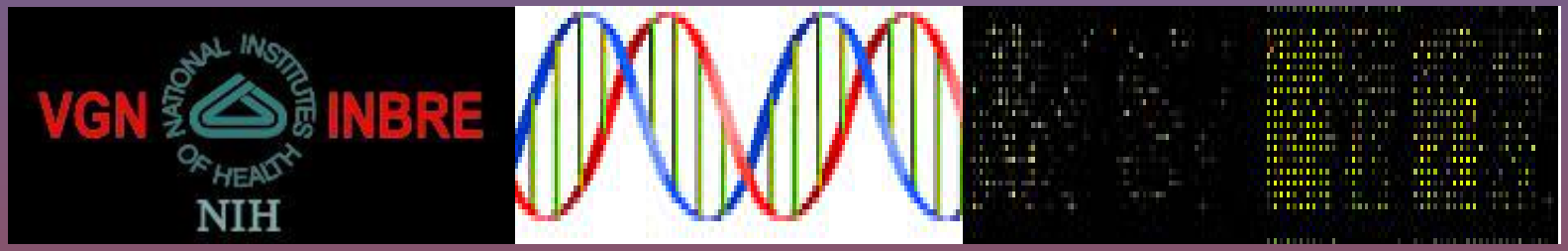
Microarray Technology



Professor

Dr. Mushtak T. S. Al-Ouqaili

Outline of the lecture

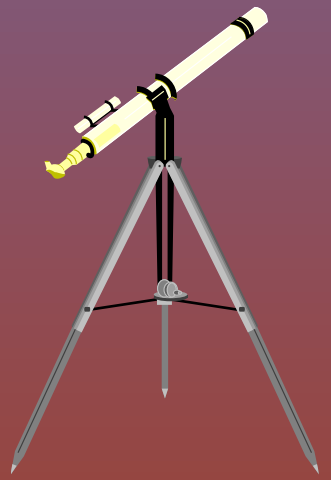
- Overview of Microarray Technology
  - Types of Microarrays
  - Manufacturing
  - Instrumentation and Software
  - Data Analysis-Basic
  - Applications

Microarray Development

- Relatively young technology

Widely adopted

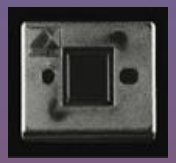
Mainly used in gene discovery



Evolution & Industrialization

1989: First Affymetrix Genechip Prototype

1994: First Commercial Affymetrix Genechip

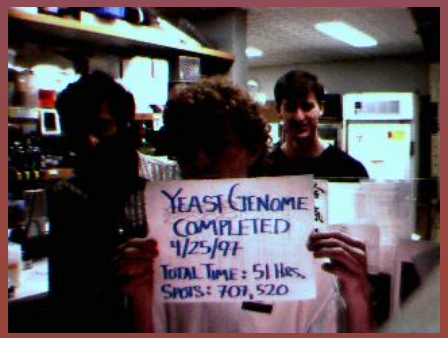


1994- First cDNAs arrays were developed at Stanford University

1994: First Commercial Scanner-Affymetrix

1996- Commercialization of arrays

1997-Genome-wide Expression Monitoring in *S. cerevisiae*



What are Microarrays?

- **Microarrays are simply small glass or silicon slides upon the surface of which are arrayed thousands of features (usually between 500 up to a million)**
- **Using a conventional *hybridization* process, the level of expression of genes is measured (for instance)**
- **Microarrays are read using laser-based fluorescence scanners**
- **The process is “high throughput”**

Why use Microarrays?

- Determine what genes are active in a cell and at what levels
- Compare the gene expression profiles of a control vs treated
- Determine what genes have increased or decreased in during an experimental condition
- Determine which genes have biological significance in a system
- Discovery of new genes, pathways, and cellular trafficking

Why analyze so many genes?

- **Just because we sequenced a genome doesn't mean we know anything about the genes. Thousands of genes remain without an assigned function.**

- **Patterns or clusters of genes are more informative regarding total cellular function than looking at one or two genes – can figure out new pathways**

The six steps in development of a DNA microarray experiment

1. Manufacturing of the microarray
2. Experimental design and choice of reference: what to compare to what?
3. Target (sample) preparation and hybridization
4. Image acquisition (scanning) and quantification of gene expression

5. Database building, filtering, and normalization

6. Bioinformatics: Statistical analysis, data mining, pathway analysis

Types of Microarrays

-Expression Arrays
-Protein microarrays (Proteomics)
-Resequencing arrays
-CGH arrays- Comparative genomic hybridization
-SNP Arrays
-Antibody Arrays
-Exon arrays-Alternative splice variant detection
-Tissue Arrays

Microarray Formats

A) Cartridge-based

- Spotted
- Electronic

B) Spotted Glass Slide

C) Tissue Section Slide

Cartridge-based Chips

-Miniaturized, high density arrays of DNA oligos within a plastic housing

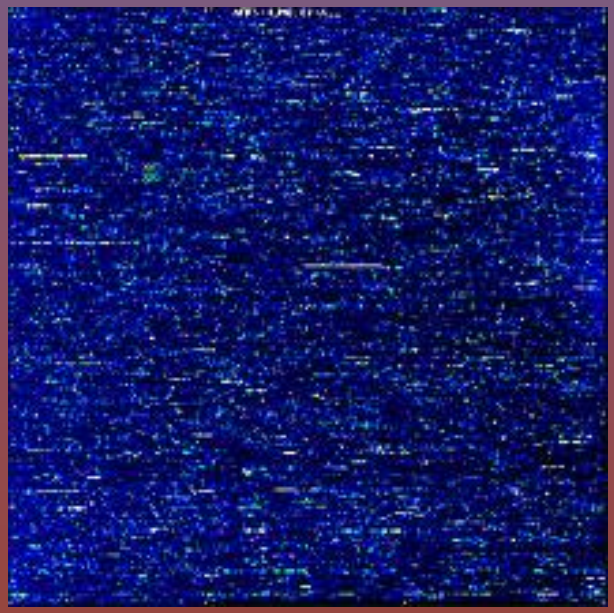
-One sample=One chip (Affymetrix, Agilent, Applied Biosystems…)

- Generally used with expression and DNA arrays

Cartridge-based Expression Microarrays

Involves Fluorescently tagged biotinylated cRNA

-One chip per sample
-Uses single fluorescent dye
-More expensive



Affymetrix GeneChip Image

Spotted Glass Arrays

Uses cDNA, Oligonucleotide, protein, antibody

-Robotically spotted cDNAs or Oligonucleotides

- Printed on Nylon, Plastic, or Glass microscope slide

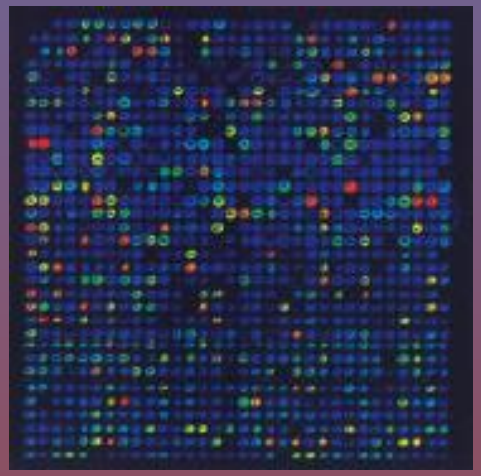
Agilent:Oligonucleotide Array

BD’s Antibody Array

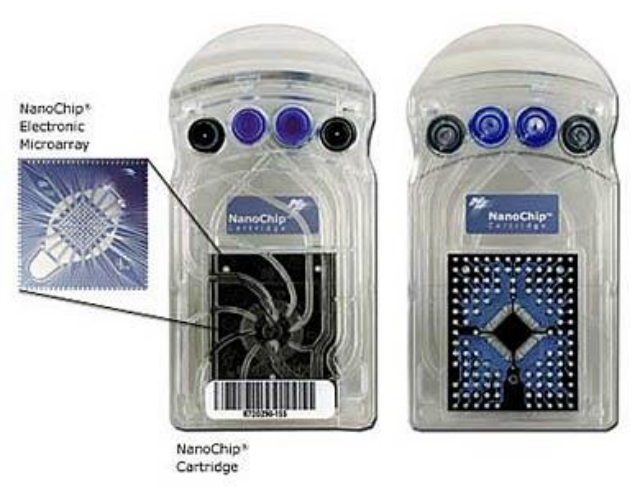
Spotted cDNA and Oligo Glass Arrays:

Involves two dyes on the same slide

- **Red dye-Cy5**
- **Green dye-Cy3**
- **Control and experimental cDNA**



Electroniconically Addressable Microarrays



Nanogen- Nanochip

Motorola- eSensor Chip

Expression Arrays

Most common type of microarray

Spotted glass, cartridge, and electronic

Involves extracting RNA from a sample and converting it to cDNA by priming off of the Poly A tail of mRNA for eukaryotes and using random hexamers for prokaryotes [WHY?]

