Lecture 2:
DNA Microarray Overview

(Some slides from Dr. Holly Dressman, Duke University
http://genome.genetics.duke.edu/STAT_talk_301.ppt)

Announcements

- Go to class web page
  http://www.cs.washington.edu/527
  - Add yourself to class list
  - Check out HW1, including last year's
- CSE 590C Org. meeting today,
  3:30 MGH 284
  http://www.cs.washington.edu/590c

New Room: EE1-031

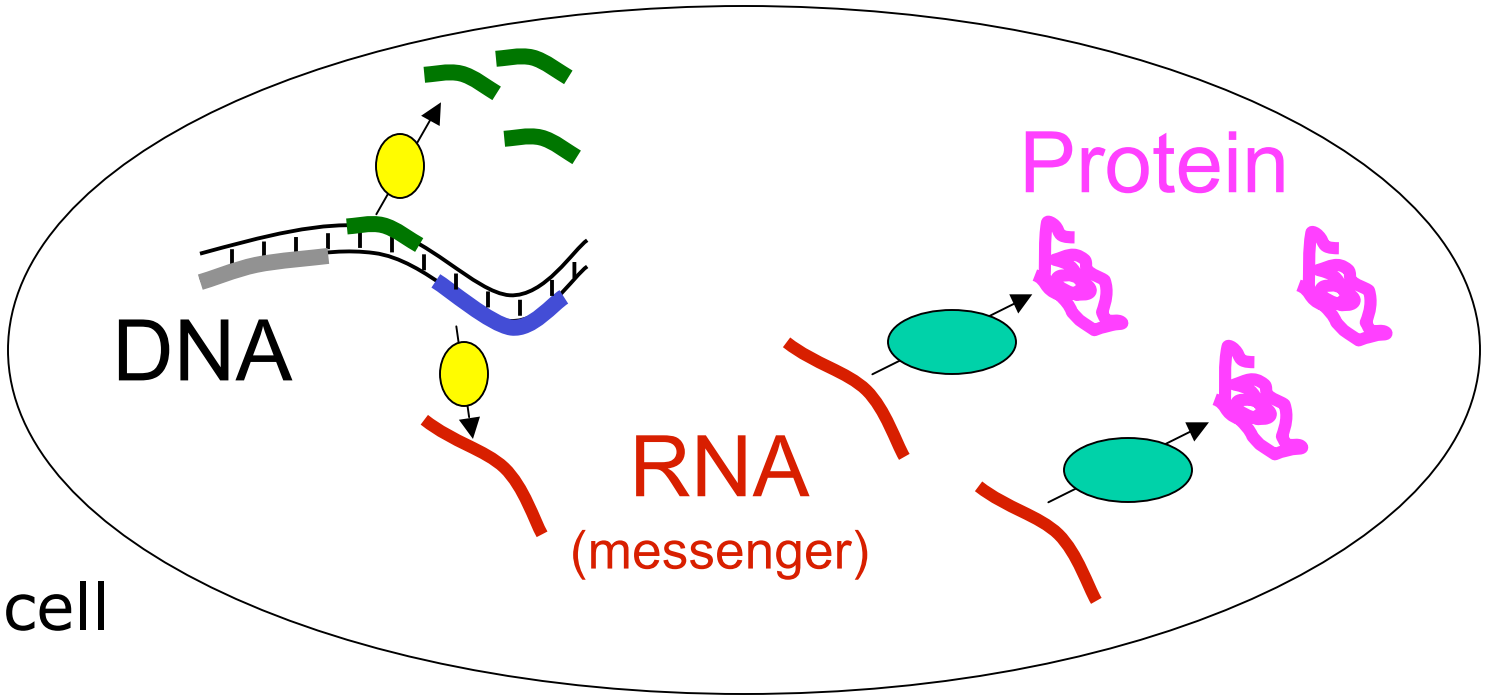
Starting Wednesday

Talks This Week

- **Combi Seminar:**
  Dr. Daniel Miranker, UTexas
  MoBIoS: A Specialized Database Management System for Biological Discovery
  Wed 1:30, K-069

Gene Expression: The "Central Dogma"

DNA → RNA → Protein

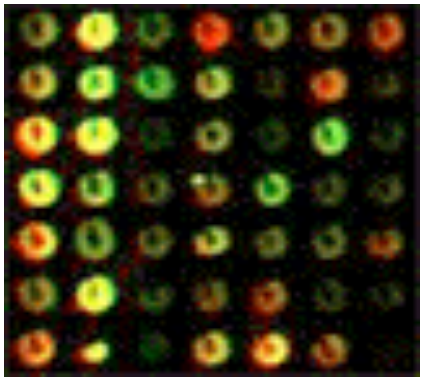


Gene Expression

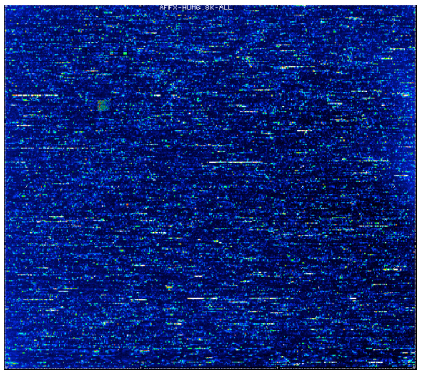
- Proteins do most of the work
- They're dynamically created/destroyed
- So are their mRNA blueprints
- Different mRNAs expressed at different times/places
- Knowing mRNA "expression levels" tells a lot about the state of the cell

Microarrays

A snapshot that captures the activity pattern of thousands of genes at once.



