

Lecture 9:

The Age of Biotechnology

Aims:

- Classical Biology vs Emerging Technology
..... for the study of the Operomics!
- The integration of engineering and biological science
Examples - Microarrays: DNA, RNA, protein, lipid
 - Cantilevers
 - Microfluidics
 - Nanotubes
- The integration of computers and biological science

The Century of Biology

1800's-Early 1900's - Chemistry
(Organic, Physical Chemistry)

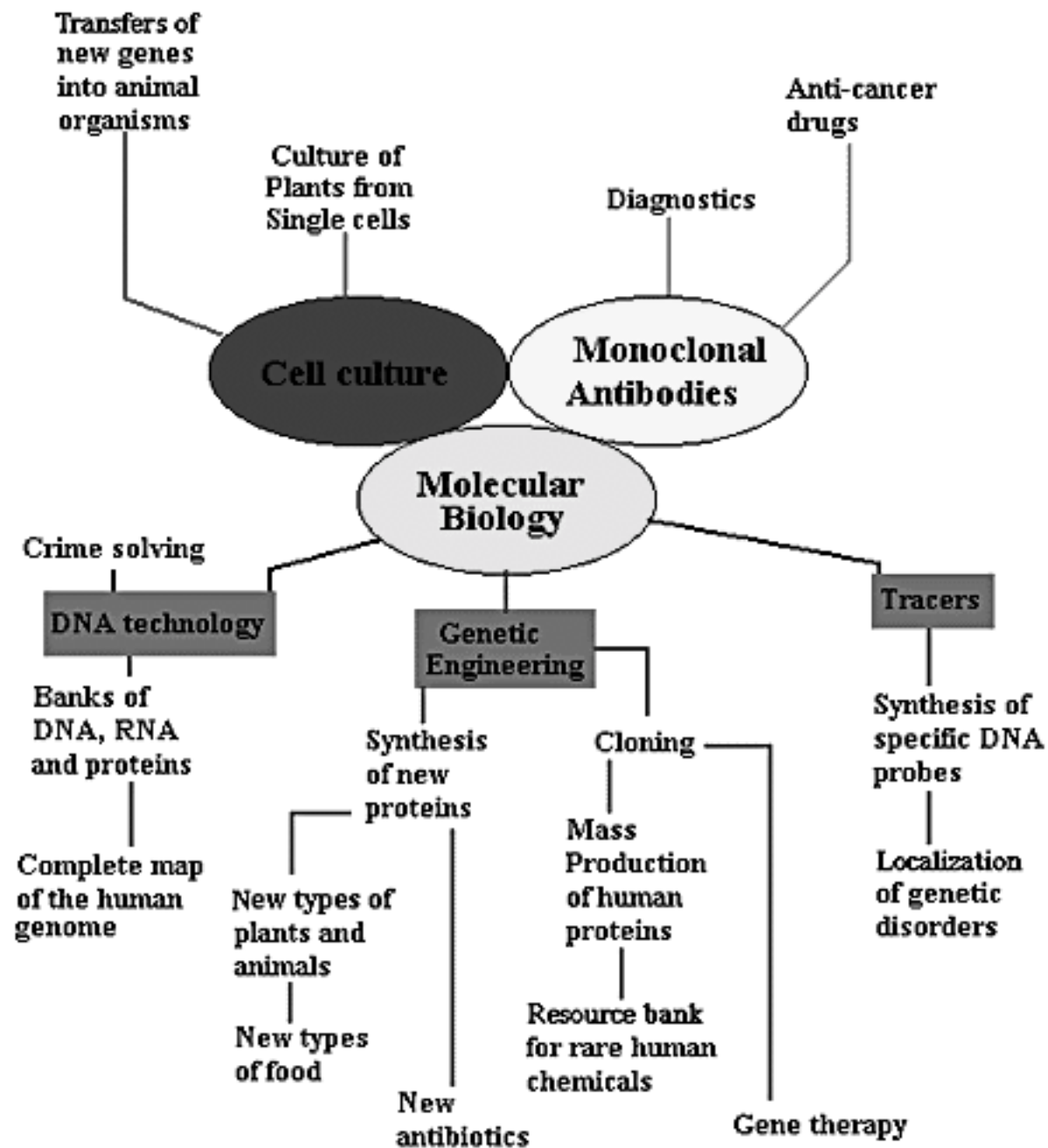
1900's - Physics
(atomic energy, semiconductors)

2000's - Biology
(Genome Sequencing, Pharmacogenomics
Stem Cell Therapeutics, Genetic Therapy)

New areas of biotechnology

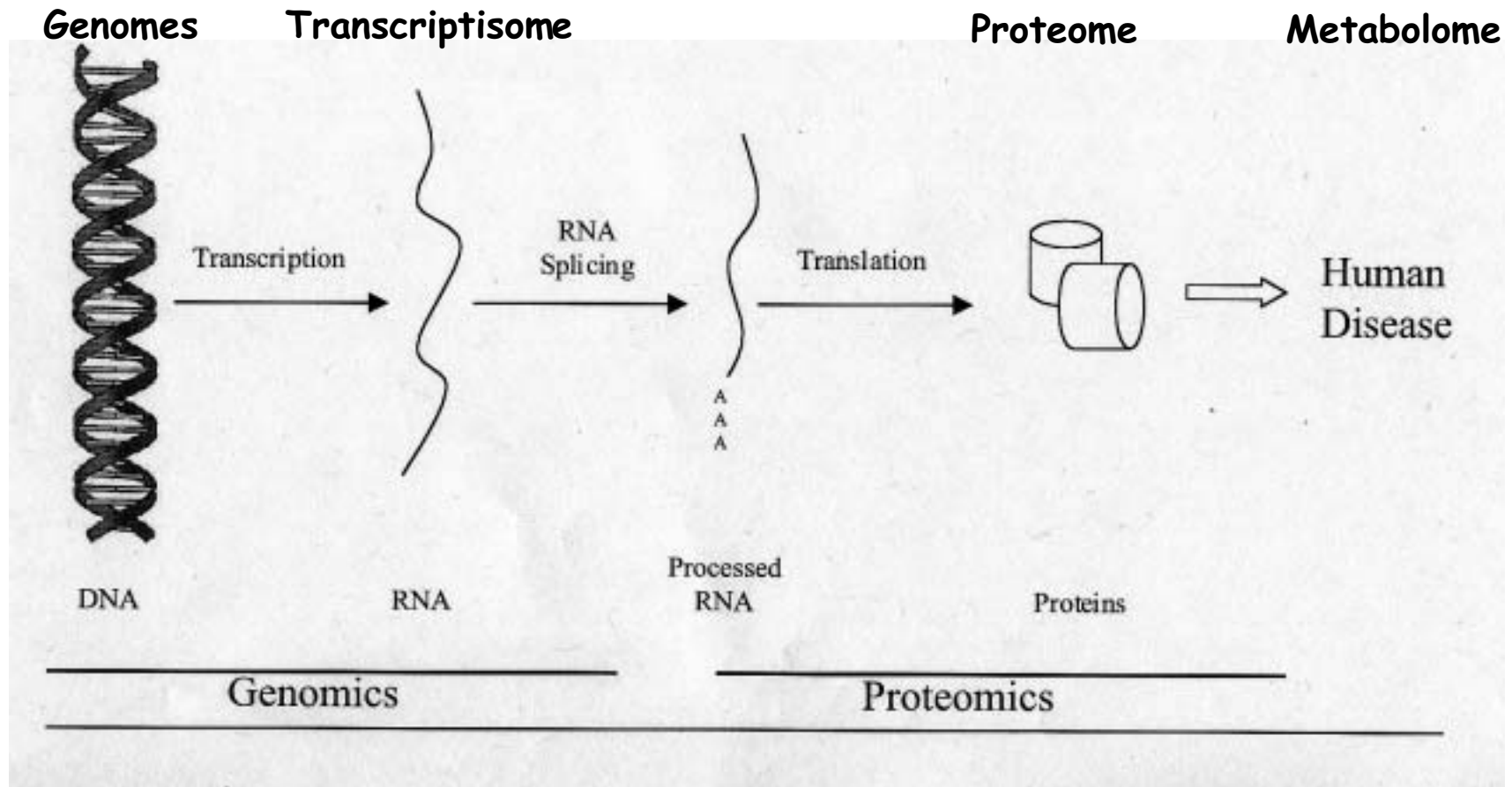
- Detecting Biological Molecules
- Understanding Biological Molecules
(Functional Genomics, Systems Biology)
- Changing Biological Molecules
(Genetic Engineering)

Areas of Applied Biotechnology:



Operomics

From Genes to Human Disease



Order of Complexity of Protein interactions:

Genome → Transcriptome → Proteome → Metabolome

Proteomics vs Genomics

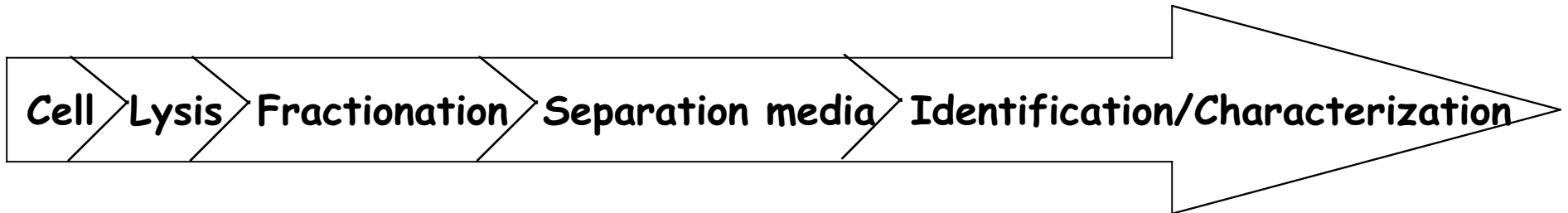
- **mRNA levels** do not necessarily represent the amount of active protein in a cell
- The gene sequence does not describe **post-translational modifications** essential for protein function and activity
- The study of the genome does not describe **dynamic cellular processes**

Techniques

Proteomic/Genomic

- **Separation & visualization of complex mixtures**
 - Gel electrophoresis (e.g. 1D, 2D for proteomics)
 - Image analysis
- **Identification:** e.g. of proteins by mass spectrometry
- **Sequencing:** amino acid, nucleotide
- **Bioinformatics:** databases and analysis software

Basic Approach For The Study of Proteins:



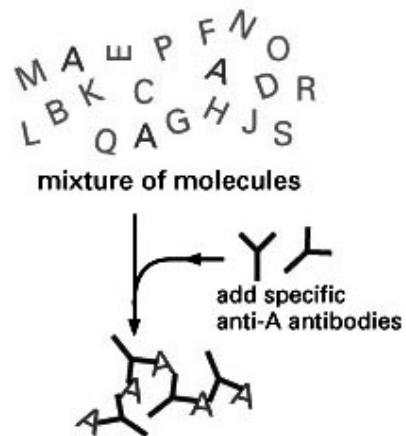
- **Source:** - cell
- **Purify molecule(s) of interest:** - fractionation, purification
- gels, chromatography
- **Perform studies for:** - characterization, functionality, specificity, etc
- **Basic “concepts” of techniques:** - binding specificity
- hybridization

Traditional Techniques for Functional/Characterization

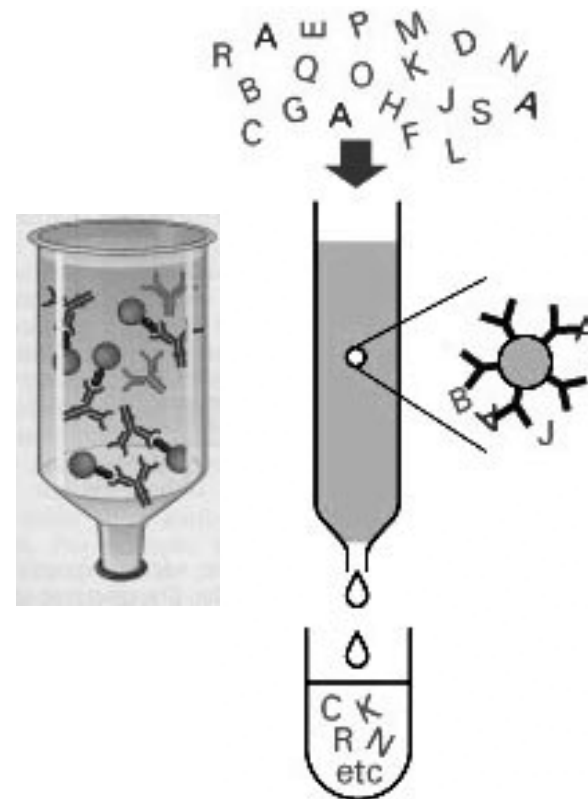
- Enzyme Linked Immunosorbent Assays (ELISA)
- Blotting: Western (Proteins), Southern (DNA), Northern (RNA)
- 2D Electrophoresis
- Mass spectrometry
- Flow cytometry

Tools For Purification

**Immunoprecipitation:
purification of antigen**



**Purification by
affinity chromatography**



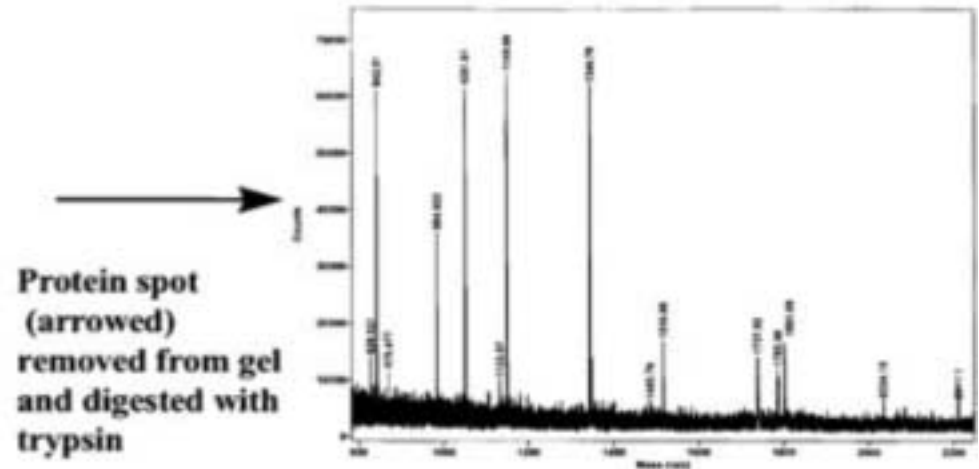
More...DNA binding proteins etc

Proteomics: Protein Data points



Coomassie blue stained 2D-gel of a total cellular protein extract of colon carcinoma

*It takes days or weeks
to do this analysis*

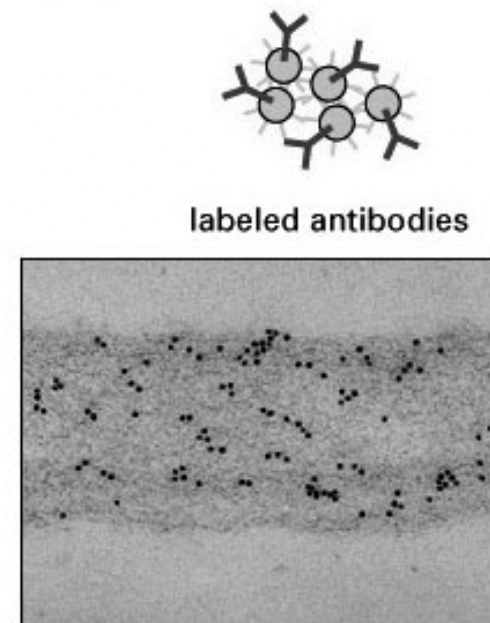
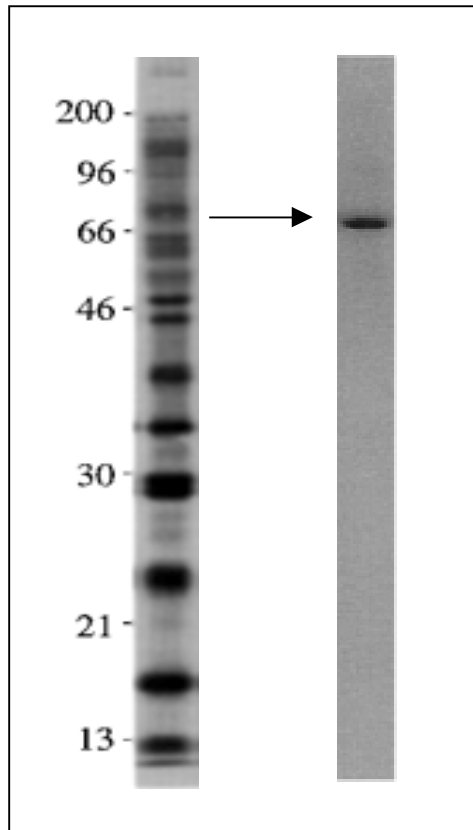


Mass spectrum of peptide fragments obtained by MALDI-TOF mass spectrometry

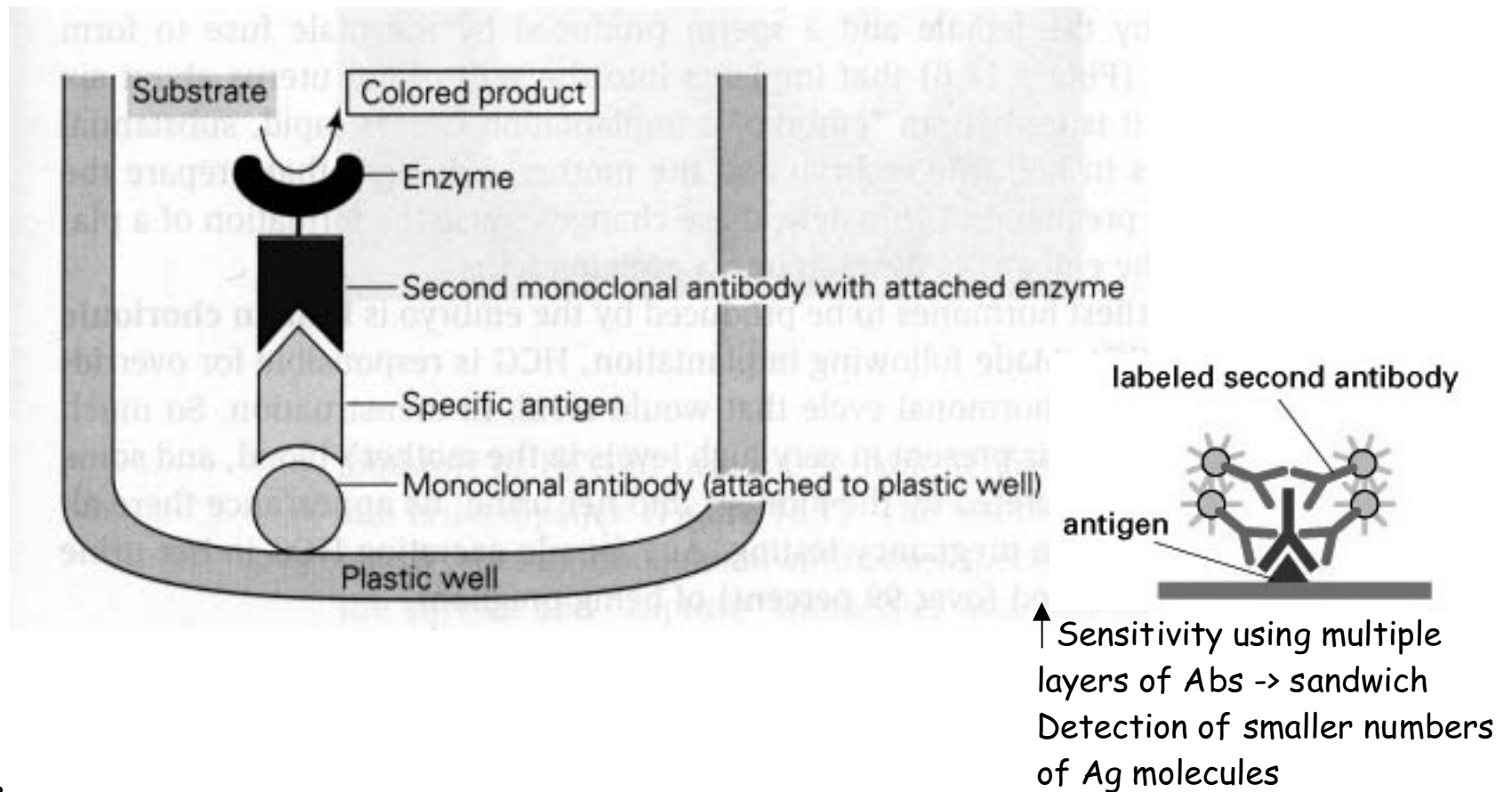
Database matching of peptide mass information identifies spot as nucleoside diphosphate kinase A (NM23)

Proteins as molecular tags:

Biochemical detection



Enzyme linked immunosorbent assay (ELISA)



But...

