NCCAVS
17 June 2009

Biophysics looking forward from the past

Dr. S Jeffrey Rosner
Agilent Technologies

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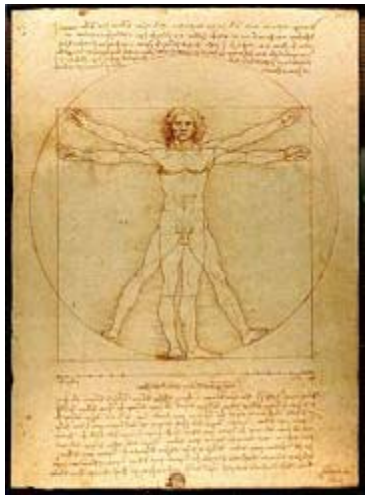
Biophysics is wide and deep – *levels of organization*

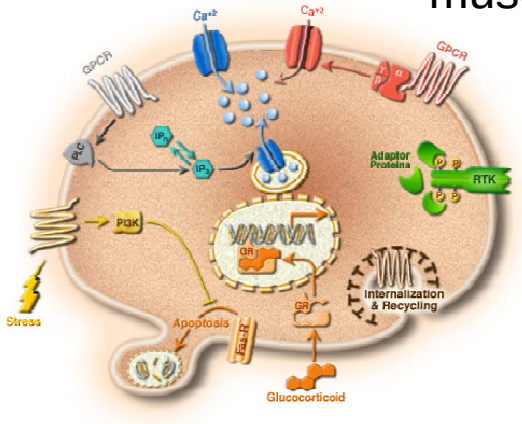
•Organisms…

– how they develop, see, hear, taste, feel and think



•Organs…

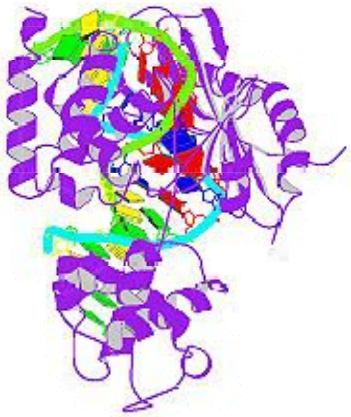
– respiration, material transport, immunology, muscle function, skeletal structure



•Cells…

– move, divide, detect and respond to signals from the environment

– materials travel into and through cells.



•Molecular biology...

– structure and behavior of the biomolecules that make up cells

– ability of molecules to perform complex biological tasks dependent on their three-dimensional shapes and dynamic properties

– the relationship of structure to function is a central question.

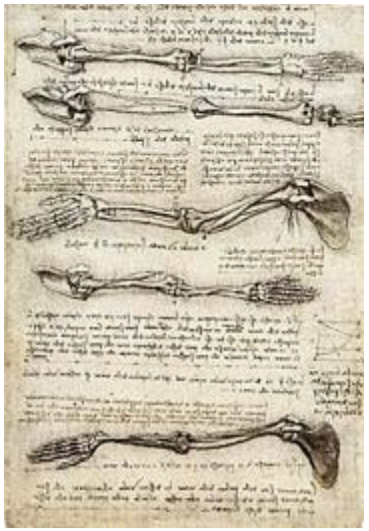
Historical perspective – Greeks to Renaissance

Physical basis of perception

- Galen -2nd century
- Pharmaceutical formulation
- Cataract surgery

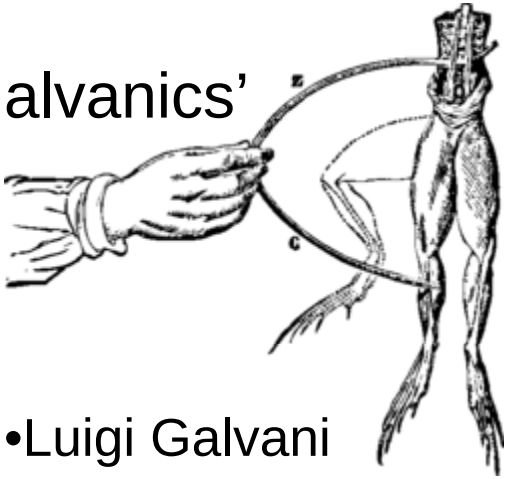
Mechanics

- Leonardo DaVinci
- Proportions and mechanics of human body



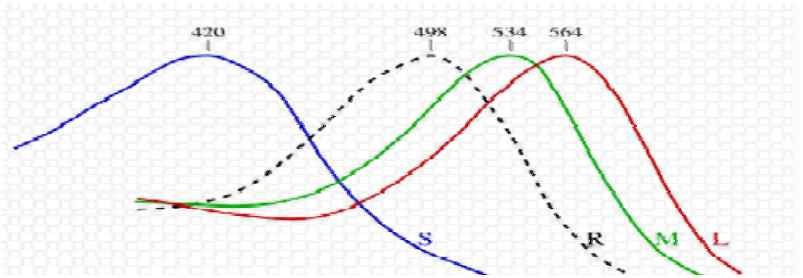
18th century

‘Galvanics’



- Luigi Galvani “animal electricity”
- Physician and physicist

Optics



- Thomas Young
- Tri-chromatic theory of human vision

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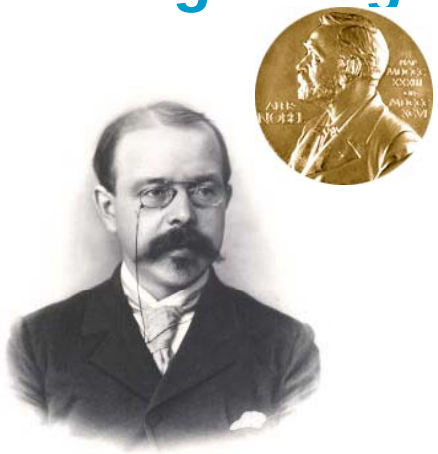
The beginnings of modern biophysics - *19th-20th century*

