

DNA arrays for early disease detection

Chem 395
Bioanalytical chemistry

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Presented by
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Outline

- ❖ What is a Microarray?
- ❖ Types of Microarray
- ❖ Steps involved in Microarray fabrication
- ❖ What happens to the Genes in disease state?
- ❖ Application of Microarray
- ❖ Summary

What is a Microarray?

A microarray is a **spatially ordered, minituarized** arrangement of multitude of **immobilized** reagents

Why Microarrays?????

- ✓ Small volume- miniaturization
- ✓ High throughput analysis
- ✓ Large information generated
- ✓ Less time required to analyze

Types of Microarrays

- ❖ **cDNA and Oligonucleotide Microarrays**

Probe: ssDNA, Oligonucleotide

Target: ssDNA, ssRNA

Principle: Hybridization



- ❖ **Protein Microarrays**

Probe: Antibody

Target: Antigen

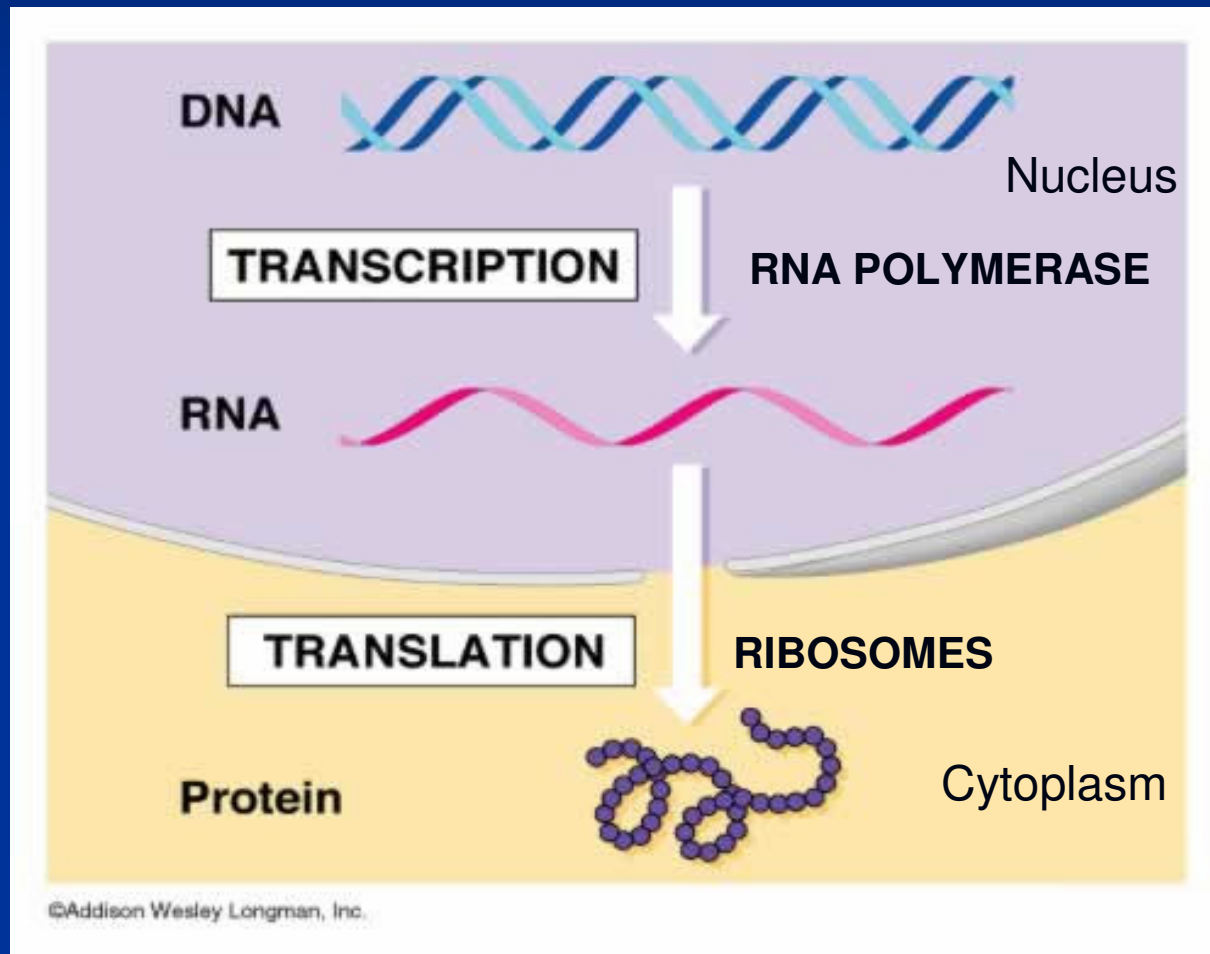
Principle: peptide chemistry



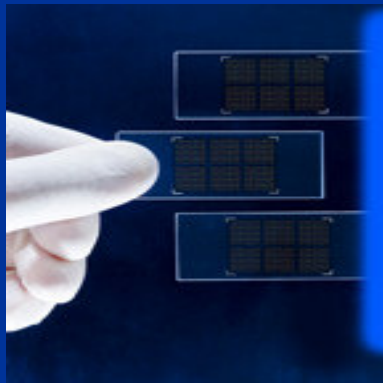
- ❖ **Others**

Tissue arrays

Gene expression



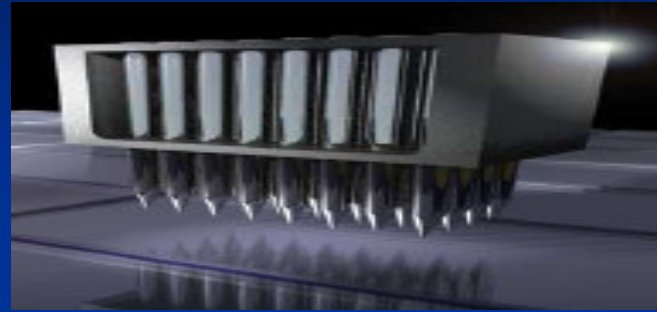
cDNA ARRAYS



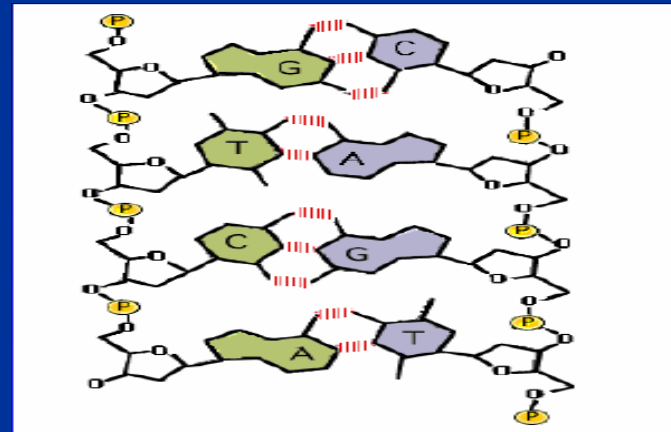
