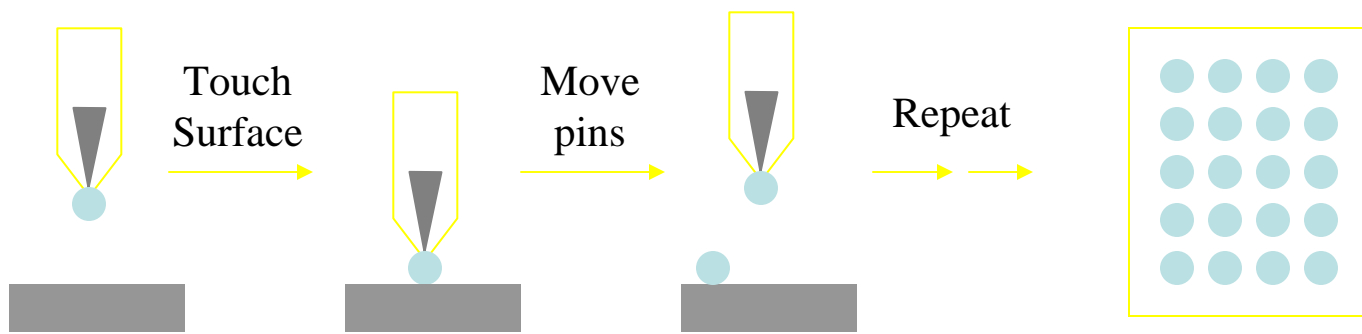
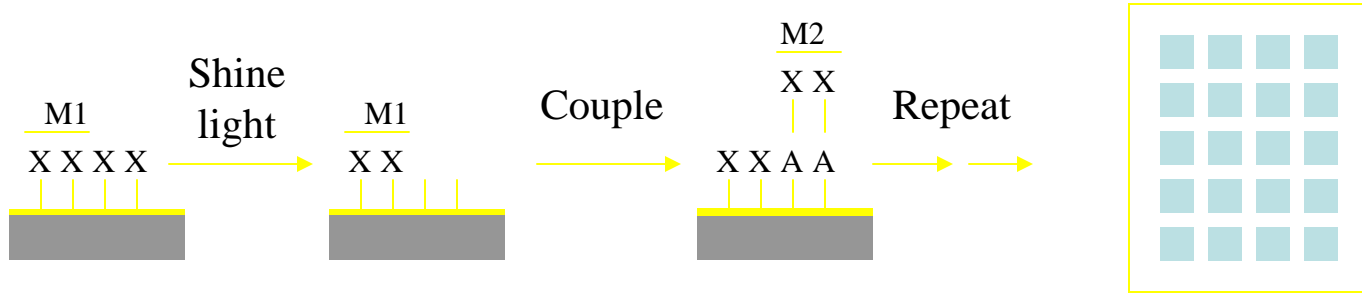
 - synthesis *in situ* (short oligos)**

 - photolithography – Affymetrix**

 - maskless digital micromirror - NimbleGen**

 - ink-jet - Agilent**

 - spotting pre-synthesized oligos (long oligos)**



Oligo vs. cDNA Arrays

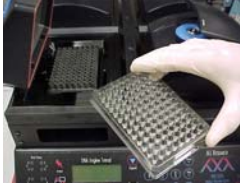
<i>Arrays</i>	<i>Pros</i>	<i>Cons</i>
<i>Oligonucleotide</i>	<ul style="list-style-type: none">• Complete control over sequence• Sequence and geometric perfection• Extremely high feature density	<ul style="list-style-type: none">• Lack of flexibility• Absolute requirement for sequence data• Uneven performance by individual array elements• Lack of oligo picking rules
<i>cDNA</i>	<ul style="list-style-type: none">• High degree of flexibility• Sequence independent• High stringency hybridization• No need for signal amplification	<ul style="list-style-type: none">• Clone handling• Clone authentication• Possible cross-hybridization

cDNA/Oligo Microarrays

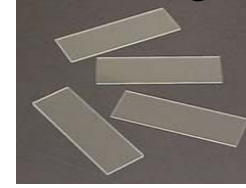
- **cDNA clones/oligos**
- **Instrumentation**
- **Array fabrication**
- **Probe preparation/hybridization**
- **Scanning arrays/image analysis**
- **Informatics**

Arraying Process

amplifying



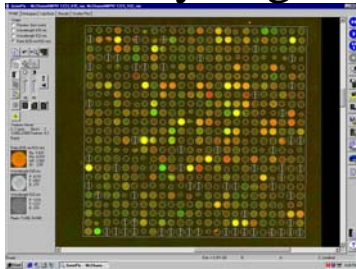
coating



interpreting



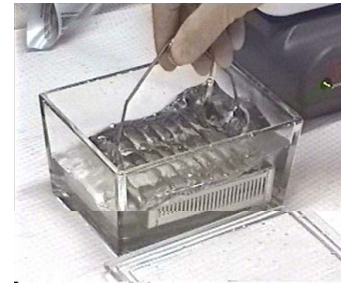
analyzing



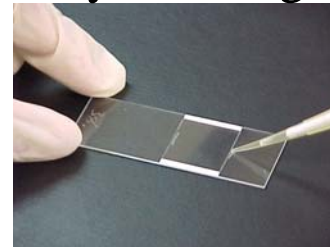
scanning



post-processing



hybridizing



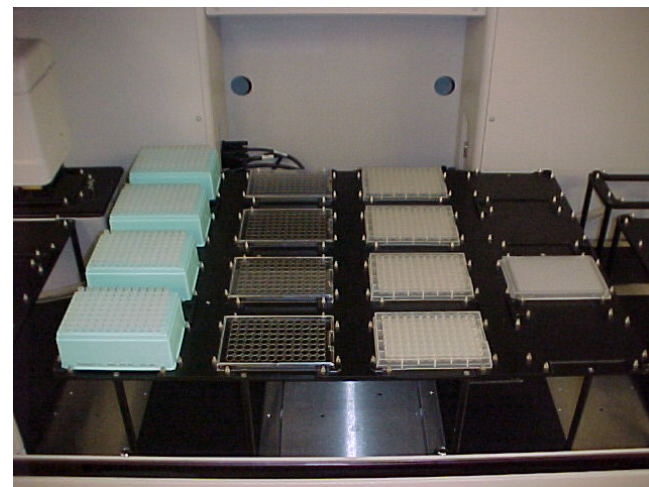
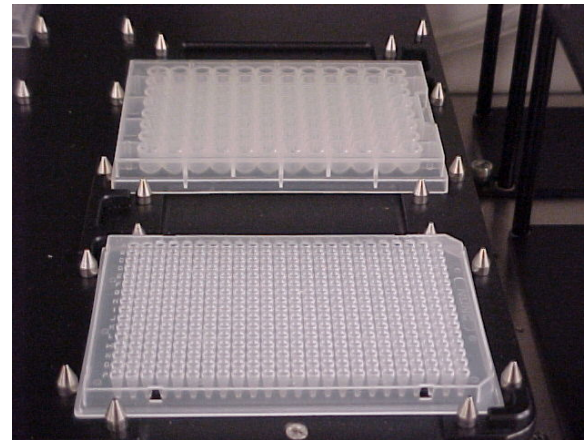
labeling



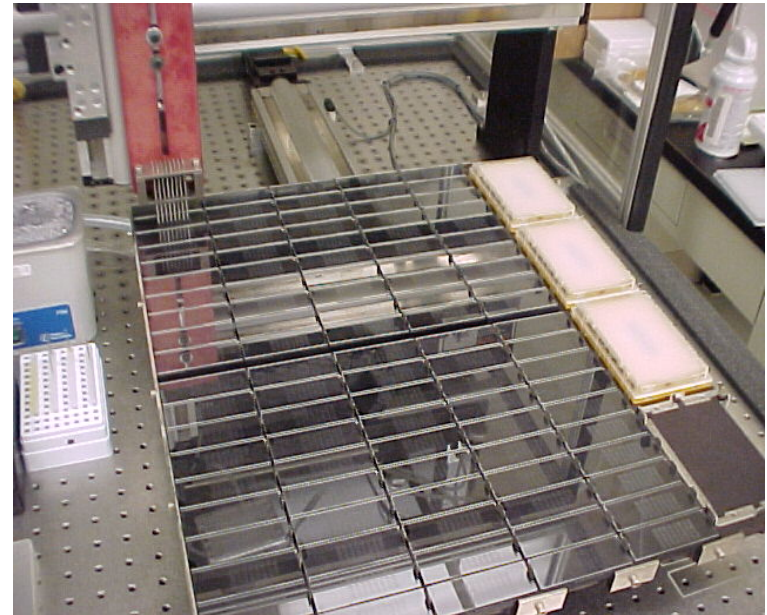
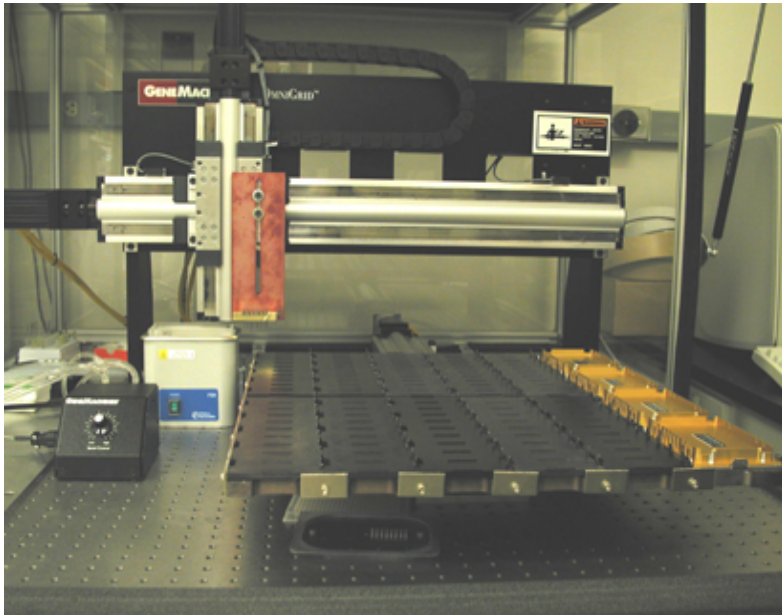
Instrumentation