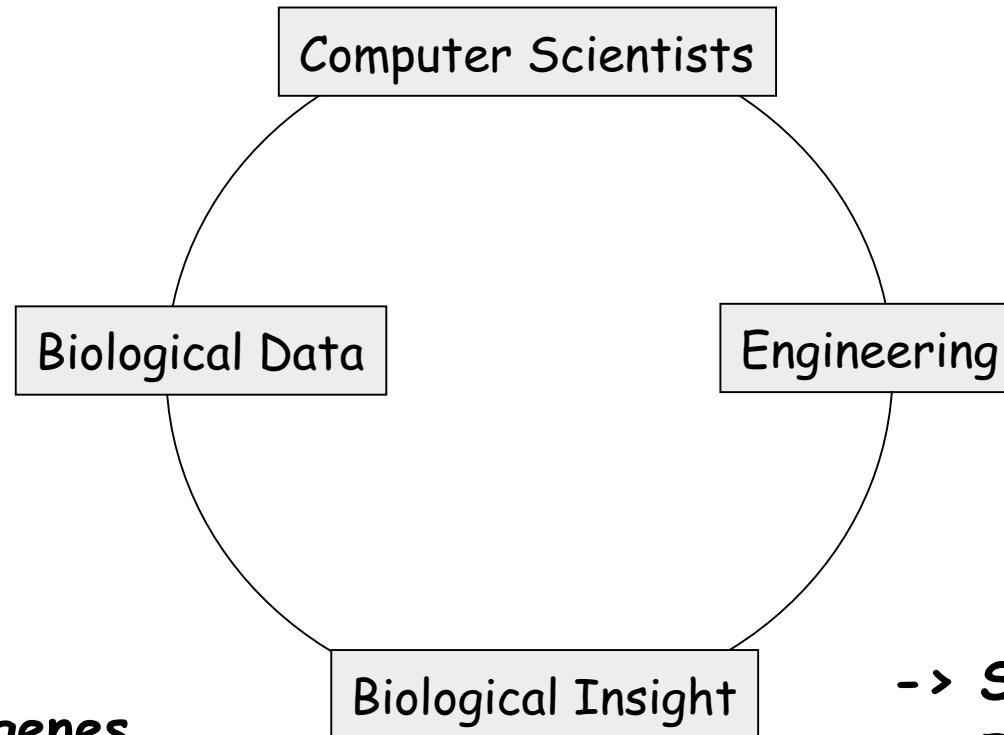
Protein Conformation Changes!

Traditional Techniques

Common biological laboratory techniques are

- Reliable
- Time inefficient
- "Simple"

Biology is becoming a Co-operative interdisciplinary science



- > 1000's genes
- > 1000's proteins

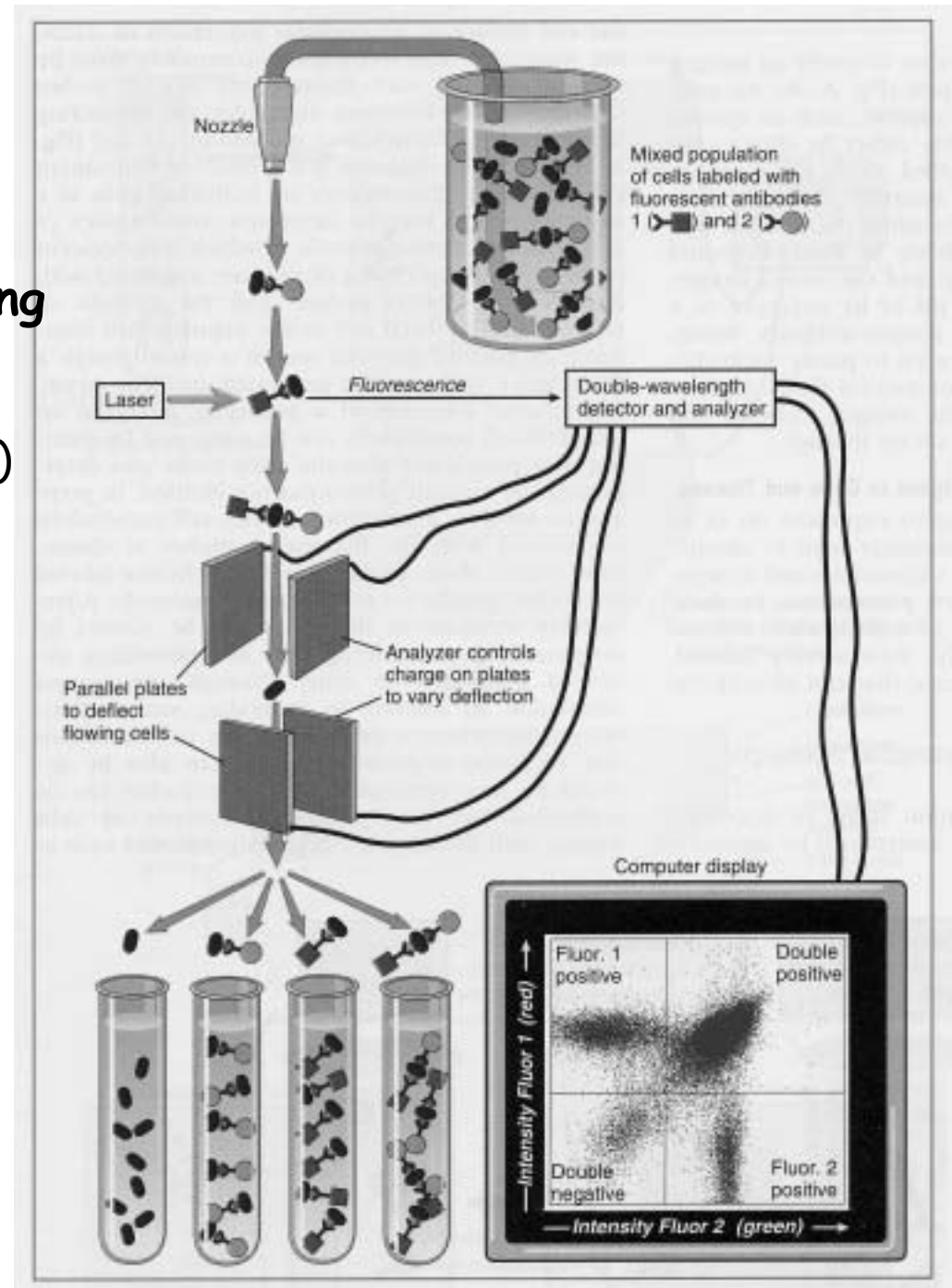
- > Structure
- > Function
- > Pathway/Network
- > Target

Engineering + Biology

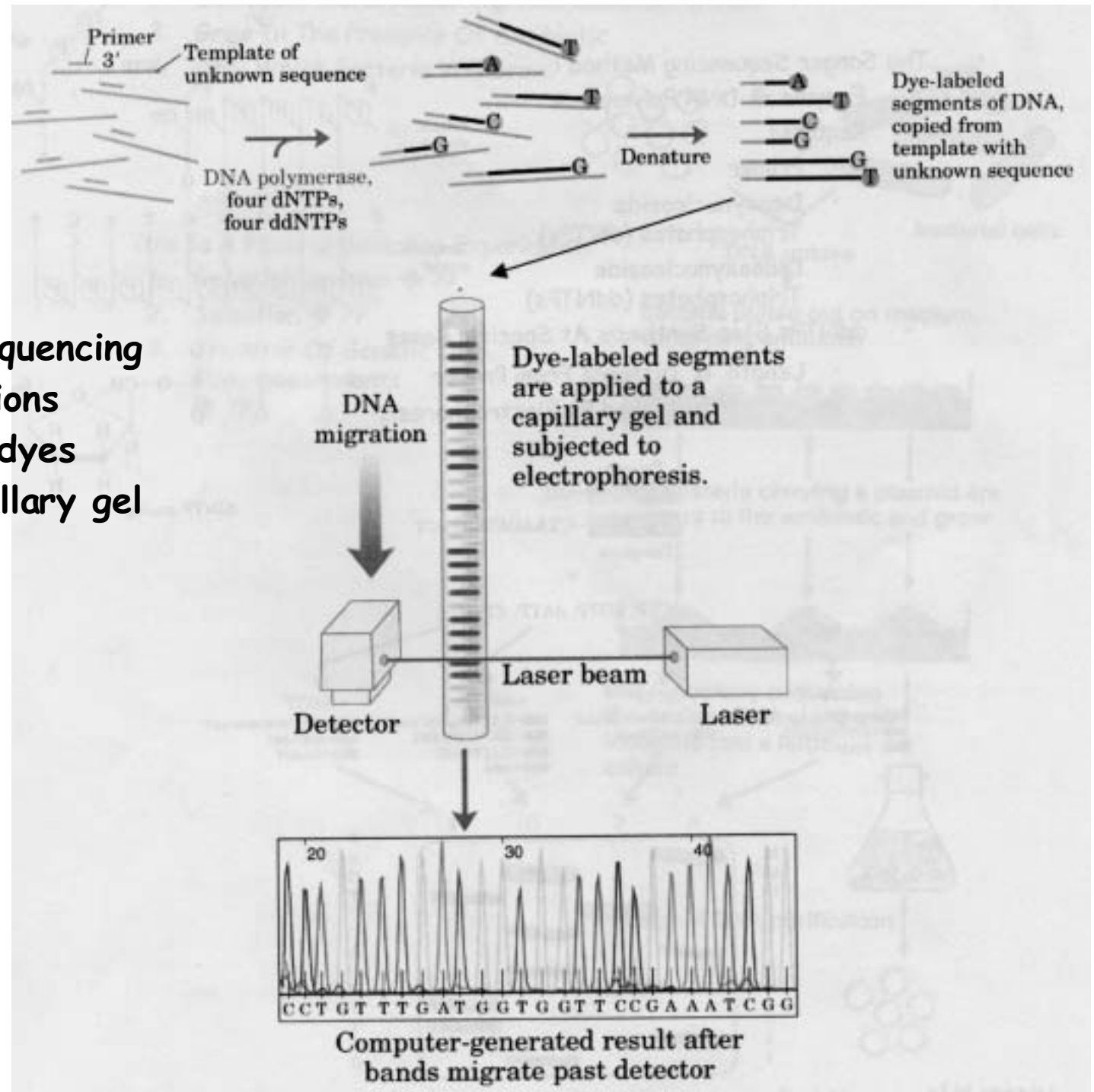
Flow cytometry and Fluorescence-activated cell sorting

- Separation based on two antigenic markers ("two-color sorting")
- Able to analyze and separate cell populations based on
 > 2 colored probes

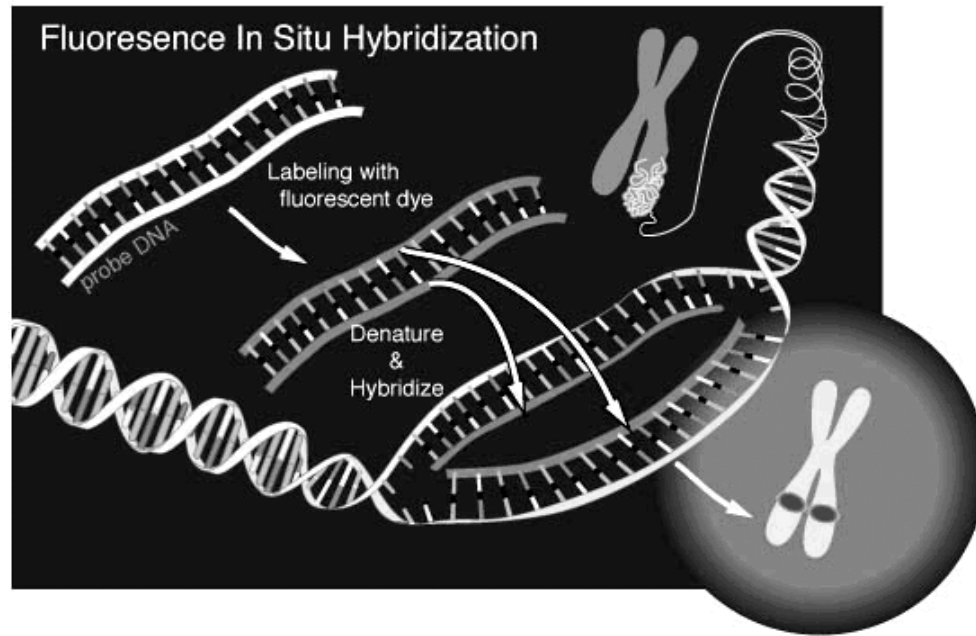
Also...



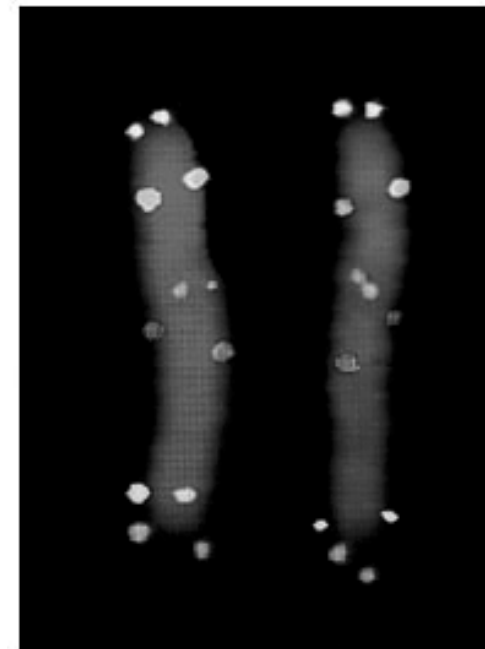
..... Automated sequencing
(nucleotide) reactions
using fluorescent dyes
Separation in capillary gel



Fluorescence In Situ Hybridization (FISH):



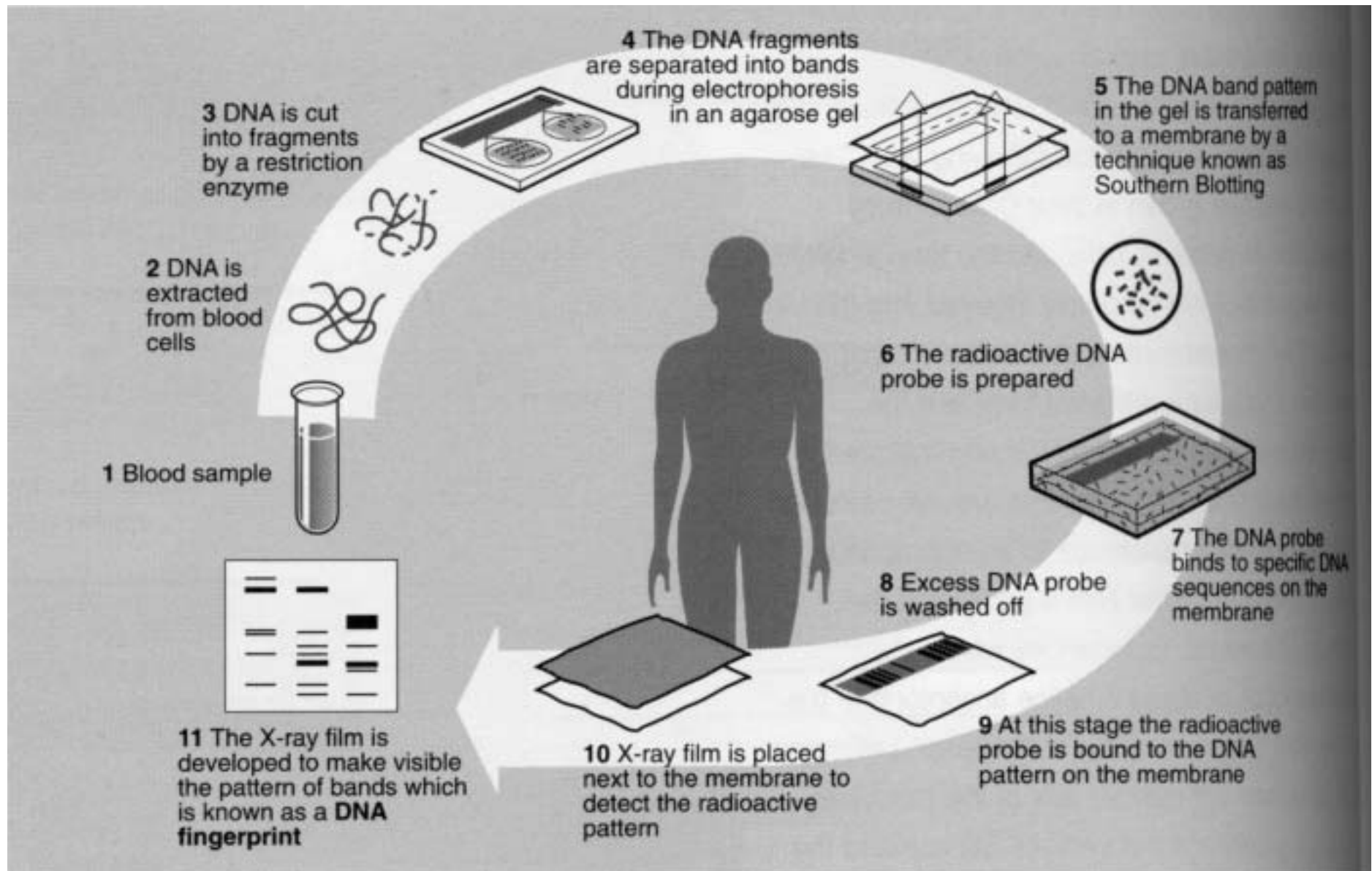
Human chromosome 5
in metaphase of mitosis.
Probe -> 2 dots on each chromosome
because already replicated DNA



