Microarrays

- Microarray technology has a profound impact on gene expression and molecular Biology research.
- One or a few genes vs thousands of genes simultaneously
- Ability to analyse complete genomes in a single experiment.
- The main advantages of microarrays technology are:
  - High throughput
  - Multiple specimen screen
  - Time-efficiency
  - Low cost
  - Multiple uses

Microarrays-cont.
Understanding the molecular mechanisms

- Which genes are active in a cell and at what levels?
- Gene expression profiles of a control vs treated
- Which genes have increased or decreased in during an experimental condition?
- Which genes have biological significance in a system?
- Discovery of new genes, pathways, and cellular trafficking
- Whole genome sequenced BUT thousands of genes remain without an assigned function.

Microarrays-cont.
Mostly Medical and Pharmaceutical App.

- Identify new genes implicated in disease progression and treatment response
- 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

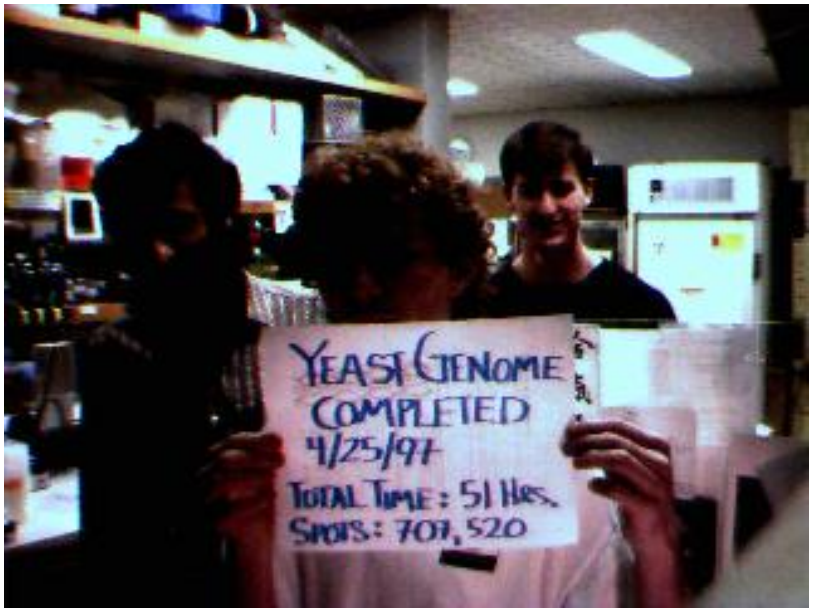
Microarrays-cont.

1989- First Affymetrix Genechip Prototype

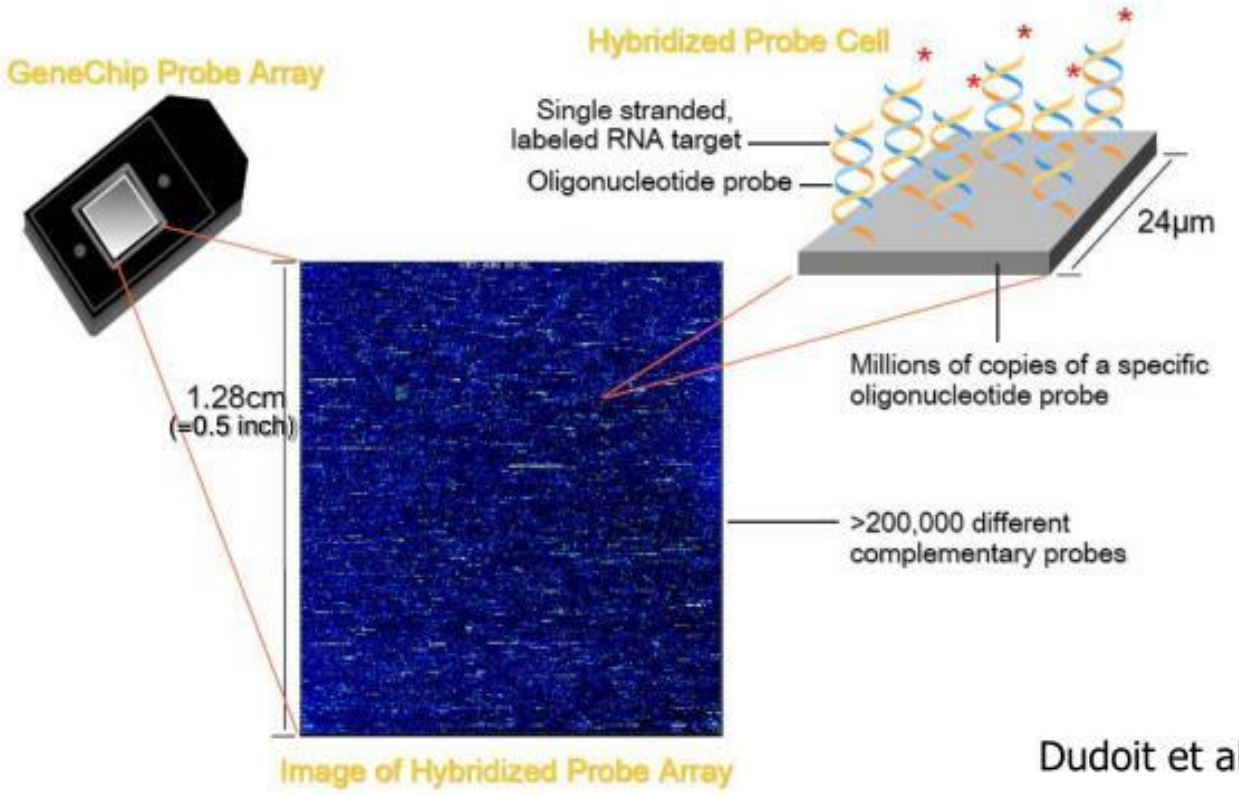
1994- First cDNAs arrays were developed at Stanford U.

1996- Commercialization of arrays

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



Affymetrix GeneChip®



Dudoit et al., 2002

Compliments of D. Gerhold

Microarray Formats

Microarray	Principle(s)	Format(s)	Density	Cost
Printed	Glass slides are used as the solid support for printing DNA probes	PCR amplicons (200-800 bp), cDNA, oligonucleotides, 25-80-bp	Moderate (~10,000-30,000)	$$$
In situ synthesized	Oligonucleotides are synthesized directly on the surface, multiple probe sets (one perfect-match probe and one mismatch probe)	Affymetrix GeneChips, 20-25-bp probes; Roche NimbleGen, 60-100-bp probes; Agilent, 60-bp probes	High (Affymetrix, $>10^6$; NimbleGen and Agilent, 15,000-$>10^6$)	$$$$
High-density bead arrays	Sequence-tagged beads are randomly assorted onto fiber-optic bundles or silicon slides	SAM, 96 samples; Sentrix BeadChip; 1-16 samples	High (~50,000-10^6)	$$$
Electronic	Electric fields are used to promote active hybridization of nucleic acids on a microelectronic device; streptavidin-biotin bonds immobilize the probes on the array surface	NanoChip 400; capture probe down; amplicon down; sandwich assays	Low (400 max)	$$

❑ **In situ synthesised (oligo)**

➢ **Cartridge-based :** Miniaturized, high density arrays of DNA oligos within a plastic housing

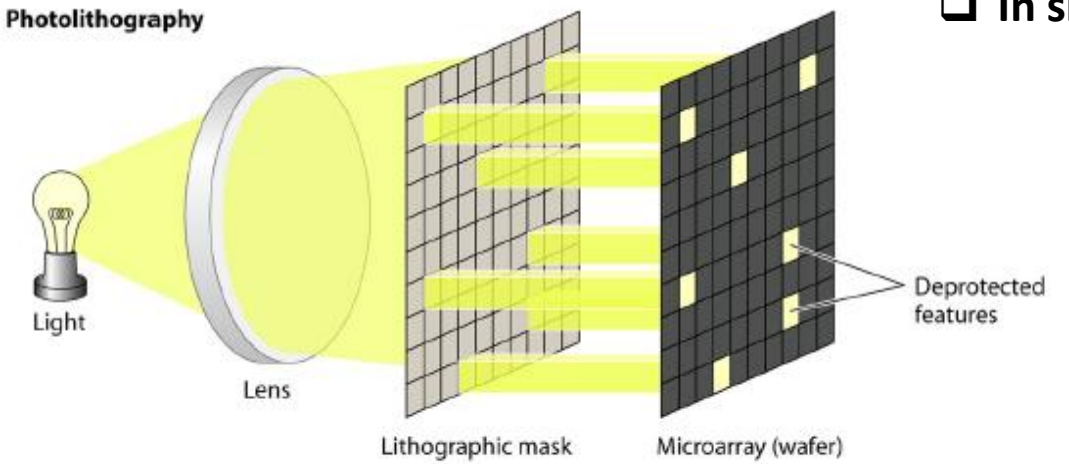
- One sample = One chip

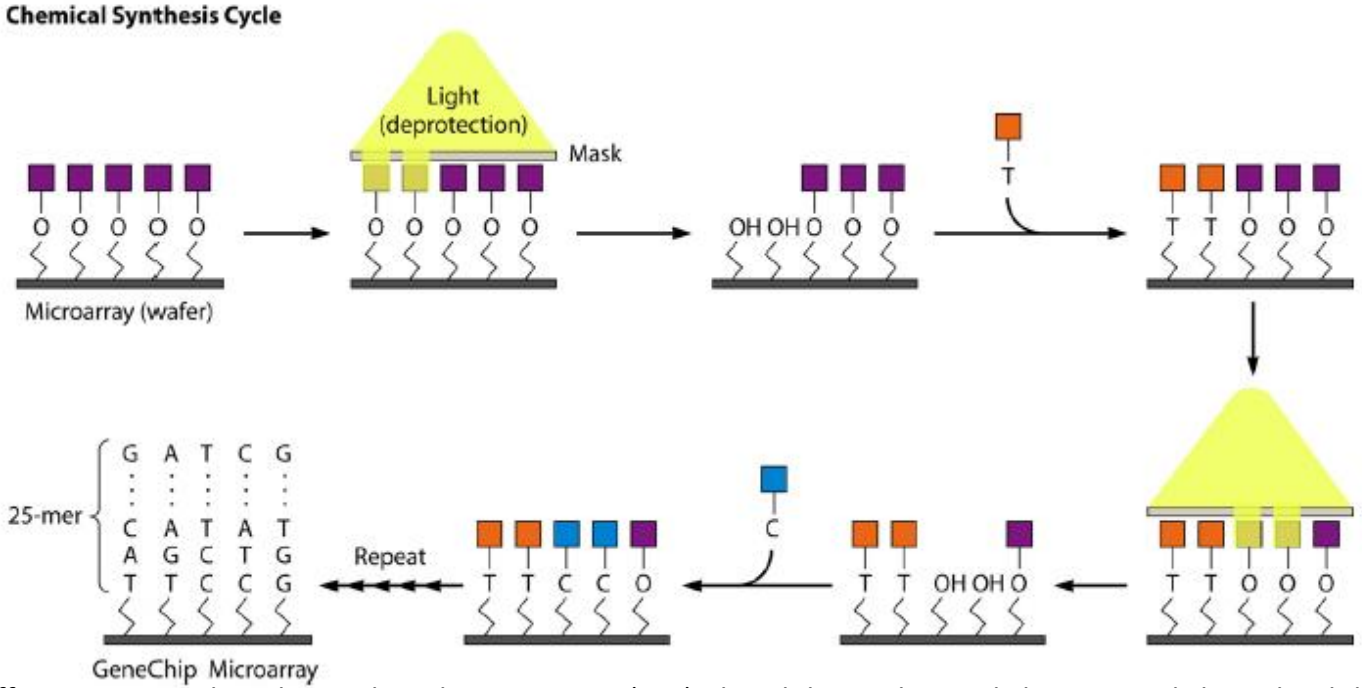
(Affymetrix, Agilent, Applied Biosystems…)