Custom spotted arrays

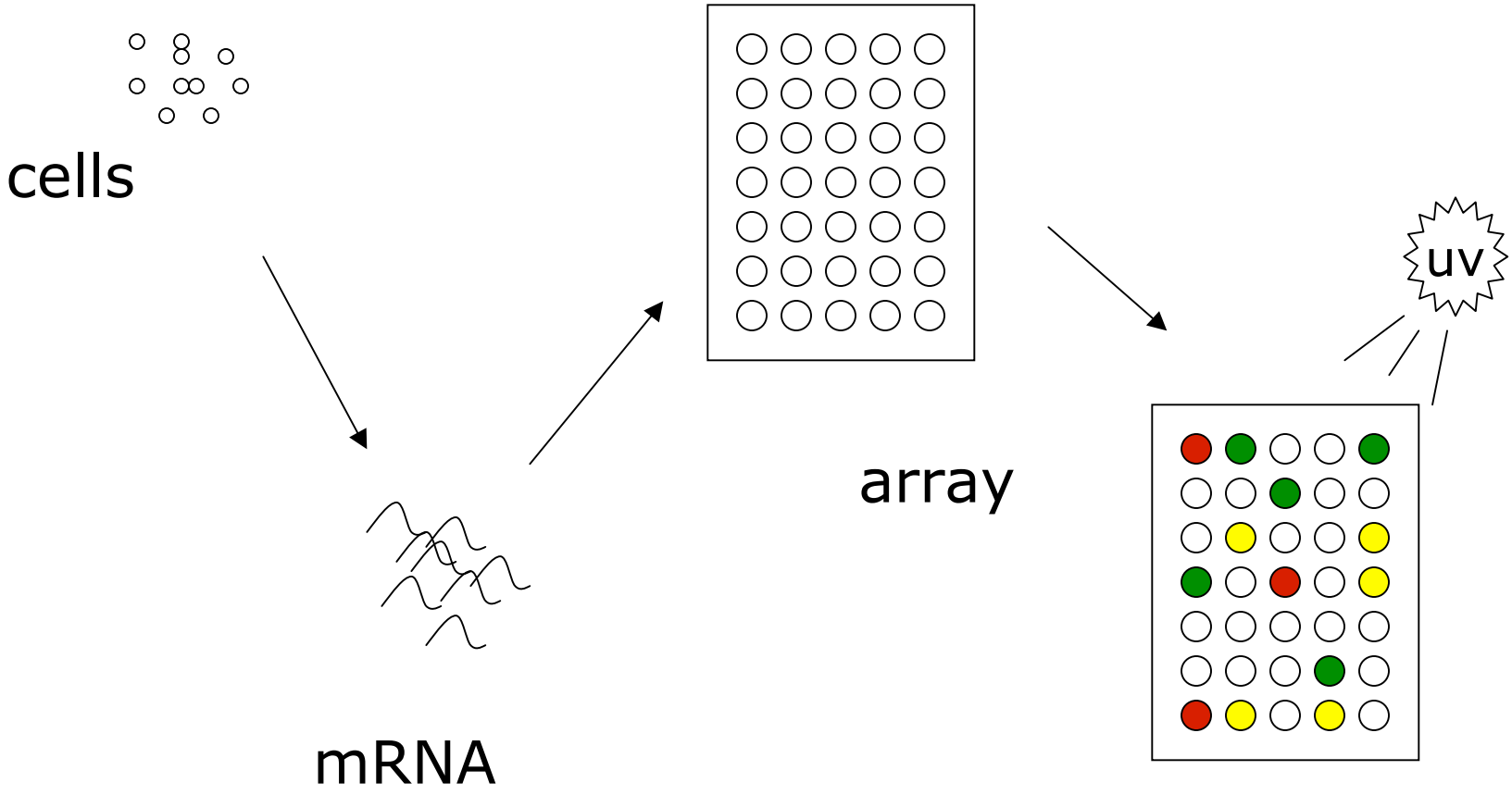


Affymetrix GeneChip

Expression Microarrays

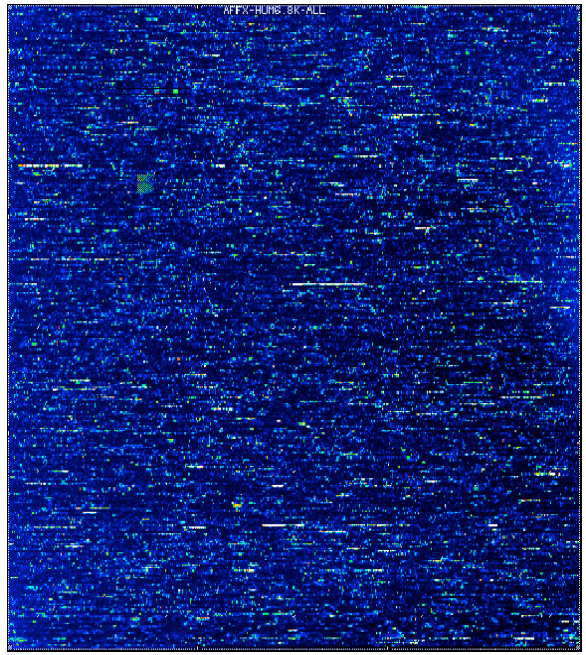
- The Array
  - Thousands to hundreds of thousands of spots per square inch
  - Each holds millions of copies of a DNA sequence from one gene
- Its Use
  - Take mRNA from cells, put it on array
  - See where it sticks – mRNA from gene x should stick to spot x

An Expression Array Experiment



An Example Application

- 72 leukemia patients
  - 47 ALL
  - 25 AML
- 1 chip per patient
- 7132 human genes per chip



Golub, et al., Science 286:531-537 (1999).

