Introduction to DNA Microarray Technologies

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

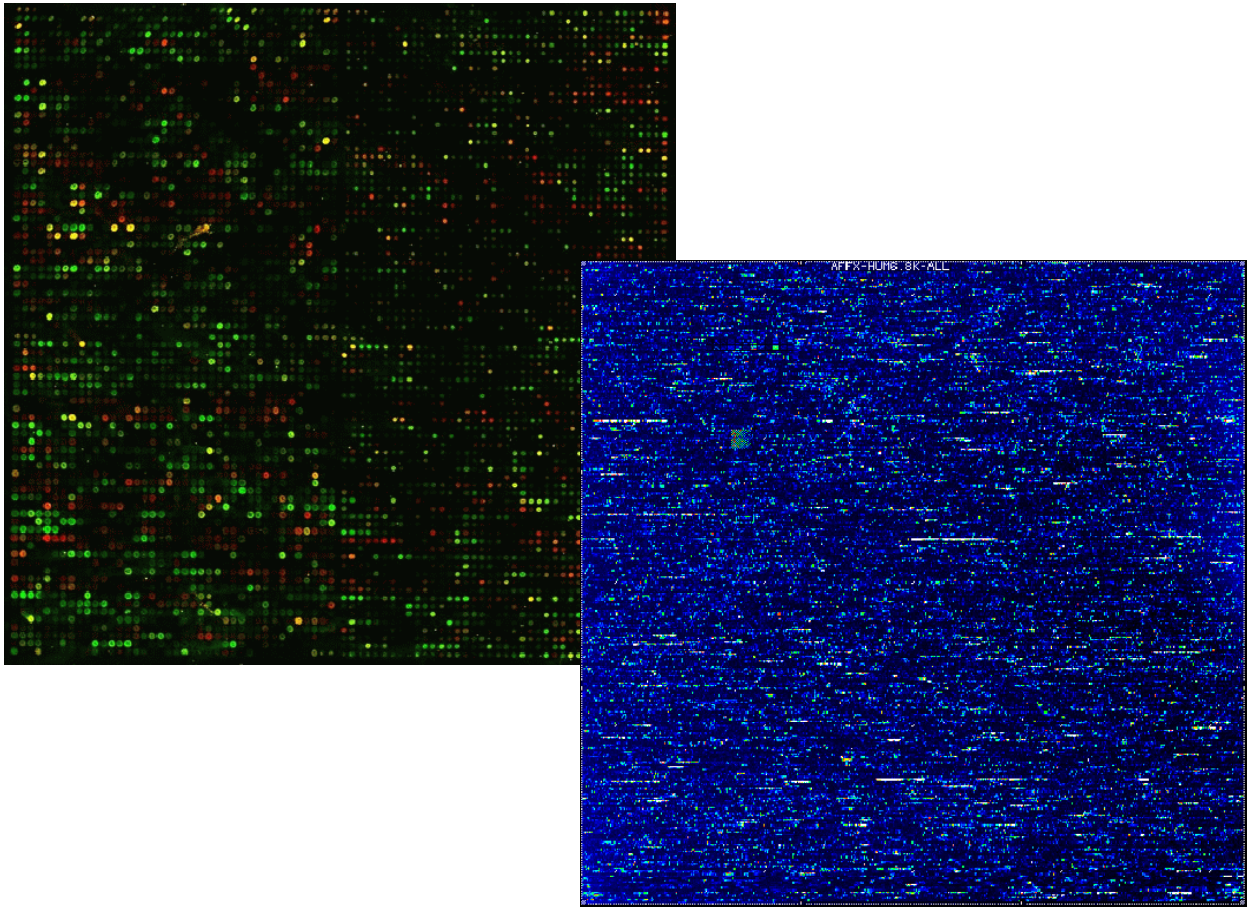
Bioconductor Short Course

Winter 2002

Outline

- Basic principles
- Spotted DNA microarrays
- Affymetrix oligonucleotide chips

DNA microarrays



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.

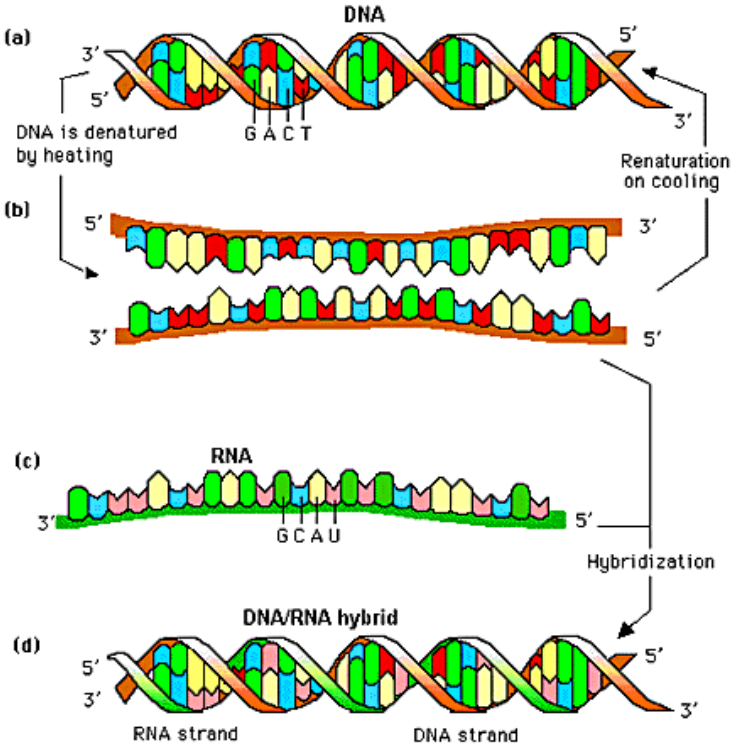
The ancestor of cDNA microarrays: the Northern blot.

Hybridization

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

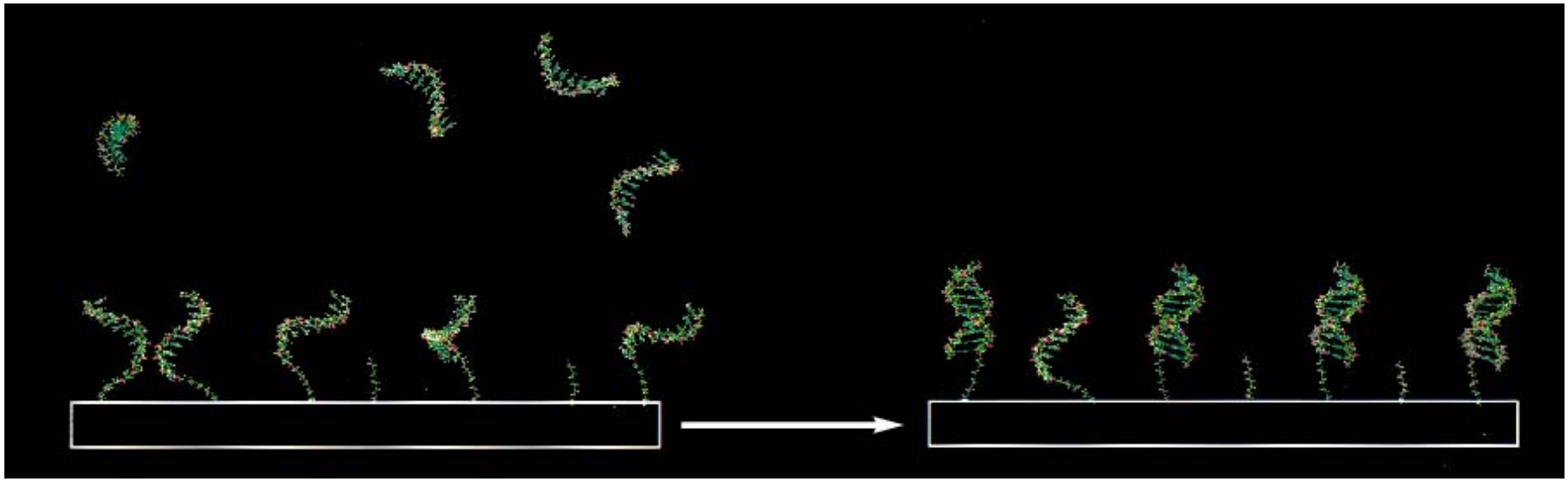
- Nucleic acid strands in a duplex can be separated, or denatured, by heating to destroy the hydrogen bonds.