- **Robot for plate replication and transfer**
Beckman Coulter - Biomek FX
- **Arrayer for printing**
GeneMachines - OmniGrid
- **Printing pins**
TeleChem - SMP3
- **Scanner for acquiring images**
Axon - Genepix 4000A/B

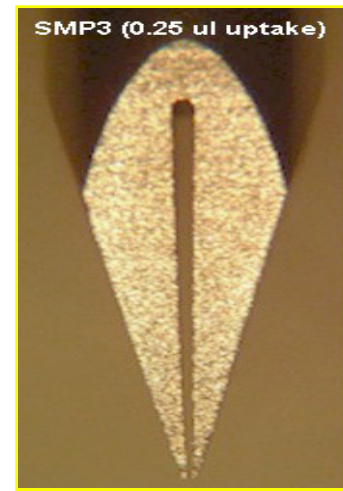
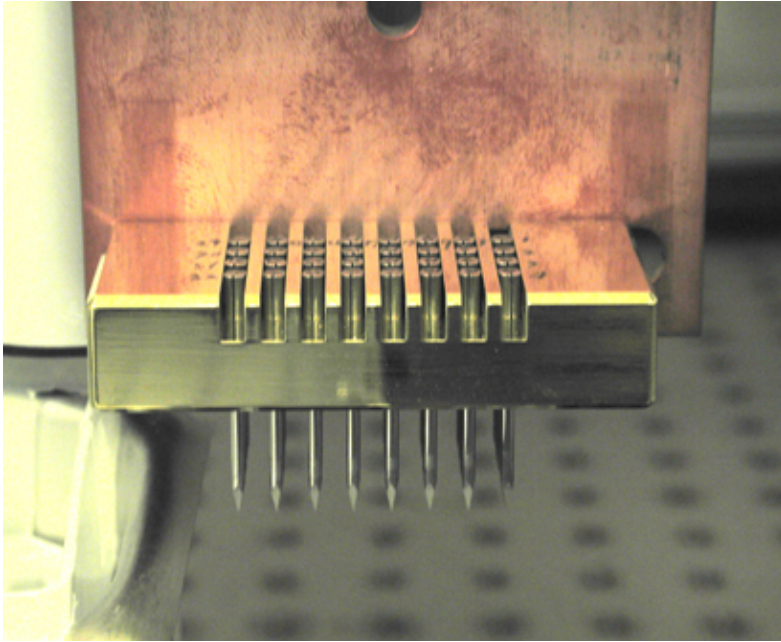
Beckman Coulter - Biomek FX