- Generally:
  - expression arrays
  - DNA arrays

❑ **In situ synthesised (oligo)**

➢ **Affimetrix GeneChip Oligonucleotide Array**

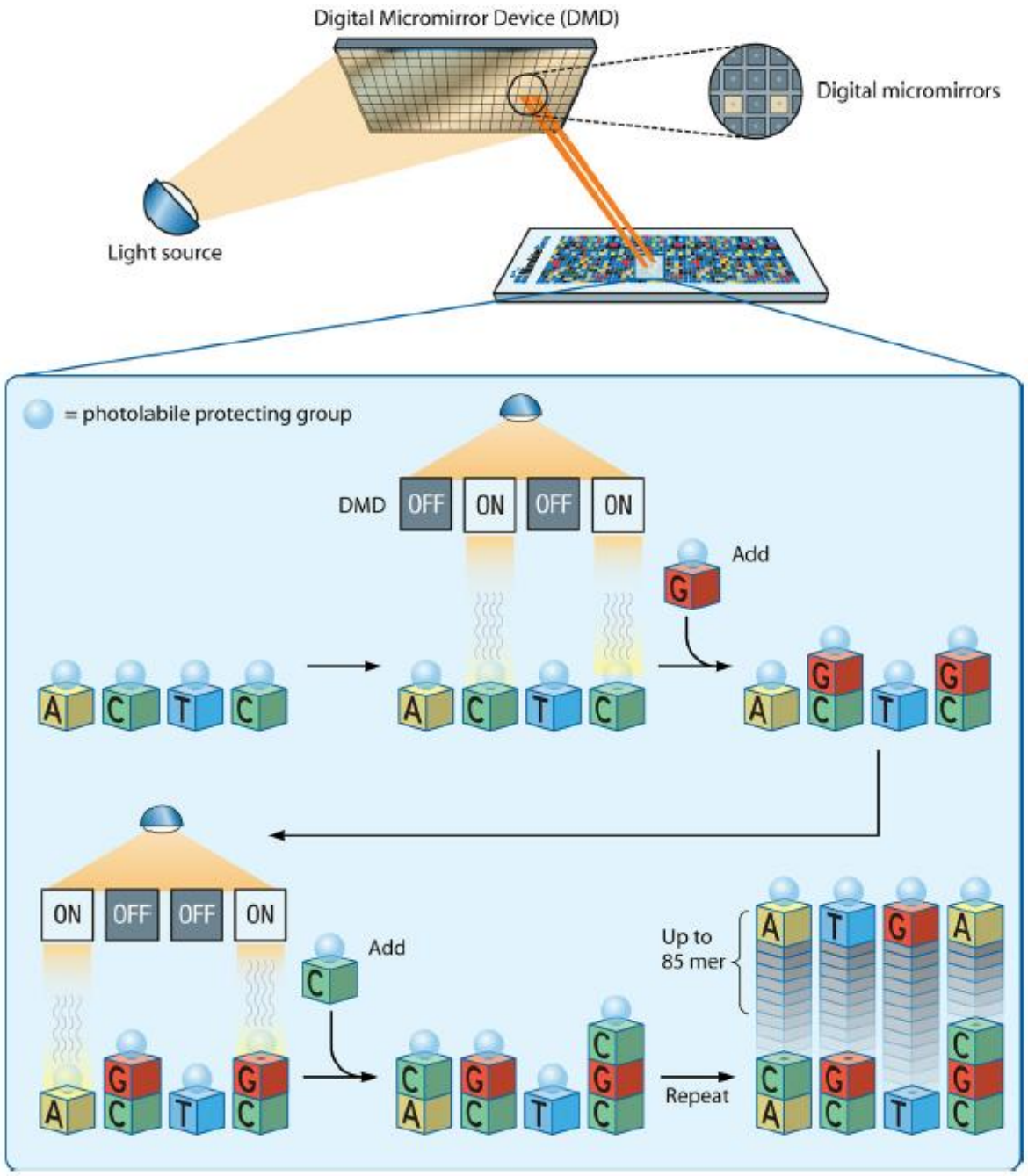




Affymetrix GeneChip oligonucleotide microarray. (Top) Photolithography. UV light is passed through a lithographic mask that acts as a filter to either transmit or block the light from the chemically protected microarray surface (wafer). The sequential application of specific lithographic masks determines the order of sequence synthesis on the wafer surface. (Bottom) Chemical synthesis cycle. UV light removes the protecting groups (squares) from the array surface, allowing the addition of a single protected nucleotide as it is washed over the microarray. Sequential rounds of light deprotection, changes in the filtering patterns of the masks, and single nucleotide additions form microarray features with specific 25-bp probes.

Roche NimbleGen oligonucleotide microarray

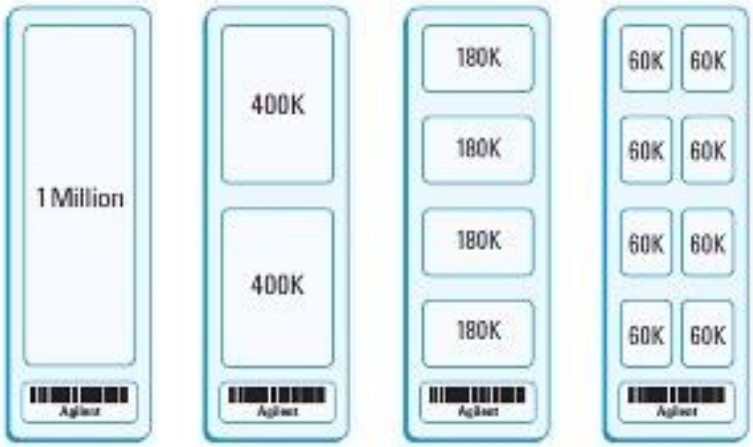
Maskless array synthesizer utilizing a digital micromirror device (DMD) to create virtual masks.

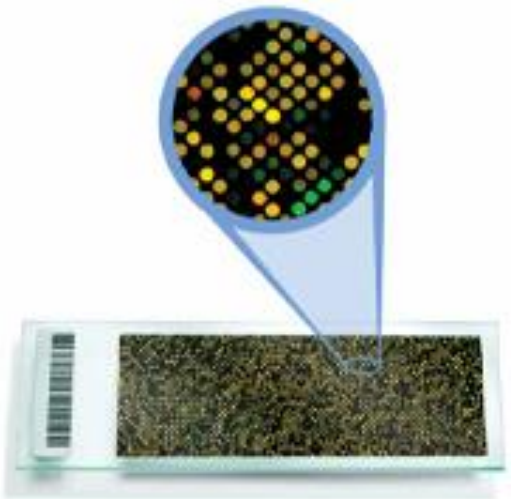


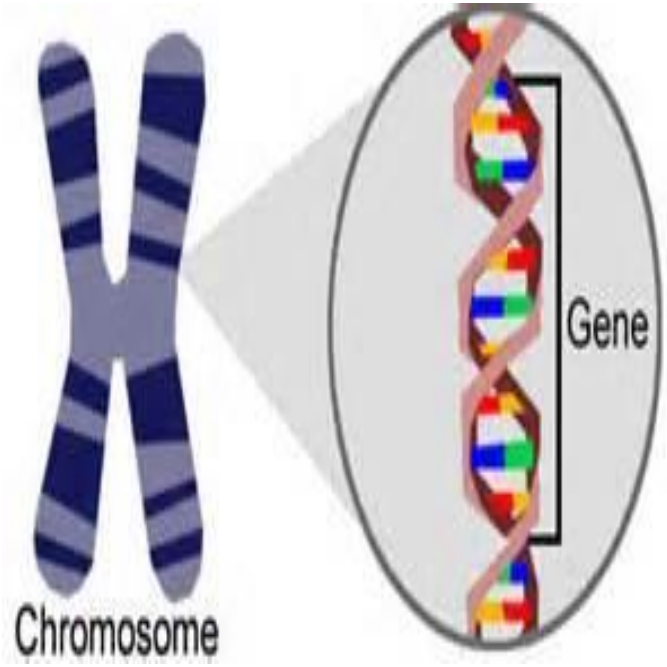
Roche NimbleGen oligonucleotide microarray. Maskless array synthesizer technology is depicted, which utilizes a digital micromirror device (DMD) to create virtual masks. The DMD forms the pattern of UV light needed to direct the specific nucleic acid addition during photo-mediated synthesis. UV light removes the photolabile protecting group (circles) from the microarray surface, allowing the addition of a single protected nucleotide to the growing oligonucleotide chain. The cycling of DMD filtering, light deprotection, and nucleotide addition creates oligonucleotide features 60 to 100 bp in length on the NimbleGen microarray

❑Spotted Glass Arrays (printed microarrays)

- Robotically spotted cDNA, Oligonucleotide, protein, antibody
- Printed on Nylon, Plastic, or Glass microscope slide

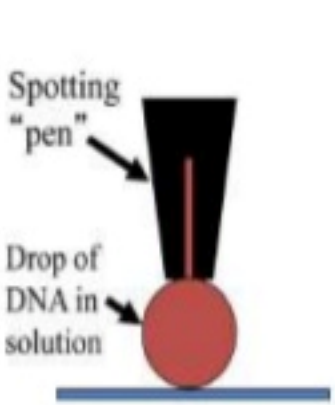






Start with individual genes

Amplify



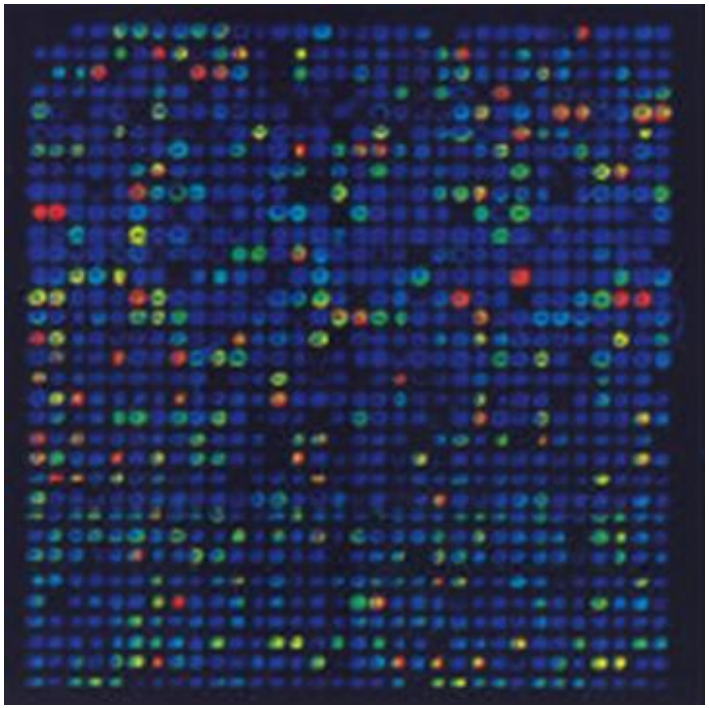
"Spot" them on a medium, e.g. an ordinary glass microscope slide usually chemically modified with poly(L-lysine) or other cross-linking chemical coating materials such as polyethyleneimine polymer, p-aminophenyl trimethoxysilane/diazotization

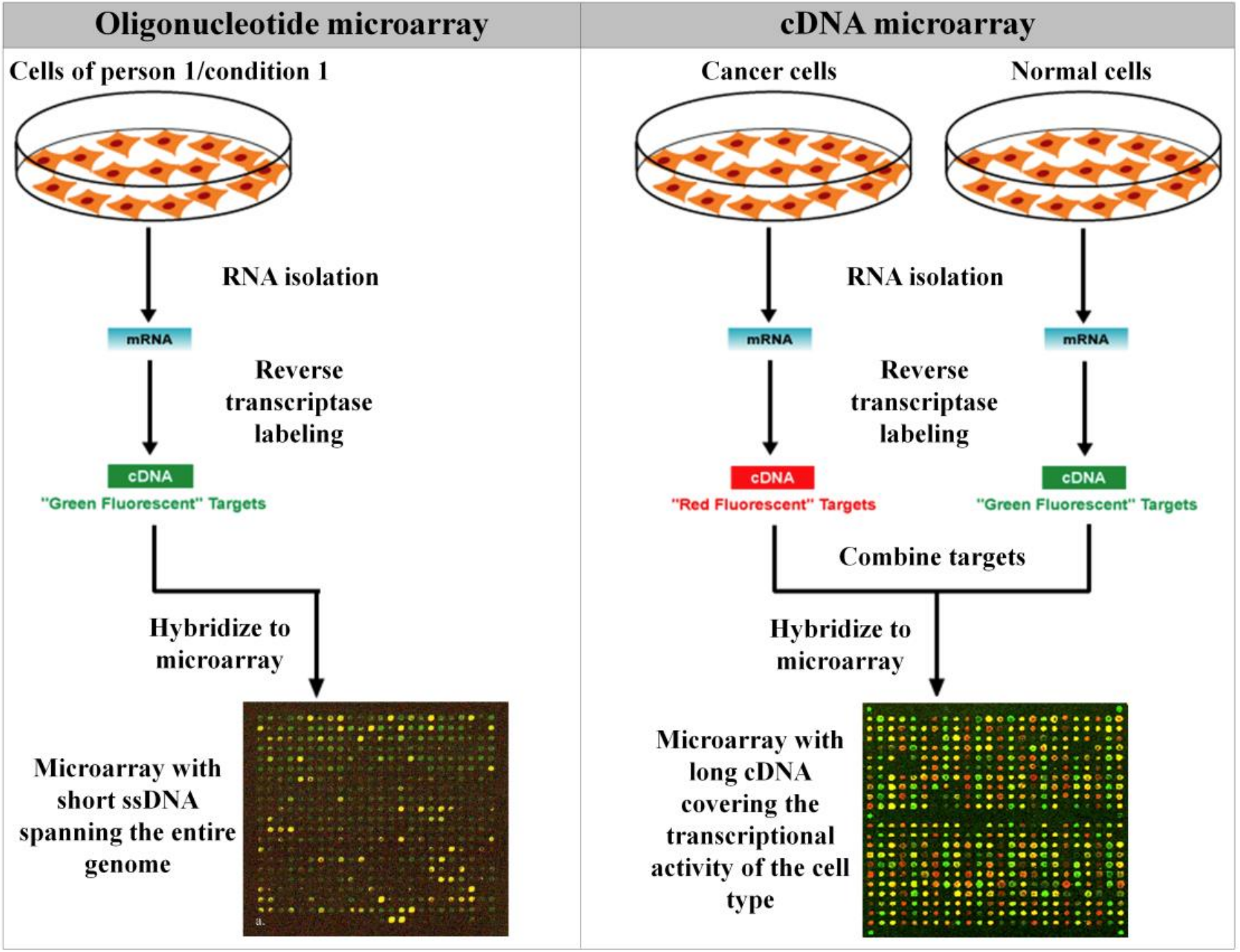
the DNA solution will be immobilised on the surface e.g. covalent or non covalent. However in the course of poly (L-lysine) the negatively charged phosphate groups in the DNA molecule, form an ionic bond with the positively charged amine-derivatised surface

