Cancer “genomics” – Technological opportunities in cancer biology and management

Joe W. Gray, PhD

Lawrence Berkeley National Laboratory

A brief survey of cancer genomics with illustrations of current technologies that are driving the field – how can we contribute?

Topics for discussion

- What goes wrong during cancer development?
- How can we understand the details?
- How can we use the information to improve cancer treatment?

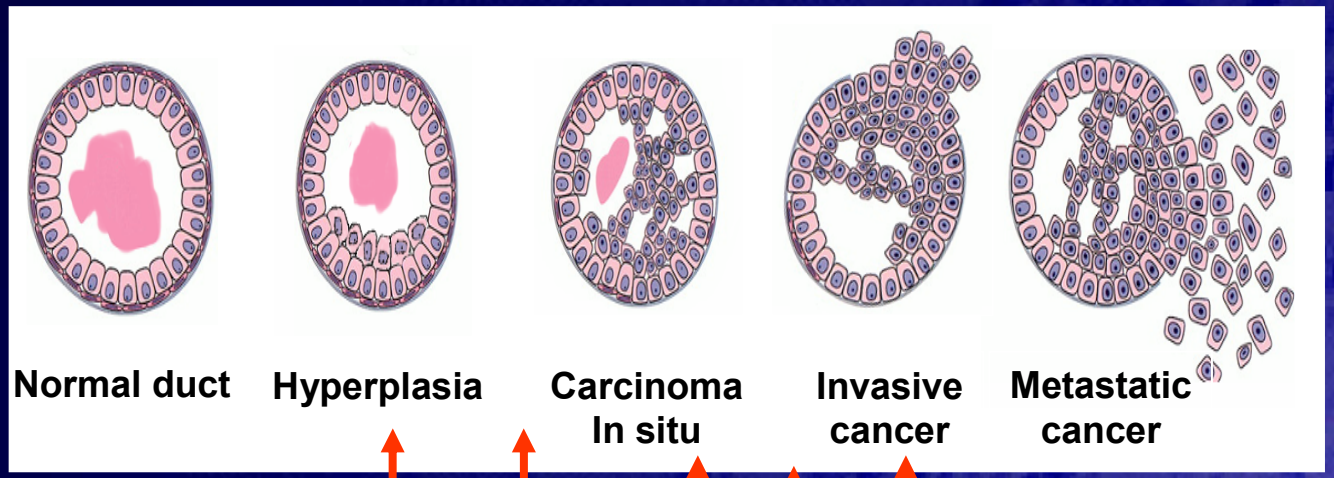
Opportunities for technology

Topics for discussion

- What goes wrong during cancer development?
- How can we understand the cancers?
- How can we use the information to improve cancer treatment?

Opportunities for technology

The “hallmarks of cancer”



Unchecked proliferation

Genome instability

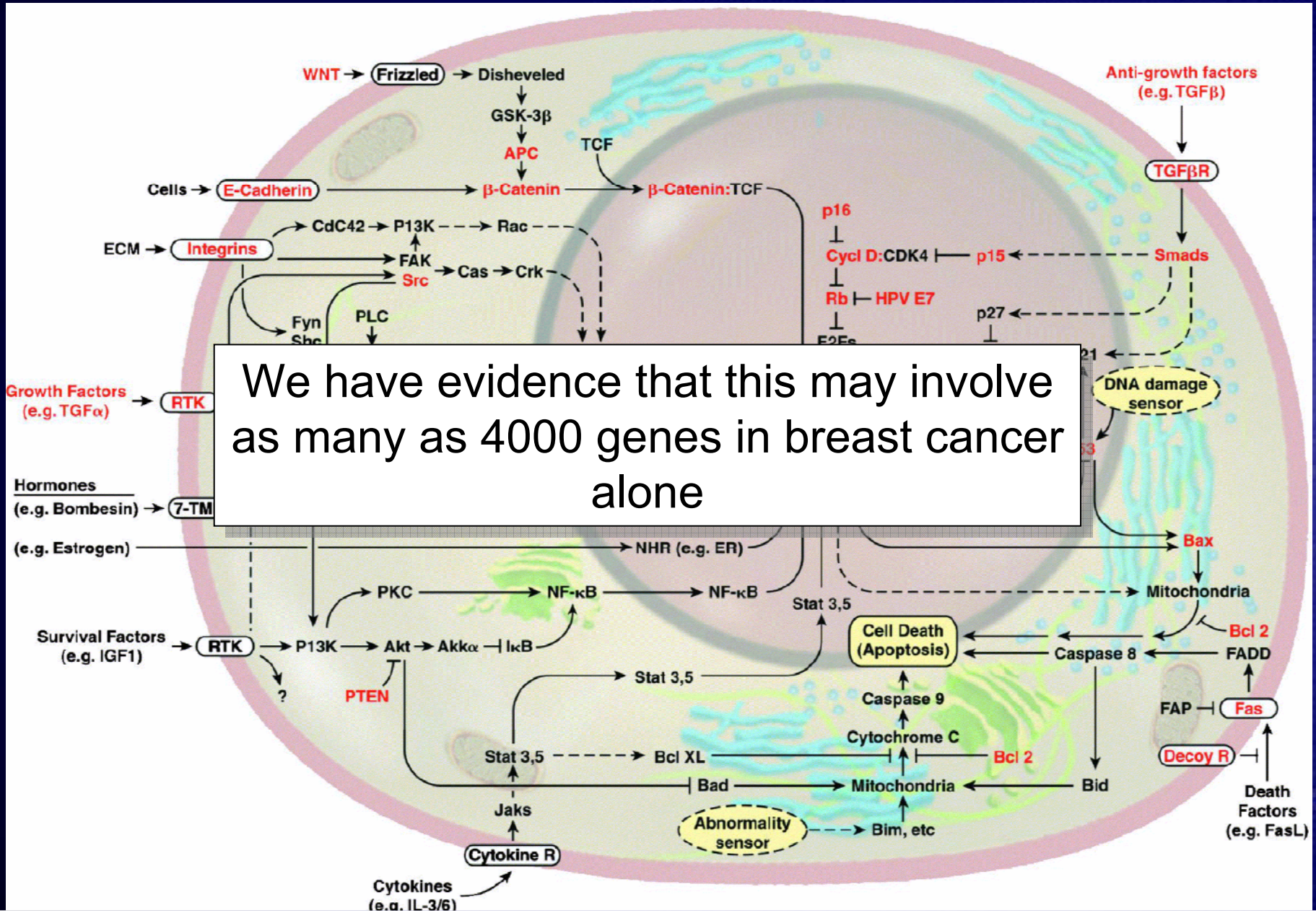
Increased angiogenesis

Increased motility

Ability to bind and proliferate In a foreign environment

Hanahan & Weinberg, Cell, 2000

This happens through deregulation of complex regulatory pathways



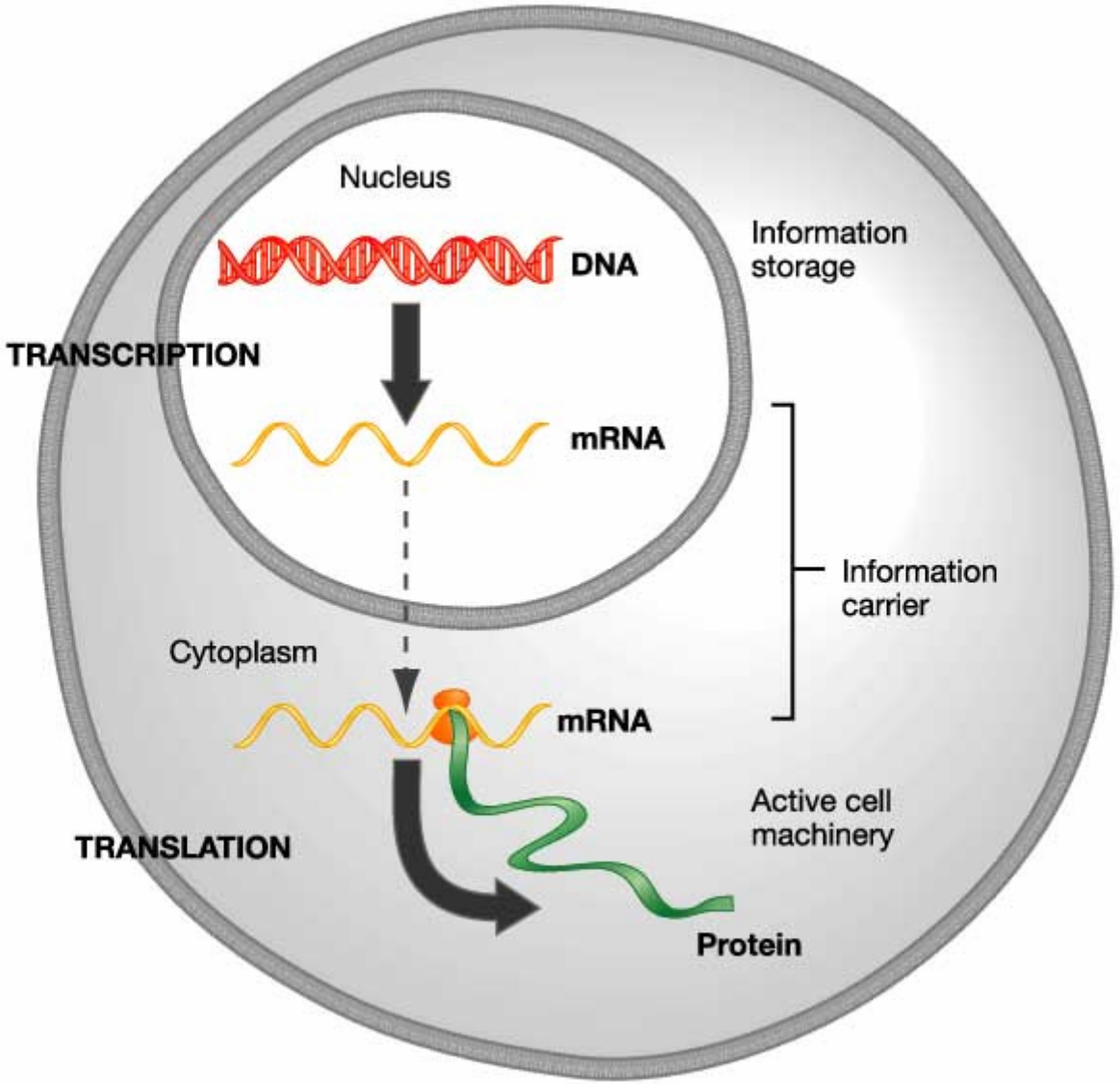
We have evidence that this may involve as many as 4000 genes in breast cancer alone

Topics for discussion

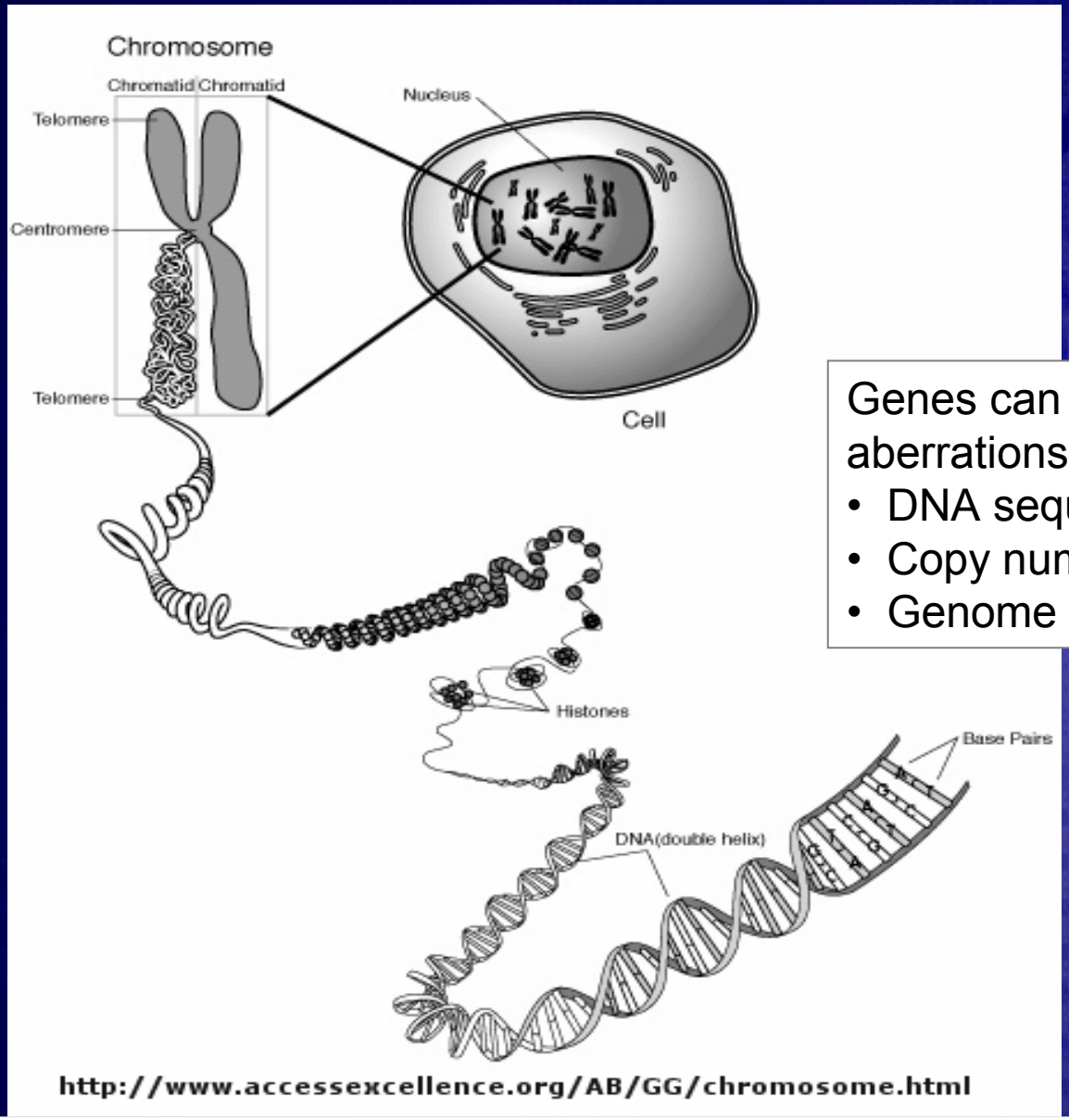
- What goes wrong during cancer development?
- How can we understand the details?
- How can we use the information to improve cancer treatment?