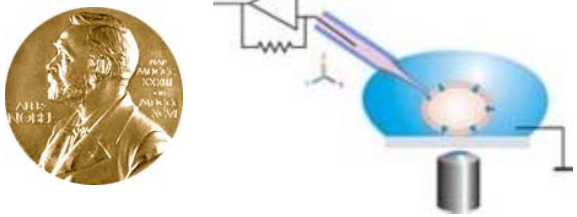


•Walther Hermann Nernst

•Membrane potentials

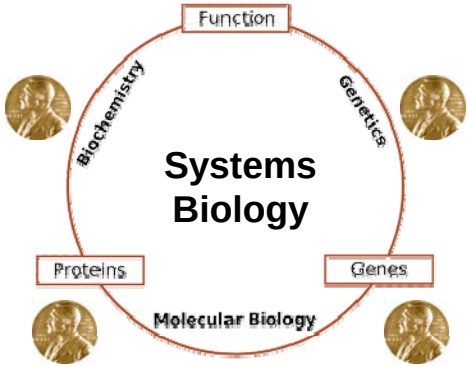
•Ion diffusion

•Origins of Electrophysiology



• Erwin Neher and Bert Sakmann

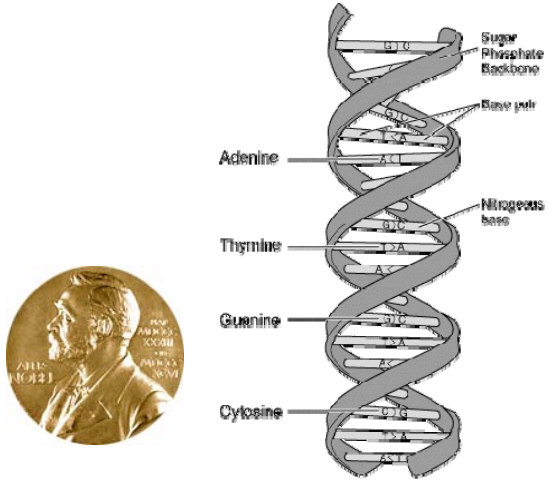
•Patch clamping



•Molecular Biology

•Chemical explanations of life

•Parallel to biophysics



•Watson, Crick and Wilkins

•X-ray diffraction

•DNA / Protein structure

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The continuing emergence of *Quantitative Biology*

Stochastic *(Historical Paradigm)*

•Progress in Life Sciences is increasingly dependent on a *systems level understanding* of functional biological processes.

•Practical progress in

- Pharmaceutical research
- AgBio
- Diagnostics
- Biomedical Measurement Technology

…depends on a fundamental understanding of these processes.

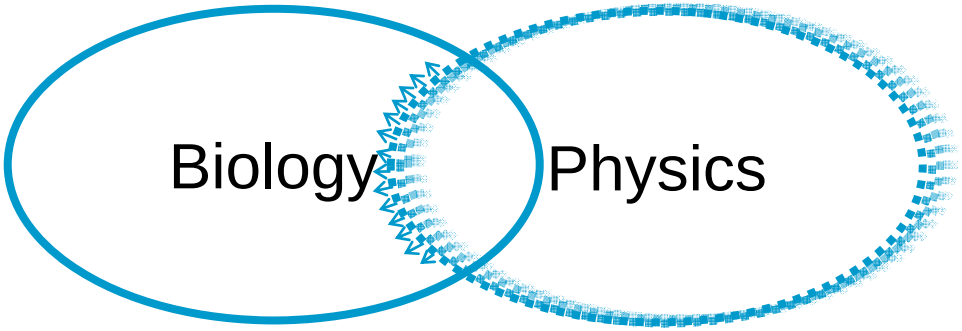
•The current paradigm in system biology is on inferring individual reaction pathways in single cells from data based on statistical populations of reactions in *populations of cells*. This can only generate *average behavior* in an average cell.

Deterministic *(Emerging Approach)*

•Direct measurements of individual *molecules in individual cells* can yield:

- actual reaction behavior as well as population variation.
- a key added dimension of information related to dynamic processes, location, interactions, interaction kinetics.

•Integration of a statistical number of individual measurements can generate a system level view of a biological process from the bottom up.



A merging of disciplines

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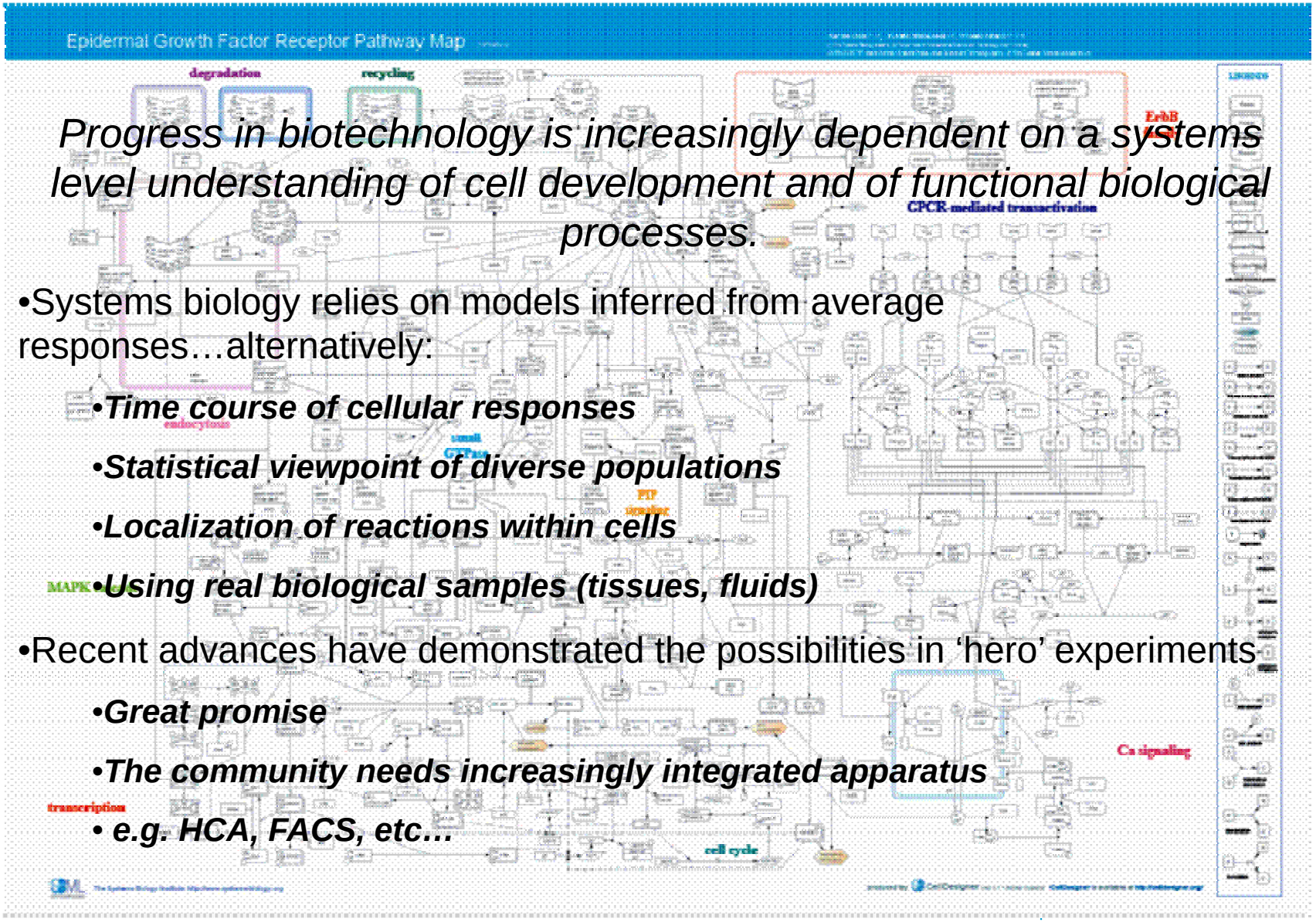
Experimental Systems Biology



Progress in biotechnology is increasingly dependent on a systems level understanding of cell development and of functional biological processes.

