Introduction to DNA microarray technologies

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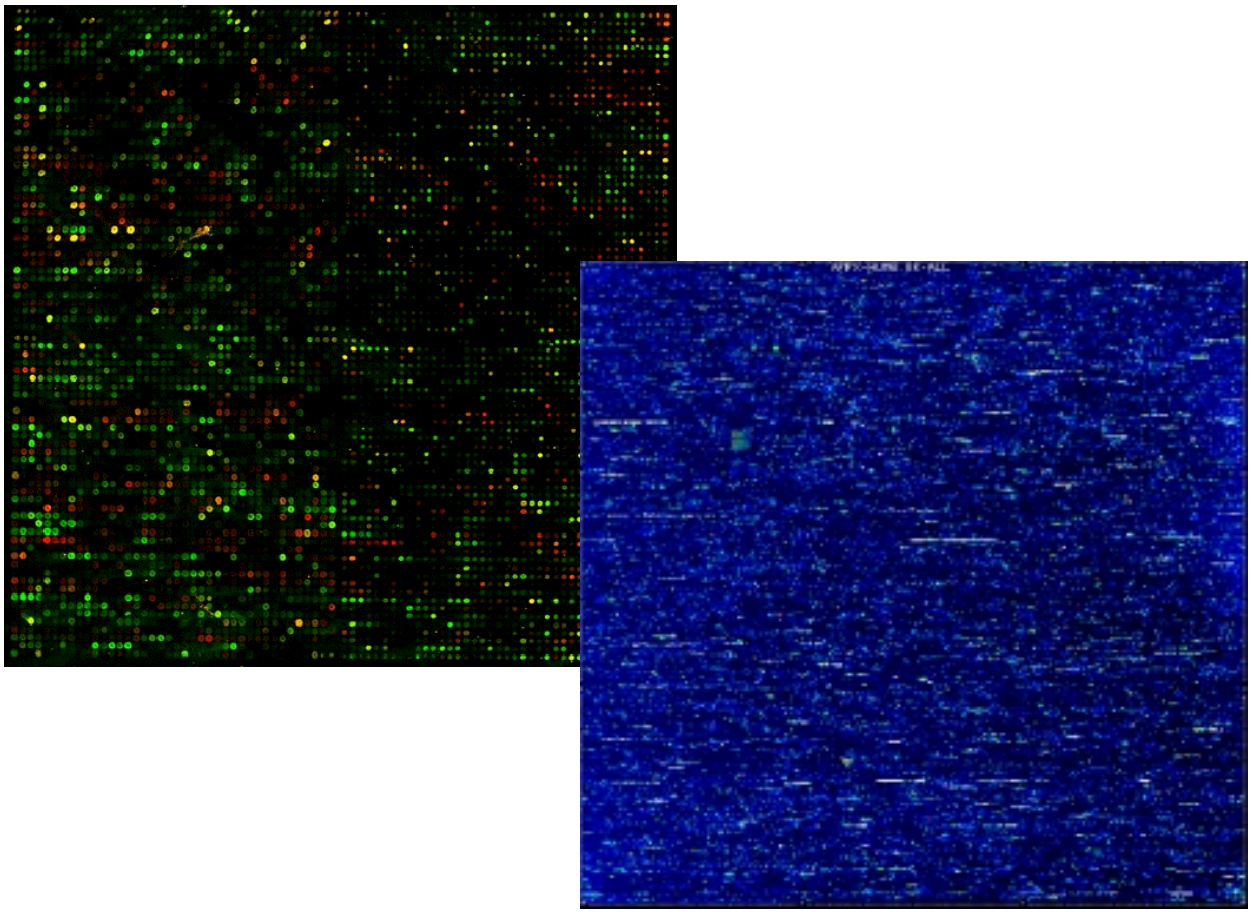
Outline

- Basic principles
- cDNA 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.

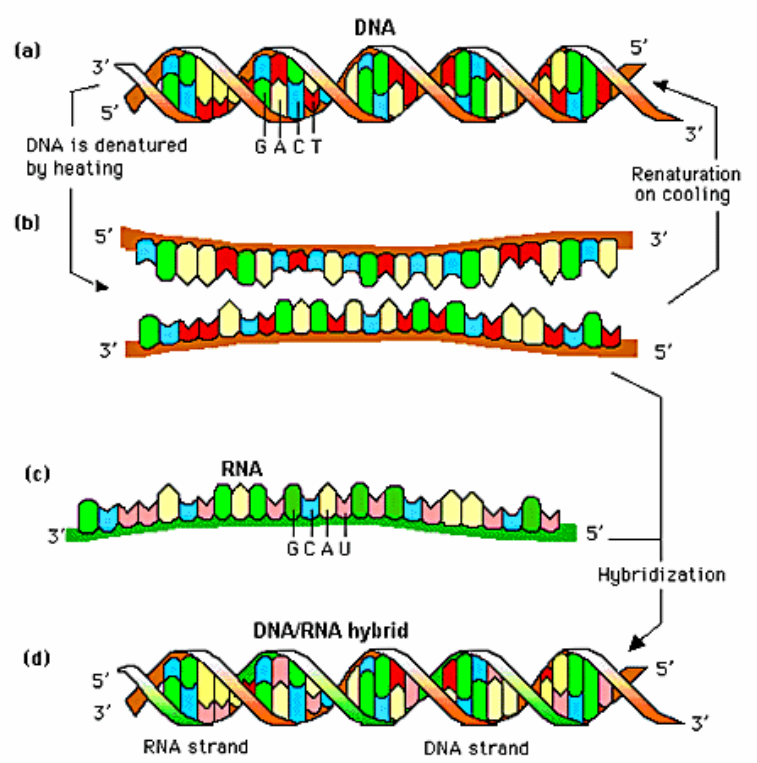
The ancestor of cDNA microarrays: the **Northern blot**.

Hybridization

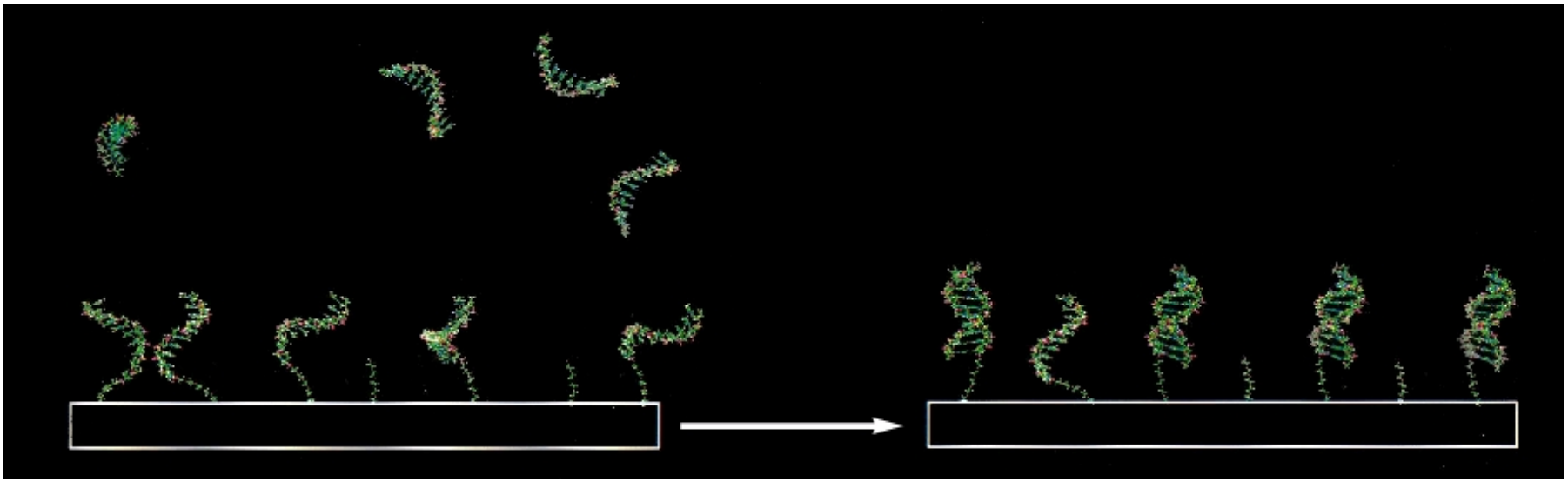
- **Hybridization** refers to the **annealing** of two nucleic acid strands following the base-pairing rules.