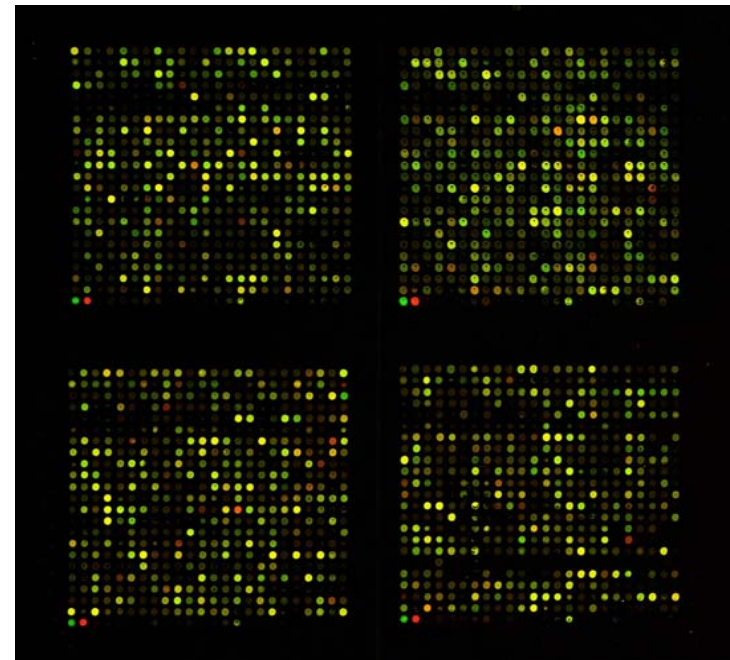
GeneMachines-OmniGrid



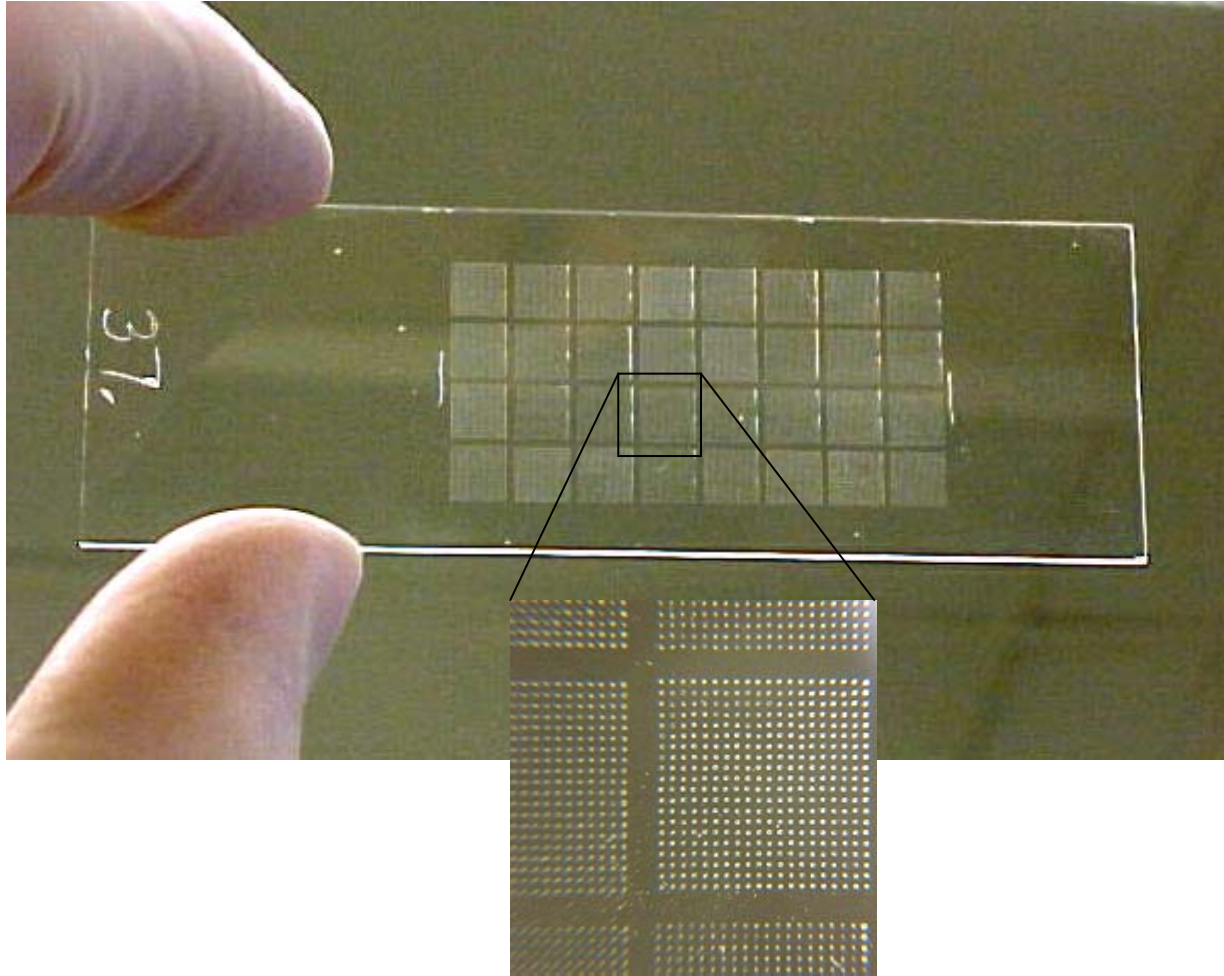
Printing tips



Axon Scanner-GenePix 4000

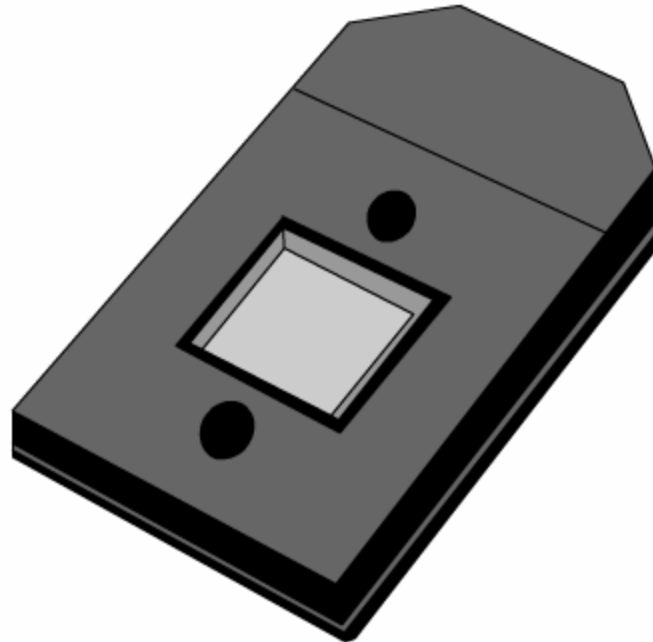


An Example of Glass Arrays



Affymetrix-GeneChip

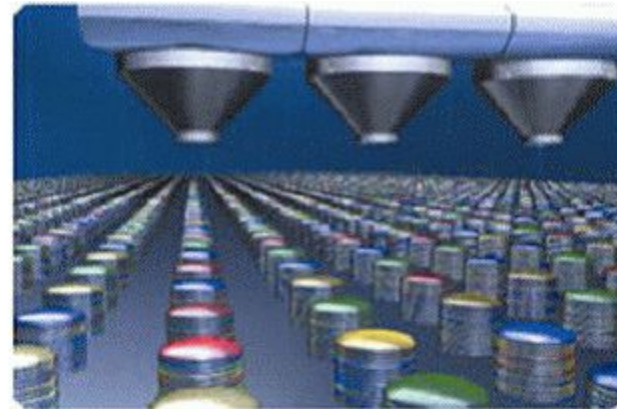
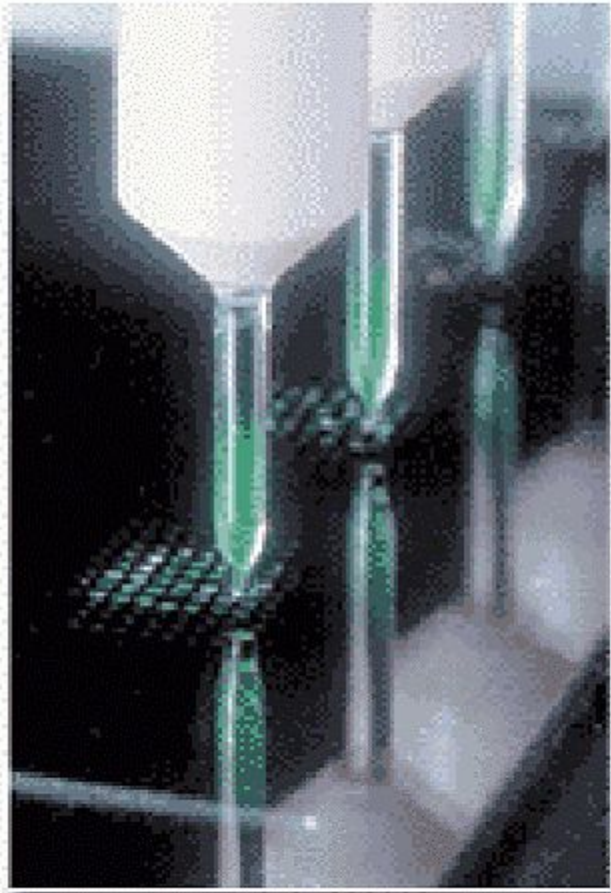
Stephen Fodor developed a technique called GeneChip® probe arrays where he built the sequences he wanted to screen. These GeneChip® arrays are printed on special glass.



GENE CHIP CARTRIDGE



Ink-jet Microarrays

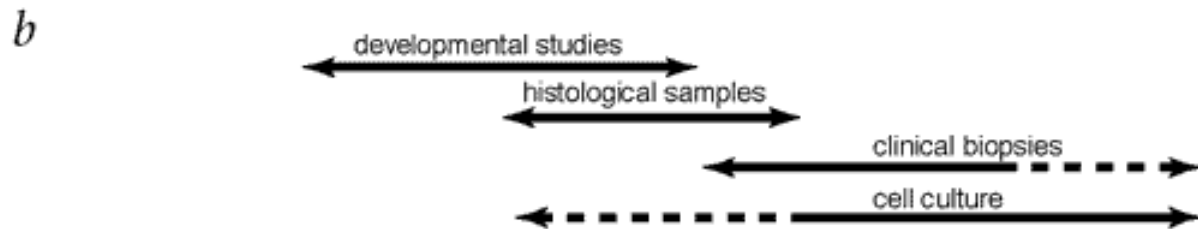
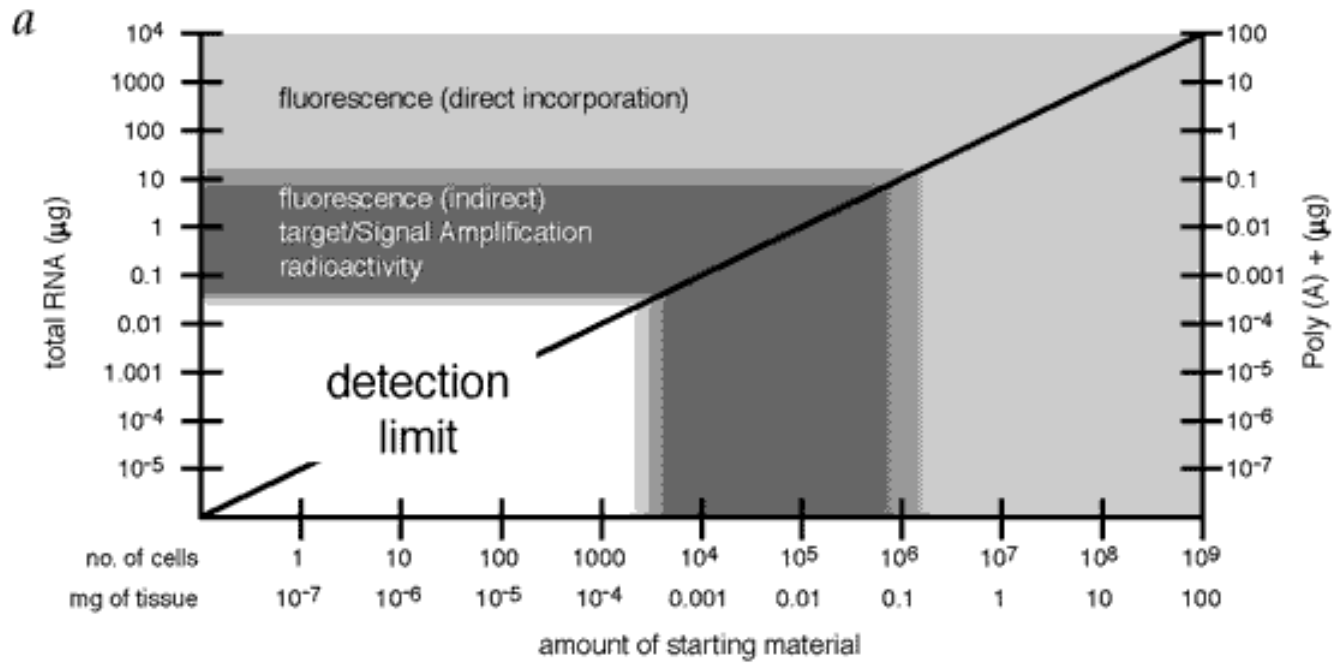


DNA Microarray Methodologies

- **Fluorescence method**
 - **Direct vs. Indirect**
- **Radioisotope method**
- **Colorimetric method**

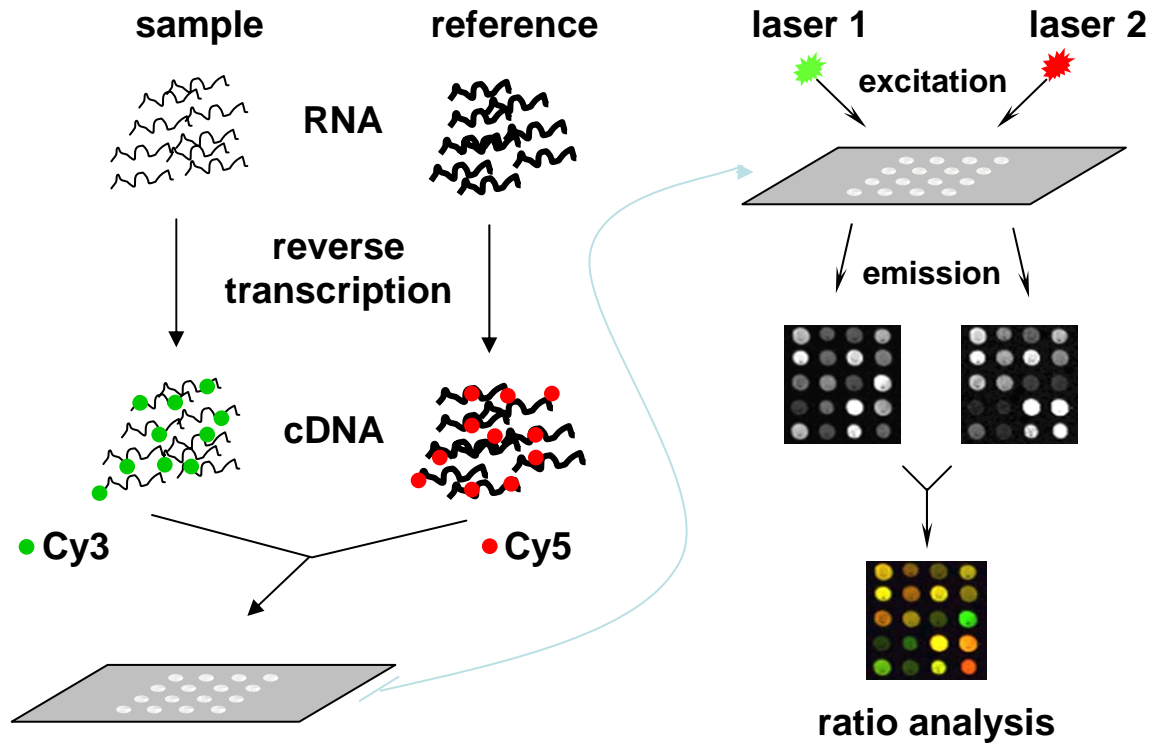
Direct vs. Indirect

	Direct	Indirect
Starting material	More	Less
Labeling efficiency for Cy3 or Cy5	Different	Equal
Time	Short	Long
Background intensity	Higher	Lower



Duggan, D.J. *et al. Nature Genet.* 21, 10-14 (1999)