Key Issue:
What's Different?

- What genes are behaving differently between ALL & AML (or other disease/normal states)?
- Potential uses:
  - Diagnosis
  - Prognosis
  - Insight into underlying biology/biologies
  - Treatment

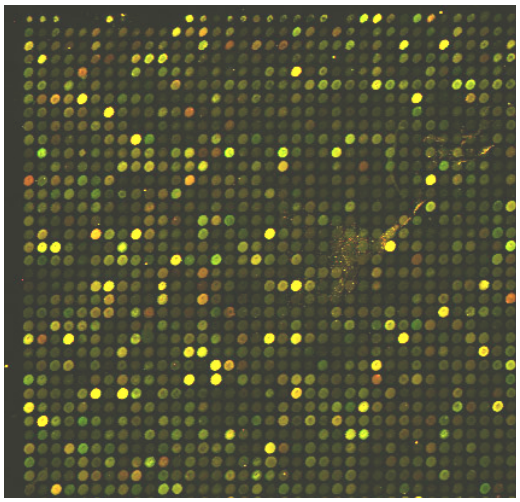
A Classification Problem

- Given an array from a new patient: is it ALL or AML?

- Many possible approaches:
  LDA, logistic regression, NN, SVM, …
- Problems:
  - Noise
  - Dimensionality

An Example Application

- Yeast “Sporulation”
- 7 time points over ~18 hours
- One array per time point
- All 6200 yeast genes on each



Chu, DeRisi, Eisen, Mulholland, Botstein, Brown, Herskowitz, “The Transcriptional Program of Sporulation in Budding Yeast,” Science, 282 (Oct 1998) 699-705

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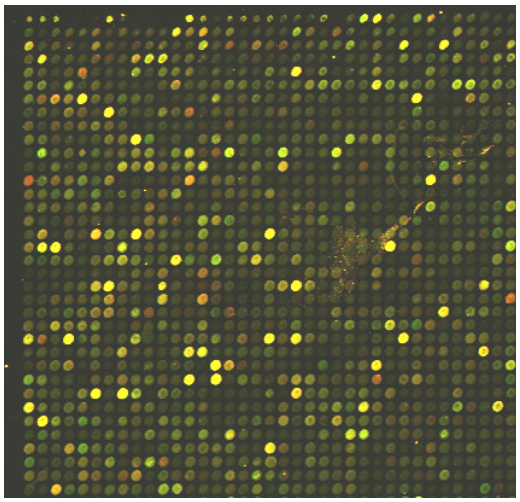


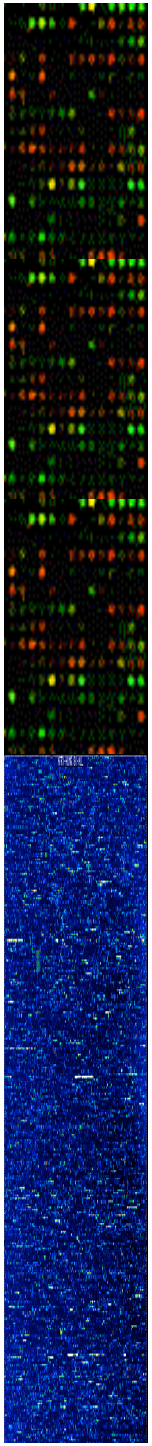
3-10x increase in number of genes known to be involved in sporulation, many with recognizable analogs in humans, presumably key players in egg/sperm formation

Chu, DeRisi, Eisen, Mulholland, Botstein, Brown, Herskowitz, “The Transcriptional Program of Sporulation in Budding Yeast,” Science, 282 (Oct 1998) 699-705

Other Applications

- Study gene function & regulation
  - Covarying ~~> coregulated?
  - Covarying ~~> common pathway?

- Refined categorization of diseases
  - E.g., "prostate cancer" is almost certainly not one disease. Are subtypes distinguishable at expression level?



Practical Applications of Microarrays

Gene Target Discovery

- Diseased vs normal cell comparison suggests sets of genes having key roles.
- Over/underexpressed genes in the diseased cells can suggest drug targets

Pharmacology and Toxicology

- Highly sensitive indicator of a drug's activity (pharmacology) and toxicity (toxicology) in cell culture or test animals.
- Screen or optimize drug candidates prior to costly clinical trials.

Diagnostics

- Potential to diagnose clinical conditions by detecting gene expression patterns associated with disease states in either biopsy samples or peripheral blood cells.