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

Hybridization



Hybridization

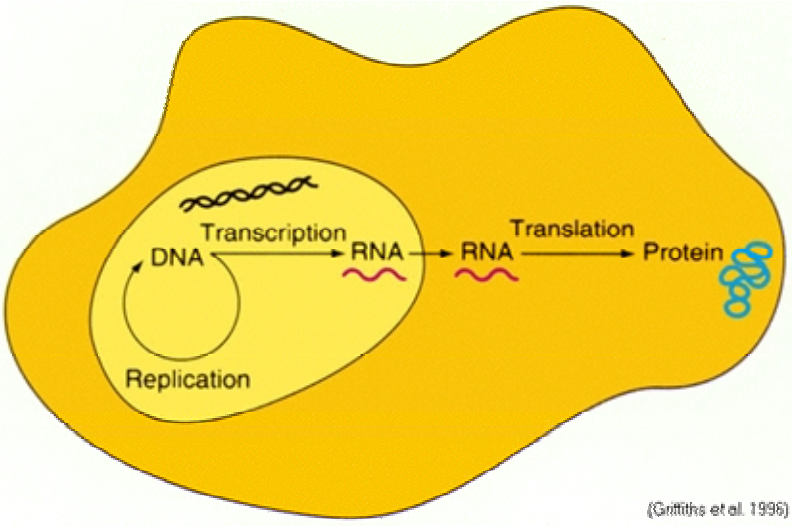


Gene expression assays

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);
- **cDNA arrays (Brown/Botstein).**

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

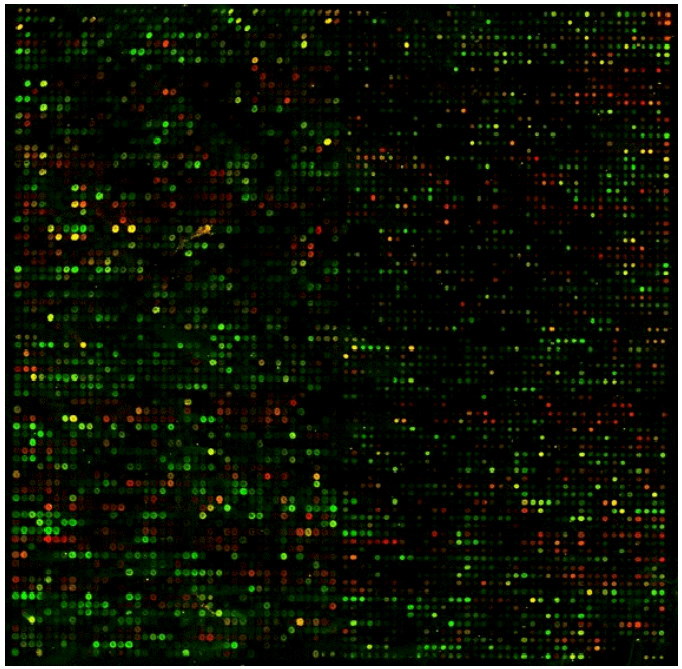
- **Cancer research:** Molecular characterization of tumors on a genomic scale

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

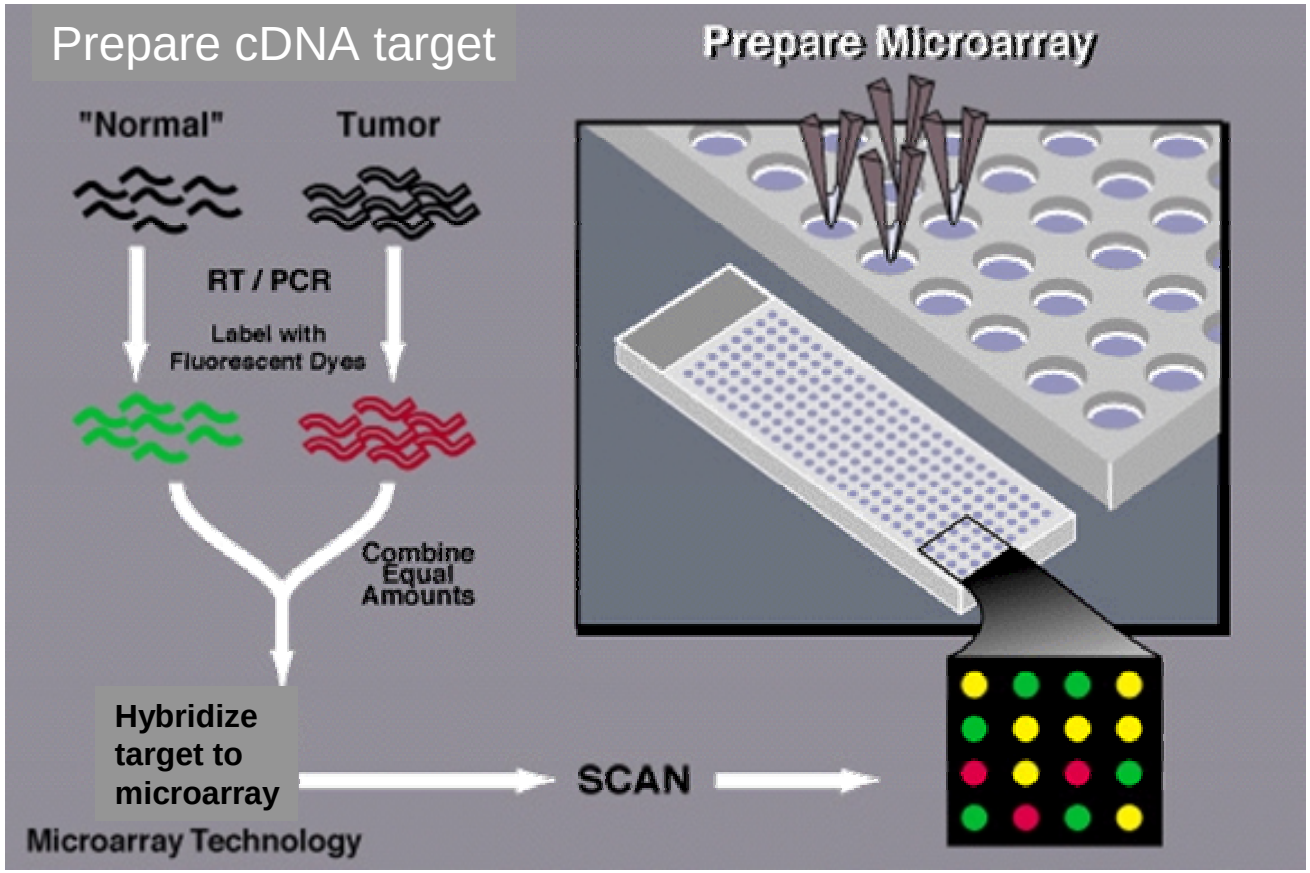
Applications of microarrays

- 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.

cDNA microarrays



cDNA microarrays



cDNA 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.