Opportunities for technology

Cancer is a disease of the genes



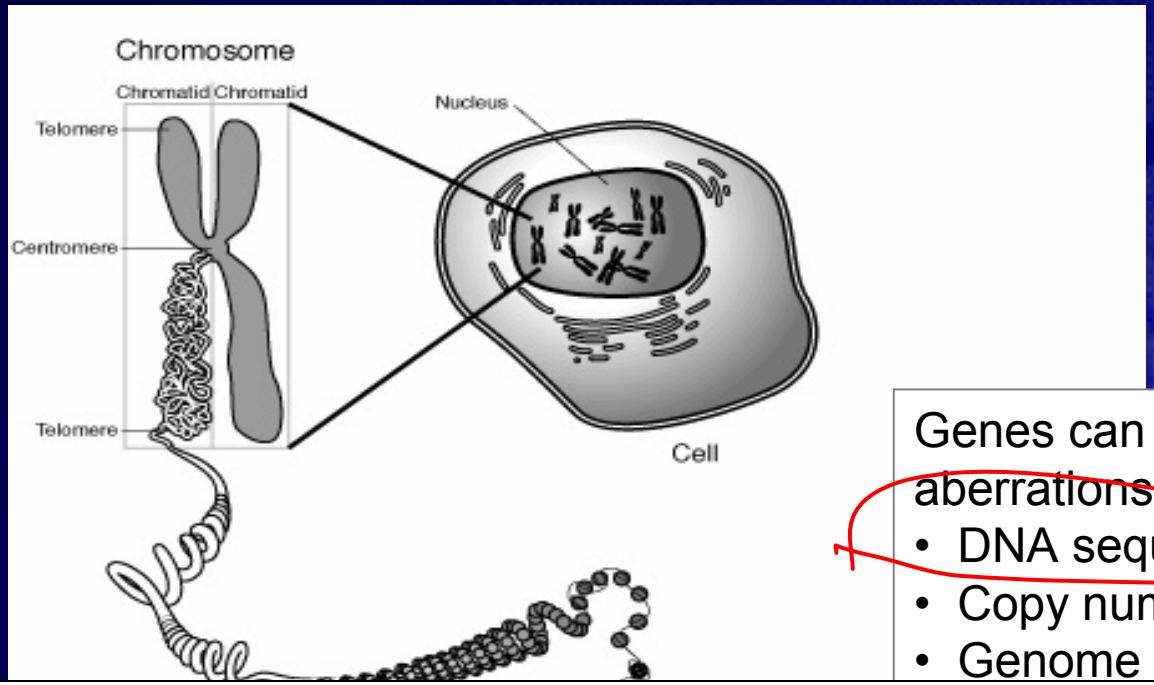
The normal genome is encoded in $3x10^9$ bp of DNA packaged into the nucleus of a cell



Genes can be deregulated by aberrations involving:

- DNA sequence
- Copy number or expression
- Genome organization

The normal genome is encoded in $3x10^9$ bp of DNA packaged into the nucleus of a cell



Genes can be deregulated by aberrations involving :

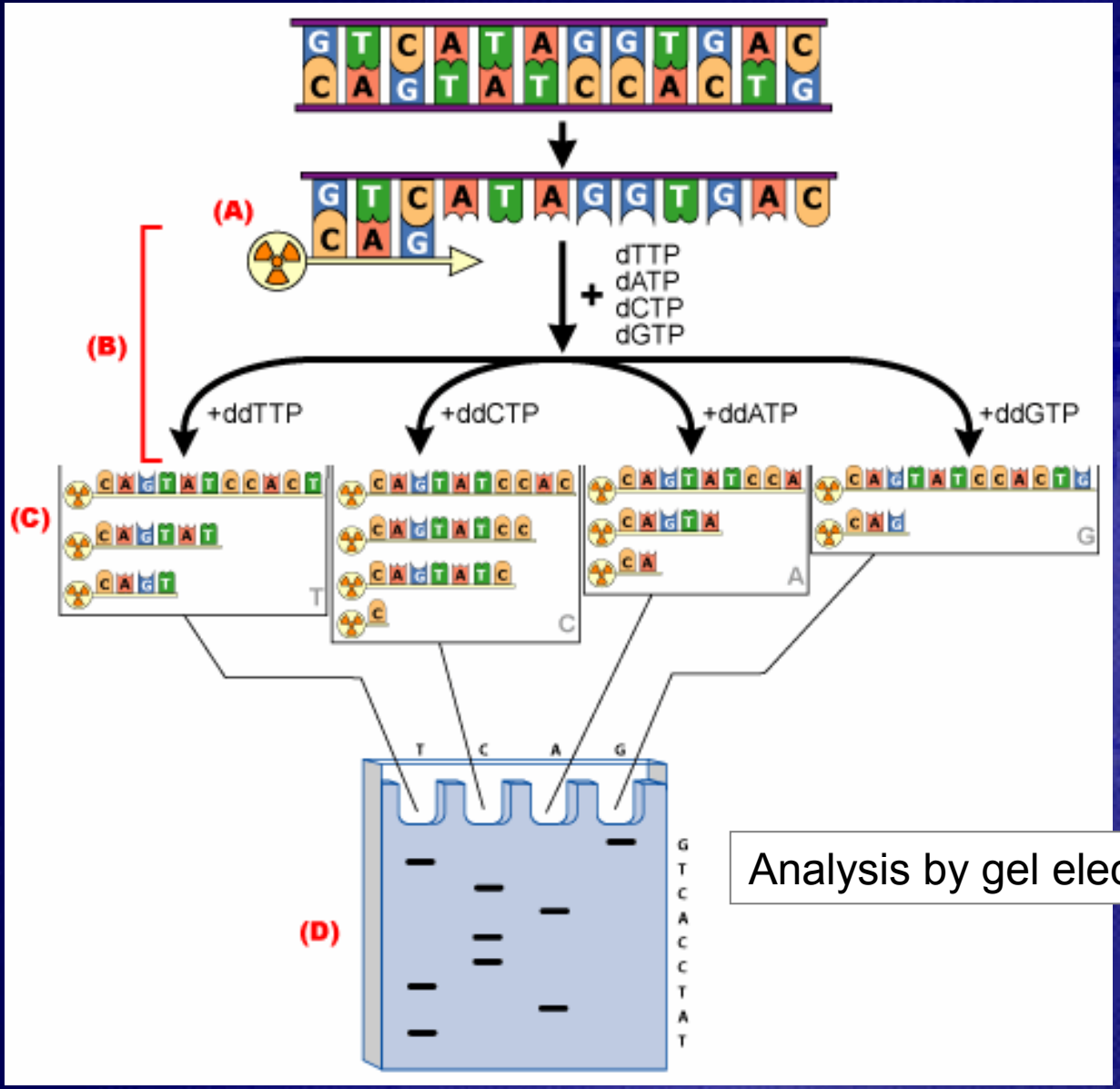
- DNA sequence
- Copy number or expression
- Genome organization

Some studies suggest the mutation rate may be as high as 10^{-5}/bp/cell (i.e. 10^4 mutations per cell)

Cancer genomes need to be scanned at the DNA sequence level to discover the mutation subset that deregulates critical genes and to ID therapy targets

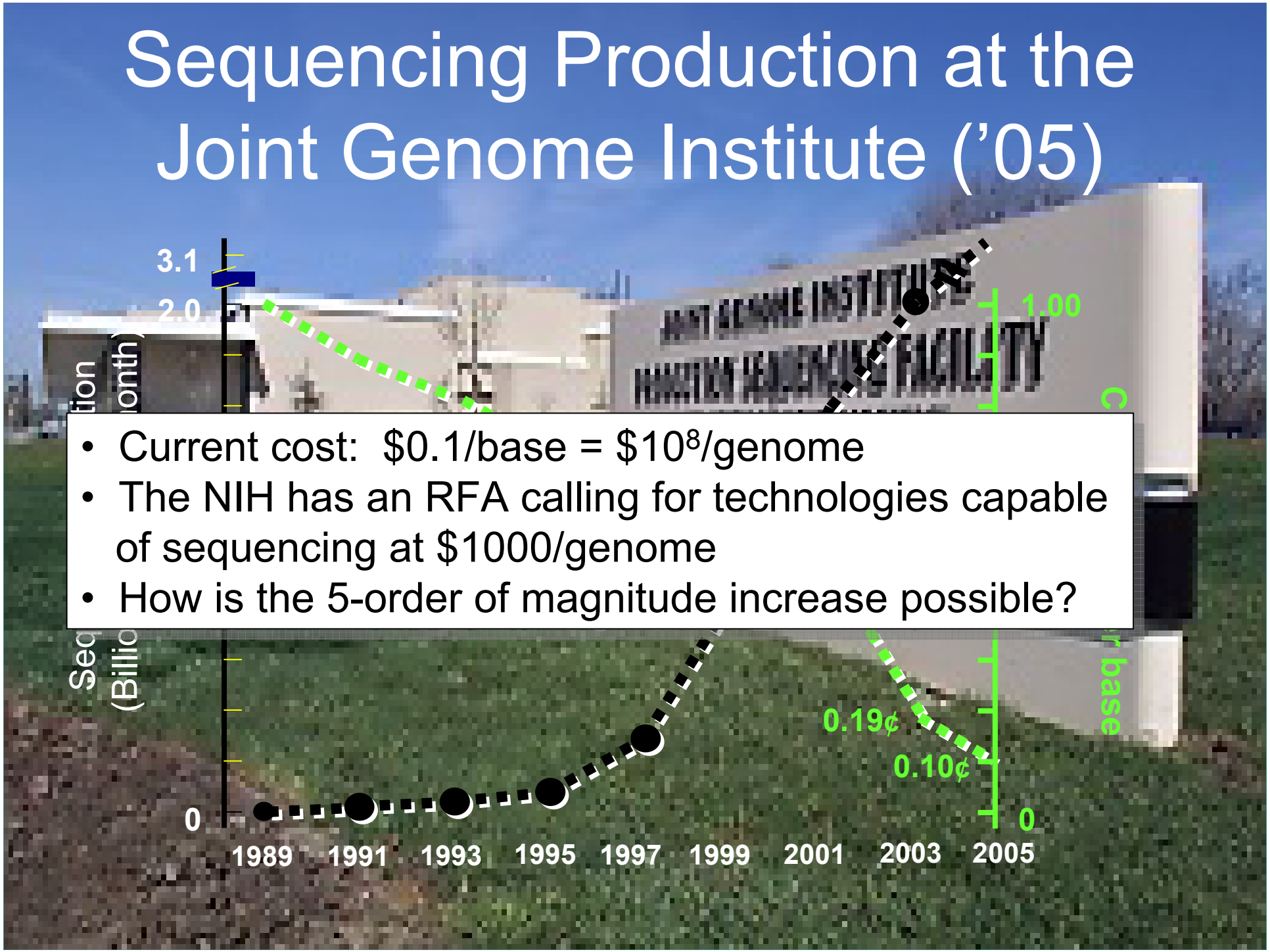
A $100M cancer genome sequencing project is underway
May expand to $1B if successful

Current sequencing technology



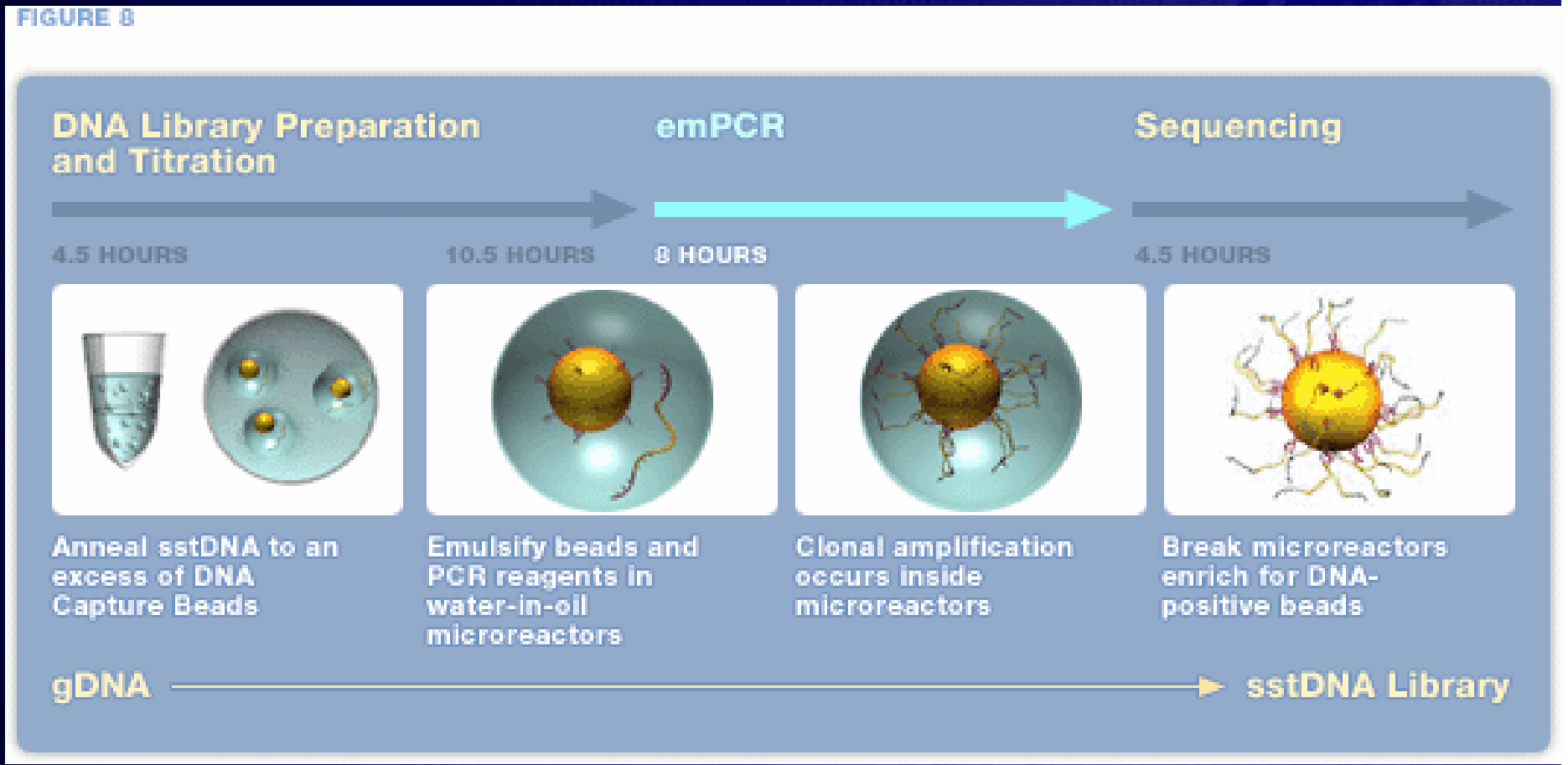
Analysis by gel electrophoresis

Sequencing Production at the Joint Genome Institute ('05)