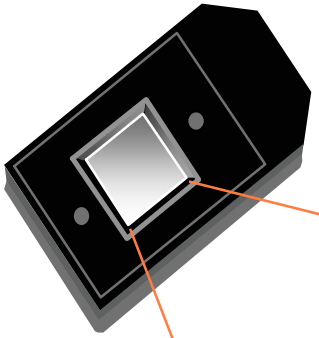
Microarray Technologies

- Oligo Arrays
  - Affymetrix -
    - one color
    - Short oligos
    - match/mismatch
  - Agilent, inter alia
    - 2 color
    - Longer oligos
- Spotted cDNA arrays
- Bead-based systems

GeneChip® Probe Array

GeneChip® Probe Arrays

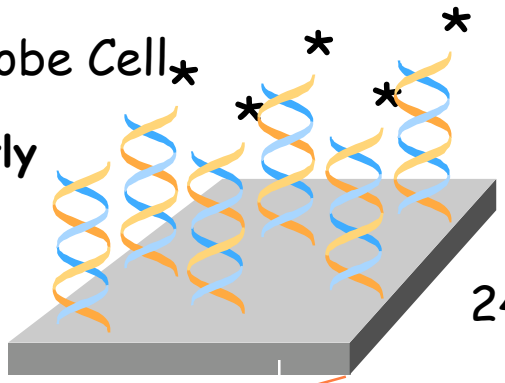
GeneChip Probe Array



Hybridized Probe Cell

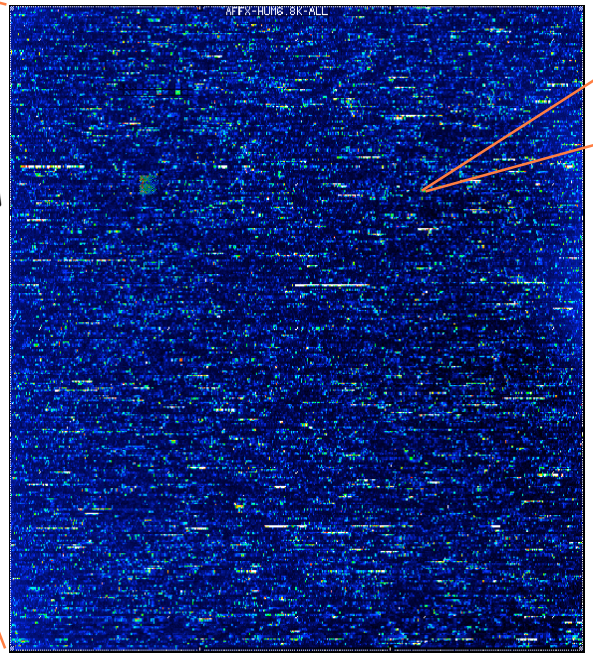
Single stranded, fluorescently labeled DNA target

Oligonucleotide probe



24μm

1.28cm



Each probe cell or feature contains millions of copies of a specific **oligonucleotide** probe

Over 250,000 different probes complementary to genetic information of interest

Image of Hybridized Probe Array

How unique is a 20-mer?

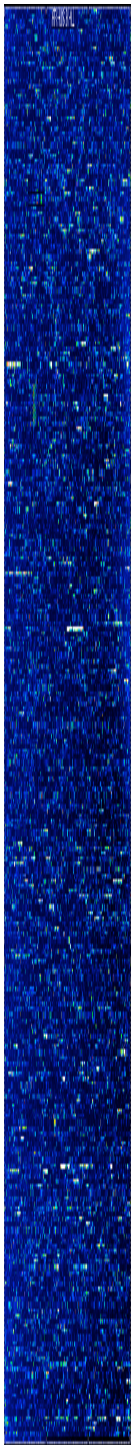
- VERY CRUDE model: DNA is random—every position is equally likely to be A, C, G, or T, independent of every other
- Then probability of a random 20-mer is

$$\left(\frac{1}{4}\right)^{20} = \left(\frac{1}{2}\right)^{40} = \left(\left(\frac{1}{2}\right)^{10}\right)^{4} = \left(\left(\frac{1}{1024}\right)\right)^{4} \approx \left(10^{-3}\right)^{4} = 10^{-12}$$