- Systems biology relies on models inferred from average responses…alternatively:
  - ***Time course of cellular responses***
  - ***Statistical viewpoint of diverse populations***
  - ***Localization of reactions within cells***
  - ***Using real biological samples (tissues, fluids)***
- Recent advances have demonstrated the possibilities in 'hero' experiments
  - ***Great promise***
  - ***The community needs increasingly integrated apparatus***
  - ***e.g. HCA, FACS, etc…***

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Scaling of biophysics techniques

Scientific demonstration *Probing the boundaries of known science*	Routine experimentation *Transferring capabilities to general scientific practice*	Routine high throughput *Transfer to commercial and large-scale academic pursuits*
•Optical Tweezers •AFM-based molecular recognition •Super-resolution optical microscopy •Mechanical property testing of cells	•Patch Clamping •Multi-variate analysis of high-content screening •Fluorescent biomarkers in histopathology •High performance fluorescence – TIRF, FRET, FRAP, FLIM, …	•Epi Fluorescence •Protein Crystallography •Array-based genomics

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Fluorescence microscopy

•British scientist Sir George G. Stokes first described fluorescence in 1852

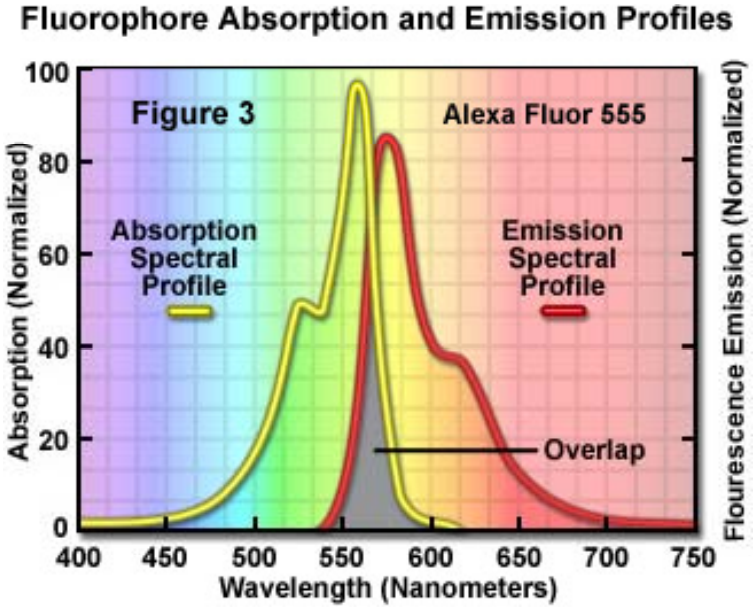
- Coined the term when he observed that the mineral fluorspar emitted red light when it was illuminated by ultraviolet excitation
- Stokes noted that fluorescence emission always occurred at a longer wavelength than that of the excitation light.

•1930s – fluorochromes first used to stain tissue, bacteria, pathogens…drove development of epifluorescence microscope

•70s→advanced techniques for:

time resolution – FRAP, FLIM
spatial resolution – TIRF (z-res)
interaction – FRET

•High Content Analysis/Screening



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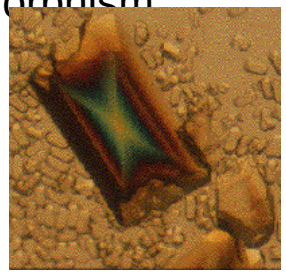
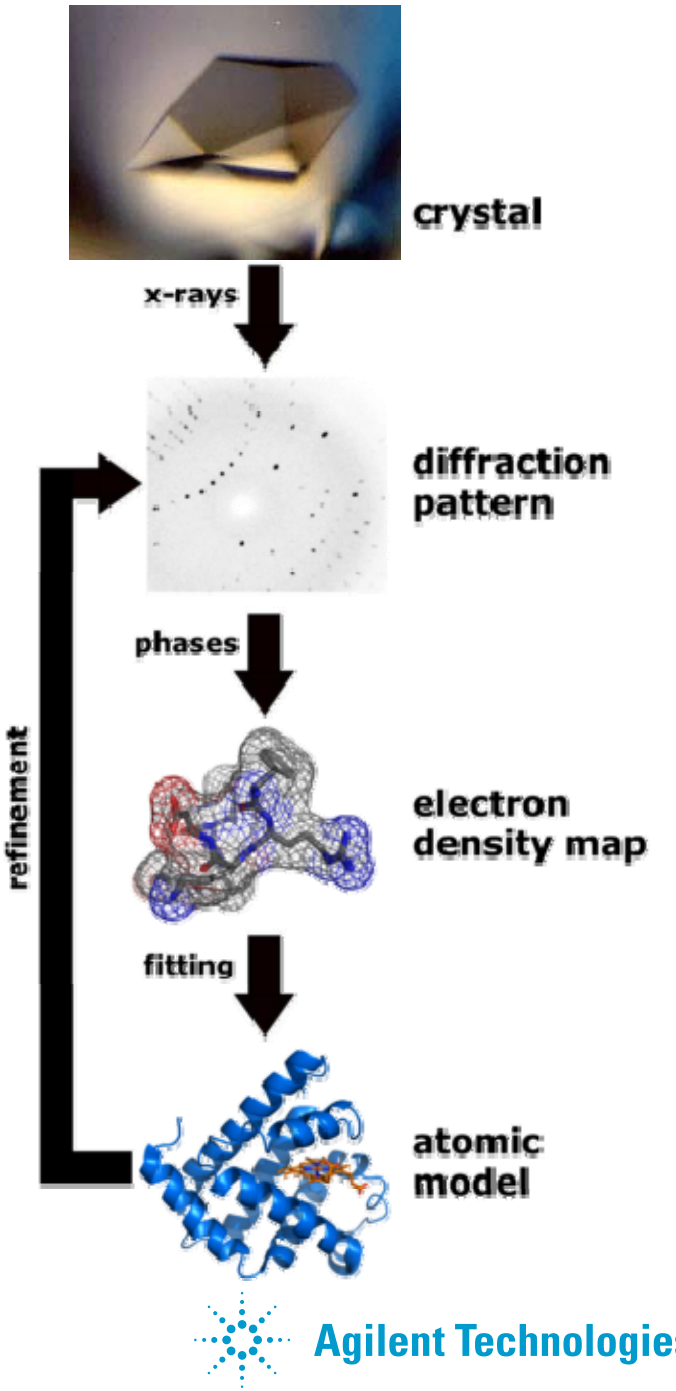
Protein Crystallography