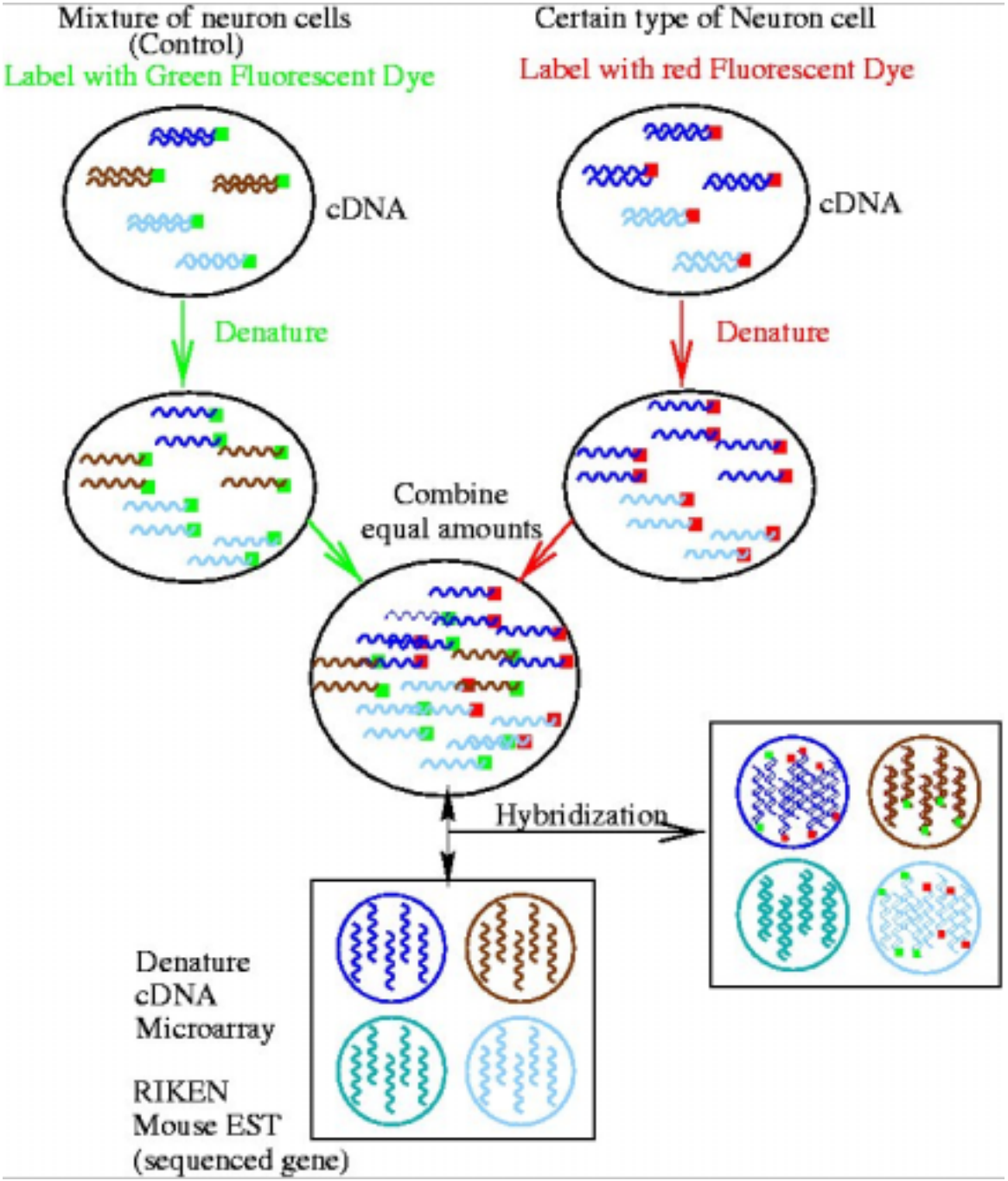
cDNA microarrays

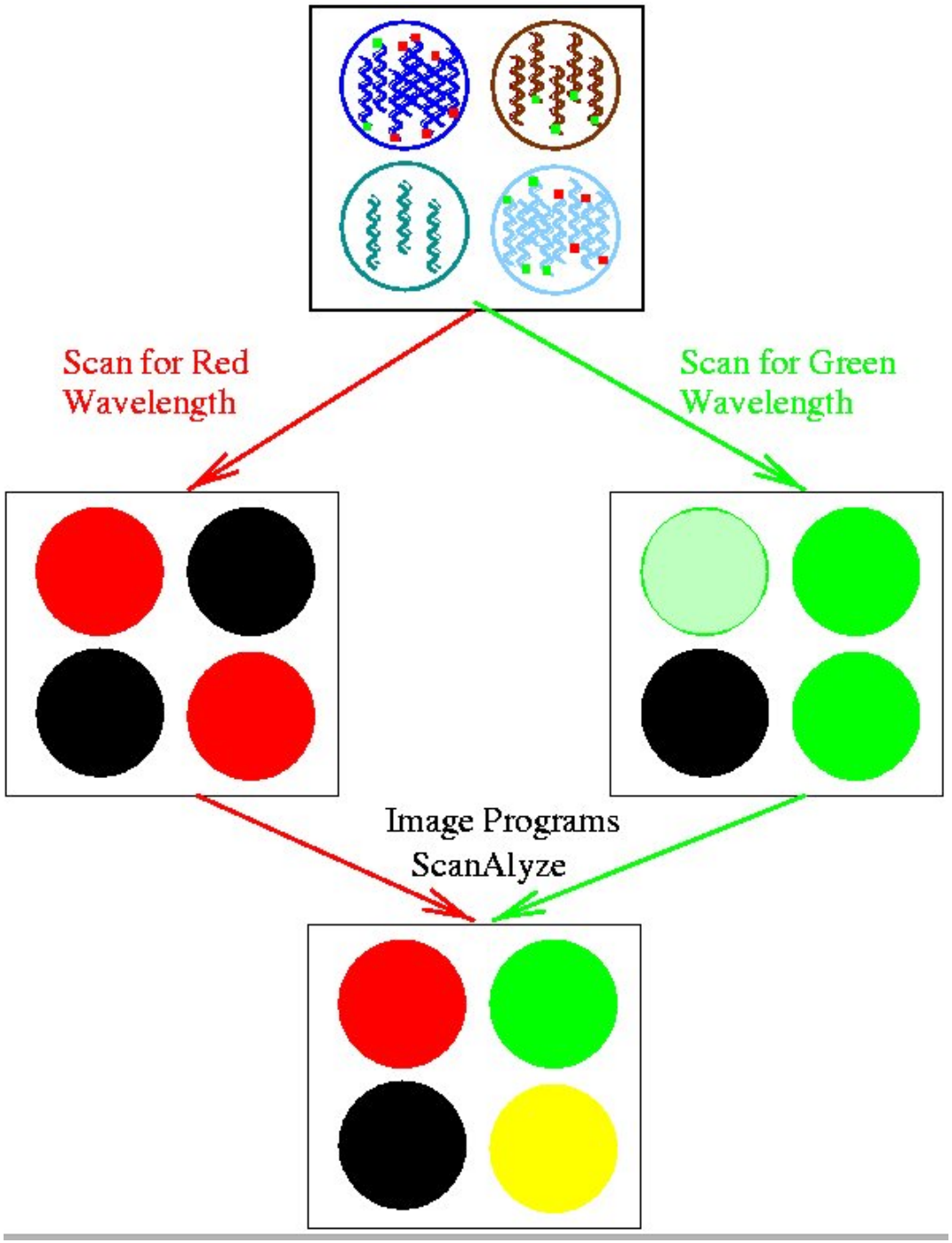
- The **ratio** of the red and green fluorescence intensities for each spot is indicative of the relative abundance of the corresponding DNA probe in the two nucleic acid target samples.

cDNA microarrays

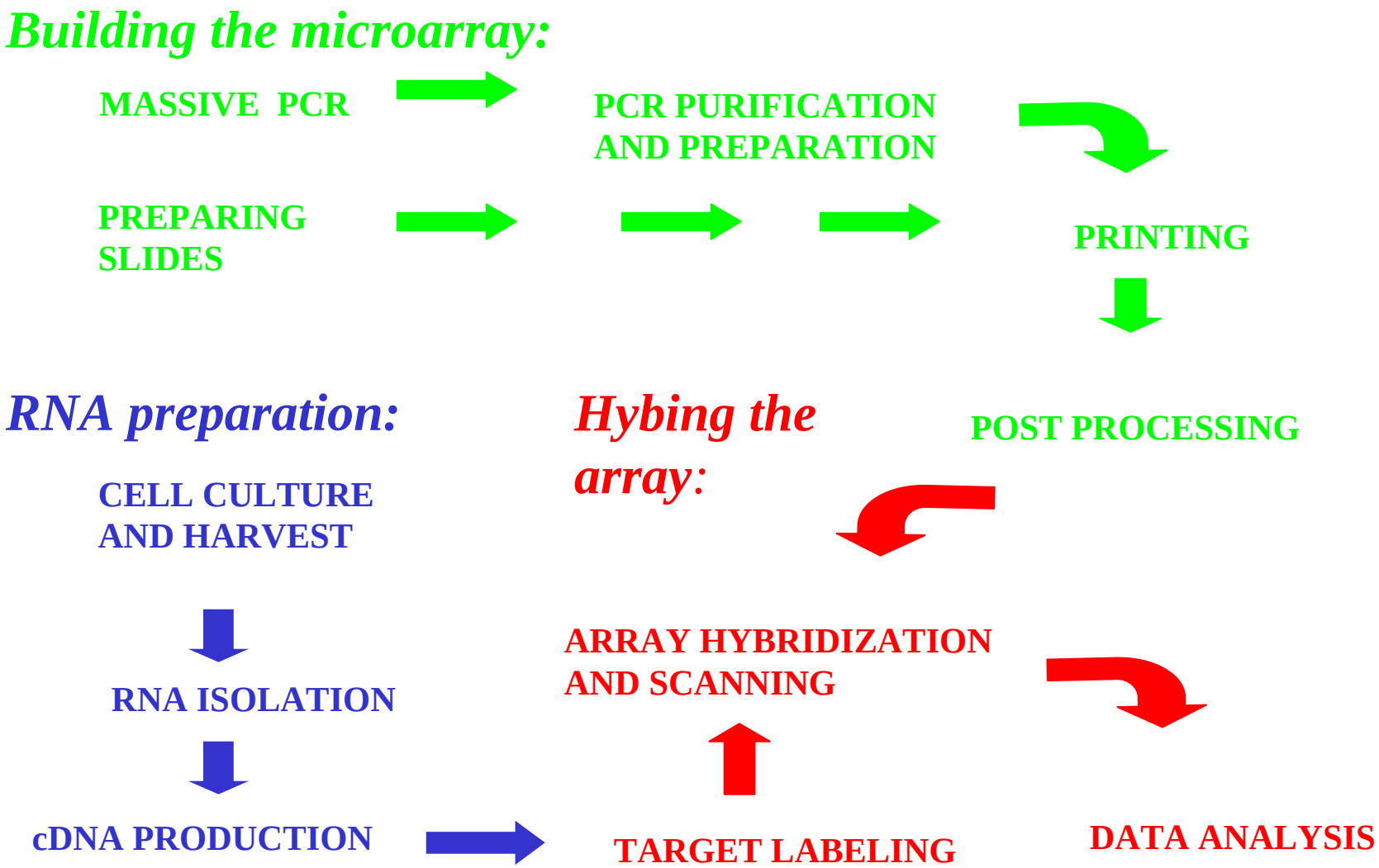
$$M = \log_2 R/G = \log_2 R - \log_2 G$$

- **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.

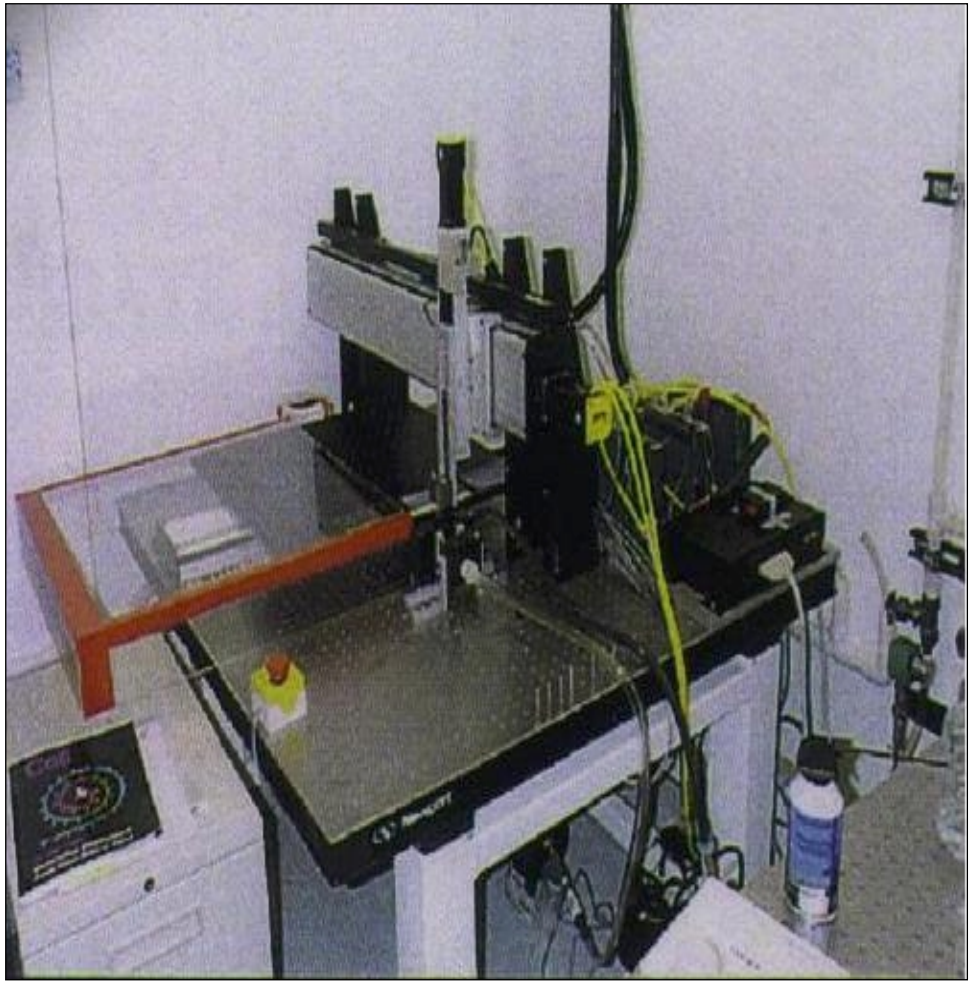

Mixture of neuron cells
(Control)
Label with Green Fluorescent Dye
Certain type of Neuron cell
Label with red Fluorescent Dye
cDNA
cDNA
Denature
Denature
Combine
equal amounts
Hybridization
Denature
cDNA
Microarray
RIKEN
Mouse EST
(sequenced gene)