Measures the amount and type of mRNA transcripts

Provides information on whether genes are up or down regulated in a specific condition

Can find novel changes in ESTs for specific conditions

Protein Microarrays

True protein microarrays are evolving very slow and only a few exist.

Technology is not straight forward due to inherent characteristic of proteins [e.g. available ligands, folding, drying…]

Most are designed to detect antibodies or enzymes in a biological system

Protein is on the microarray

Some detect protein-protein interaction by surface plasmon resonance other use a fluorescence based approach

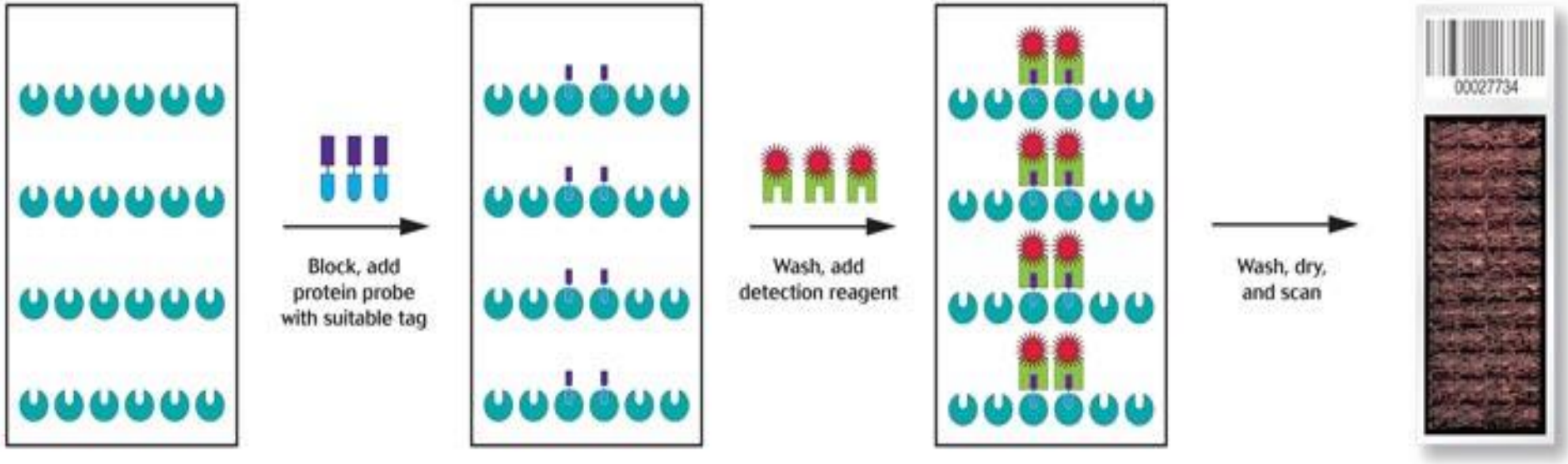
Protein Microarrays

The Invitrogen Human Protein Microarray is a high-density microarray

It contains thousands of unique human proteins

[kinases, phophatases, GPCRs, nuclear receptors, and proteases]

Figure 2—Using the ProtoArray™ Human Protein Microarray is simple



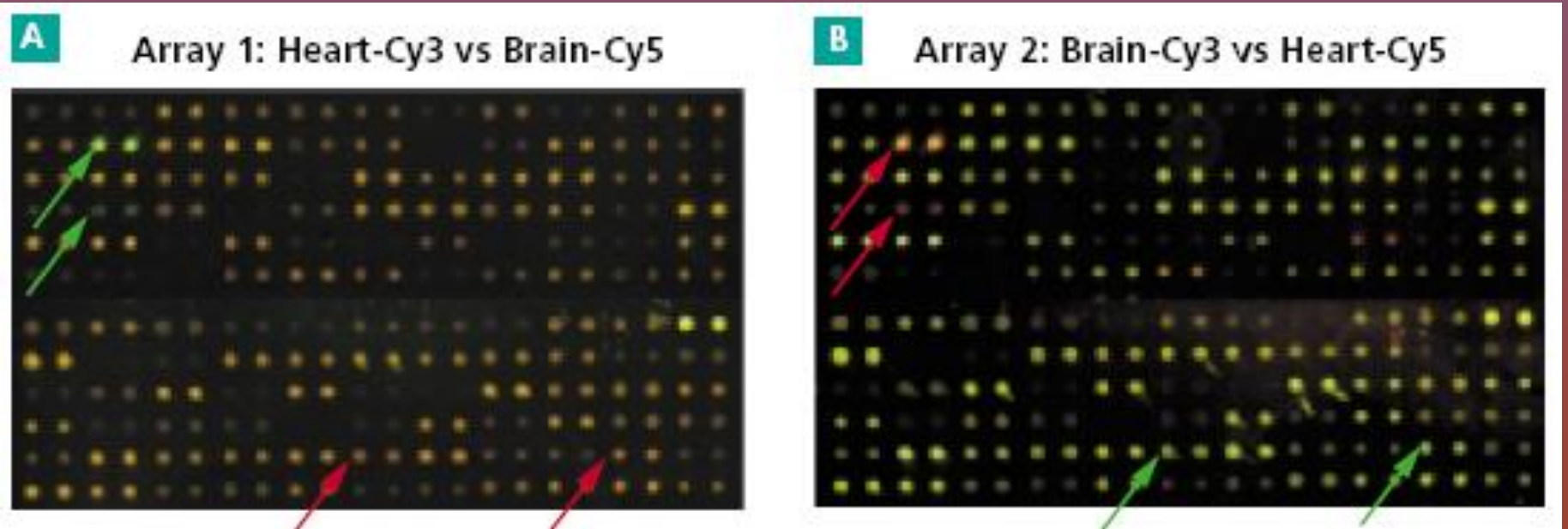
Antibody Arrays

-Assay hundreds of native proteins simultaneously

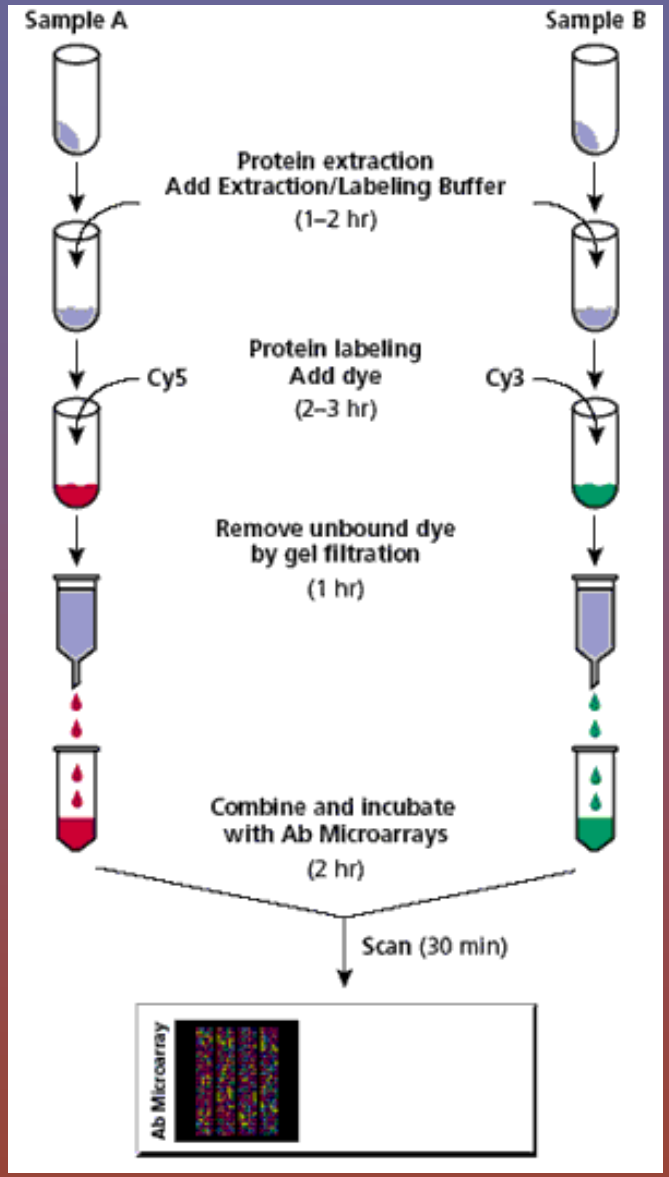
-Compare protein abundances in a variety of biological samples