Hybridization



Nucleic Acid Hybridization

Hybridization



Gene expression assays

The main types of gene expression assays:

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

Applications of microarrays

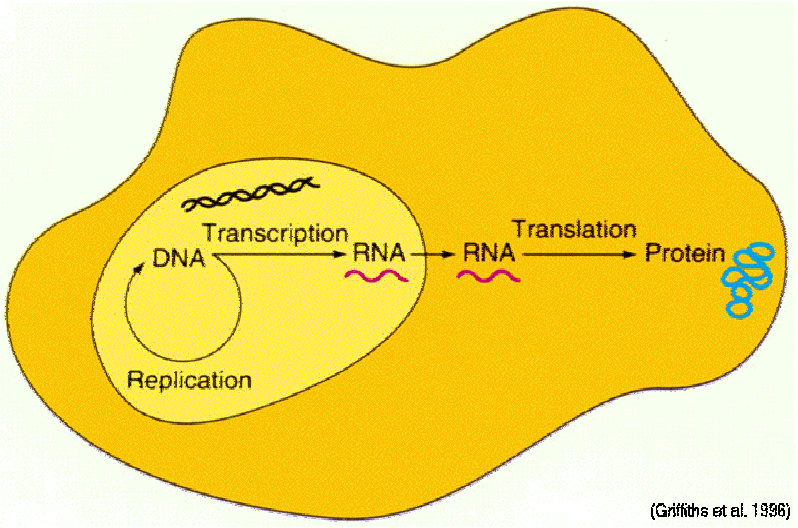
- Measuring transcript abundance (cDNA arrays);
- Genotyping;
- Estimating DNA copy number (CGH);
- Determining identity by descent (GMS);
- Measuring mRNA decay rates;
- Identifying protein binding sites;
- Determining sub-cellular localization of gene products;
- …

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.
- **…**

Transcriptome



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

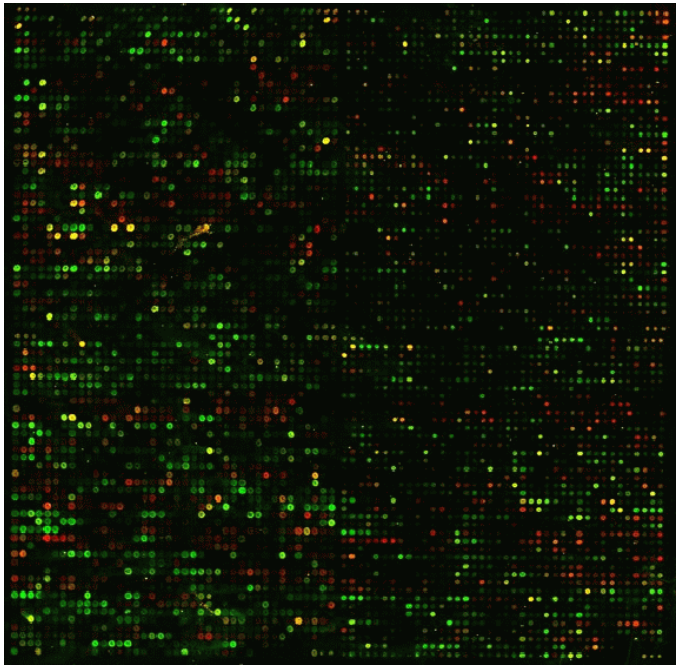
Transcriptome

- 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.

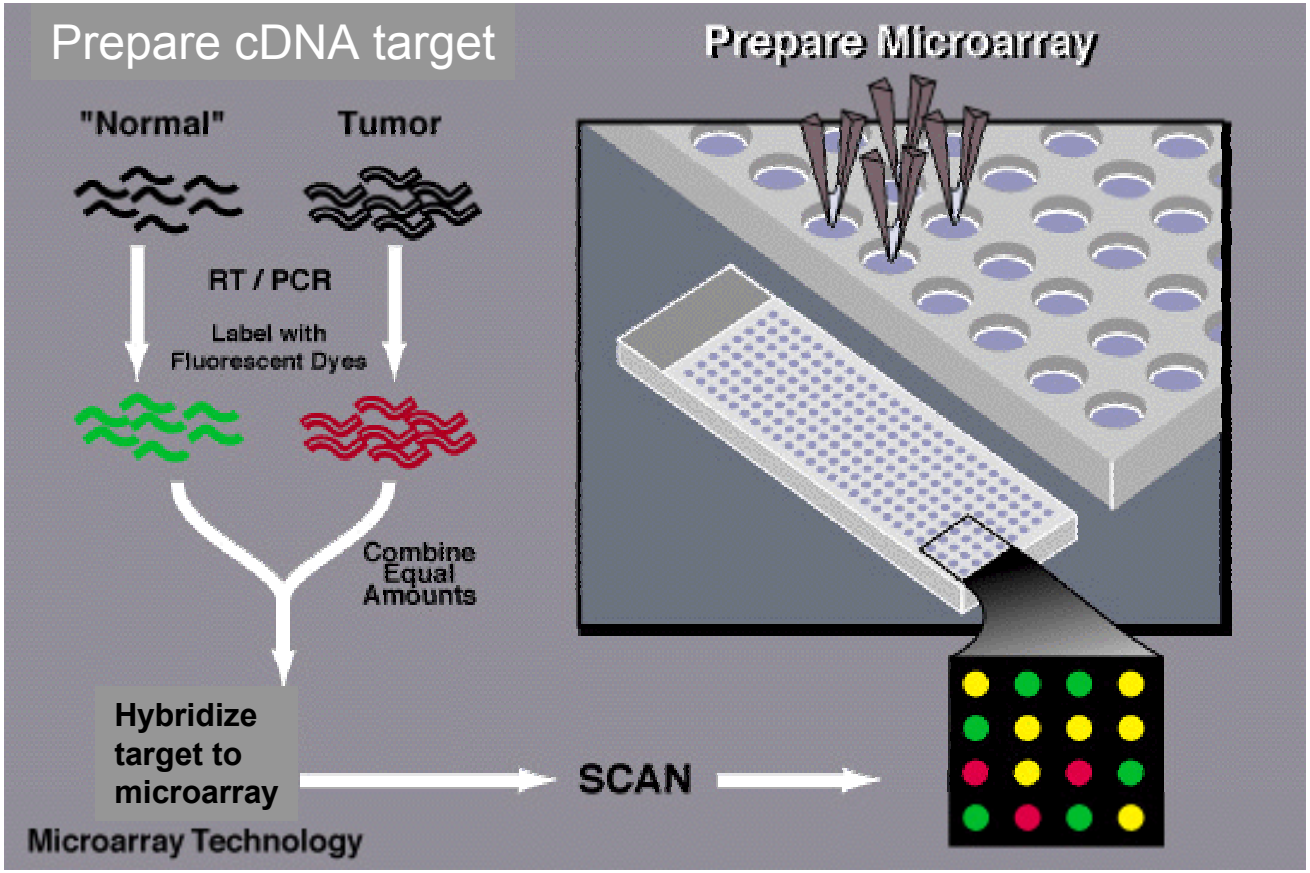
Applications of microarrays

- 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.

Spotted DNA microarrays



Spotted DNA microarrays



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.