Spotted Glass cDNA or oligo Arrays

Two dyes on the same slide

- Red dye-Cy5
- Green dye-Cy3
- ➢ For control and experimental cDNA





https://www.youtube.com/watch?v=6ZzFihESjp0

A comparison between cDNA and oligonucleotides arrays

cDNA Microarrays

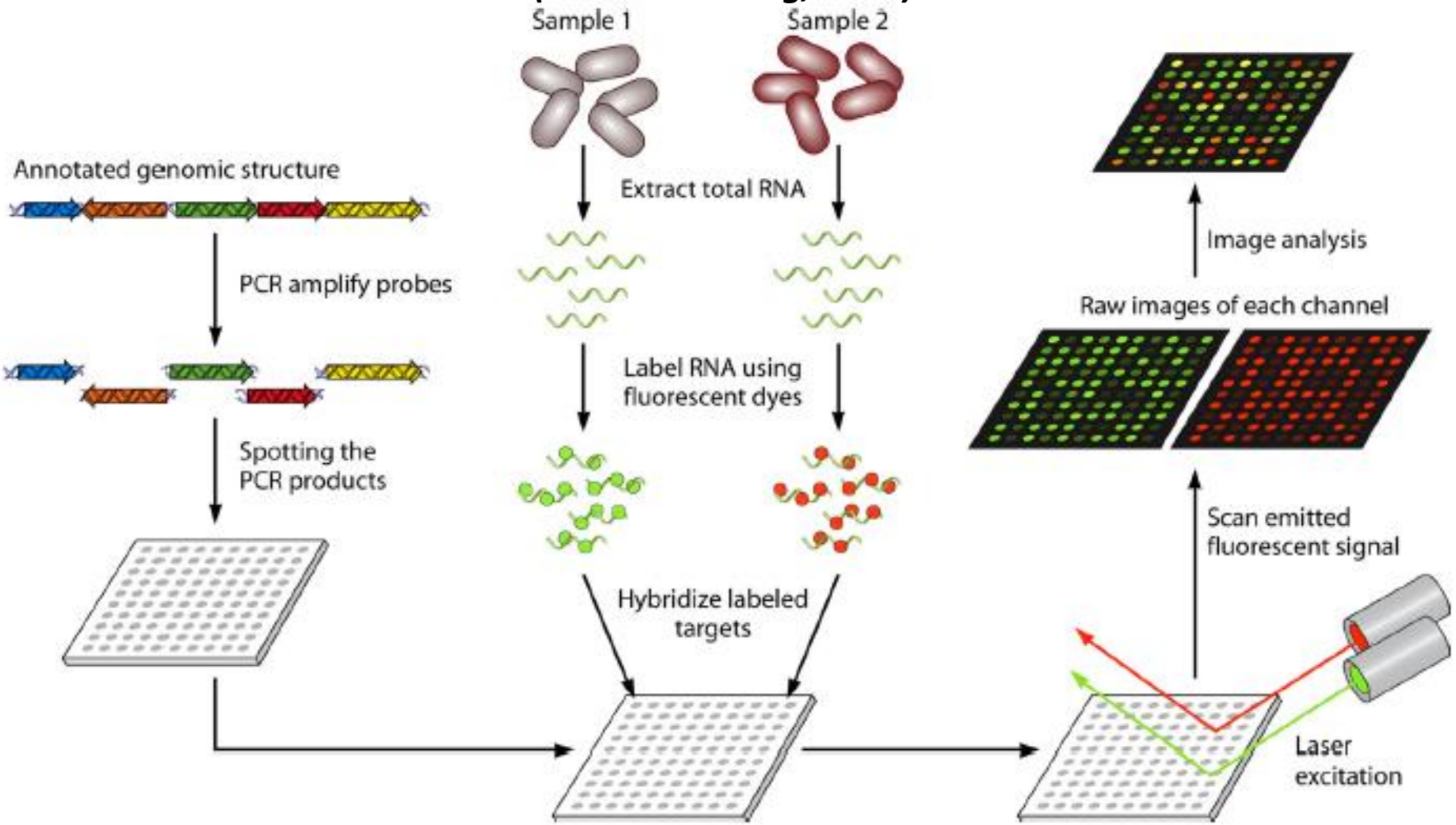
- Immobilisation of whole cDNA
- cDNA is obtained via becteria
- Spotted by inkjet technology
- cDNA libraries are needed

oligonucleotide Microarrays

- Immobilisation of characteristc sequences
- Sequences are designed
- Sequences are synthesized on the chip
- Several commercial products are available

General workflow of printed arrays

(Miller and Tang, 2009)



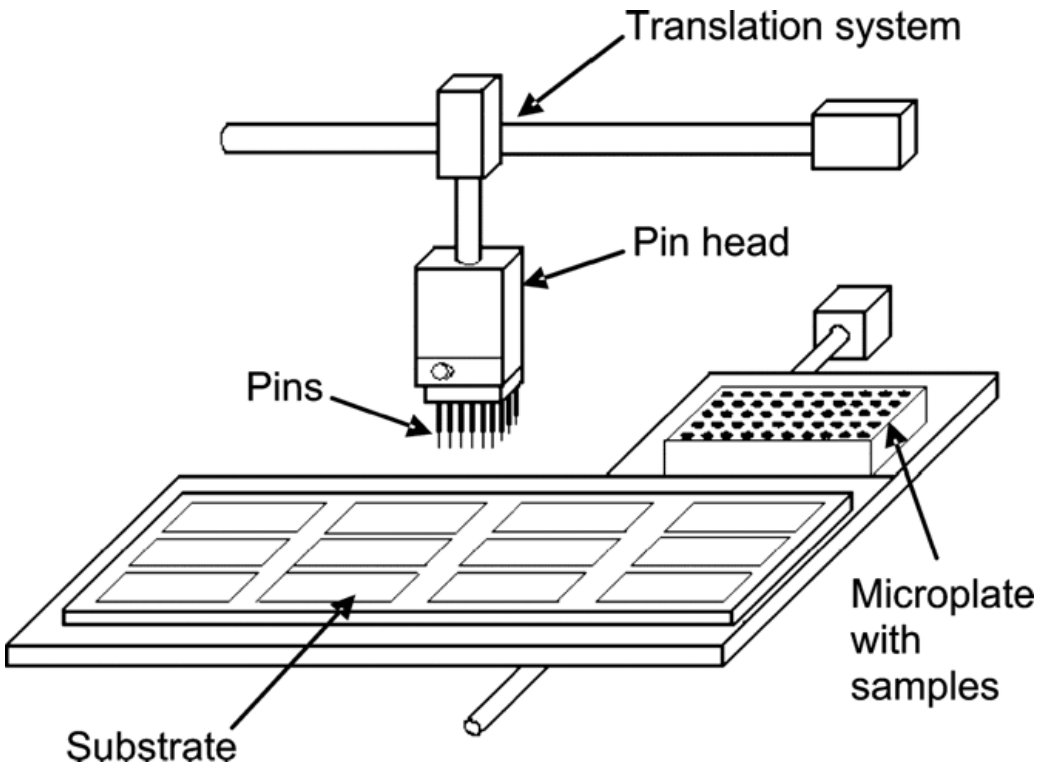
Workflow summary of printed microarrays. Probes are PCR amplified (or oligonucleotides are synthesized) and subsequently spotted onto a glass slide. In this example, two samples to be compared undergo RNA extraction, cDNA production, and differential fluorescent labeling. Hybridization of labeled target nucleic acids to the probe array allows fluorescent scanning to provide data for analysis.

Printing Arrays

Barbulovic-Nad I, et al. Critical Reviews in Biotechnology 26: 237-259, 2006

Pin printing: widely used for most non-commercial microarray fabrication

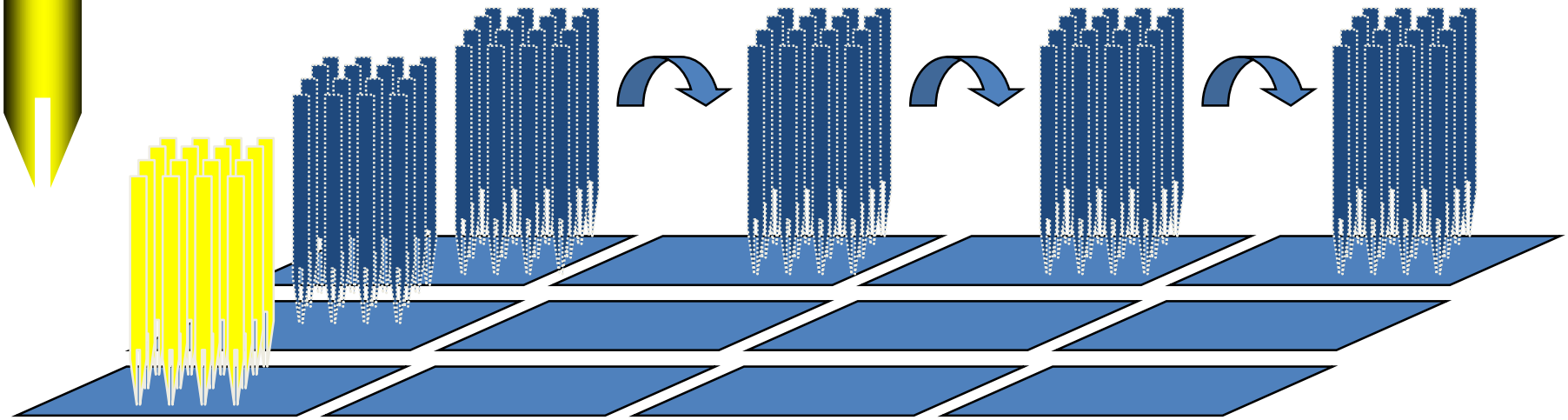
- The typical substrates: microscope slides treated with poly-lysine, amino silanes or amino-reactive silanes that enable DNA or protein to bind to the surface
- Alternatives: polymer-coated glass or plastics