- Nobel Prize in Chemistry, 1962
  - Max Perutz and Sir John Kowdery Kendrew solved structure of sperm whale myoglobin
- Protein structure determination
  - 90% by x-ray diffraction
  - 9% by NMR (includes secondary structure)
  - …dichroism, cryo-electron microscopy, etc..
- Scaling challenges
  - Purification of sufficient volume
  - Some proteins don't like to crystallize – entropy, high conformation flexibility, polymorphism
  - Undisturbed crystallization
    - Environmental control
    - *Space experiments since 1991 (STMV @right)*



  - Computation – accommodation of twinning, polymorphs, etc…
  - Syrrx, Inc., Structural Genomics…automation of the process in the 90s.
    - Location near prime synchrotrons!!!



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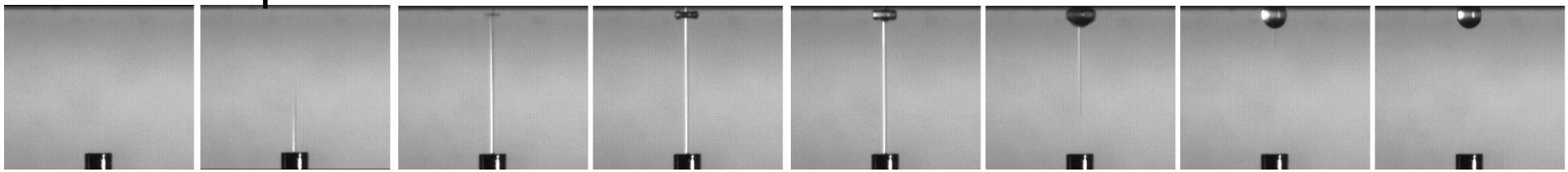
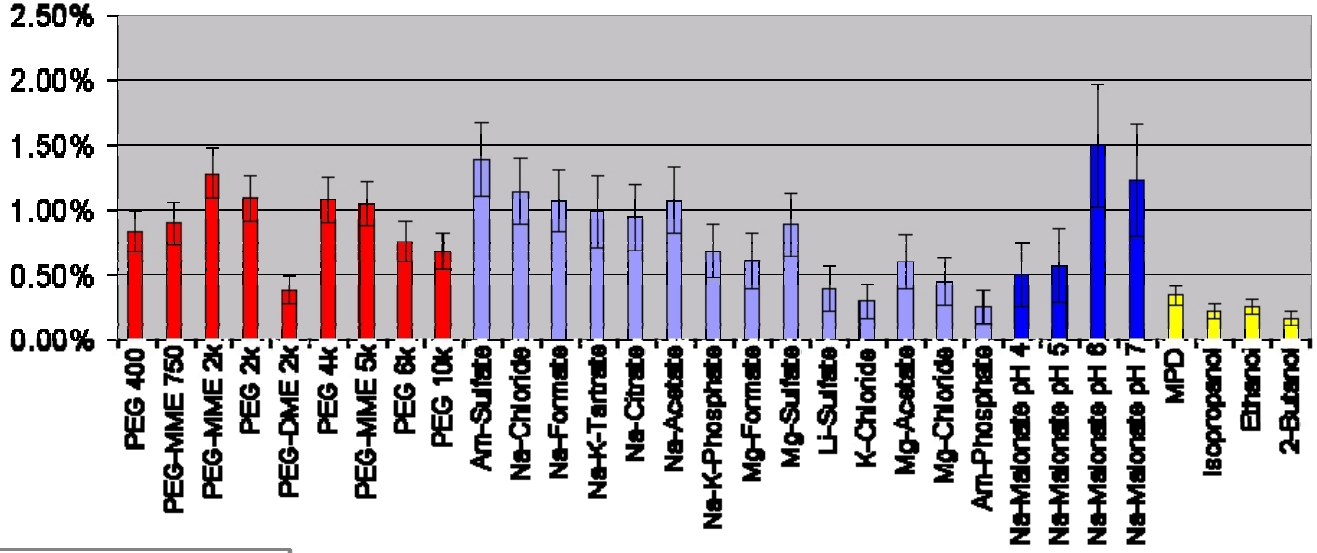
Automation of combinatorial protein crystallization

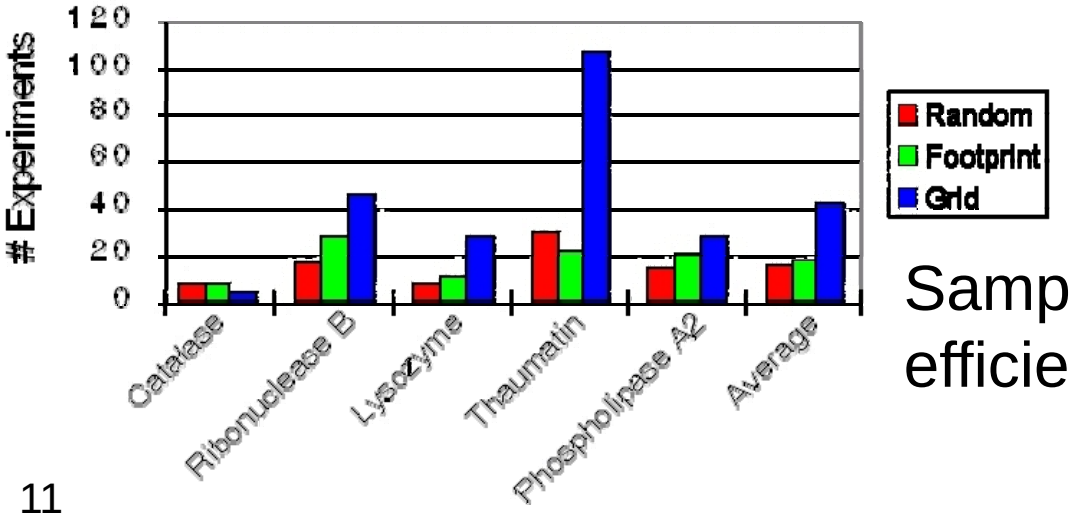
100 nL Dispense at 1000 Frames/Second



Innovadyne Technologies

Solvent sensitivity





Sampling efficiency

A rich set of variables that can be optimized by careful combinatorics

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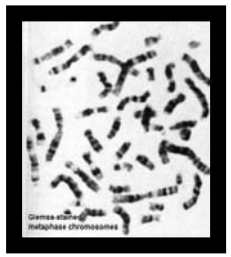
DNA Microarrays Enable Genome-wide DNA Copy Number Measurements