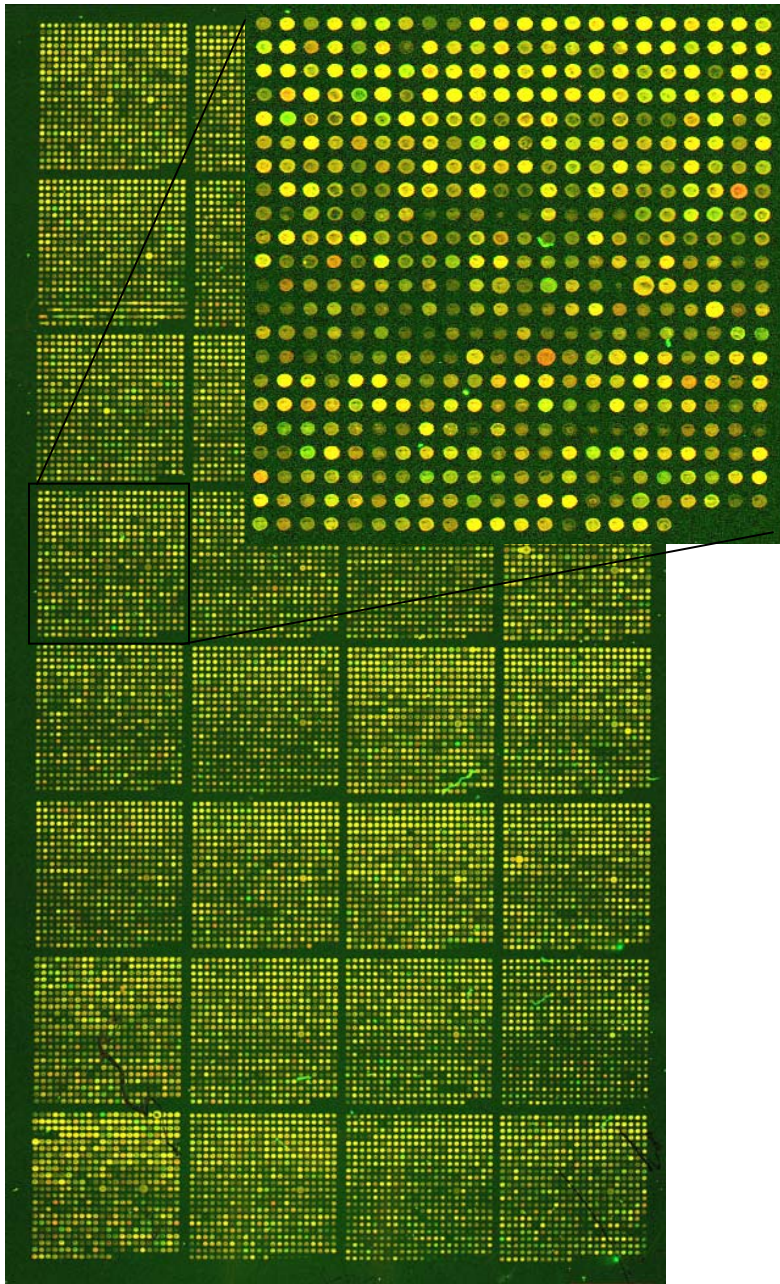
Schematic of Array Use



* adapted from Duggan, et. al., Nature Genetics (supplement), vol. 21, 1999.

Probe preparation/hybridization

- **Total RNA isolation**
- **RNA amplification**
- **Probe making**
- **Probe cleanup**
- **Hybridization setup**
- **Hybridization washes**



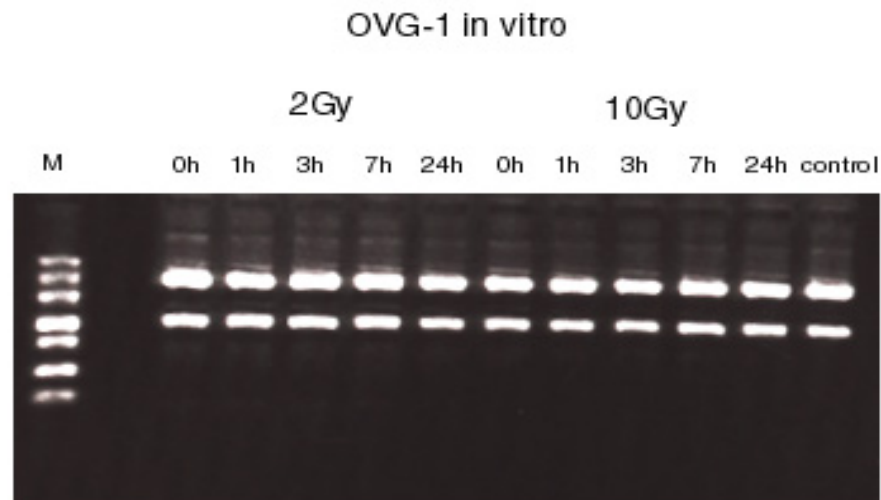
**An Image of
15K Array Chip**

Confounding Factors for Microarrays

- **Array quality**
- **RNA quality**
- **Labeling efficiency**
- **Probe cleanup**
- **Hybridization conditions**
- **Washes**
- **Scanning**