steel

spotting pin

Spotting is done by a robot such as inkjet printing



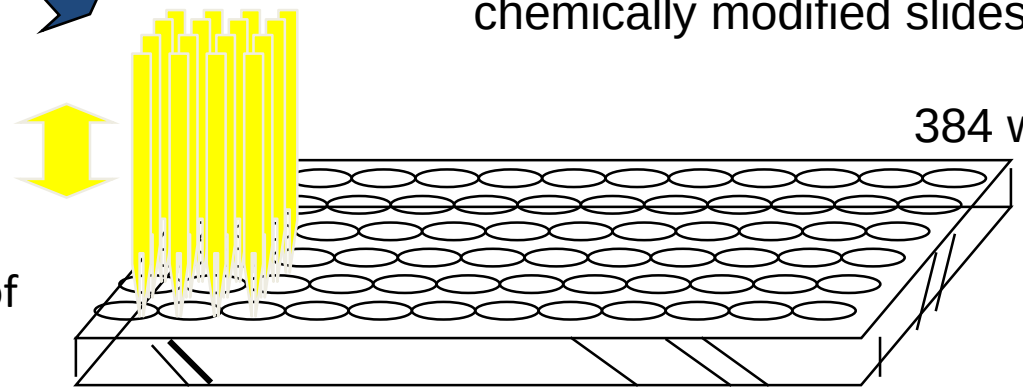
1 nanolitre spots

chemically modified slides

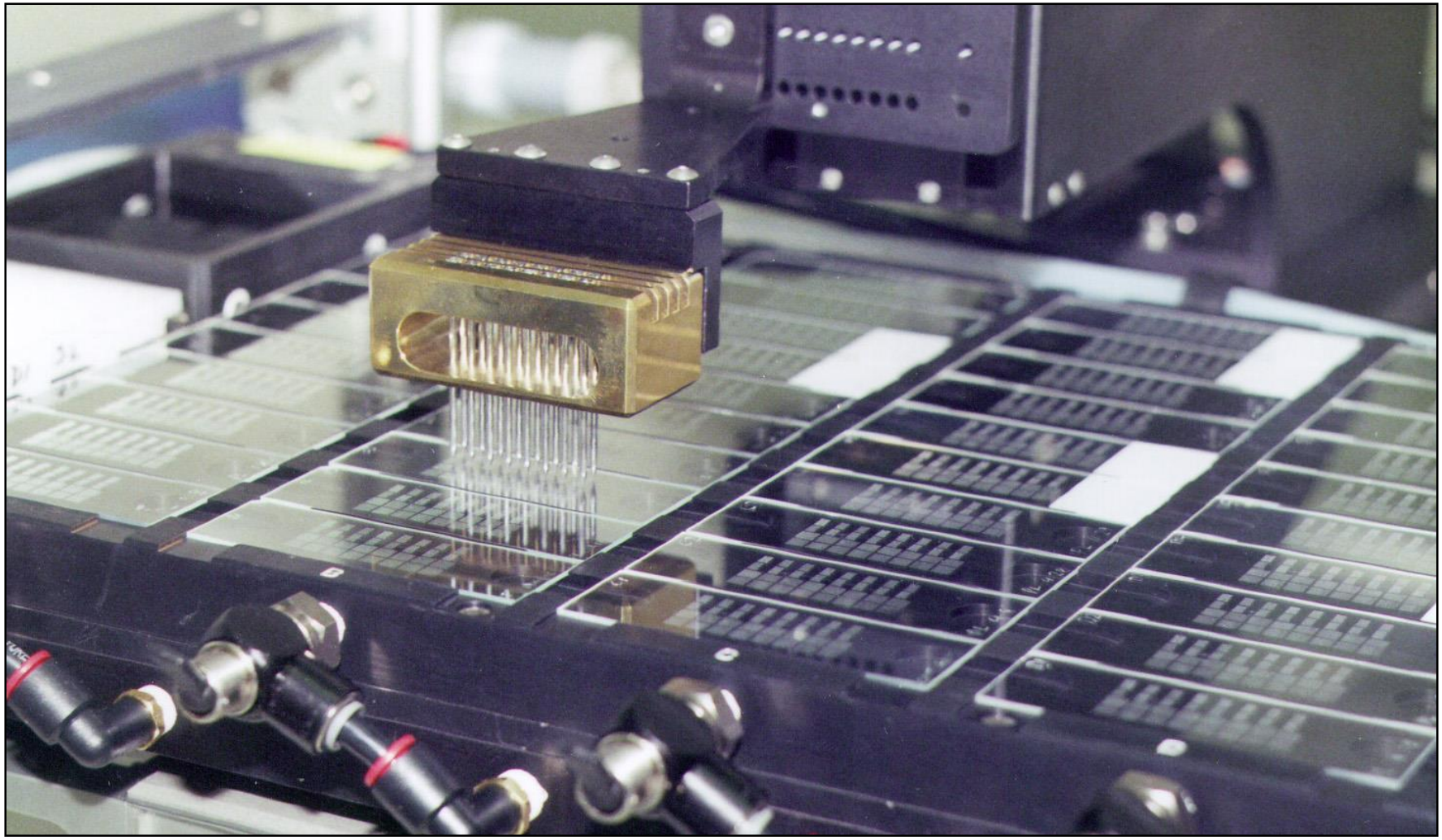
90-120 µm diameter

concentration of 100-500 µg/ml

384 well source plate

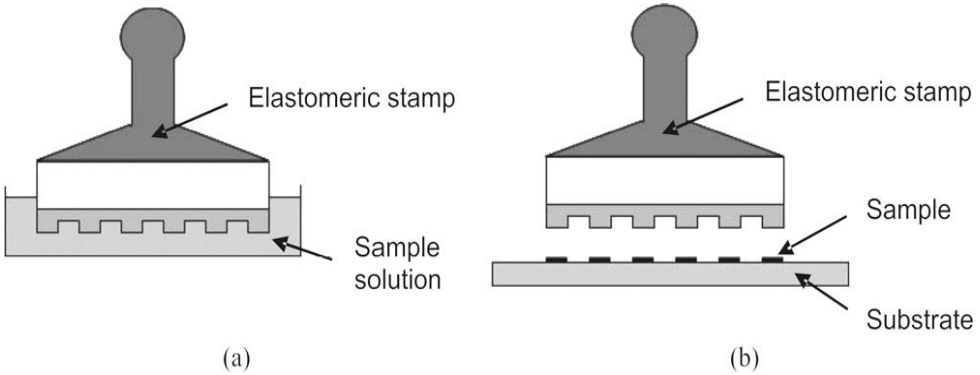


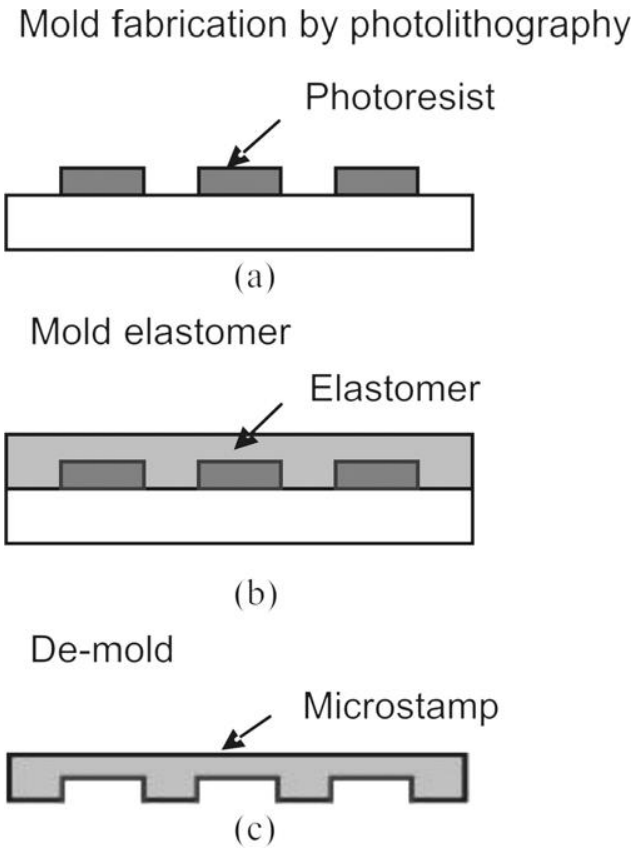
Printing Arrays on 50 slides

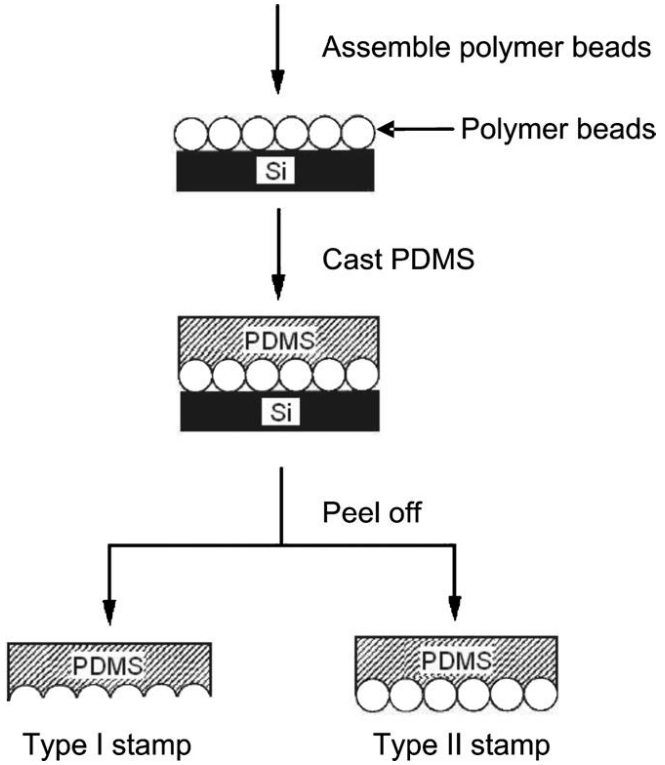


Printing Arrays

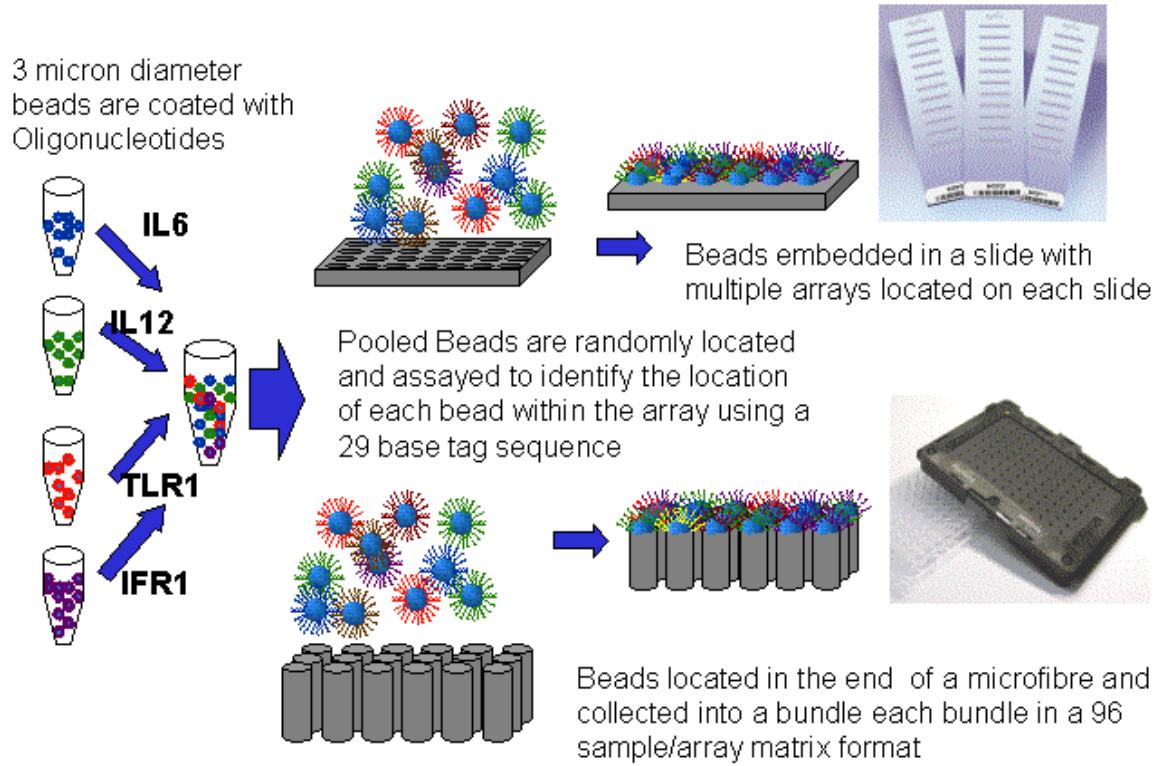
Microstamps: hundreds of spots are printed in parallel, enabling high-throughput microarray fabrication