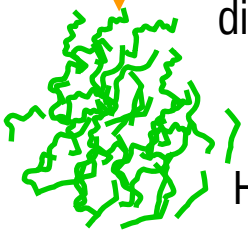


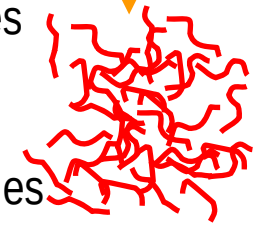
Isolate DNA from cells

Normal genome

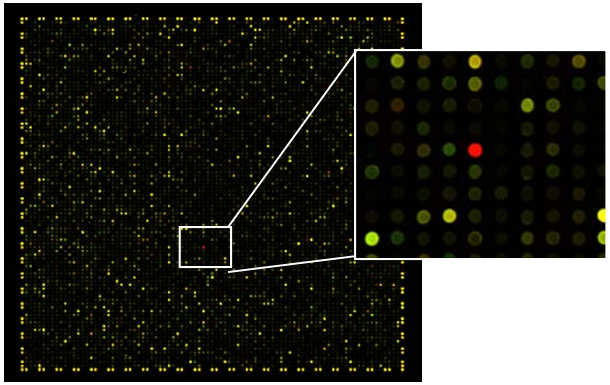
Cancer genome

Label samples with different fluorochromes





Hybridize both samples to microarray

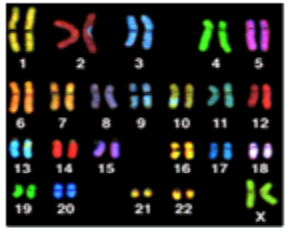


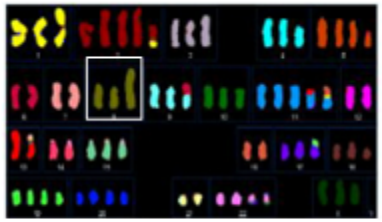
Array- based CGH

Comparative Genomic Hybridization

Cancer

Widespread genomic instability

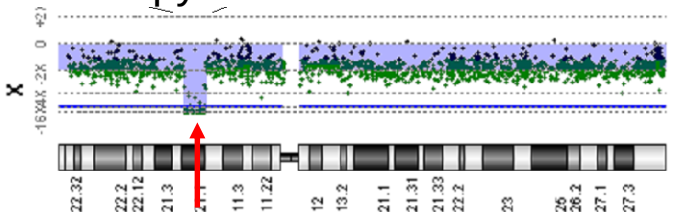




Normal Cancer Genome

Developmental Disorders

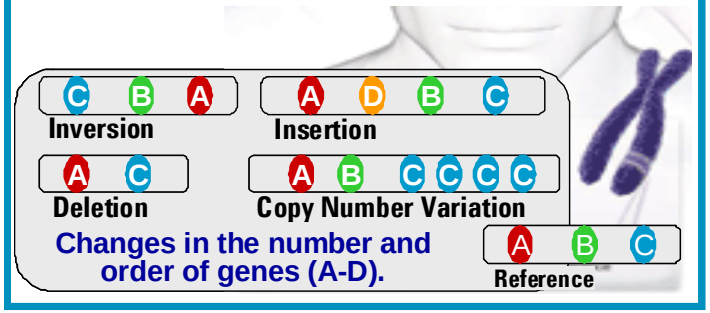
DNA copy number aberrations



Muscular Dystrophy: deletions in the DMD gene on chromosome X

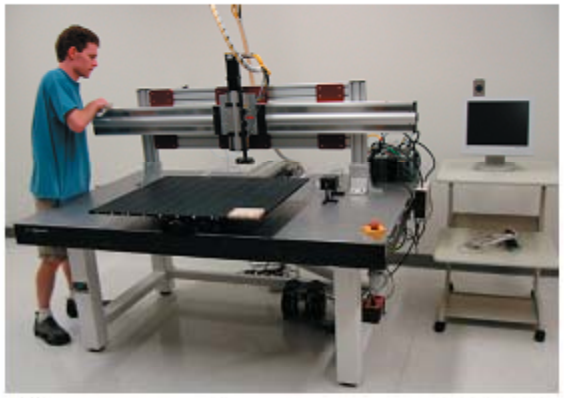
Copy Number Variations

major source of genetic diversity



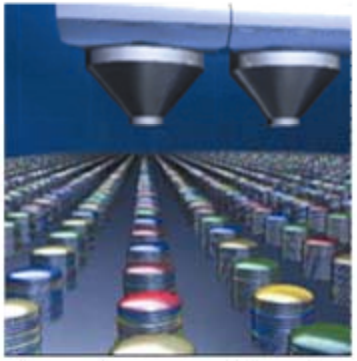
Breakthroughs in DNA Copy Number Measurements