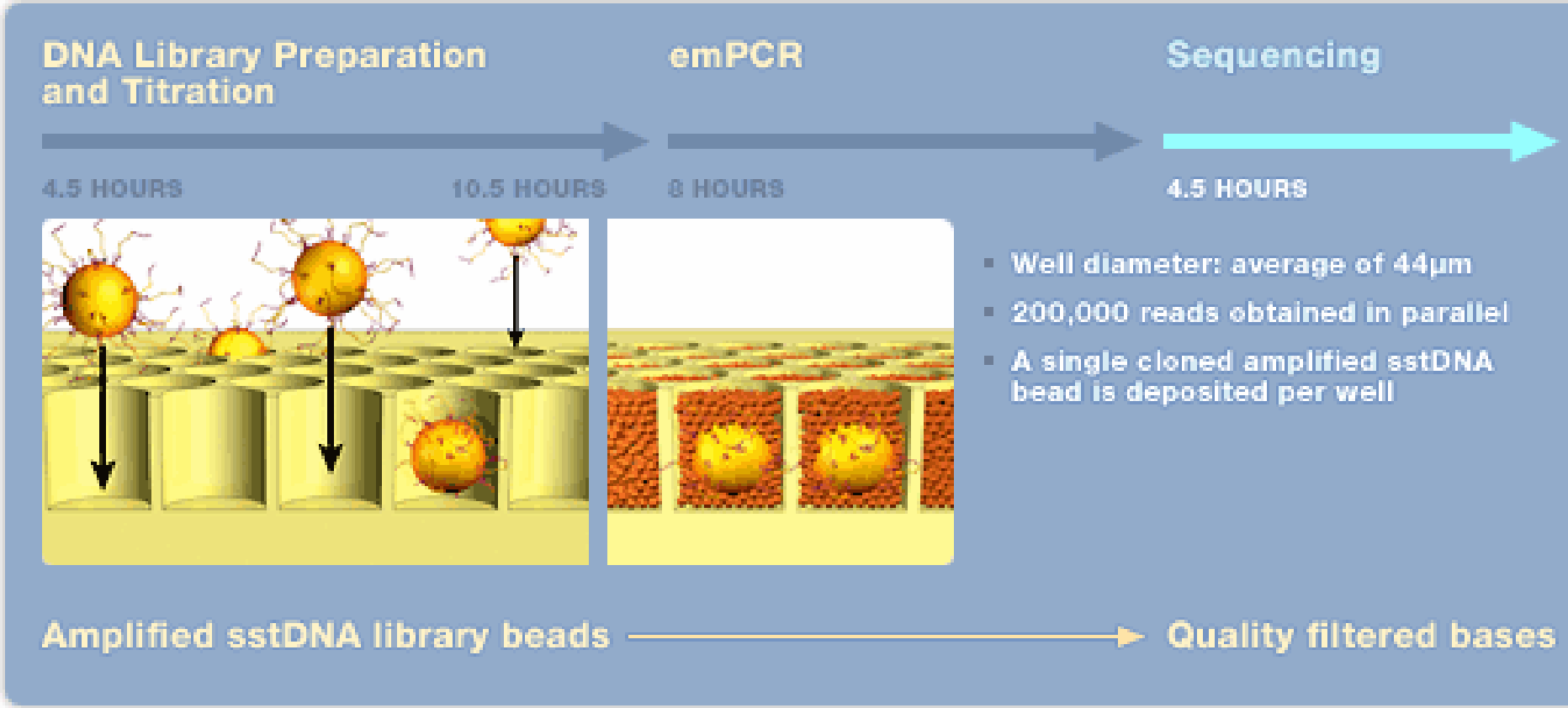
- Current cost: $0.1/base = 10^8/genome
- The NIH has an RFA calling for technologies capable of sequencing at $1000/genome
- How is the 5-order of magnitude increase possible?

Sequencing one molecule at a time

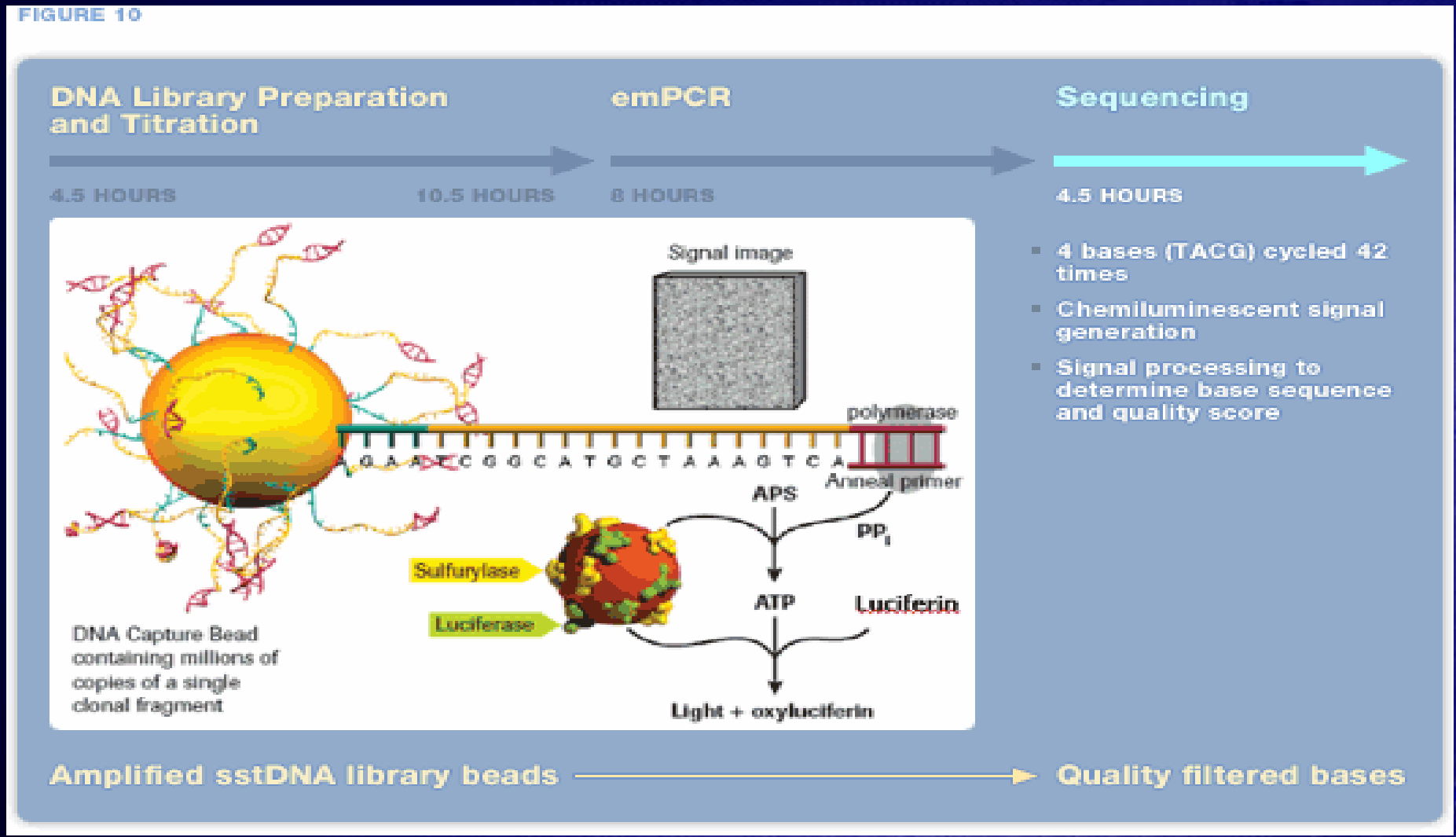


Sequencing one molecule at a time

FIGURE 9



Sequencing one molecule at a time

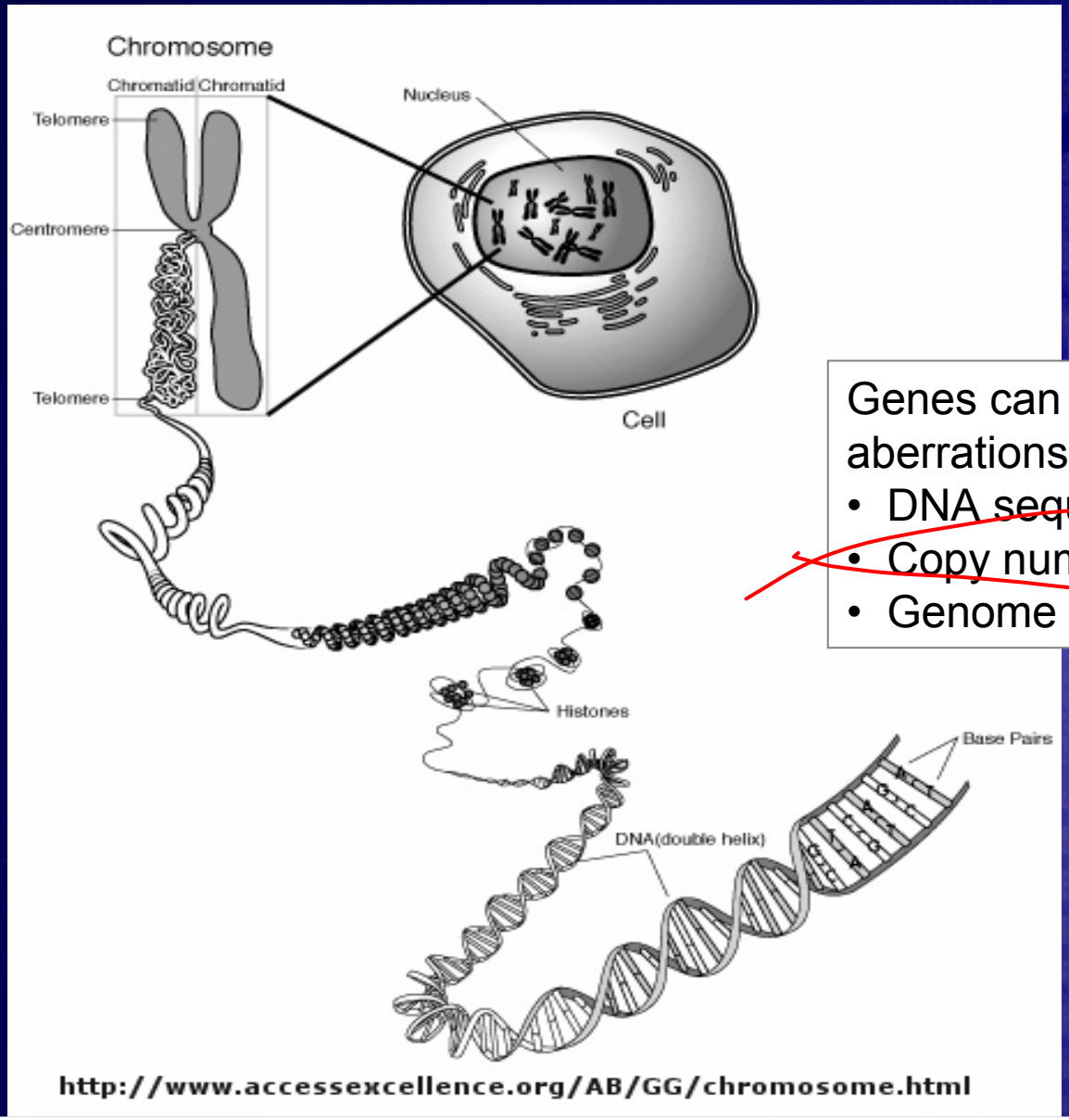


454 Technology

There are many variations on "polony" sequencing

- Future work will involve
  - Increasing polony density
  - Decreasing reagent costs
  - Improving sequencing fidelity

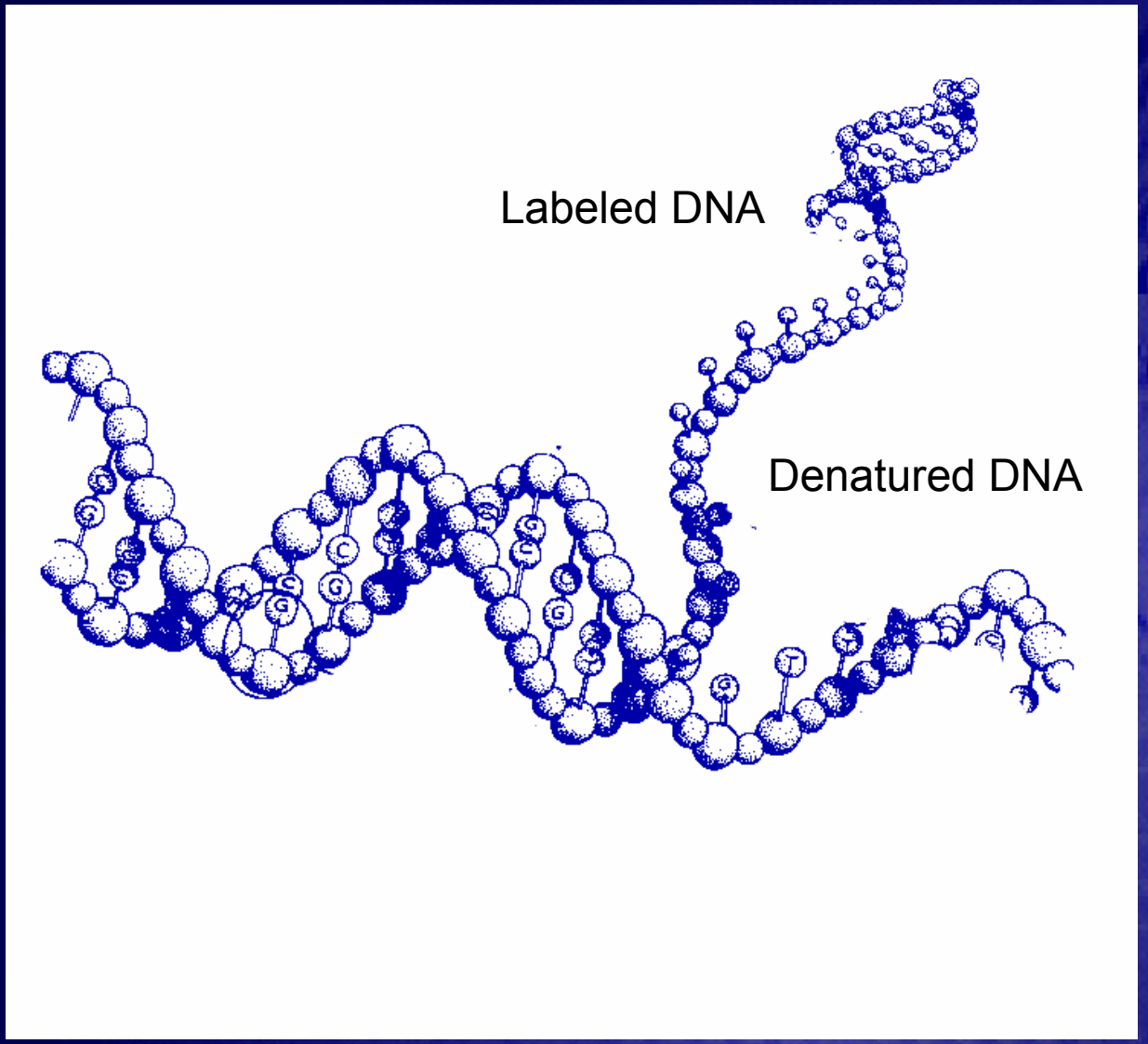
The normal genome is encoded in $3x10^9$ bp of DNA packaged into the nucleus of a cell