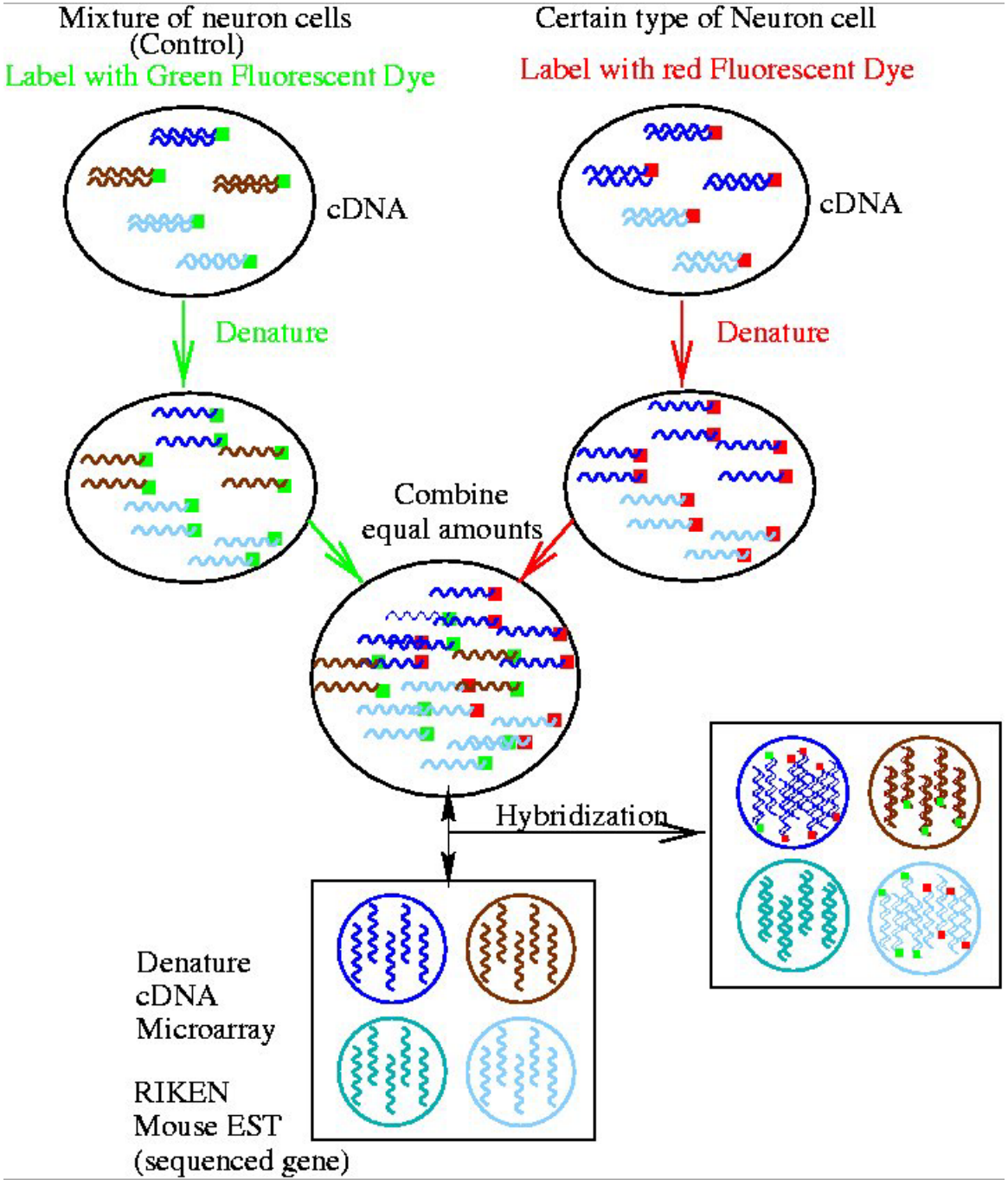
Spotted DNA microarrays

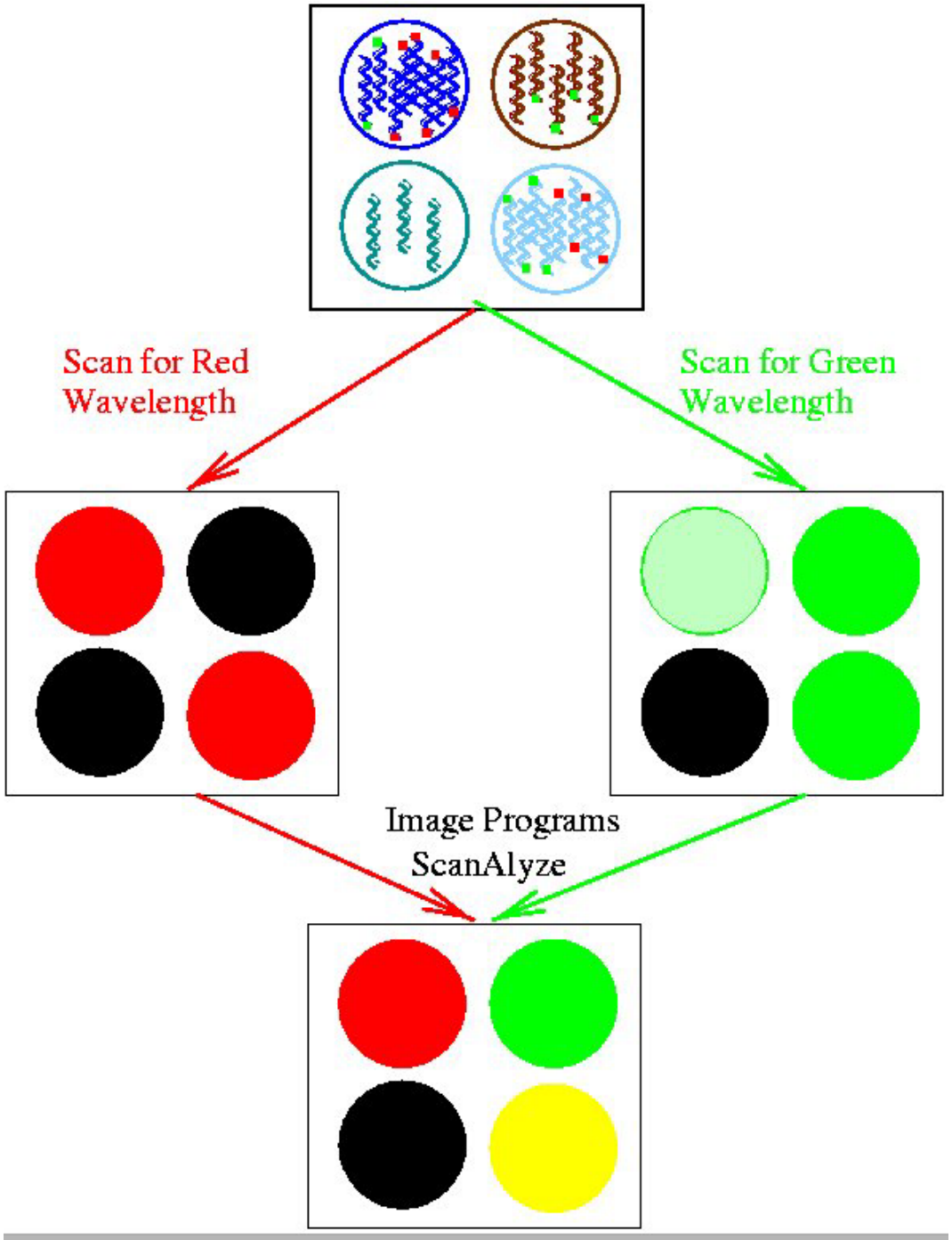
- The ratio of the red and green fluorescence intensities for each spot is indicative of the relative abundance of the corresponding DNA probe in the two nucleic acid target samples.

Spotted DNA microarrays

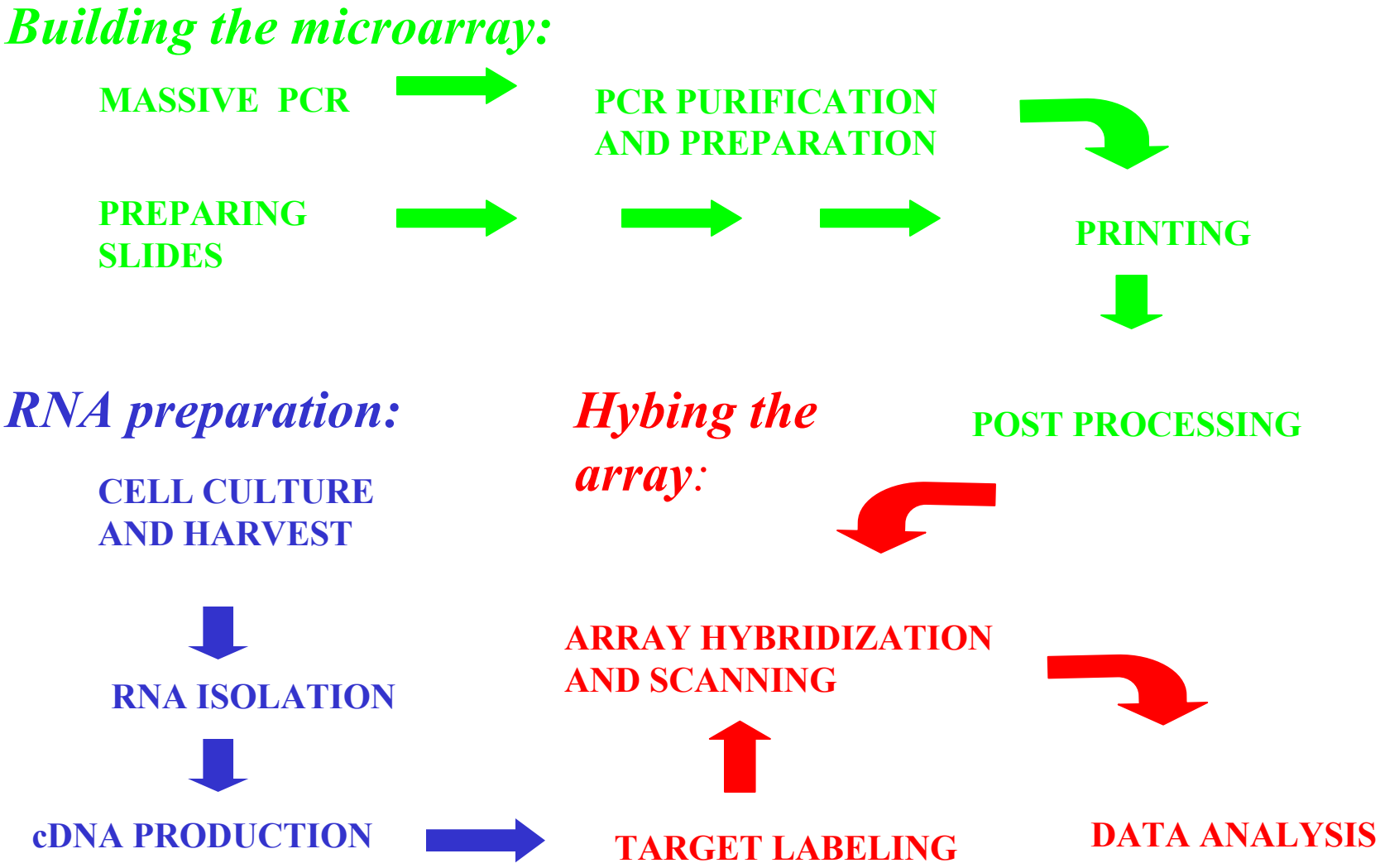
$$M = \log_2 R/G = \log_2 R - \log_2 G$$

- **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.