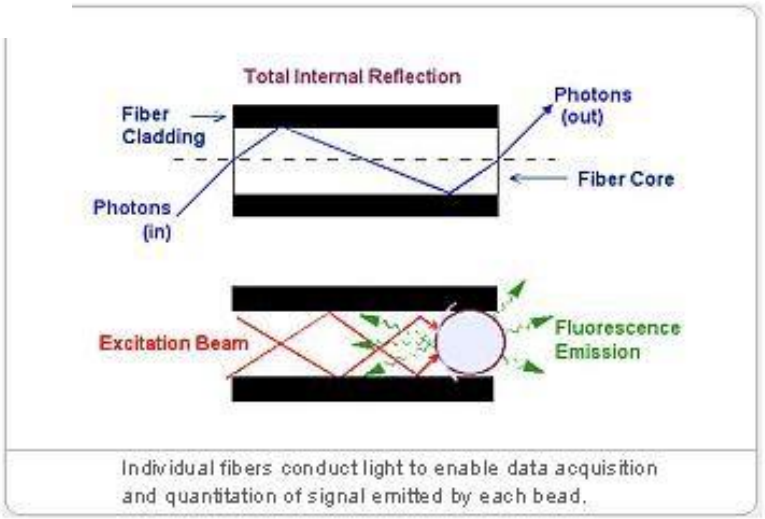






❑ Bead Arrays



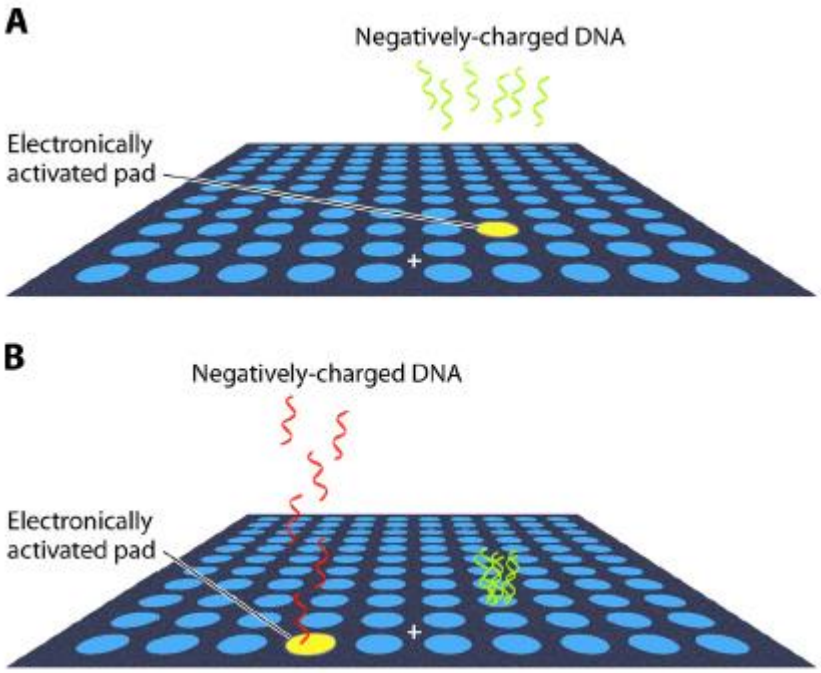


https://www.youtube.com/watch?v=lVG04dAAyvY

Microarrays Formats

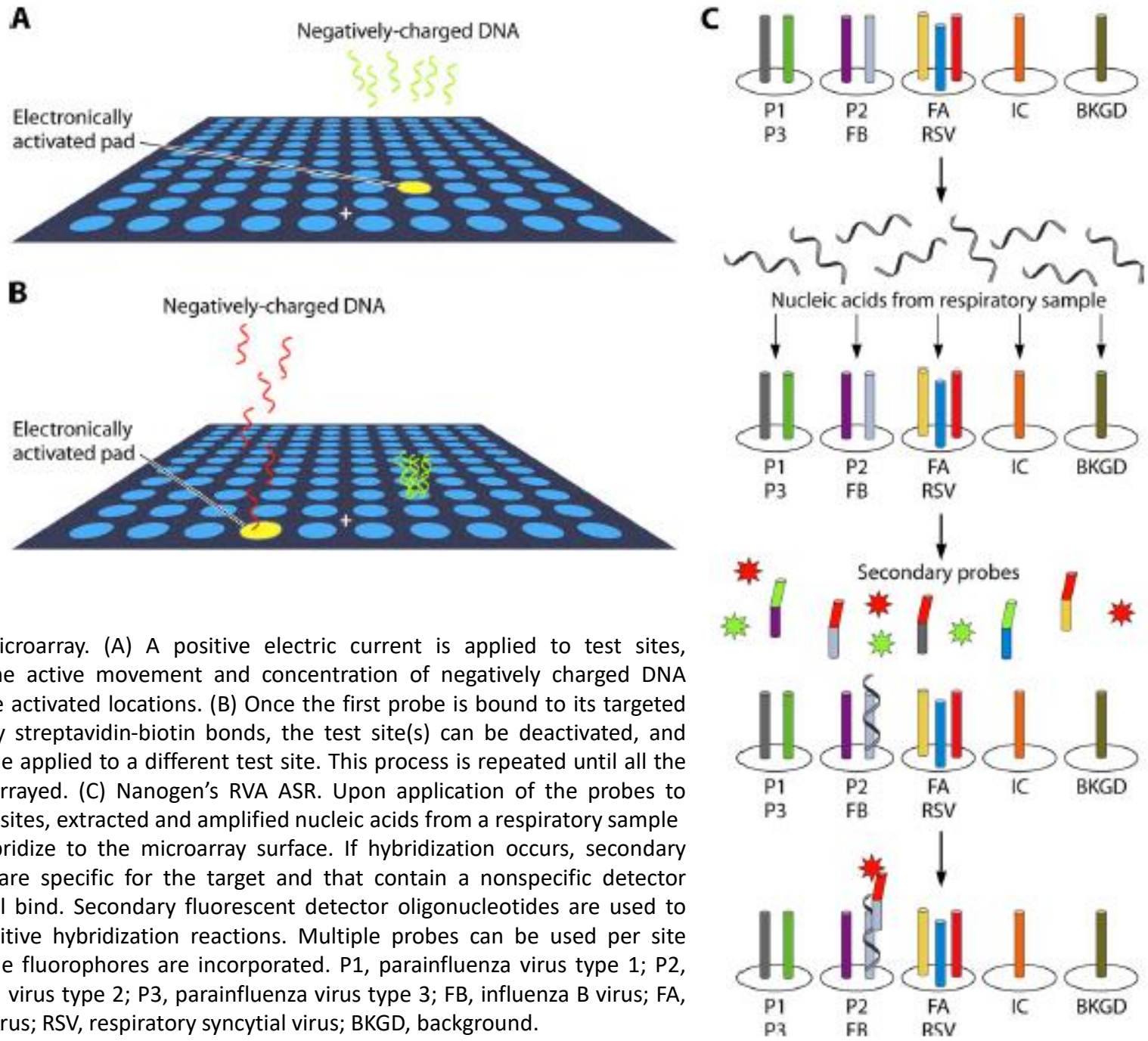
Electronic Microarrays

- Active control of nucleic acid transport.
- Microelectronic cartridges (NanoChip 400; Nanogen) use complementary metal oxide semiconductor technology
- (-)ly charged nucleic acids are transported to specific sites by applying (+) current and captured by SA-biotin interaction
- Typically, target DNA passively hybridizes with the immobilized probes on the microarray but can also be concentrated electronically



☺ Universal blank chip: content specified directly by the user → more flexibility

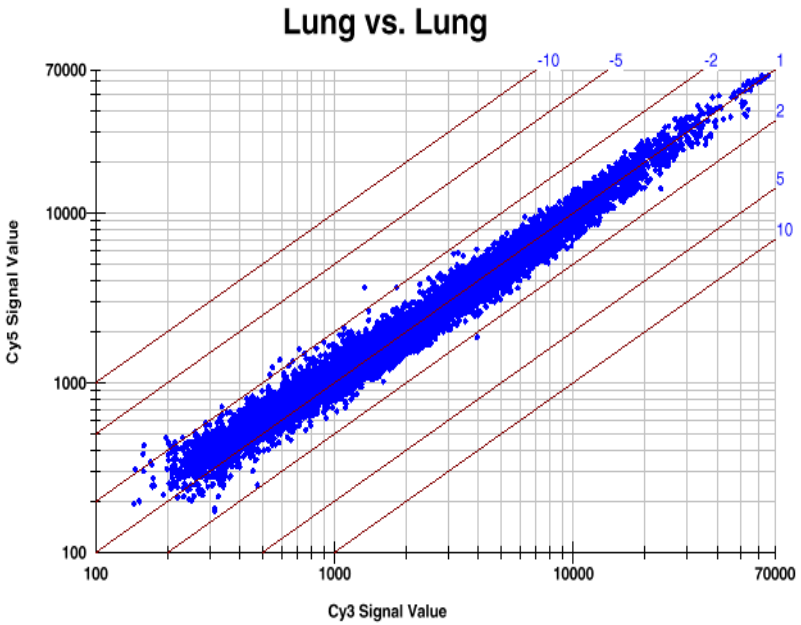
☺ Density is currently limited to 400 spots but this is sufficient for the majority of diagnostic applications.



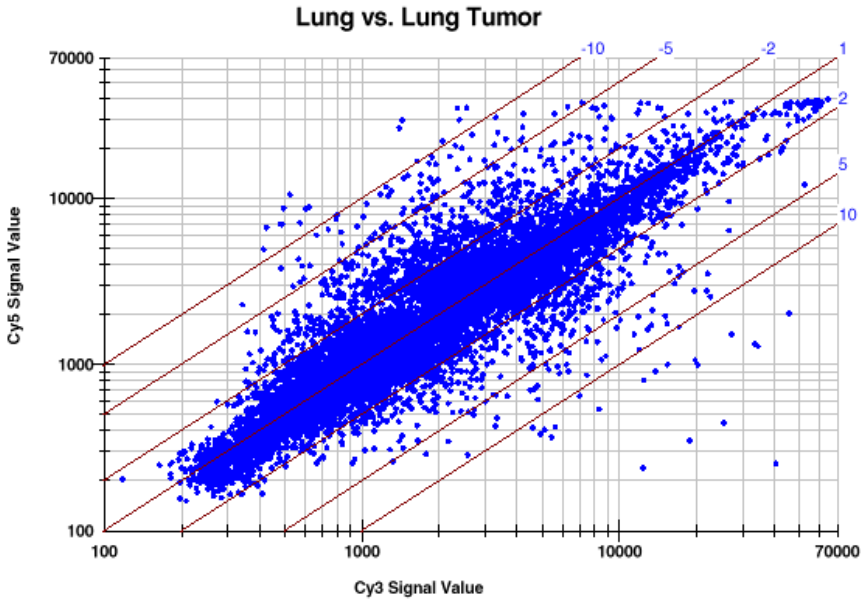
Electronic microarray. (A) A positive electric current is applied to test sites, facilitating the active movement and concentration of negatively charged DNA probes to the activated locations. (B) Once the first probe is bound to its targeted location(s) by streptavidin-biotin bonds, the test site(s) can be deactivated, and current can be applied to a different test site. This process is repeated until all the probes are arrayed. (C) Nanogen's RVA ASR. Upon application of the probes to targeted test sites, extracted and amplified nucleic acids from a respiratory sample passively hybridize to the microarray surface. If hybridization occurs, secondary probes that are specific for the target and that contain a nonspecific detector sequence will bind. Secondary fluorescent detector oligonucleotides are used to measure positive hybridization reactions. Multiple probes can be used per site when multiple fluorophores are incorporated. P1, parainfluenza virus type 1; P2, parainfluenza virus type 2; P3, parainfluenza virus type 3; FB, influenza B virus; FA, influenza A virus; RSV, respiratory syncytial virus; BKGD, background.

Overall quality of microarray?