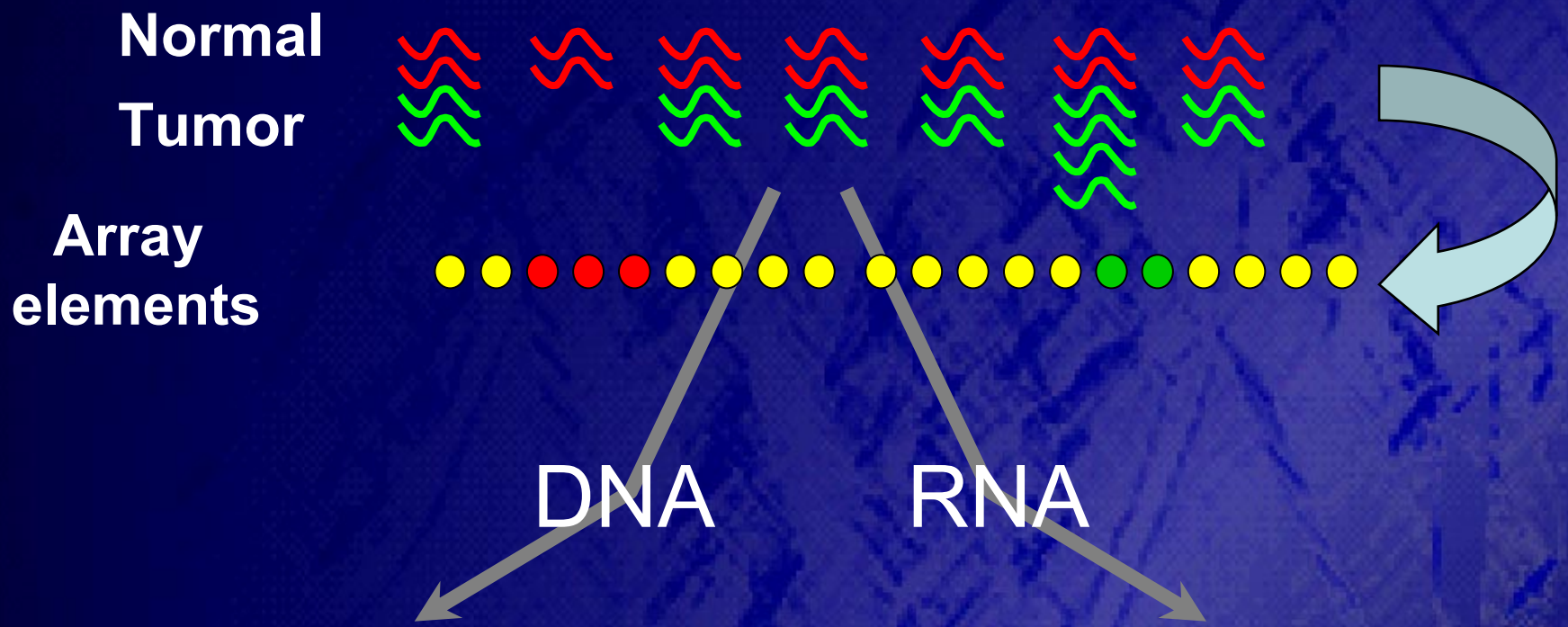
Genes can be deregulated by aberrations involving :

- DNA sequence
- Copy number or expression
- Genome organization

Staining DNA with DNA



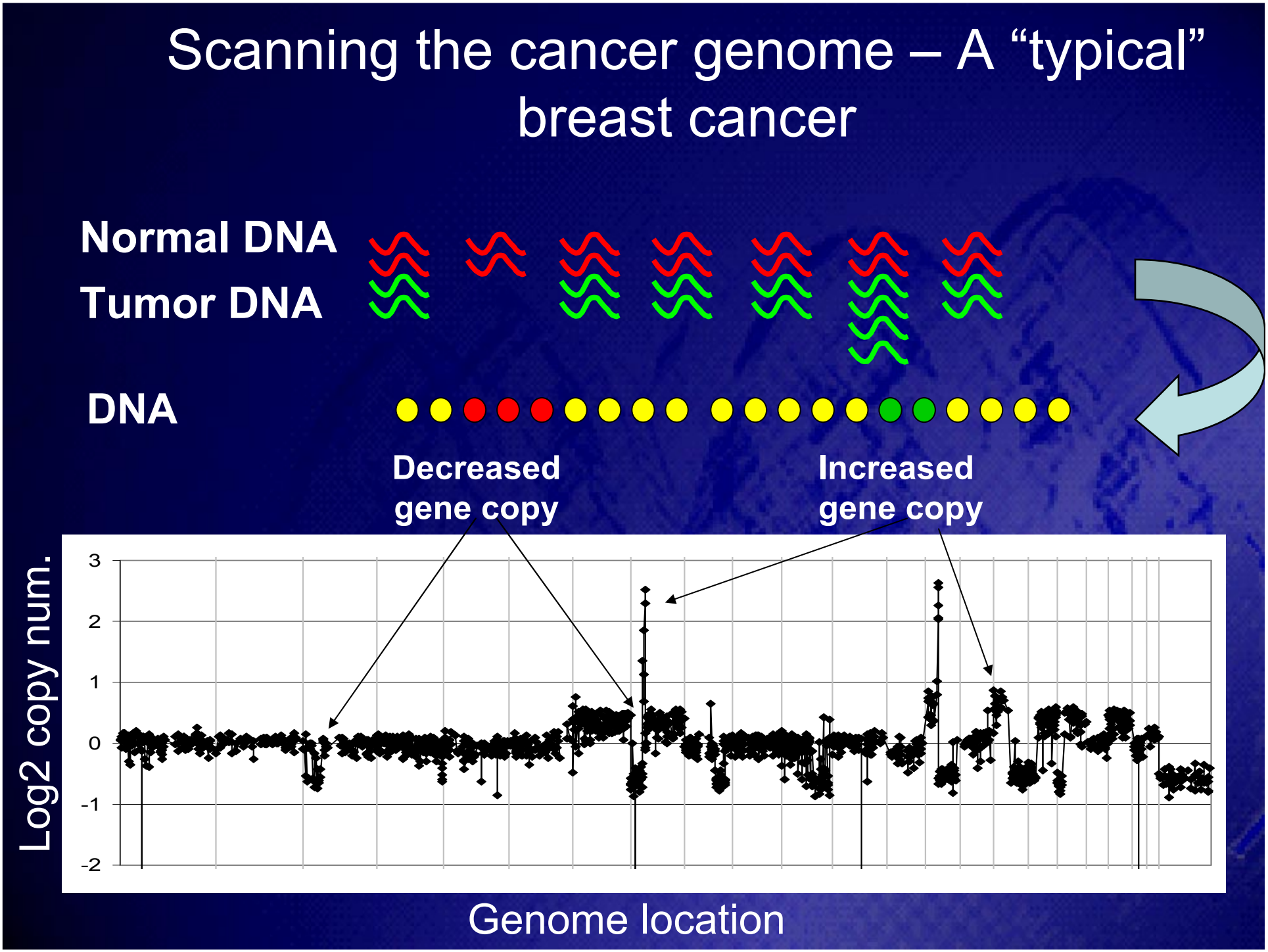
Scanning for genomic aberrations that alter gene expression



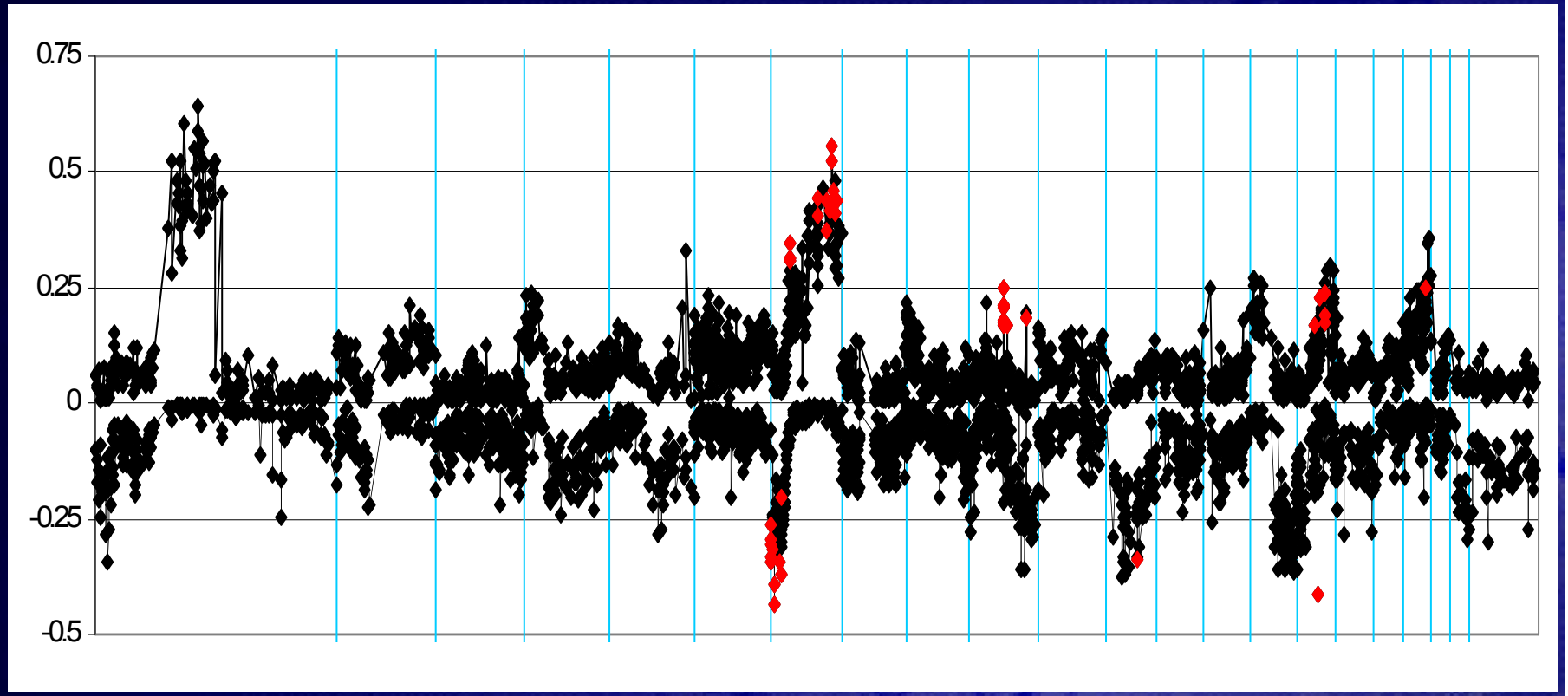
Array manufacture and readout is key

- Array manufacture
  - Spotted DNA
  - Photolithography
  - Micromirror based synthesis
- Hybridization efficiency
- Detection
- Issues
  - Array “probe” density
  - Cost
  - Amount of material required

Scanning the cancer genome – A "typical" breast cancer



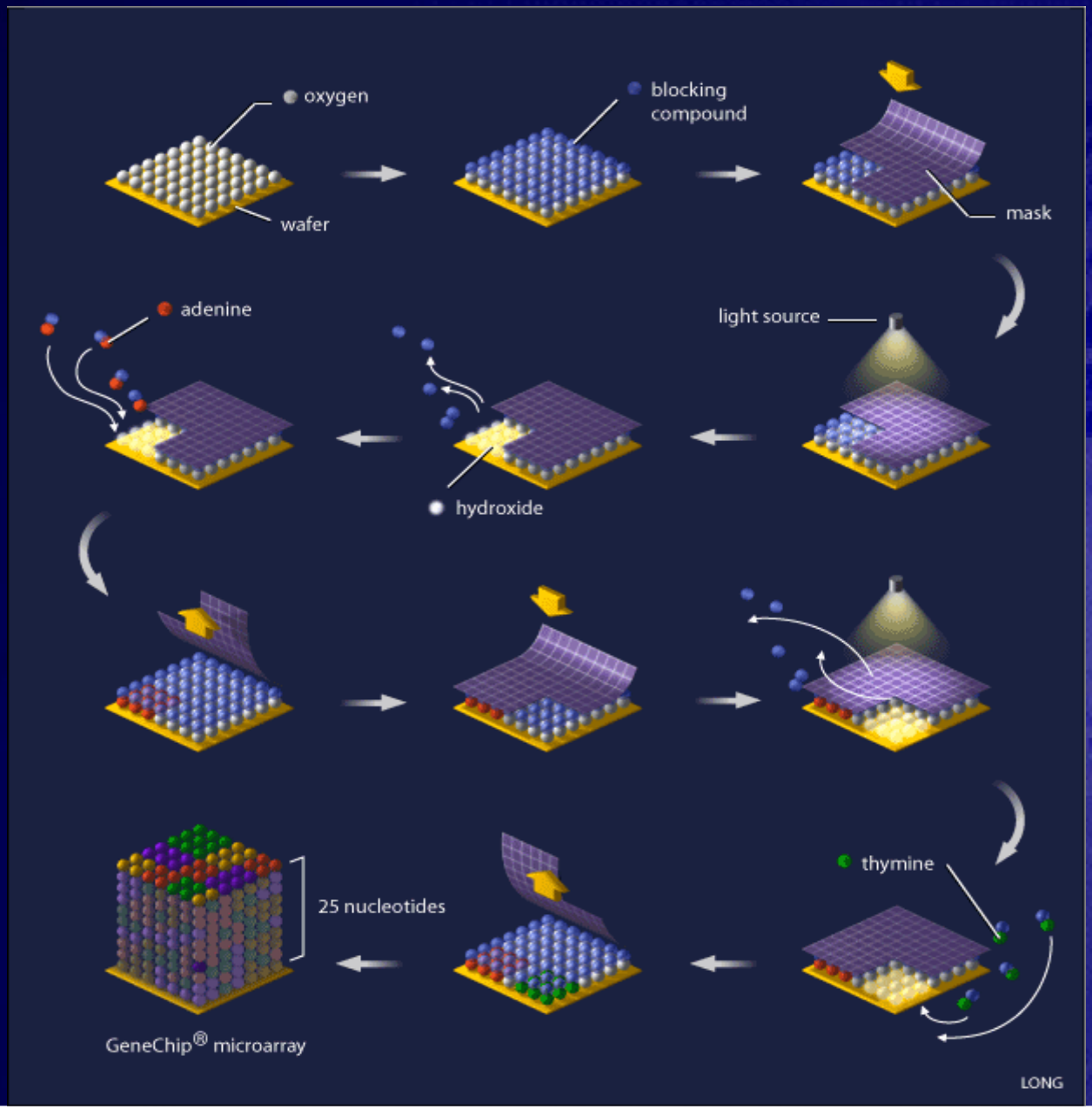
Recurrent aberrations in breast cancers – markers for poor outcome-