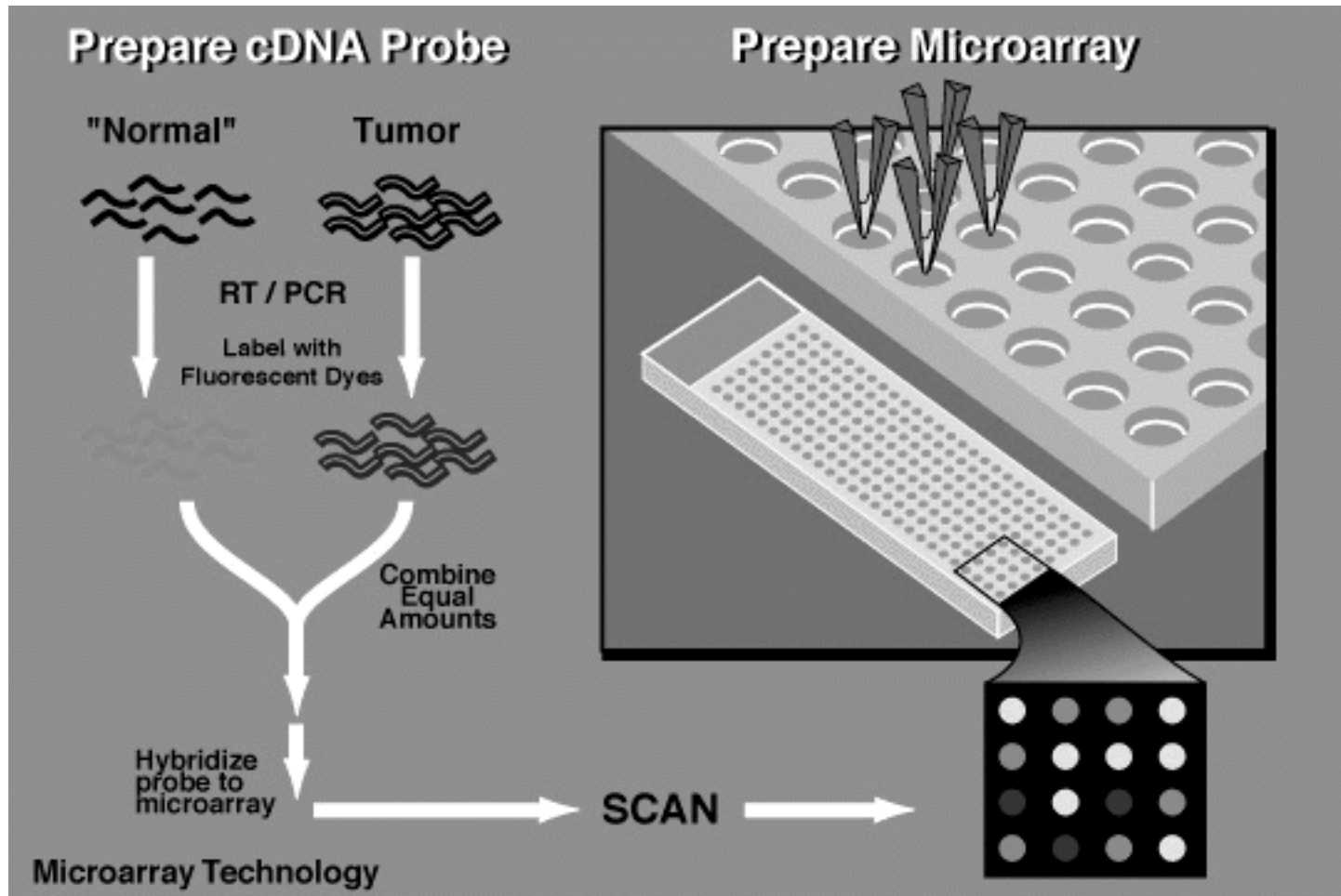
2 μ m

DNA fingerprinting

- a combination of techniques



Microarray Technology

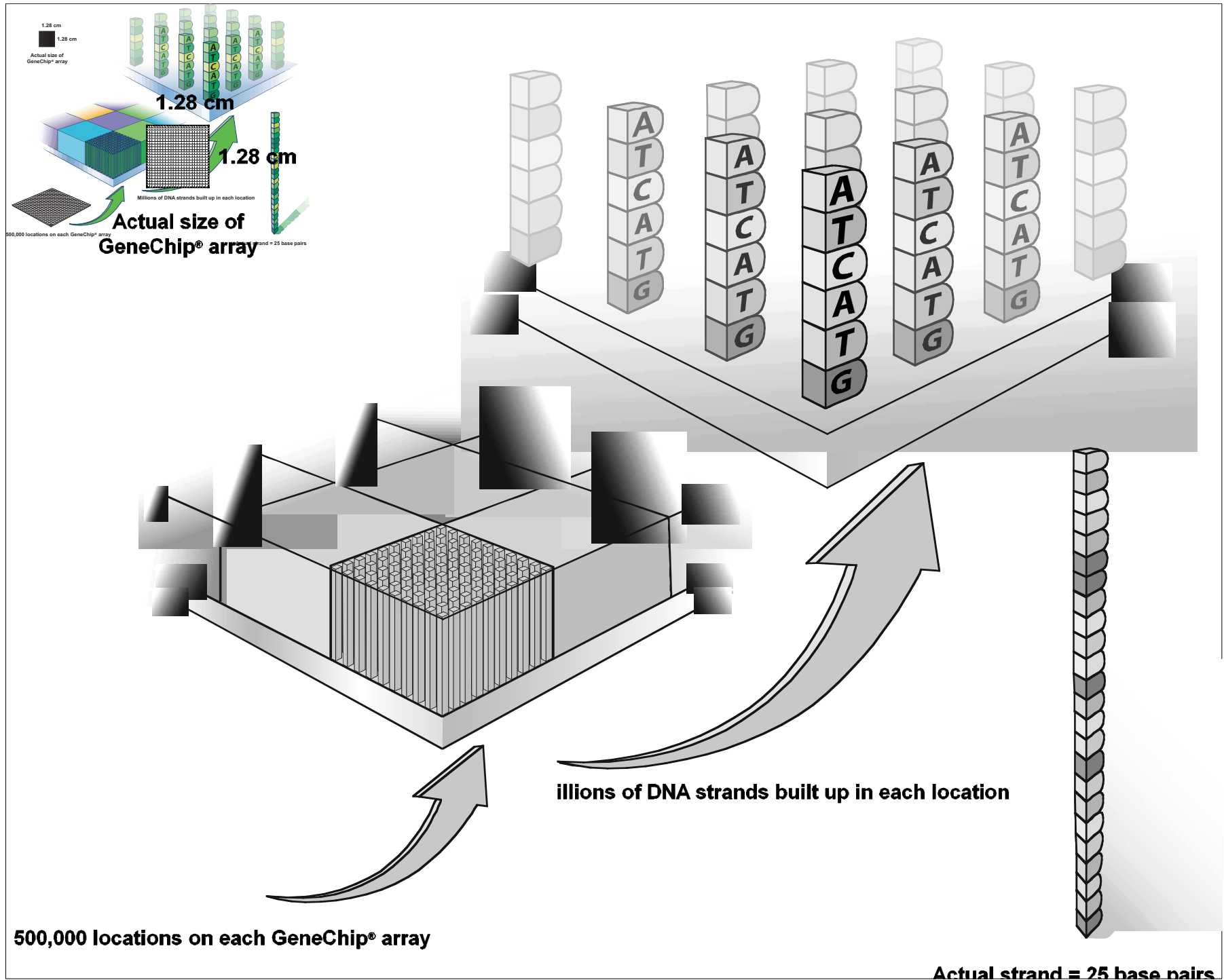


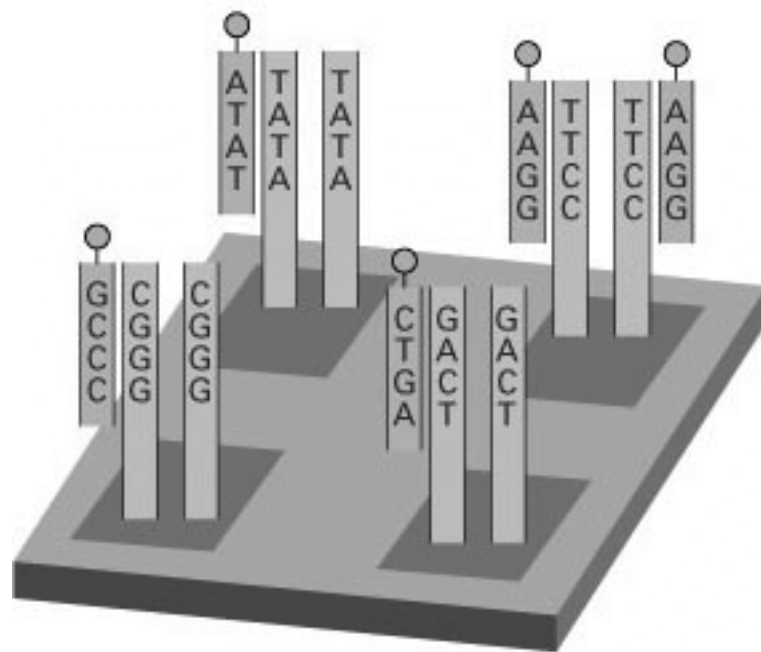
Fabrication

- Oligos/cDNA can be synthesized and stored
- Oligo seqs can be synthesized on a slide or chip using various commercial technologies.
 - E.g ink-jet technology similar to that used in color printers.
 - Separate cartridges for the four bases (A, C, G, T) are used to build nucleotides on a slide.
- Photolithography

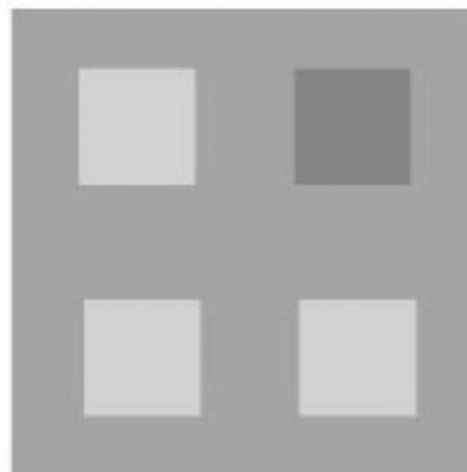
Method

- Each "gene" (i.e., sequence of interest or *feature*) is represented by multiple short (25 nucleotide) oligo probes
- mRNA extracted from a biological sample is labeled (dye) and hybridized to a chip

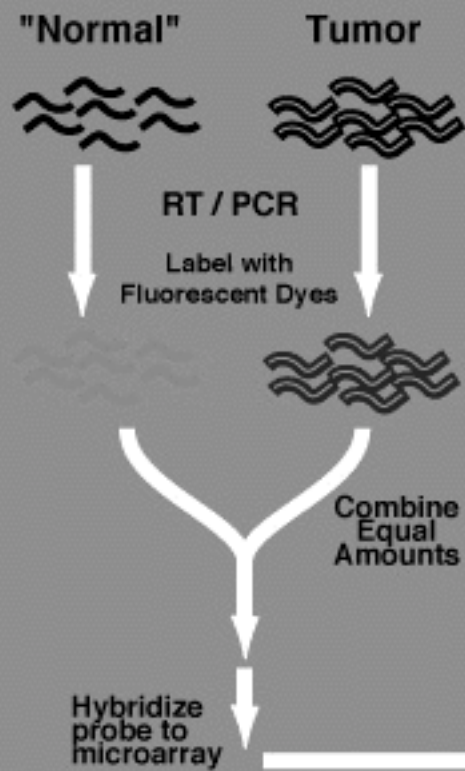




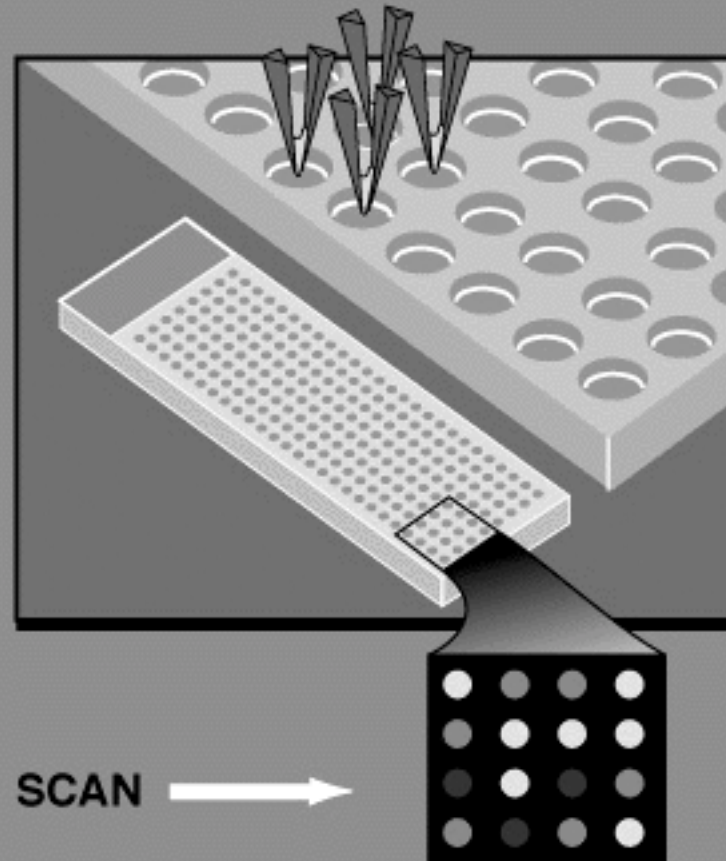
examine microarray
with fluorescent
scanner



Prepare cDNA Probe



Prepare Microarray



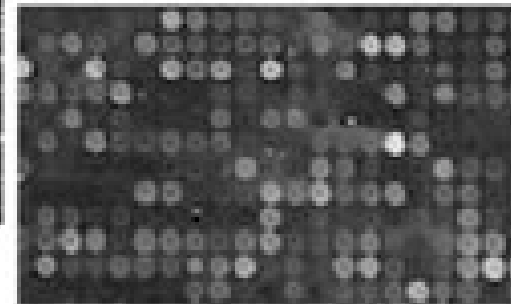
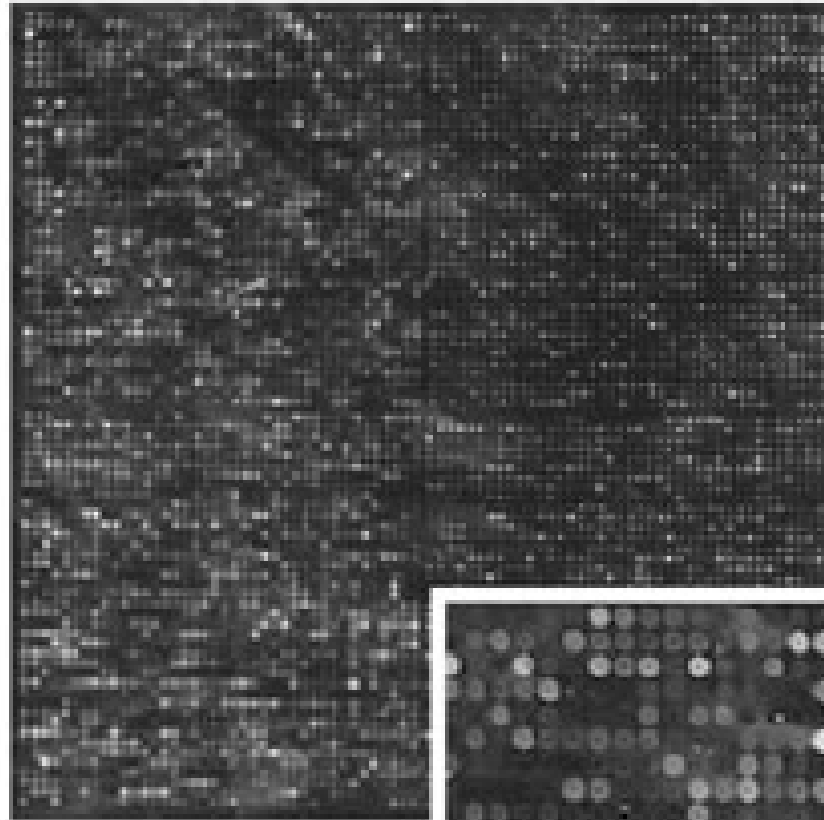
SCAN