Marketed By: Agilent technologies

Steps Involved in Microarrays

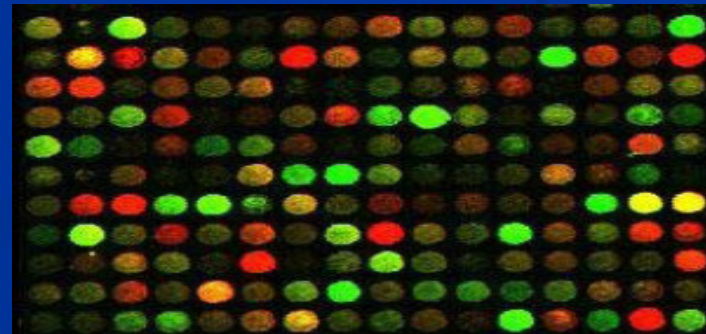
- ❖ Microarray printing



- ❖ Hybridization

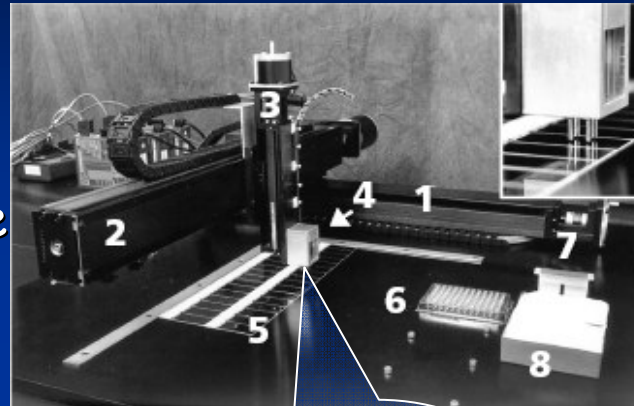


- ❖ Detection

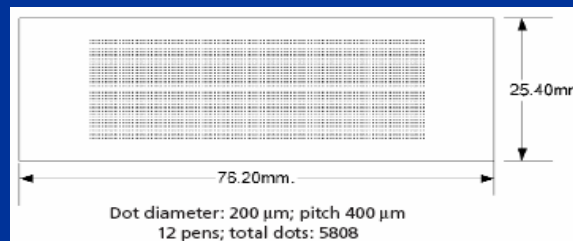
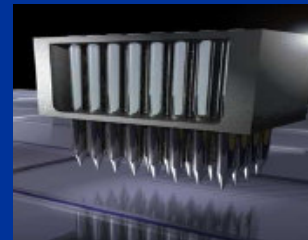


DNA-Microarray printing

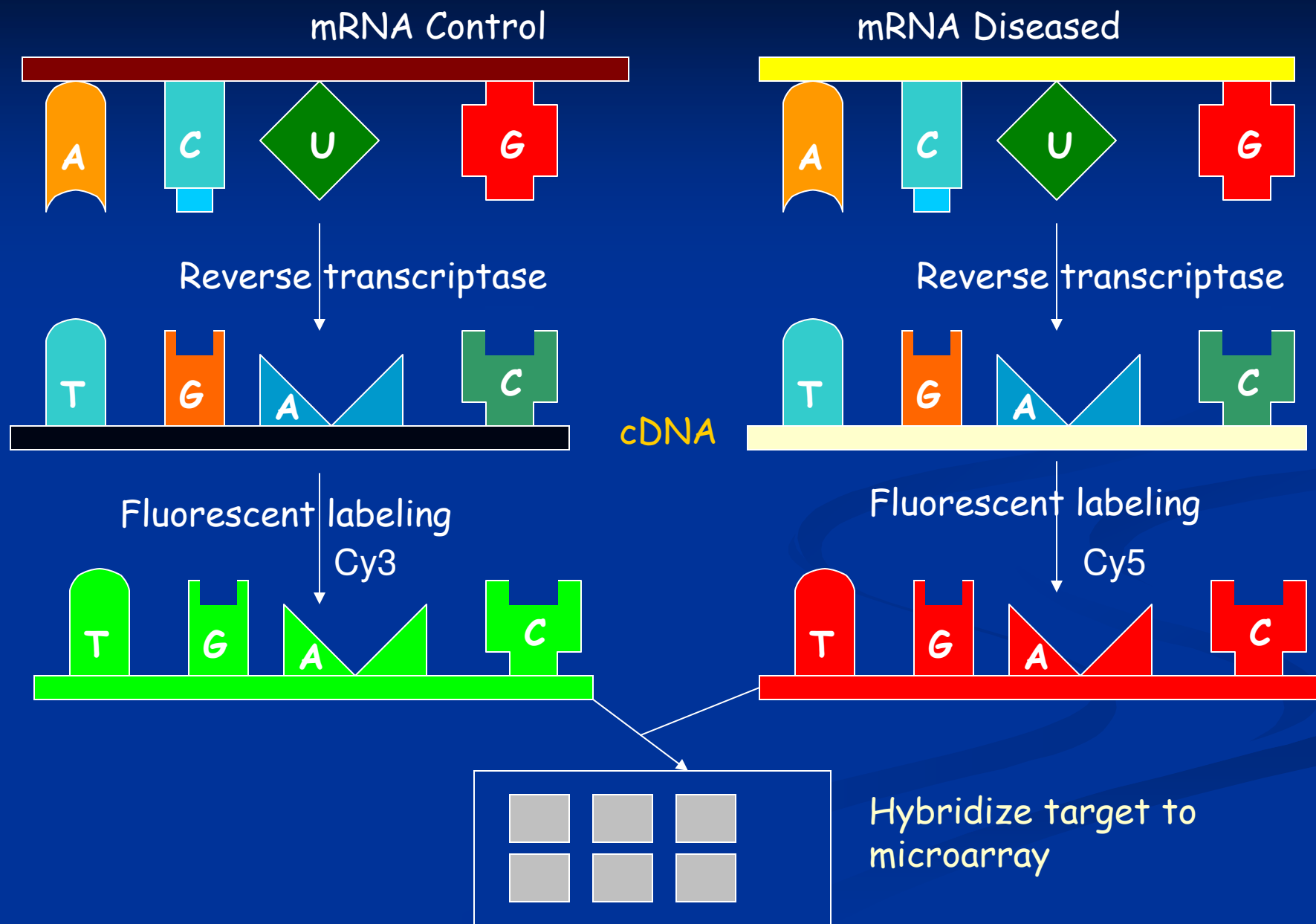
- 100-10000 spots
- Glass slide used as substrate
- DNA is attached covalently to glass slide
- 96-384 well microtitre plates used
- Spot Volume 0.25-1nl.
- Spot size 100-150 μ m in diameter