Scan for Red
Wavelength
Scan for Green
Wavelength
Image Programs
ScanAlyze

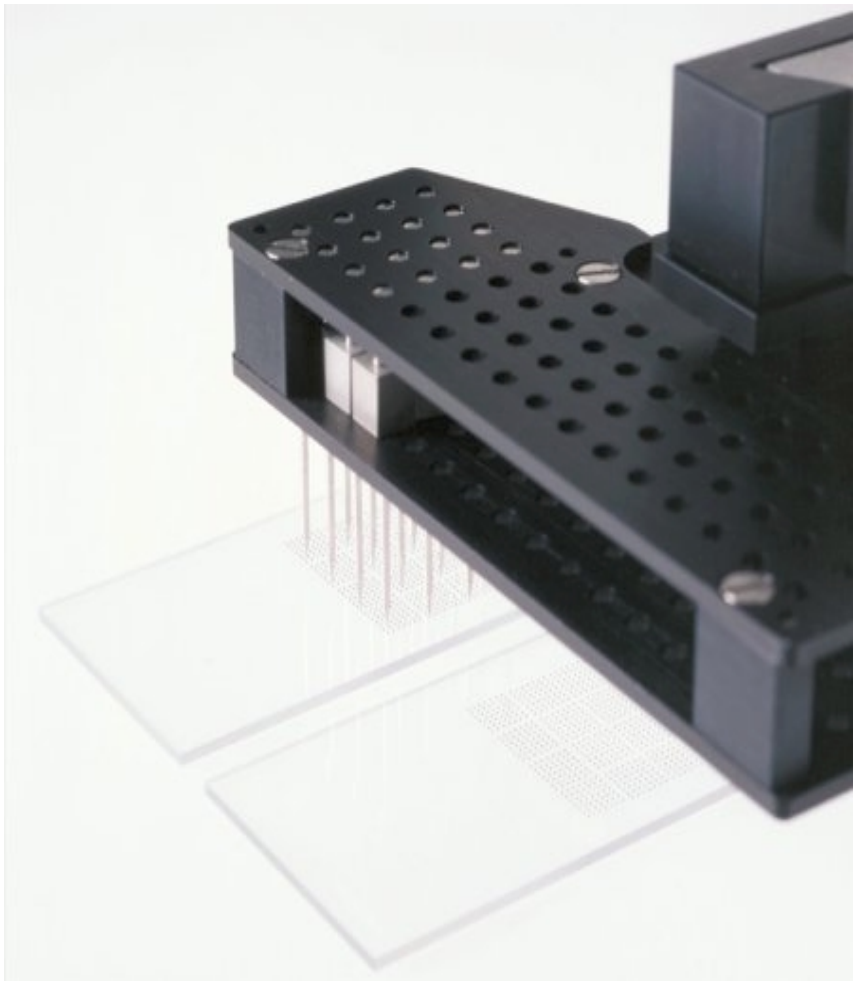
The process


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:
ARRAY HYBRIDIZATION AND SCANNING
TARGET LABELING
DATA ANALYSIS

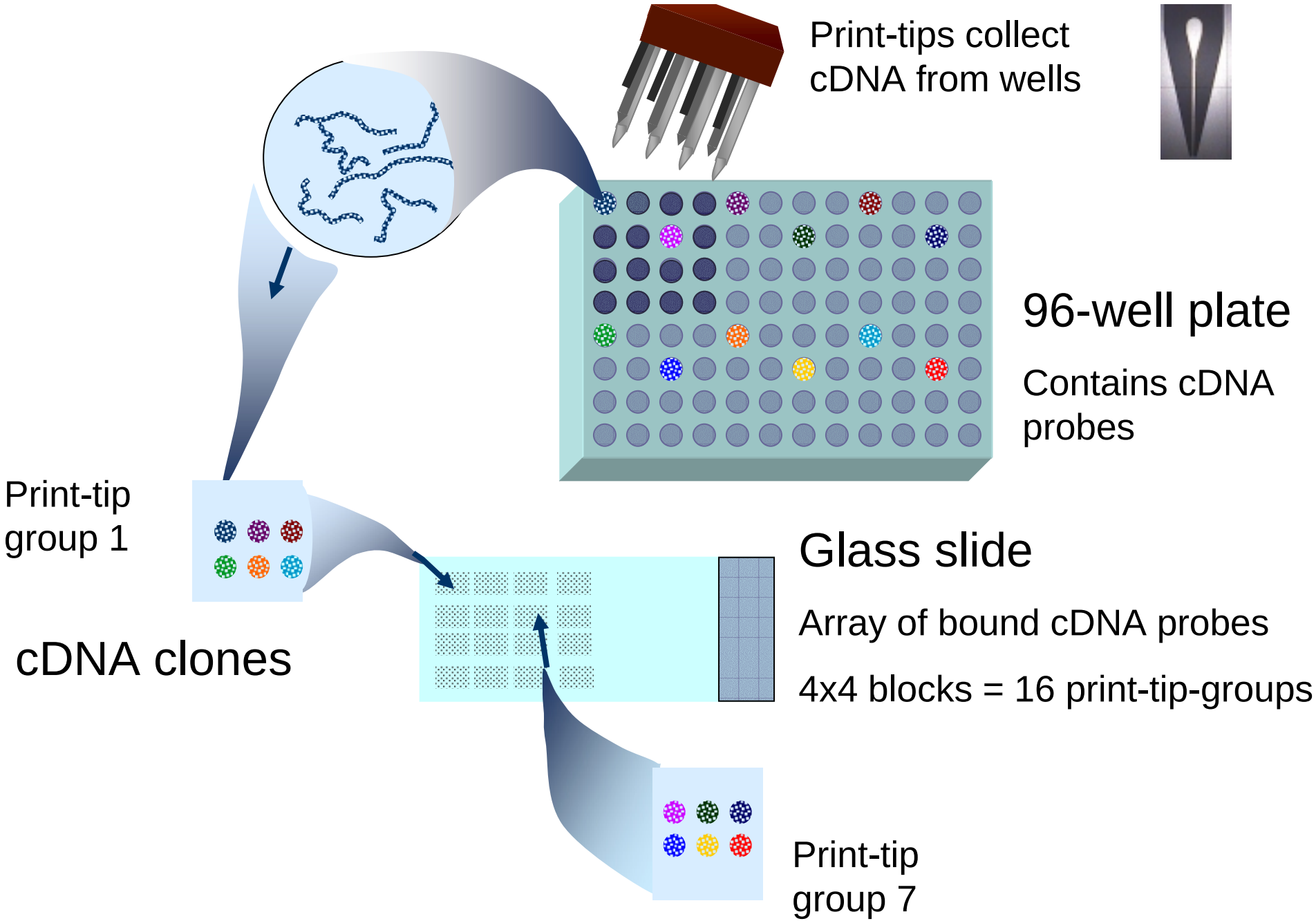
The arrayer



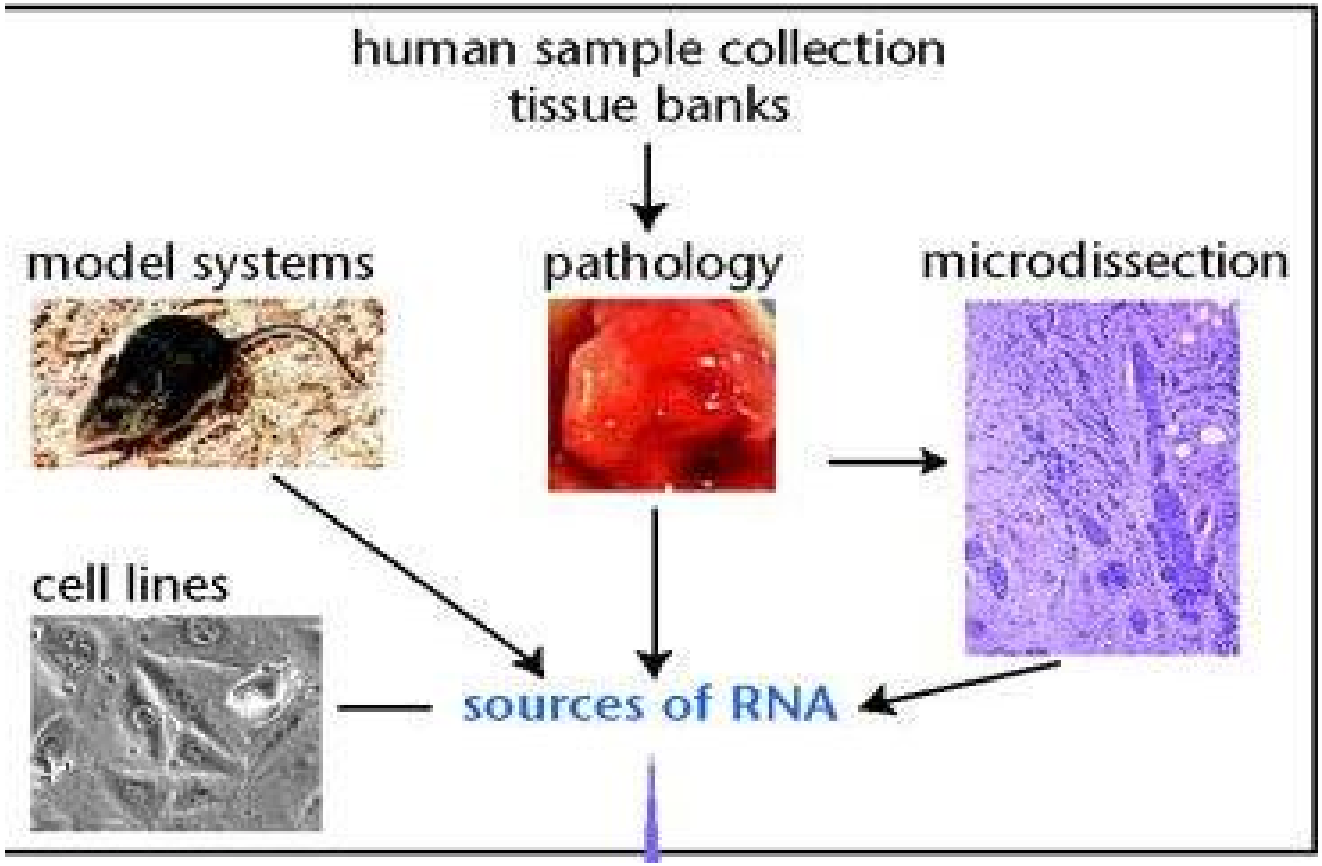
Ngai Lab arrayer, UC Berkeley



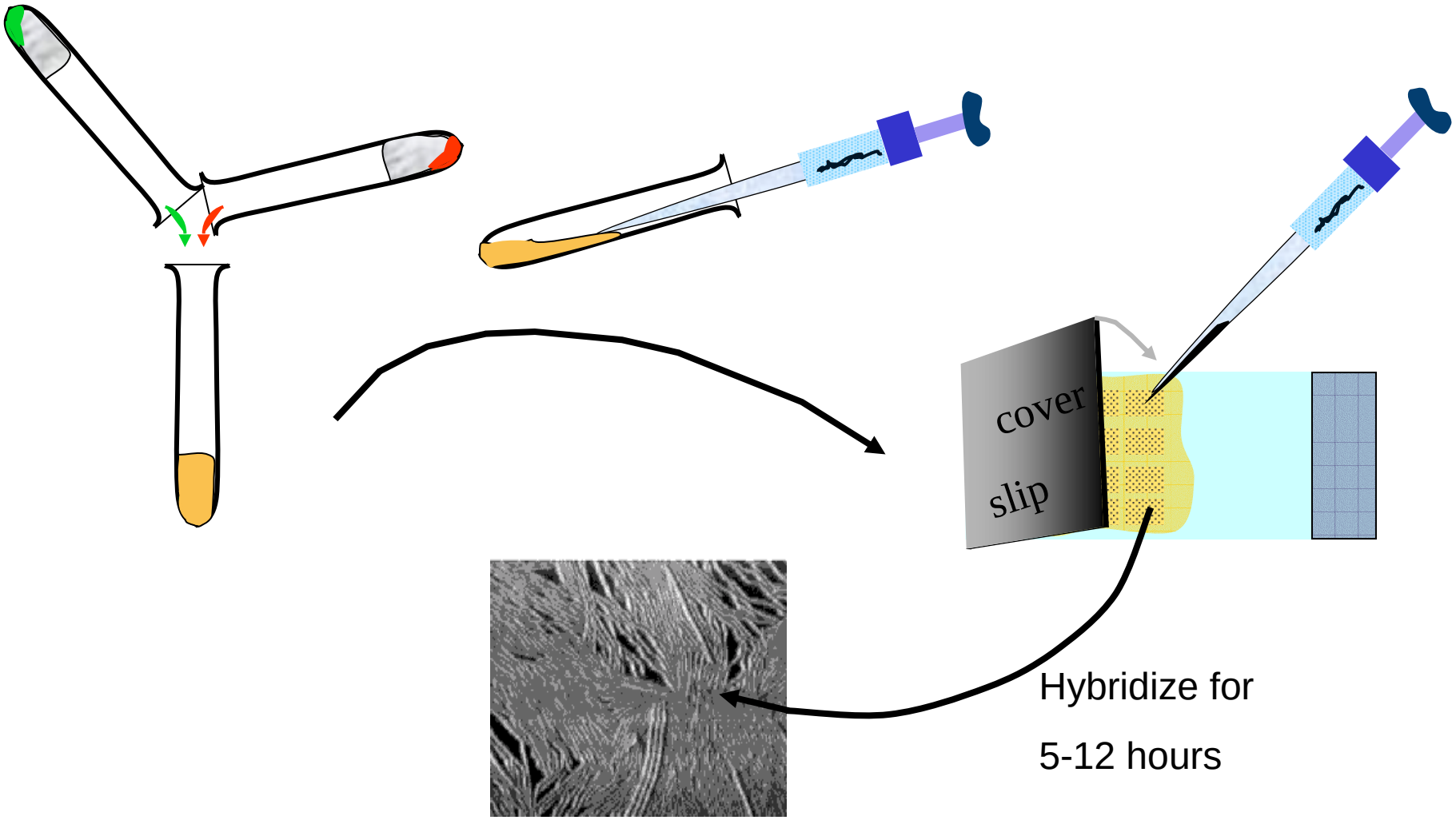
Print-head


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

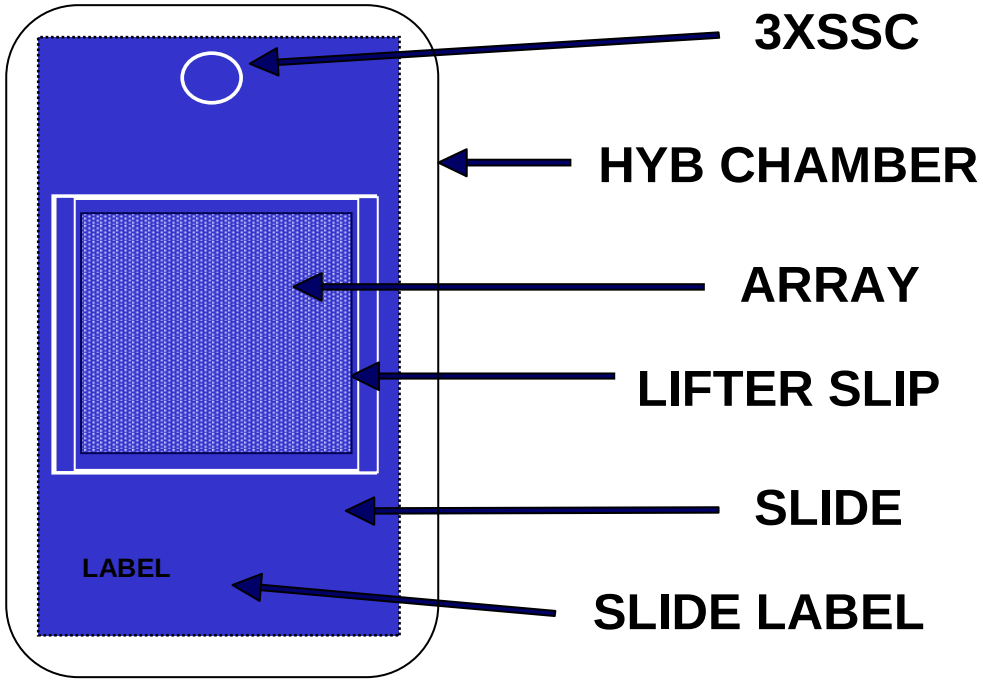