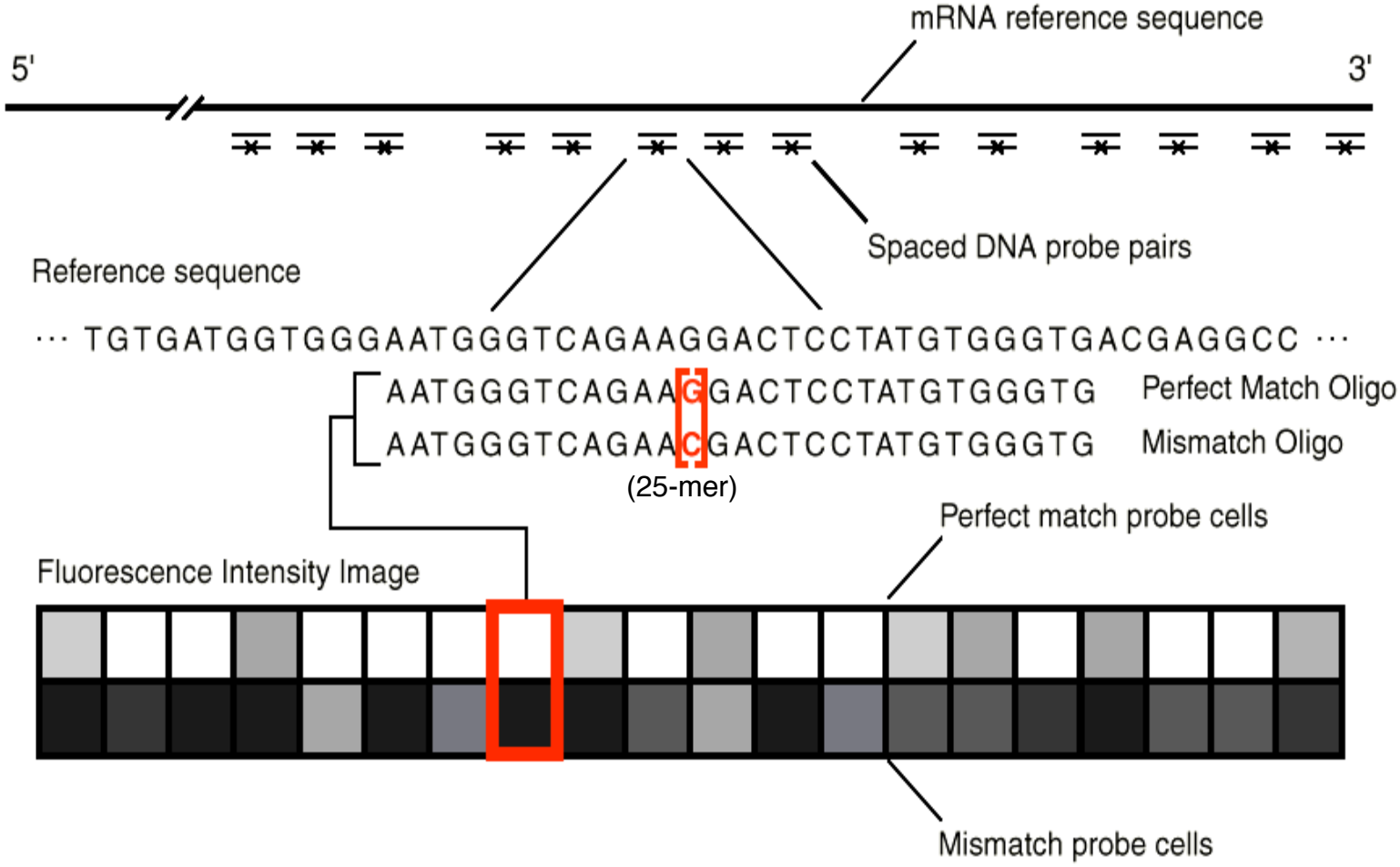
- So, a specific 20-mer occurs in random human-sized DNA sequence with probability about $3 \times 10^{9} \times 10^{-12} = .003$

How Random is a Genome?

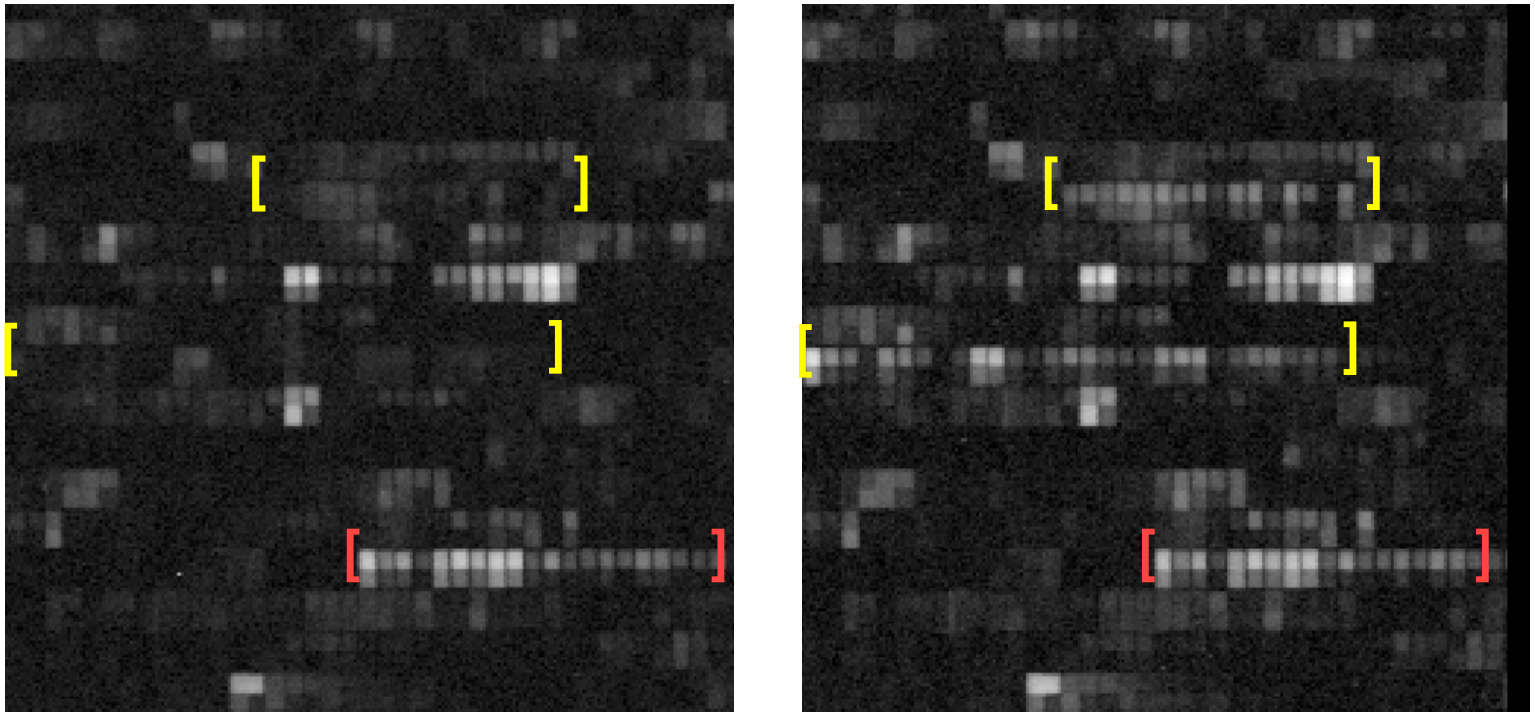
- G/C content can vary from ~40-60% across and within organisms ("isochores")
- Adjacent pairs not independent
- Adjacent triples not independent (esp. in genes)
- …
- Many large-scale repeats, e.g.
  - similar genes, domains within genes
  - transposons & other junk
    - within primates, ~ 5% of all DNA is composed of (noisy) copies of a 300bp ALU sequence
- Nevertheless, crude model above is a useful guide