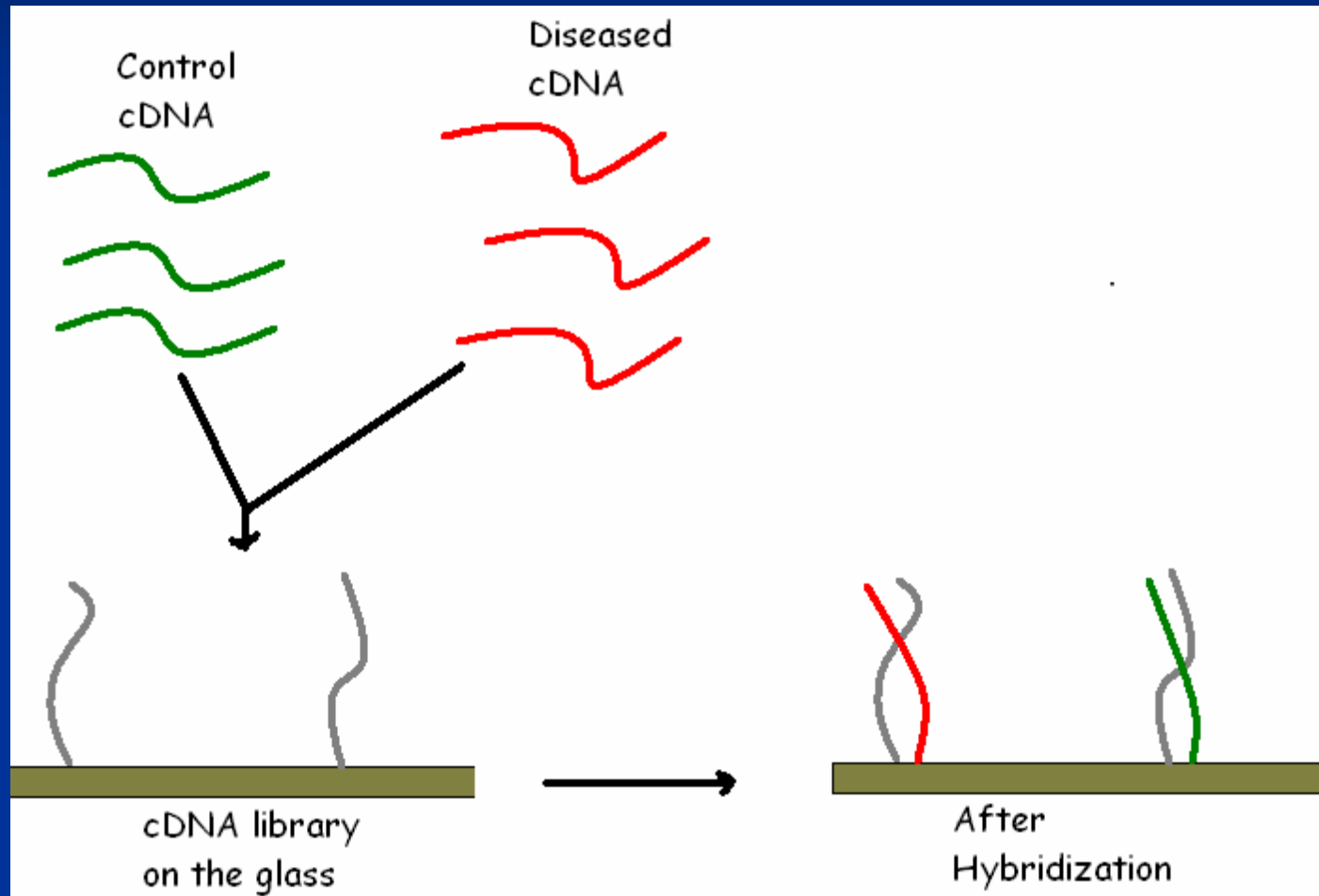
- 1,2,3-X,Y,Z direction
- 4-print head
- 5-glass slide
- 6-Microtitre plate
- 7-Distilled water
- 8- Drying