Hybridization



Binding of cDNA target samples to cDNA probes on the slide

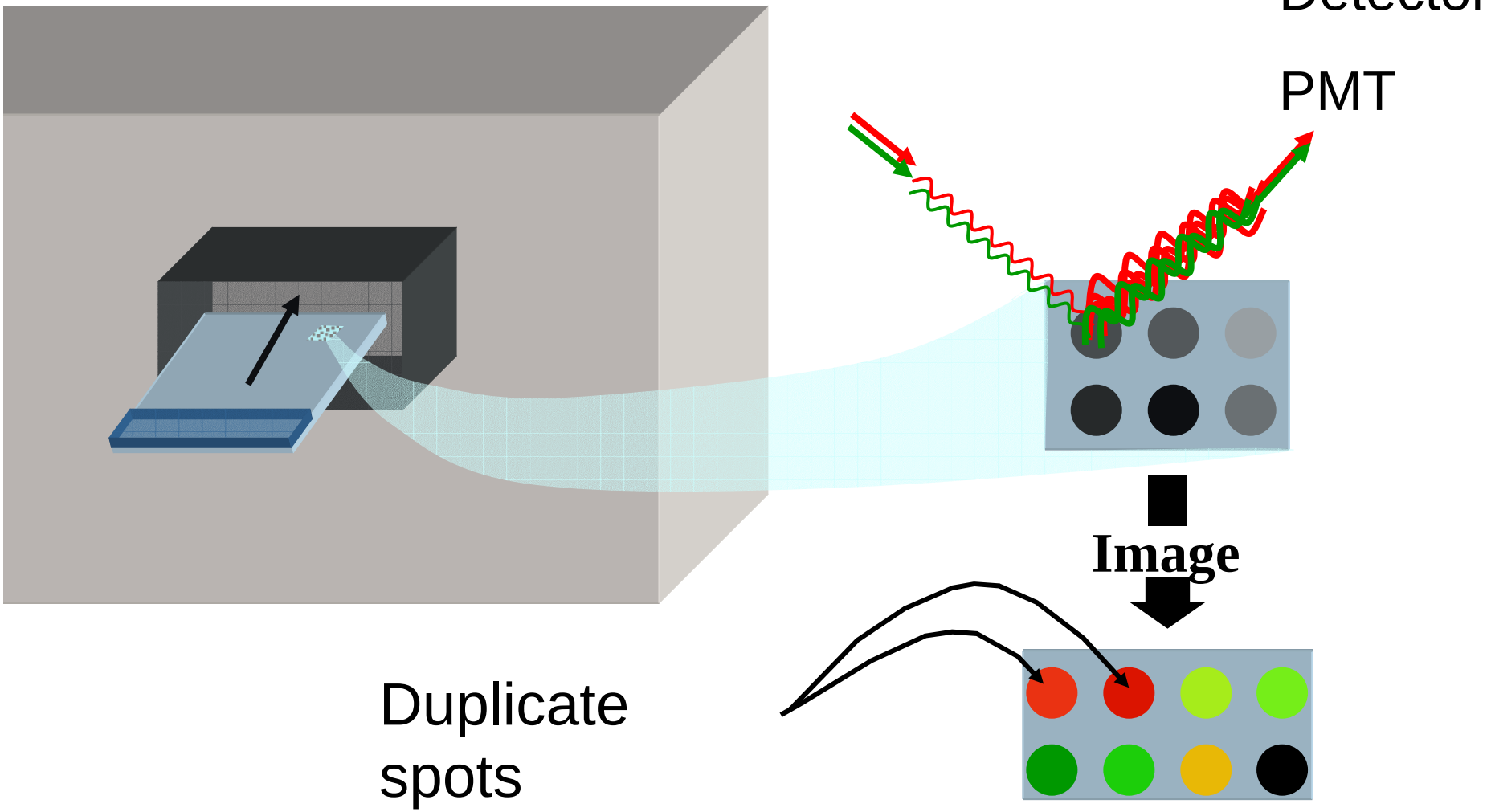
Hybridization chamber



- Humidity
- Temperature
- Formamide

(Lowers the Tmp)

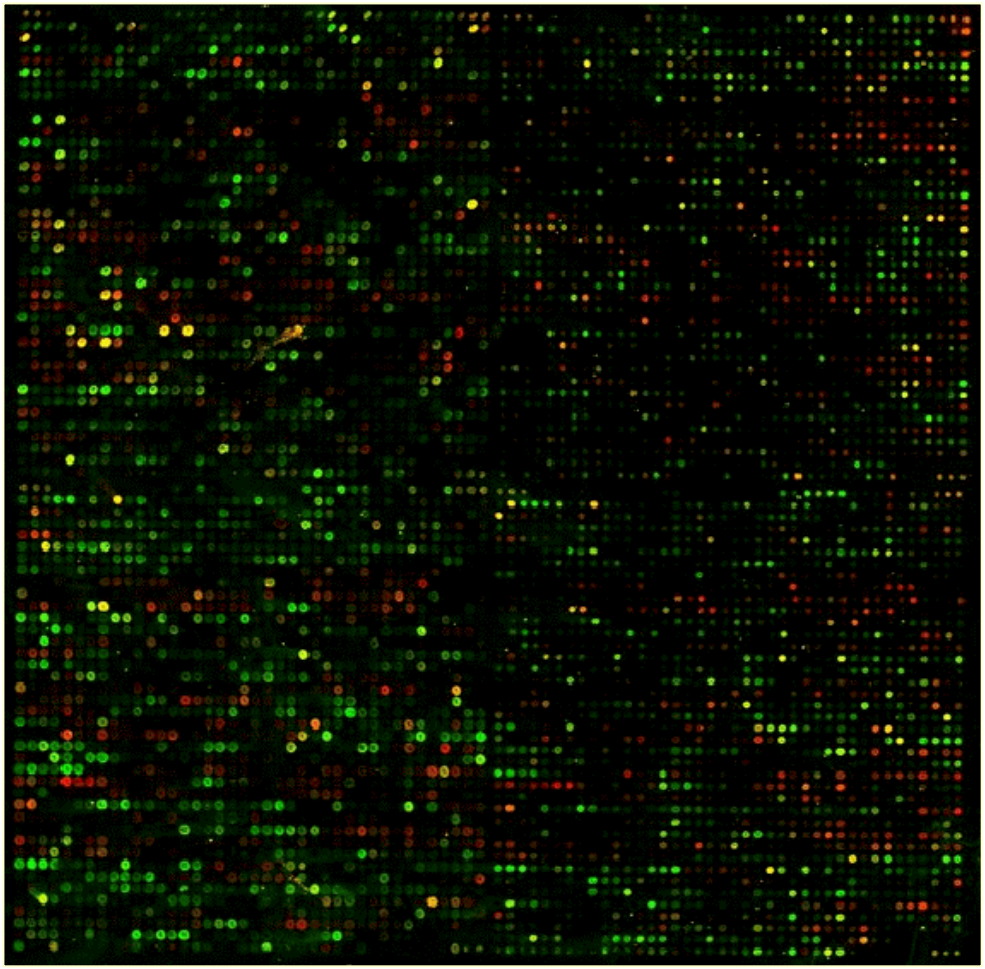
Scanning



Duplicate spots

Cy5: 635nm
Cy3: 532nm

RGB overlay of Cy3 and Cy5 images

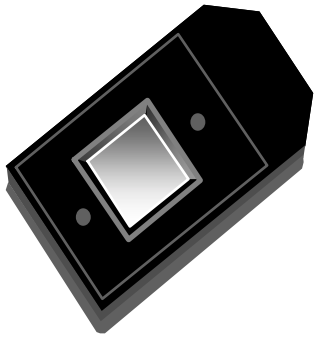


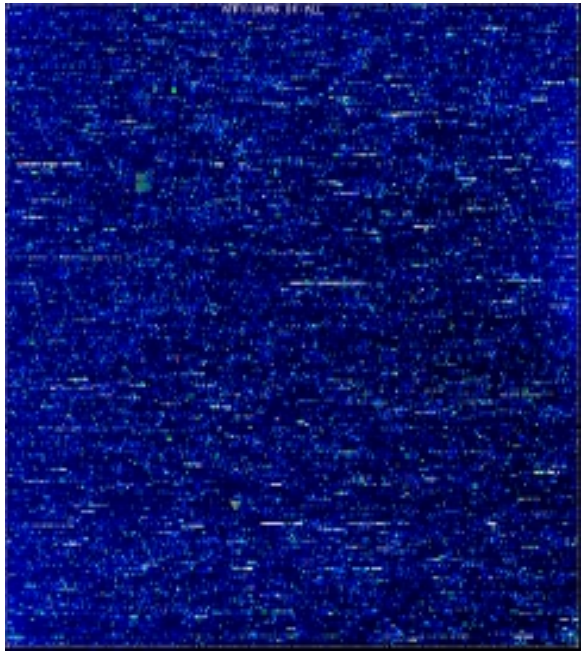
Raw data

E.g. Human cDNA arrays

- ~43K spots;
- 16–bit TIFFs: ~ 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.

Oligonucleotide chips





Probe sets

- Each gene is represented by 16-20 oligonucleotides of 25 base-pairs, i.e., 25-mers.
- **Perfect match probe**, **PM**: A 25-mer complementary to the reference sequence.
- **Mismatch probe**, **MM**: same as PM but with a single homomeric base change for the middle (13^{th}) base.
- **Probe pair.** A (PM,MM) pair.
- **Probe set.** 16-20 probe pairs.
- The purpose of the MM probe design is to measure non-specific binding and background noise.