"homebrew" microarray printing



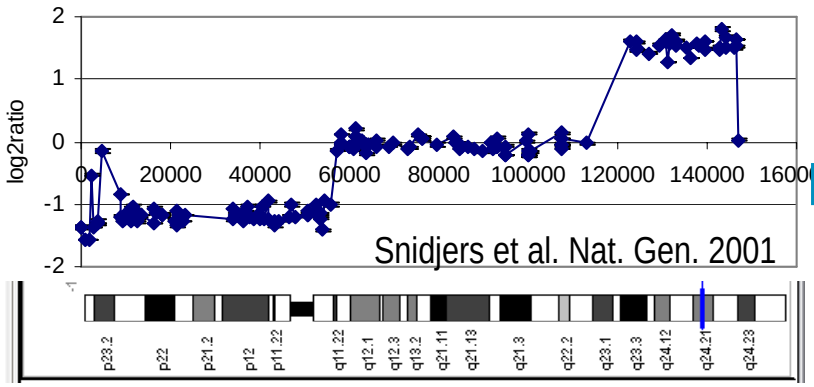
Servo motor-powered DNA microarrayer

Industrialized manufacturing of custom oligonucleotide arrays



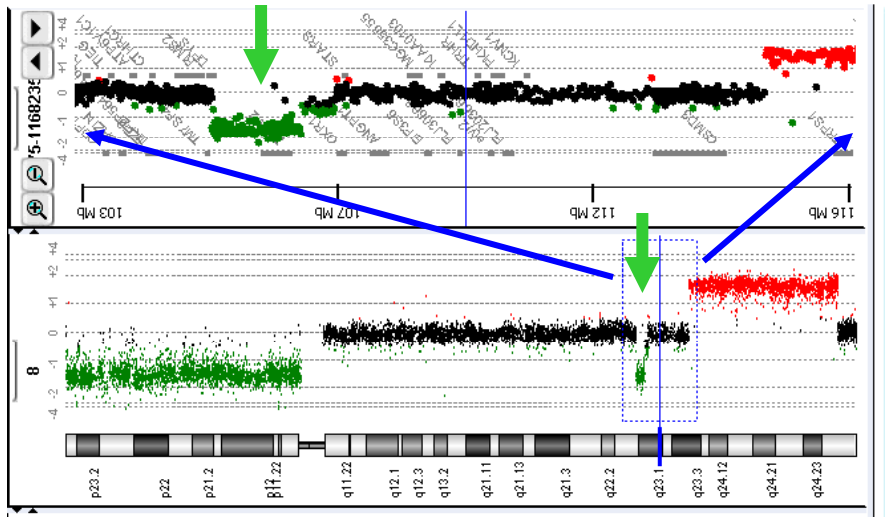
in situ synthesis of oligonucleotides using Ink Jets

2001 BAC array CGH



Copy number losses and gains on chromosome 8 in colon tumor cell line.

2006 Agilent oligo array CGH



Scaling of biophysics techniques

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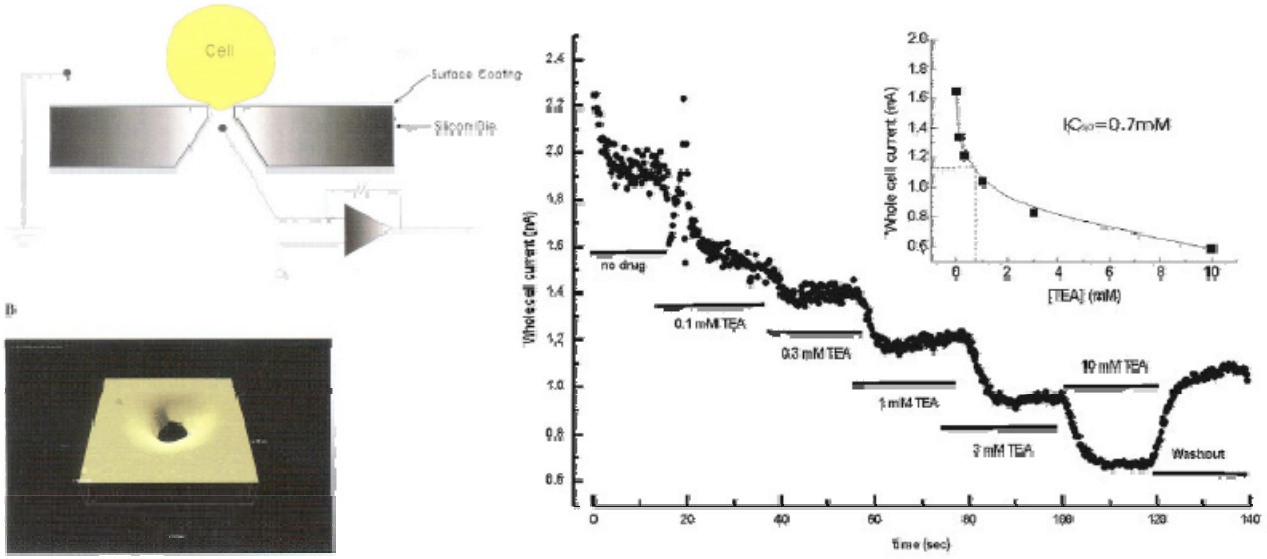
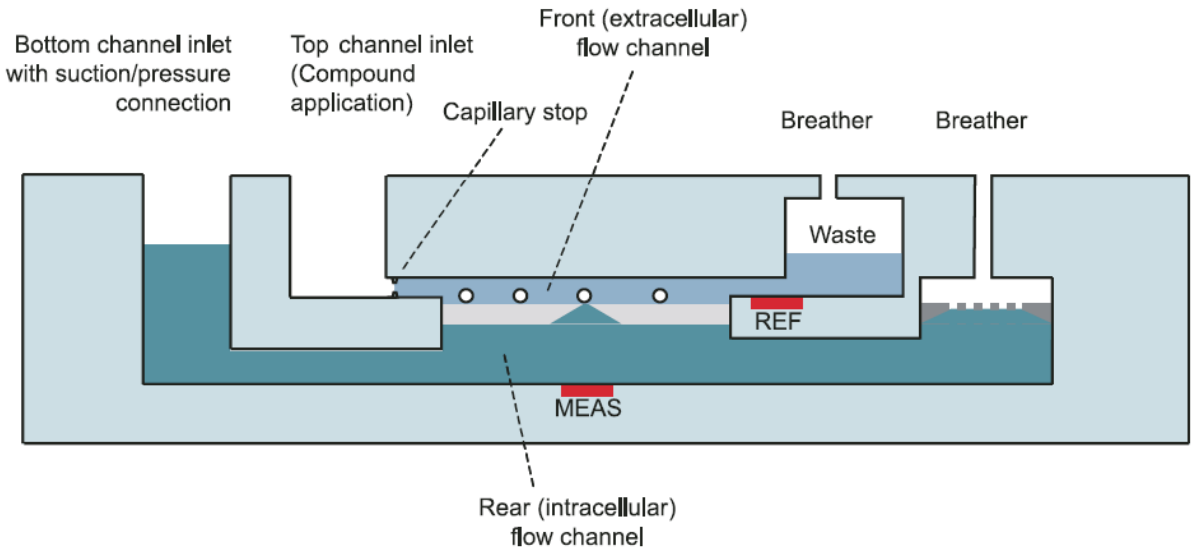
Automated patch clamping