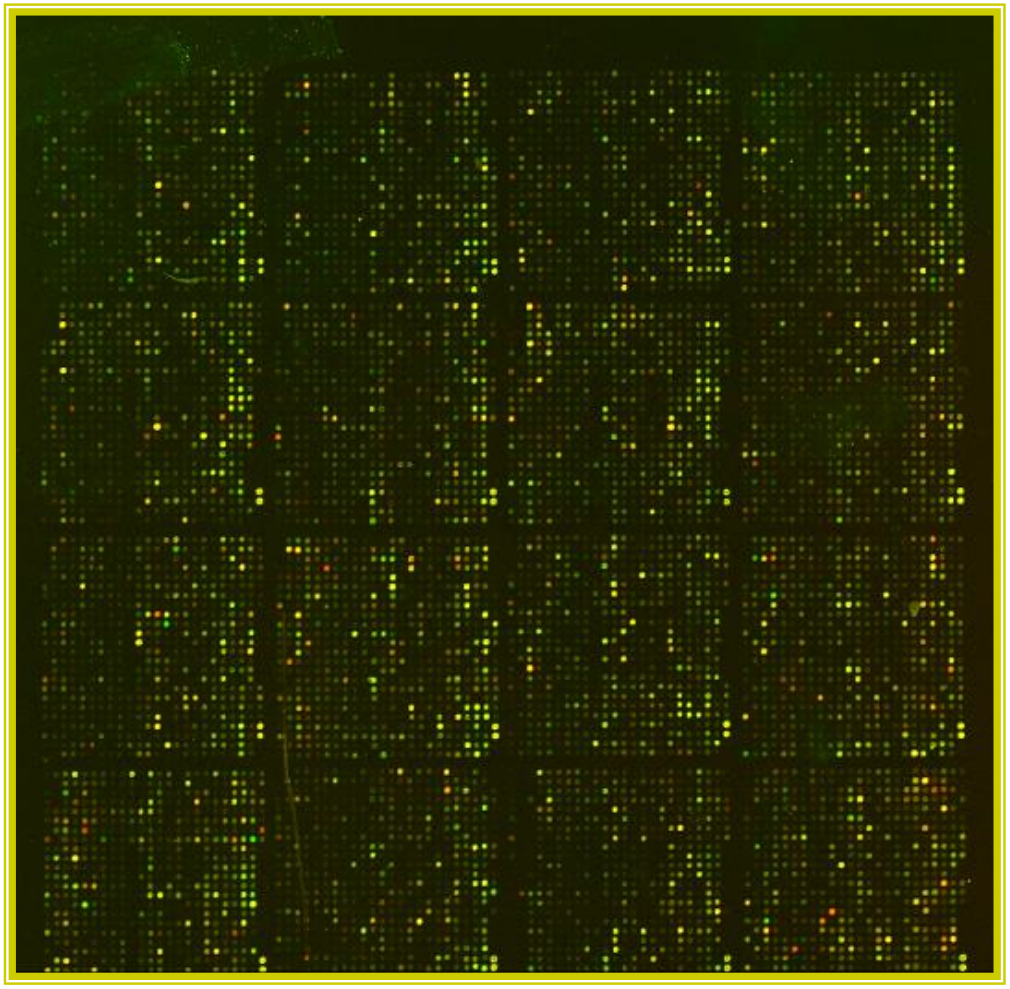
Normal vs. Normal



Normal vs. Tumor



Microarrays promise a lot but…



Scientist are often challenged by the huge quantities of data produced

We should able to interpret the meaning behind these complex expression patterns in microarray data

Software…

Well-designed, user-friendly software is the key to tracking, integrating, qualifying, and ultimately deriving scientific insight from the experimental results.

Silicon Genetics

GeneSifter
Lab Edition

Looking to understand the biology behind your microarray data?
See the forest *and* the trees.™

"I just did two hours of analysis that took me two months to do with another program."
— *Brian J. Rybarczyk, Ph.D., UNC-Chapel Hill*

BioDiscovery

From Images to Answers™
the total solution for microarray and high-density array image analysis

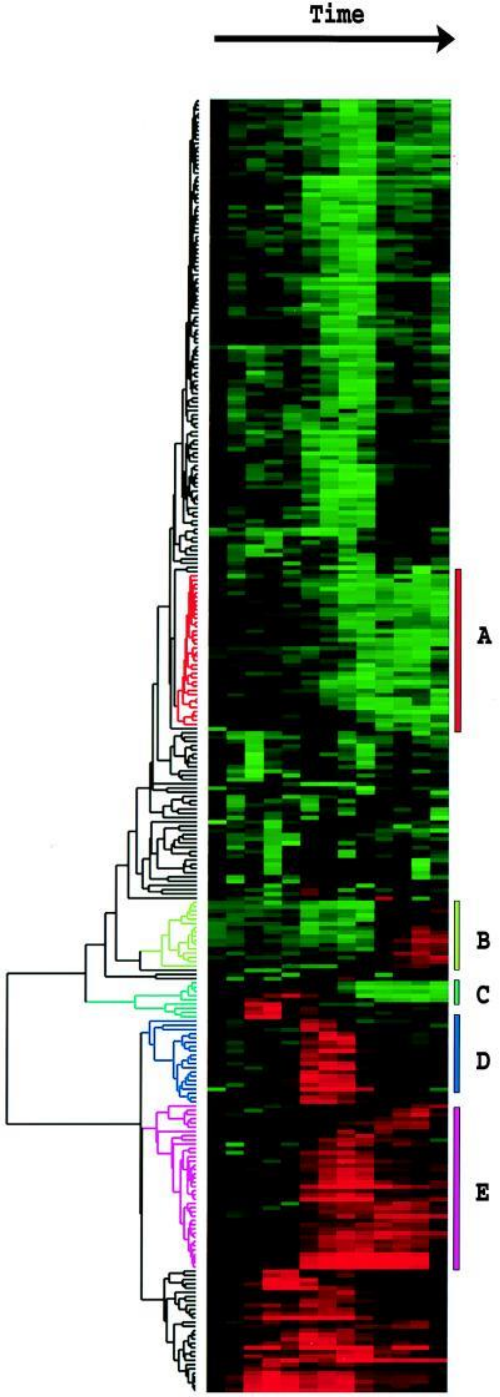
"I should have used GeneDirector!"

Data Mining??

- Data volumes are too large for traditional analysis methods
- Only small portion of data is analyzed
- Decision support process becomes more complex

Therefore

- prediction, classification, deviation detection, segmentation needed
- Data mining generates more sophisticated summaries and reports to aid understanding of the data – find clusters, partitions in data



Clustered display of data from time course of serum stimulation of primary human fibroblasts.

A Cholesterol Biosynthesis

B Cell Cycle

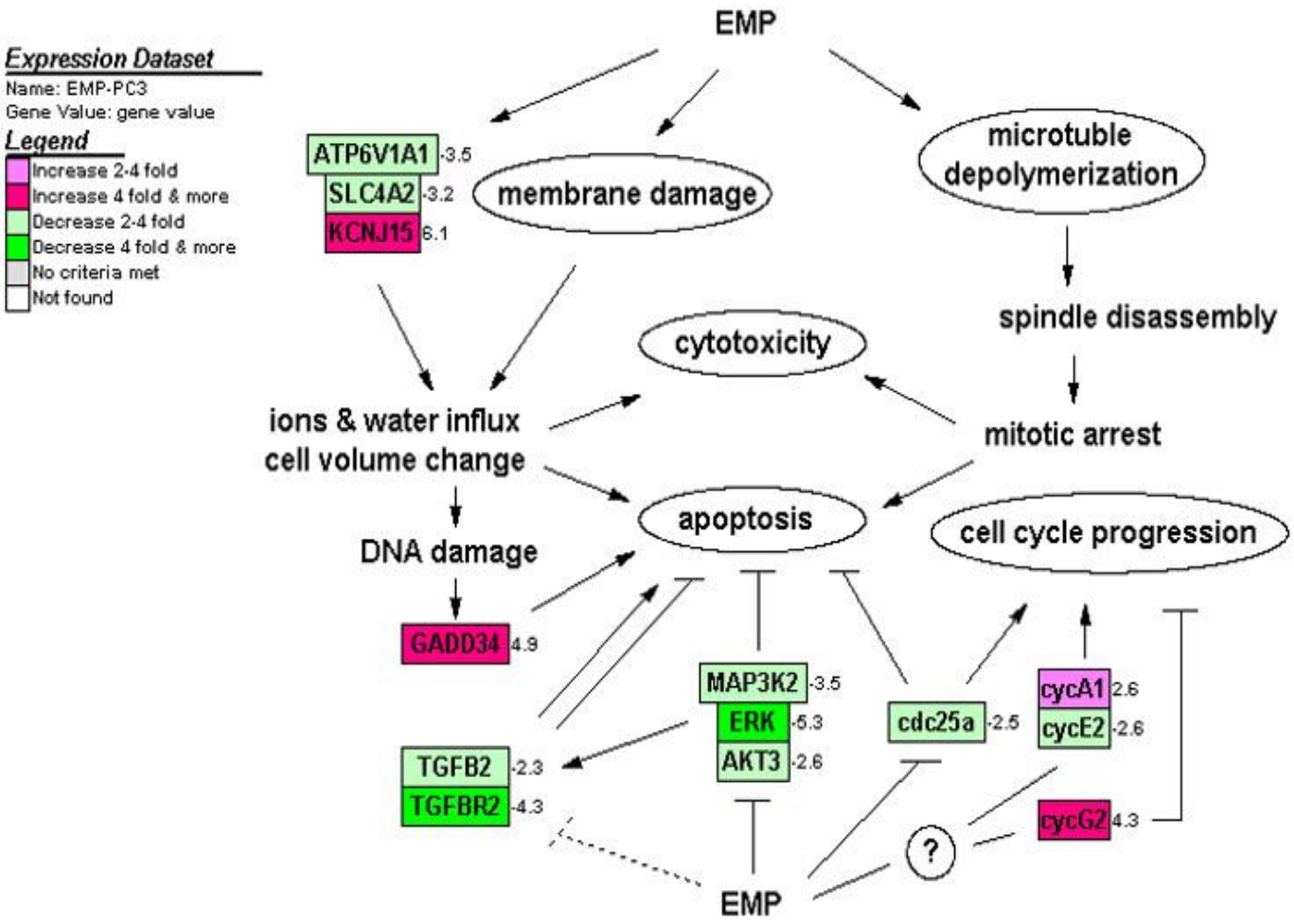
C Immediate Early Response

D Signaling and Angiogenesis

E Wound Healing and Tissue Remodeling

Eisen *et al.*
***Proc. Natl. Acad. Sci. USA 95 (1998) pg* 14865**

Bioinformatics:
A biological meaning from the raw data



Validation

Two types of validation

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

2. Validating the biological phenomenon with new samples and same experimental conditions

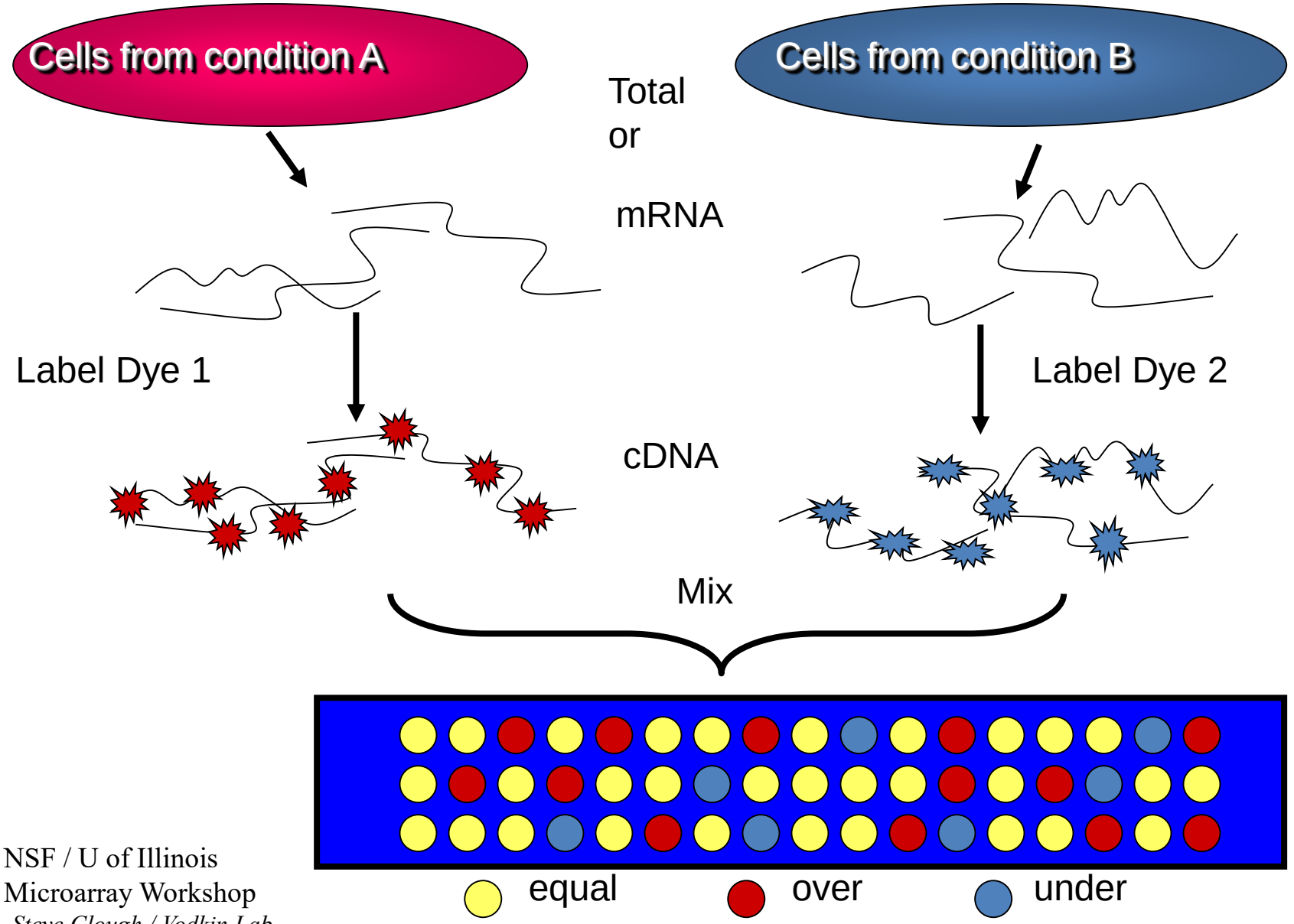
- There's no way that all of your microarray data can be validated.
- It's strongly recommended that any key findings be verified by independent means.