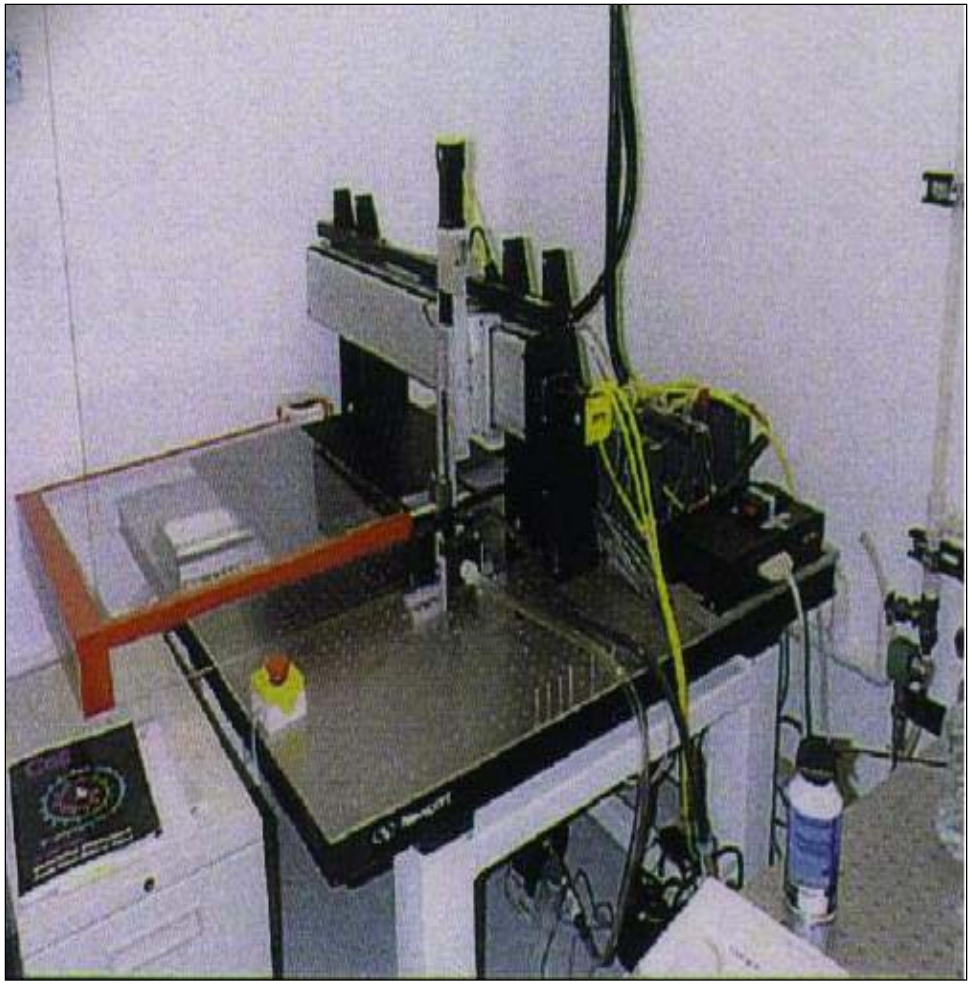

Mixture of neuron cells
(Control)
Label with Green Fluorescent Dye
Certain type of Neuron cell
Label with red Fluorescent Dye
cDNA
cDNA
Denature
Denature
Combine
equal amounts
Hybridization
Denature
cDNA
Microarray
RIKEN
Mouse EST
(sequenced gene)


Scan for Red
Wavelength
Scan for Green
Wavelength
Image Programs
ScanAlyze

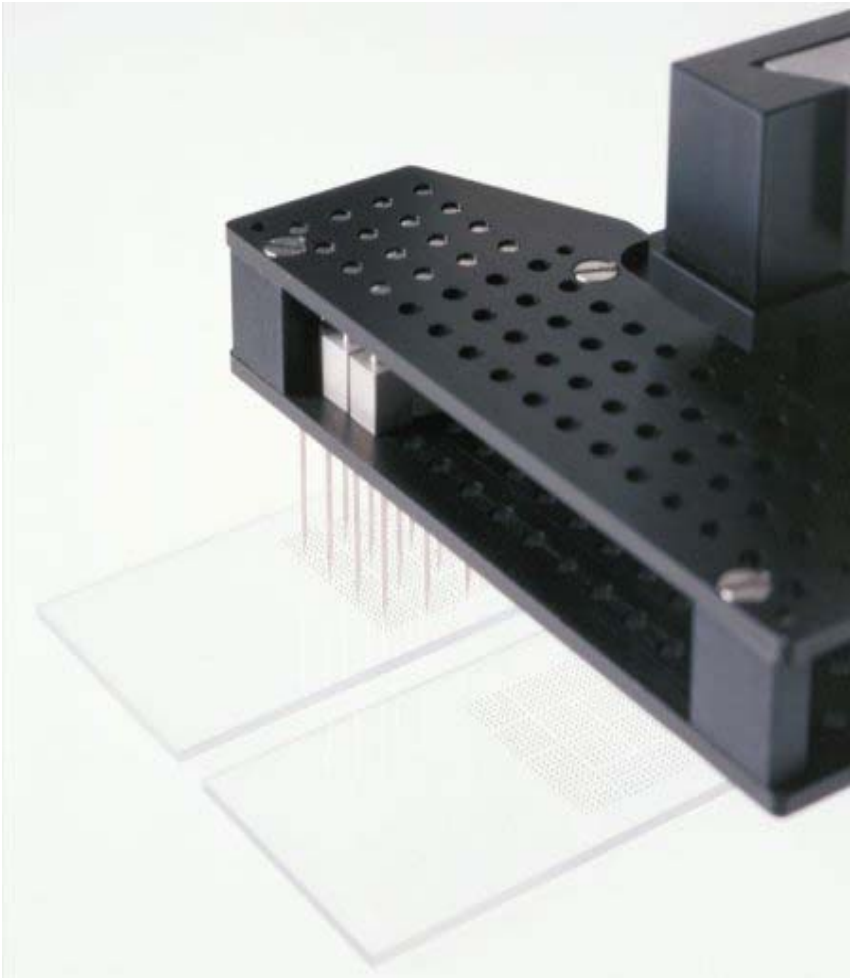
The process


Building the microarray:
MASSIVE PCR
PCR PURIFICATION AND PREPARATION
PREPARING SLIDES
PRINTING
RNA preparation:
Hybing the array:
POST PROCESSING
CELL CULTURE AND HARVEST
ARRAY HYBRIDIZATION AND SCANNING
RNA ISOLATION
cDNA PRODUCTION
TARGET LABELING
DATA ANALYSIS

