Statistical Methods and Software for the Analysis of Microarray Experiments

www.stat.berkeley.edu/~sandrine/Docs/Talks/MBI04/mbi.html

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Ohio State University, Columbus, OH
September 20--24, 2004

Lecture 1:
Introduction to DNA Microarray Technologies

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Acknowledgments

Slides from

Bioconductor Short Courses

www.bioconductor.org

Sandrine Dudoit, Robert Gentleman, Rafael Irizarry, and Yee Hwa Yang.

Outline

- Basic principles.
- Two-color spotted DNA microarrays.
- Affymetrix oligonucleotide chips.

Basic principles

Differential expression

- Each cell contains a complete copy of the organism's genome.
- Cells are of many different types and states
  E.g. Blood, nerve, and skin cells, dividing cells, cancerous cells, etc.
- What makes the cells different?
- Differential gene expression, i.e., when, where, and how much each gene is expressed.
- On average, 40% of our genes are expressed at any given time.

Central dogma

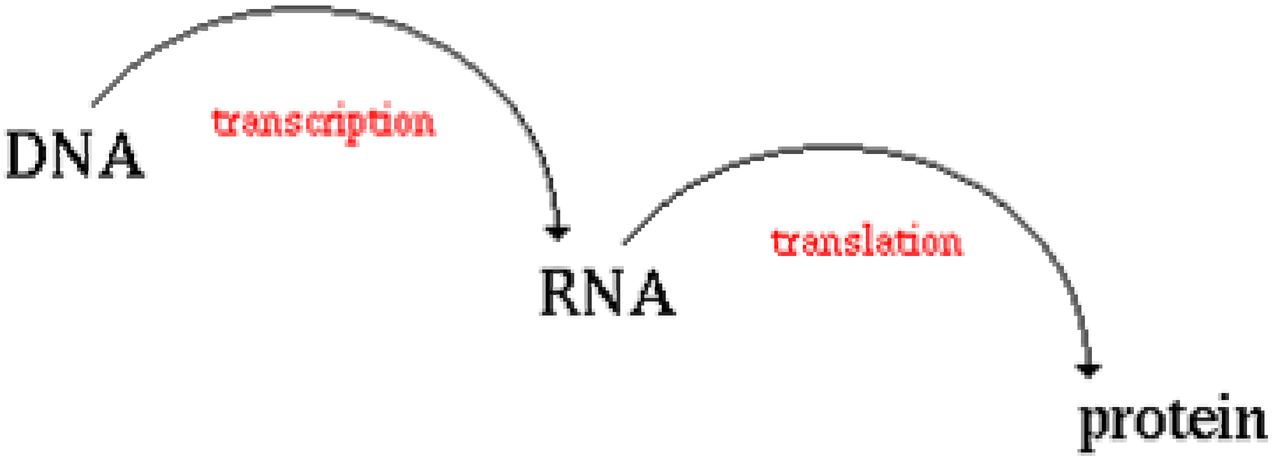
The expression of the genetic information stored in the DNA molecule occurs in two stages:

- (i) transcription, during which DNA is transcribed into mRNA;
- (ii) translation, during which mRNA is translated to produce a protein.

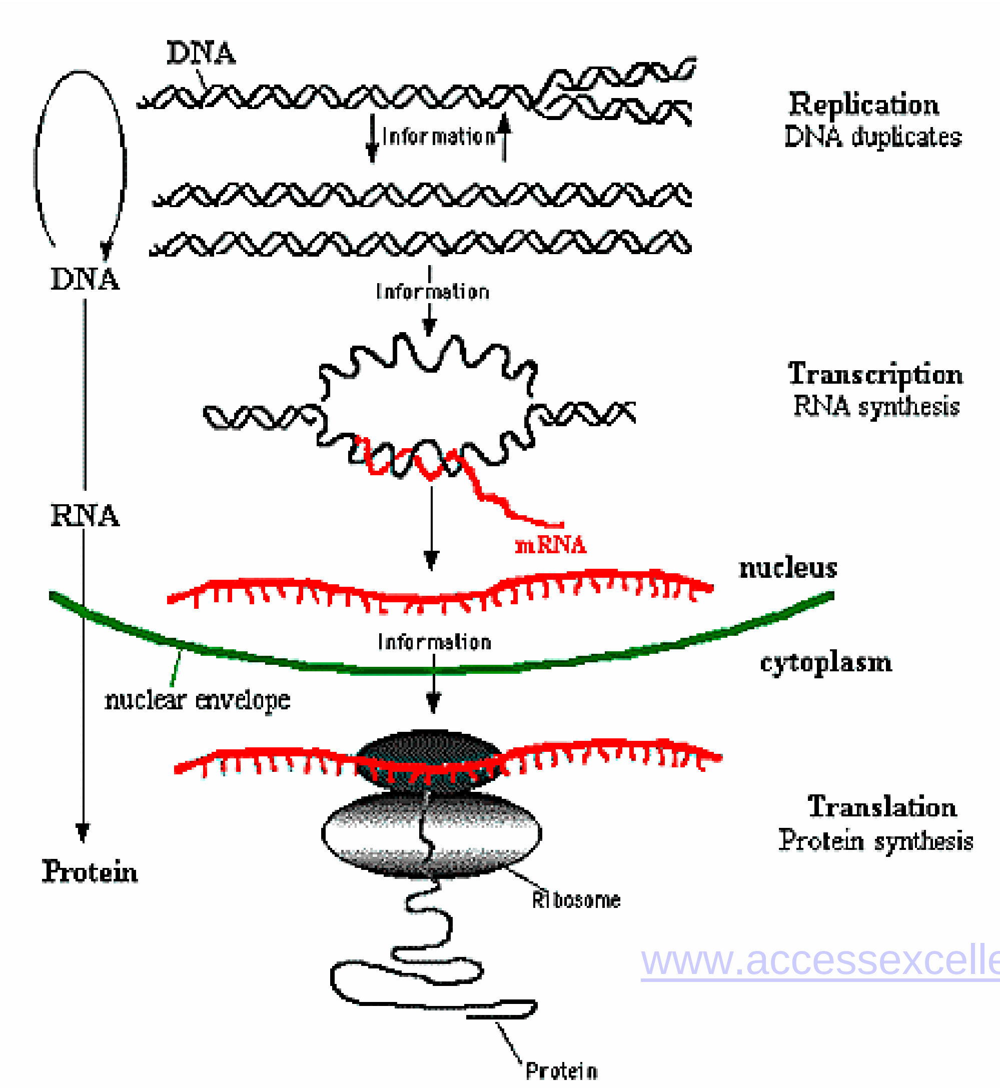
DNA → mRNA → protein

Other important aspects of gene regulation: methylation, alternative splicing, etc.

Central dogma



Central dogma



www.accessexcellence.com/AB/GG/

Functional genomics

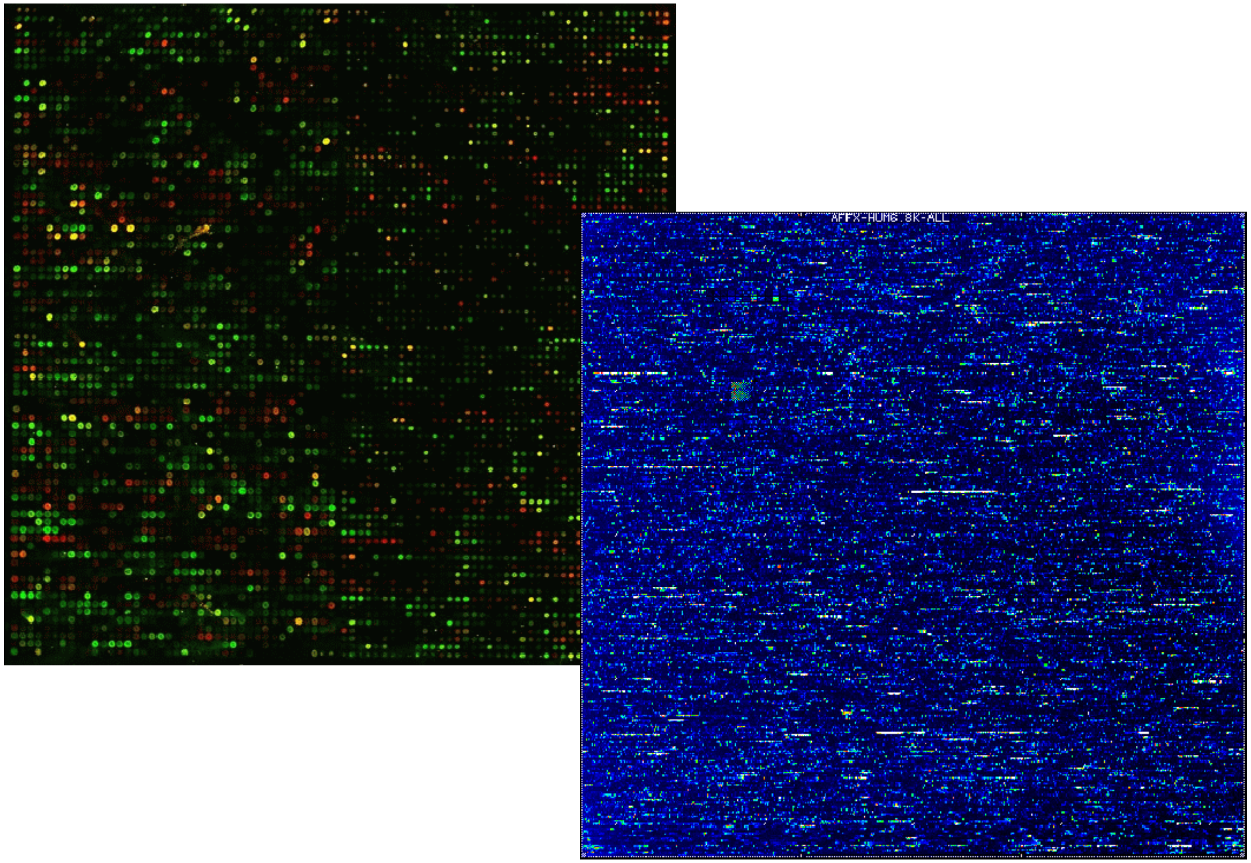
- The various genome projects have yielded the complete DNA sequences of many organisms.

  E.g. Human, mouse, yeast, fruitfly, etc.

  Human: 3 billion base-pairs, ~30-40 thousand genes.

- Challenge: **go from sequence to function**, i.e., define the role of each gene and understand how the genome functions as a whole.

DNA microarrays



DNA microarrays

- DNA microarray experiments are high-throughput biological assays for measuring the abundance of DNA or RNA sequences in different types of cell samples for thousands of sequences simultaneously.
- Exploit the availability of sequence data to get information on gene expression in different types of cells.

DNA microarrays

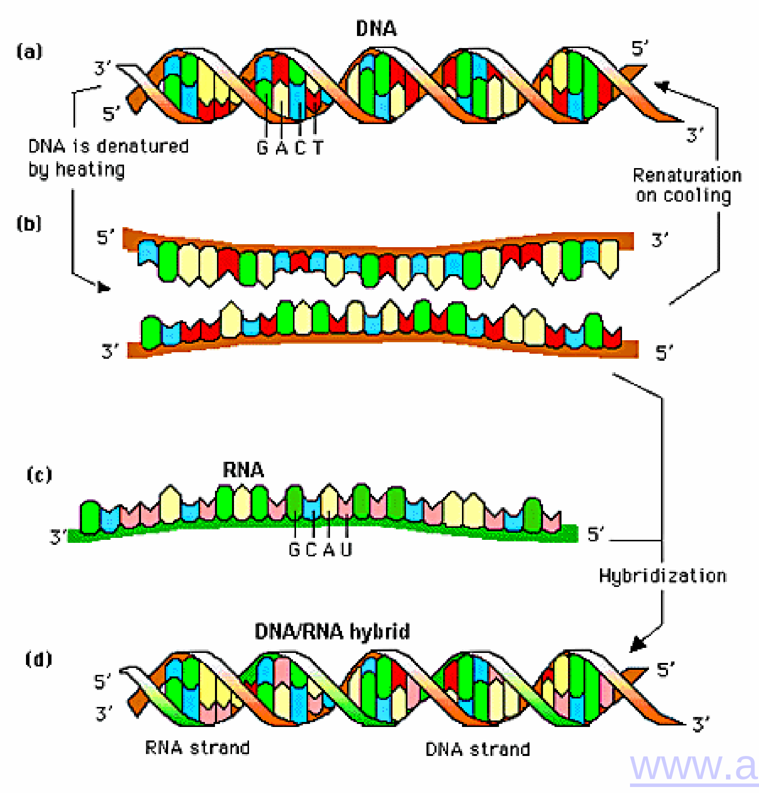
- DNA microarrays rely on the hybridization properties of nucleic acids to monitor DNA or RNA abundance on a genomic scale in different types of cells.
- The ancestor of cDNA microarrays: the Northern blot.