e.g. Immunohistochemistry, Western Blot, *in silico,* quantitative real-time PCR, etc

Expression Arrays

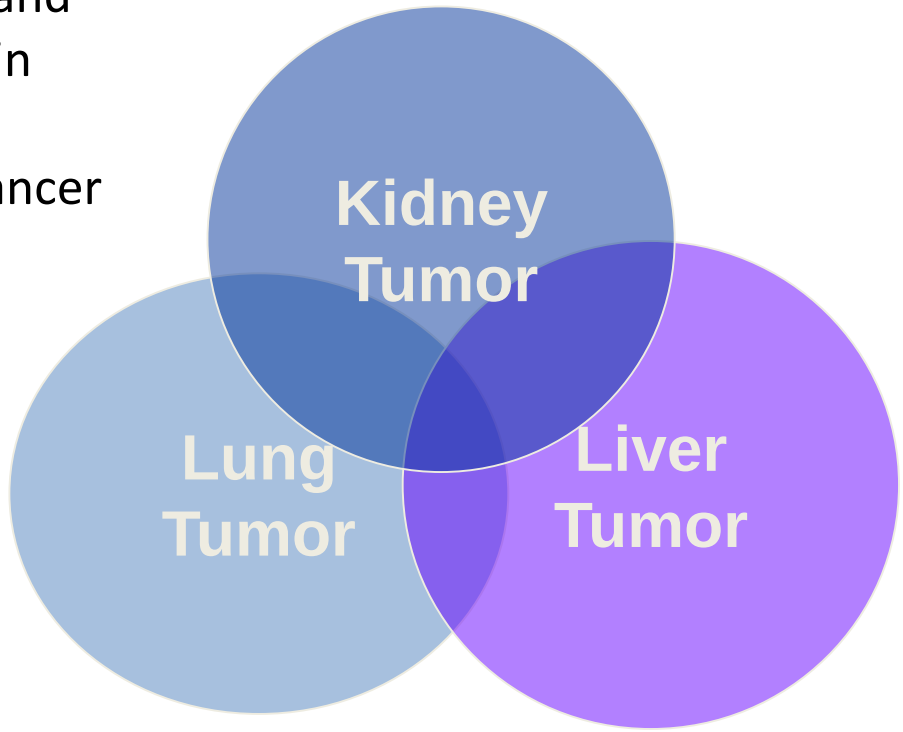
- Most common type of microarray
- Involves extracting RNA from a sample and converting it to cDNA
- Measures the amount and type of mRNA transcripts
- Provides information on whether genes are up or down regulated in a specific condition

Ratio of expression of genes from two sources



Profiling Gene Expression

Similarities and differences in expression profile, in cancer



Same cancer type, but different expression profiles → subtypes

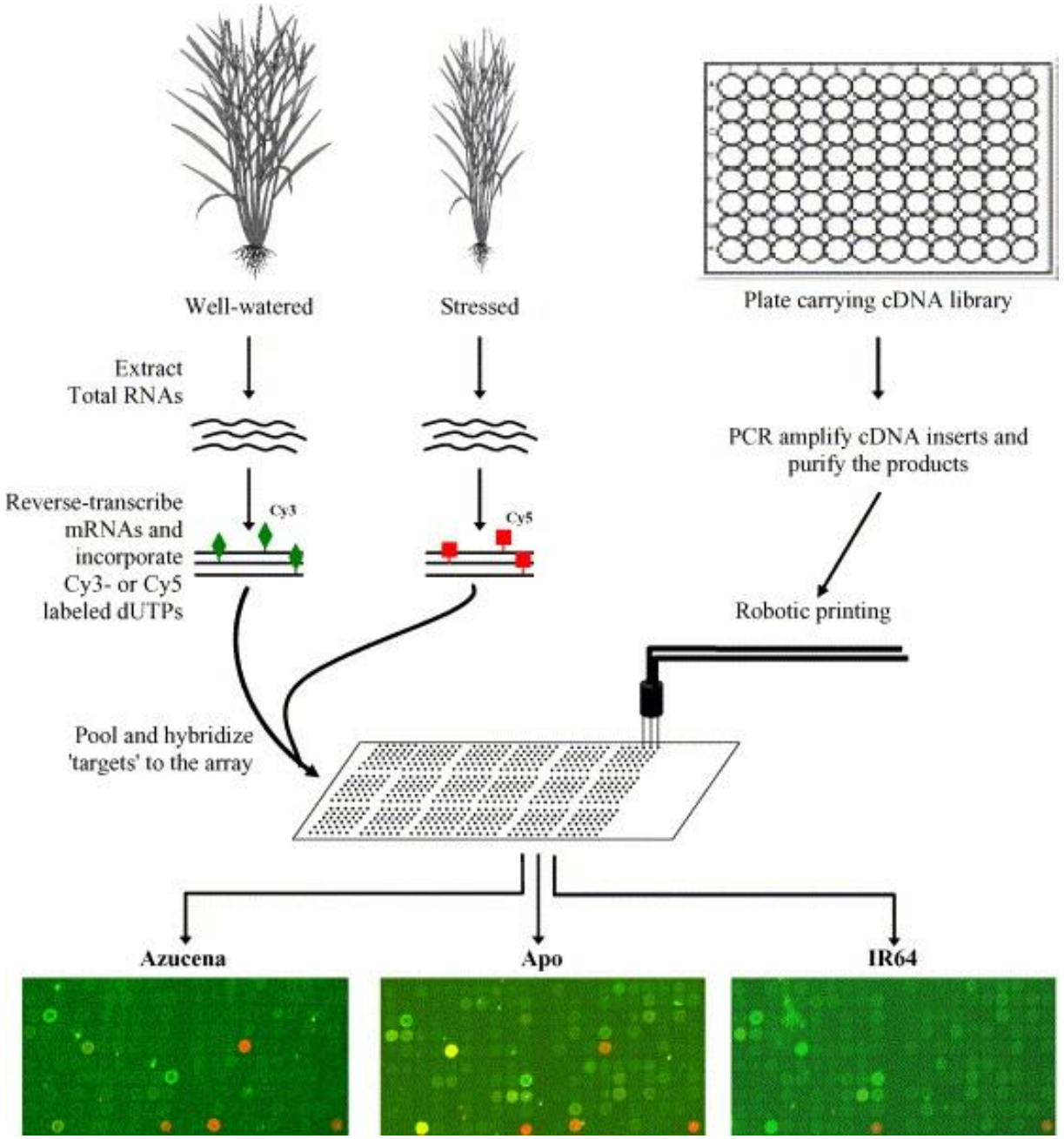
Different tissues, different expression profile

For accurate diagnosis and therapy

In Agriculture:

Cellular responses to specific signals:

- Disease states
- Chemicals/Drugs
- Environmental conditions

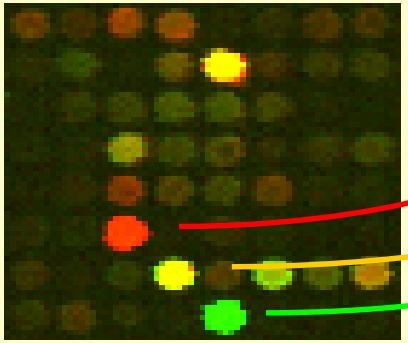


Example- Reading an array

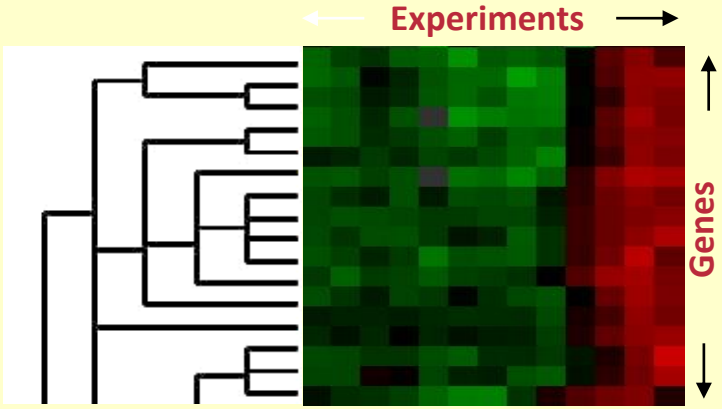
Block	Column	Row	Gene Name	Red	Green	Red:Green Ratio
1	1	1	tub1	2,345	2,467	0.95
1	1	2	tub2	3,589	2,158	1.66
1	1	3	sec1	4,109	1,469	2.80
1	1	4	sec2	1,500	3,589	0.42
1	1	5	sec3	1,246	1,258	0.99
1	1	6	act1	1,937	2,104	0.92
1	1	7	act2	2,561	1,562	1.64
1	1	8	fus1	2,962	3,012	0.98
1	1	9	idp2	3,585	1,209	2.97
1	1	10	idp1	2,796	1,005	2.78
1	1	11	idh1	2,170	4,245	0.51
1	1	12	idh2	1,896	2,996	0.63
1	1	13	erd1	1,023	3,354	0.31
1	1	14	erd2	1,698	2,896	0.59

Campbell & Heyer, 2003

Image Analysis & Data Visualization

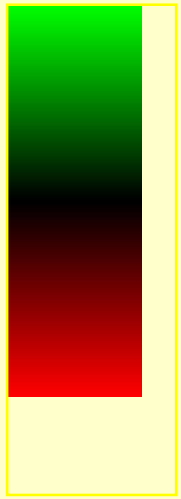


Cy3	Cy5	$\frac{Cy5}{Cy3}$	$\log_2\left(\frac{Cy5}{Cy3}\right)$
~~200~~	10000	50.00	5.64
~~4800~~	4800	1.00	0.00
~~9000~~	300	0.03	-4.91



Underexpressed

Overexpressed



8
4
2
2
4
8

Protein-Binding Microarrays

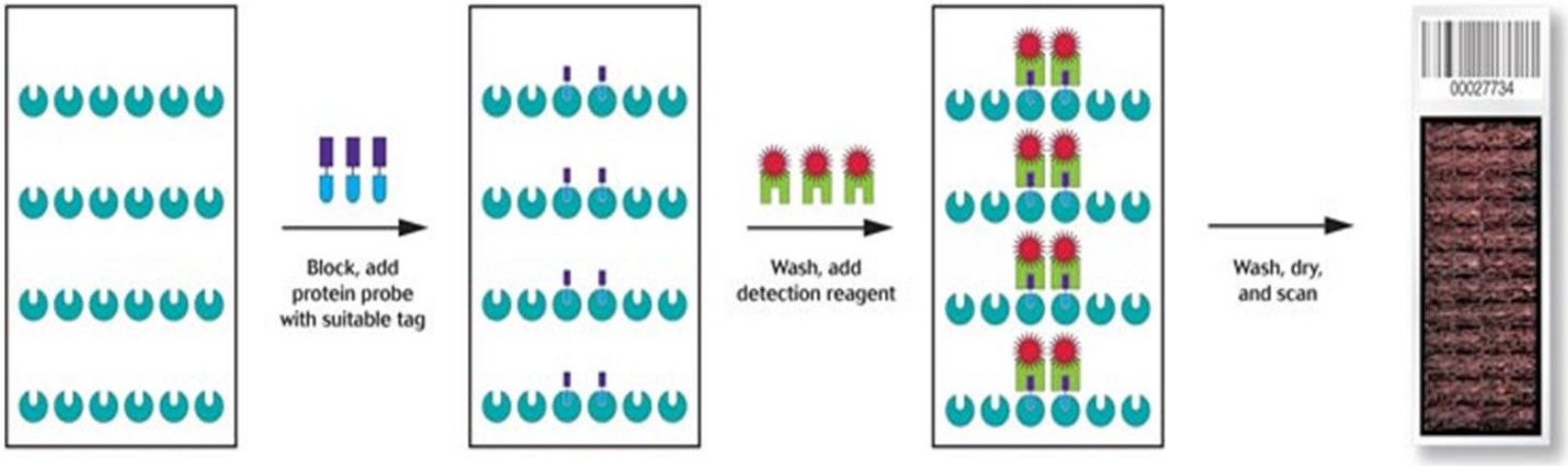
- dsDNA probes used on array
- purified protein hybridized to array detected by antibody to protein or to epitope tag
- They can be used to
  - identify transcription factor (TF) binding sequences (motifs) and target genes
  - characterize DNA-binding specificity of large number of TFs,