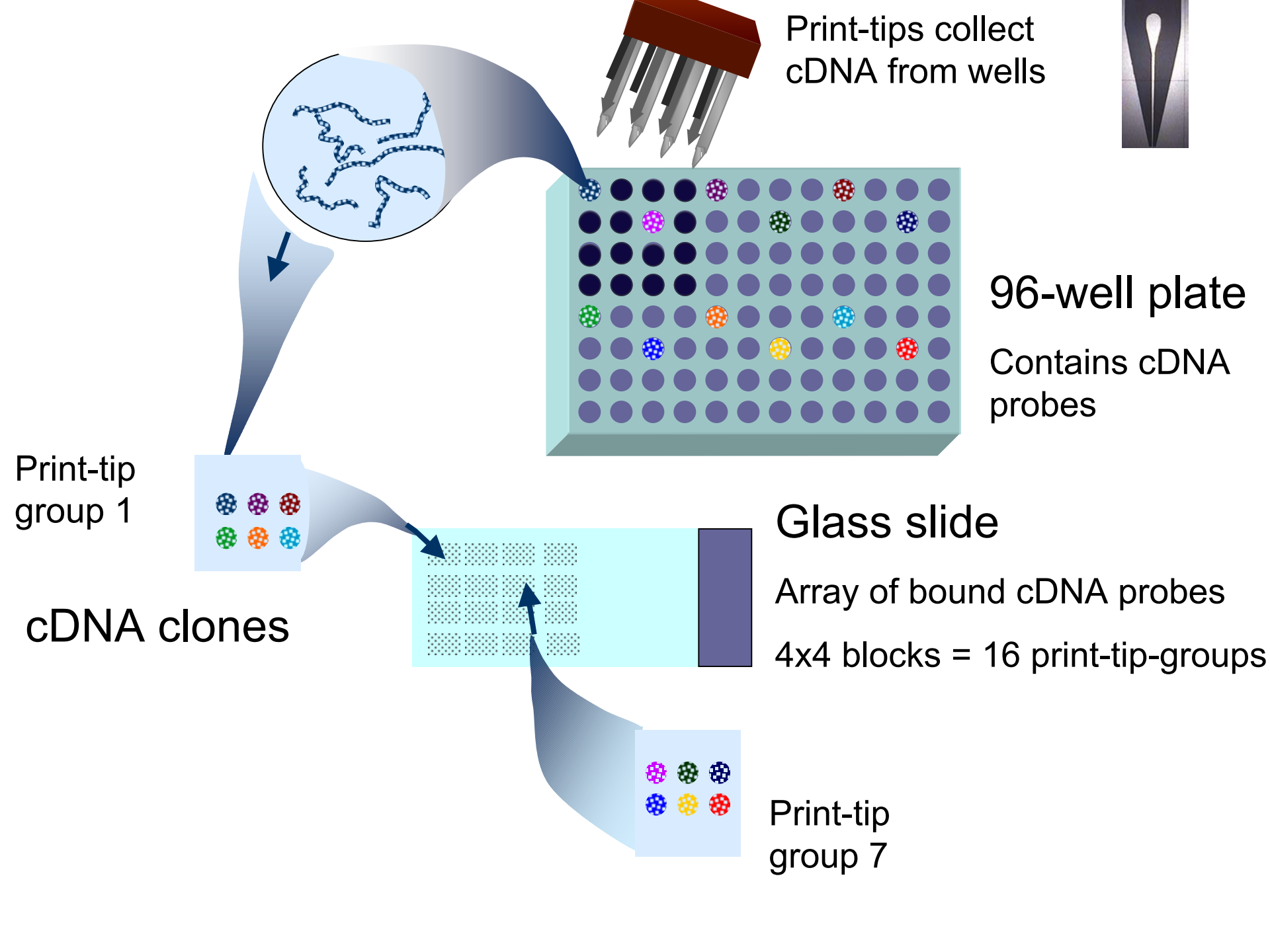
The arrayer



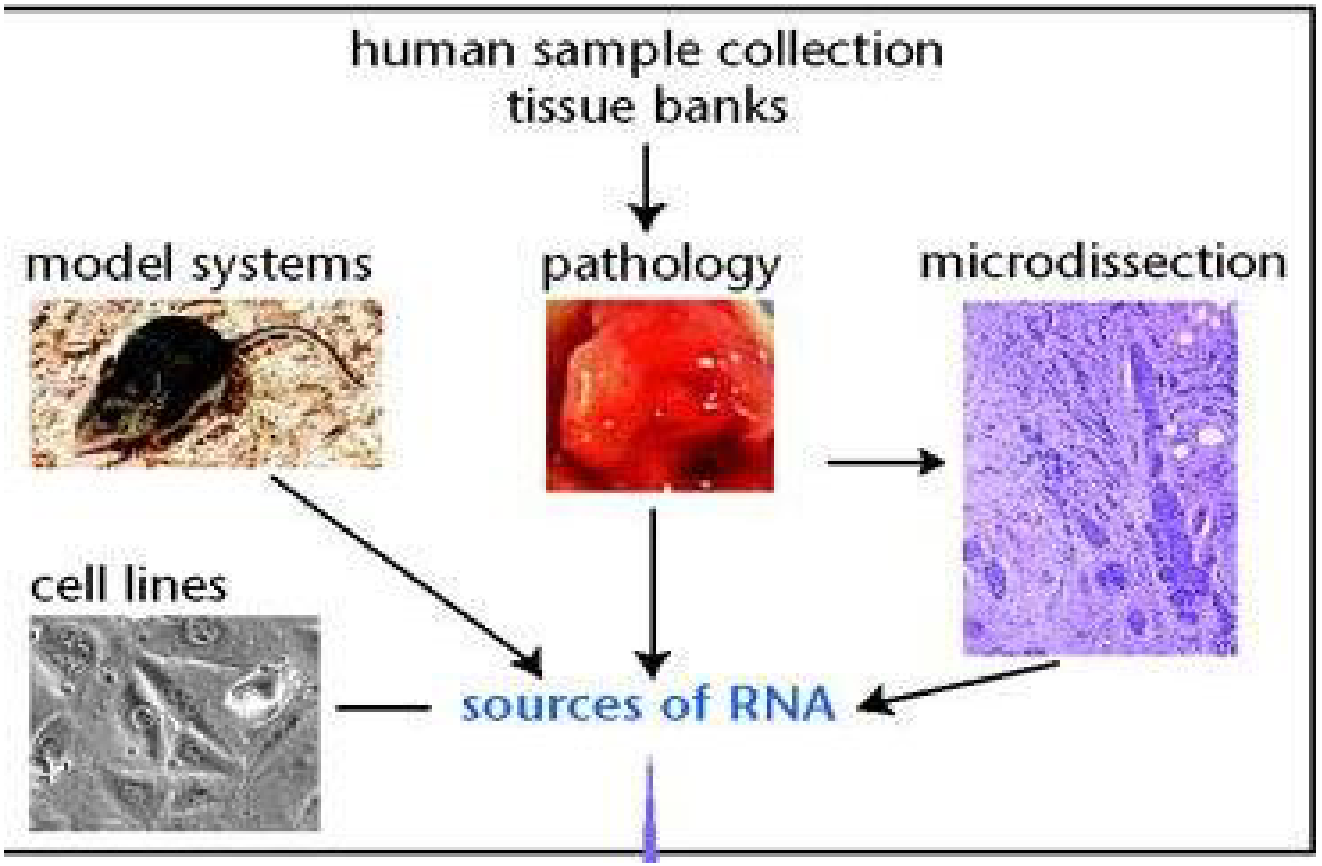
Ngai Lab arrayer, UC Berkeley



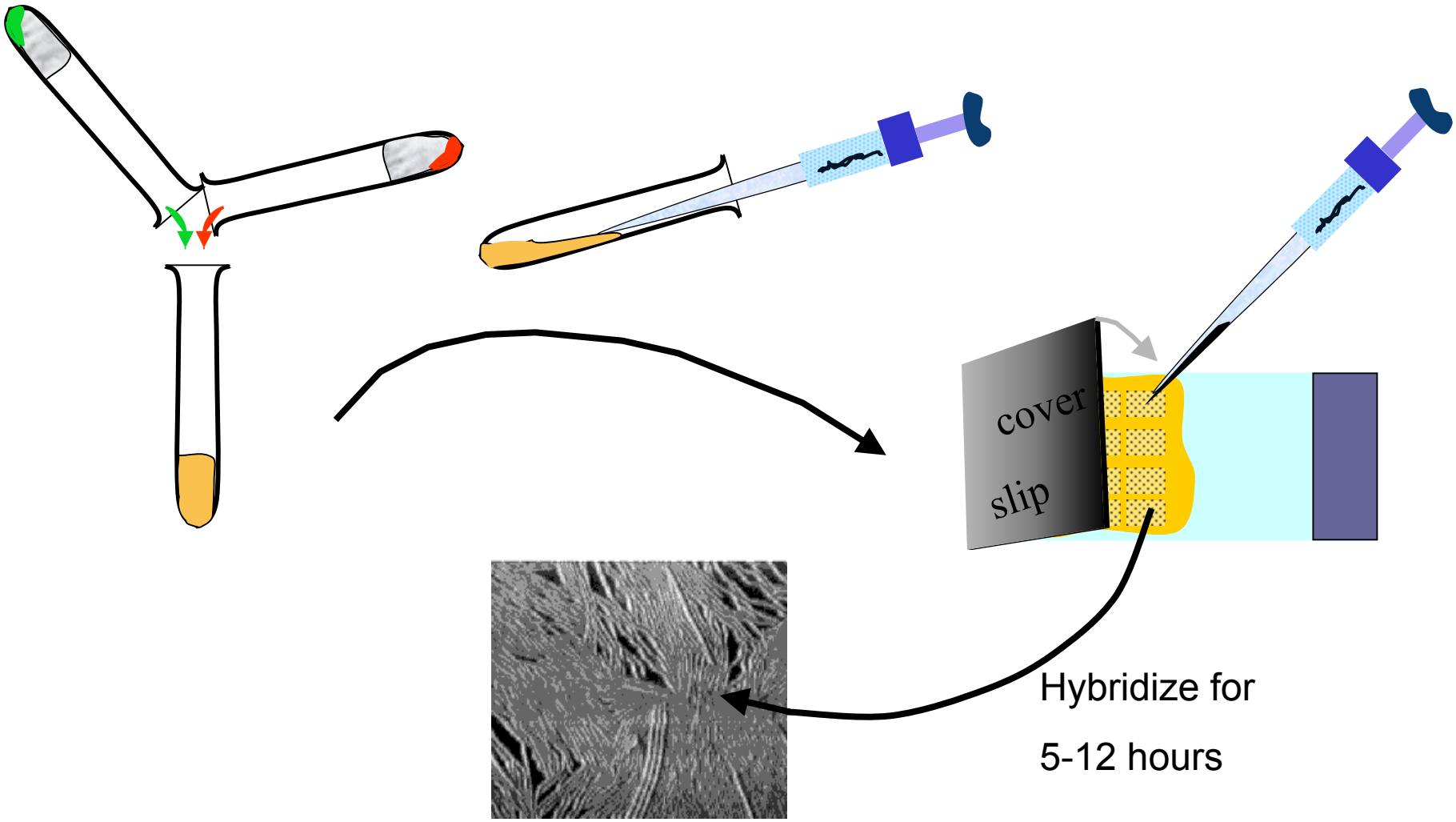
Print-head


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

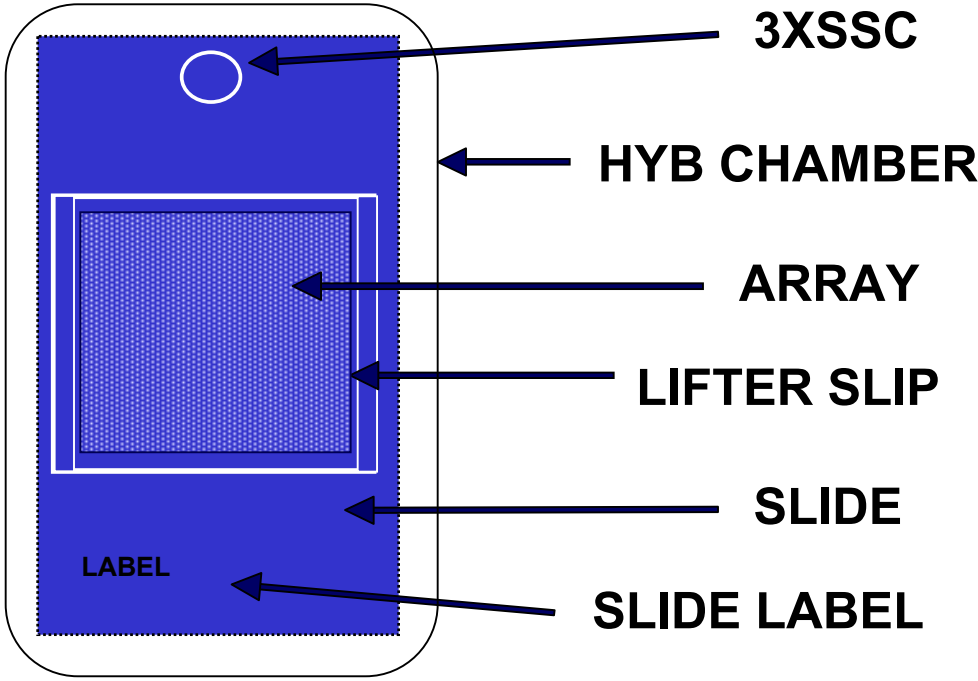


Hybridization



Binding of cDNA target samples to cDNA probes on the slide

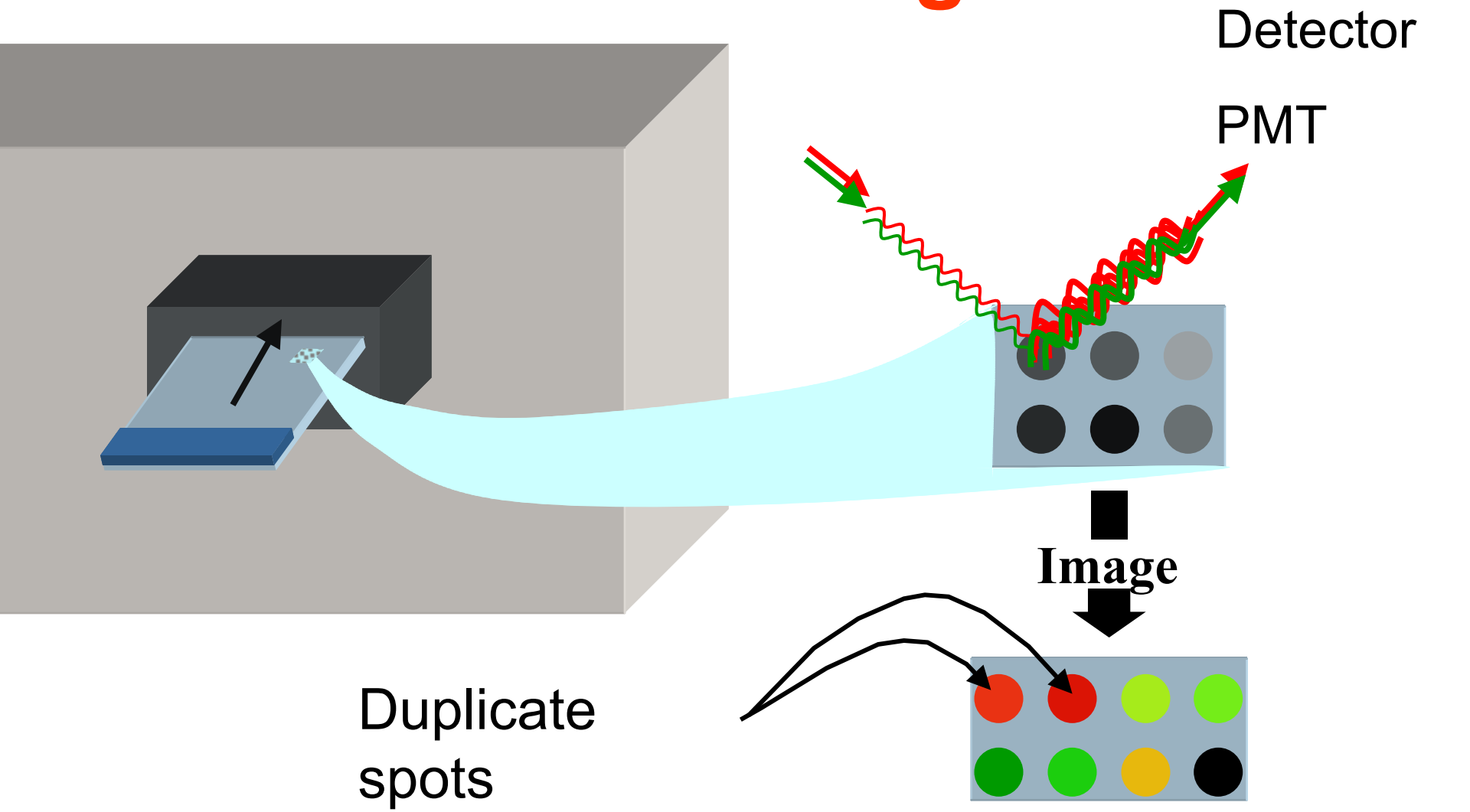
Hybridization chamber



- Humidity
- Temperature
- Formamide

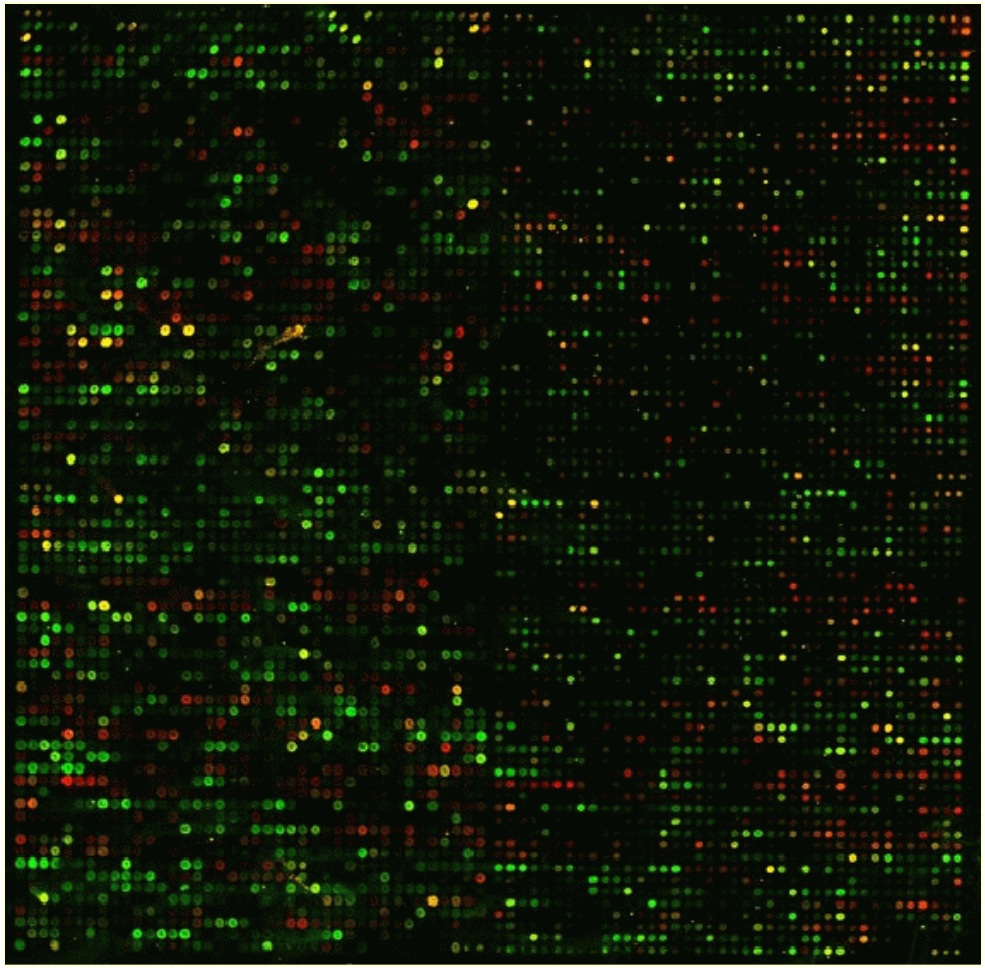