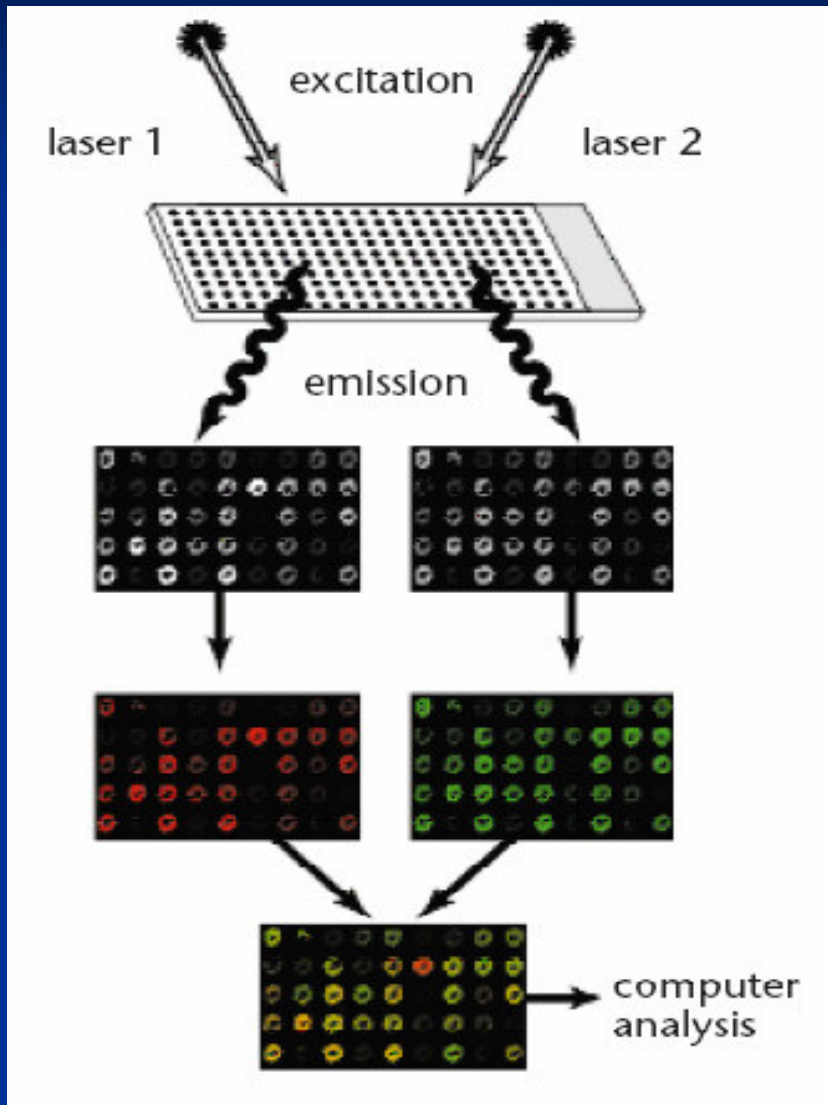
Hybridization



Hybridization



Detection:



The slide is scanned twice

- Once to measure **red intensity**

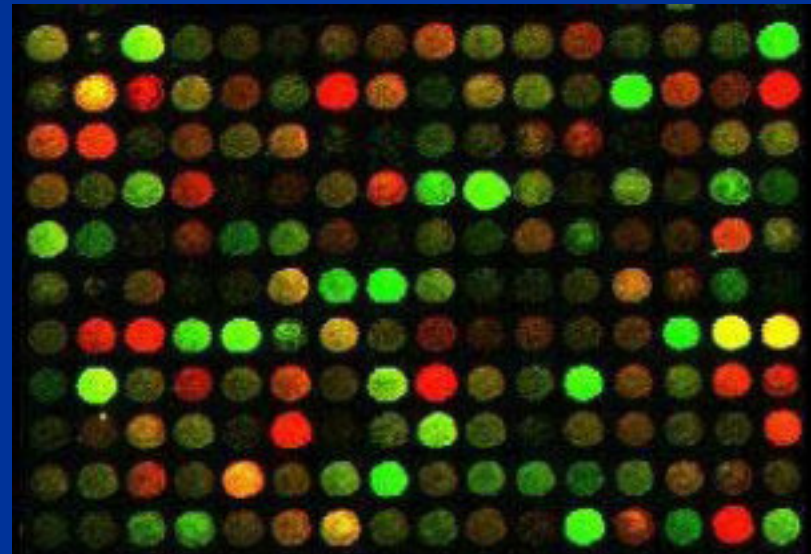
- Once to measure **green intensity**

The images are overlaid to produce one image

Scanning

$$M = \log R/G = \log R - \log G$$

- $M < 0$: gene is over-expressed in green-labeled sample compared to red-labeled sample.
- $M = 0$: gene is equally expressed in both samples.
- $M > 0$: gene is over-expressed in red-labeled sample compared to green-labeled sample.



What happens to Genes in (Cancer) Disease State

- ❖ Certain genes undergo overexpression.
- ❖ No. of copies of particular genes may increase.
- ❖ Gene mutation.

Changes in gene expression levels

- ✓ Gene is overexpressed in a certain **disease state**,
- ✓ More cDNA(target) will hybridizes to probe, as compared to control cDNA,
- ✓ In turn, the spot will fluorescence red with greater intensity than it will be with green.
- ✓ Expression patterns of various genes is characterized involved in many diseases,
- ✓ Compare expression pattern of the gene from the individual with the expression pattern of a known disease.

Genomic gains and losses

- ✓ Number of copies of a particular target gene has increased.
- ✓ Large amount of (Disease) sample DNA will hybridize to those spots on the microarray compared to (normal) control DNA hybridizing to those same spots.
- ✓ Those spots containing the sample DNA will fluoresce red with greater intensity than they will fluoresce green, indicating that the number of copies of the gene involved in the disease has gone up.