Hybridization

- Hybridization refers to the annealing of two nucleic acid strands following the base-pairing rules.

- Nucleic acid strands in a duplex can be separated, or denatured, by heating to destroy the hydrogen bonds.

Hybridization



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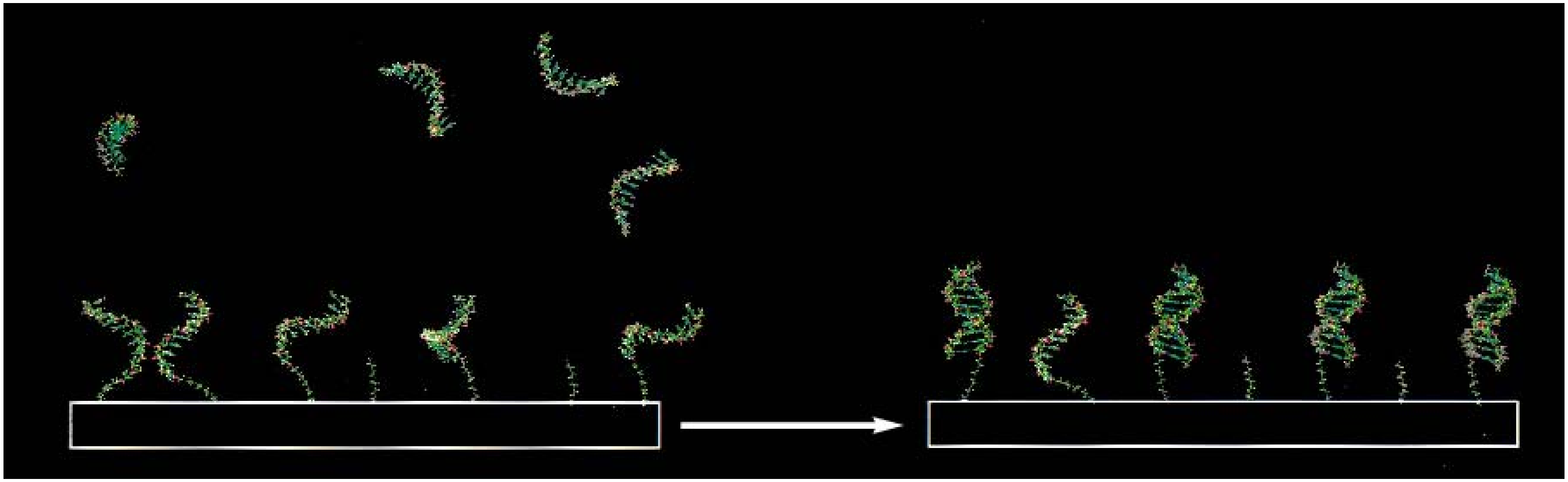
Nucleic Acid Hybridization

Hybridization



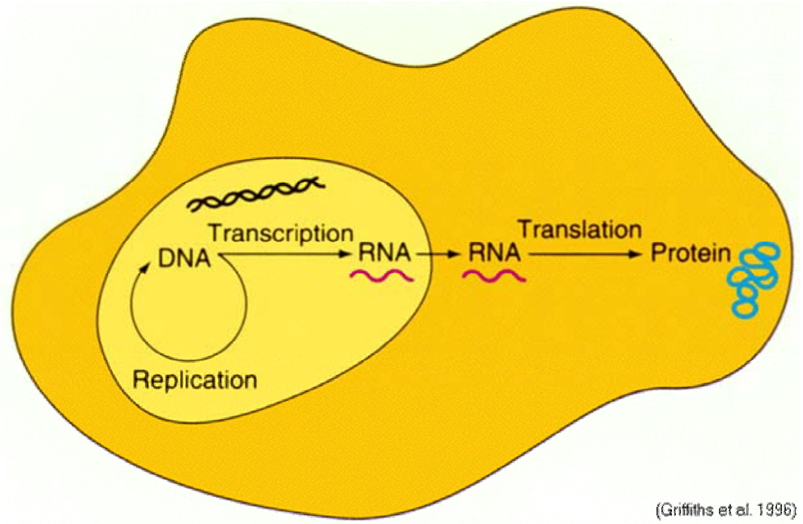
Gene expression assays

- Spotted cDNA arrays (Brown/Botstein);
- Short oligonucleotide arrays (Affymetrix);
- Long oligonucleotide arrays (Agilent Inkjet);
- Fibre optic arrays (Illumina);
- Serial analysis of gene expression (SAGE);
- Etc.

Applications of microarrays

- Measuring transcript abundance (cDNA arrays);
- Genotyping;
- Estimating DNA copy number (CGH);
- Determining identity by descent (GMS);
- Measuring mRNA decay rates;
- Identifying protein binding sites;
- Determining sub-cellular localization of gene products;
- Etc.

Transcriptome



(Griffiths et al. 1996)

- mRNA or transcript levels sensitively reflect the state of a cell.
- Measuring protein levels (translation) would be more direct but more difficult.

Transcriptome

- The transcriptome reflects
  - Tissue source: cell type, organ.
  - Tissue activity and state:
    - Stage of development, growth, death;
    - Cell cycle;
    - Disease vs. healthy;
    - Response to therapy, stress.

Applications of microarrays

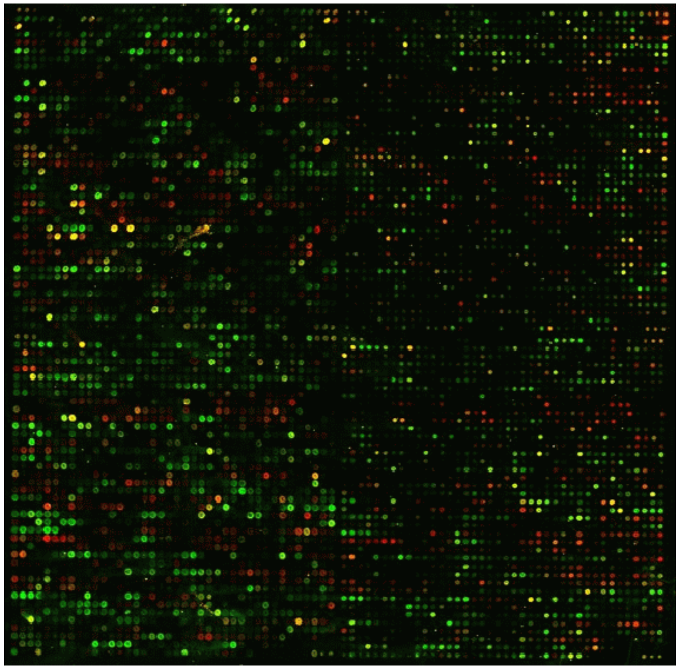
- **Cancer research:** Molecular characterization of tumors on a genomic scale

  → more reliable diagnosis and effective treatment of cancer.
- **Immunology:** Study of host genomic responses to bacterial infections.
- Etc.

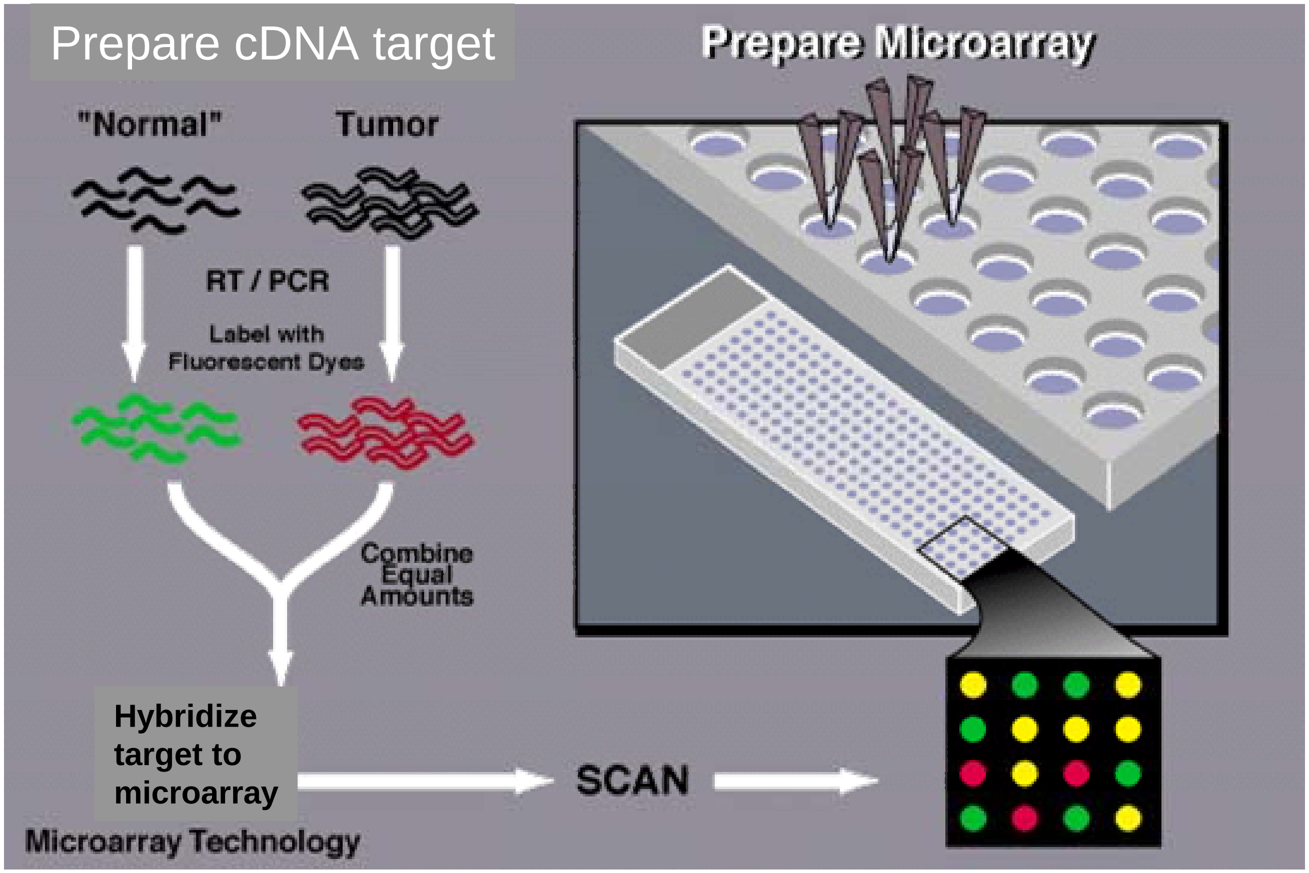
Applications of microarrays

- Compare mRNA (transcript) levels in different types of cells, i.e., vary
  - Tissue: liver vs. brain;
  - Treatment: drugs A, B, and C;
  - State: tumor vs. non-tumor, development;
  - Organism: different yeast strains;
  - Timepoint;
  - etc.

Two-color spotted DNA microarrays



Spotted DNA microarrays



www.accessexcellence.com/AB/GG/

Spotted DNA microarrays

- The relative abundance of a spotted DNA sequence in two DNA or RNA samples may be assessed by monitoring the differential hybridization of these two samples to the sequence on the array.
- Probes: DNA sequences spotted on the array, immobile substrate.
- Targets: Nucleic acid samples hybridized to the array, mobile substrate.