(Lowers the Tmp)

Scanning



Cy5: 635nm
Cy3: 532nm

RGB overlay of Cy3 and Cy5 images



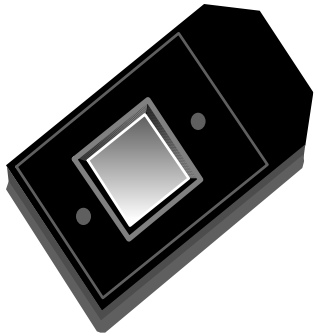
Raw data

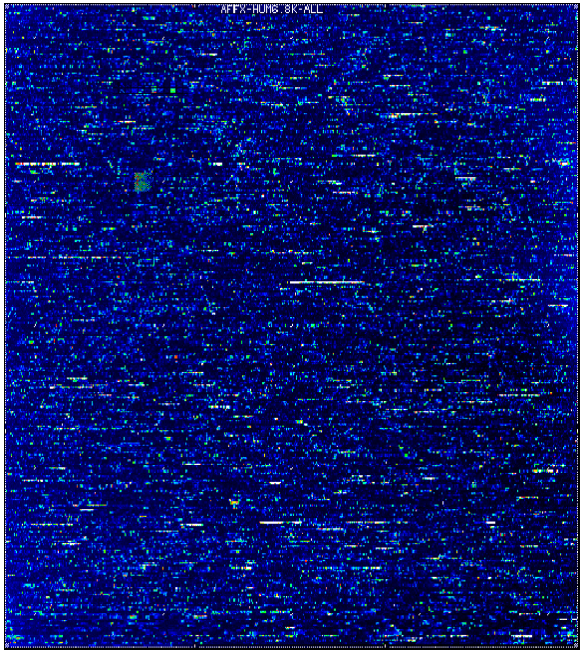
- Pairs of 16–bit TIFFs, one for each dye.
- E.g. Human cDNA arrays:
  - ~43K spots;
  - ~ 20Mb per channel;
  - ~ 2,000 x 5,500 pixels per image;
  - spot separation: ~ 136um.
- For a “typical” array, the spot area has
  - mean = 43 pixels,
  - med = 32 pixels,
  - SD = 26 pixels.