Microarray Technology

DNA microarray

Experimental conditions →

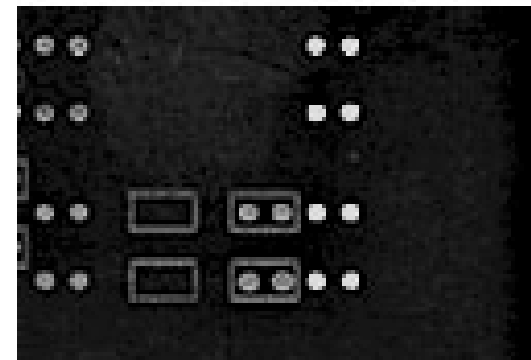
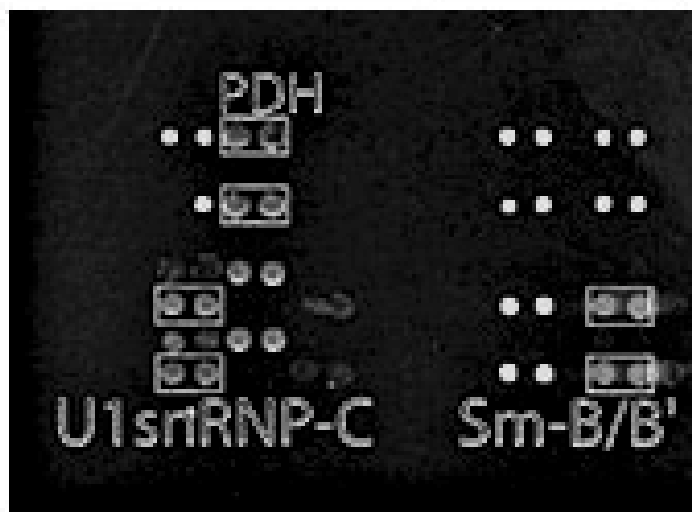
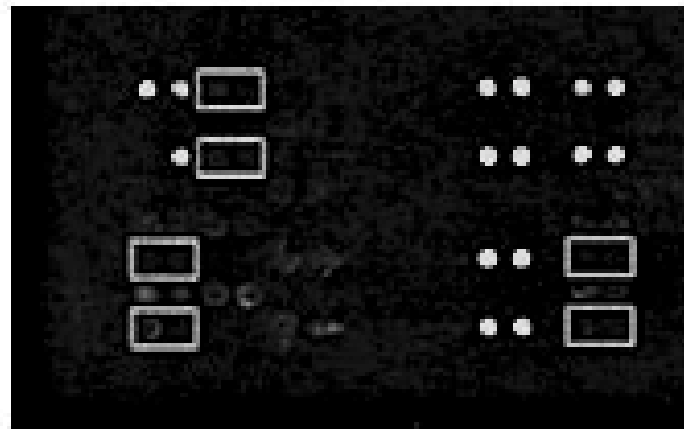
Gene ↓

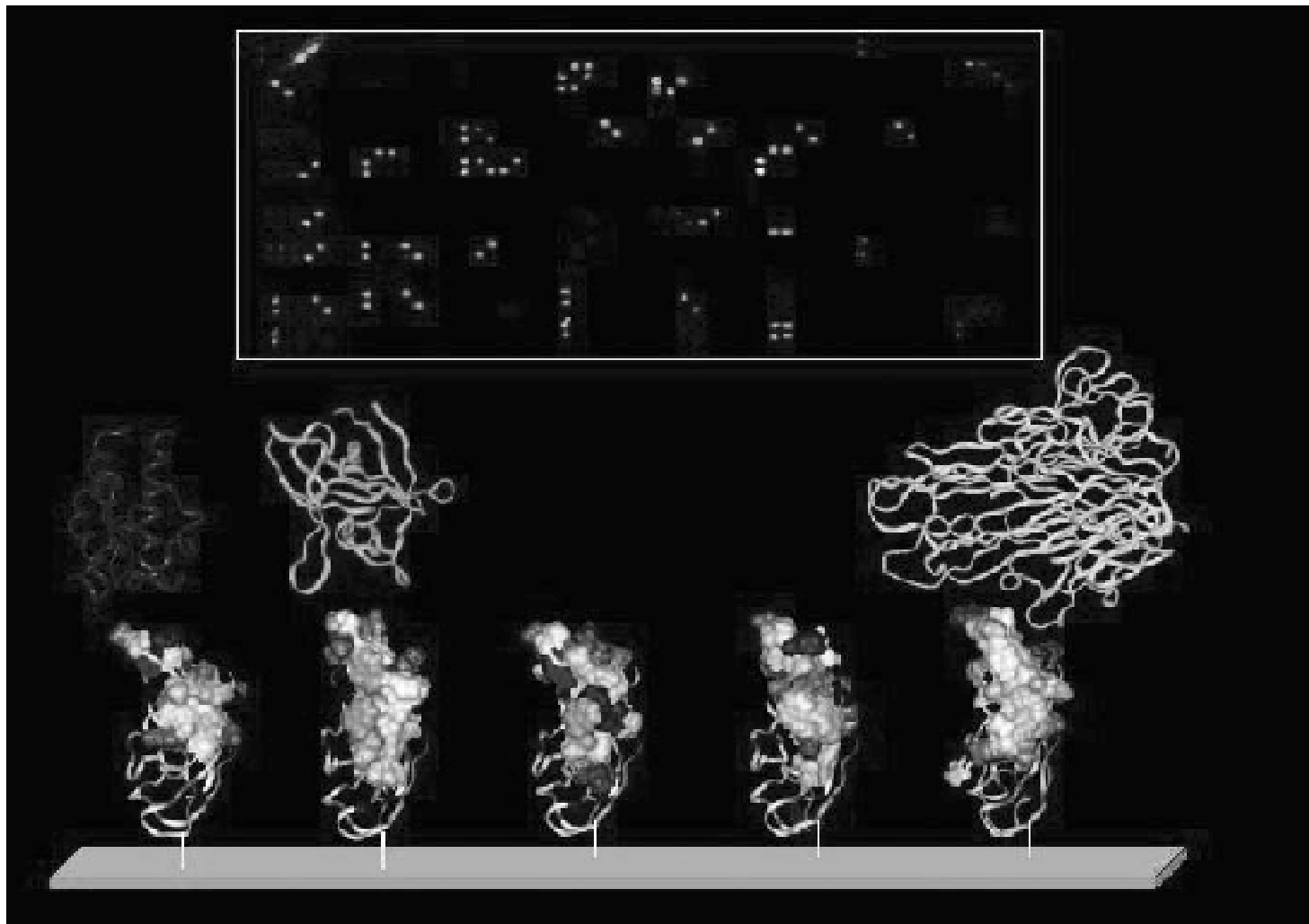


0.2 cm

Protein Array

Control





Protein-chip: capture molecules immobilized on a surface bind to specific target proteins (red, green and yellow), giving a signal that can be read by viewing the chip (top)

Arrays -

Advantages:

- Time
- High throughput
- Small volume, concentration

Disadvantages:

- No "optimized" algorithm for analysis
 - time, reliability, normalisation, statistics
- Huge experimental variability
 - cell cycle/phase
- Accessibility

Biacore

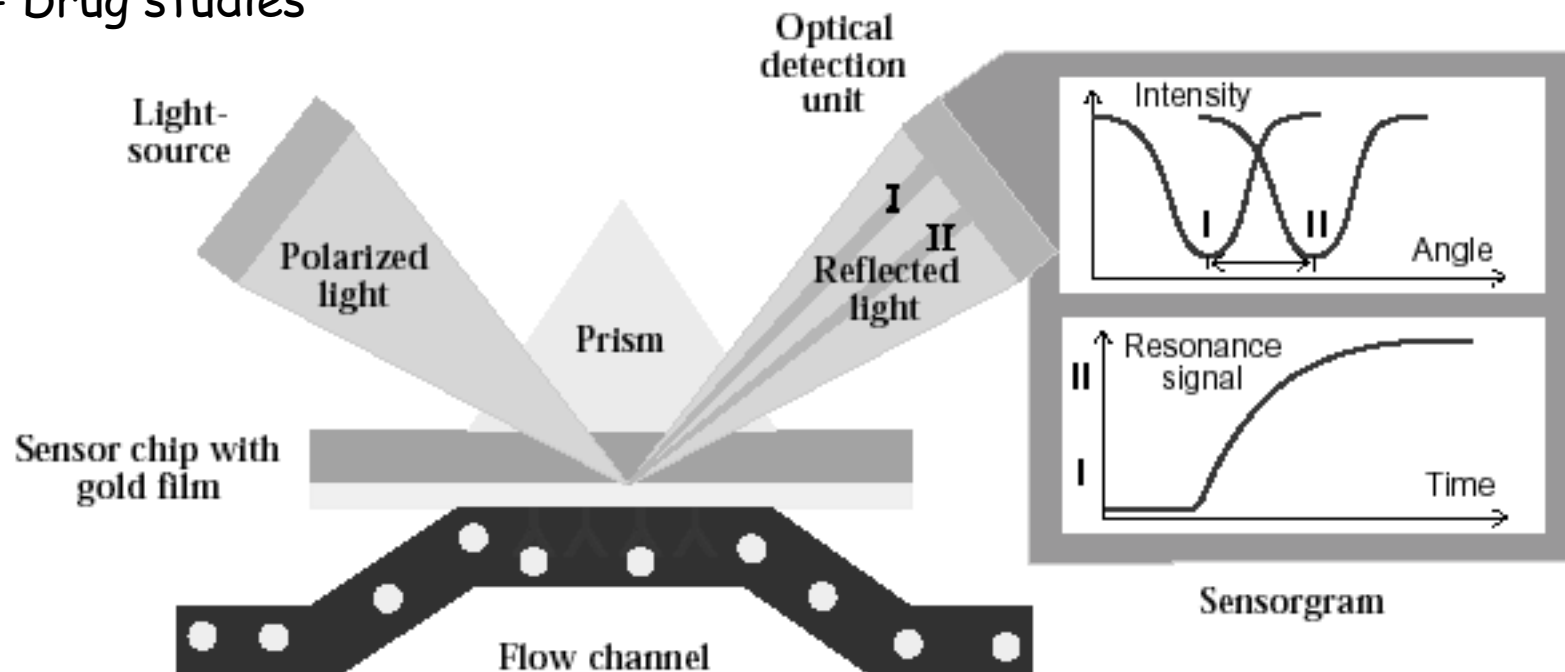
SPR (surface plasmon resonance) detection system

- detects changes in mass in aqueous monolayer close to sensor chip surface by measuring changes in refractive index

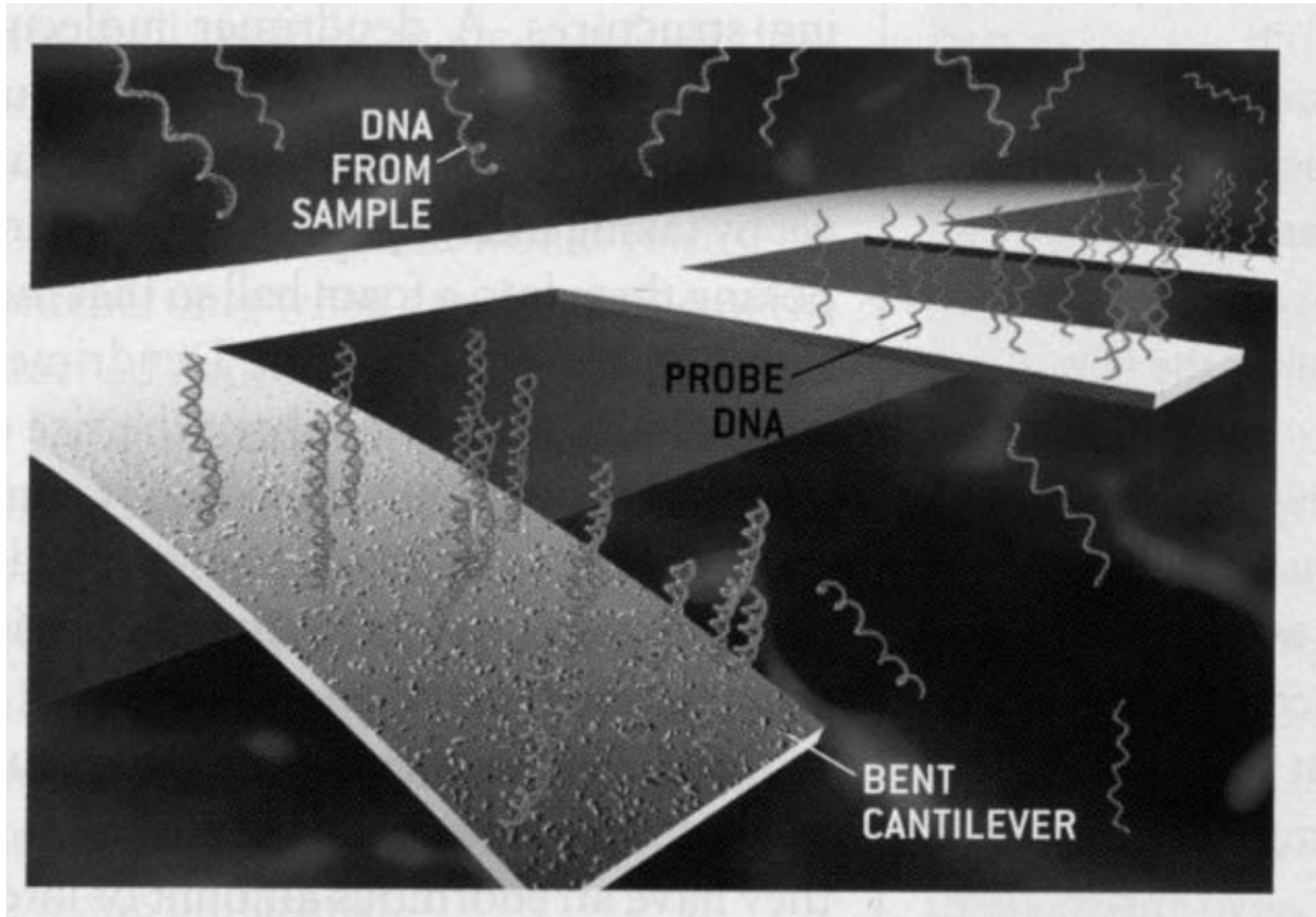
Uses:

- Reaction binding

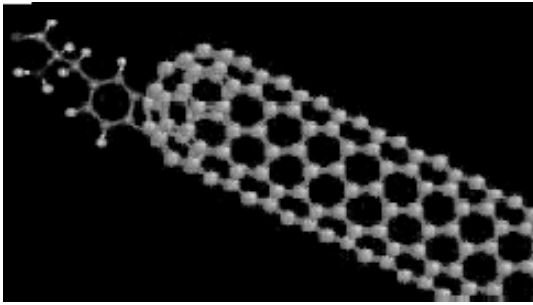
- How much binding, strength (affinity), specificity
- Kinetic studies
- Drug studies



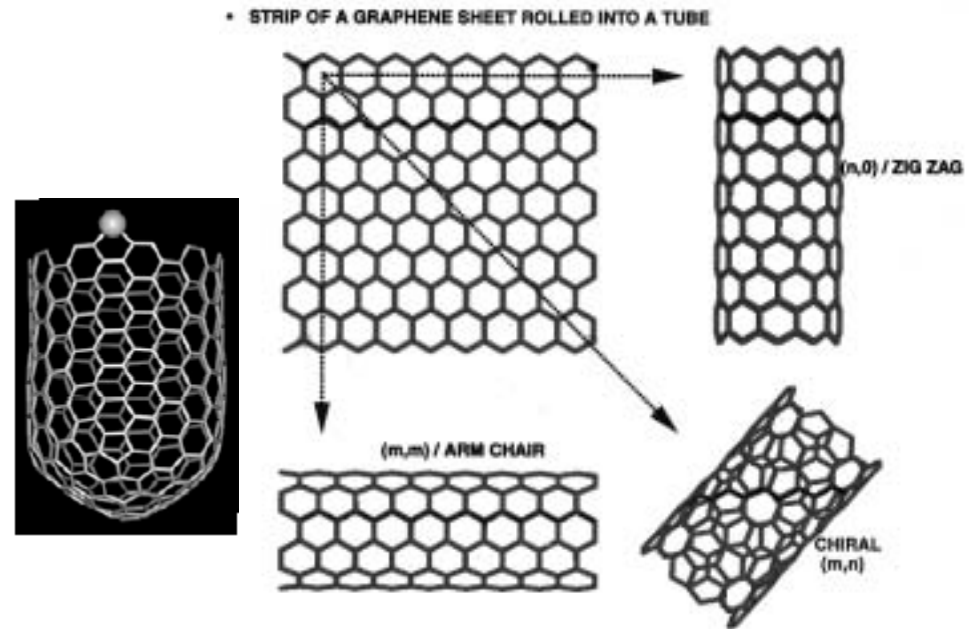
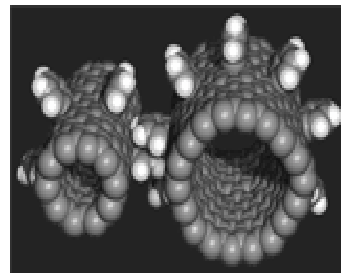
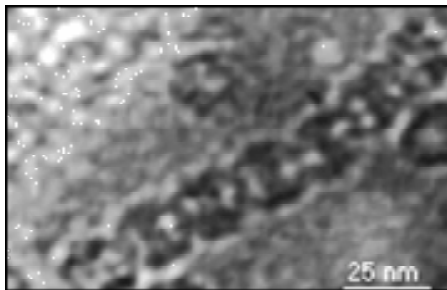
Cantilevers



Carbon nanotube technology (CNT)



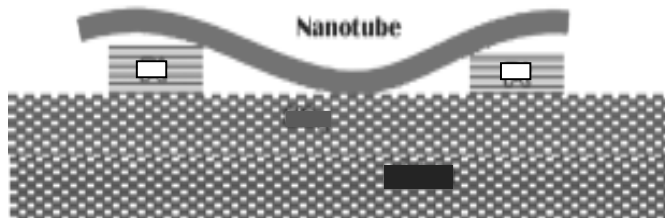
- Configurationally equivalent to a 2D graphene sheet rolled into a tube.
- Tubular form of carbon with diameter as small as 1nm & length of few nm to microns.
- Stiff & tensile strength
- Semiconducting