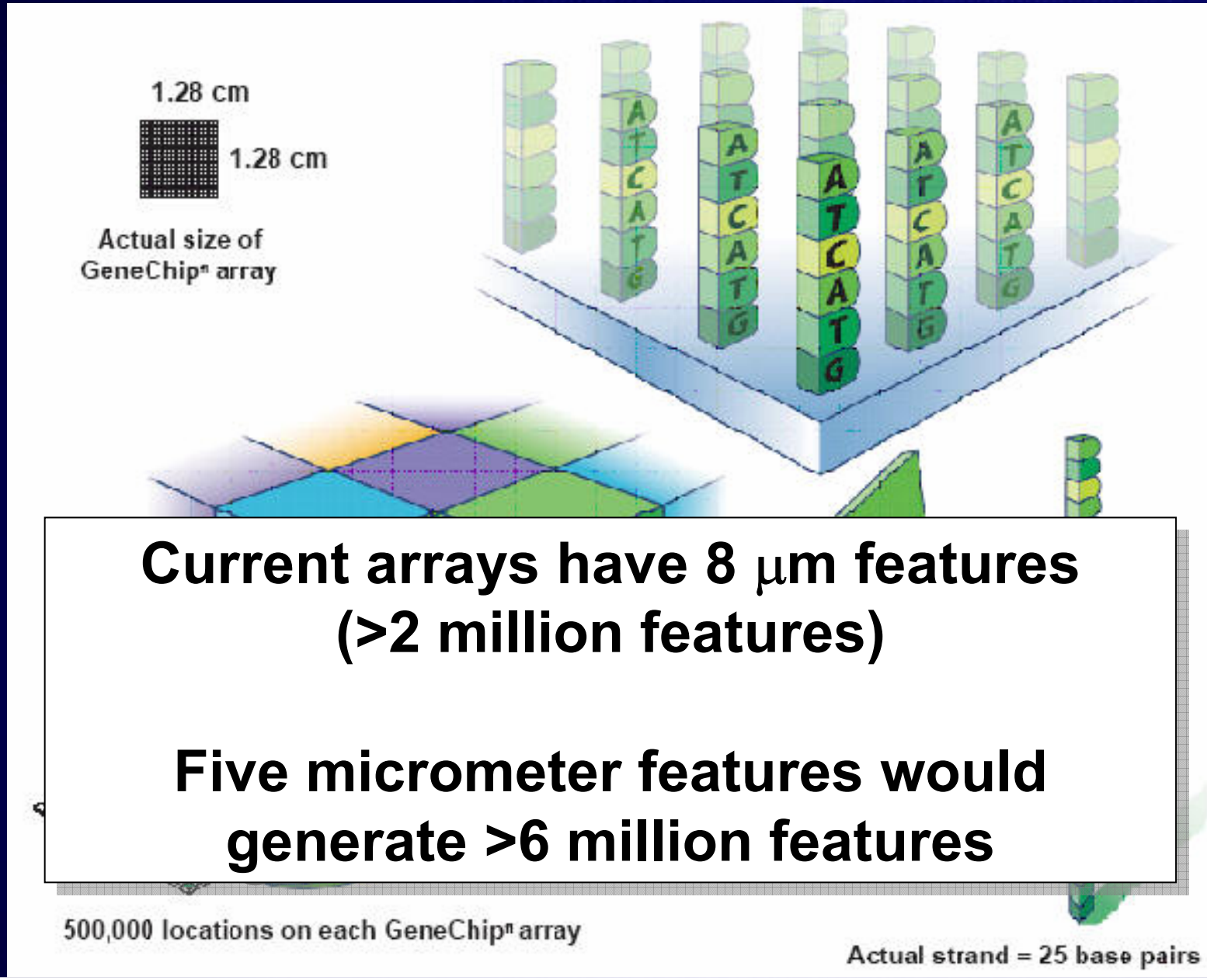
High level amplifications are associated with reduced survival

Technologies are needed to read out at higher resolution and lower cost while using less material

Array synthesis using photolithography



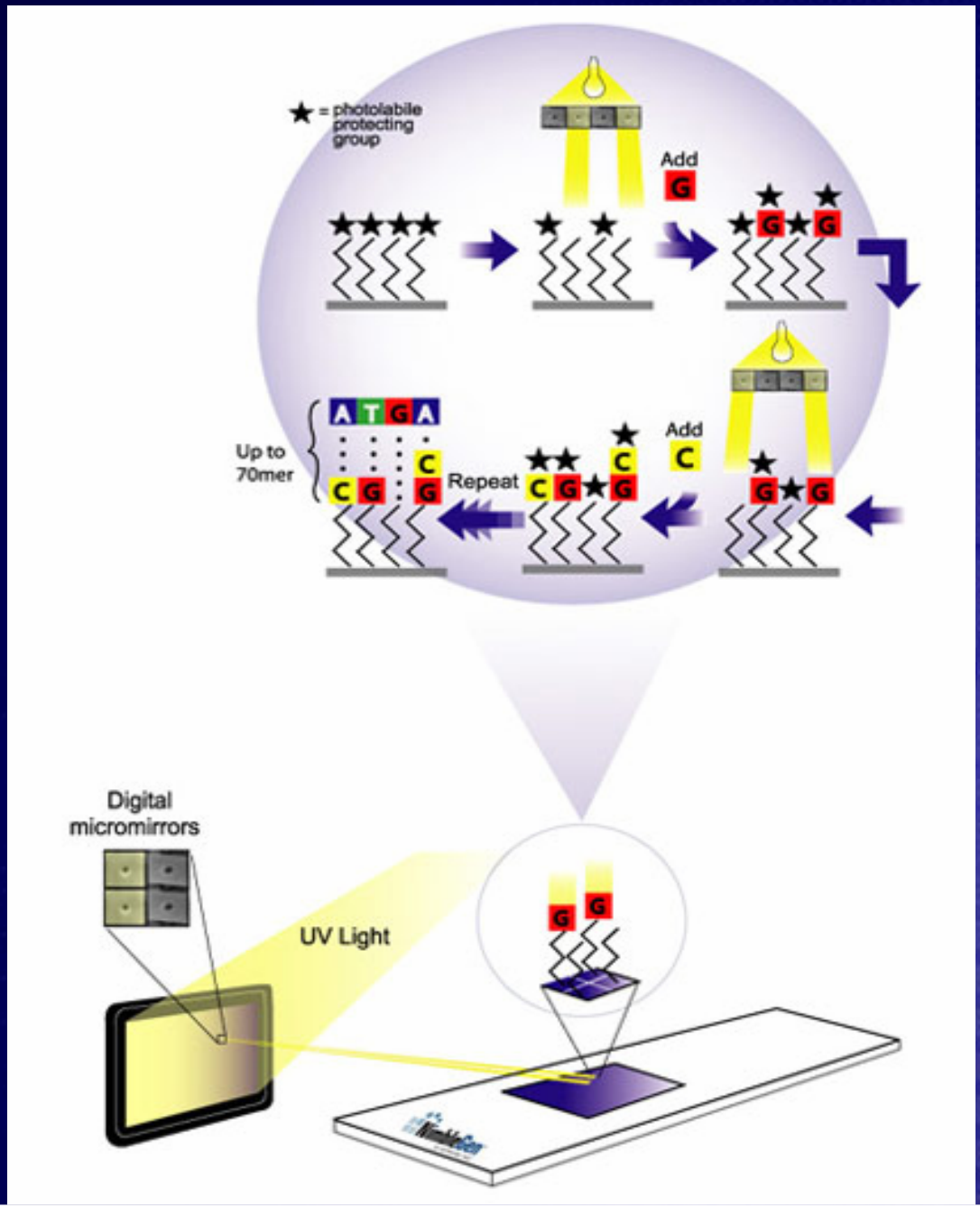
Affymetrix



Current arrays have 8 μm features (>2 million features)

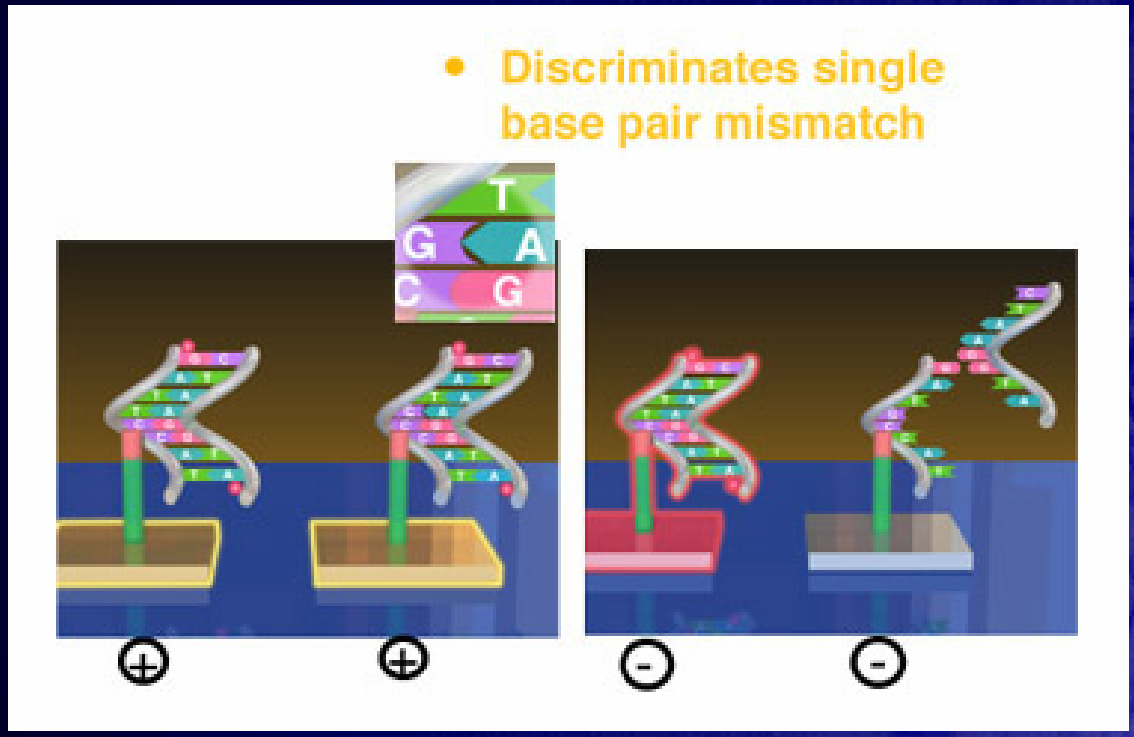
Five micrometer features would generate >6 million features

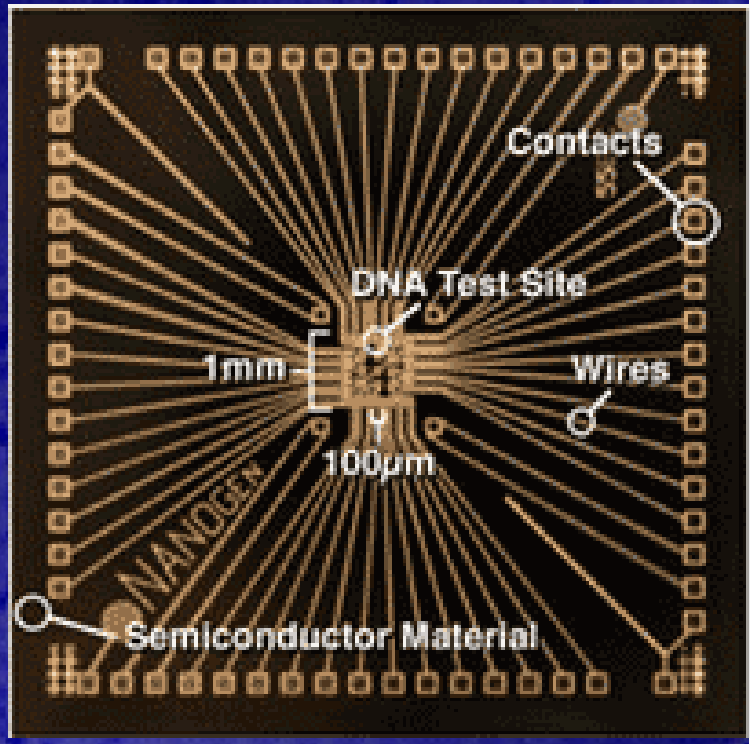
Synthesis one pixel at a time



Nimblegen

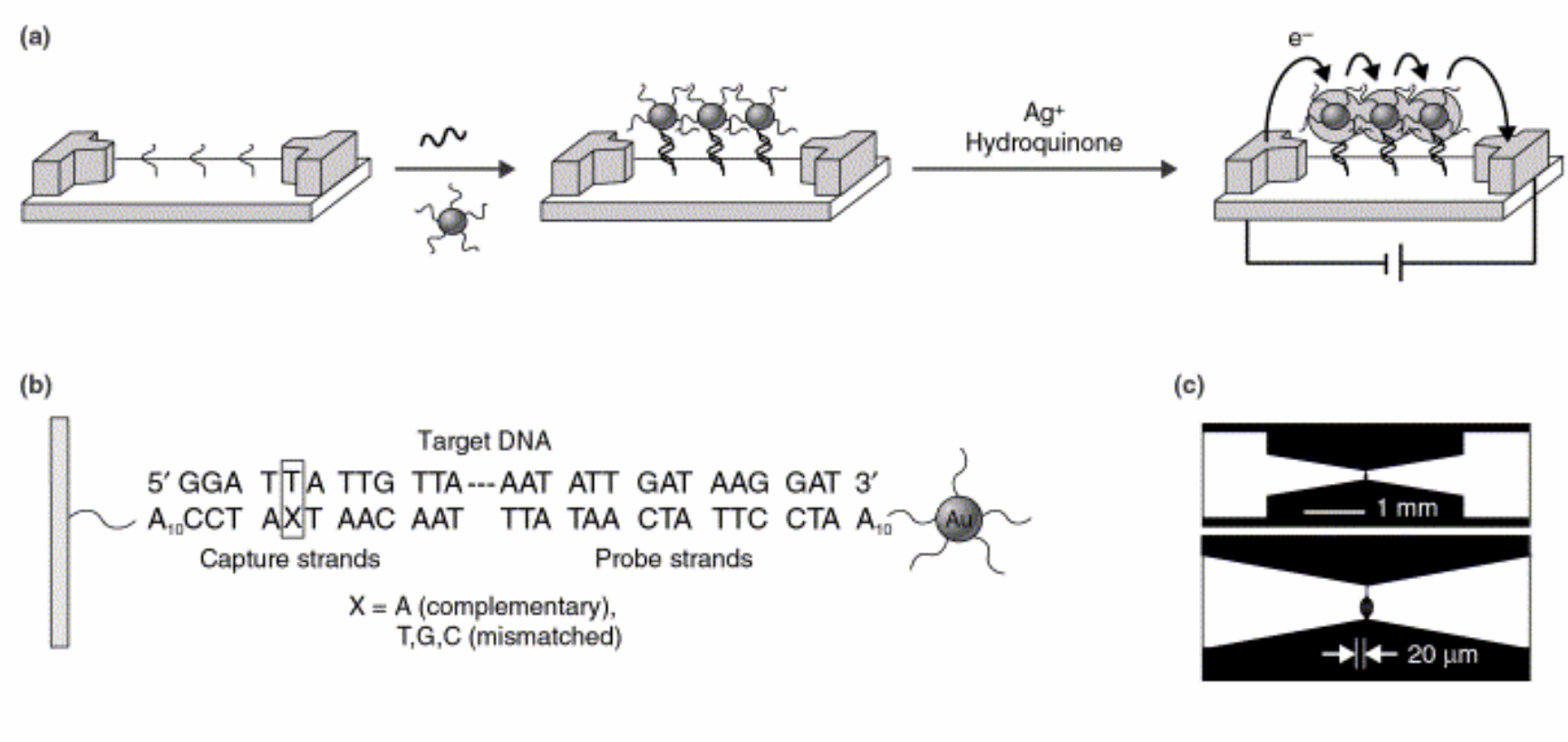
Electrostatic hybridization to improve efficiency and specificity





Nanogen

Electrochemical detection to facilitate readout?