-GenTel and BD biosciences

-Antibody or ligand is on the microarray



Antibody Arrays-labeling scheme



SNP, Genotyping, and DNA Mapping Arrays

Targets DNA not RNA like expression

Requires amplification of target DNA

Uses multiple probes sets to determine base change at a specific nucleotide position in the genomic DNA.

Use thousand of oligos that “tile” or span the genomic DNA for characterization.

Provides sequence and genotyping data including LOH, Linkage analysis and single nucleotide polymorphisms

Resequencing Arrays [Affy]

Enable the analysis of up to 300,000+ bases of double-stranded sequence (600,000 bases total) on a single Affy array

Used for large-scale resequencing of organisms genome and organelles

Faster and cheaper than sequencing but very limited to few organisms and/or organelles

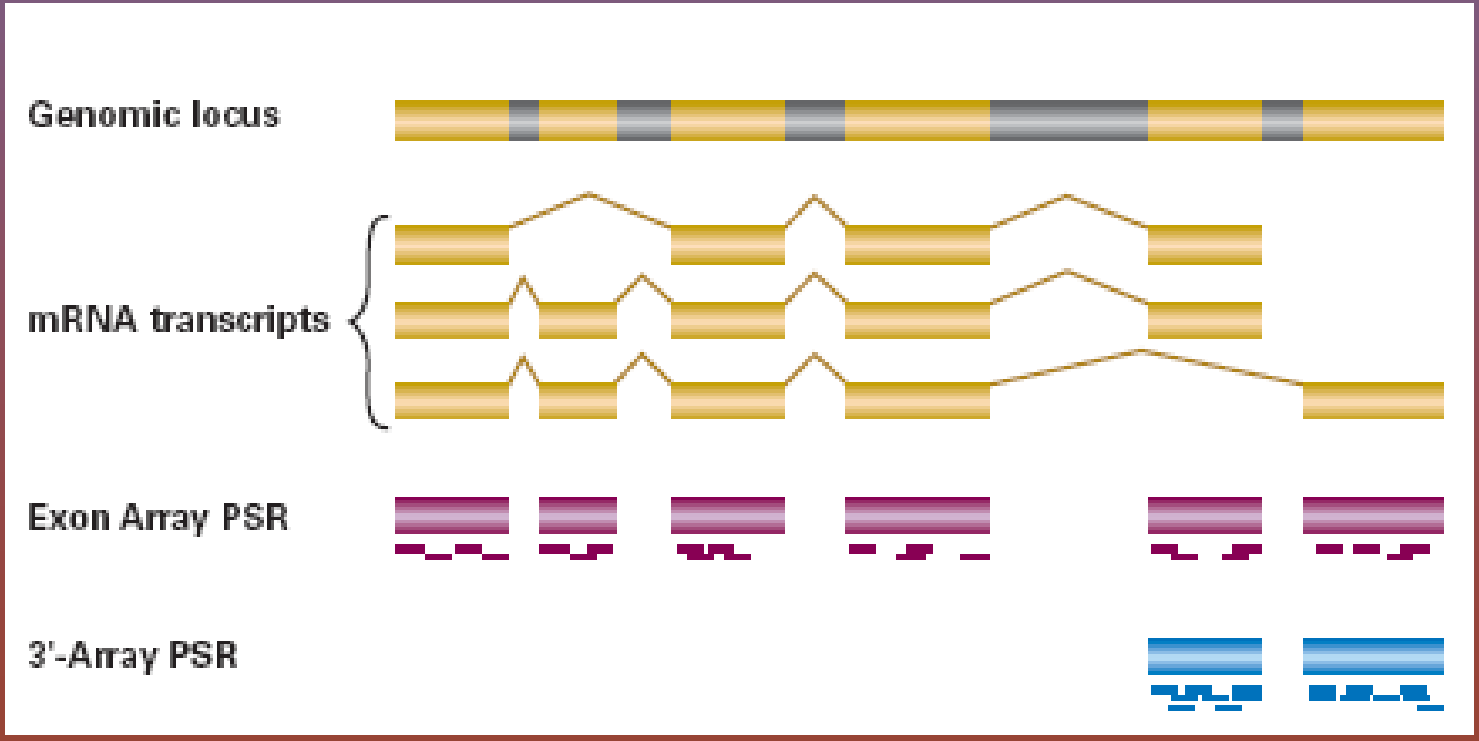
Large potential

Exon Arrays-Alternative splice variant detection

Probes are designed for hybridizing to individual exons of genomic DNA

Tissue or development specific splicing leads to normal or expected protein diversity

Defective splicing can lead to disease



CGH Arrays- Comparative Genomic Hybridization

Provides DNA and chromosomal information

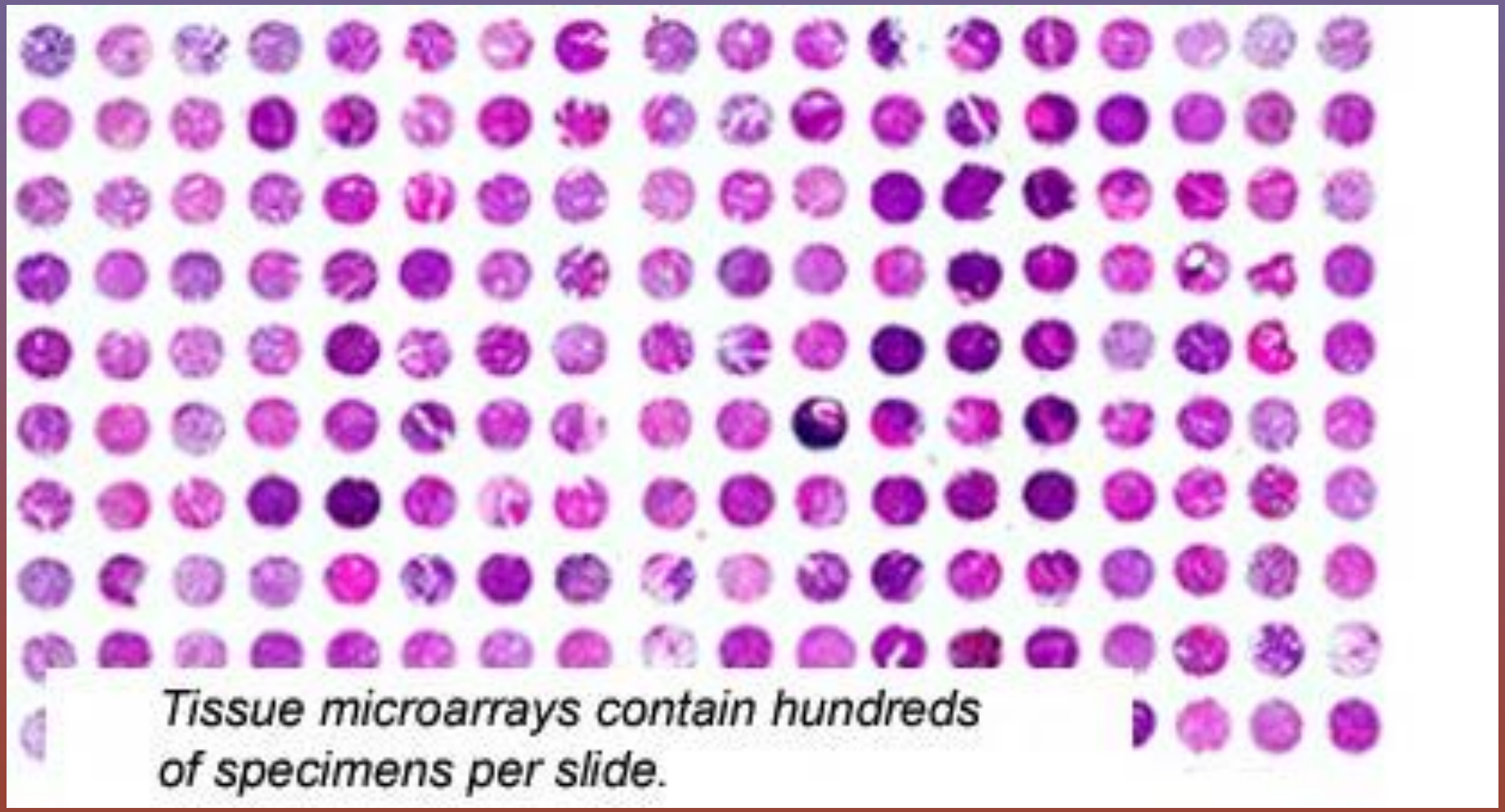
DNA Copy number and allele-specific information