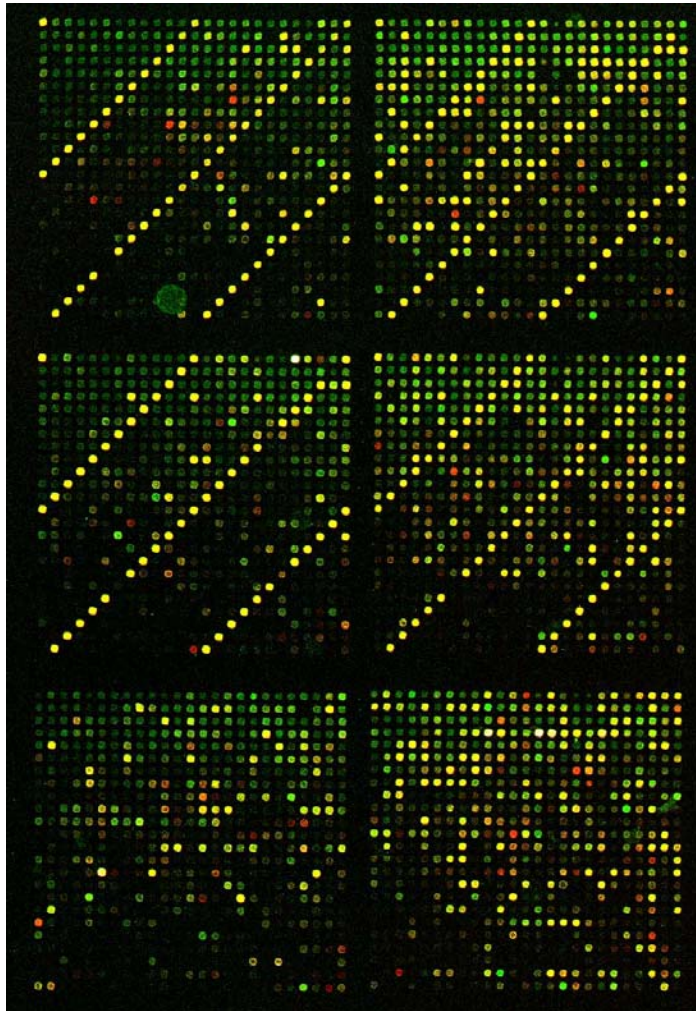
The general requirement of RNA

- **2.0 > ratio (OD 260/280) > 1.80**
- **Gel check RNA: clear 18s, 28s**

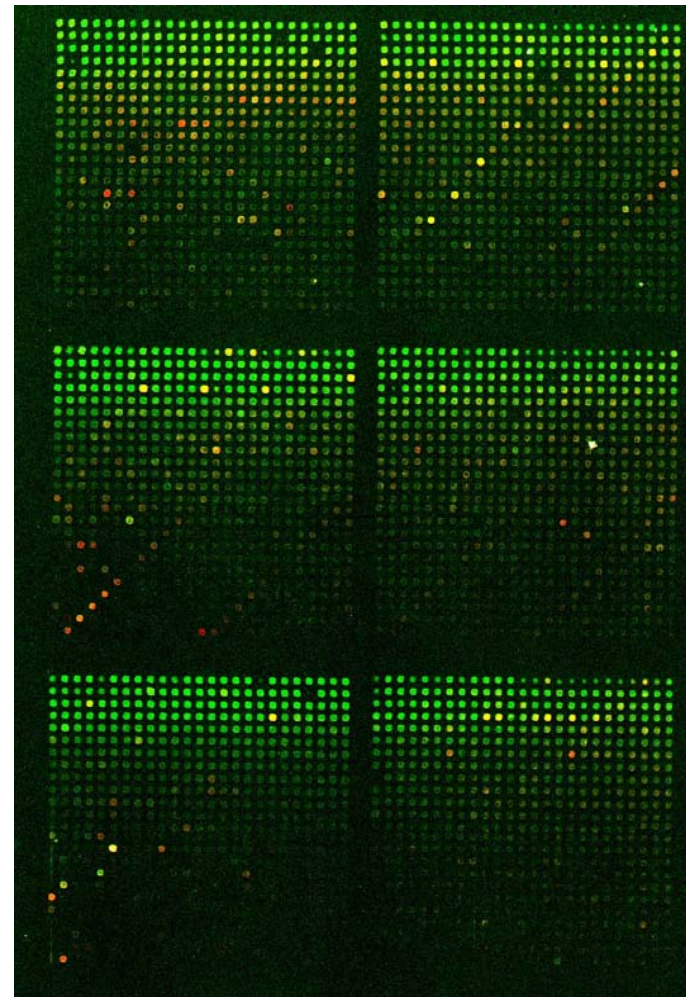


Different Hybridization Conditions

42°C

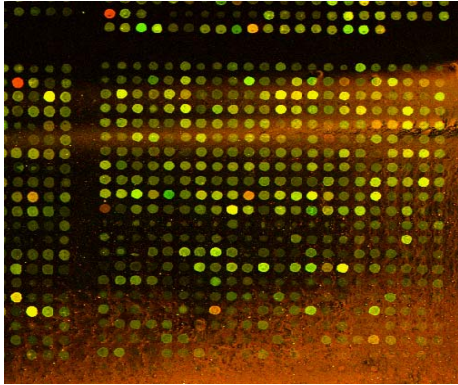


65°C

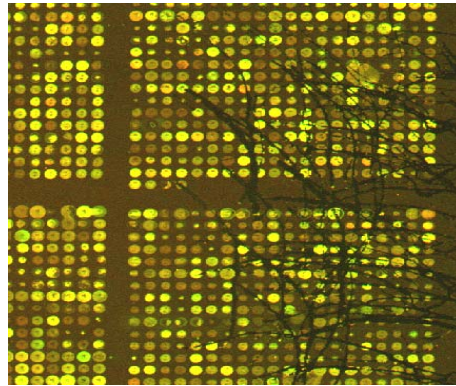


Common Artifacts

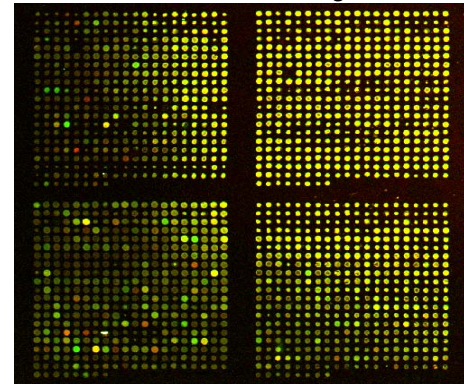
background



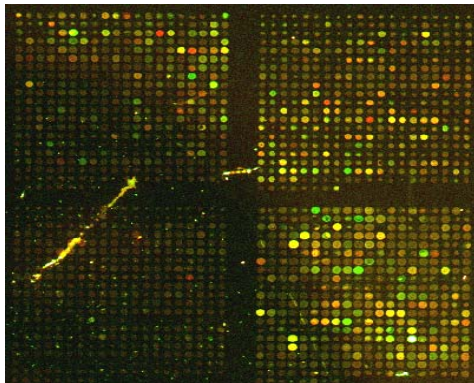
scratches



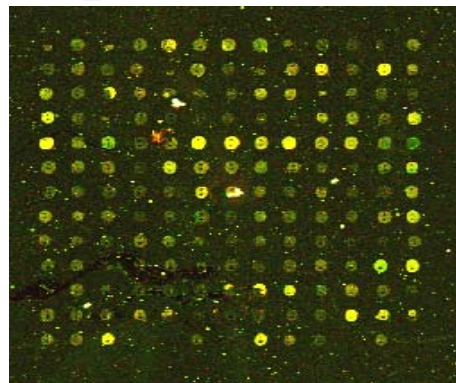
uneven hybridization



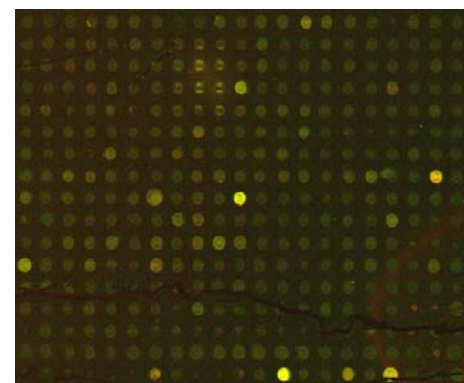
dim region



particulates



low detection



Experimental Design

- **Array**
 - **intra-array replicates**
 - **housekeeping genes or spiked control**
- **Biology**
 - **replicates**
 - **references**
 - **reciprocal labeling**

Validation of Array Data

- **Sequencing**
- **Northern Blot Analysis**
- **TaqMan**
- **RT-PCR**
- **Western Blot Analysis**

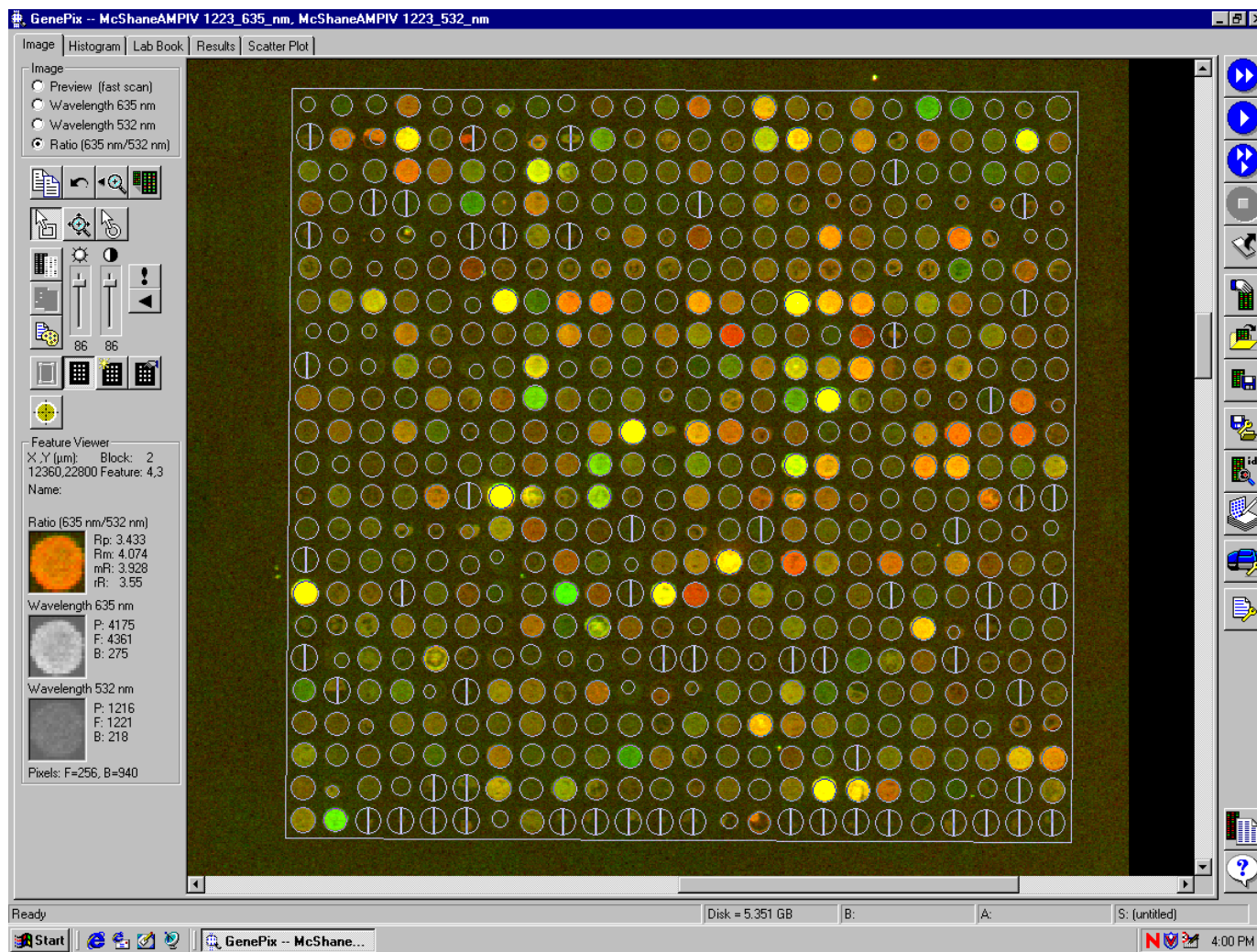
Informatics

- **Array image analysis**
- **Array data management**
- **Array data analytical tools**

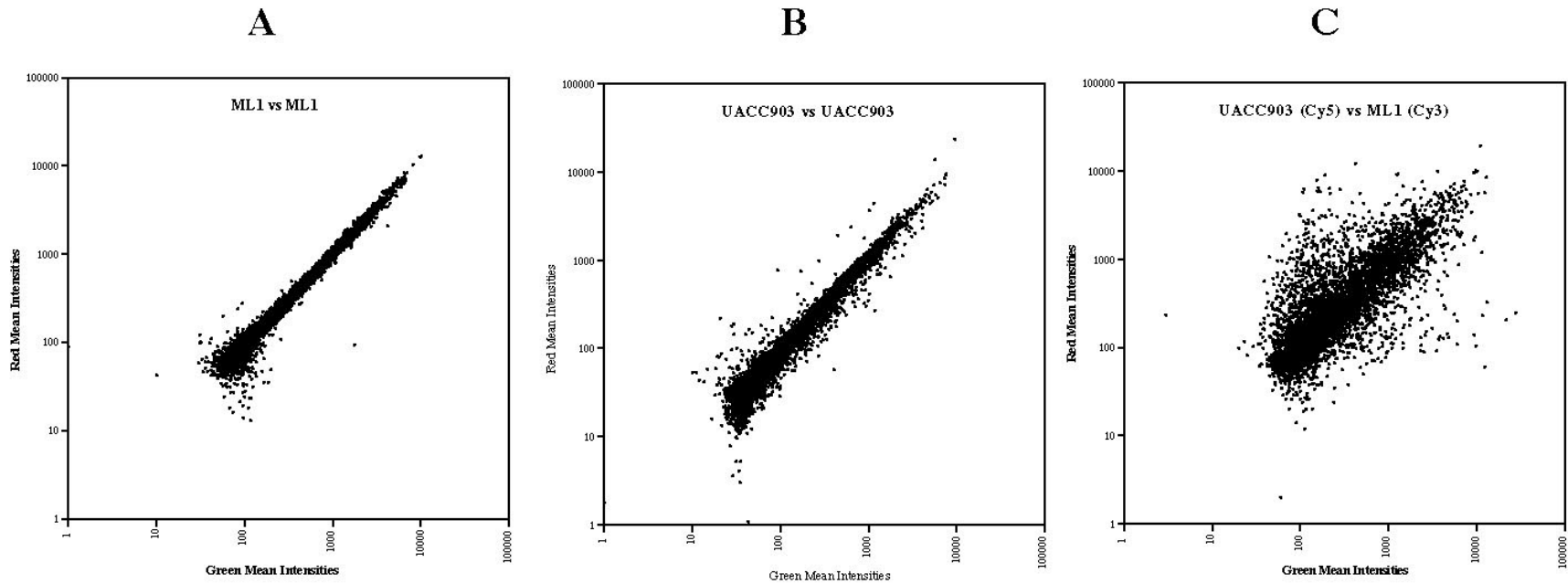
Microarray Data Analysis

- **Microarray Image Analysis: Target, signal, quality, etc**
 - **GenePix Pro - Axon**
 - **ArraySuite – NHGRI**
- **Gene expression clustering and classification**
- **Gene-gene interaction/Function analysis**