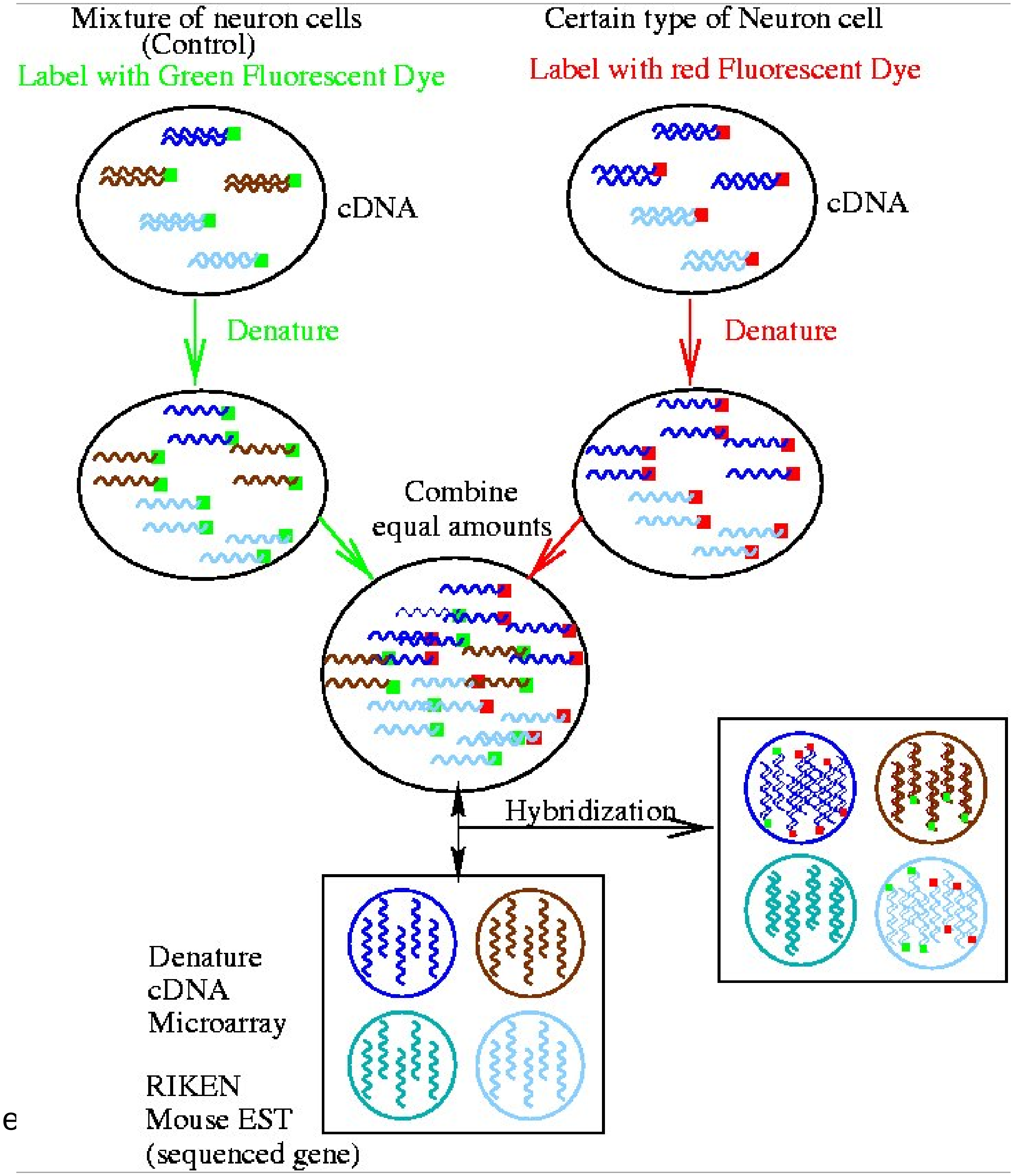
Spotted DNA microarrays

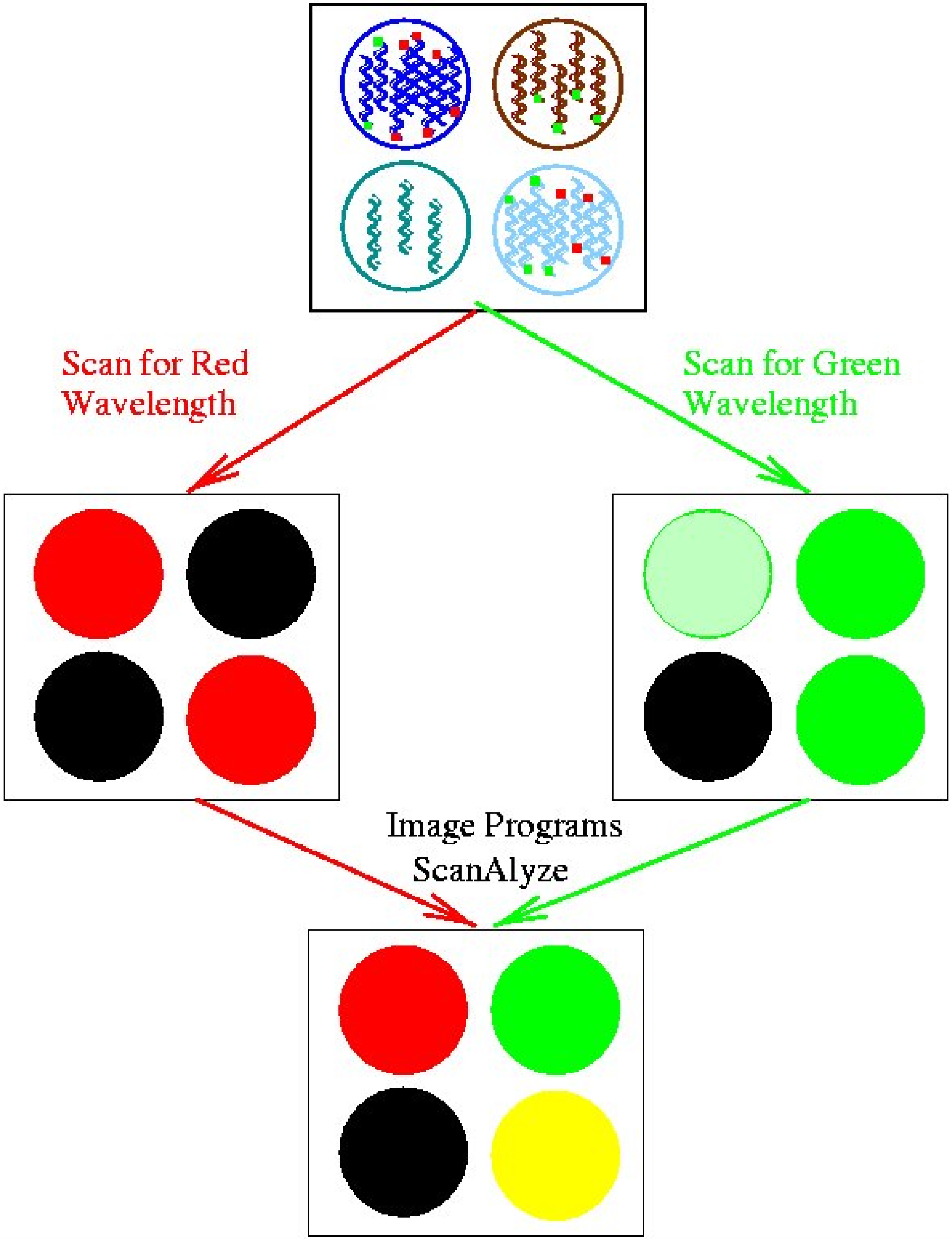
- The ratio of the red and green fluorescence intensities for each spot is indicative of the relative abundance of the corresponding DNA probe in the two nucleic acid target samples.

Spotted DNA microarrays

$$M = \log_2 R/G = \log_2 R - \log_2 G$$

- **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.


Mixture of neuron cells
(Control)
Label with Green Fluorescent Dye
Certain type of Neuron cell
Label with red Fluorescent Dye
cDNA
cDNA
Denature
Denature
Combine
equal amounts
Hybridization
Denature
cDNA
Microarray
RIKEN
Mouse EST
(sequenced gene)

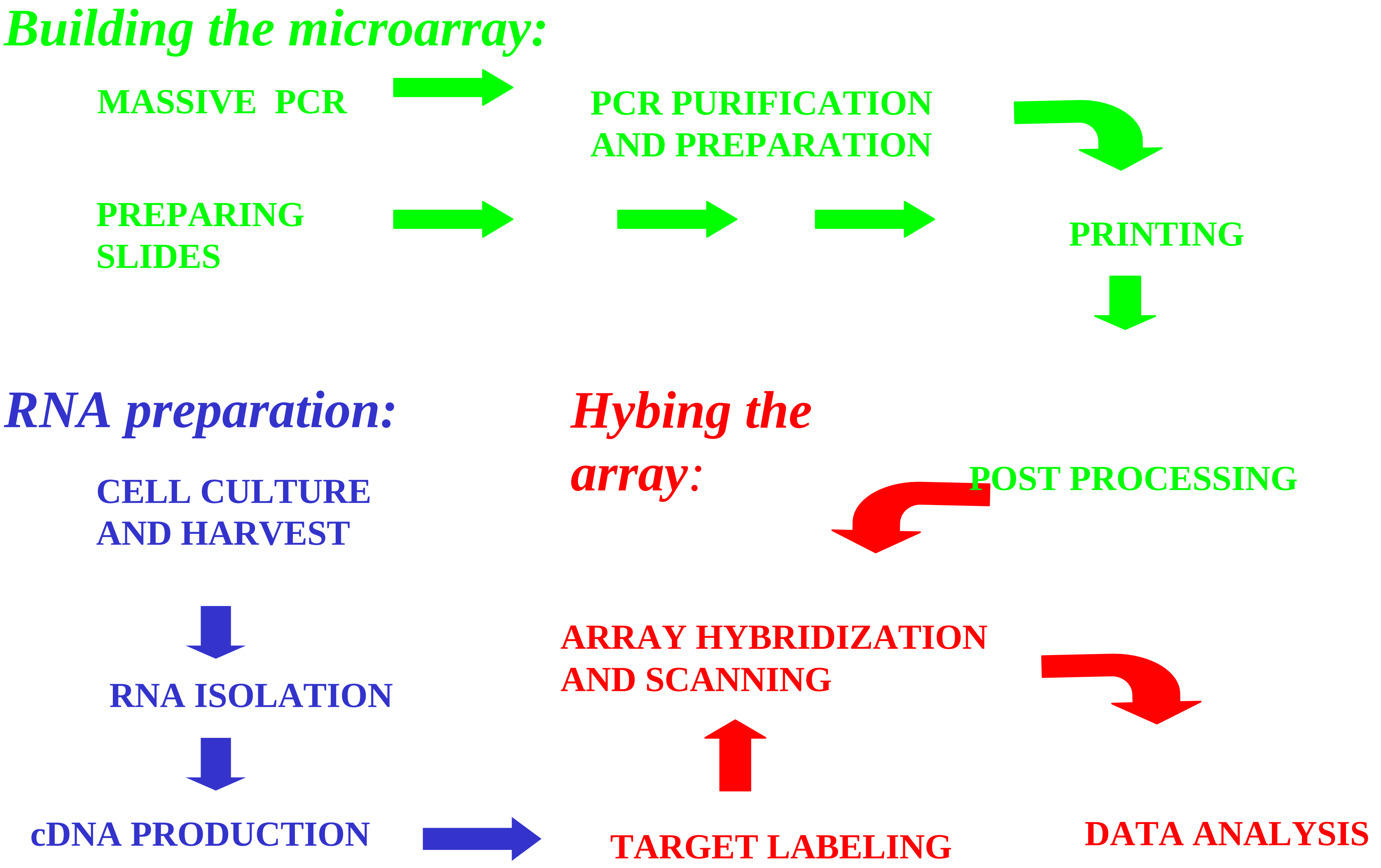


Scan for Red Wavelength

Scan for Green Wavelength

Image Programs ScanAlyze

The process



Building the microarray:

MASSIVE PCR

PCR PURIFICATION
AND PREPARATION

PREPARING
SLIDES

PRINTING

POST PROCESSING

RNA preparation:

CELL CULTURE
AND HARVEST

RNA ISOLATION

cDNA PRODUCTION

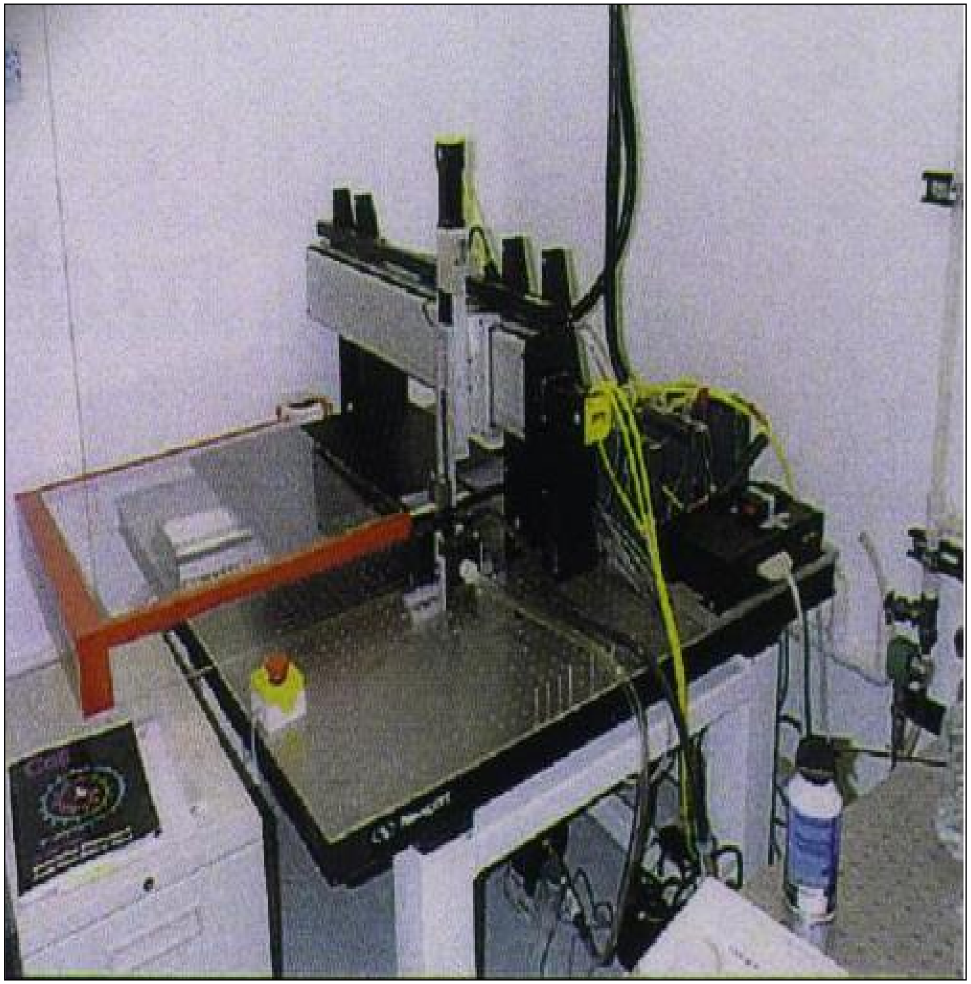
Hybing the array:

TARGET LABELING

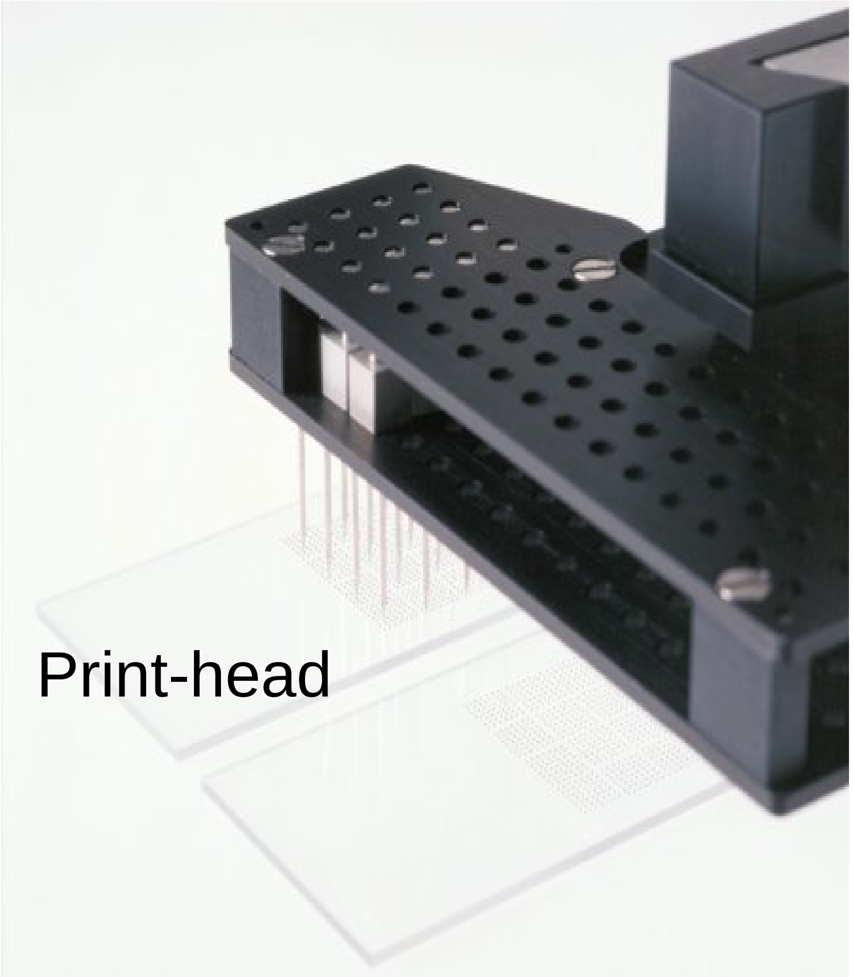
ARRAY HYBRIDIZATION
AND SCANNING

DATA ANALYSIS

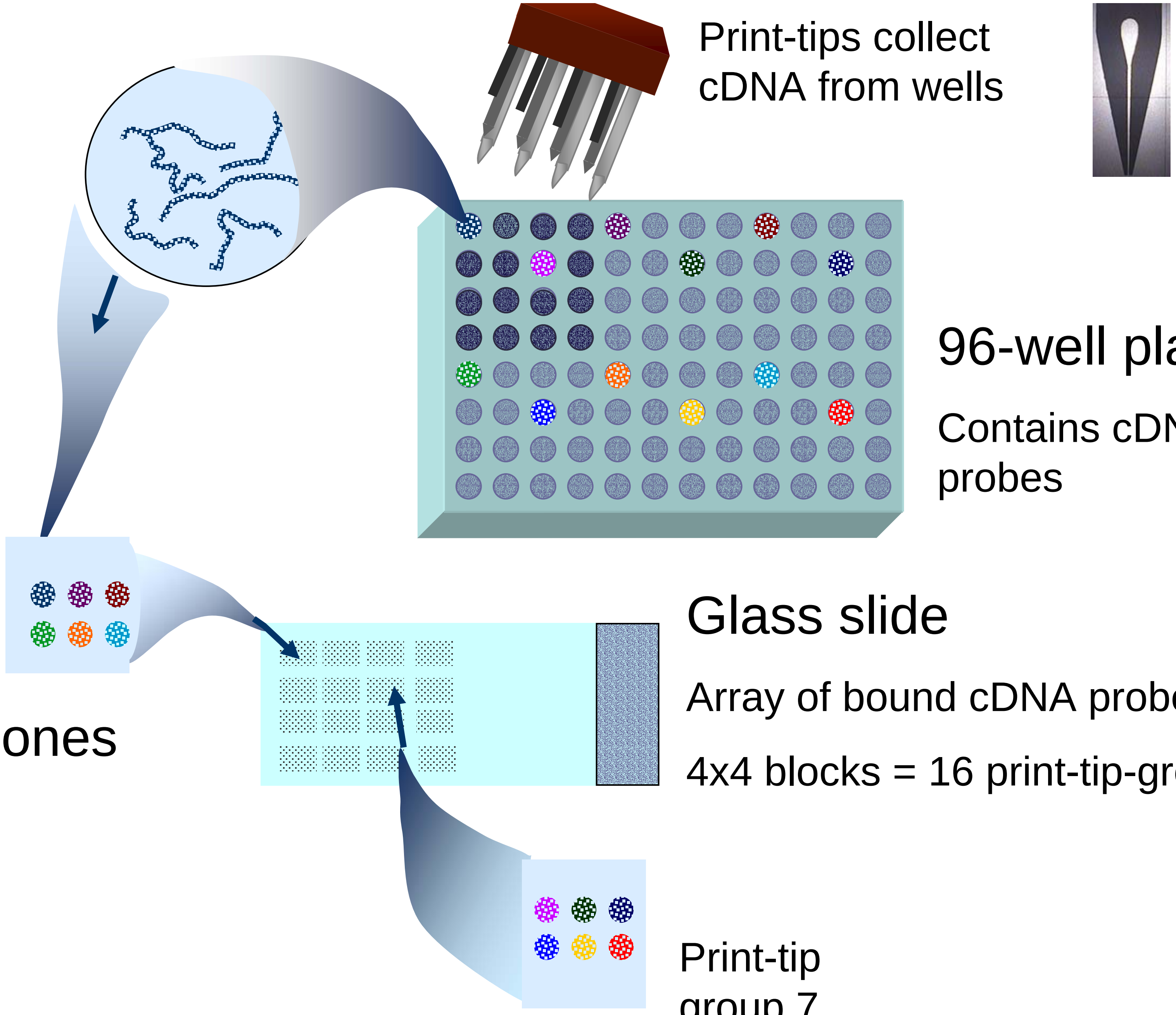
The arrayer



Ngai Lab arrayer, UC Berkeley



Print-head



Print-tips collect cDNA from wells

96-well plate

Contains cDNA probes

Print-tip group 1

cDNA clones

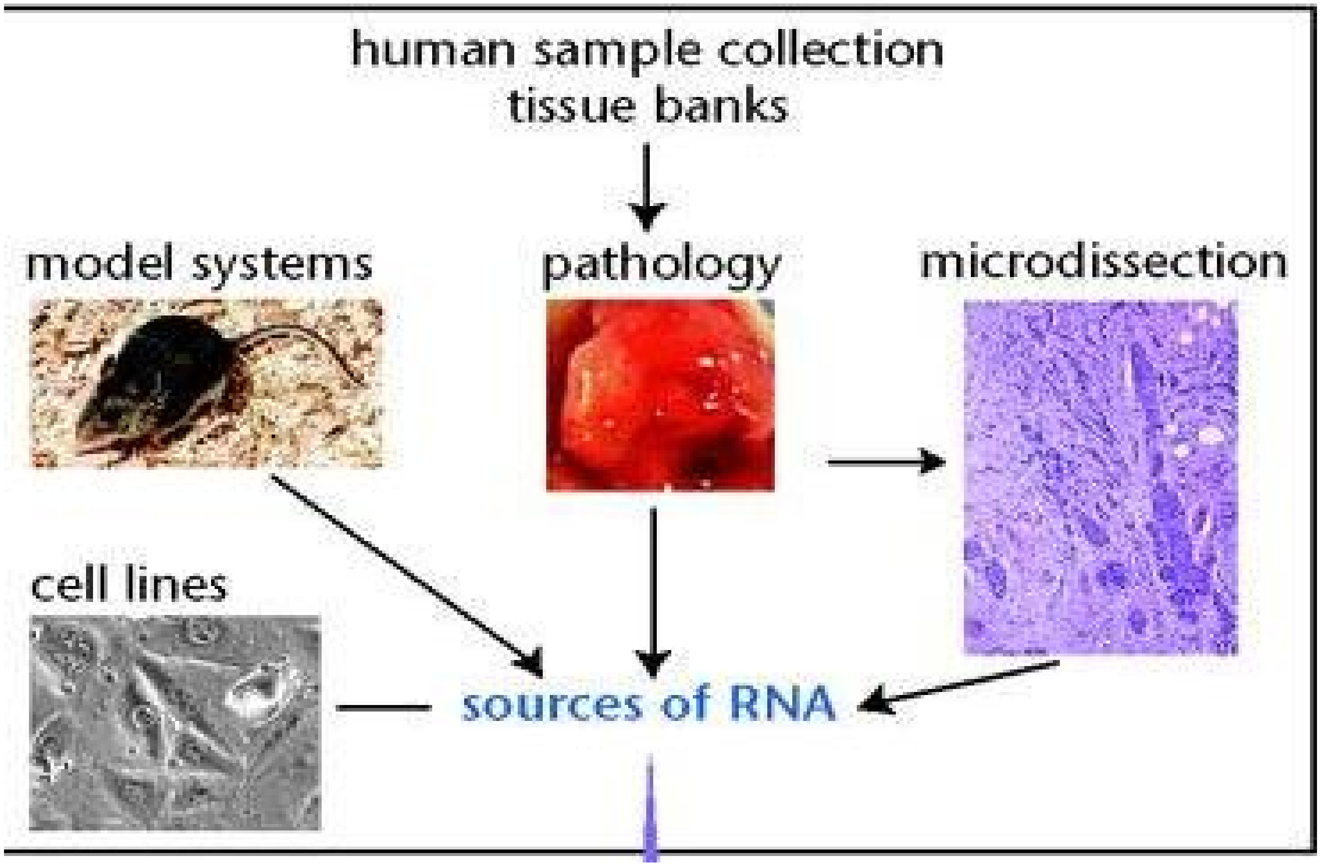
Glass slide

Array of bound cDNA probes

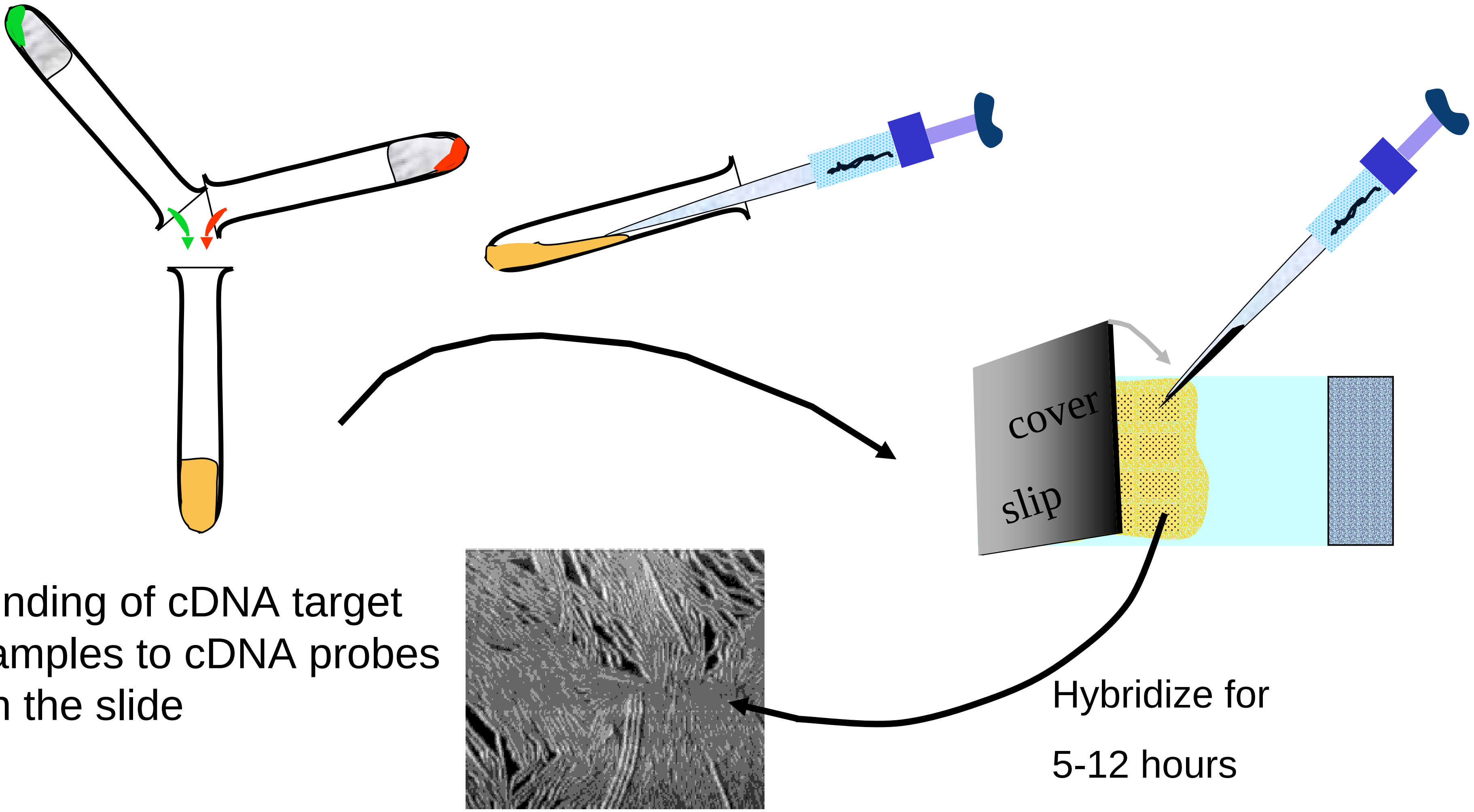
4x4 blocks = 16 print-tip-groups

Print-tip group 7

Sample preparation



Hybridization

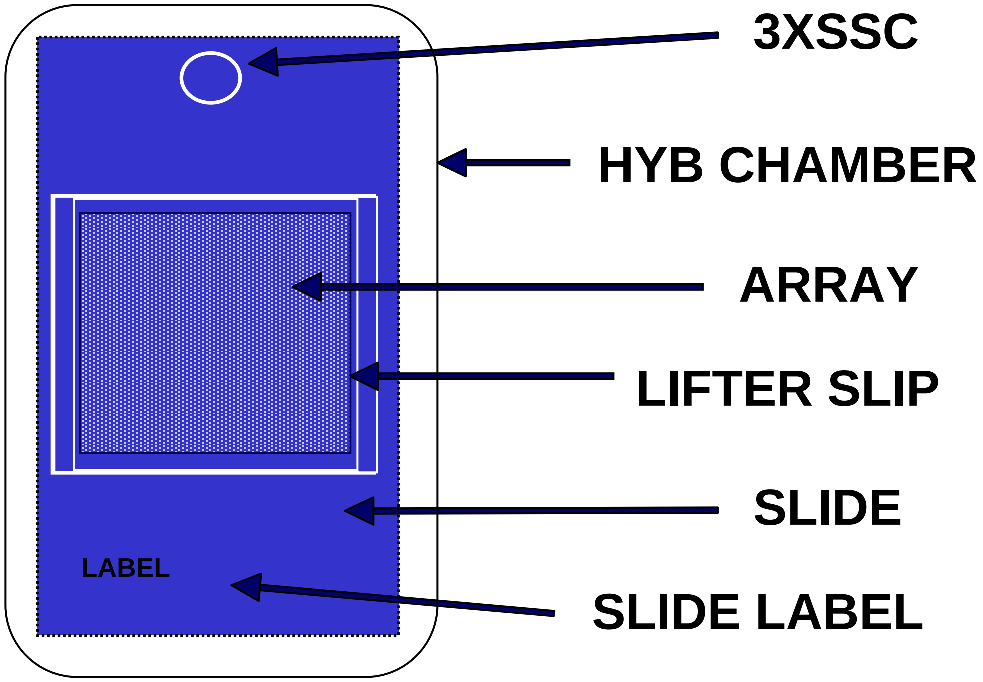


Binding of cDNA target samples to cDNA probes on the slide

Hybridize for

5-12 hours

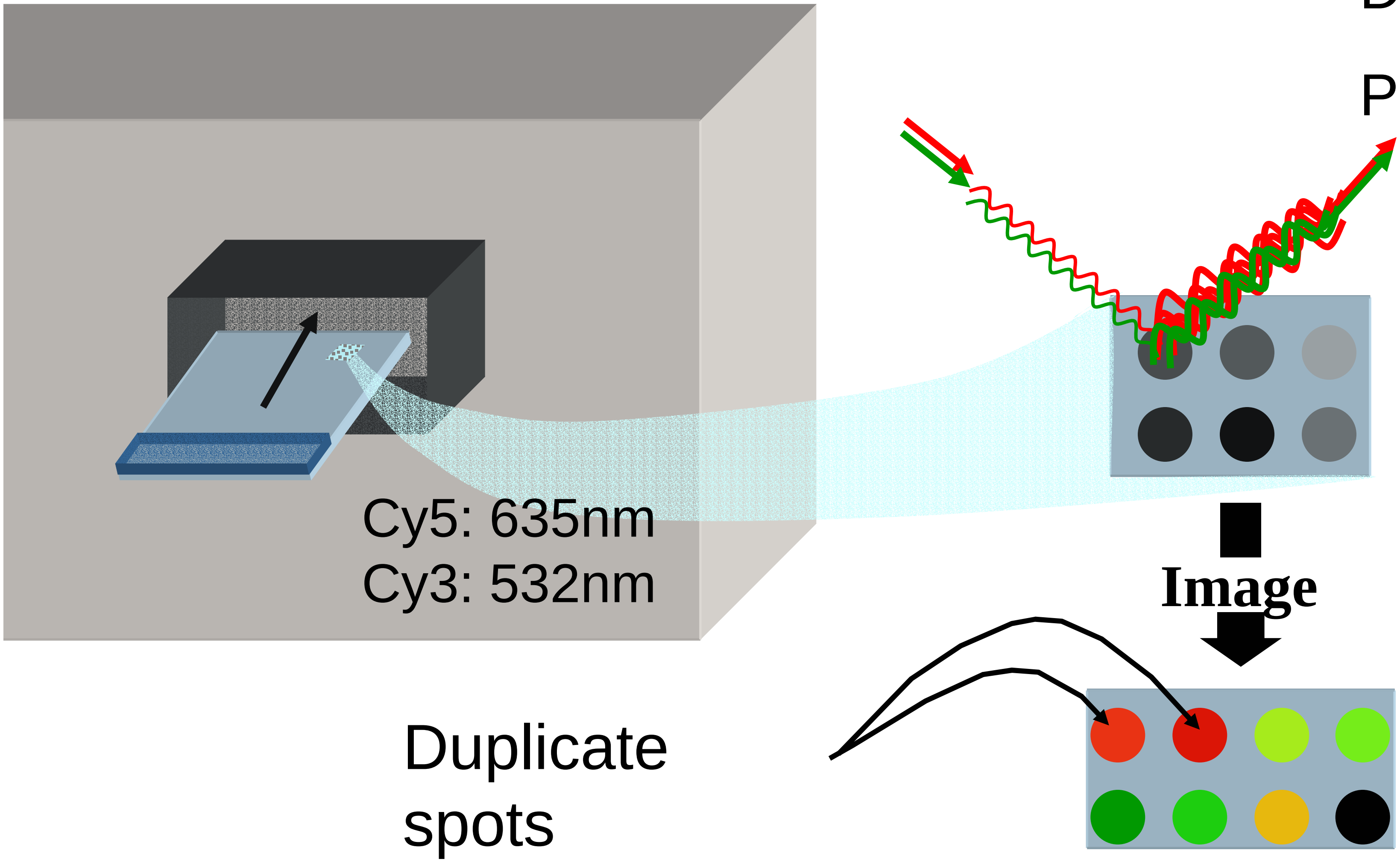
Hybridization chamber



- Humidity
- Temperature
- Formamide

(Lowers the Tmp)