Determine regions of chromosomal deletion (LOH) or amplification

Enables the identification of critical gene(s) that have altered copy number and may be responsible for the development and progression of a particular disease.

Tissue Arrays

Slide based “spotted” tissues (not really)



Tissue microarrays contain hundreds of specimens per slide.

Assembling Tissue Arrays

Coring of embedded paraffin tissues and plugging or inserting into new paraffin block

Sectioning and deposition onto a slide

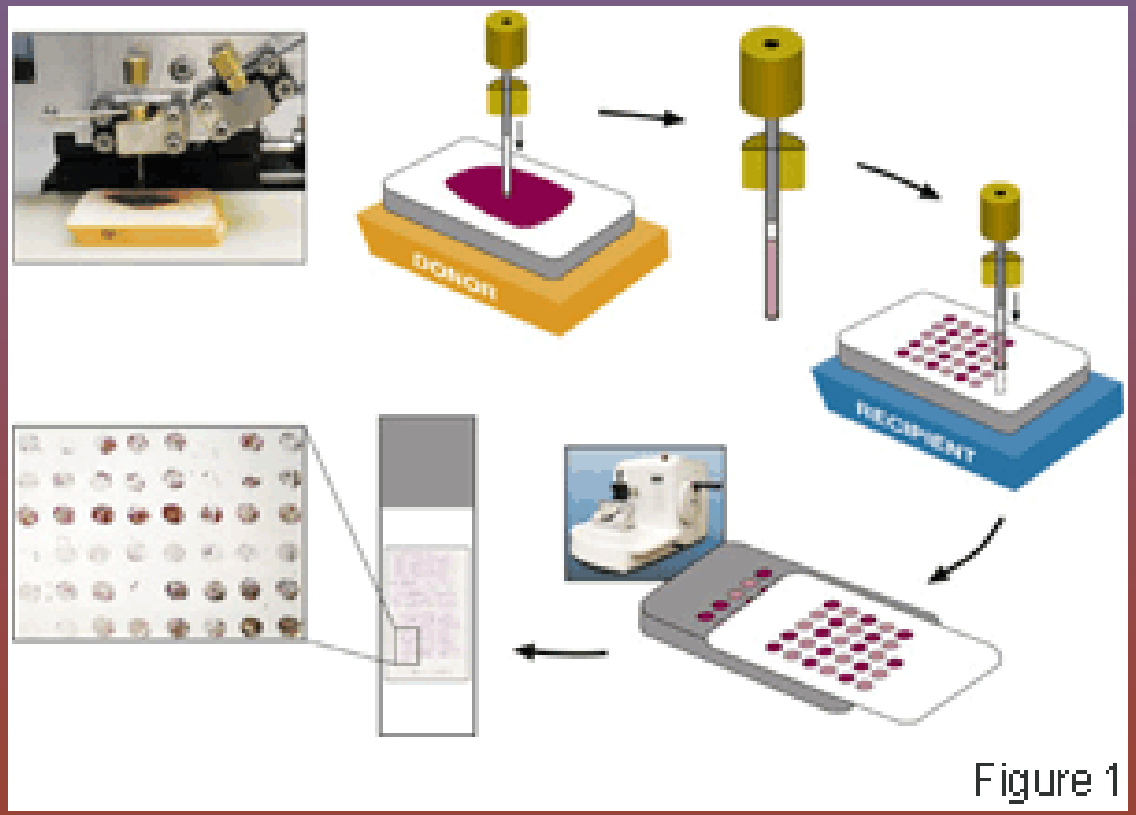


Figure 1

GeneChip Technology
Affymetrix Inc

Miniaturized, high density arrays of 1,300,000 DNA oligos 1-cm by 1-cm

Manufacturing Process:

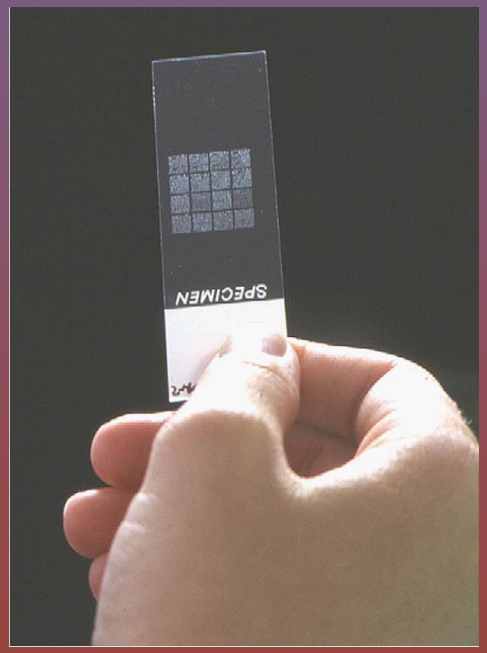
Solid-phase chemical synthesis and Photolithographic fabrication techniques employed in semiconductor industry

WE WILL DISCUSS THIS IN DETAIL IN ANOTHER PPT

Printed cDNA or Oligonucleotide Arrays

- **Robotically spotted cDNAs (50mer) or Oligonucleotides (70mers) vs. Affymetrix's that uses 25mers**