Probe sets

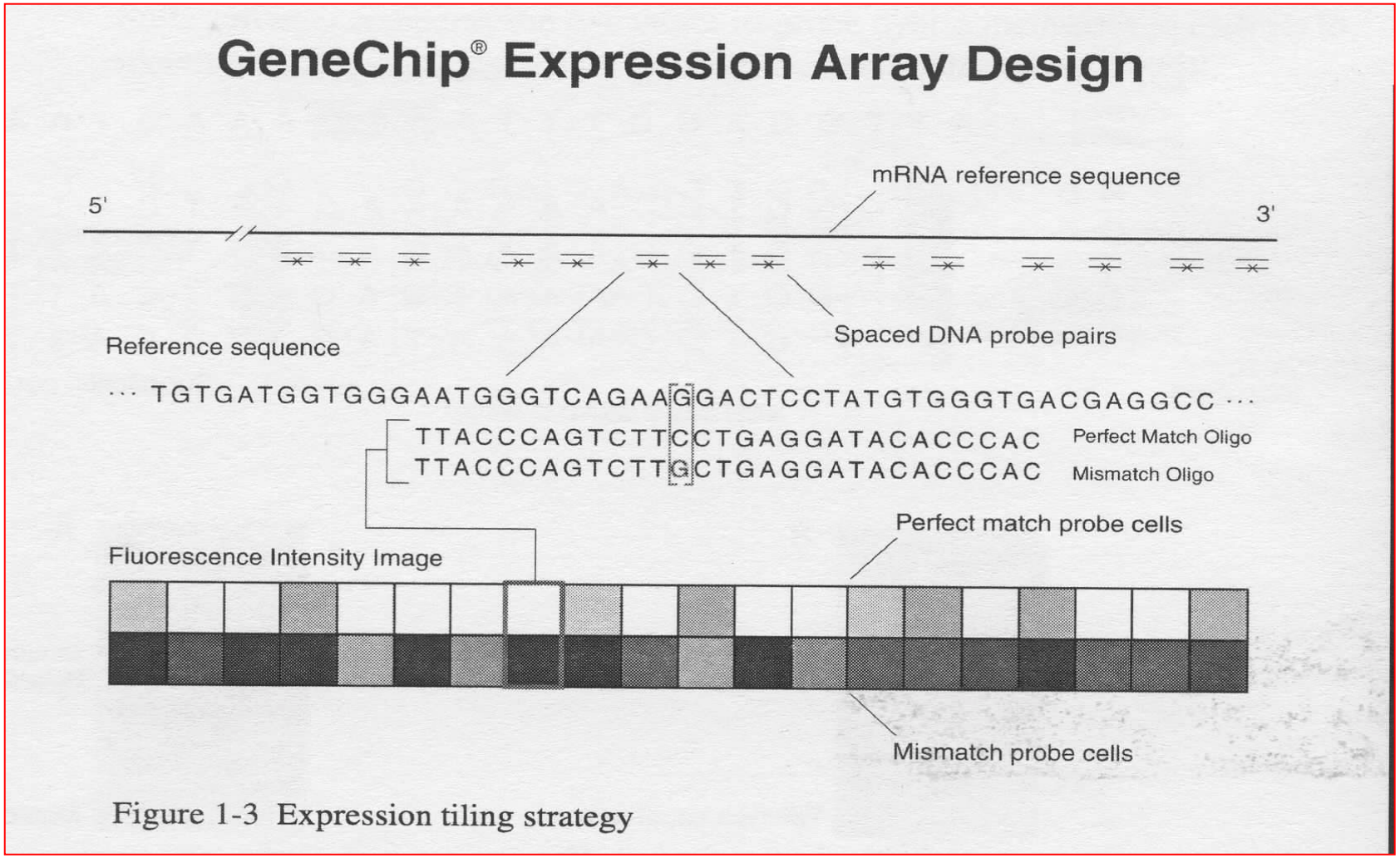
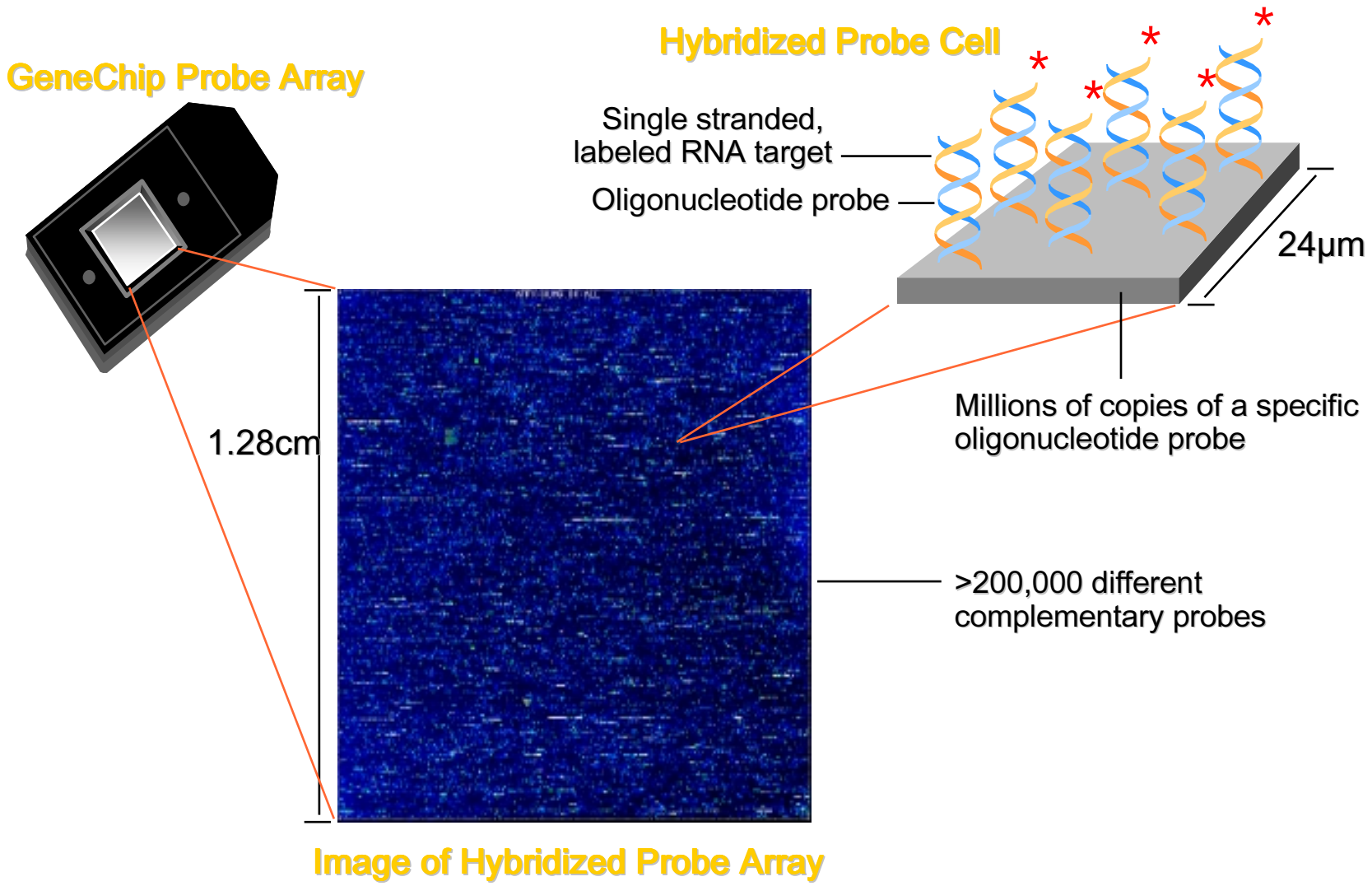