Probe Tiling Strategy
Gene Expression



Gene Expression Tiling Strategy

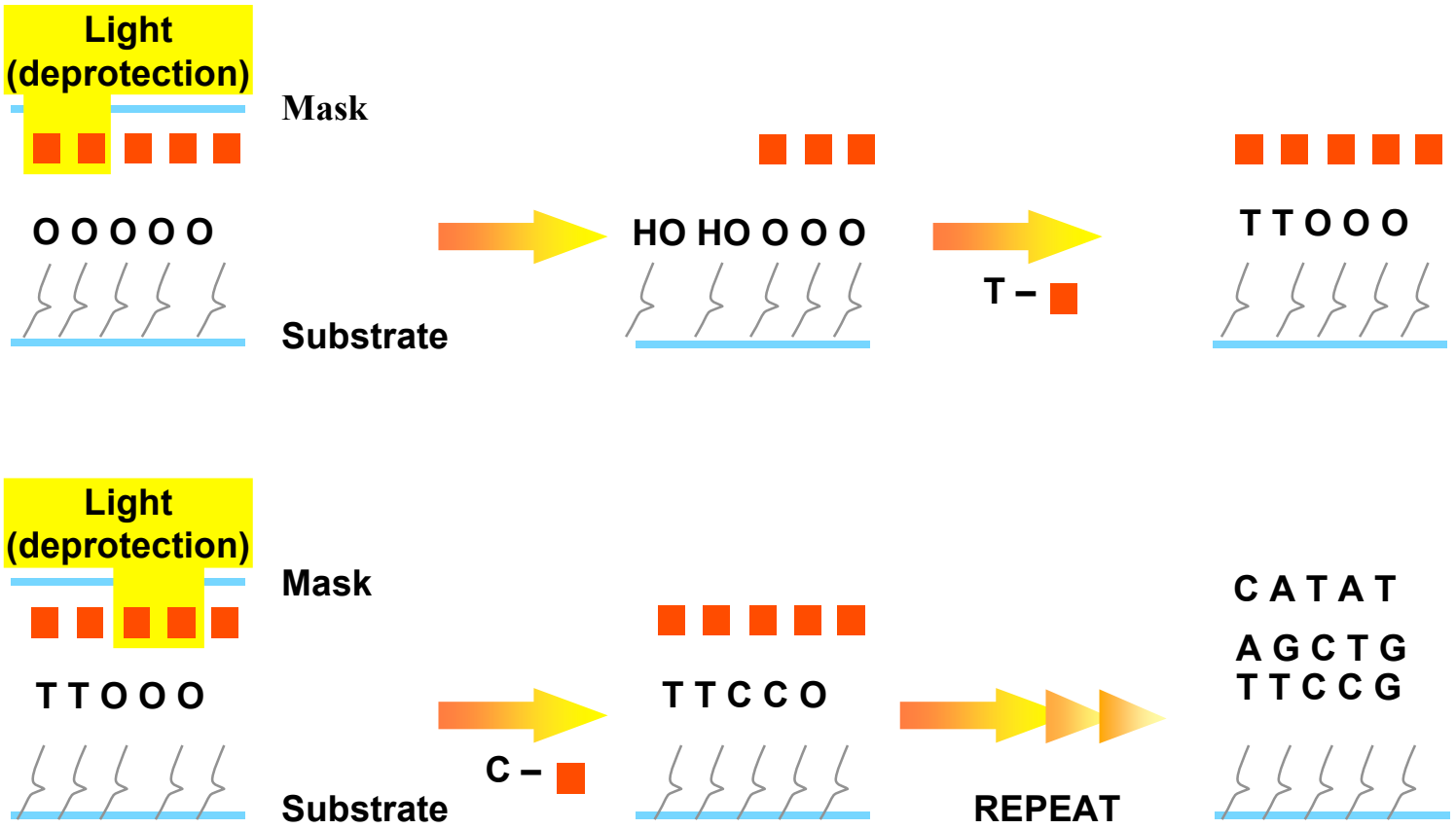


Uninduced Induced

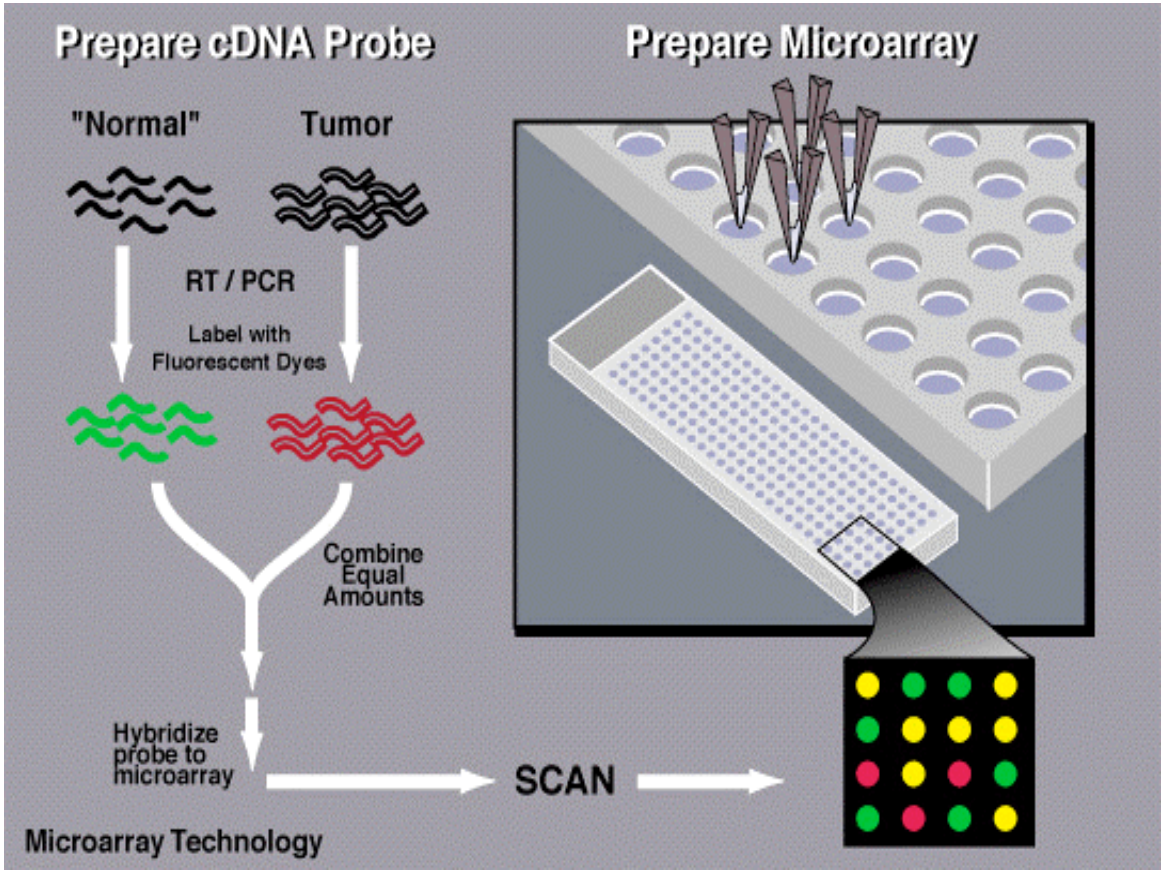
40 separate hybridization events are involved in determining the presence or absence of a transcript