- **Printed on Nylon, Plastic, or Glass surface**



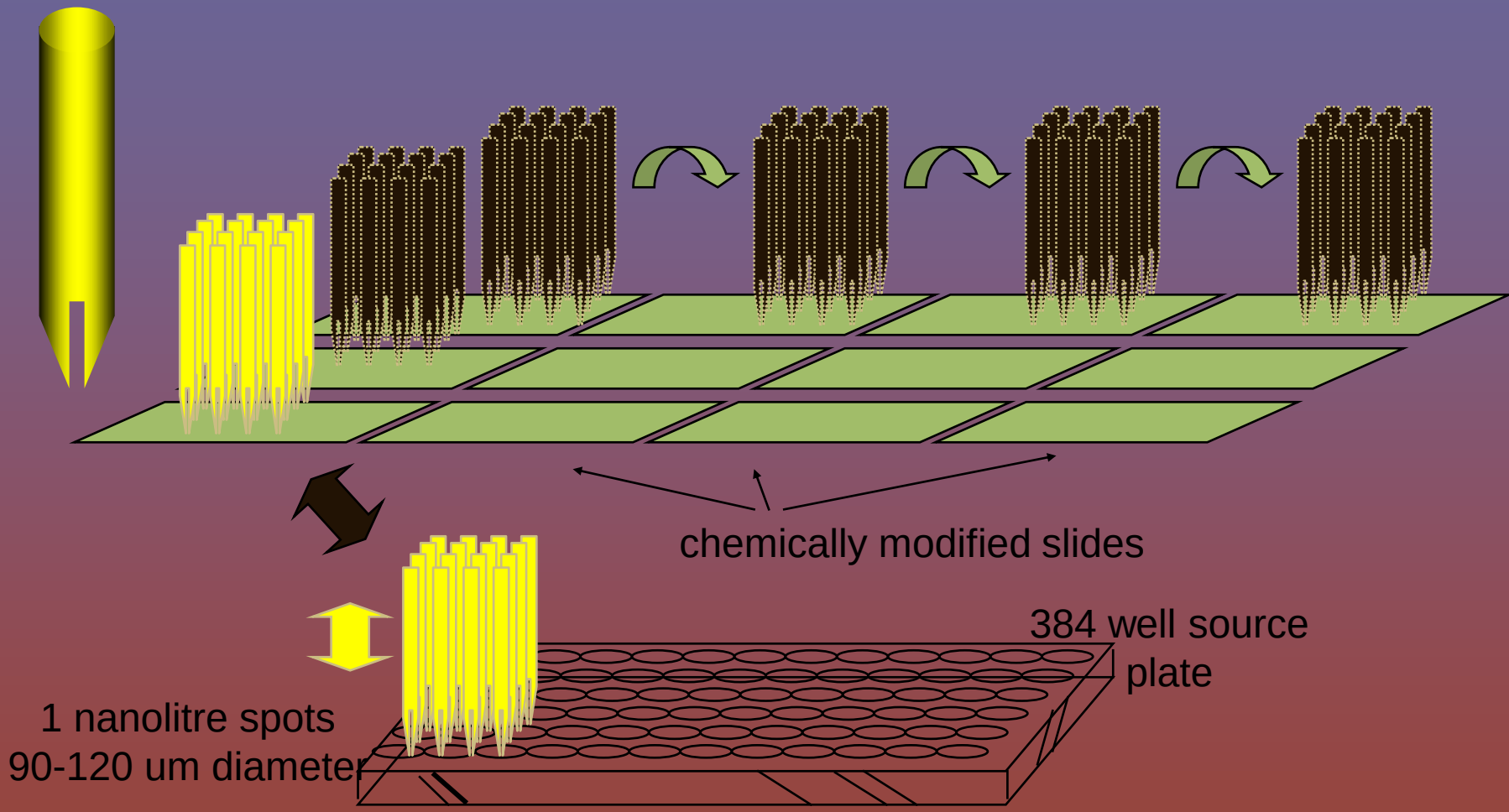
cDNA Array

Microarray of thousands of Oligos on a glass slide

Spotted arrays

steel

spotting pin



The process

Building the chip:

MASSIVE PCR → PCR PURIFICATION and PREPARATION → PREPARING SLIDES → PRINTING

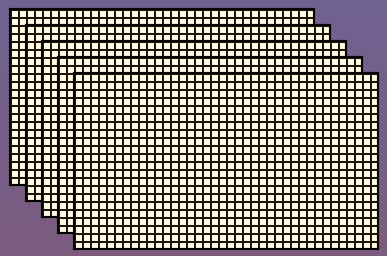
RNA preparation:

CELL CULTURE AND HARVEST → RNA ISOLATION → cDNA PRODUCTION

Hybing the chip:

PROBE LABELING → ARRAY HYBRIDIZATION → POST PROCESSING → DATA ANALYSIS

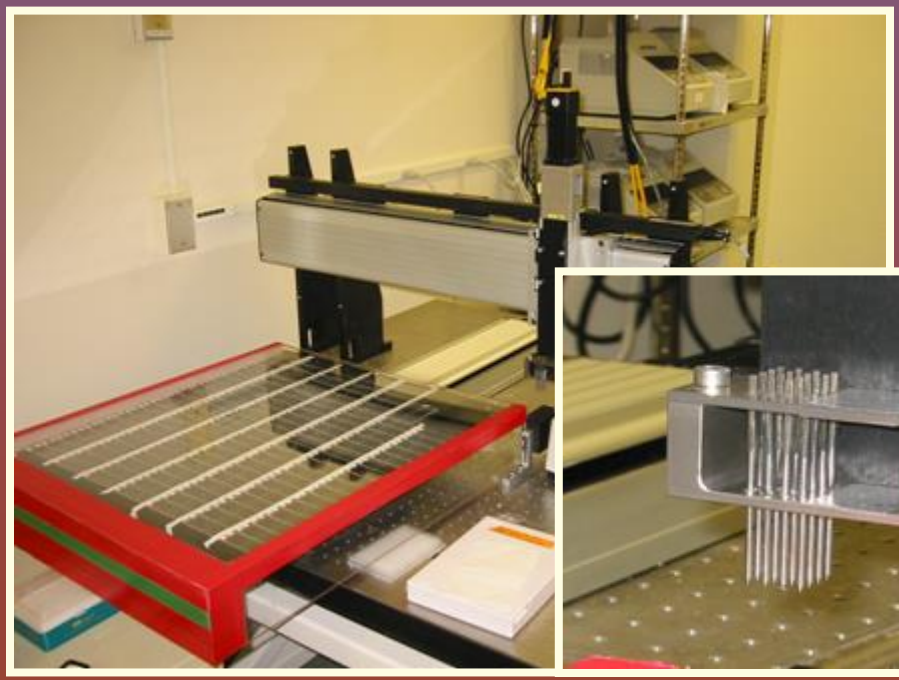
Building the chip



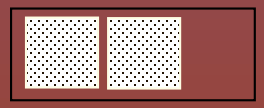
Arrayed Library
(96 or 384-well plates of bacterial glycerol stocks)