Figure 1-3 Expression tiling strategy

Oligonucleotide chips

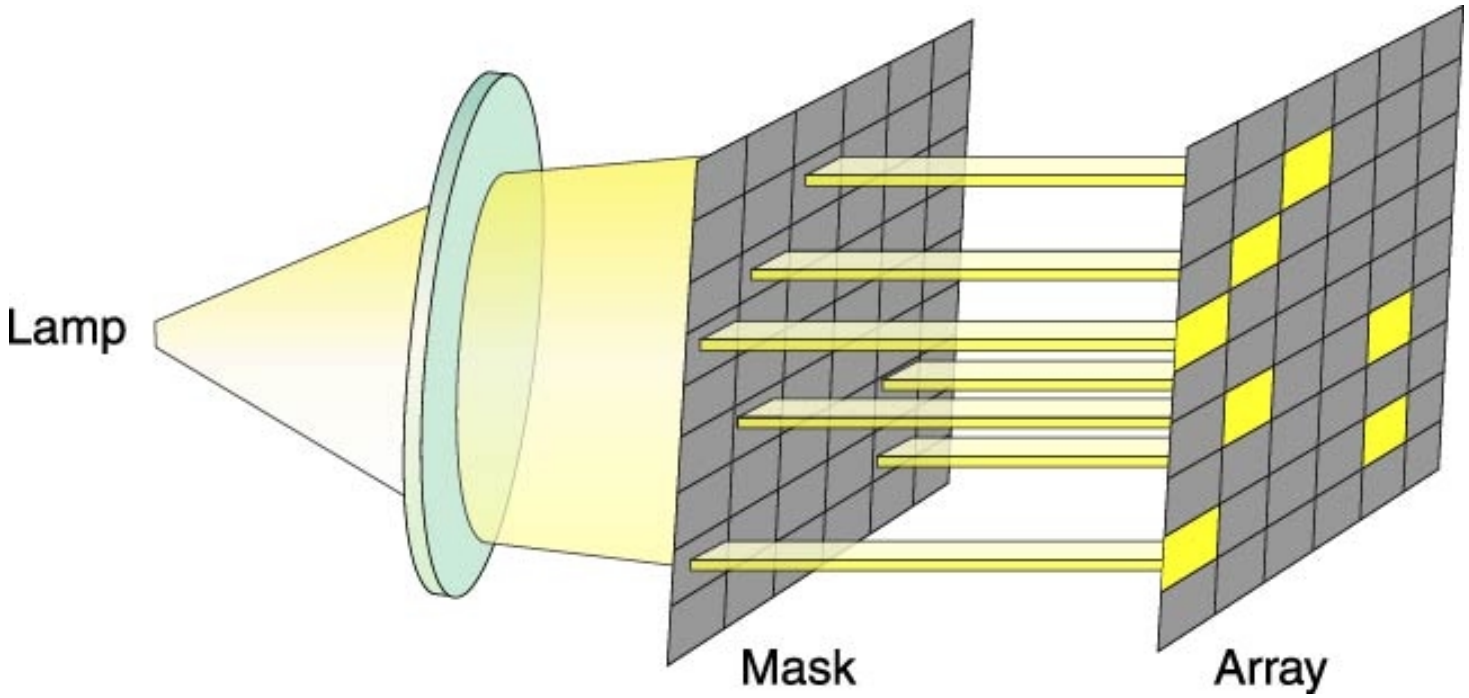


Compliments of D. Gerhold

Oligonucleotide chips

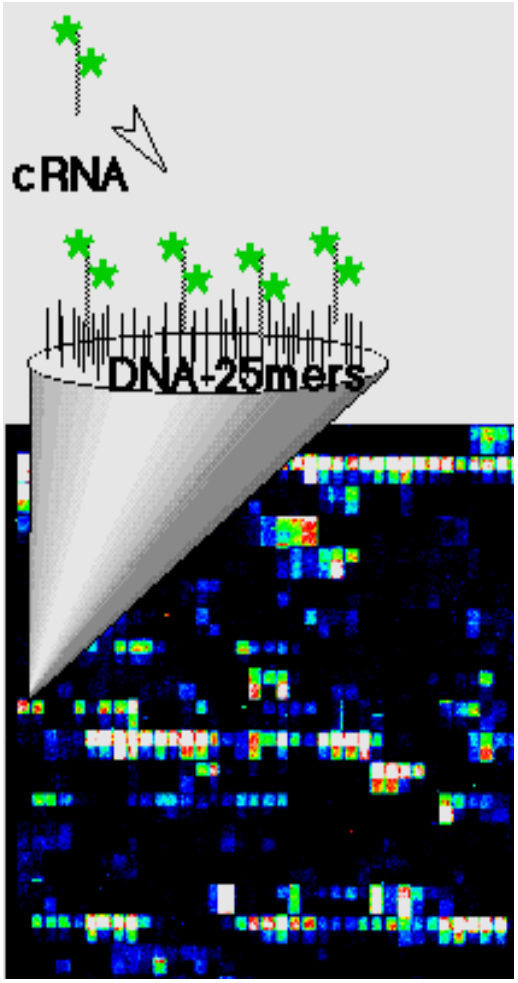
- The probes are synthesized *in situ*, using combinatorial chemistry and photolithography.
- **Probe cells** are square-shaped features on the chip containing millions of copies of a single 25-mer probe. Sides are 18-50 microns.

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.

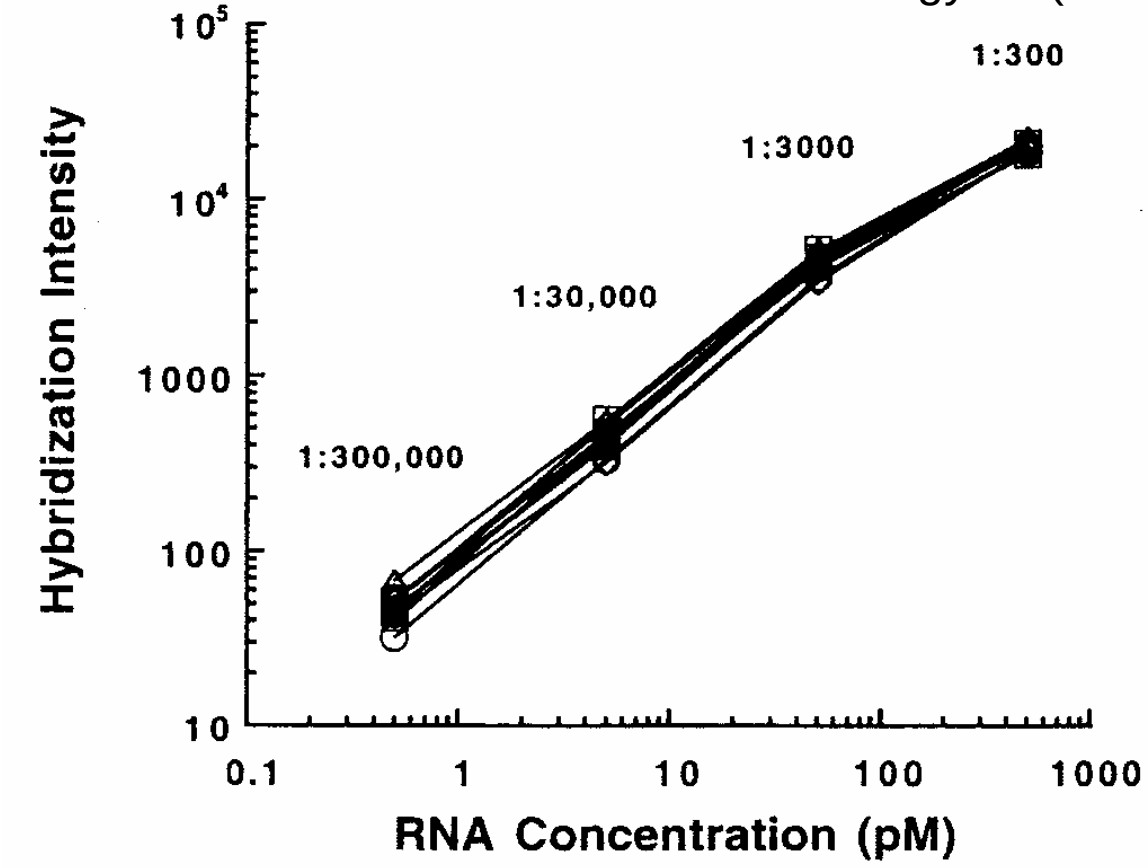
Expression measures

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

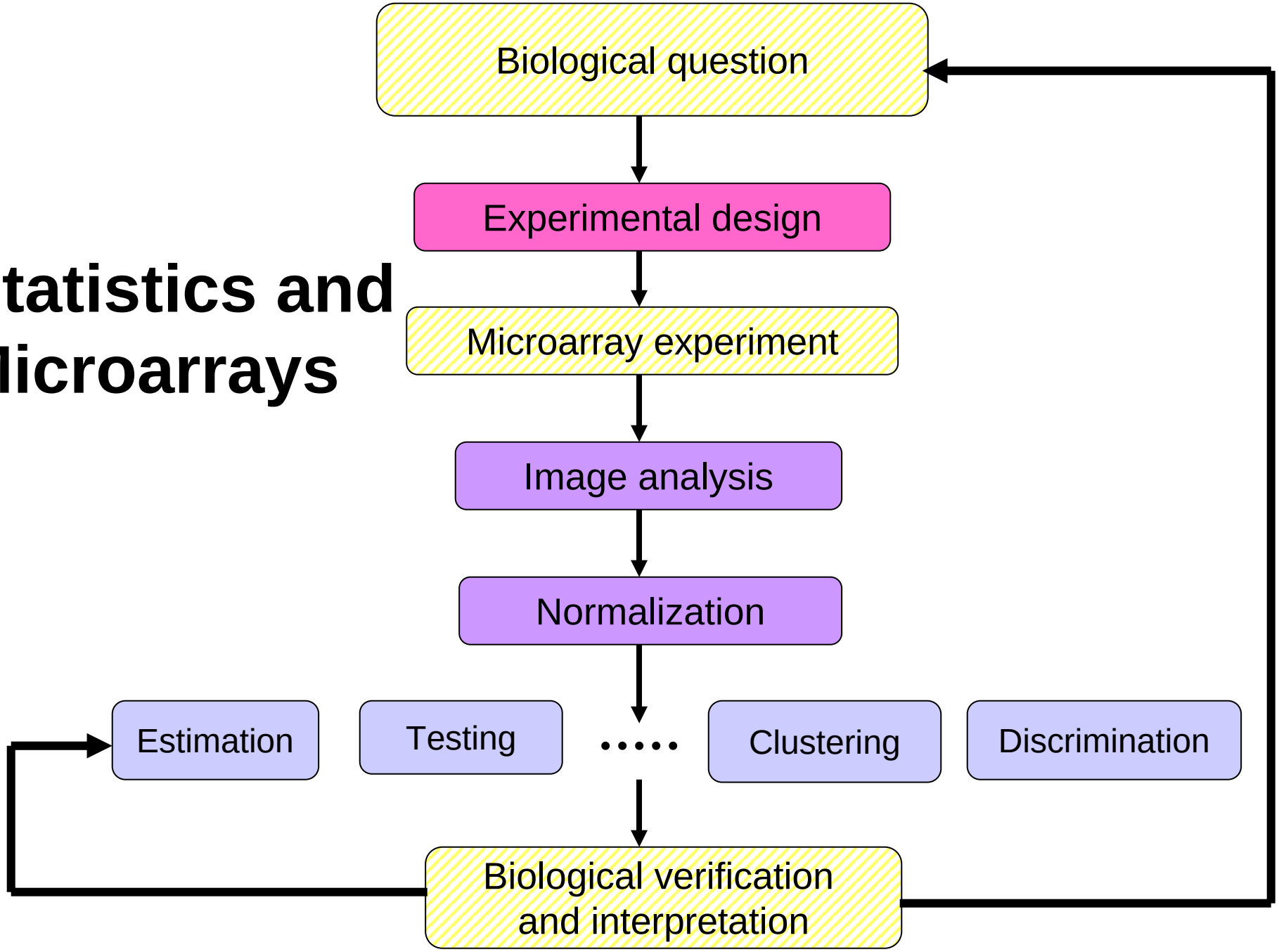
  AvDiff = average of PM-MM.
- Problem: MM may also measure signal.
- More on this in lecture *Pre-processing in DNA microarray experiments.*

What is the evidence?

Lockhart et. al. Nature Biotechnology 14 (1996)



Statistics and Microarrays



Statistical computing

Everywhere …

- for statistical design and analysis:
  - pre-processing, estimation, testing, clustering, prediction, etc.
- for integration with biological information resources (in house and external databases)
  - gene annotation (GenBank, LocusLink);
  - literature (PubMed);
  - graphical (pathways, chromosome maps).

Integration of 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 in DNA microarray experiments

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