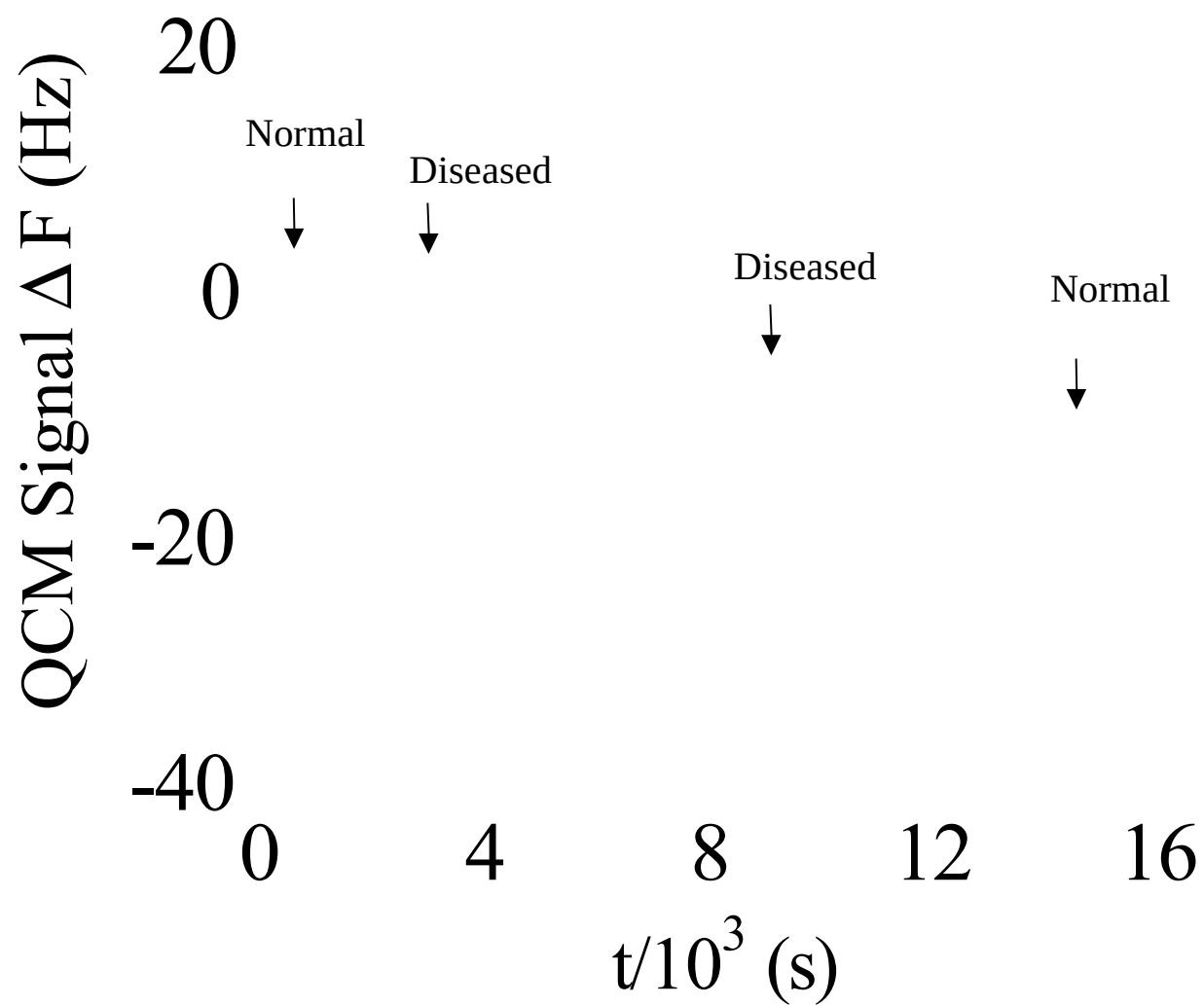
Uses: cables, multifunctional materials, functionalized as polymer backbone, filter membranes, supports, body armor (NASA), quantum wire interconnectors, molecular devices.

Biomedical: *in vivo* real time monitoring, lab-on-a-chip, drug delivery, DNA sequencing, artificial muscles, bone replacement.

Many other molecules forced into "tubes" structures e.g., hsp70.

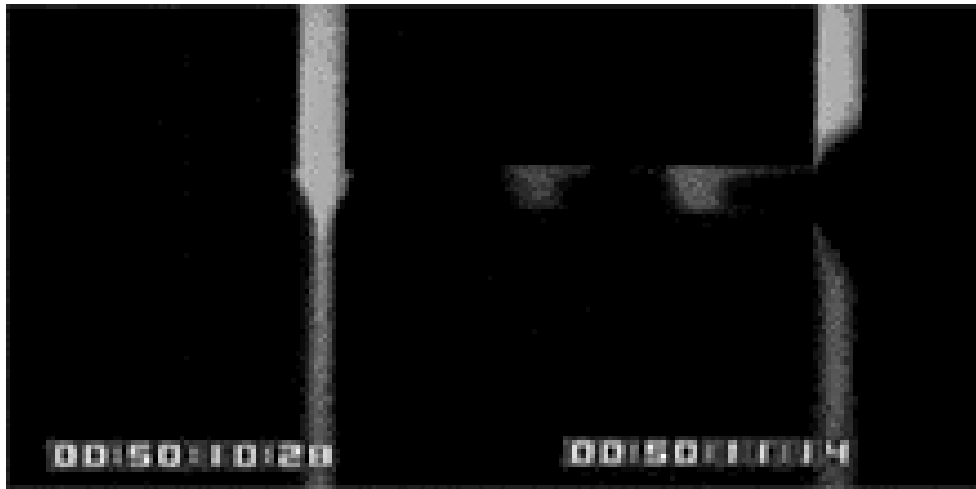
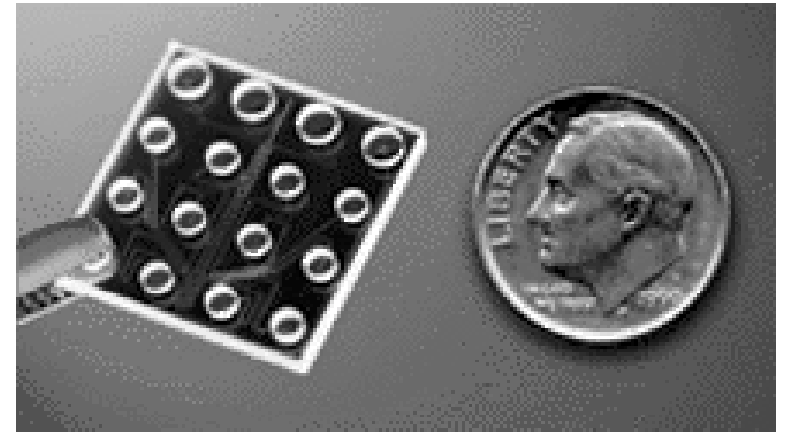


Human Serum



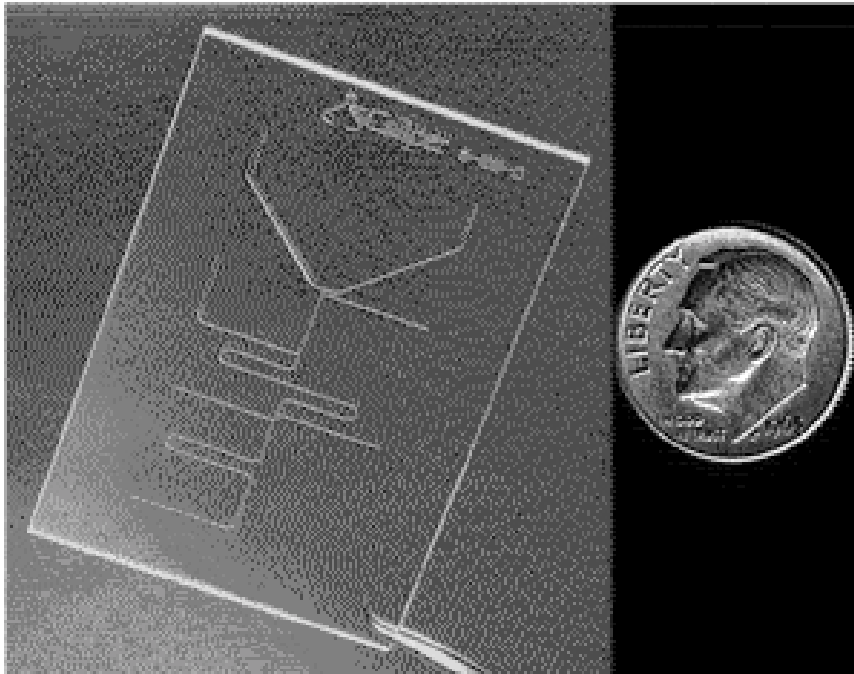
Microfluidics

Interconnected channels etched into glass
Volumes : few picoliters

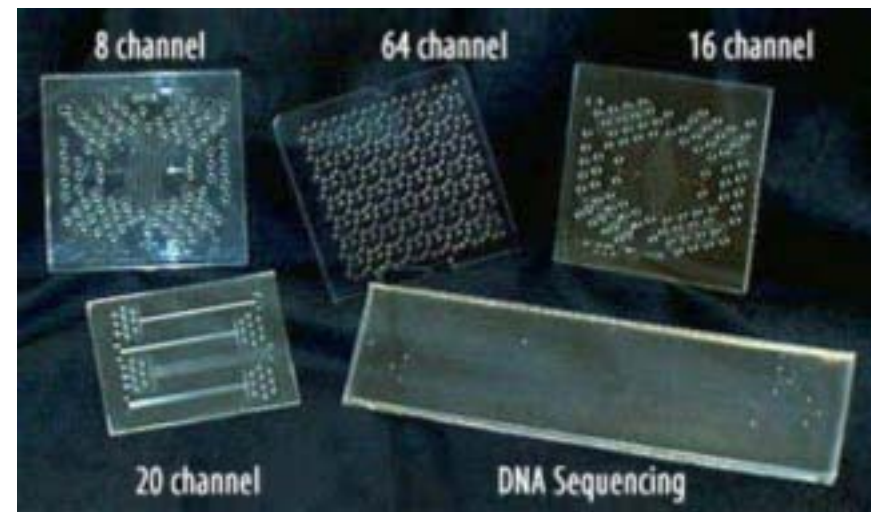


- Fluorescent material electroosmotically pumped from top to bottom.
- Channels 80 microns wide & 10 microns deep at the rate of ~ 1 nanoliter per sec.
- Fluid flow at intersection is electrokinetically controlled from all 4 directions simultaneously.
- Volume at the intersection is 50 picoliters.
- Electric field is changed so volume at intersection is "dispensed" in leftward direction.

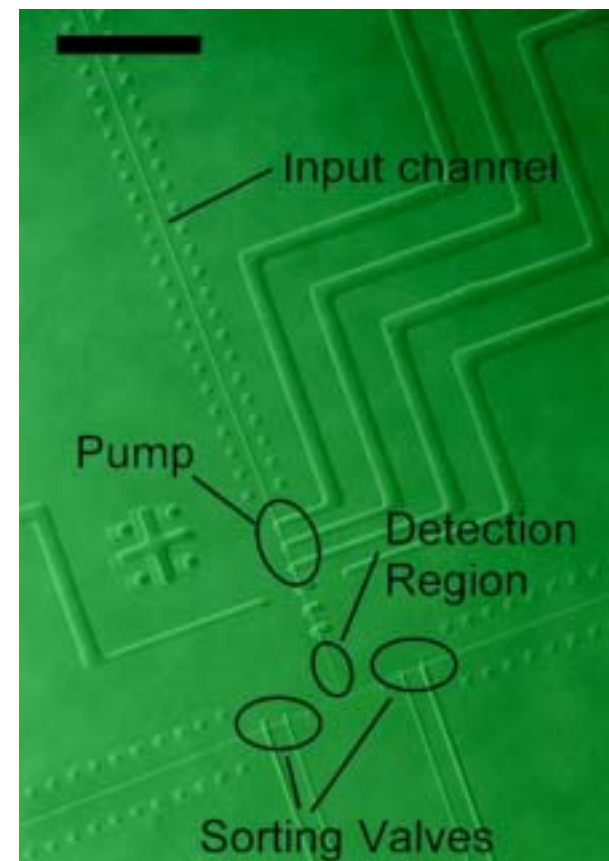
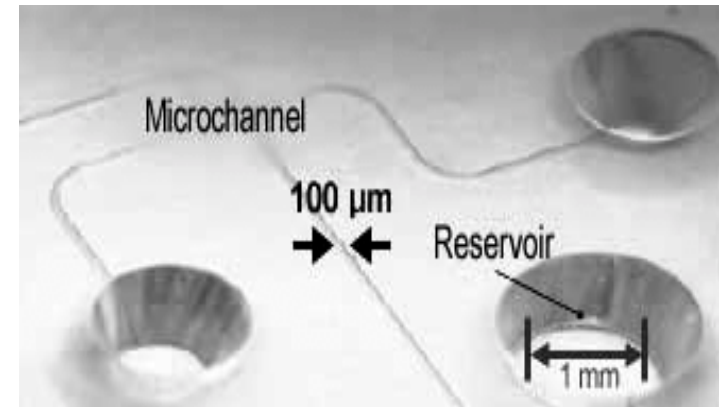
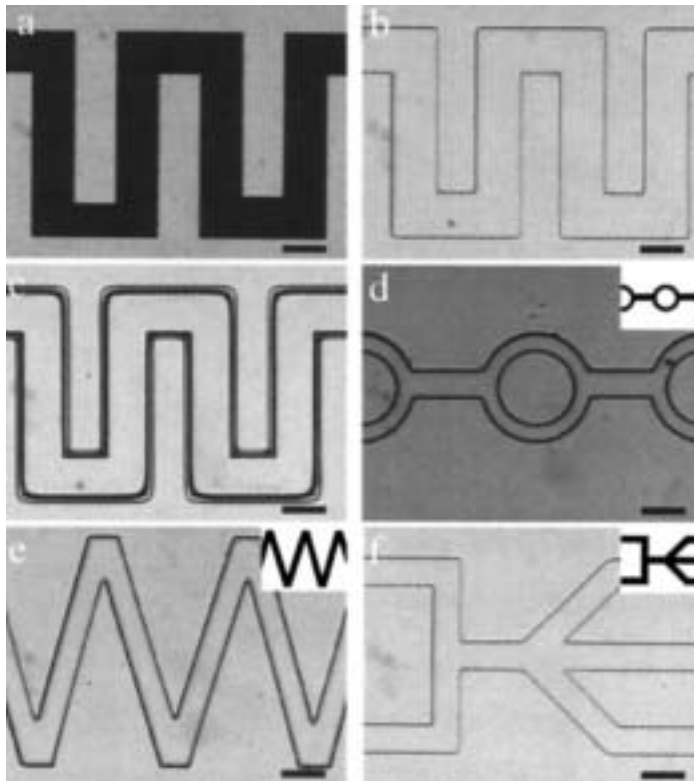
Caliper's Lab-on-a-chip



Aclara's Lab Cards

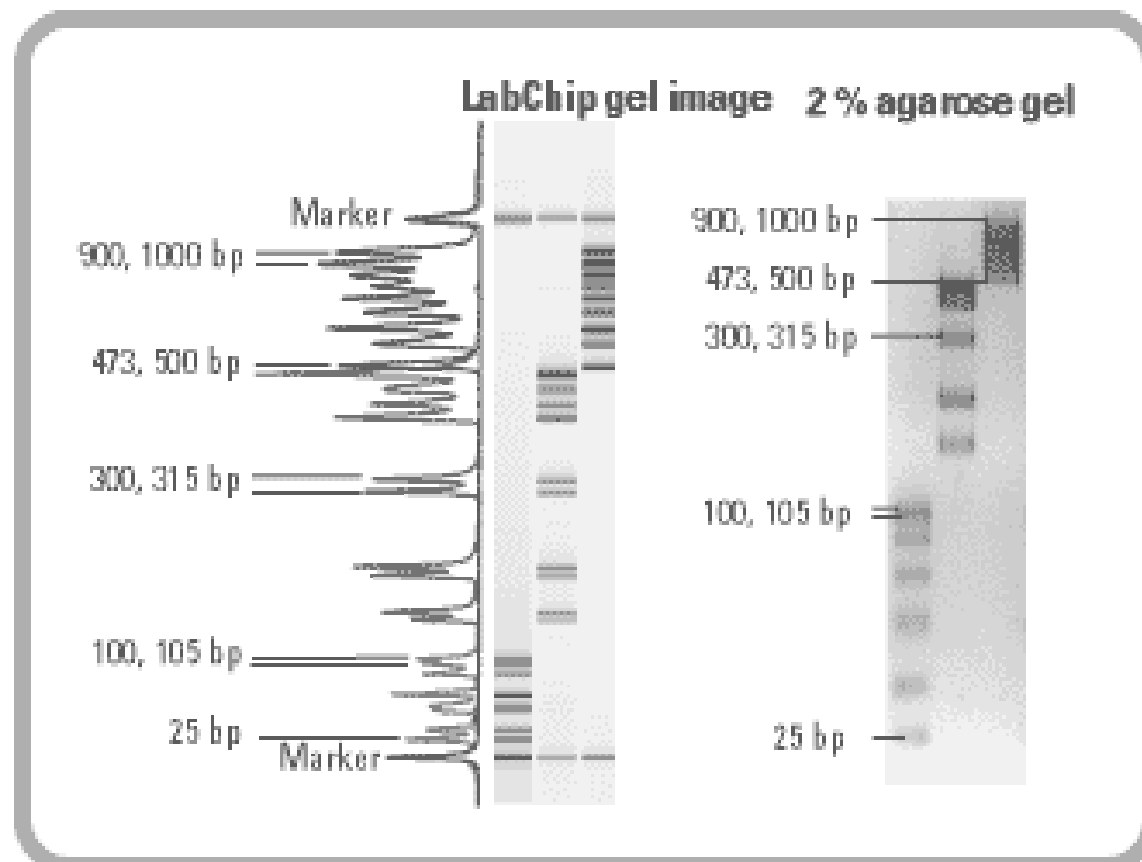


Microchannels



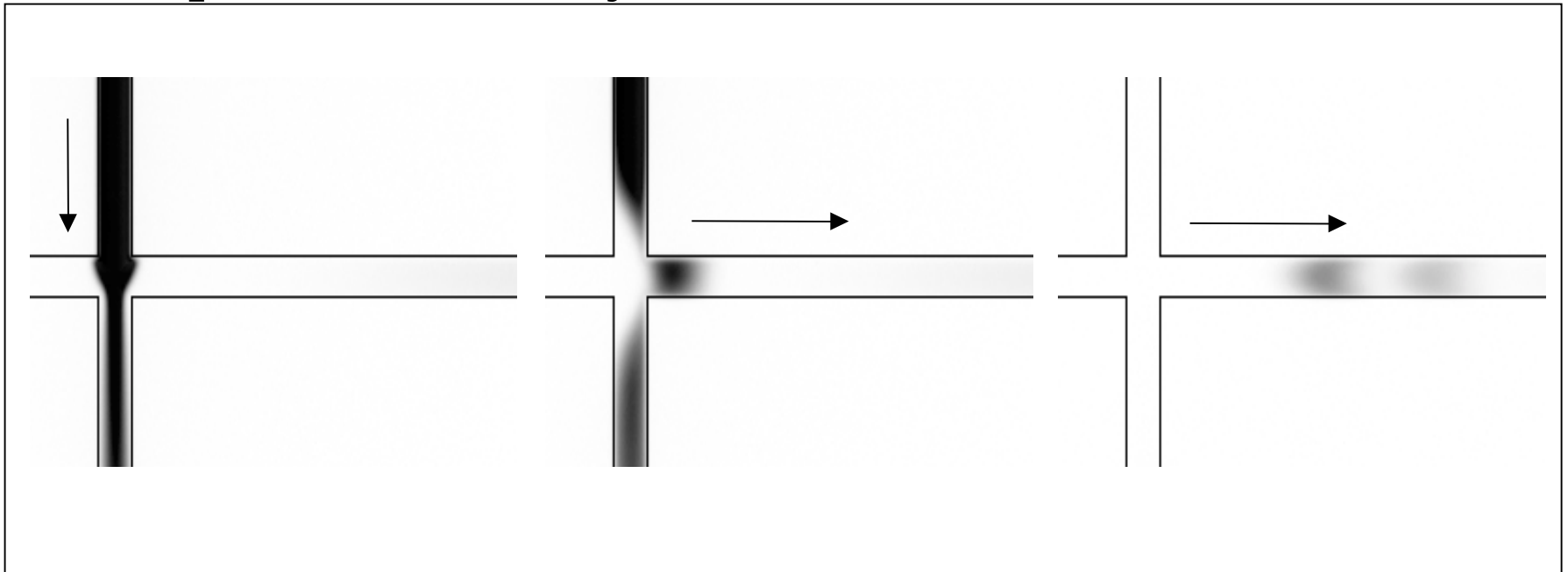
Microfluidics:

- Some applications:
- PCR
 - DNA, RNA, protein analysis
 - Cell studies
 - On-chip reactions



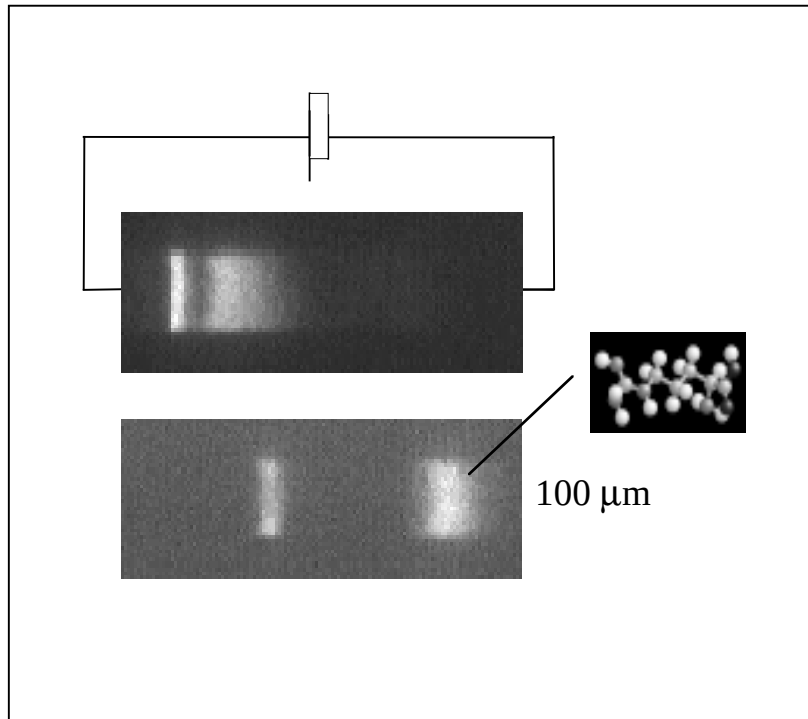
Electrophoresis on a Microchip

Sample Stacked Assays



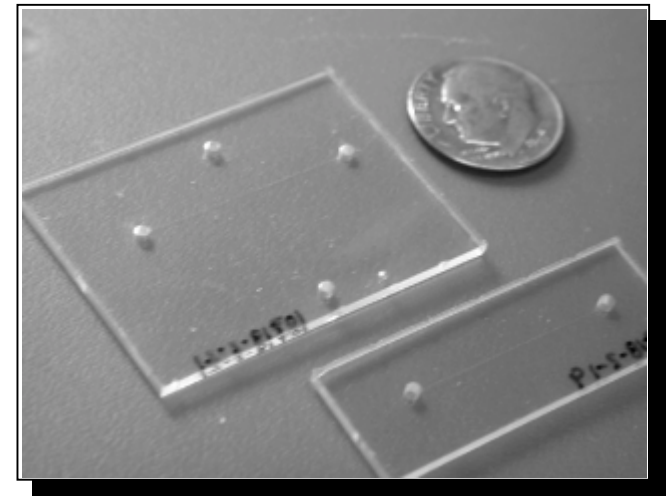
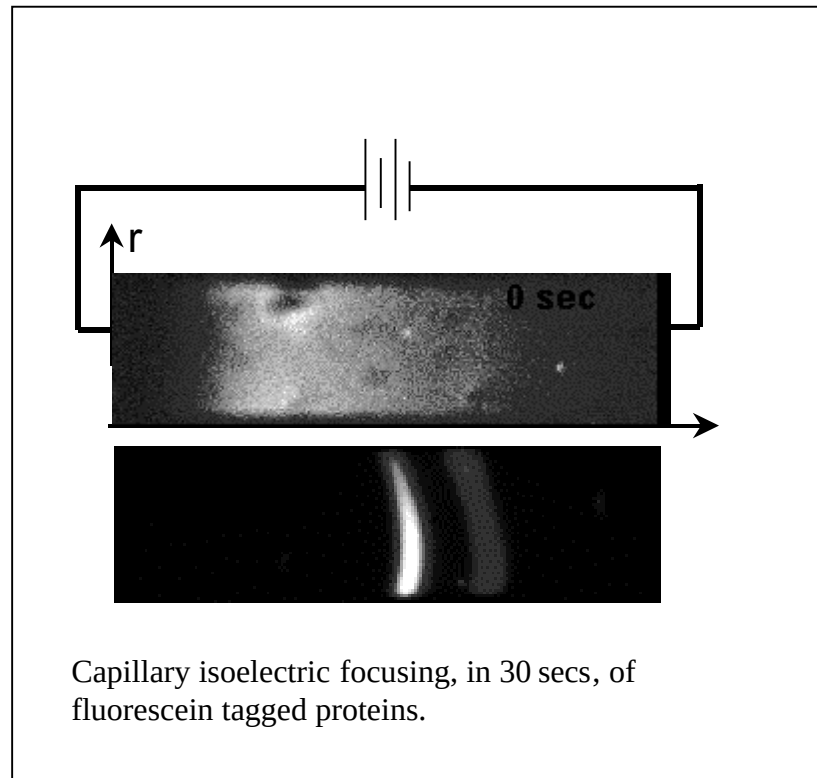
Over-speeding of two separate bands.
Arrows indicate direction of flow.

Electrokinetics



- Protein-Protein interactions
- Enzymatic assays
- Research
- Diagnostics
- Drug screening

Isoelectric Focusing

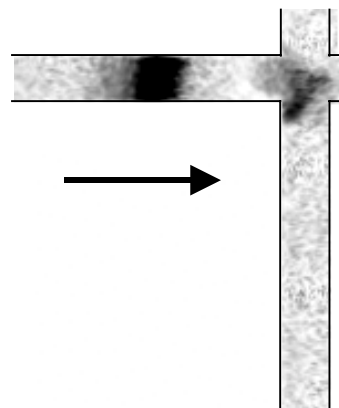
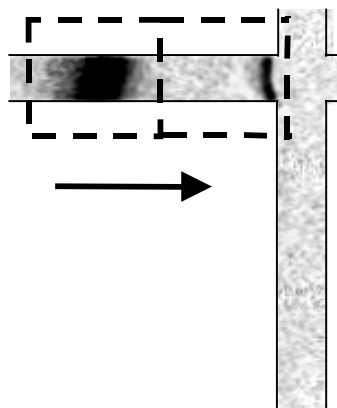


Microchannels are:

- 200 μm wide by 20 μm deep
- 2.5 – 3 cm long

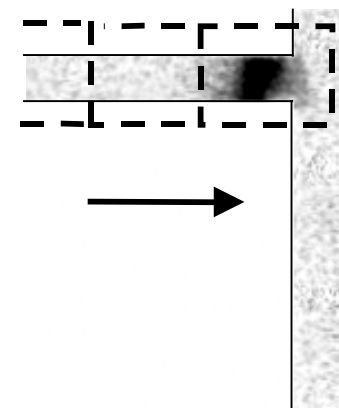
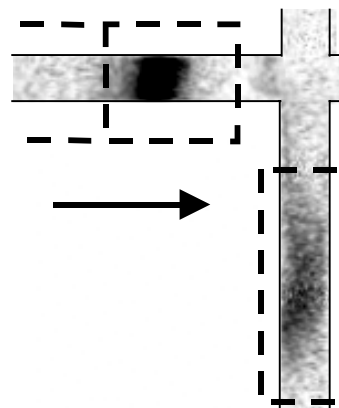
- Improved IEF
- Improved 2D

-> Improved protein separation



Switch

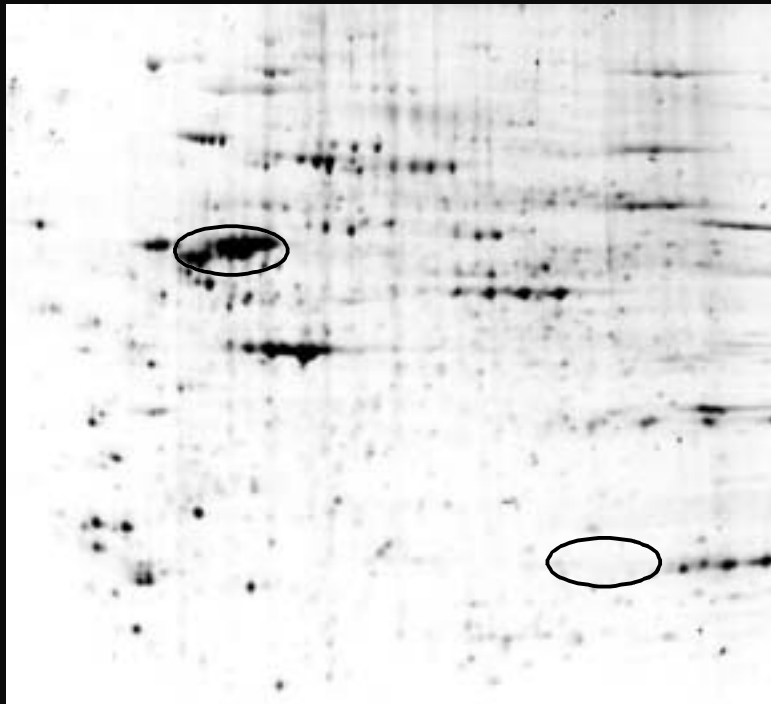
$\Delta t = 3s$



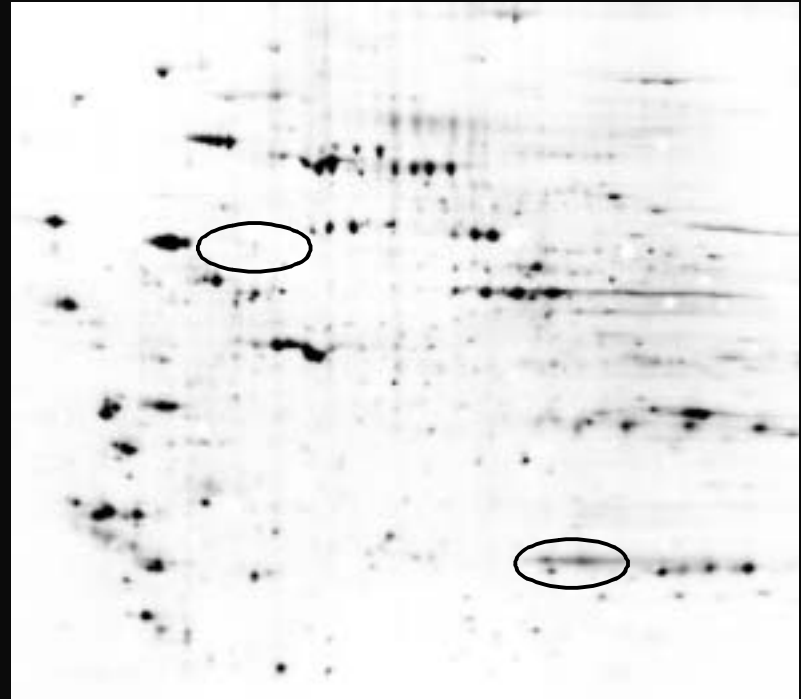
CE

$\Delta t < 20s$

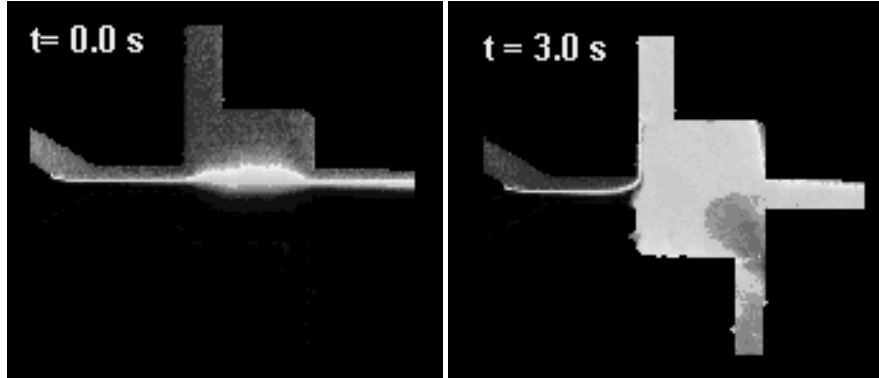
Sypro Ruby



Silver Stain Plus



On-Chip Mixing



Reaction mixing done in 3 secs.
What took 6 mins!

- Protein interactions
- Protein-DNA/RNA interactions
- Enzymatic reactions

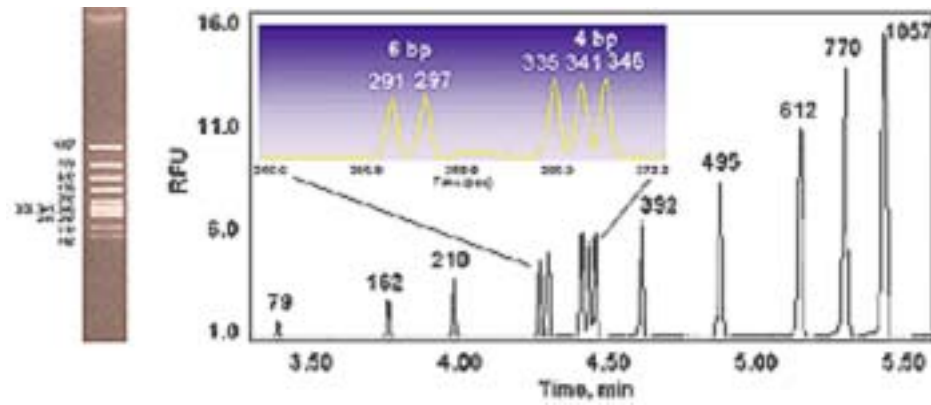
-> Faster

Properties of microfluidics

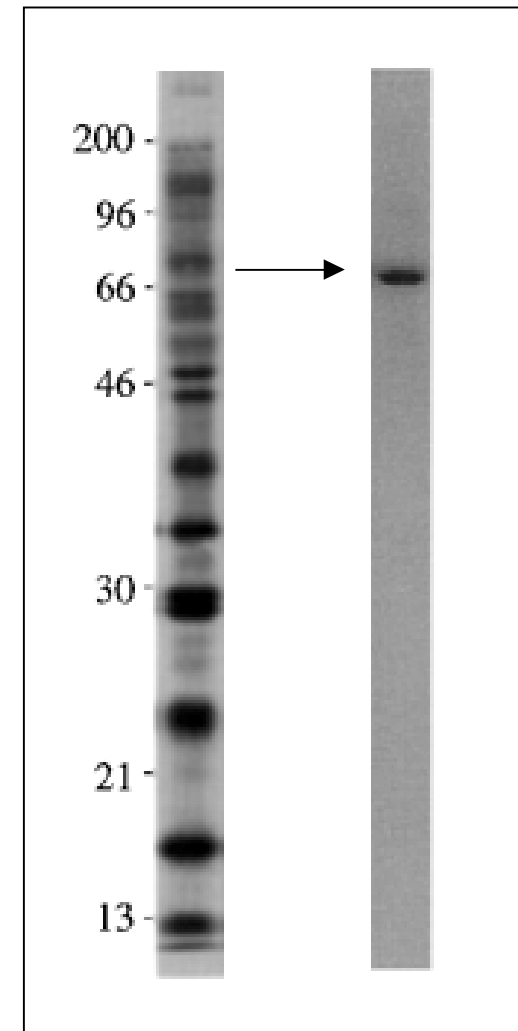
- Versatility: size
- Reproducibility
- Time efficient
- Sample size
- Physiological

Conventional analysis at the:

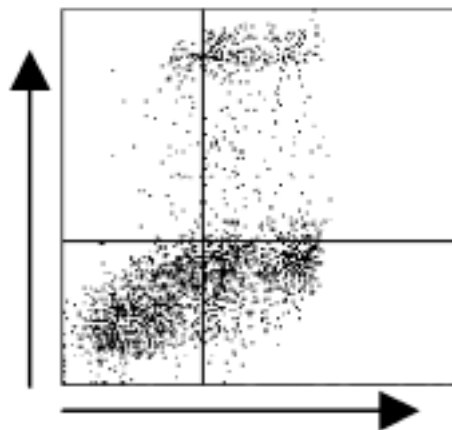
Gene level



Protein level

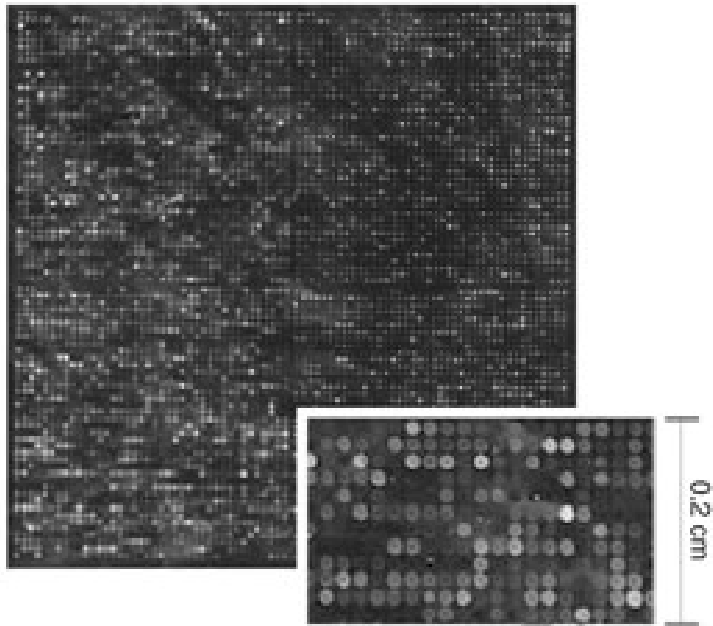


Cell level

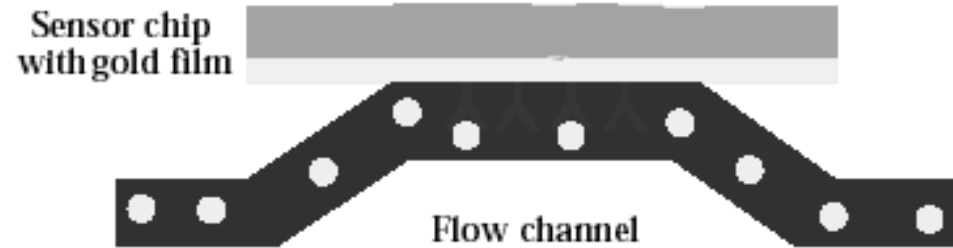


New Technologies:

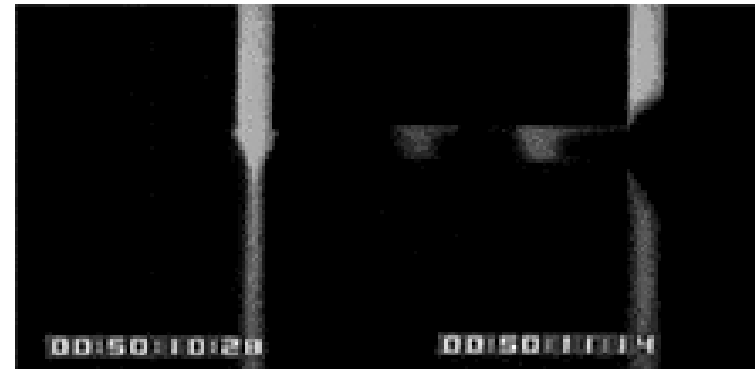
DNA/mRNA/Protein microarray

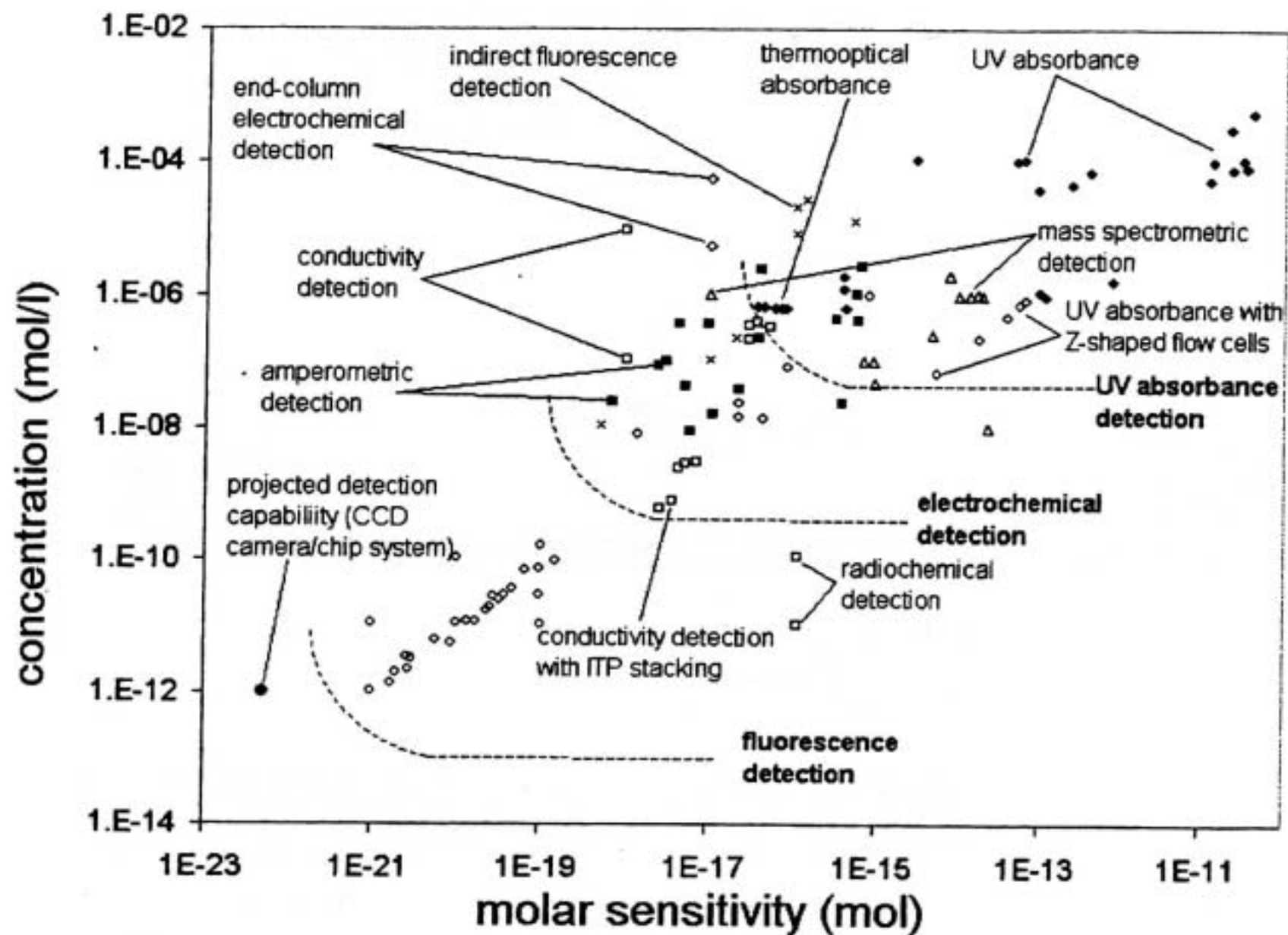


Biacore



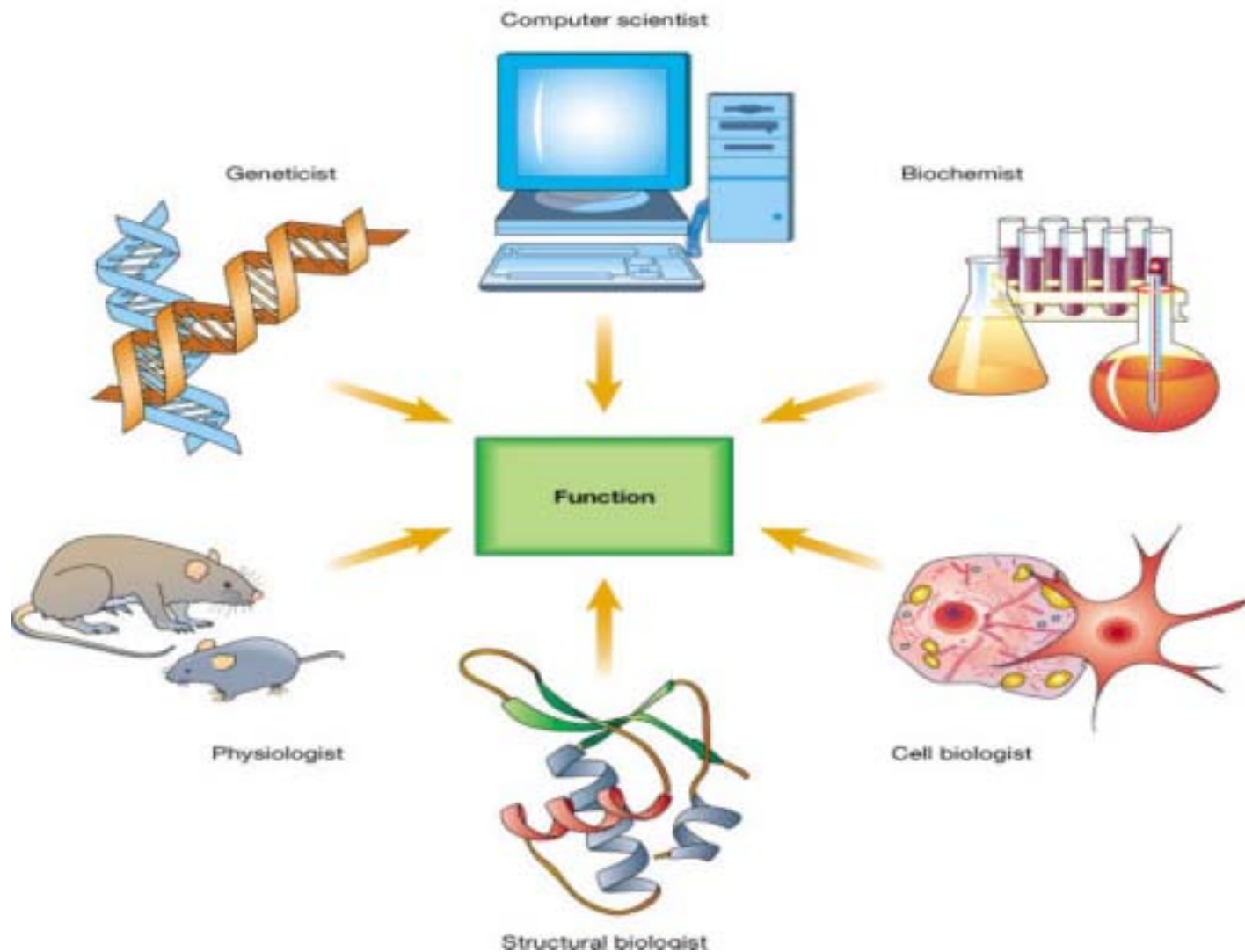
Microfluidics



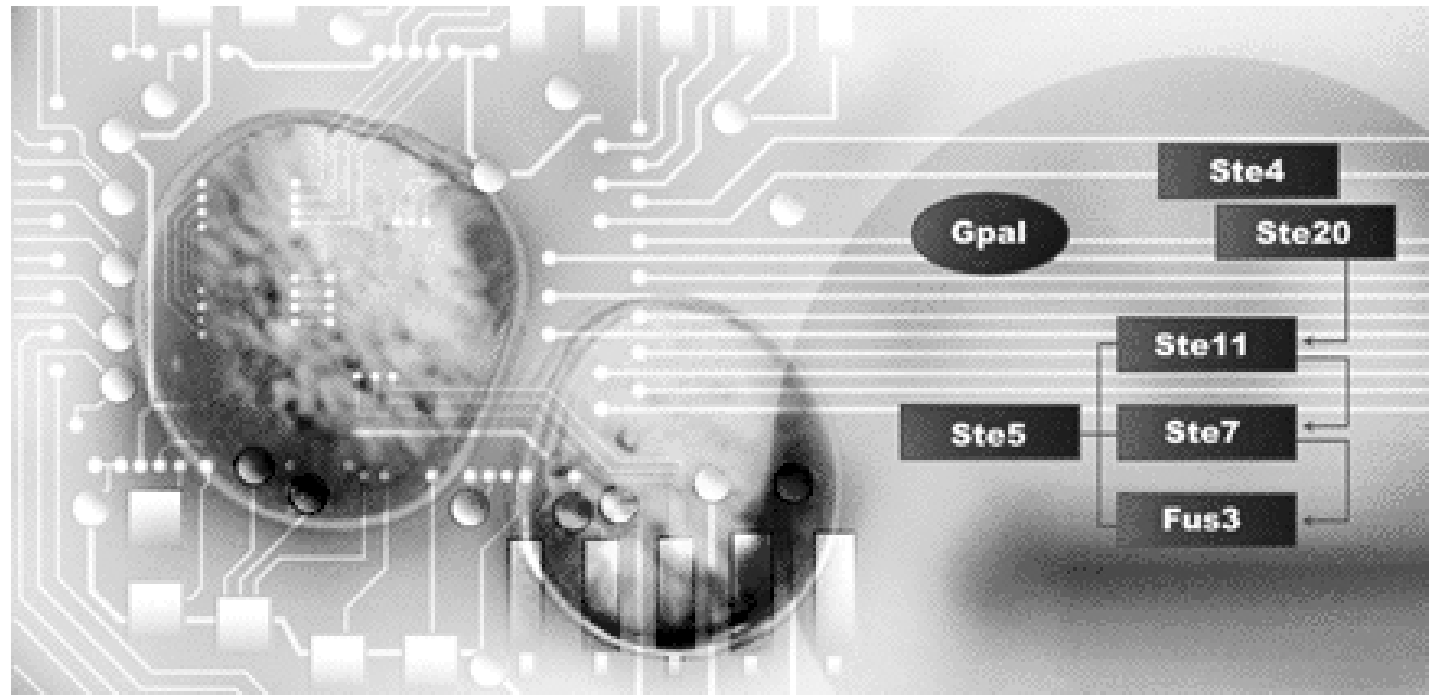


Understanding Function

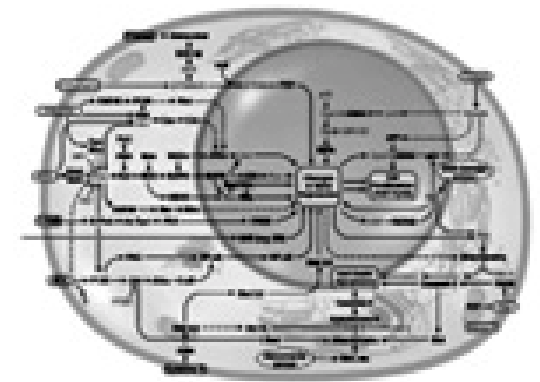
Understanding Function



Tools for studying digital signaling networks



.... could be applied to study cellular signaling networks (e.g yeast alpha-factor signal transduction pathway).



Techniques/Technology

Common biological laboratory techniques

- Reliable
- Time inefficient
- "Simple"

Integration of engineering and biological science -> New Biotechnology

Advantages and Desirable Properties:

- Versatility: size
 - Reproducibility
 - Time efficient
 - Sample size
 - Physiological
 - High throughput screening
- > Development of new drugs, organisms, even amino acids

The integration of computers and biological science

The management of information --> Bioinformatics - *in silico*

Basic approach of study: is still followedbut more efficient

.....Techniques
Proteomic/Genomic cont.

Bioinformatics - databases and analysis software

Informatics

The ability to utilize available information to make an informed "statement"

→ The integration of Computers with Biology

Bioinformatics = Biology + Information

Uses computers to solve molecular biology problems

Analysis of genetic/protein information to predict biological function

in silico Biology

Bioinformatics attempts to Model the pathway:

gene ----> protein ----> cellular interactions

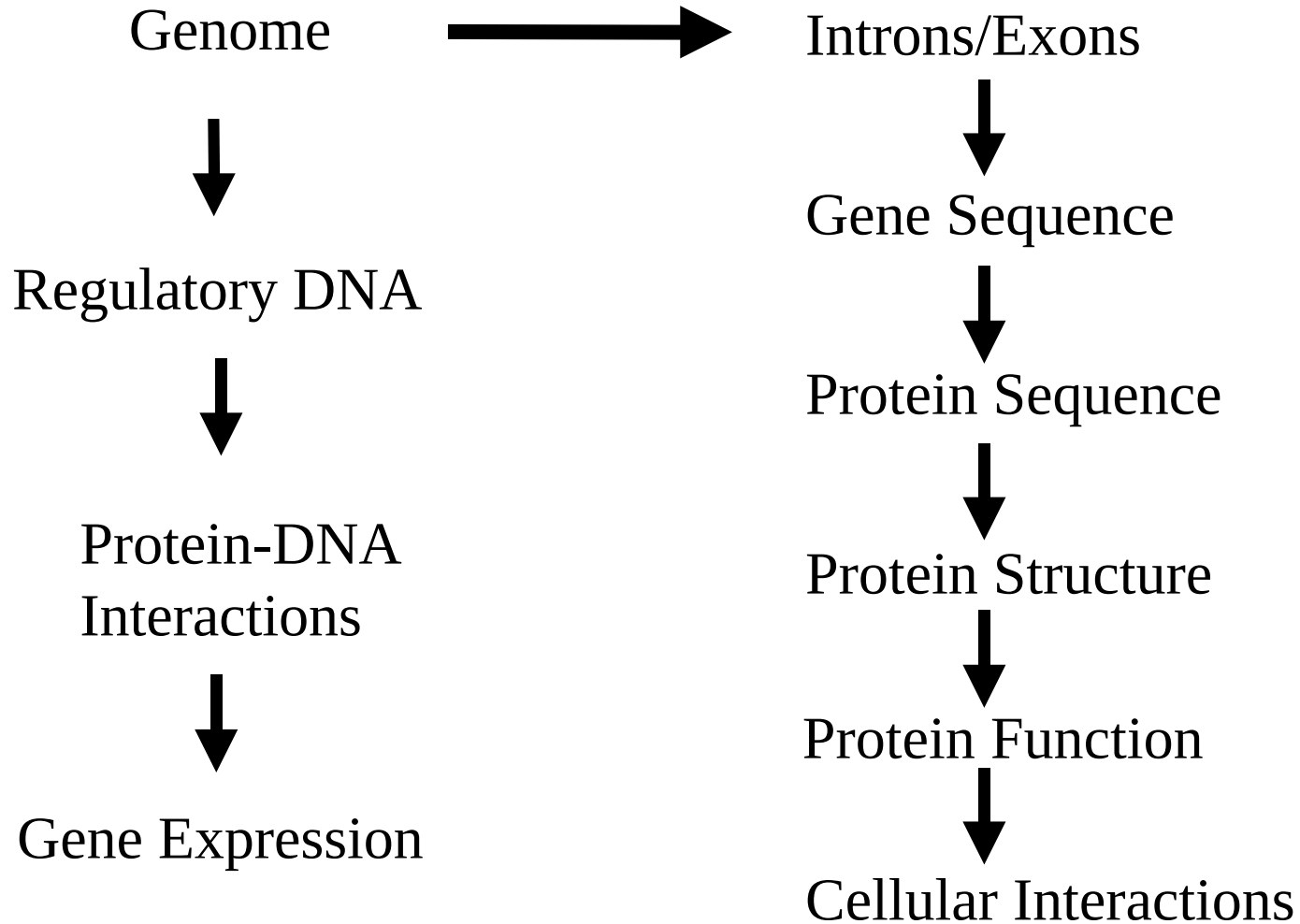
DNA —————> RNA —————> Protein

Sequence —————> Structure —————> Function

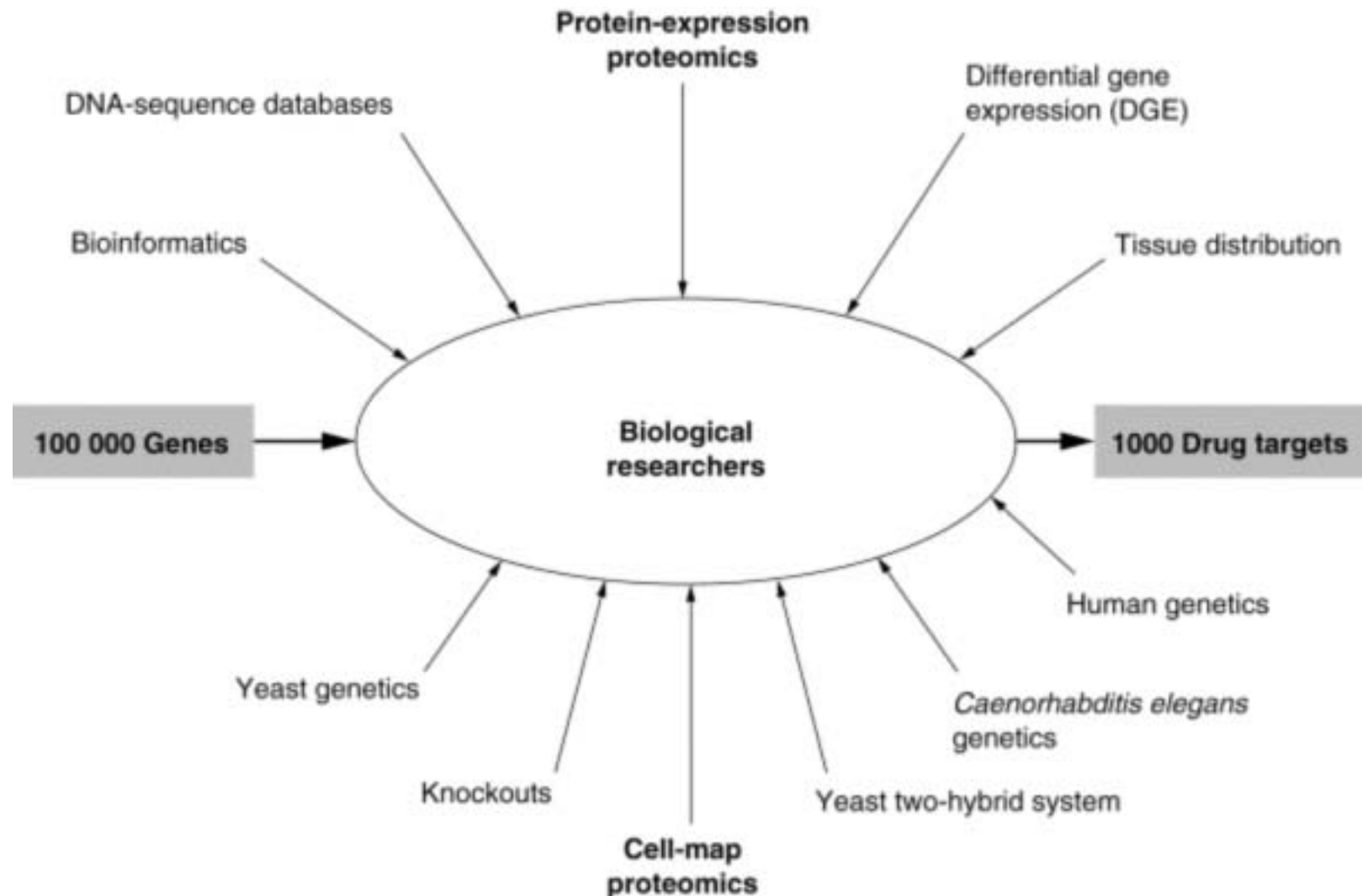
High sequence similarity (DNA, RNA, or amino acid sequences)
usually implies significant functional or structural similarity

The Opposite Is Not True

Biological Information Flow



Target Validation & Functional Analysis



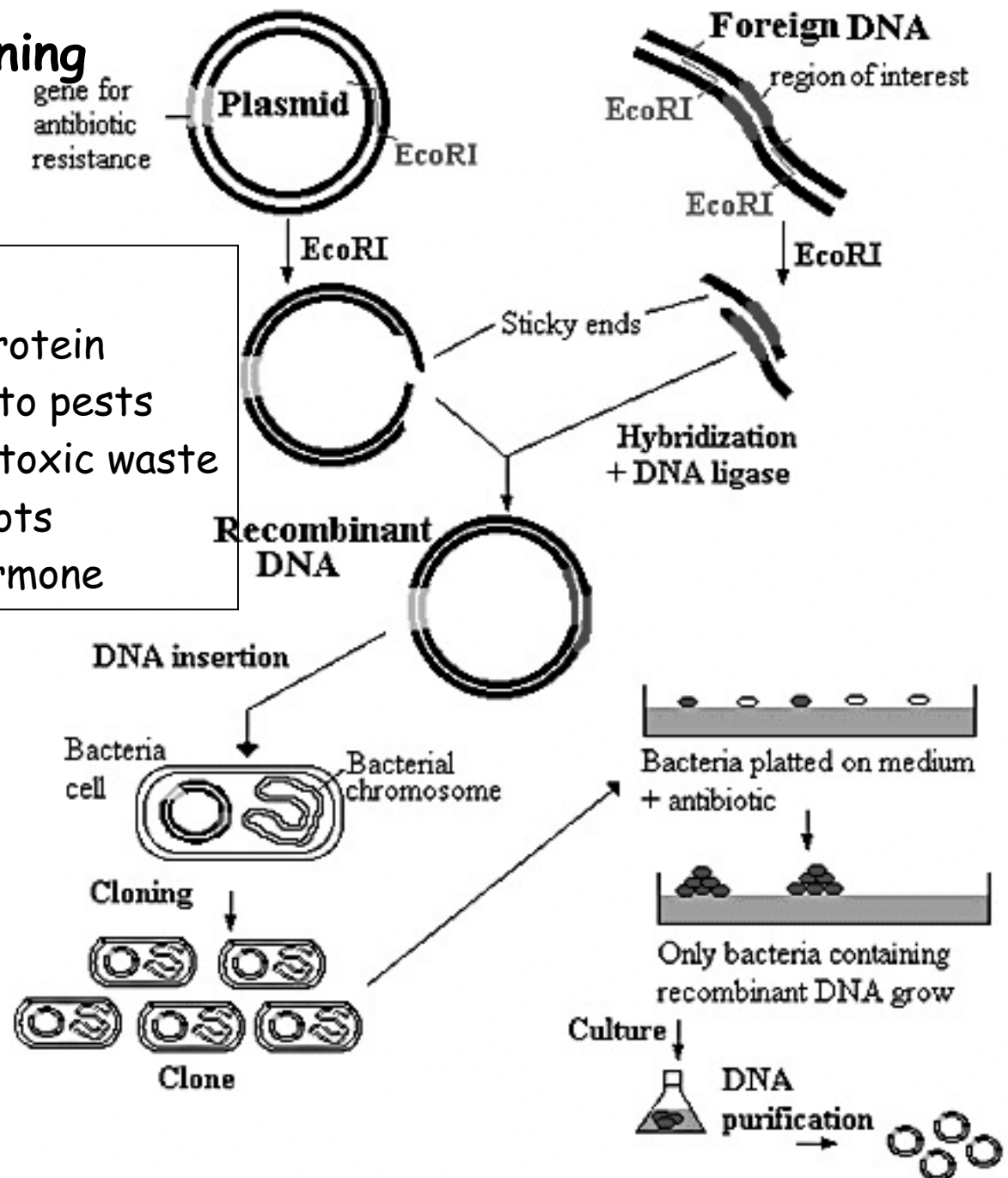
Changing Biological Molecules

Genetic Engineering: Cloning

Cloning into a plasmid:

Uses:

- Making lots of a particular protein
- Agriculture - gene resistant to pests
- Industry - bacteria clean up toxic waste
- Medicine - dissolves blood clots
- human growth hormone

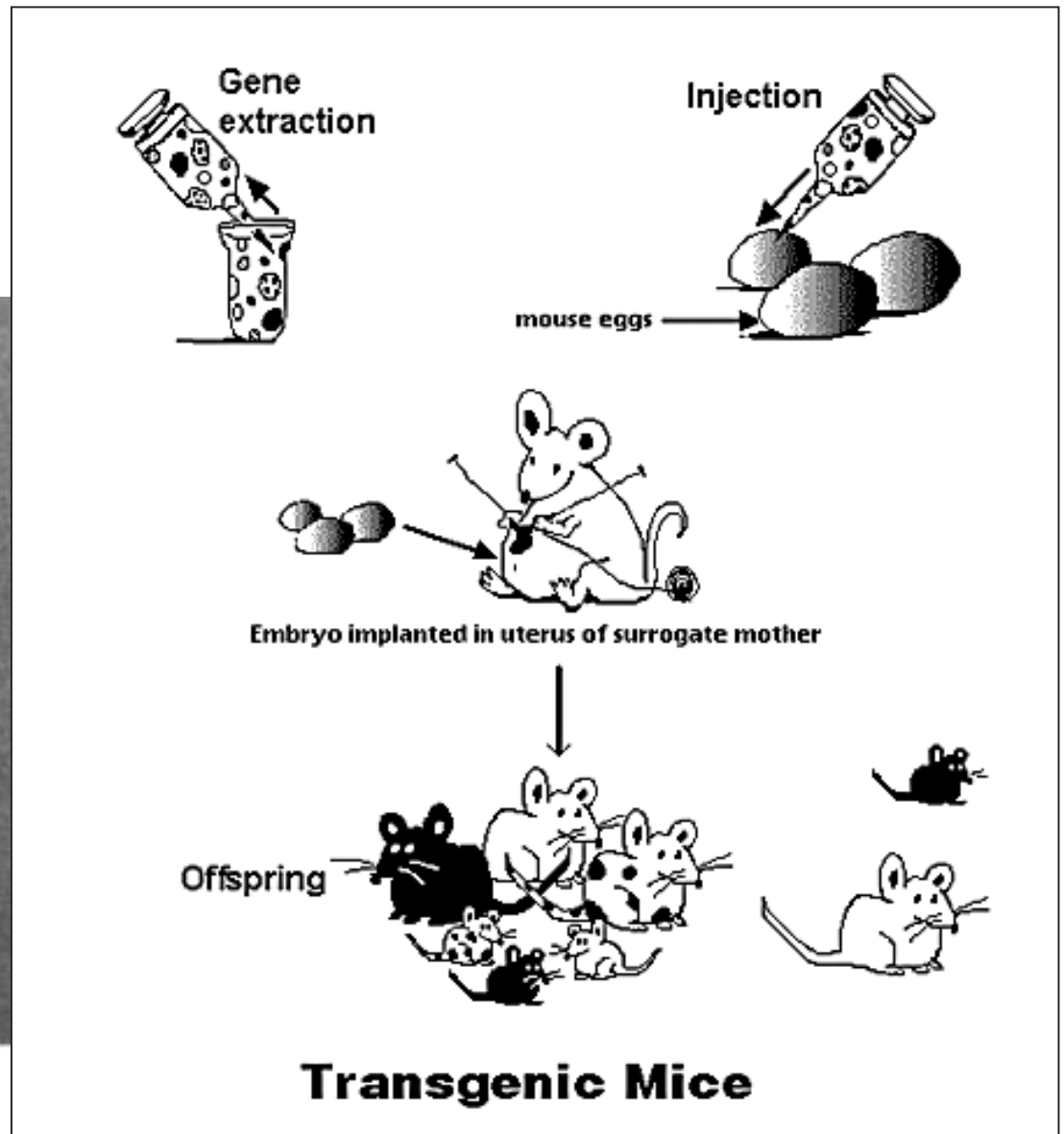


- Gene Knockout
- Gene Addition
- Gene Replacement

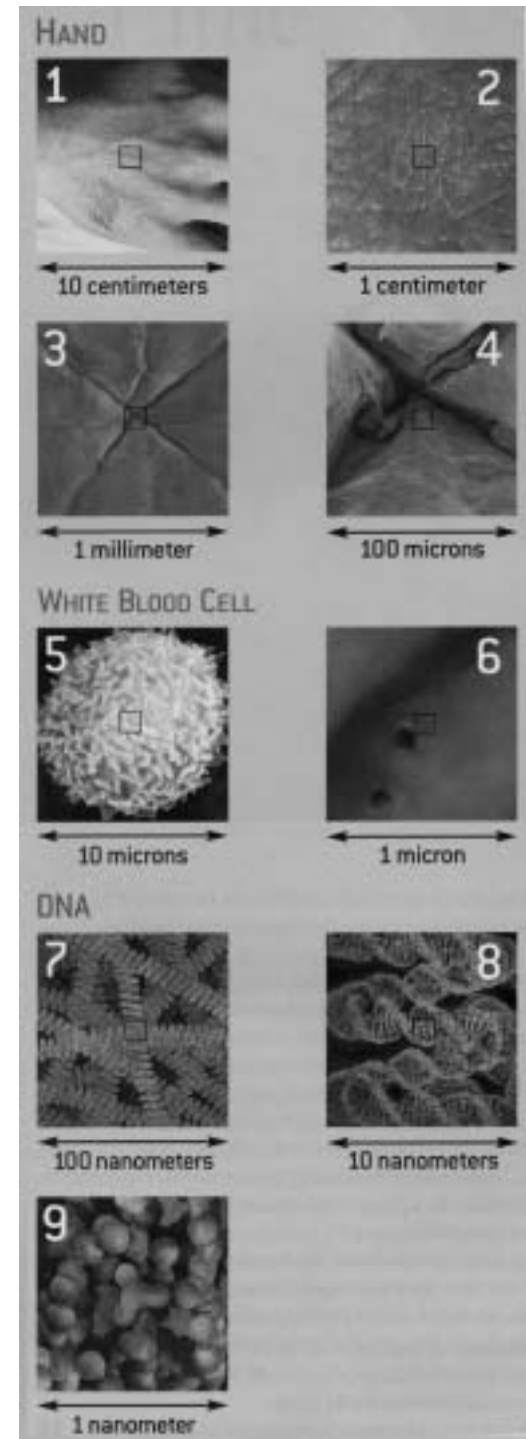


growth hormone

Precursor to Gene Therapy



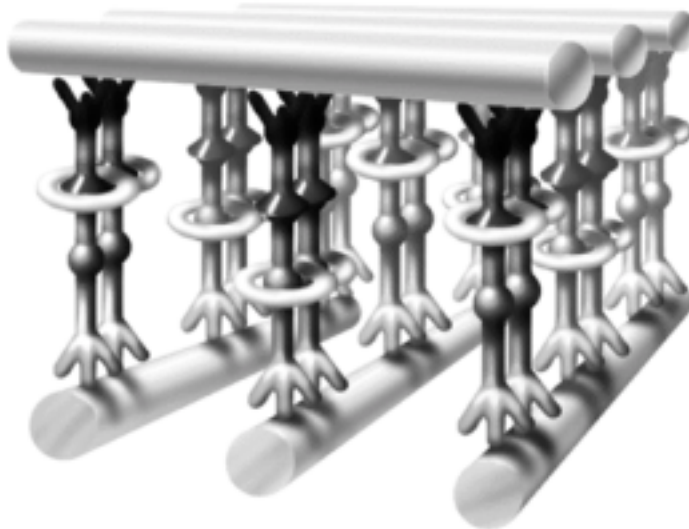
**We can now modify biological organisms ...
from Macro to Micro to Nano!**



Ultimate Goal

- Self-Assembling Nanotechnology Devices
- Computer Modeling of Cell/Organism

Assembling molecules into working circuits



Constructing real molecular chips will be a big challenge.

Are there currently any self
assembling nanotechnology
"devices"?

Web resources.....MANY!

Companies and Organizations by industry:

http://www.biospace.com/company_index.cfm

Good general source of info.....www.biospace.com

Neat Genomics research and Bioinformatics site:

<http://ublib.buffalo.edu/libraries/units/sel/bioinfo/humangenome.html>

Some neat Proteomics sites:

http://www.biochem.abdn.ac.uk/jef/Protein_Lab.html

<http://www.abdn.ac.uk/mmb023/2dhome.htm>

<http://www.mann.embl-heidelberg.de/Default.html>

<http://www.expasy.ch/>

<http://www.harefield.nthames.nhs.uk/nhli/protein/>

<http://biobase.dk/cgi-bin/celis>

<http://iupucbio1.iupui.edu/frankw/molan.htm>

<http://www.mpiib-berlin.mpg.de/2D-PAGE/>

<http://prospector.ucsf.edu/>

<http://www.proteome.com/databases/index/html>

Biotech companies:

General Purpose: Genentech, Chiron, Amgen, Biogen, Berlex

Anti Aging

Geron
Centageneticx

Cancer

EntreMed
Onyx

Cardiovascular

CV Therapeutics
Scios
Collateral Therapeutics
Cardiac Science

Diabetes

Metabolex

Immunology

InterMune
Immunex
Biomune

Neurobiology

BioMedica
Rinat Neuroscience
Neurogen

Signal Transduction

Tularik
SUGEN

Genomic Mining

Exelexis
Incyte
Human Genome Sciences
Sequenom

Drug Delivery Methods

Inhale
Alkermes
Targeted Molecules Corp
Cell Genesys
Alza

Drug Screening

Rigel
Lynx Therapeutics
Millenium
Darwin
Xenocor

Bioinformatics:

Databases

Incyte
Hyseq
Celera

Tools

Accelrys
Tripos
InforMax
Silicon Genetics

Structural

Structural Bioinformatics
Astex Technology

A glimpse into the future

Foresight Institute
<http://www.foresight.org>