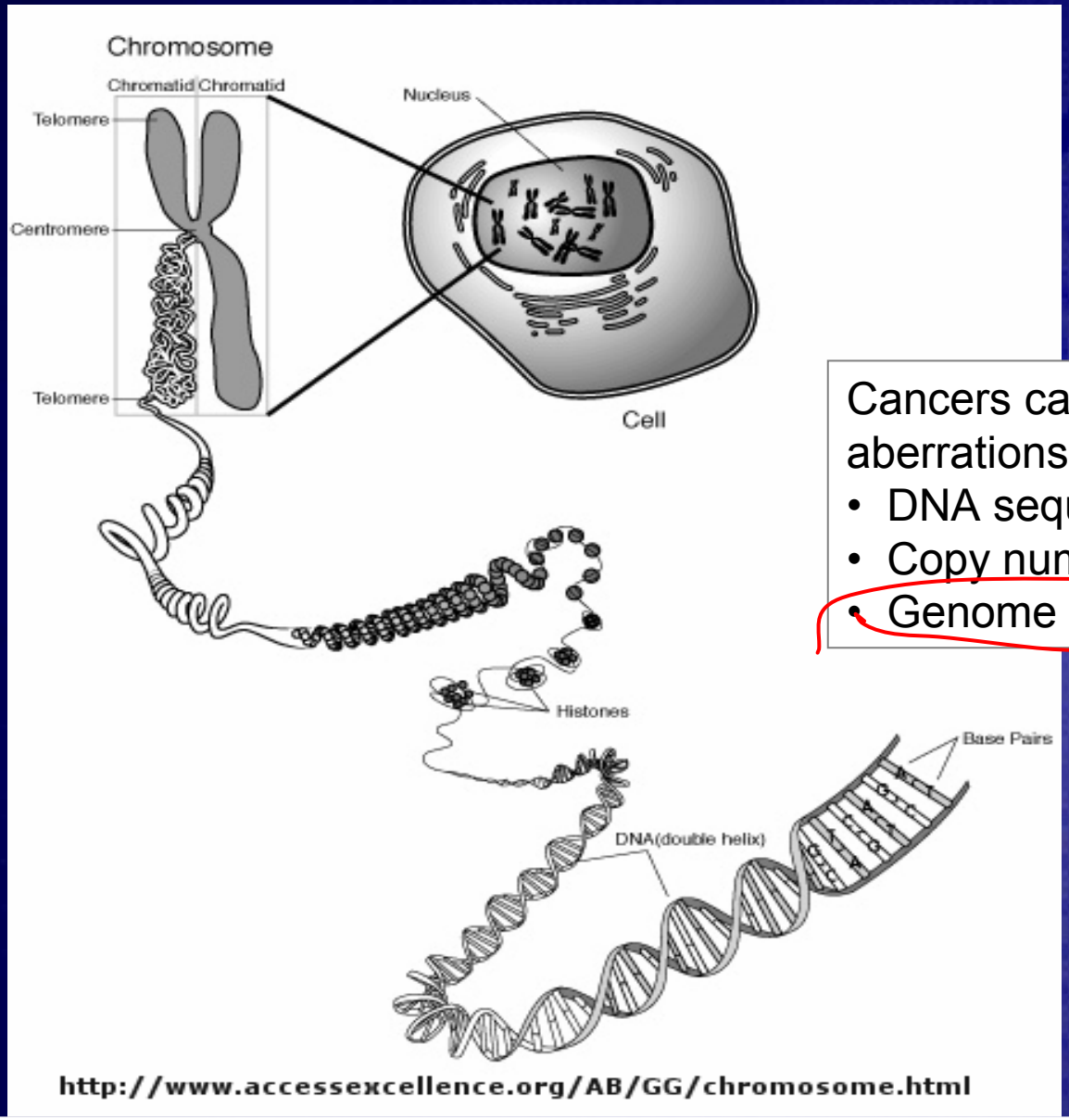


Curr Opin Chem Biol. 2002 Dec;6(6):816-22.

More powerful technologies are clearly possible

The current challenge is cost ($150/array)

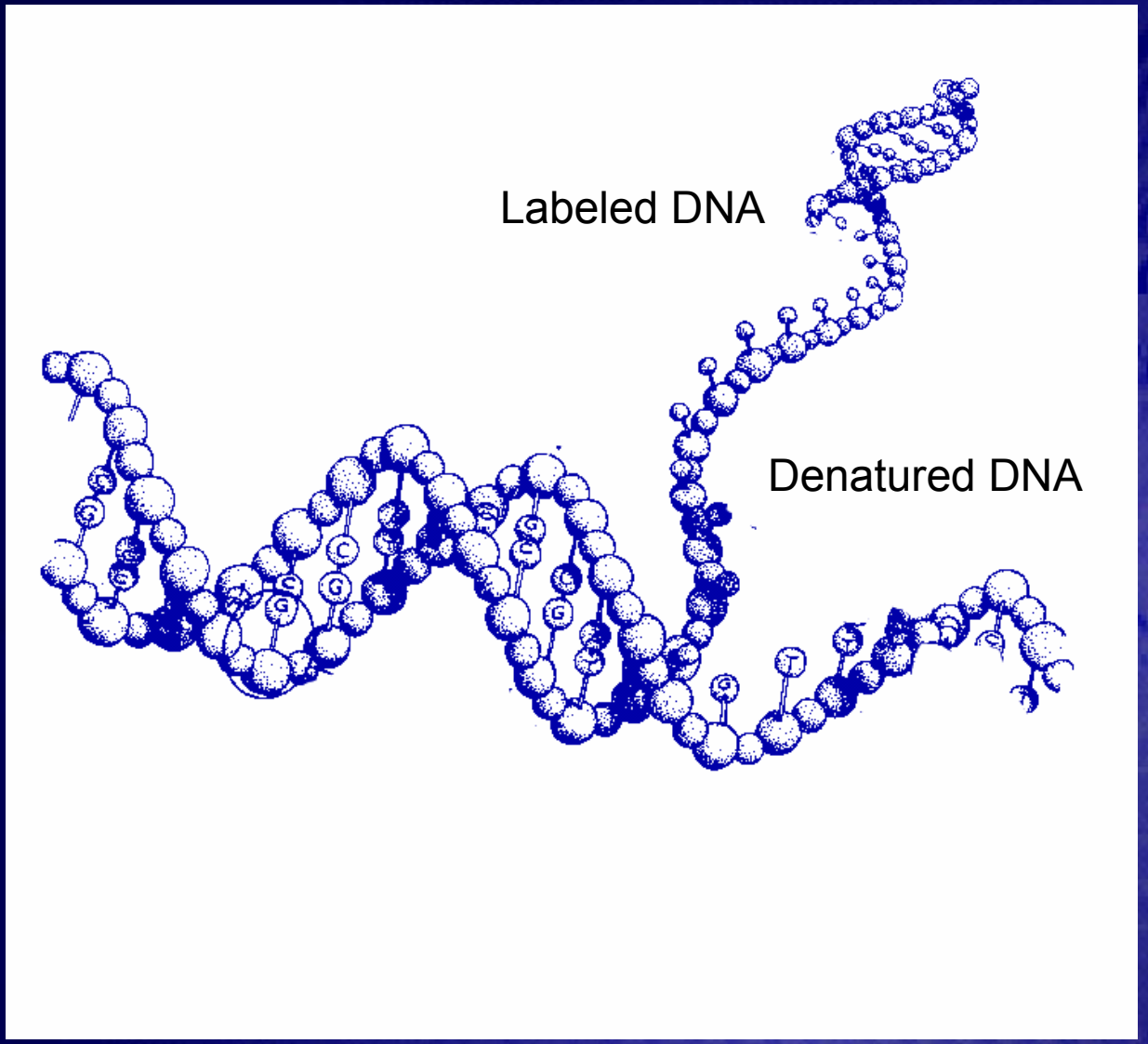
The normal genome is encoded in $3x10^9$ bp of DNA packaged into the nucleus of a cell



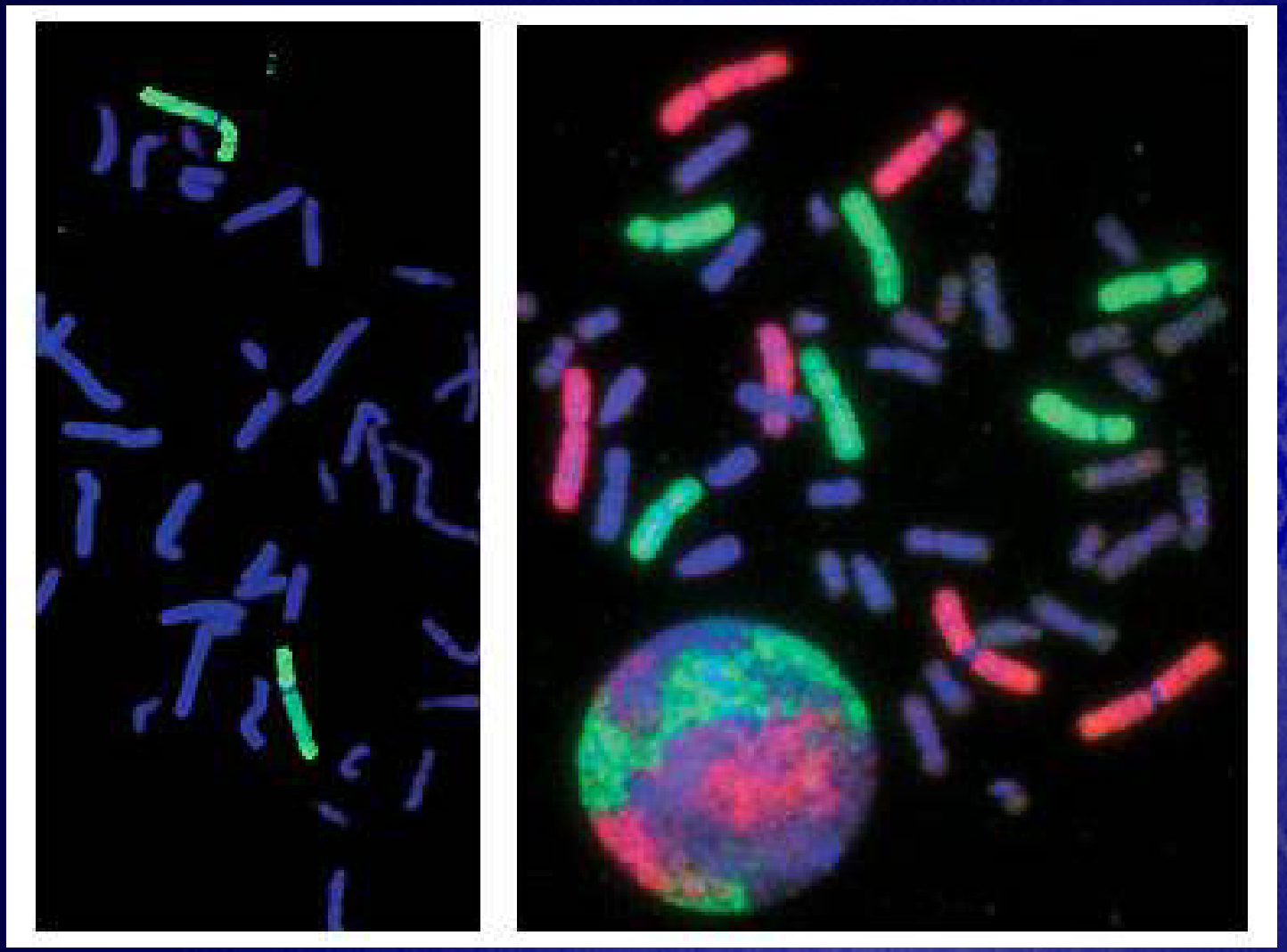
Cancers can arise from aberrations involving:

- DNA sequence
- Copy number or expression
- Genome organization

Staining DNA with DNA

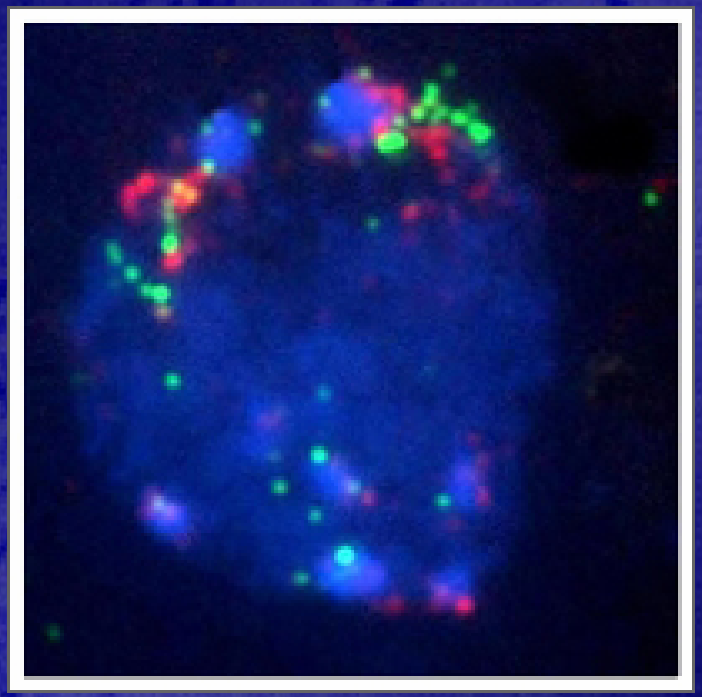


Fluorescence in situ hybridizaiton



Technical need - Advanced microscopy to assess 3D organization

- Currently assessed using confocal microscopy
- Resolution limited to ~0.2 μm
- Limited temporal resolution
- Multicolor analysis
- Software for 3D visualization