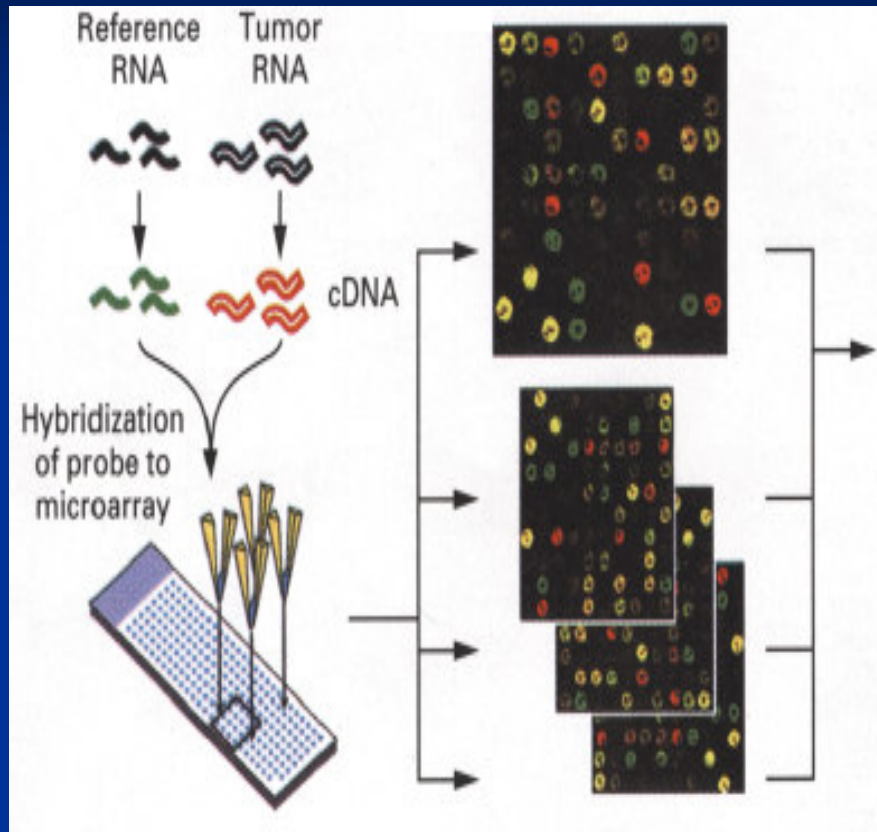
Gene Expression profile analysis in Human hepatocellular carcinoma by cDNA microarray

- ❖ Eun Jung Chung, Young Kwan Sung, MohammadFarooq, Younghee Kim, Sanguk Im, Won Young Tak, Yoon Jin Hwang, Yang Il Kim, Hyung Soo Han, Jung-Chul Kim, and Moon Kyu Kim., Mol. Cells, Vol. 14, pp382-387, 2002

HCC (Hepatocellular carcinoma)