Image Analysis-GenePix Pro

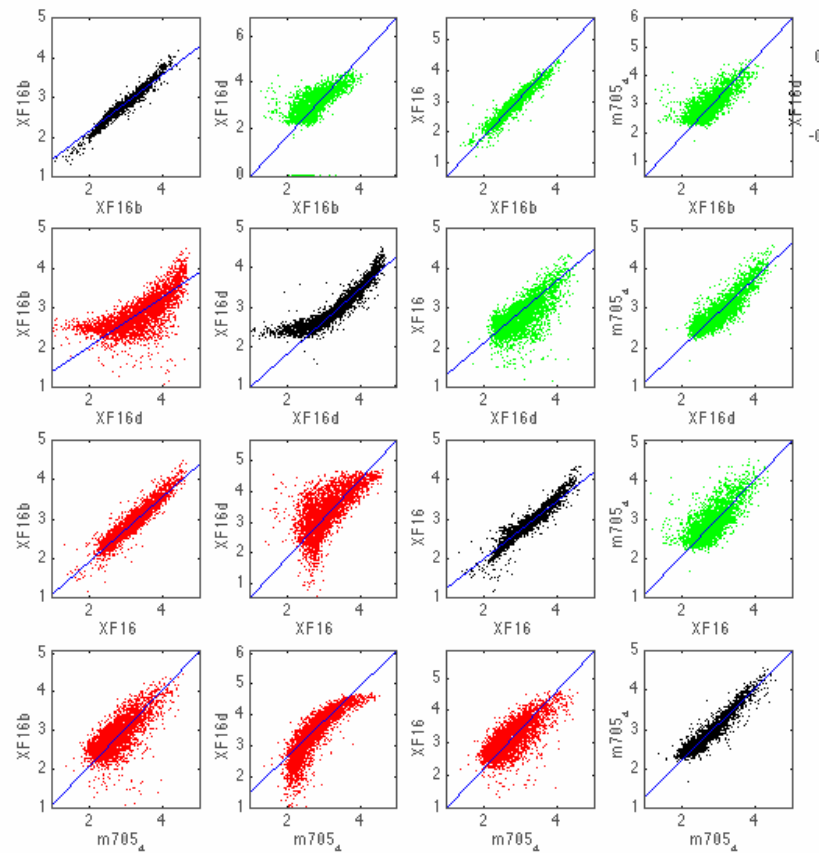


Scatter Plots



Levels of correlation between the same and different samples.

Global Normalization (between-slides normalization)

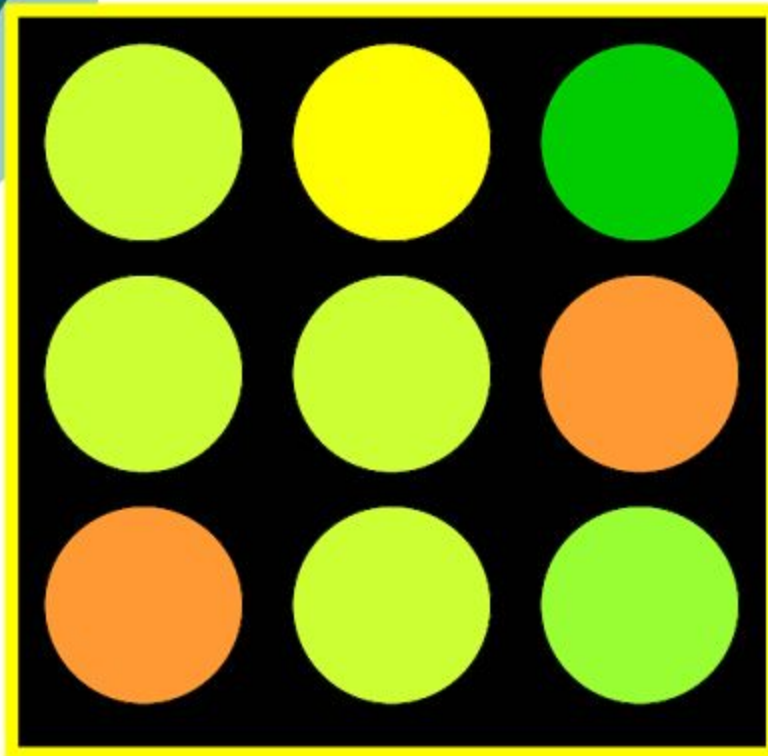


Data Normalization

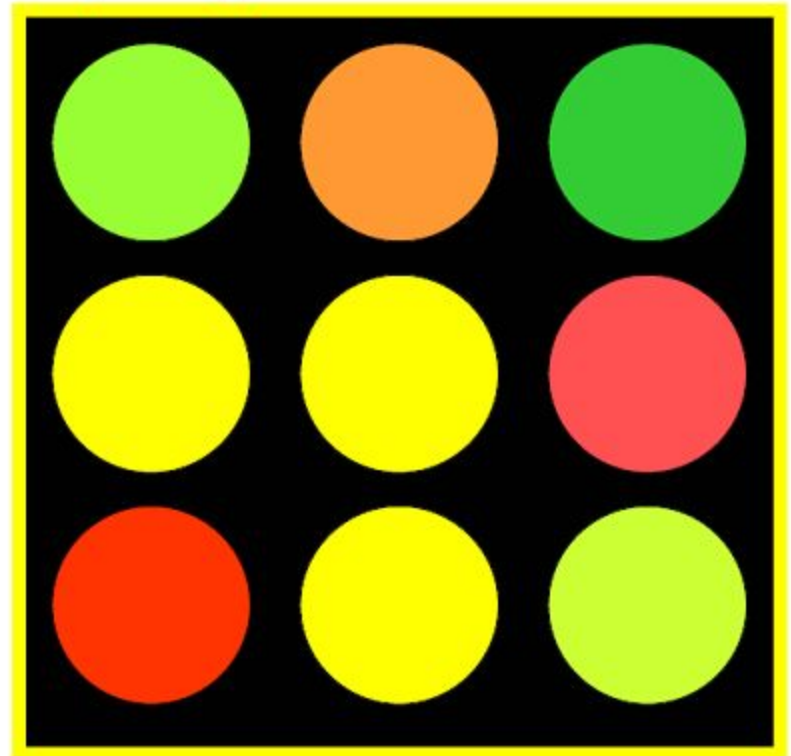
- **To correct**
 - **Dye bias**
 - **Location bias**
 - **Intensity bias**
 - **Pin bias**
 - **Slide bias**