Scanning



Detector

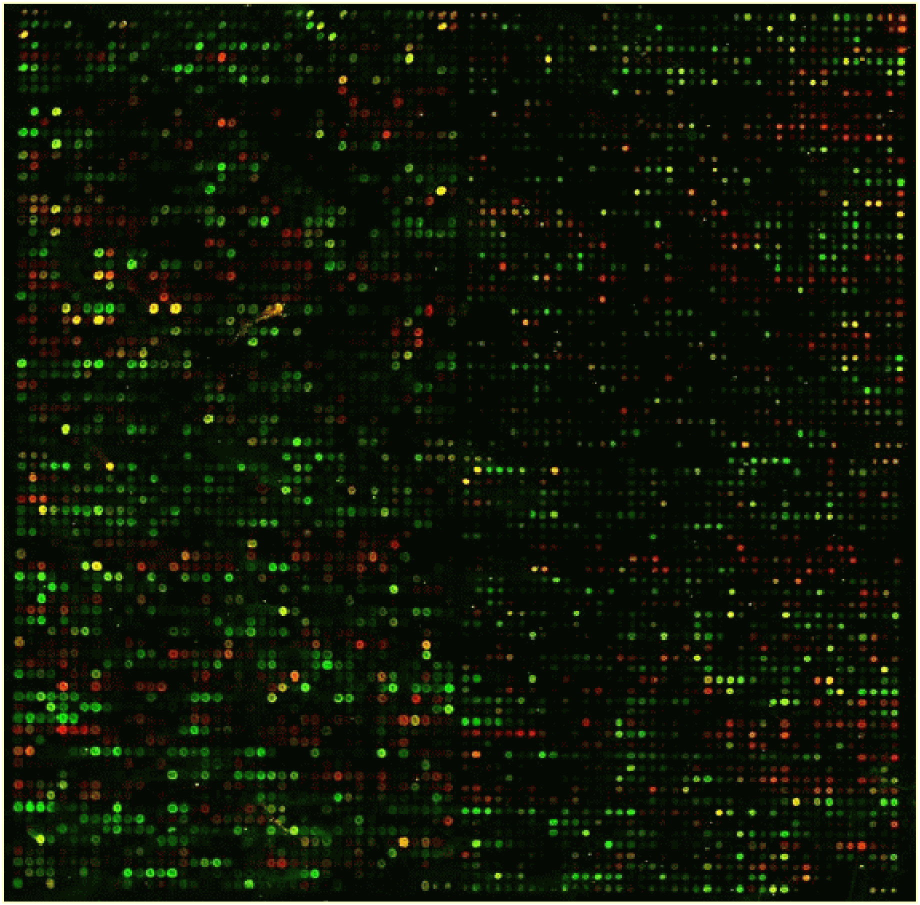
PMT

Cy5: 635nm
Cy3: 532nm

Image

Duplicate spots

RGB overlay of Cy3 and Cy5 images



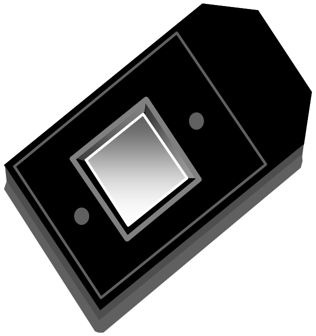
Raw data

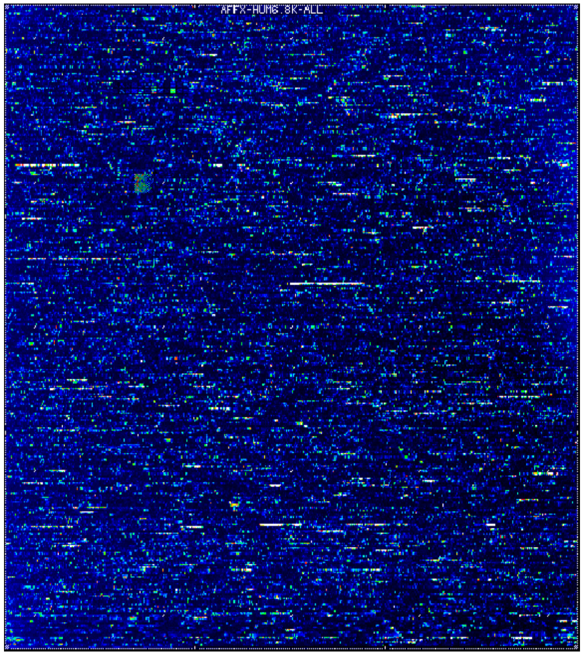
- Pairs of 16–bit TIFFs, one for each dye.
- E.g. Human cDNA arrays:
  - ~43K spots;
  - ~ 20Mb per channel;
  - ~ 2,000 x 5,500 pixels per image;
  - spot separation: ~ 136um.
- For a "typical" array, the spot area has
  - mean = 43 pixels;
  - med = 32 pixels;
  - SD = 26 pixels.

Animation

http://www.bio.davidson.edu/courses/genomics/chip/chip.html

Affymetrix oligonucleotide chips





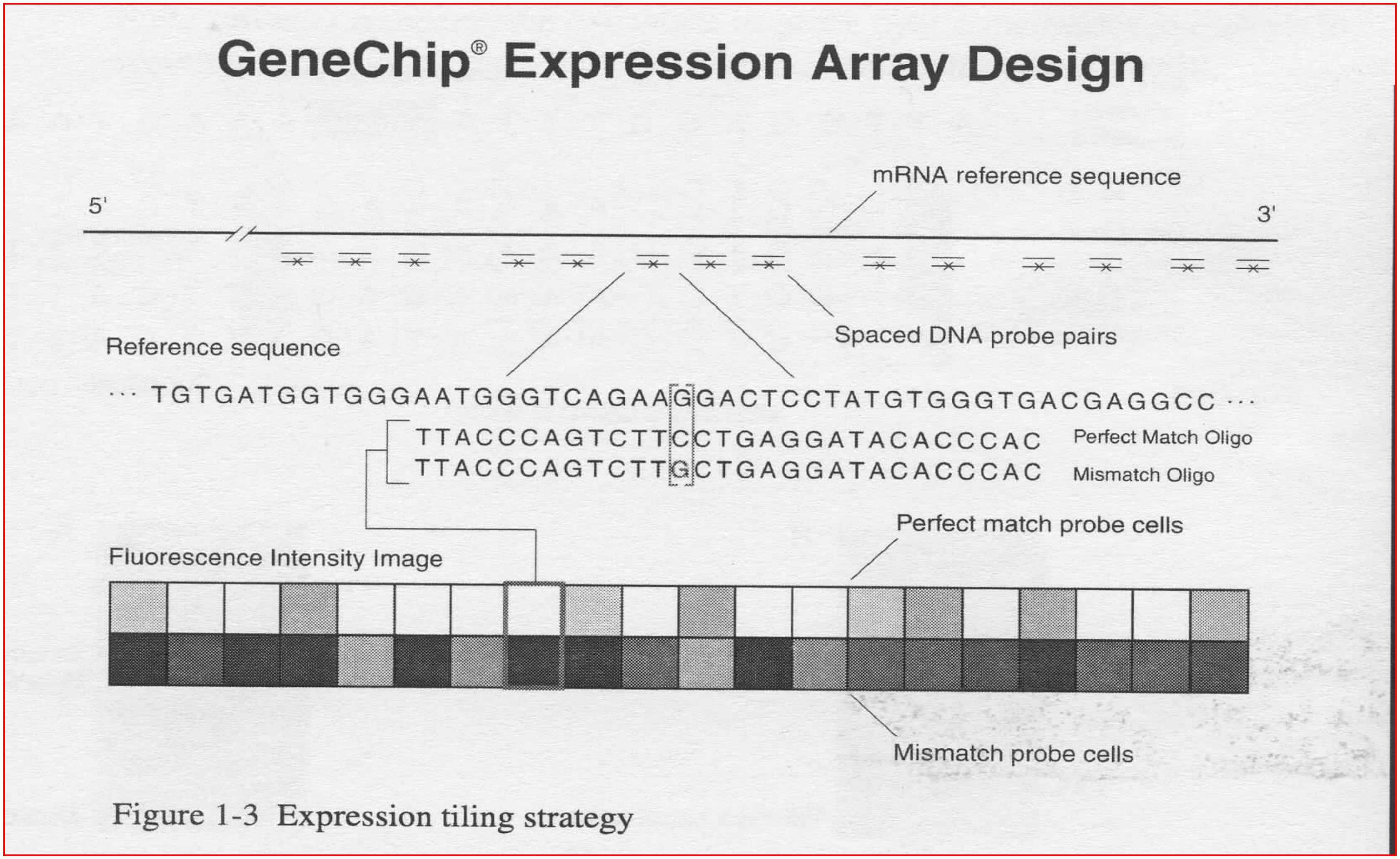
www.affymetrix.com

Terminology

- Each gene or portion of a gene is represented by 11 to 20 oligonucleotides of 25 base-pairs.

- Probe: an oligonucleotide of 25 base-pairs, i.e., a 25-mer.
- Perfect match (PM): A 25-mer complementary to a reference sequence of interest (e.g., part of a gene).
- Mismatch (MM): same as PM but with a single homomeric base change for the middle (13th) base (transversion purine <-> pyrimidine, G <->C, A <->T) .
- Probe-pair: a (PM,MM) pair.
- Probe-pair set: a collection of probe-pairs (11 to 20) related to a common gene or fraction of a gene.
- Affy ID: an identifier for a probe-pair set.
- The purpose of the MM probe design is to measure non-specific binding and background noise.

Probe-pair set



GeneChip® Expression Array Design

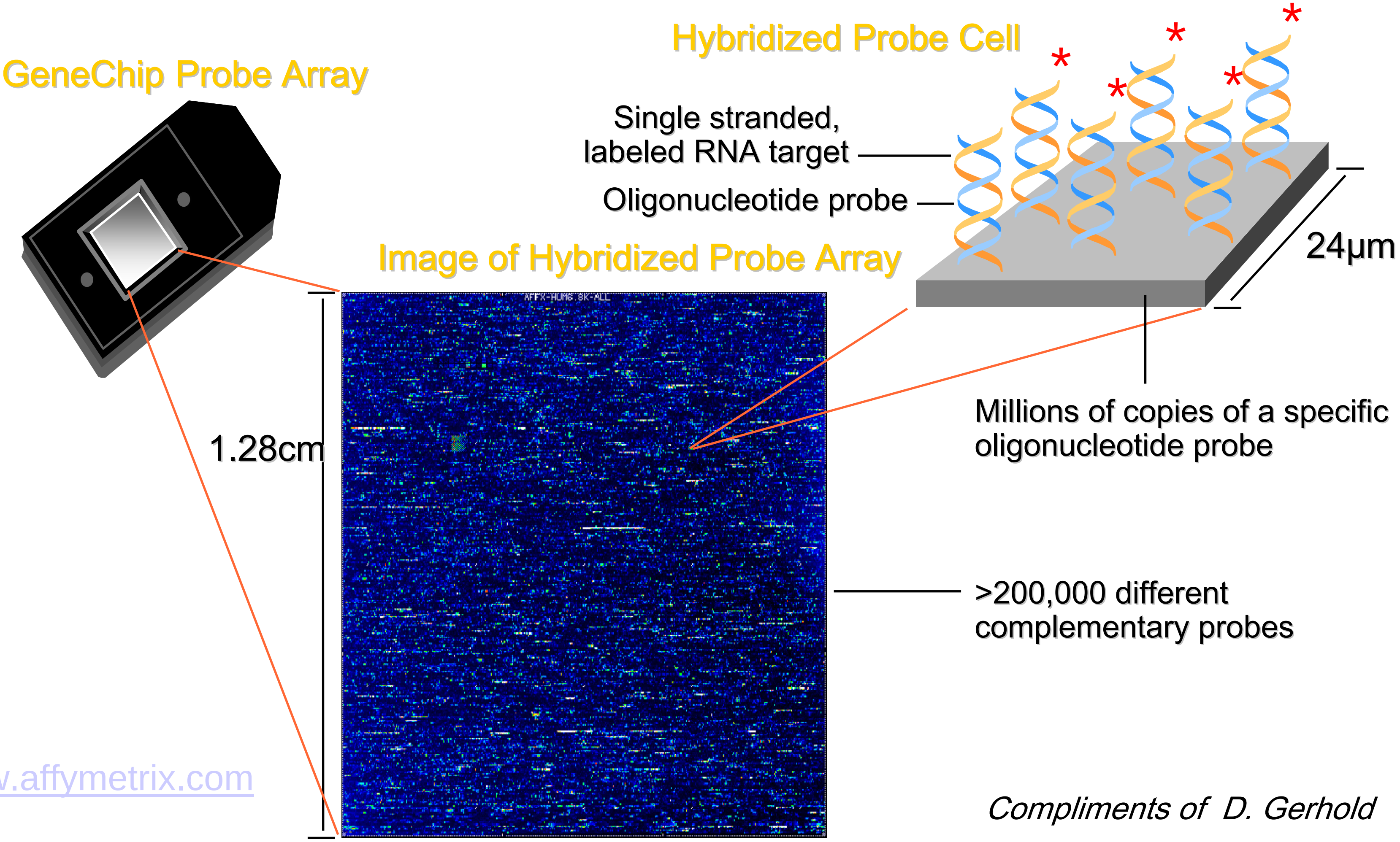
mRNA reference sequence
5'
3'
Spaced DNA probe pairs
Reference sequence
··· TGTGATGGTGGGAATGGGTCAGAAGGACTCCTATGTGGGTGACGAGGCC ···
TTACCCAGTCTTCCTGAGGATACACCCAC
Perfect Match Oligo
TTACCCAGTCTTGCTGAGGATACACCCAC
Mismatch Oligo
Perfect match probe cells
Fluorescence Intensity Image
Mismatch probe cells