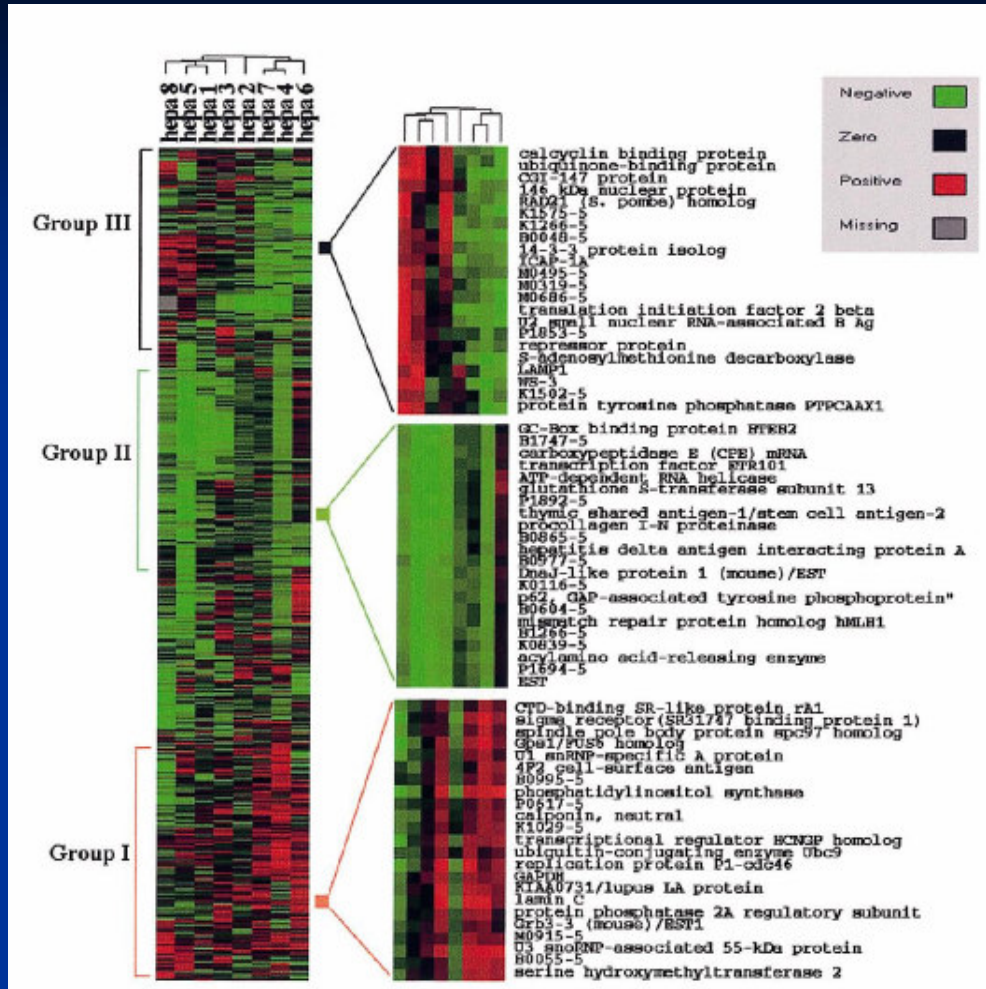
- ❖ Primary liver cancer (HCC)
- ❖ Somatic mutations and activation of certain oncogenes.
- ❖ These events Lead to expression changes in genes.
- ❖ cDNA arrays are used to analyze expression patterns

cDNA arrays



Computer analysis

3063 human cDNA
8 different samples of HCC



Up-regulated genes in HCC

- ❖ Galectin-3
- ❖ Serine/threonine kinase
- ❖ Fibroblast growth factor receptor
- ❖ Ribosomal protein L35A

Down-regulated genes in HCC

- ❖ mRNAs of Nip3
- ❖ Decorin
- ❖ Insulin-like growth factor binding protein-3

Gene expression patterns of 8 hepatocellular carcinomas. The genes were primarily classified into three groups, based on their clustering pattern.

Application of Microarrays

	Application
cDNA array.	Tumor classification, risk assessment, and prognosis prediction.
Expression analysis.	Drug development, drug response, and therapy development

Summary

- Data can be generated in a high throughput, parallel fashion.
- Less time required for analysis.
- If gene expression data is already known for a certain disease. Then we can compare the gene expression data of the individual with the known, and predict the disease

References

- David J. Duggan, Michael Bittner, Yidong Chen, Paul Meltzer & Jeffrey M. Trent, *Nature genetics supplement*, volume 21, january 1999.
- Sunil R. Lakhani, Michael J. O'hare & Alan ashworth, *Nature medicine*, volume 7, number 4, april 2001.
- Vivian G. Cheung, Michael Morley, Francisco Aguilar, Aldo Massimi, Raju Kucherlapati & Geoffrey Childs, *Nature genetics supplement*, volume 21, january 1999.
- <http://www.ncbi.nlm.nih.gov/>

Acknowledgement

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- Chem 395 students