Example of Data Normalization

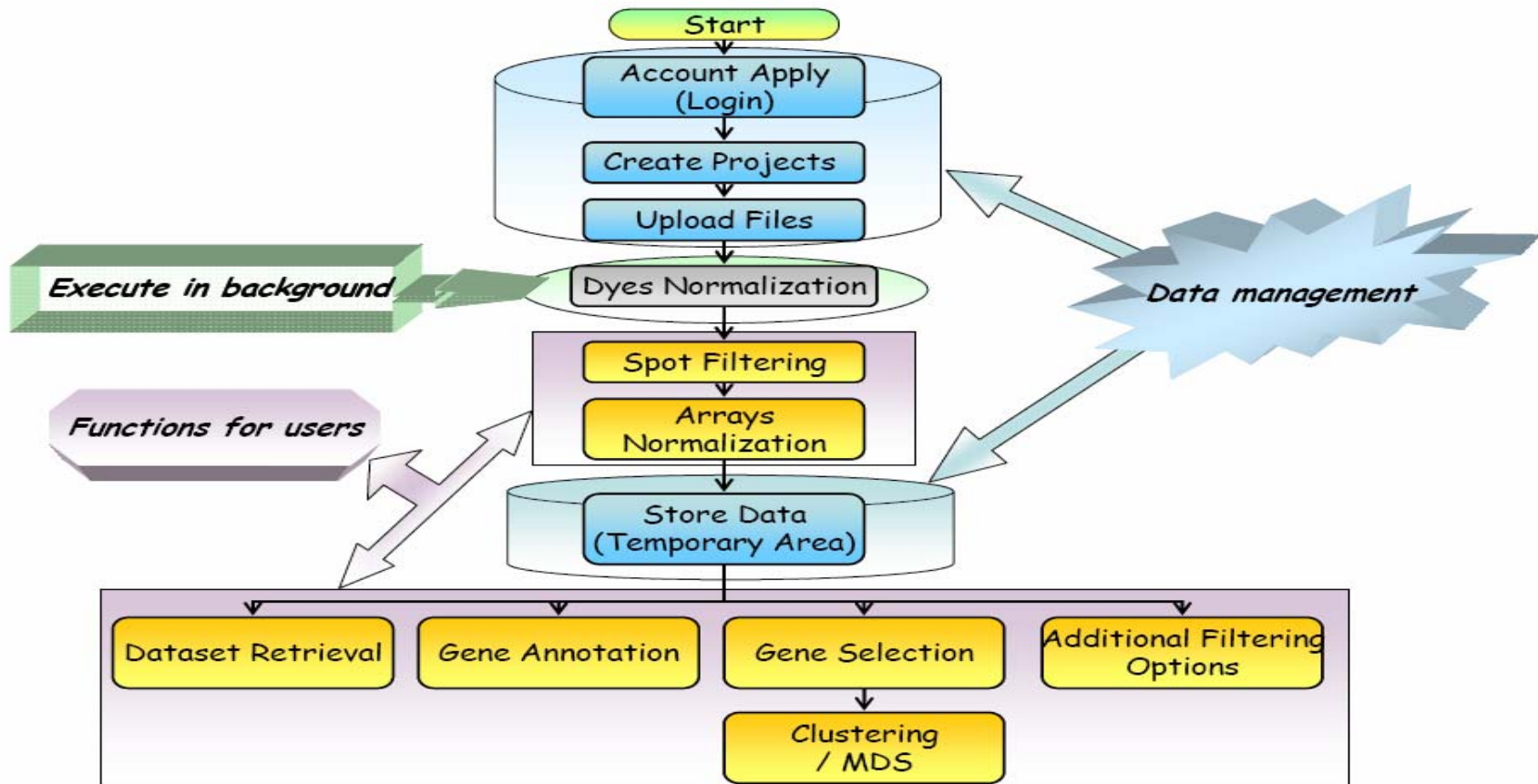
Uncalibrated, red light under detected



Calibrated, red and green equally detected

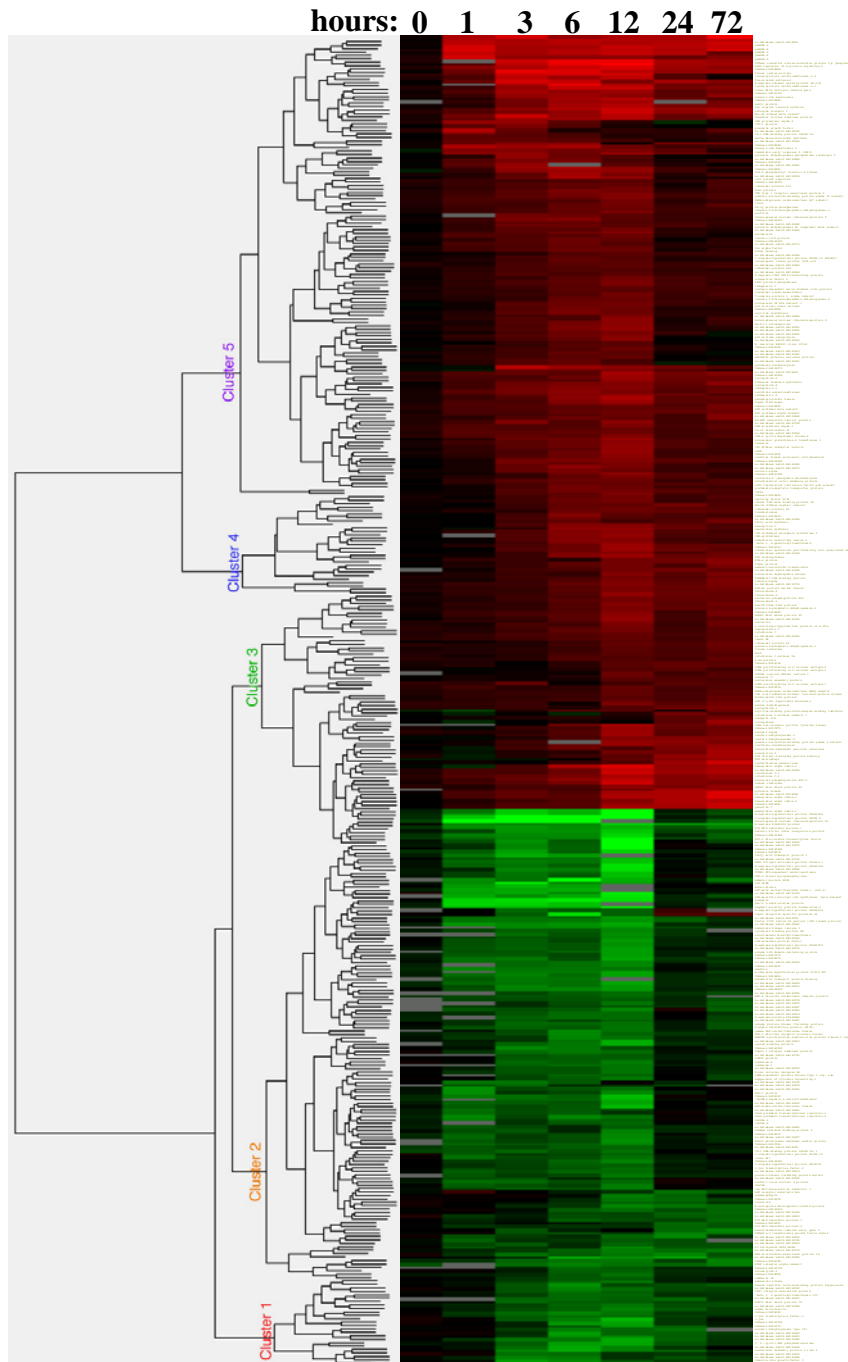


NTU Center for Genomic Medicine Web-based System for Microarray Analysis



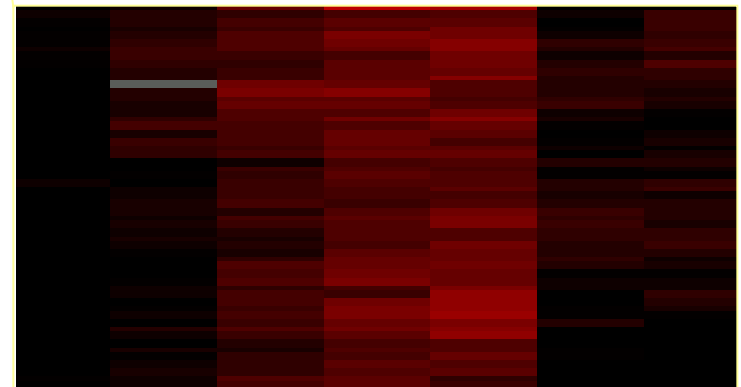
Clustering Analysis

- **Hierarchical Clustering**
- **K-means Clustering**
- **SOM Clustering**

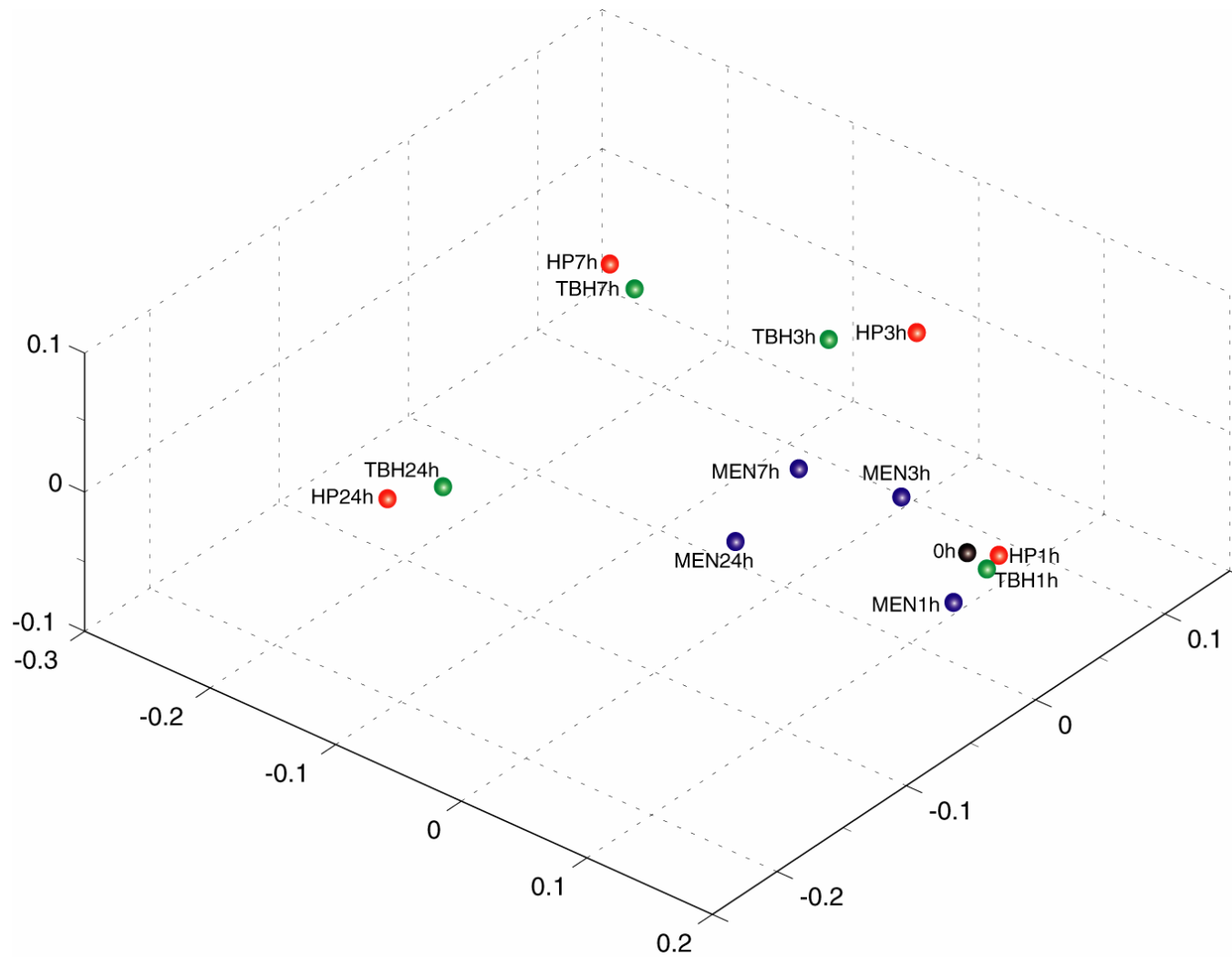


Database Tools: Hierarchical Clustering

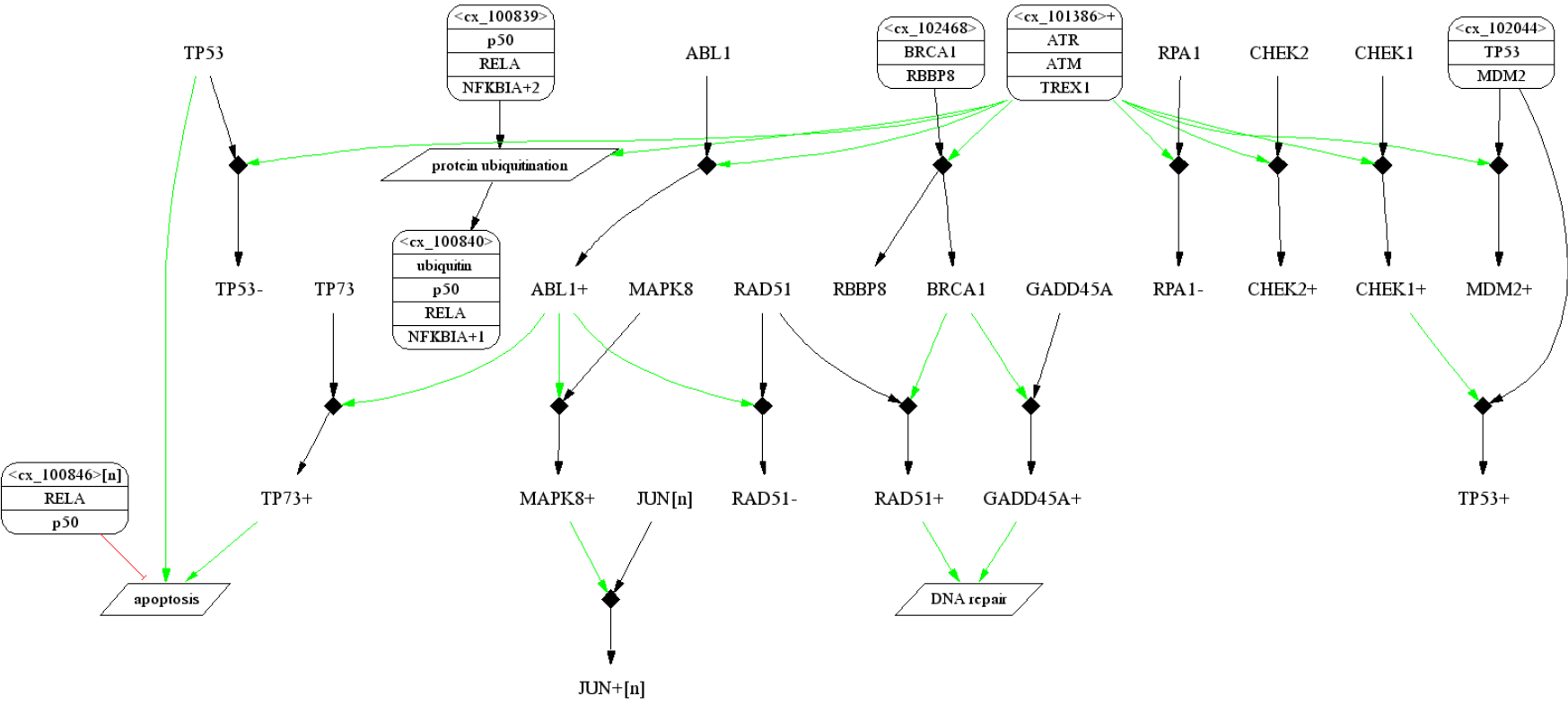
>8 >4 >2 1:1 >2 >4 >8



Database Tools: Multi-Dimensional Scaling



Gene-Gene Interaction/Function Analysis