Protein Arrays

E.g. The Invitrogen Human Protein Microarray

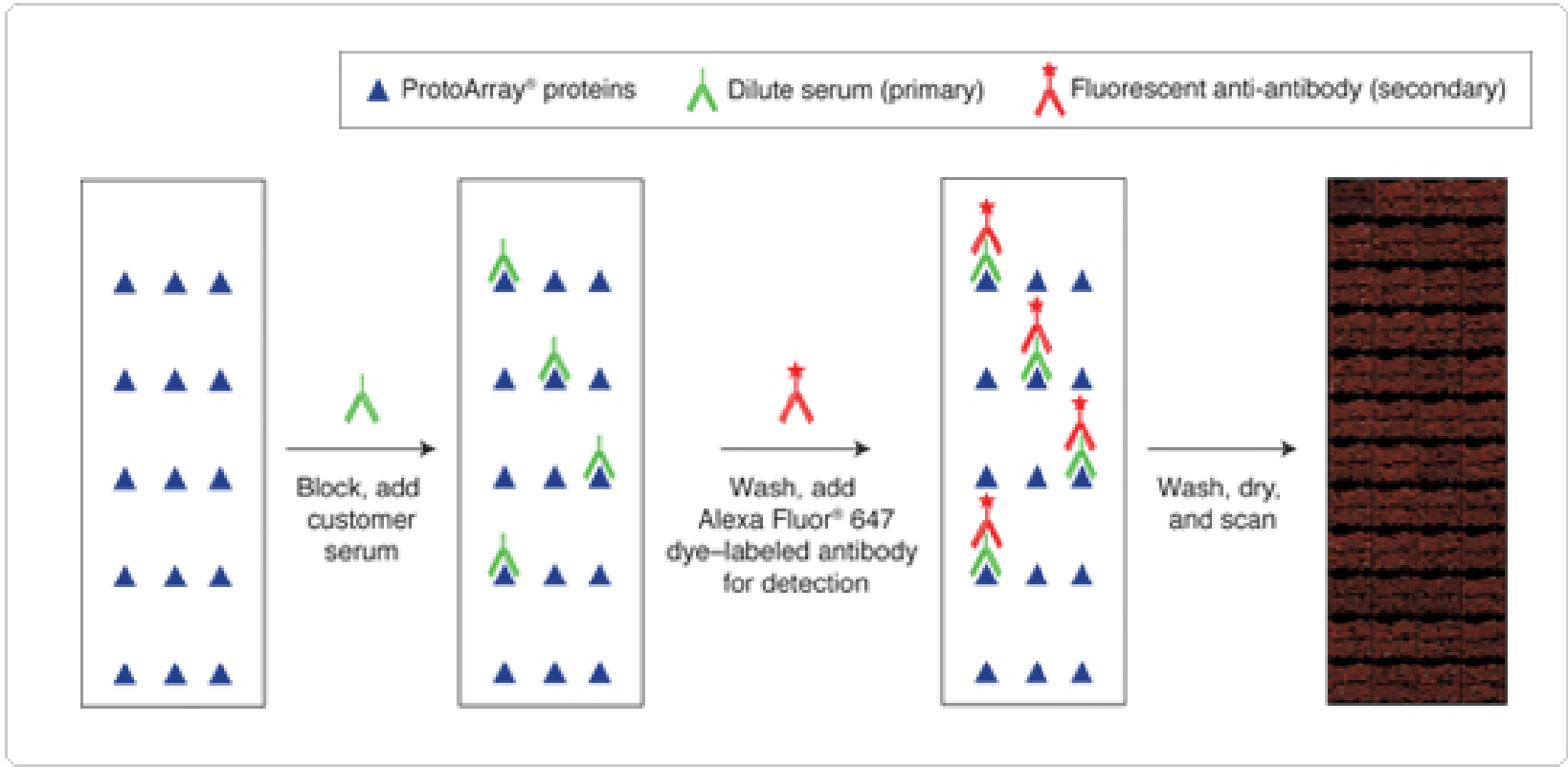
- a high-density microarray
- It contains thousands of unique human proteins
- [kinases, phophatases, GPCRs, nuclear receptors, and proteases]

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



Protein Arrays

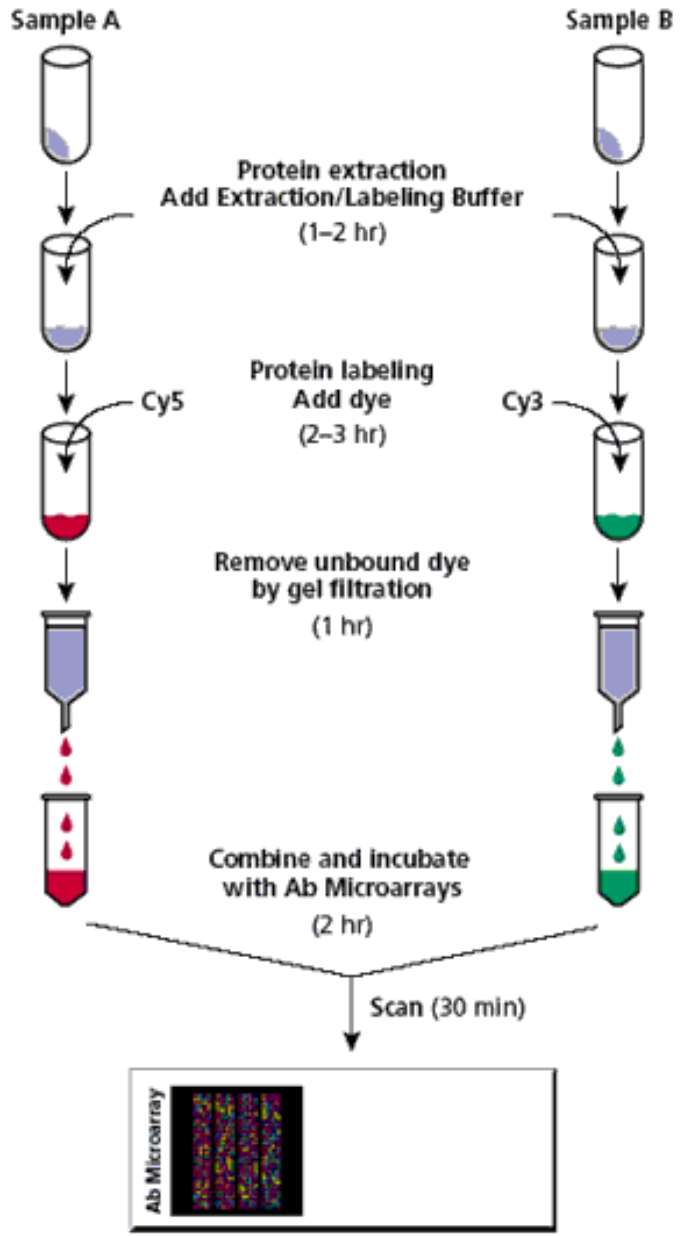
The ProtoArray® Immune Response Biomarker Profiling application
To uncover novel autoantibody markers for cancer, autoimmune disorders, and other diseases



Antibody Arrays

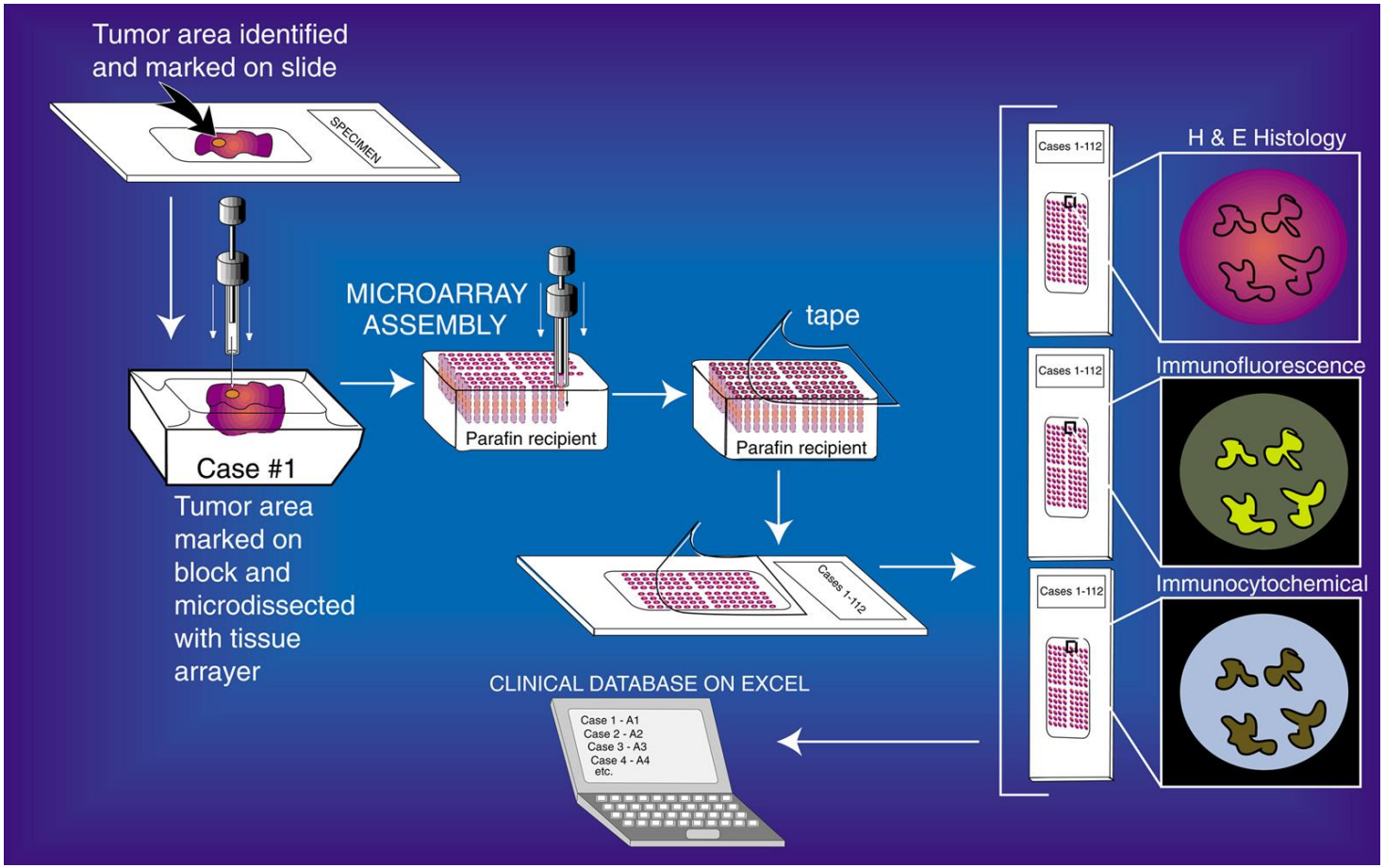
- Assay hundreds of native proteins simultaneously
- Compare protein abundances in a variety of biological samples
- Antibody or ligand is on the microarray

Antibody Arrays-labeling scheme



Tissue Arrays

- A cylindirical section from embedded paraffin tissues were taken and pluged or inserted into new paraffin block
- Sectioning and deposition onto a slide
- These sections are then stained using standard laboratory metods.



QC Dots Cancer Array (CA) control slides are blank charged microscopic slides pre-spotted with the selected tumor and normal cells.

- Those control cell spots provide on slide positive/negative control staining of the antibody used in the experiment.
- QC Dots® CA slide is designed for routine use in immunohistochemistry (IHC) and In Situ Hybridization (ISH) applications.
- Simply mount a tissue sample next to the cell dots and perform all the staining procedure with control cell dots.