Figure 1-3 Expression tiling strategy

Spotted vs. Affymetrix arrays

Spotted arrays	Affymetrix arrays
One probe per gene	11 – 20 probe-pairs per gene
Probes of varying length	Probes are 25-mers
Two target samples per array	One target sample per array

Oligonucleotide chips



GeneChip Probe Array

Hybridized Probe Cell

Single stranded, labeled RNA target

Oligonucleotide probe

24µm

Image of Hybridized Probe Array

1.28cm

Millions of copies of a specific oligonucleotide probe

>200,000 different complementary probes

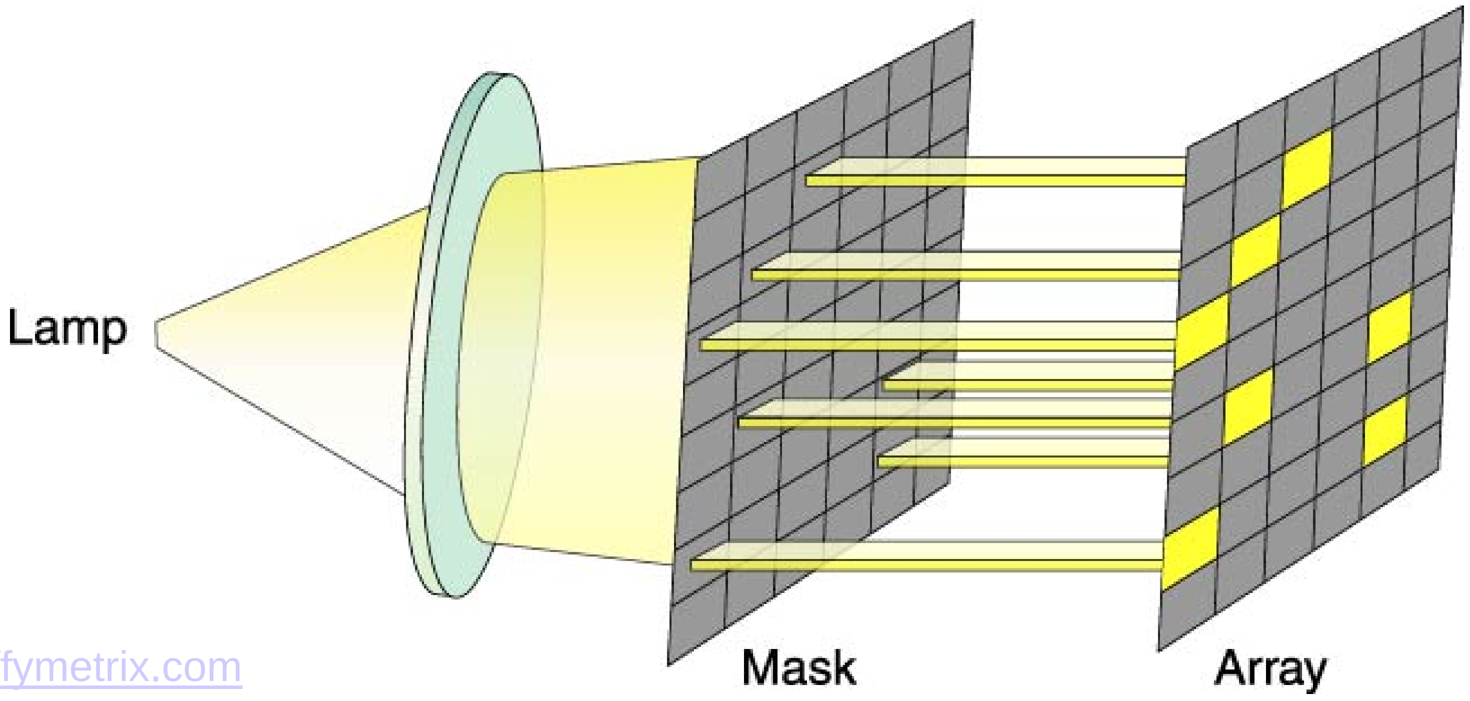
www.affymetrix.com

Compliments of D. Gerhold

Oligonucleotide chips

- The probes are synthesized *in situ*, using combinatorial chemistry and photolithography.
- Probe cells are square-shaped features on the chip containing millions of copies of a single 25-mer probe. Sides are 18-50 microns.

Oligonucleotide chips

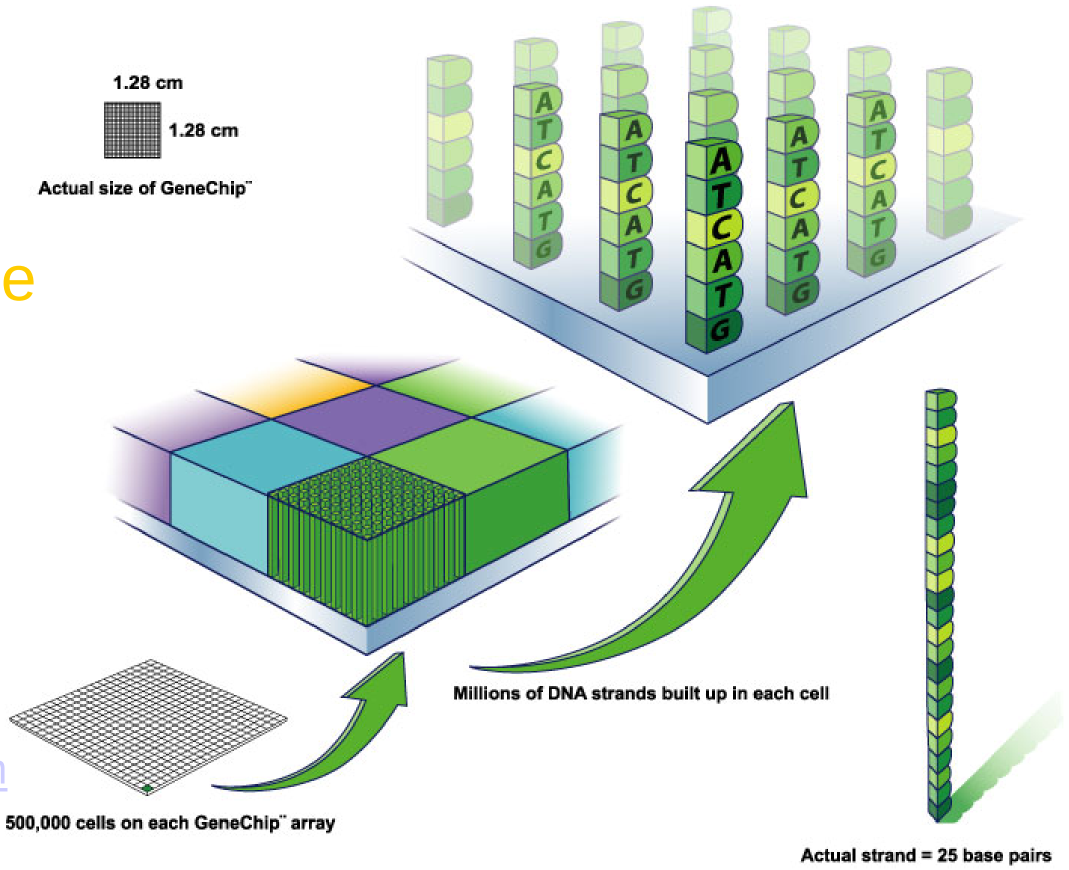


www.affymetrix.com

The manufacturing of GeneChip® probe arrays is a combination of photolithography and combinational chemistry.

Oligonucleotide chips

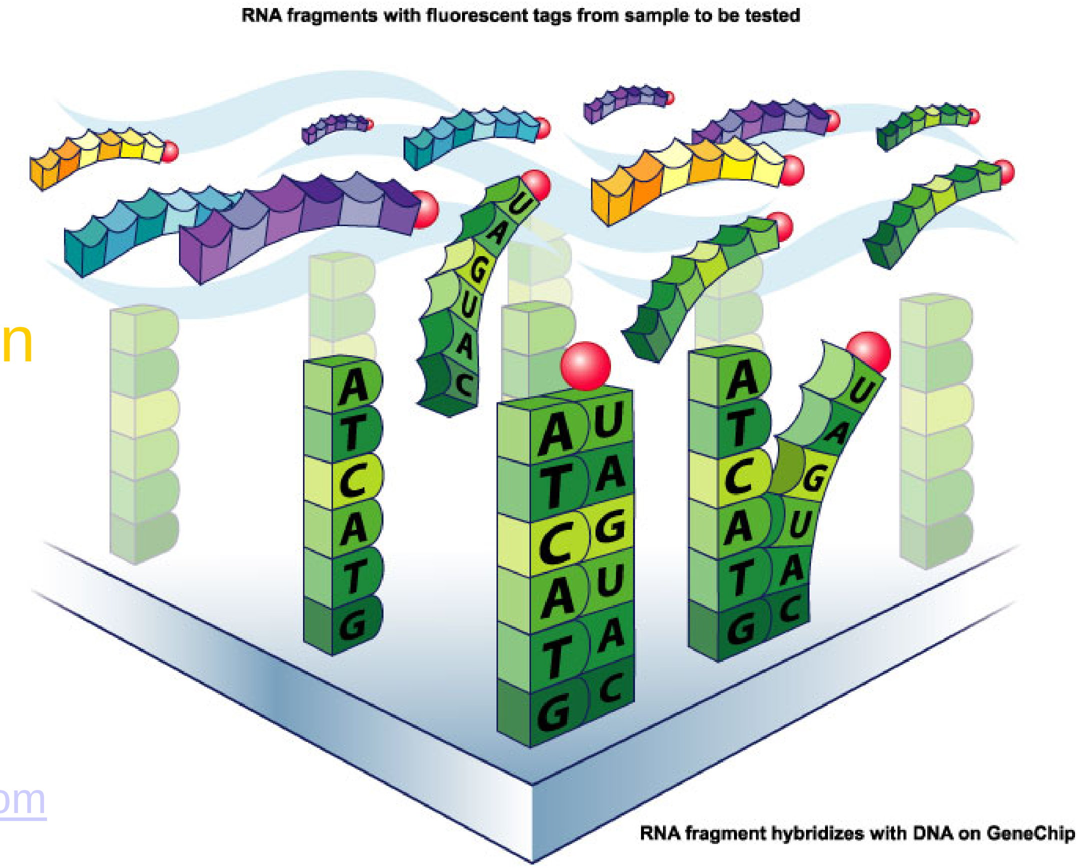
Single feature


1.28 cm
1.28 cm
Actual size of GeneChip™
A
T
C
A
T
G
Millions of DNA strands built up in each cell
500,000 cells on each GeneChip™ array
Actual strand = 25 base pairs