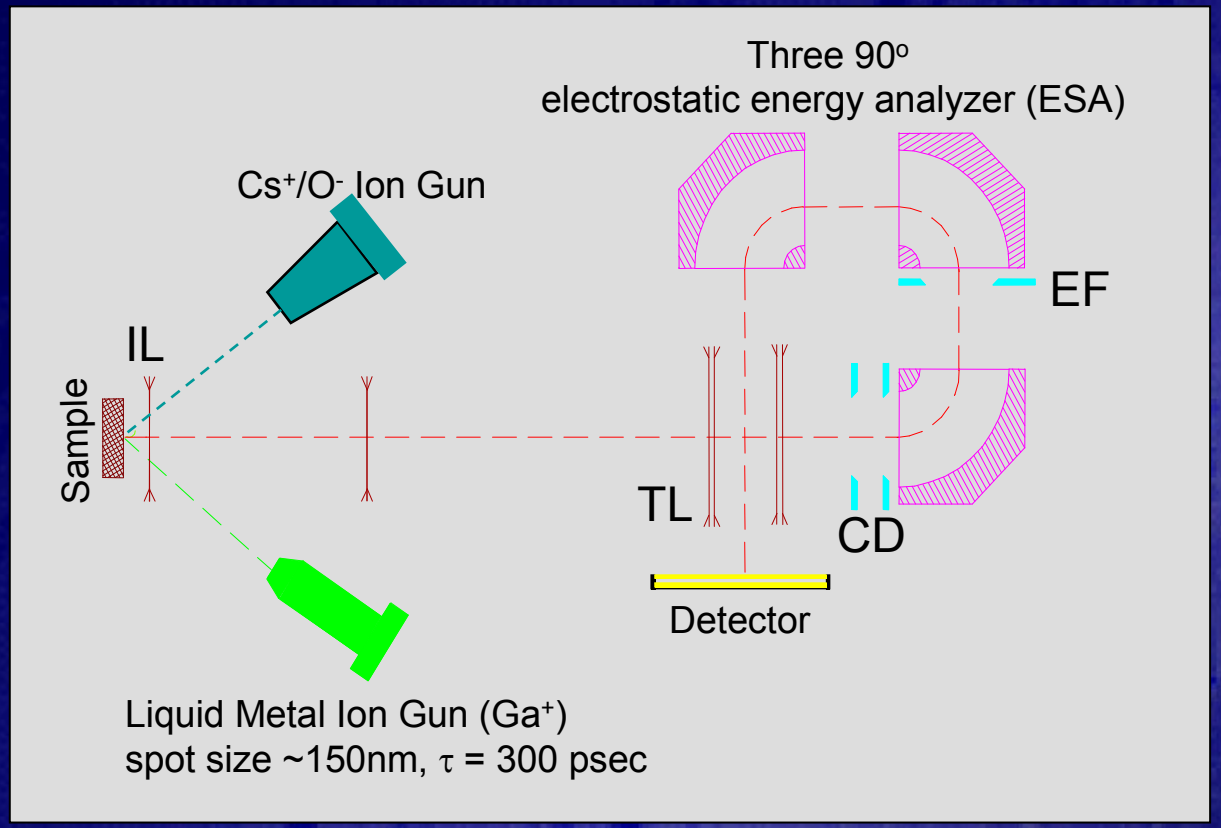
Scanned ion probe mass spectrometry

Characteristics

- Ion transmission ~ 100%
- Ion microprobe and ion microscope
- Mass range: unlimited
- Mass resolution: m/Δm~ 8000

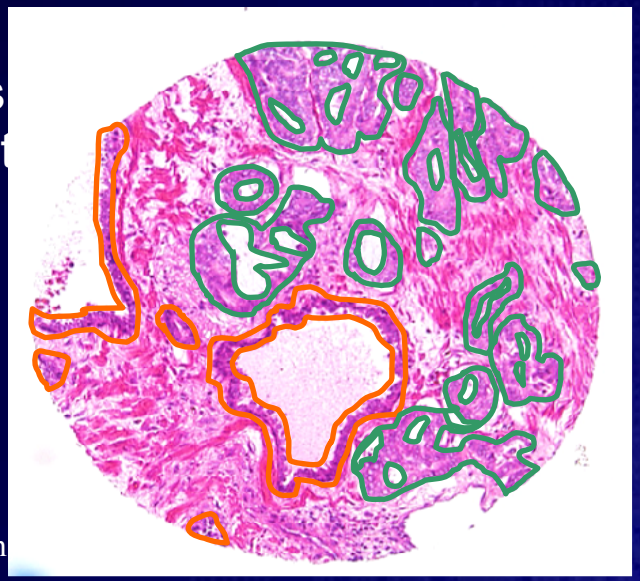
Secondary ion formation depends on:

- Primary ions
- Matrix effects
- Sample preparation

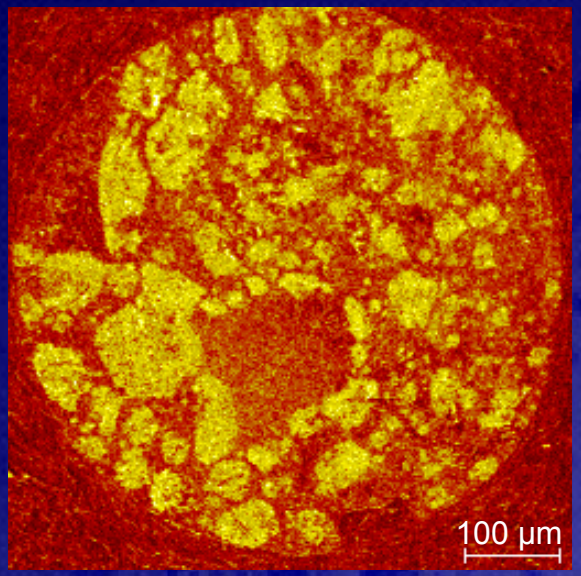


ToF-SIMS image analysis of a prostate cancer tissue section

Cancerous fixed prostat tissue

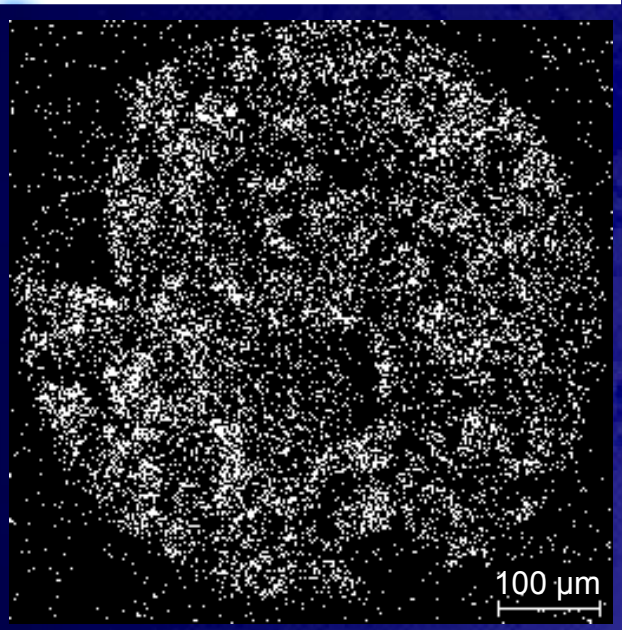


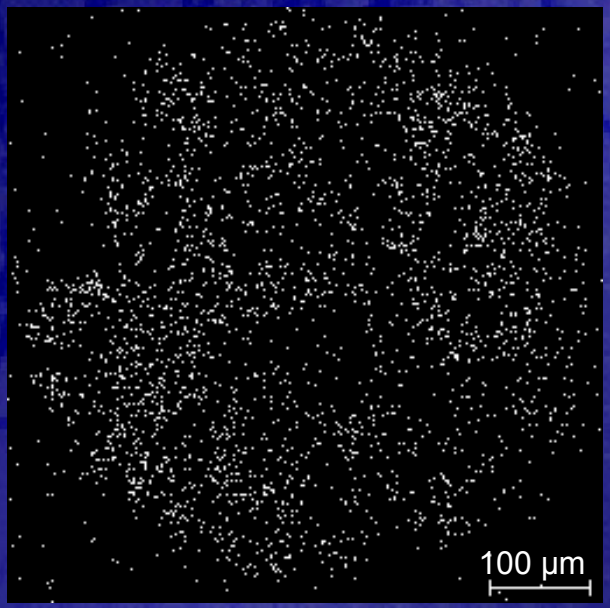
David Seligson



Total ion image

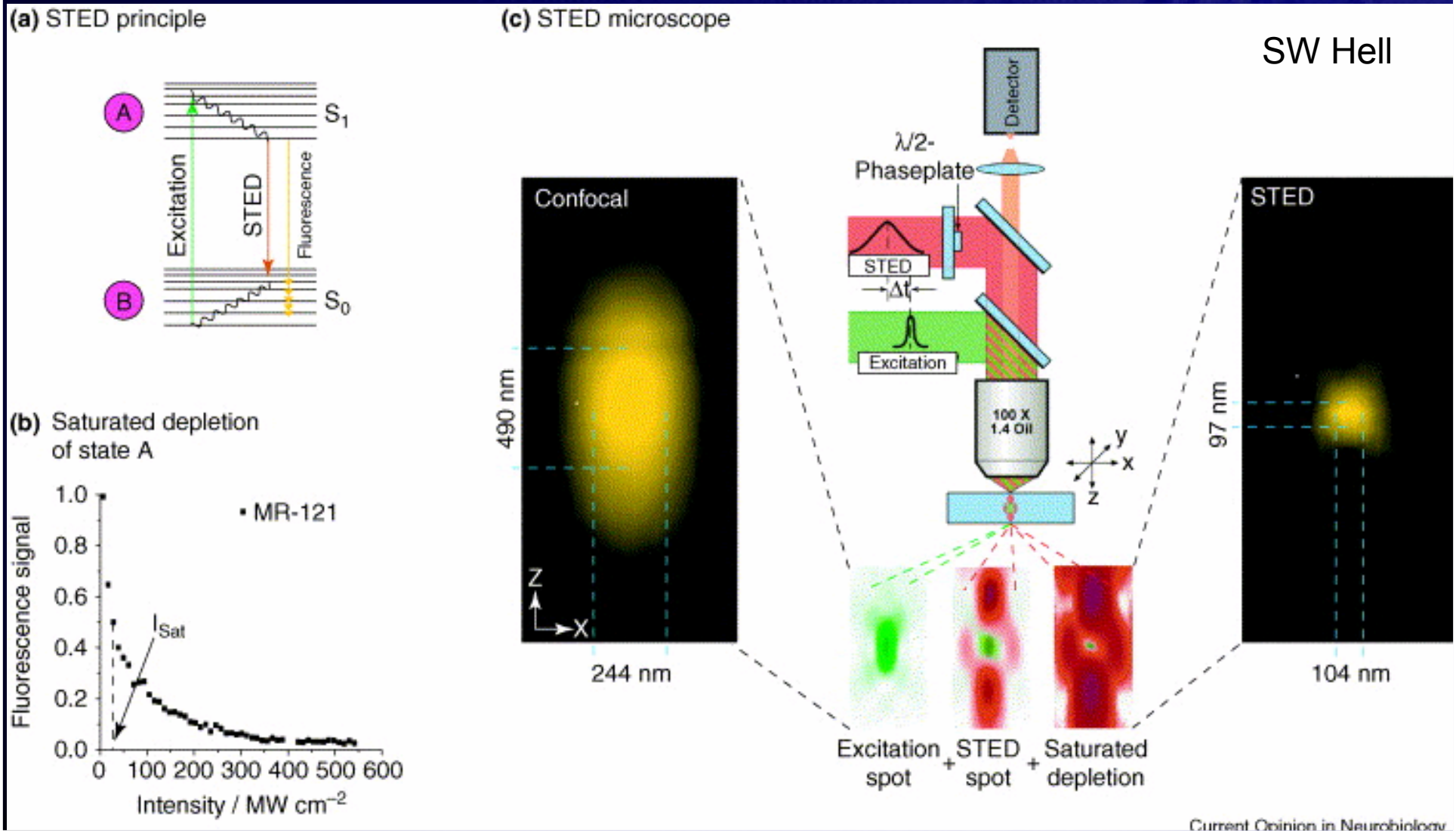
M/z = 221





M/z = 184 (phosphocholine head group)

Super-resolution microscopy
Stimulated emission depletion



Topics for discussion

- What goes wrong during cancer development?
- How can we understand the [illegible]?
- How can we use the information to improve cancer treatment?

Opportunities for technology

The details of gene deregulation differ between individual cancers – even cancers that appear the same to a pathologist

Detailed molecular analyses may identify molecular features that will predict tumor behavior including response to therapy

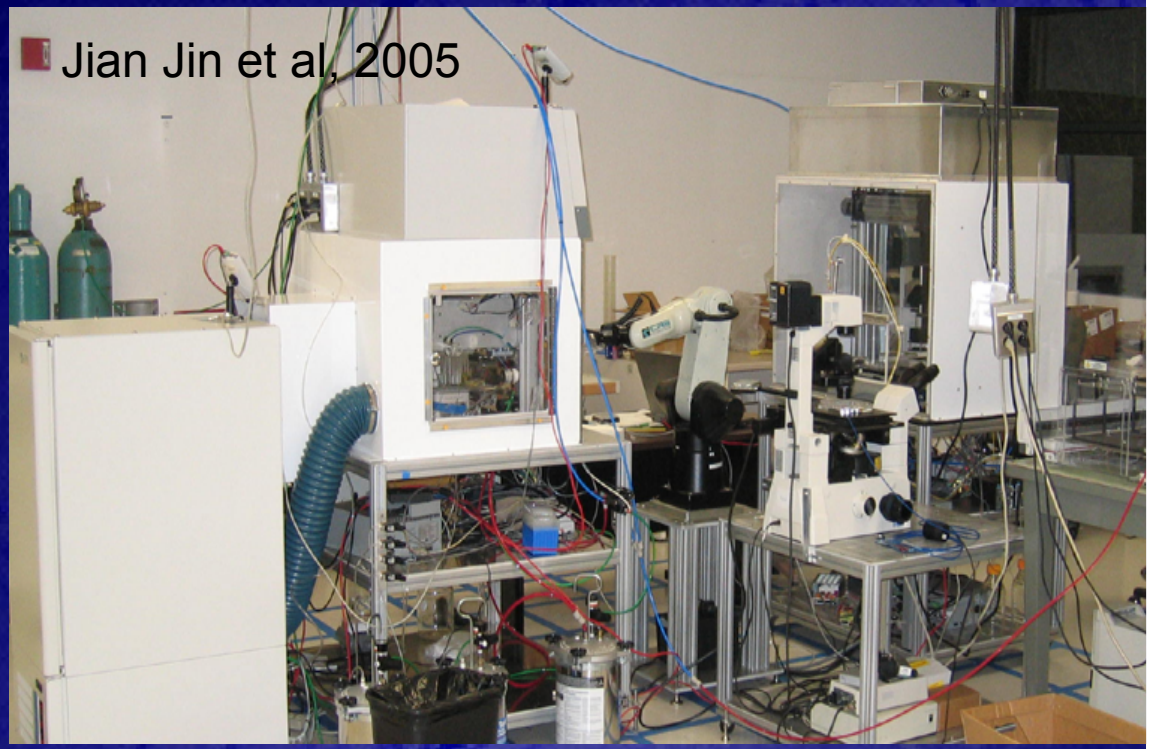
Assessing drug responses

- Identify cell lines with drug target present
- Apply genome analysis technologies to identify molecular features of each cell line
- Measure responses
- Correlate pre-treatment molecular features with cellular responses to identify response *predictors*

Hundreds of drugs are now in the development pipeline
We need to know who will respond before we evaluate them in patients