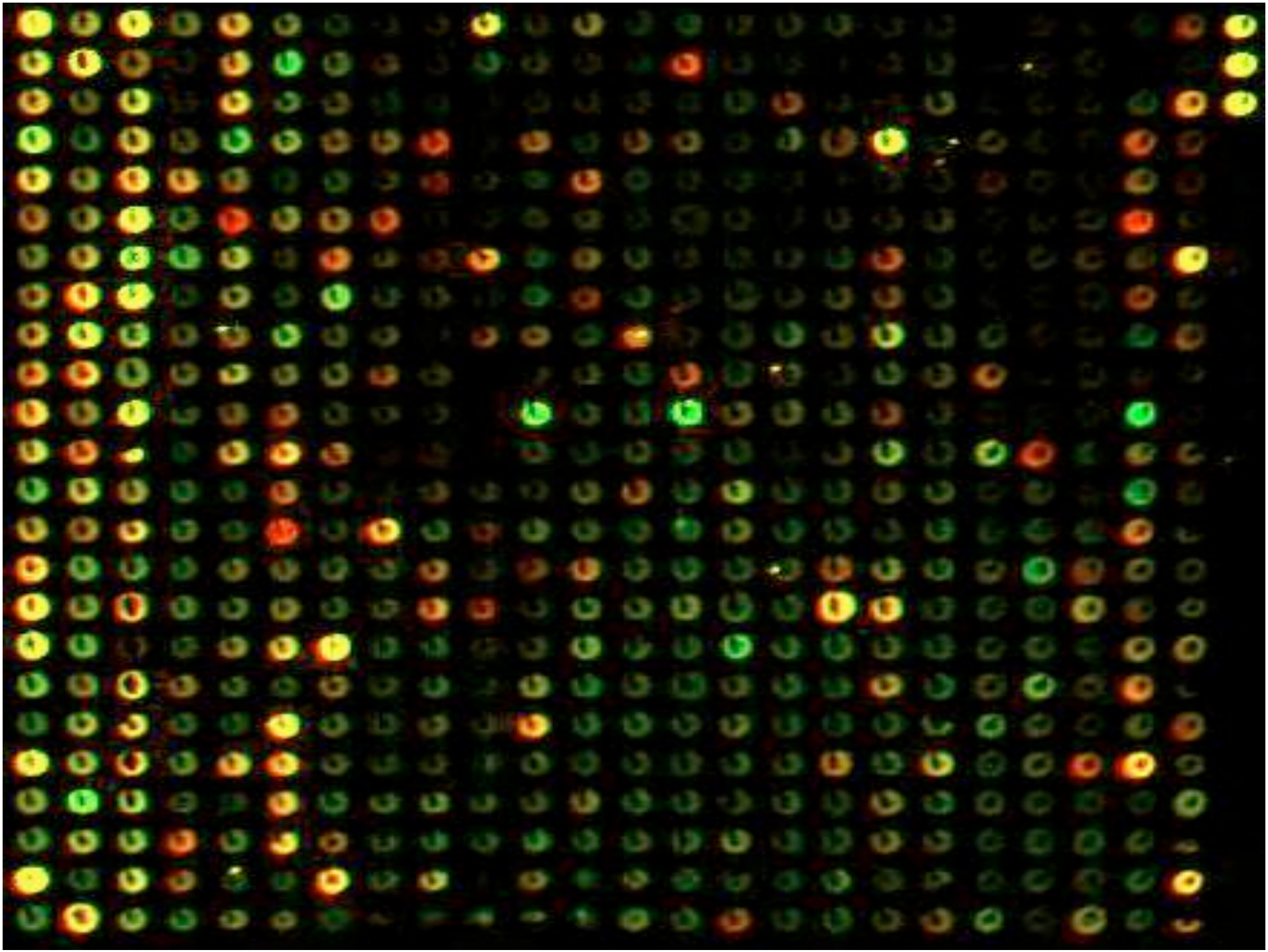
80 separate hybridization events are involved determining differential gene expression between two samples

Synthesis of Ordered Oligonucleotide Arrays



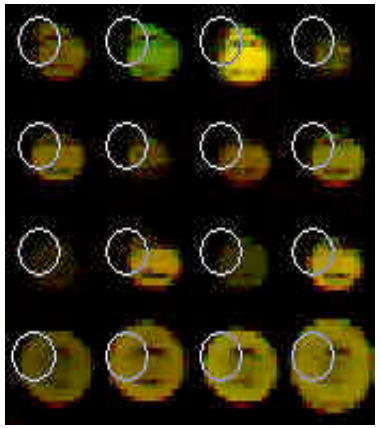
Spotted Microarray Process



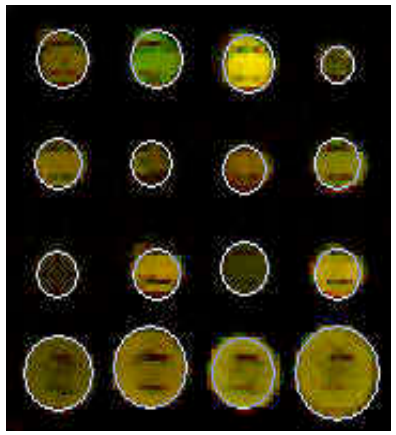


GenePix Pro Features

- Auto Align



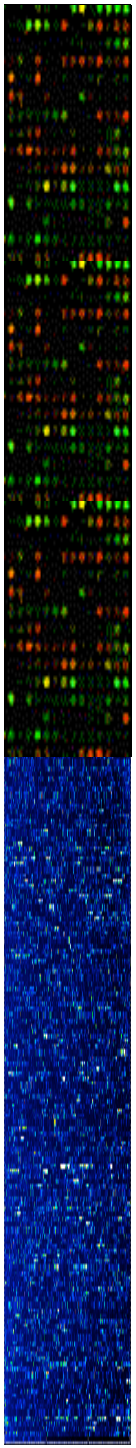
Before Auto Align



After Auto Align

Micro Array Noise Sources

- Lot-to-lot variation (chips, reagents,…)
- Experiment-to-experiment variation
  - cell state, culture purity
  - sample preparation, hybridization conditions
- Spot-to-spot variation
  - unequal dye incorporation
  - dye nonlinearity/saturation
  - uneven spot sizes
  - self- & cross-hybridization
  - Image capture & processing (spot finding, quantization, sensors)
- …



Challenges in analyzing Microarray Data

- Amount of DNA in spot is not consistent
- Spot contamination
- cDNA may not be proportional to that in the tissue
- Low hybridization quality
- Measurement errors
- Spliced variants
- Outliers
- Data are high-dimensional "multi-variant"
- Biological signal may be subtle, complex, non linear, and buried in a cloud of noise
- Normalization
- Comparison across multiple arrays, time points, tissues, treatments
- How do you reveal biological relationships among genes?
- How do you distinguish real effect from artifact?

Microarray Summary

- Lots of variations
  - Glass, nylon, beads,…
  - Long, short DNA molecules
  - Fab via photolithography, ink jet, robot
  - Radioactive vs fluorescent readout
  - Relative vs absolute intensity
- Leads to diverse sensitivity, bias, noise, etc.
- But same bottom line:
  unprecedented global insight into cellular state and function