www.affymetrix.com

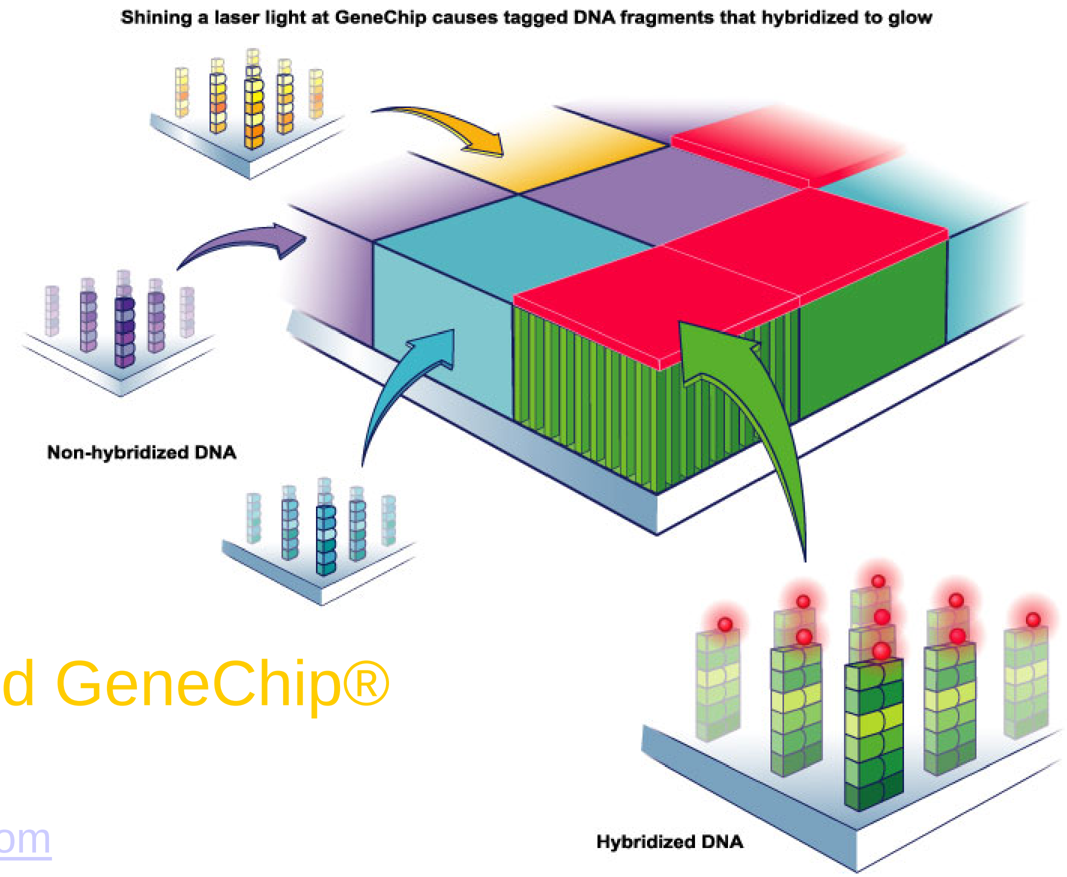
Oligonucleotide chips

Hybridization


RNA fragments with fluorescent tags from sample to be tested
U A G U A C
A T C A T G
A T C A T G
U A G U A C
A T C A T G
U A G U A C
RNA fragment hybridizes with DNA on GeneChip

www.affymetrix.com

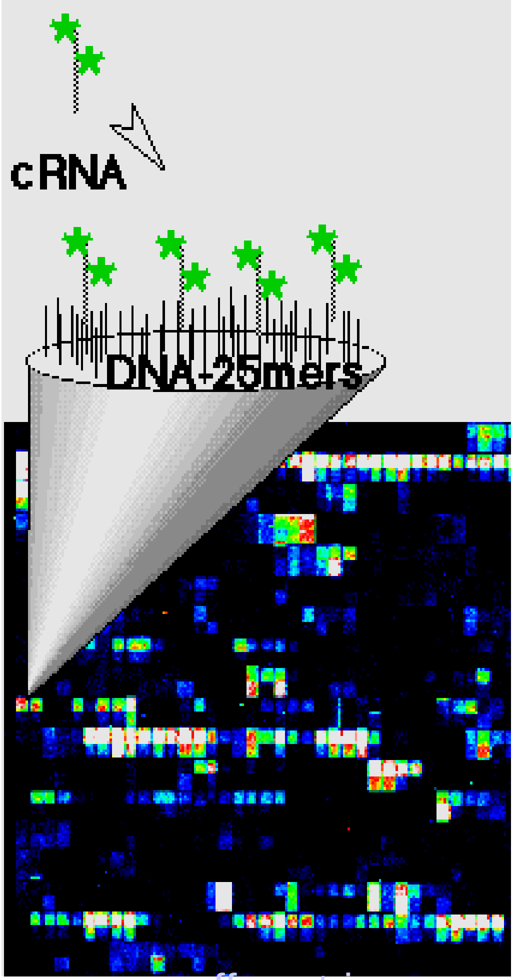
Oligonucleotide chips


Shining a laser light at GeneChip causes tagged DNA fragments that hybridized to glow
Non-hybridized DNA
Hybridized DNA

Hybridized GeneChip®

www.affymetrix.com

Image analysis



www.affymetrix.com

- About 100 pixels per probe cell.
- These intensities are combined to form one number representing the expression level for the probe cell oligo.
- → CEL file with PM or MM intensity for each cell.

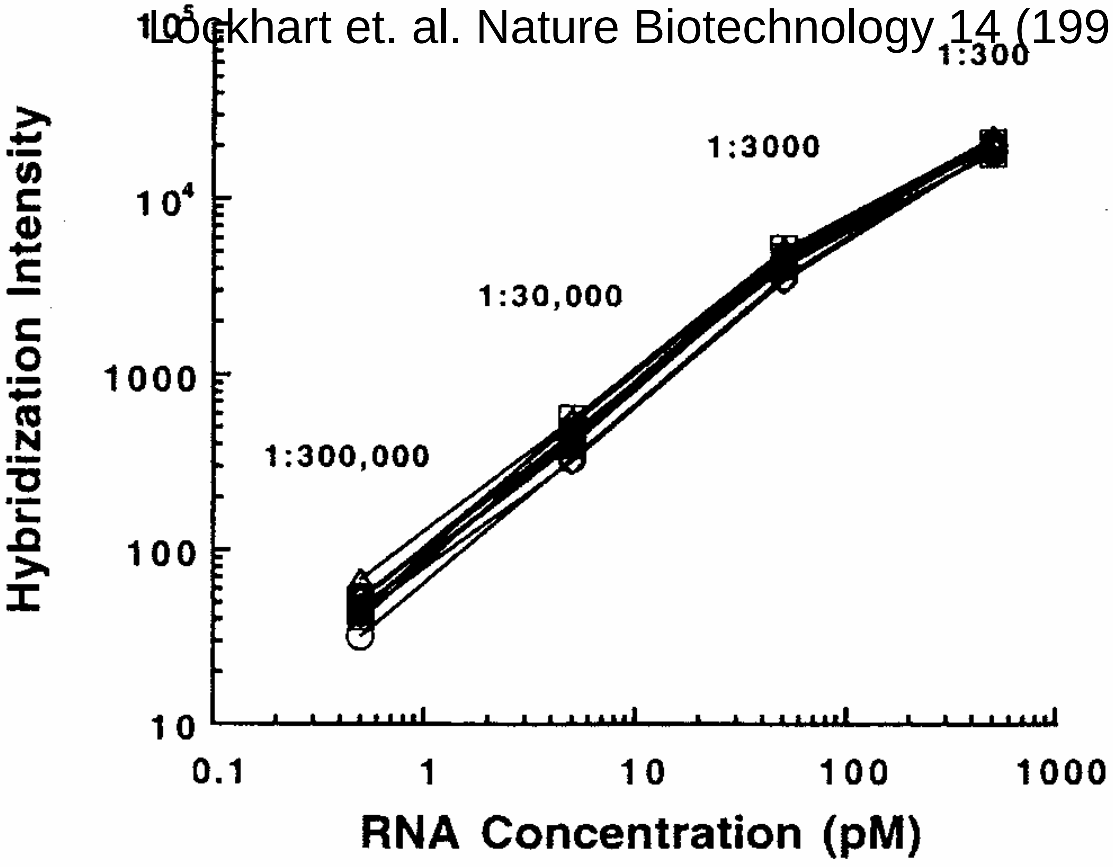
Expression measures

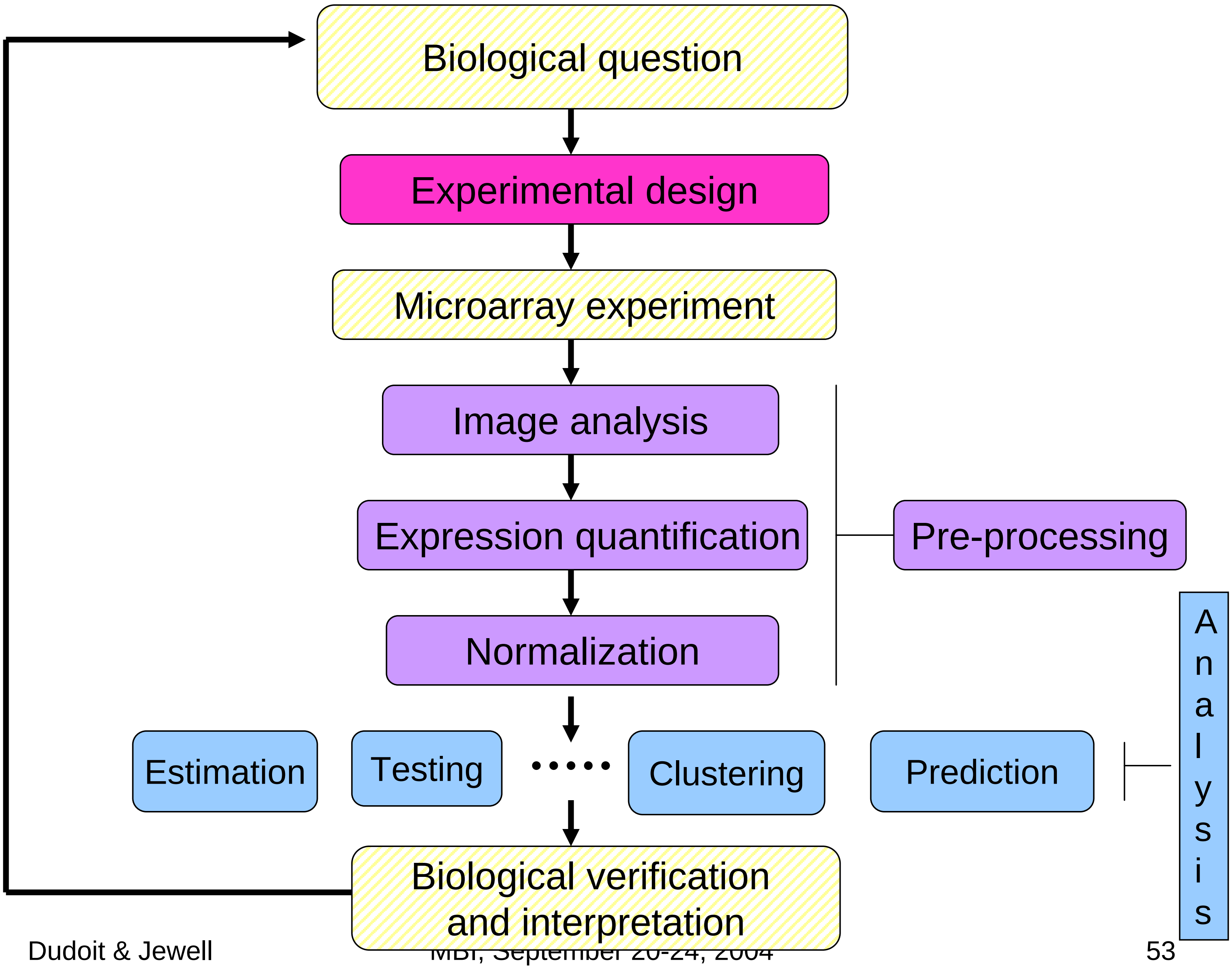
- Most expression measures are based on differences of **PM-MM**.
- The intention is to correct for background and non-specific binding.
- E.g. *MarrayArray Suite*® (MAS) v. 4.0 uses Average Difference Intensity (ADI) or

  AvDiff = average of PM-MM.
- Problem: MM may also measure signal.
- More on this in lecture *Pre-processing DNA Microarray Data.*

What is the evidence?

Lockhart et. al. Nature Biotechnology 14 (1996)





Biological question

Experimental design

Microarray experiment

Image analysis

Expression quantification

Normalization

Pre-processing

Estimation

Testing

•••••

Clustering

Prediction

Analysis

Biological verification and interpretation

Statistical computing

Everywhere …

- Statistical design and analysis:
  - image analysis, normalization, estimation, testing, clustering, prediction, etc.
- Integration of experimental metadata with biological metadata from WWW-resources
  - gene annotation (GenBank, LocusLink);
  - literature (PubMed);
  - graphical (pathways, chromosome maps).

Integration of experimental and biological metadata

- Phenotypes, microarray gene expression measures, sequence, structure, annotation, literature.
- Integration will depend on our using a common language and will rely on database methodology as well as statistical analyses.
- This area is largely unexplored.

WWW resources

- **Complete guide to “microarraying”**
  http://cmgm.stanford.edu/pbrown/mguide/
  http://www.microarrays.org
  - Parts and assembly instructions for printer and scanner;
  - Protocols for sample prep;
  - Software;
  - Forum, etc.
- **cDNA microarray animation**
  http://www.bio.davidson.edu/courses/genomics/chip/chip.html
- **Affymetrix**
  http://www.affymetrix.com