Animation

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

Oligonucleotide chips





Terminology

- Each gene or portion of a gene is represented by 16 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 (13^{th}) base (transversion purine <-> pyrimidine, G <->C, A <->T) .
- Probe-pair: a (PM,MM) pair.
- Probe-pair set: a collection of probe-pairs (16 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

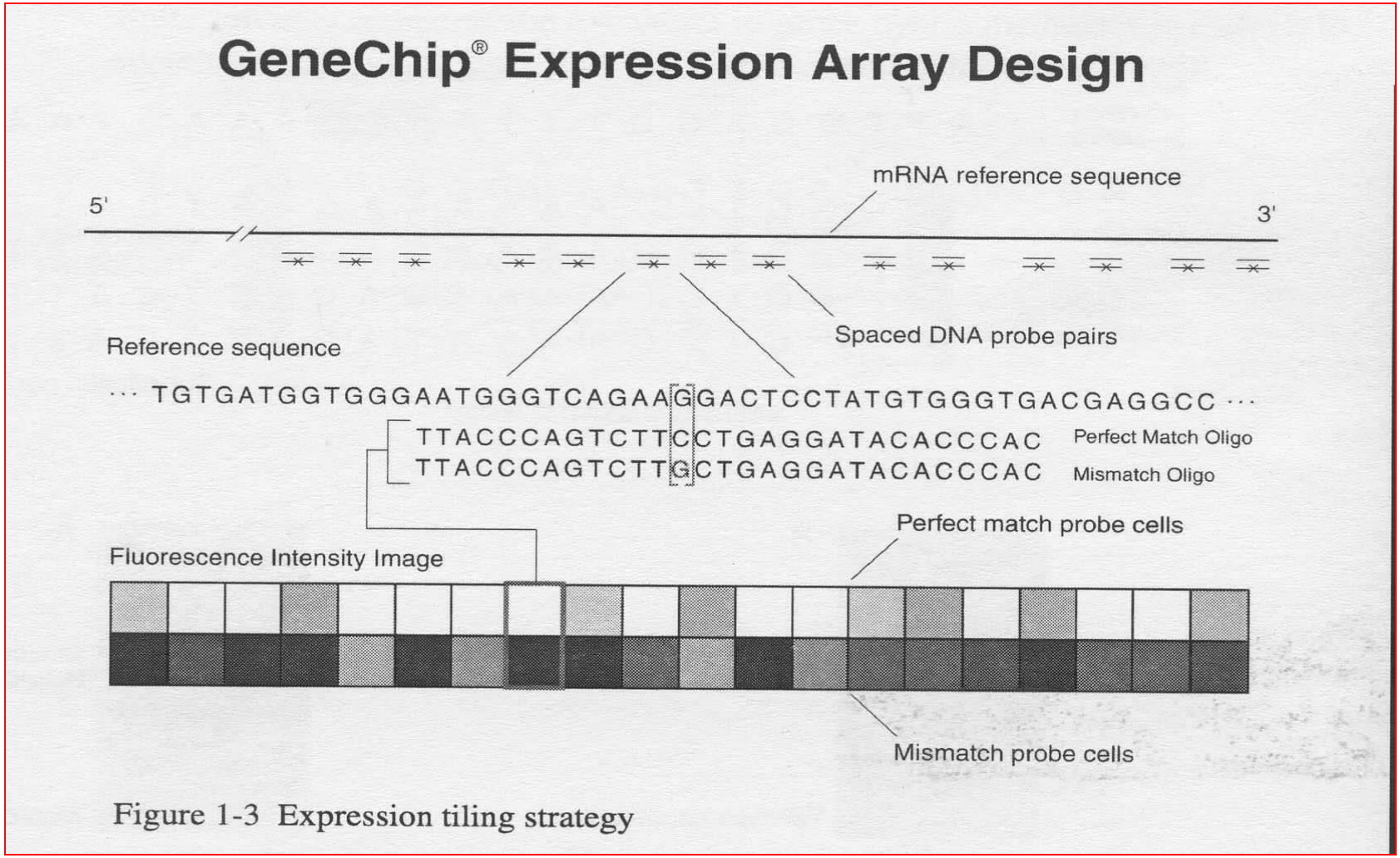
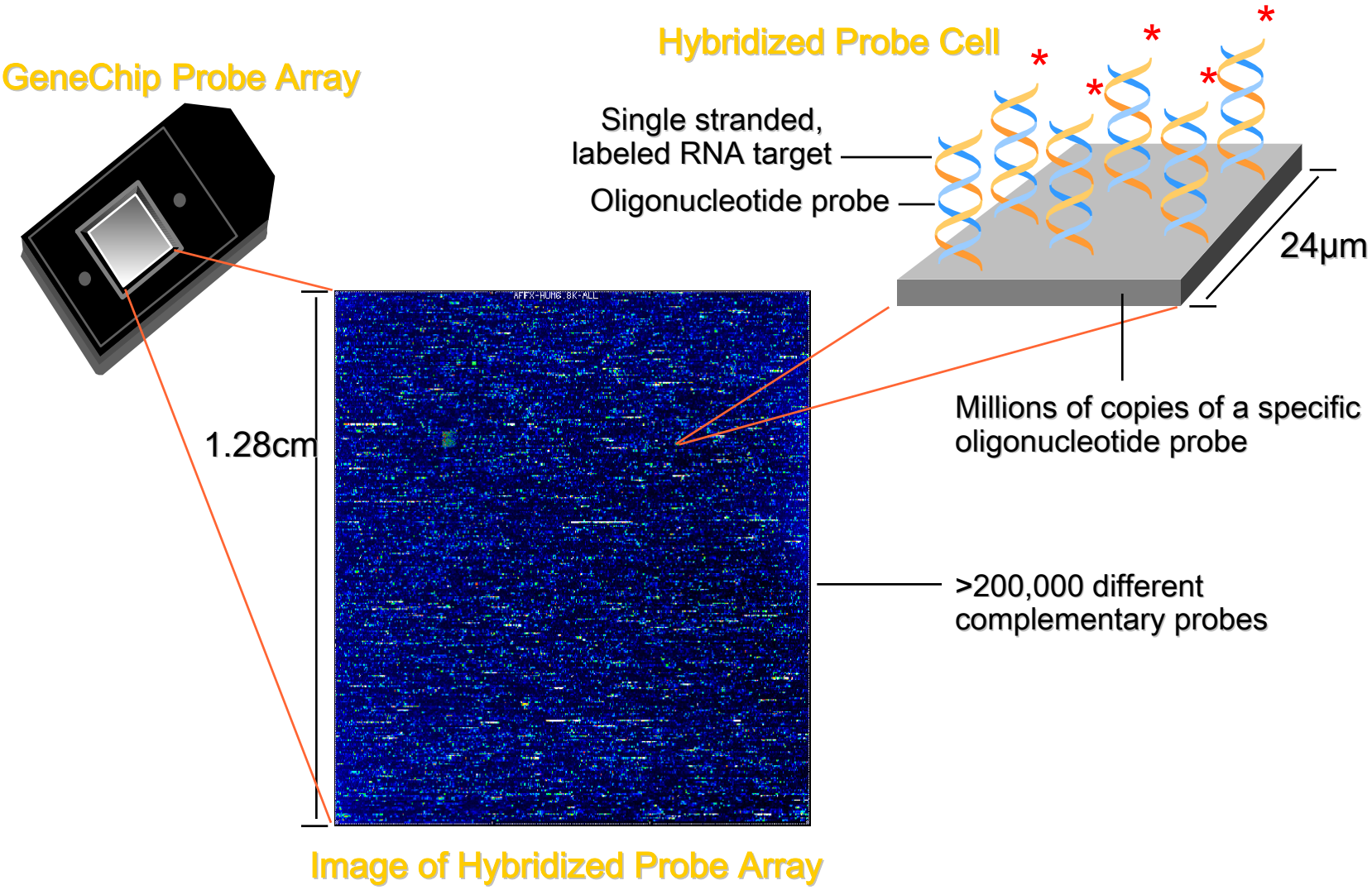


Figure 1-3 Expression tiling strategy

Spotted vs. Affymetrix arrays

Spotted arrays	Affymetrix arrays
One probe per gene	16 – 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