PCR amplification
Directly from colonies with SP6-T7 primers in 96-well plates

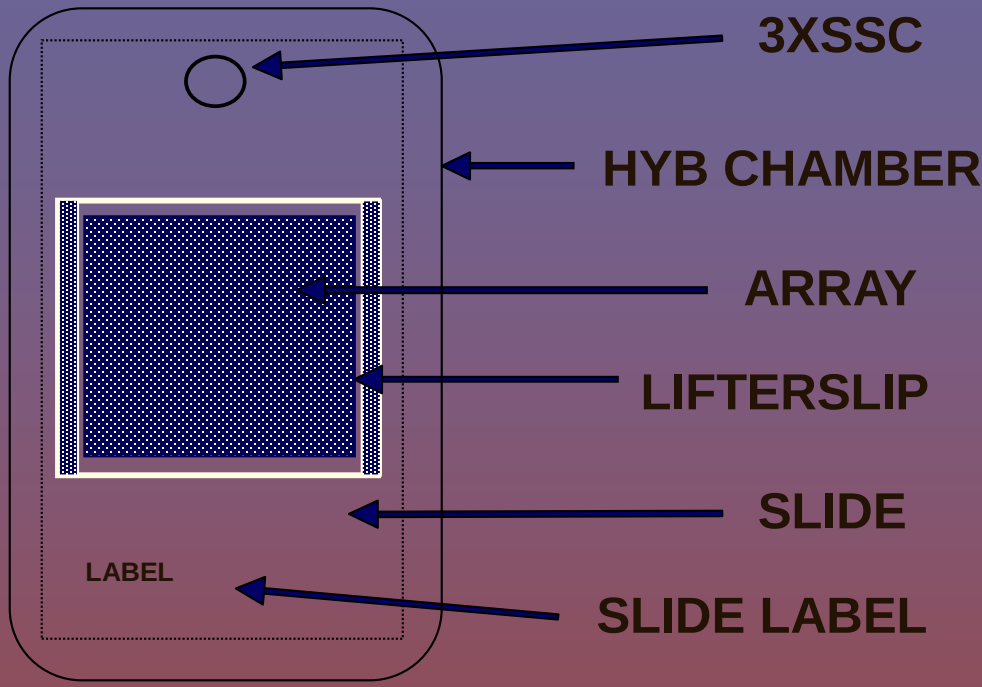
Consolidate into 384-well plates



Spot as microarray on glass slides



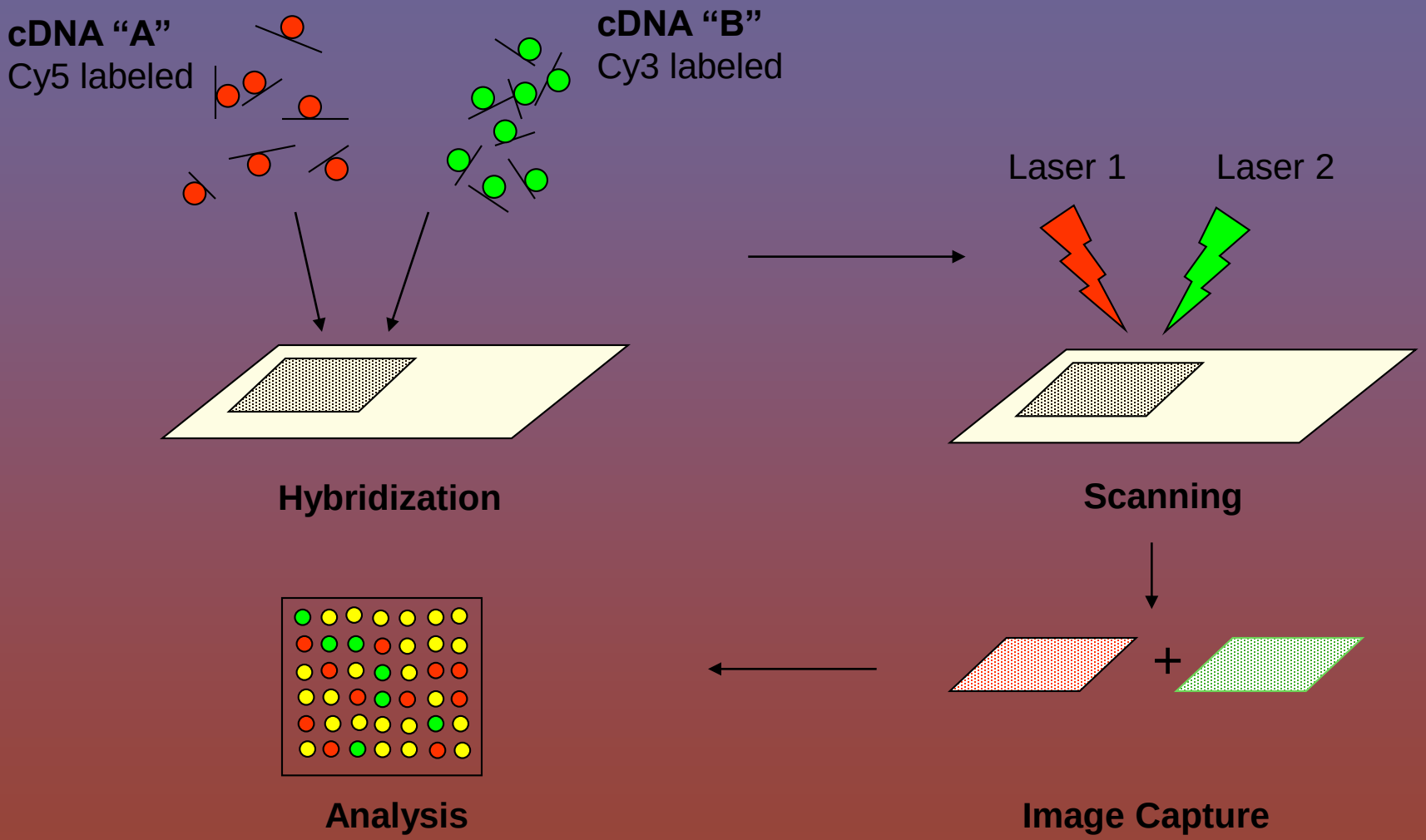
Hybridization chamber



- Humidity
- Temperature
- Formamide

(Lowers the Tm)

Expression profiling with cDNA microarrays



DNA Microarray Technology

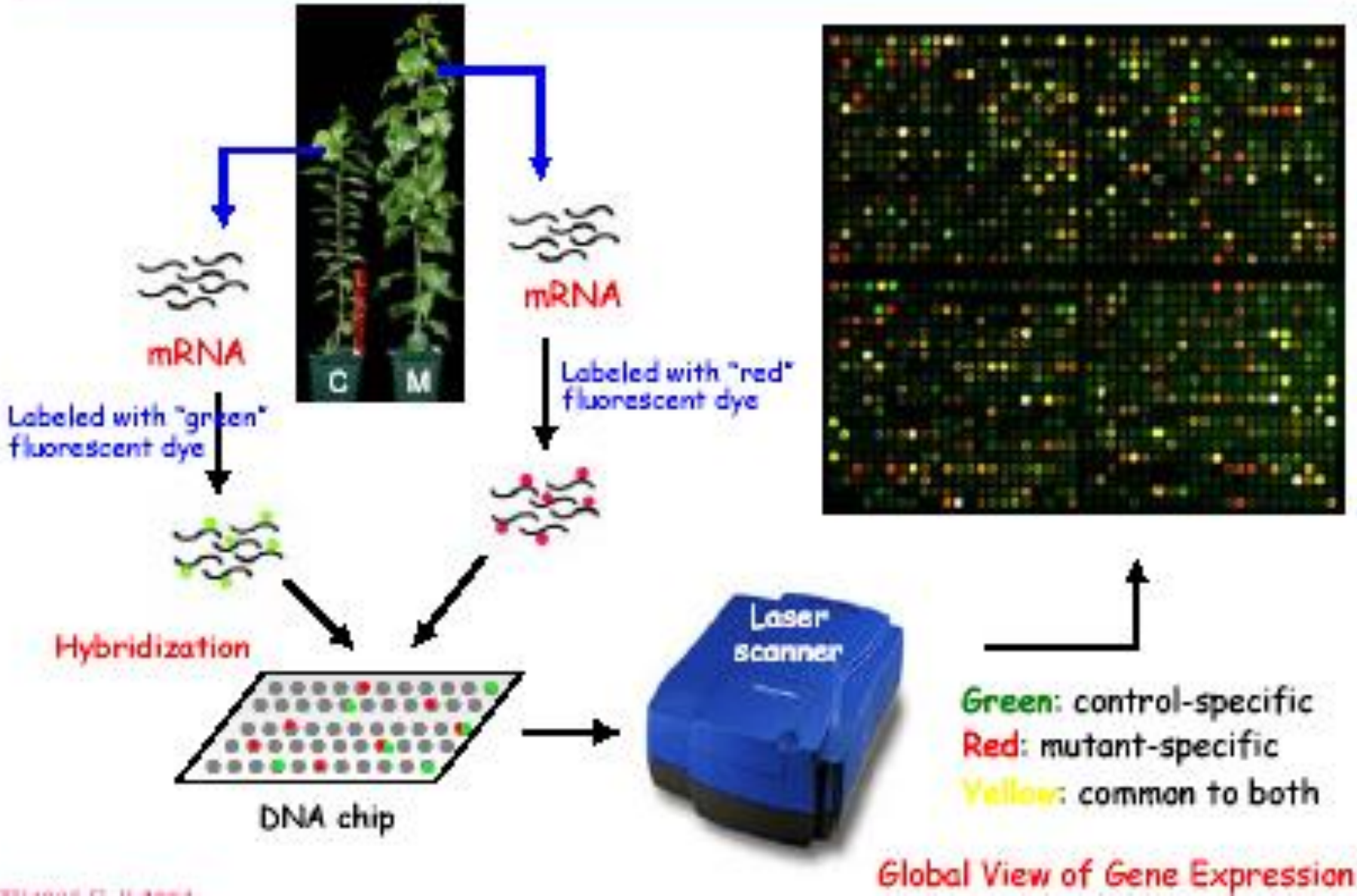
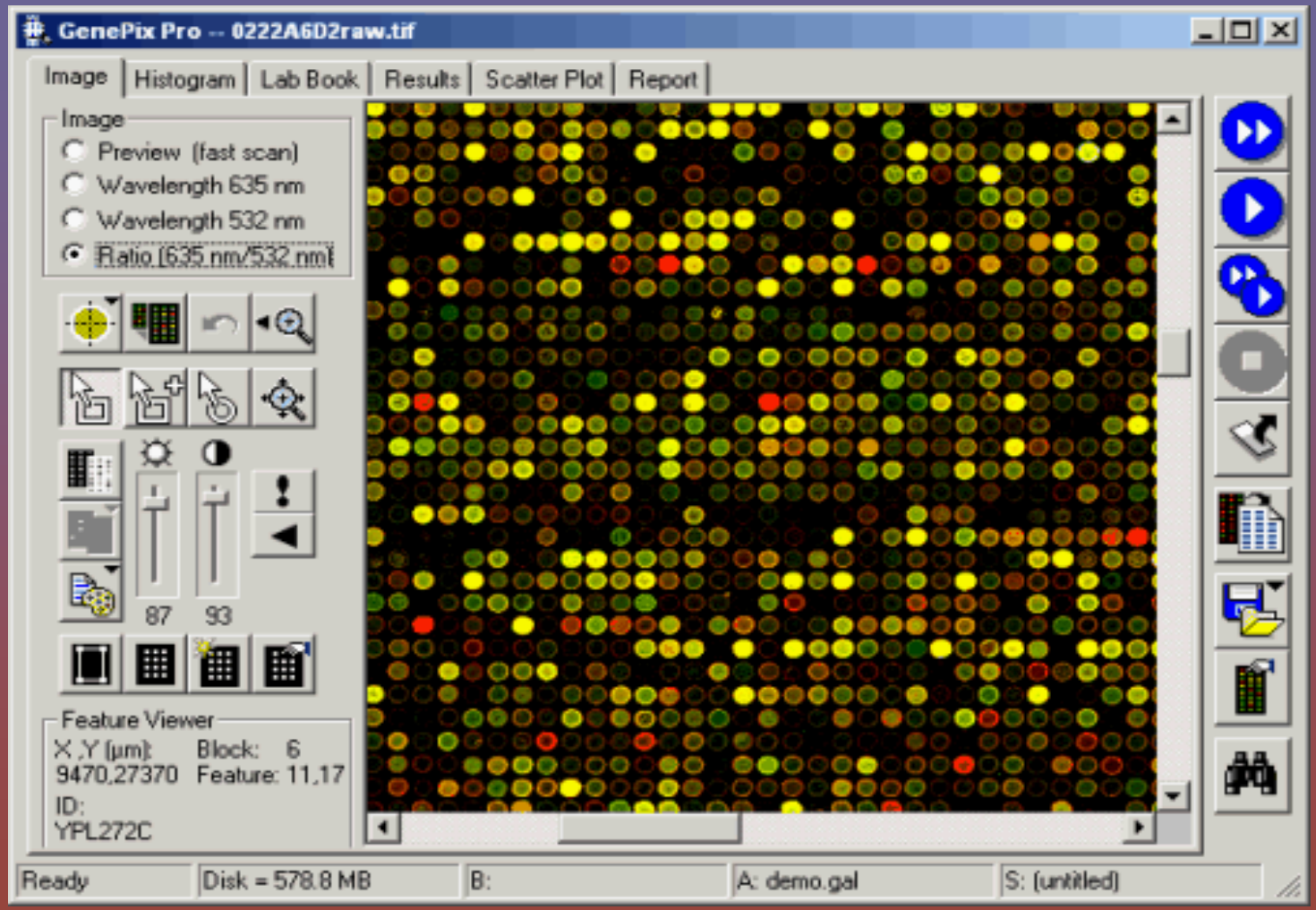