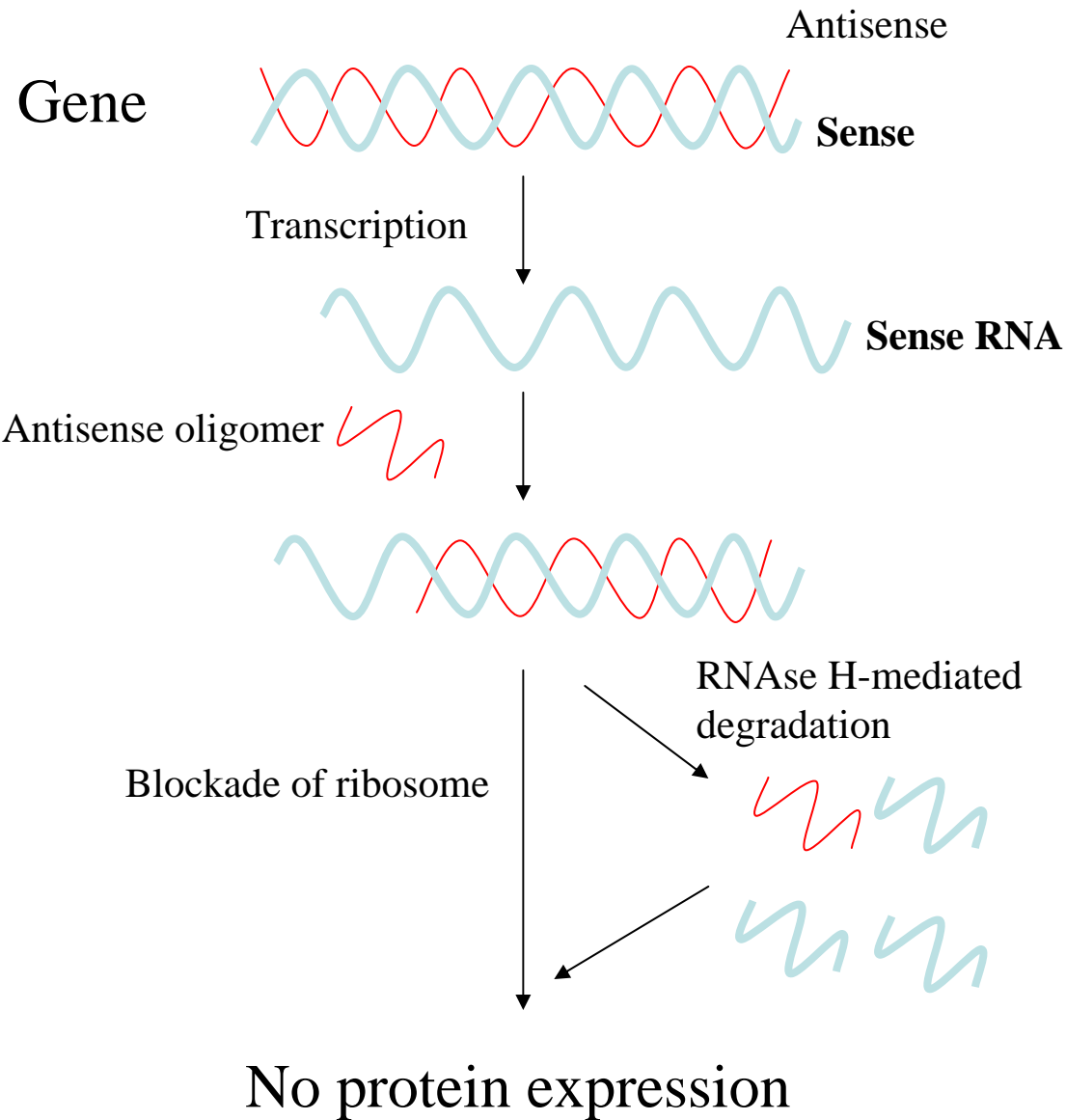
Profiling Gene Expression

- **Clinical research**
 - tumor classification
 - tumor vs. normal tissue
- **Basic research**
 - treated vs. untreated
 - wild-type vs. mutant/knockout

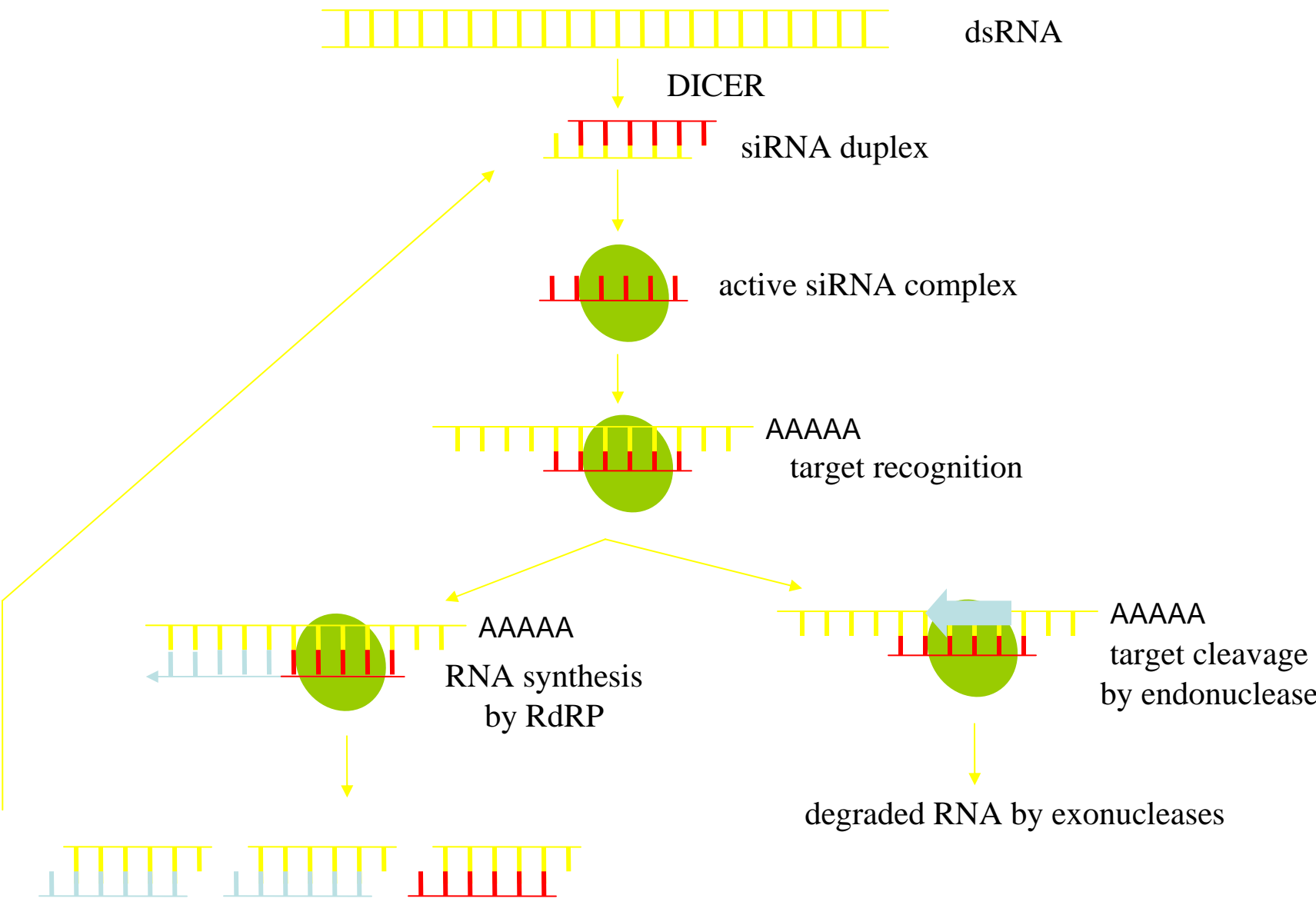
Functional Study

- **Overexpression**
- **Suppression**
 - **Anti-sense**
 - **Small interfering RNA (siRNA)**
 - **Knockout**
 - *In vitro*
 - *In vivo*

Anti-sense RNA



siRNA



Secondary siRNA by DICER

Adopted from *Plasterk Science* 296, 1263-6 (2002)

Gene expression profiles



Biological function/pathway analysis



Identify interesting genes



Functional study



Molecular targets



New drug discovery