Sophion Biosciences



- High thruput
- Parallelism
- Excellent quality of measurement



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Multi-variate analysis

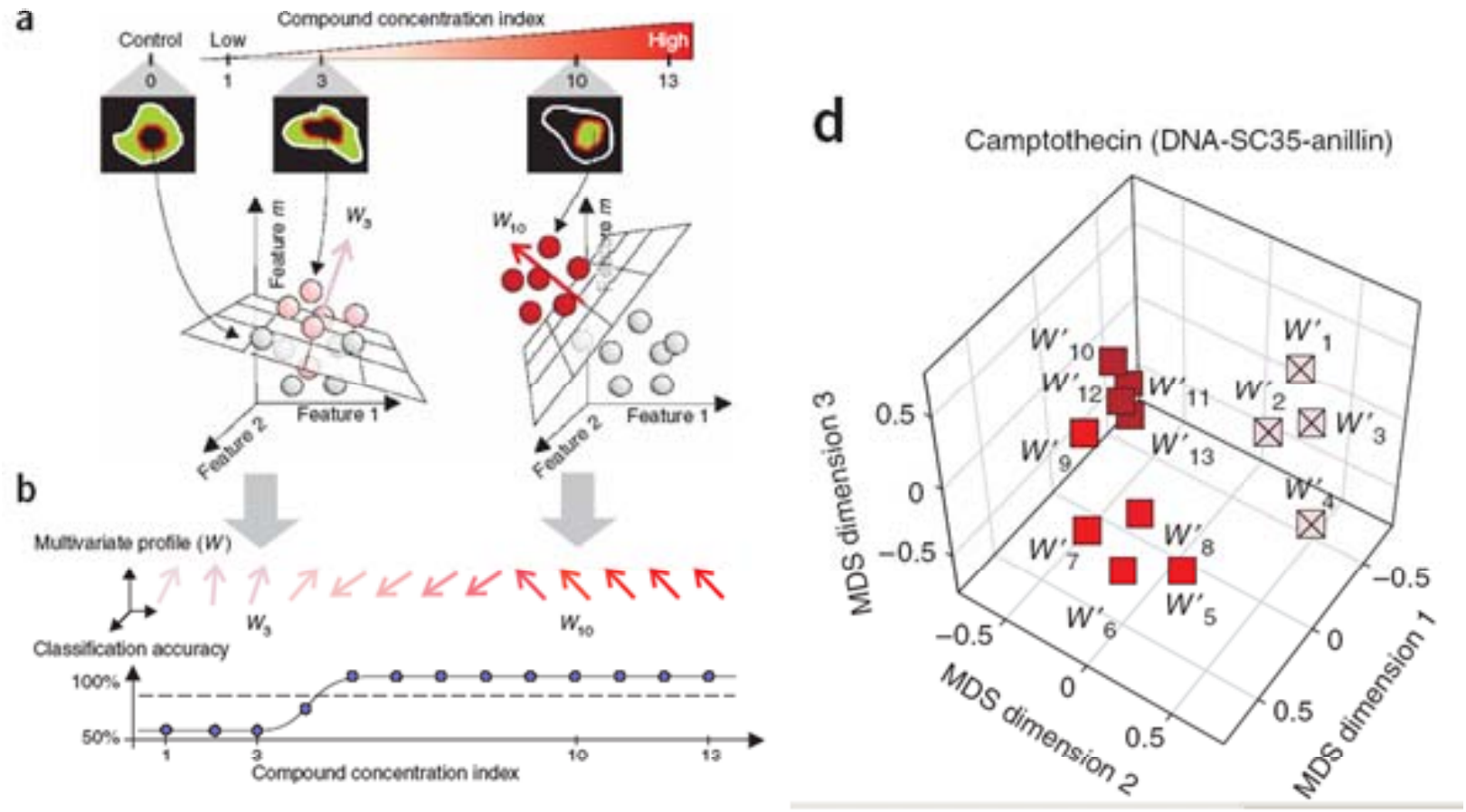

Image-based multivariate profiling of drug responses from single cells
Lit-Hsin Loo, Lani F Wu & Steven J Altschuler
NATURE METHODS | VOL.4 NO.5 | MAY 2007

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Fluorescent biomarkers in histopathology

PathVysion®

Abbott Molecular

Abbott
A Promise for Life

FDA Approved

Product Description

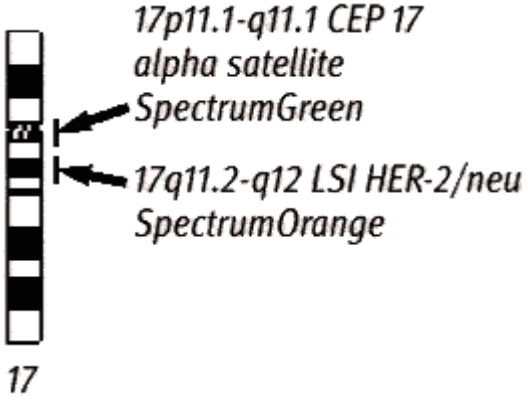
Vysis® LSI® HER-2/neu	17q11.2-12	SpectrumOrange™
Vysis CEP™ 17	17p11.1-q11.1 Alpha Satellite DNA	SpectrumGreen™

Intended Use

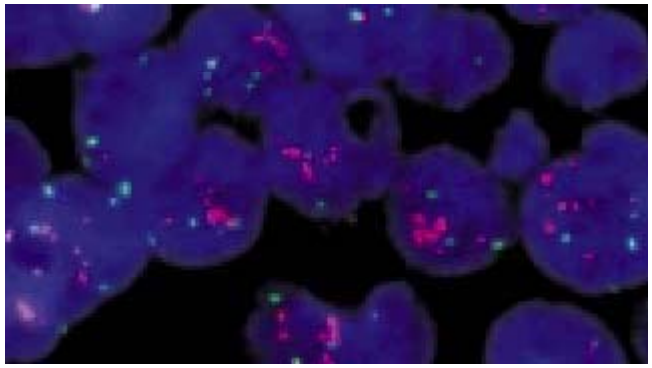
The PathVysion HER-2 DNA Probe Kit (PathVysion Kit) which is FDA approved is designed to detect amplification of the HER-2/neu gene via fluorescence in situ hybridization (FISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens. Results from the PathVysion Kit are intended for use as an adjunct to existing clinical and pathologic information currently used as prognostic factors in stage II, node-positive breast cancer patients. The PathVysion Kit is further indicated as an aid to predict disease-free and overall survival in patients with stage II, node positive breast cancer treated with adjuvant cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) chemotherapy.

The PathVysion Kit is indicated as an aid in the assessment of patients for whom HERCEPTIN® (Trastuzumab) treatment is being considered (see HERCEPTIN package insert).

HER-2/neu, also known as c-erbB2 or HER-2, is a gene that has been shown to play a key role in the regulation of cell growth. The gene codes for a 185 kd transmembrane cell surface receptor that is a member of the tyrosine kinase family. HER-2 has been shown to be amplified in human breast, ovarian, and other cancers.