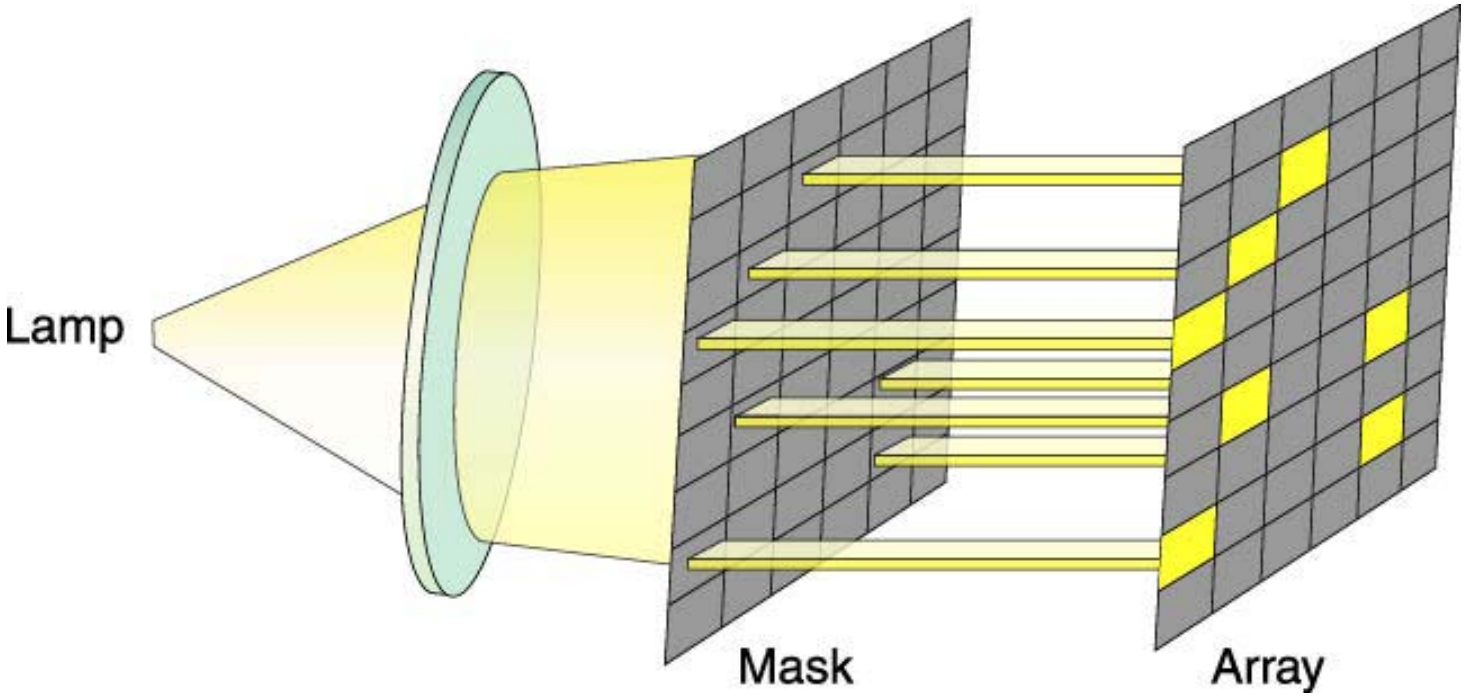


Compliments of D. Gerhold

Oligonucleotide chips

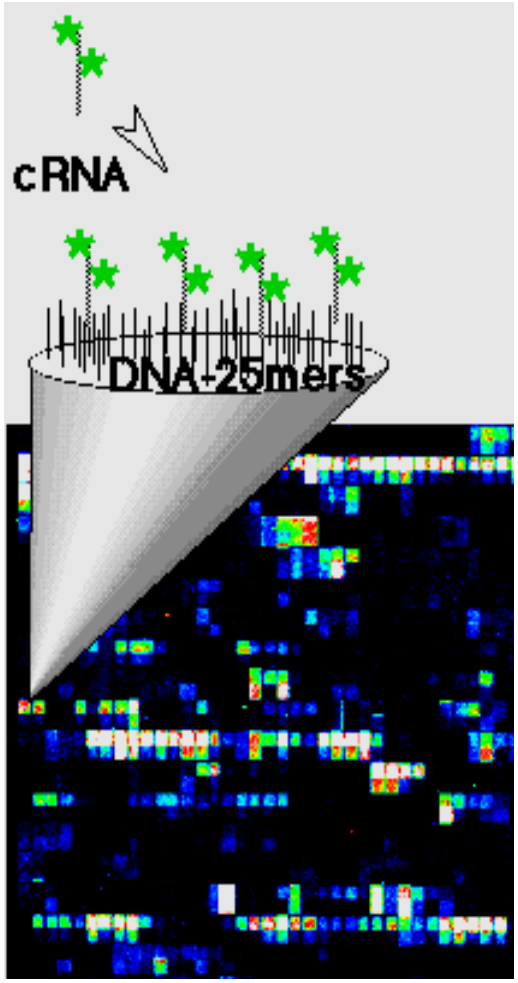
- The probes are synthesized *in situ*, using combinatorial chemistry and photolithography.
- Probe cells are square-shaped features on the chip containing millions of copies of a single 25-mer probe. Sides are 18-50 microns.

Oligonucleotide chips



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

Image analysis



- 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.

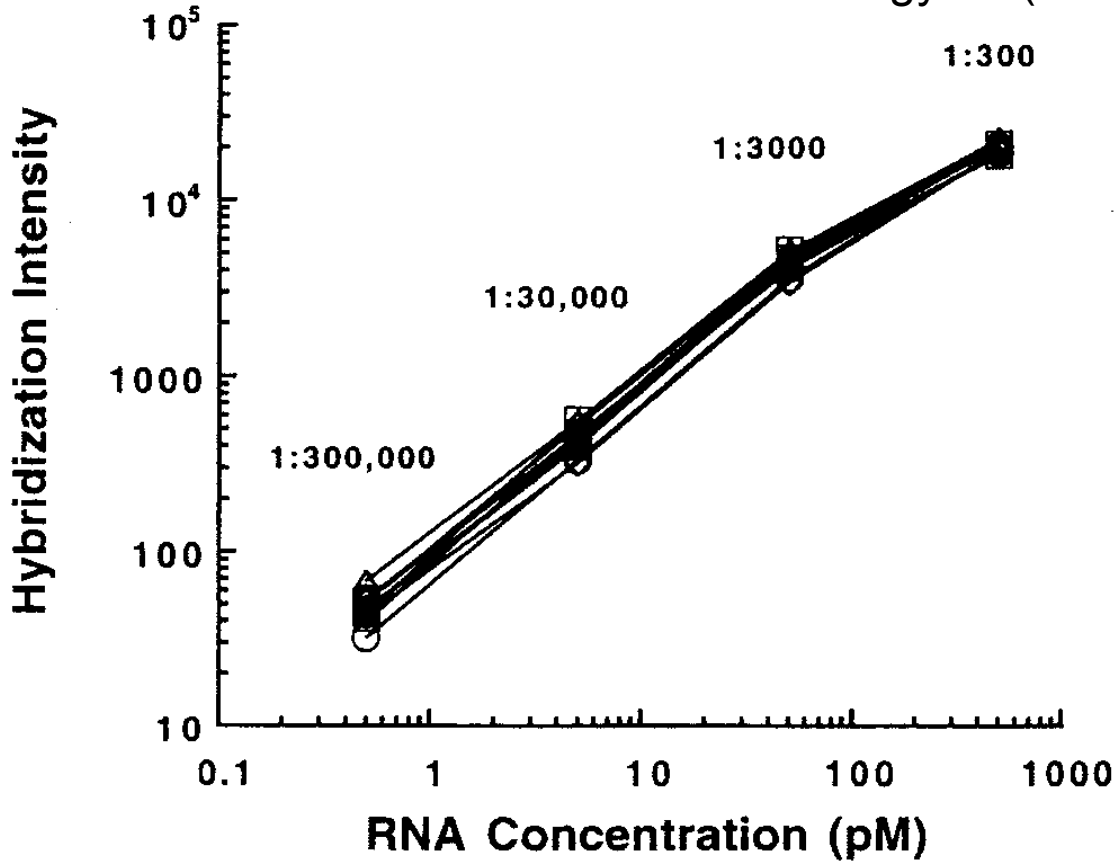
Expression measures

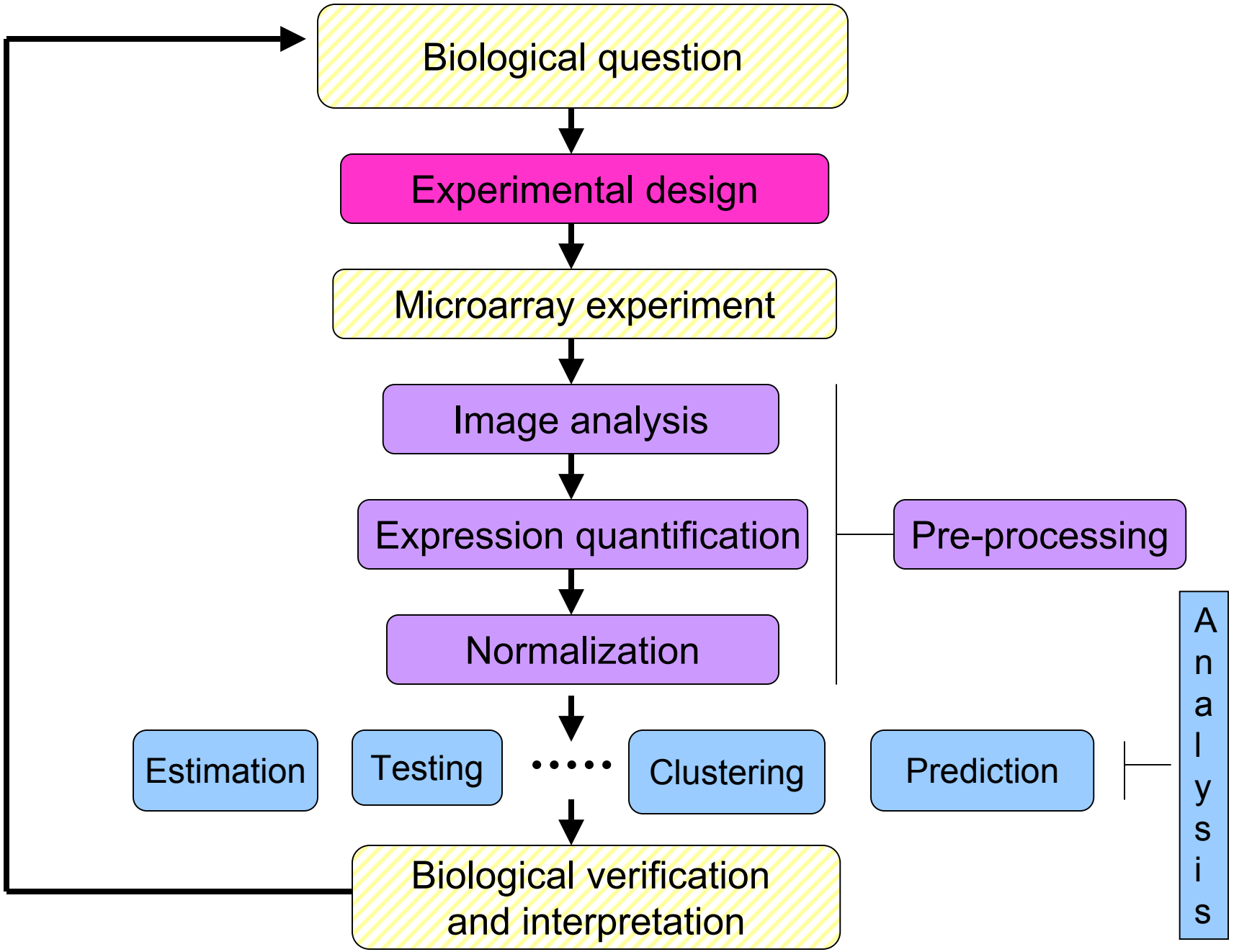
- Most expression measures are based on differences of **PM-MM**.
- The intention is to correct for background and non-specific binding.
- E.g. *MarrayArray Suite*® (MAS) v. 4.0 uses Average Difference Intensity (ADI) or

  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

- Expression, 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

Next …

Pre-processing DNA Microarray Data

- Spotted DNA microarrays
  - Image analysis;
  - Normalization.
- Affymetrix oligonucleotide chips
  - Image analysis;
  - Normalization;
  - Expression measures.