Image analysis of cDNA array



Spotted cDNA microarrays

Advantages

-Lower price and flexibility

-*Simultaneous* comparison of two related biological samples (tumor versus normal, treated versus untreated cells)

Disadvantages

-Needs sequence verification

-Measures the *relative* level of expression between 2 samples

-Features can come off the surface-poor adhesion

-Labor Intensive, requires designated staff and equipment

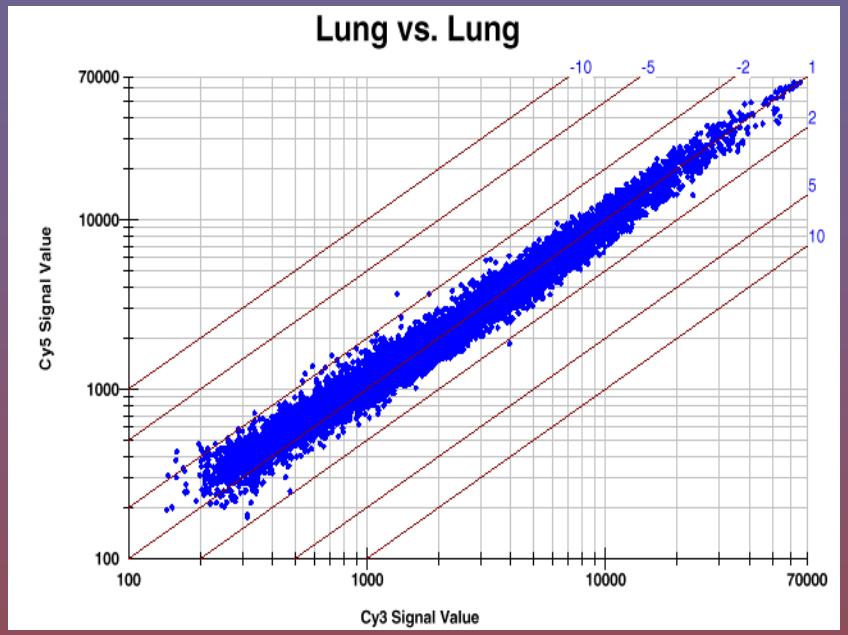
- Data is can be variable

Microarray data analysis

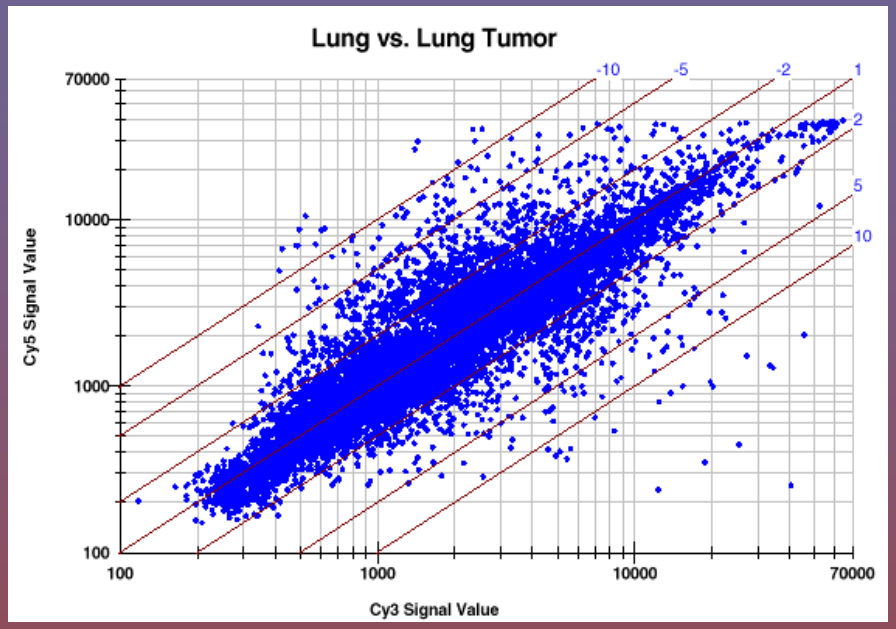
Scatter plots, significance analysis, clustering, pathway analysis… to name a few

- **Intensities of experimental samples versus normal samples**
- **Quick look at the changes and overall quality of microarray**