An in vitro system for assessment of function or Rx response

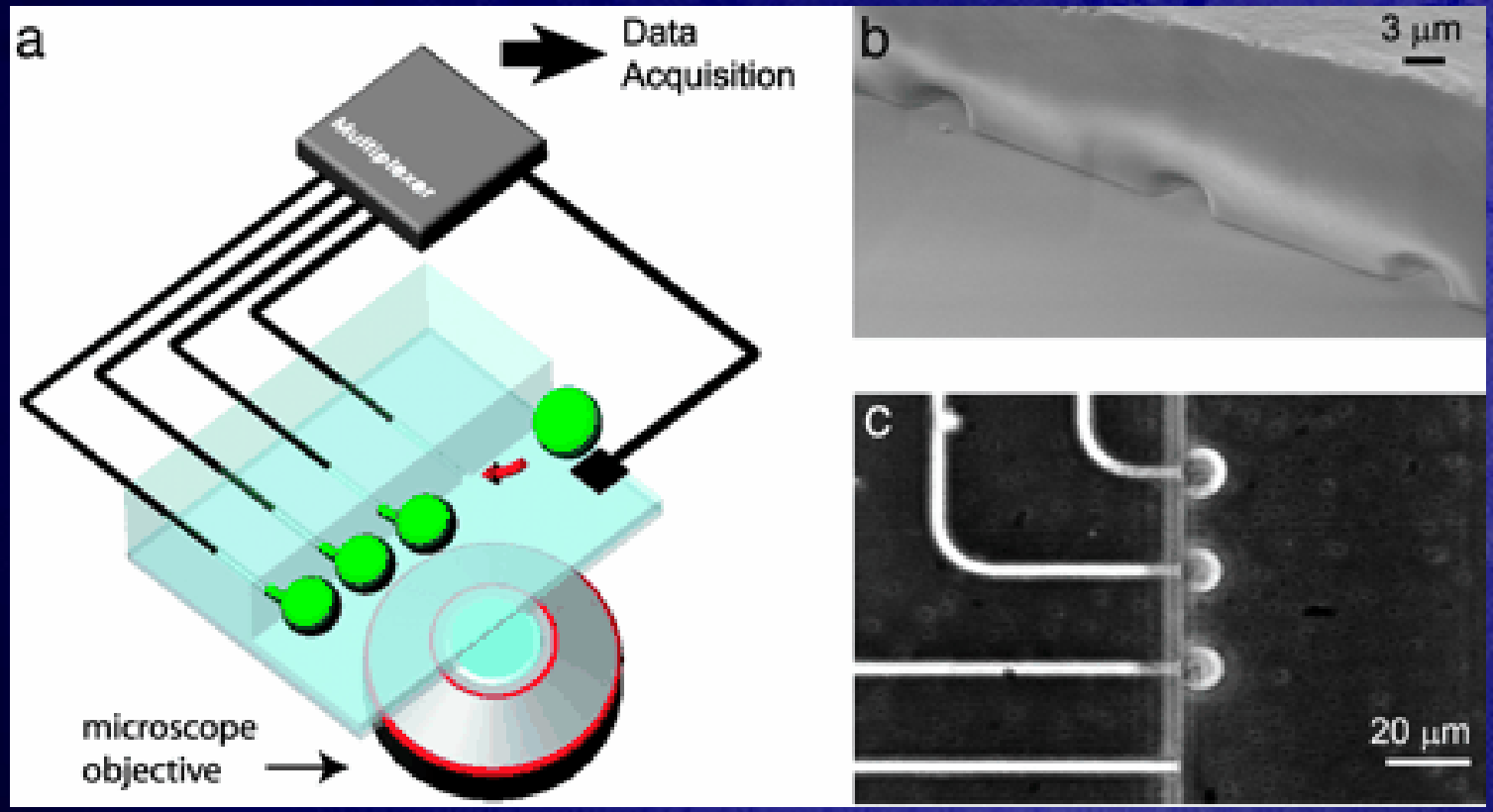
- ~60 breast cancer cell lines in 2D and 3D culture
- Molecular profiling
  - DNA, RNA, methylation, protein
  - DNA sequence
- Semi-automated cell culture
- High content imaging
  - Apoptosis
  - Motility
  - Proliferation
  - Protein localization



Supported by the NIH, Genentech, GalaxoSmithKline and Affymetrix

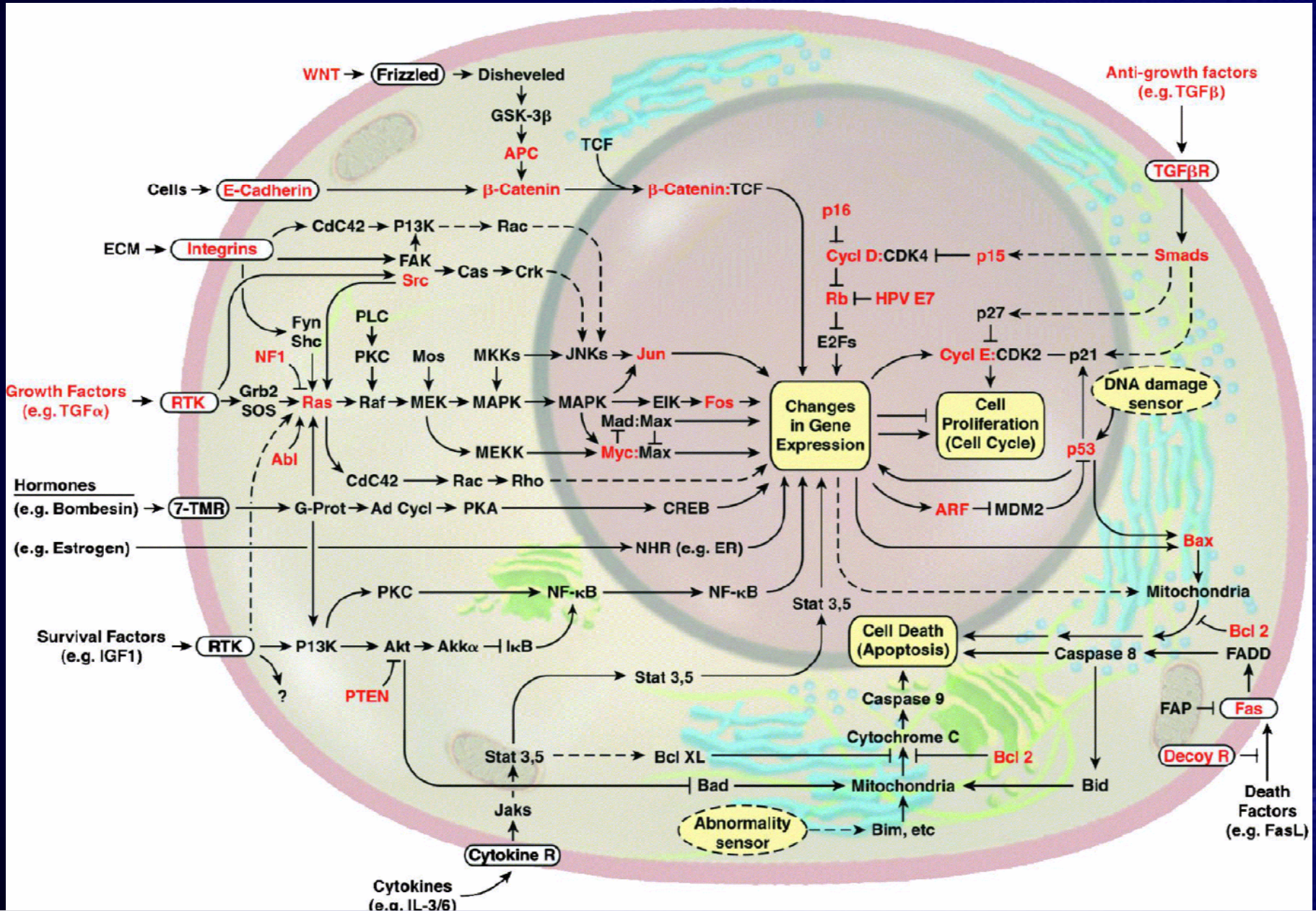
Technological opportunities

Current system is too expensive and slow to test thousands of compounds – Microfluidics and detectors (e.g. Luke Lee at UCB)

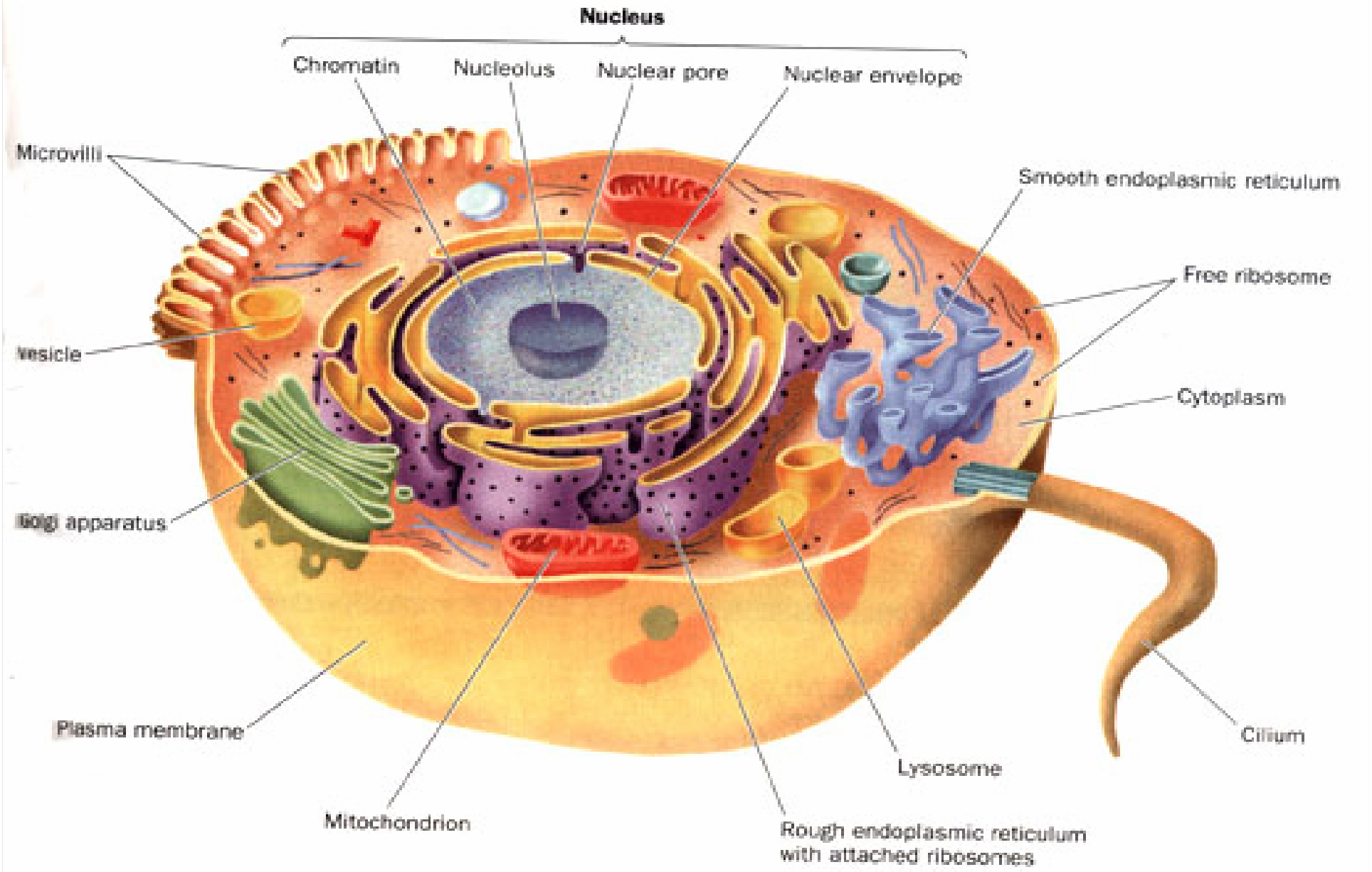


PNAS 2005

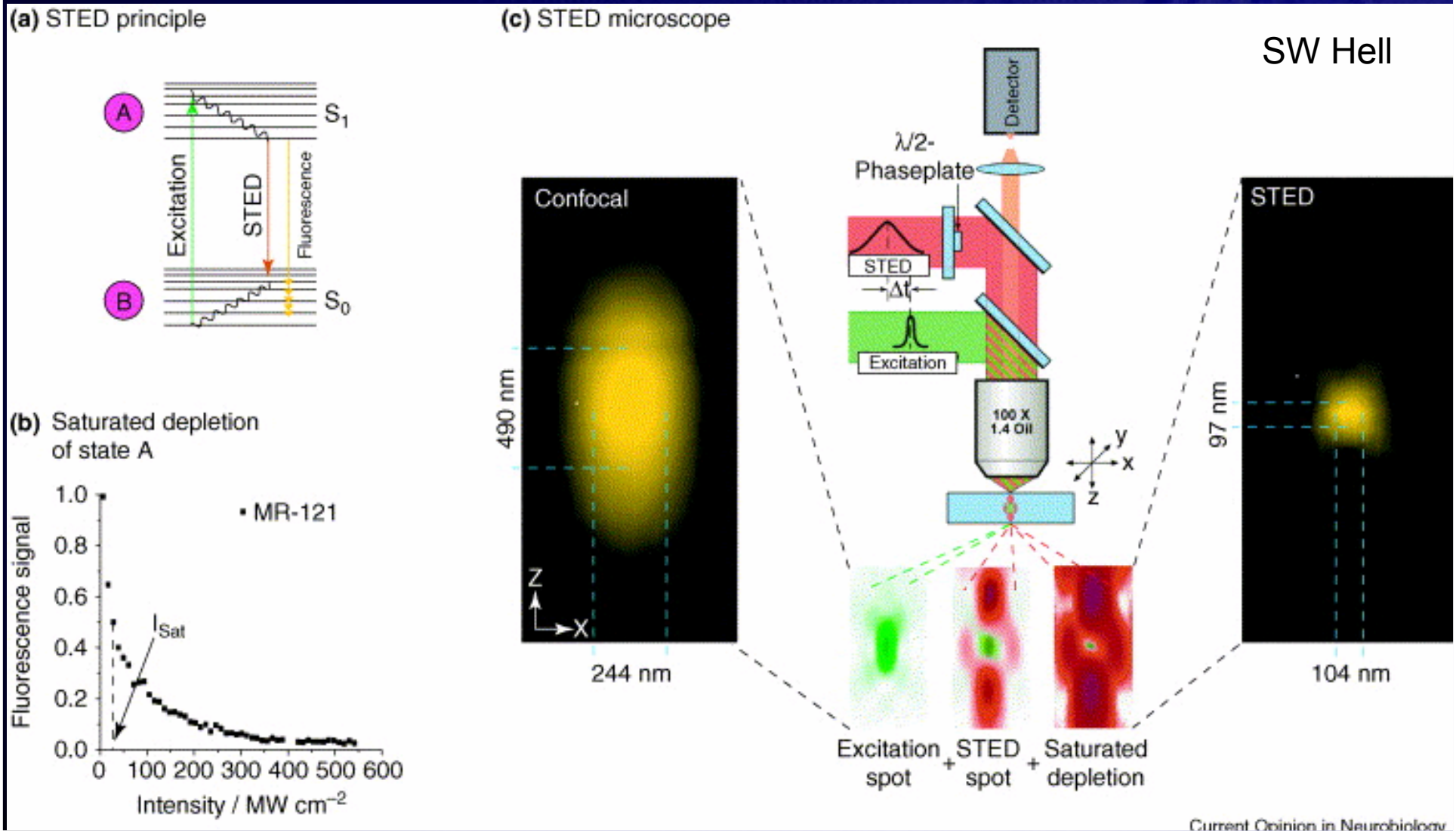
We need to be able to “see” how gene deregulation affects the signaling pathways



Cells are not “bags of chemicals”



Super-resolution microscopy for real time assessment of signal propagation



Conclusions

- Technologies now exist to interrogate the sequence, copy number, structure and expression of essentially all genes
- This information is driving the development of individualized medicine
- Challenges now are to reduce cost, increase analysis speed and enable analysis “in tissue context”