Normal vs. Normal



Normal vs. Tumor



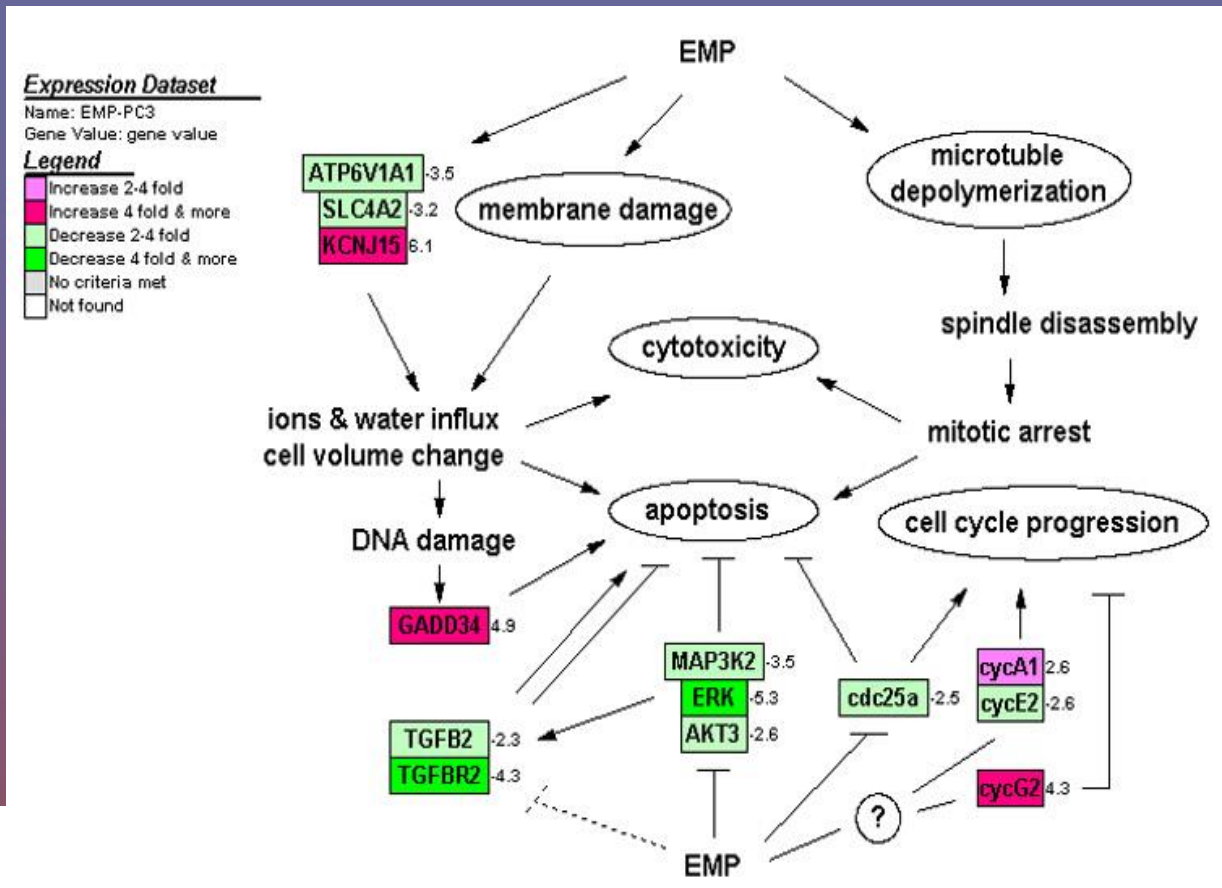
Bioinformatics : Microarray data analysis

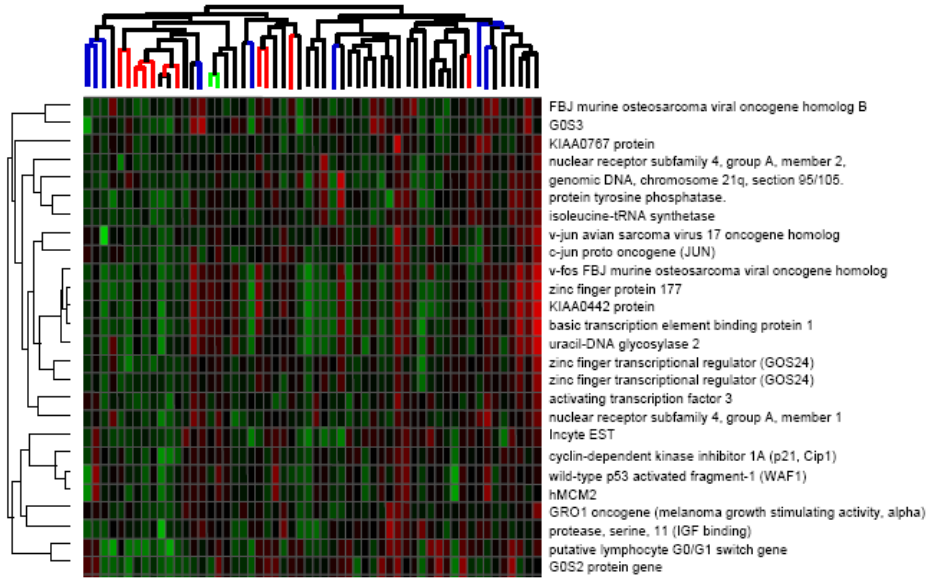
- Often is a stand alone dept. within an institute (such as the case at UVM), but works very closely with a microarray facility

- A whole field by itself

- Involves extensive knowledge of gene ontology, biochemical pathways, gene annotation, cell signaling, cellular trafficking , ….

- Uses special software such as Spotfire, Genesifter, Genespring…to name a few.


Expression Dataset
Name: EMP-PC3
Gene Value: gene value
Legend
Increase 2-4 fold
Increase 4 fold & more
Decrease 2-4 fold
Decrease 4 fold & more
No criteria met
Not found
EMP
ATP6V1A1 -3.5
SLC4A2 -3.2
KCNJ15 6.1
membrane damage
microtuble depolymerization
spindle disassembly
cytotoxicity
ions & water influx
cell volume change
mitotic arrest
apoptosis
cell cycle progression
DNA damage
GADD34 4.9
MAP3K2 -3.5
ERK -5.3
AKT3 -2.6
cdc25a -2.5
cycA1 2.6
cycE2 -2.6
TGFB2 -2.3
TGFBR2 -4.3
cycG2 4.3
?
EMP


FBJ murine osteosarcoma viral oncogene homolog B
G0S3
KIAA0767 protein
nuclear receptor subfamily 4, group A, member 2,
genomic DNA, chromosome 21q, section 95/105.
protein tyrosine phosphatase.
isoleucine-tRNA synthetase
v-jun avian sarcoma virus 17 oncogene homolog
c-jun proto oncogene (JUN)
v-fos FBJ murine osteosarcoma viral oncogene homolog
zinc finger protein 177
KIAA0442 protein
basic transcription element binding protein 1
uracil-DNA glycosylase 2
zinc finger transcriptional regulator (GOS24)
zinc finger transcriptional regulator (GOS24)
activating transcription factor 3
nuclear receptor subfamily 4, group A, member 1
Incyte EST
cyclin-dependent kinase inhibitor 1A (p21, Cip1)
wild-type p53 activated fragment-1 (WAF1)
hMCM2
GRO1 oncogene (melanoma growth stimulating activity, alpha)
protease, serine, 11 (IGF binding)
putative lymphocyte G0/G1 switch gene
G0S2 protein gene

Validating Microarray Expression Data

Microarray data are not stand alone results and requires validation by second method

Microarray data is only semi-quantitative because of a limited dynamic range.

True quantitative results must be determined with another technique such as Quantitative real-time PCR

Microarray Validation

Two types of validation

1] Validating the instrument data using the same RNA (confirming a result)

And most importantly

2] Validating the biological phenomenon with new samples same experiment conditions

Methods

Northern Blots, RPA's, Immunohistochemistry,Western Blot, *in silico*

PCR- i.e.Quantitative real-time PCR

**DNA mapping Arrays or CGH may also help indicate where or why a change is occuring

Microarray Applications

- Identify new genes implicated in disease progression and treatment response (90% of our genes have yet to be ascribed a function)
- Assess side-effects or drug reaction profiles
- Extract prognostic information, e.g. classify tumors based on hundreds of parameters rather than 2 or 3.
- Identify new drug targets and accelerate drug discovery and testing

Microarray Applications

- Gene Discovery-
  - Assigning function to sequence
  - Discovery of disease genes and drug targets
  - Target validation
- Genotyping
  - Patient stratification (pharmacogenomics)
  - Adverse drug effects (ADE)
- Microbial ID

Microarray Future

Diagnostics -[Affy, Nanogen only at this time]

- Disease detection
- Tumor classification
- Patient stratification
- Intervention therapeutics

Treatment and Customized Medicine

Clinical arrays currently available are the AmpliChip CYP450 by Affymetrix and Roche. Used for predictive phenotyping in defects of the cytochrome P450 Genes

Conclusion

- Technology is evolving rapidly
- Blending of biology, automation, and informatics
- New applications are being pursued
  - Beyond gene discovery into screening, validation, clinical genotyping, etc
- Microarrays are becoming more broadly available and accepted
  - Protein Arrays, tissue arrays, etc
  - Diagnostic Applications


HEY YOU!
Thanks FOR Listening
YOU ARE PRETTY WONDERFUL!