Antibody based markers are now entering the market after FDA approval, primarily for guidance in therapeutics…



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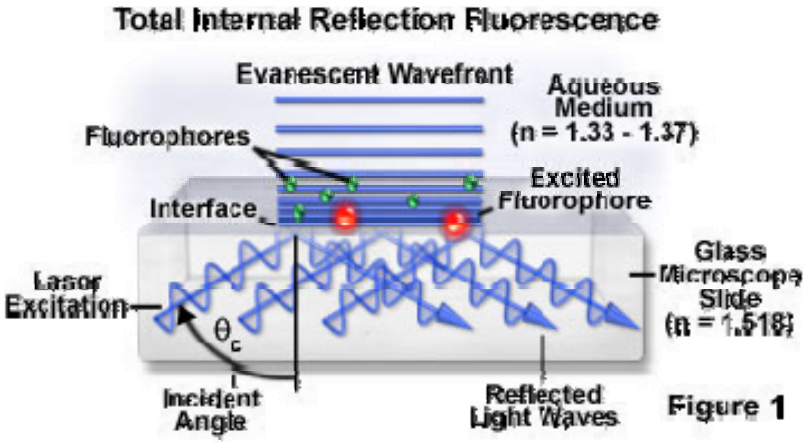
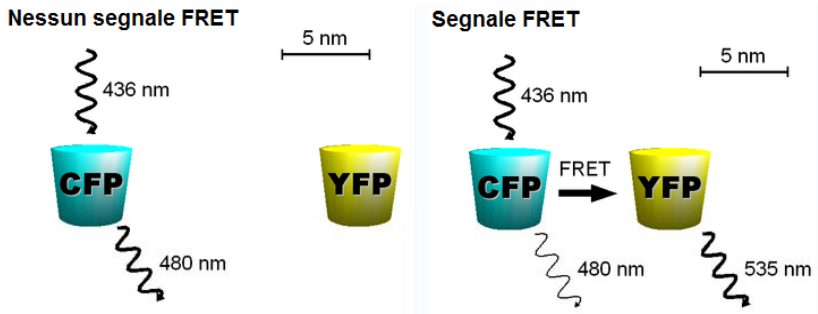
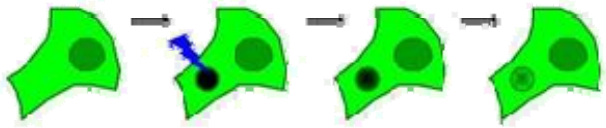
High Performance Fluorescence

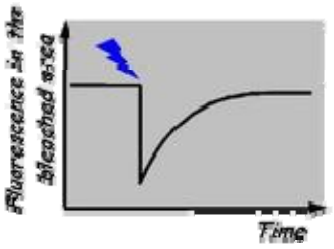
- **TIRF**-Total internal reflection fluorescence
  - Examine the material attached to a surface using the evanescent field
- **FRET**-Fluorescence resonance energy transfer
  - Fluorescence that can identify proximity of single molecules
- **FRAP**-Fluorescence recovery after photobleaching
  - Measure diffusion rates of molecules to which fluorophores are attached
- **FLIM**-Fluorescence lifetime imaging
  - Provides information about the local environment and specific fluorophores

. . .









FRAP

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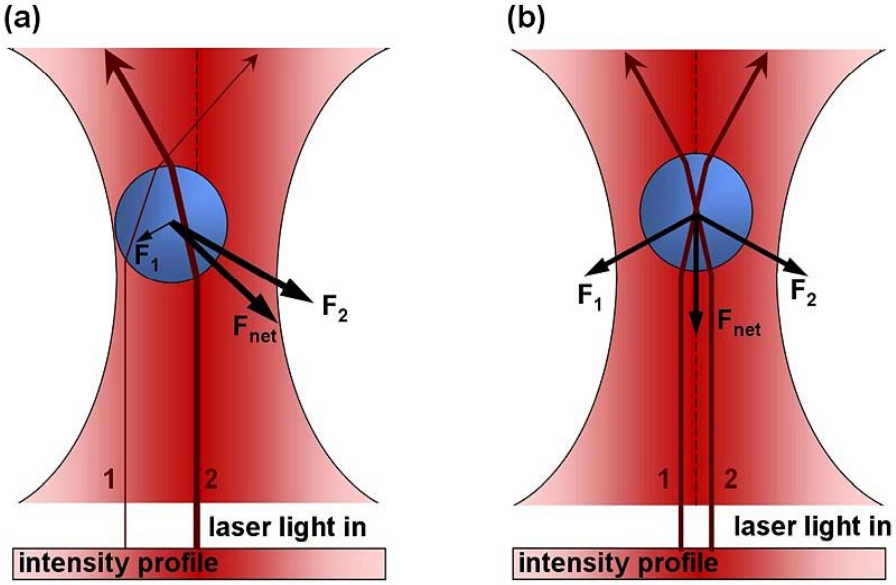
Optical Tweezers



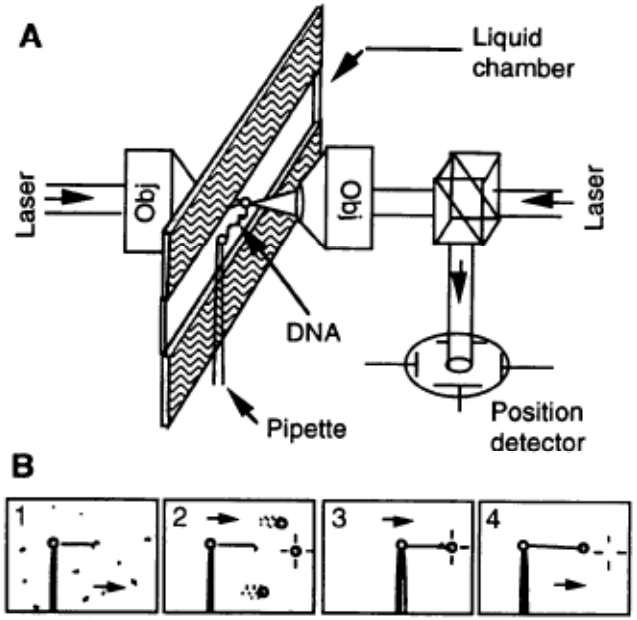
Ray optics explanation.

- ***When the bead is displaced from the beam center, as in (a), the larger momentum change of the more intense rays cause a net force to be applied back toward the center of the trap.***
- ***When the bead is laterally centered on the beam, as in (b), the net force points toward the beam waist.***

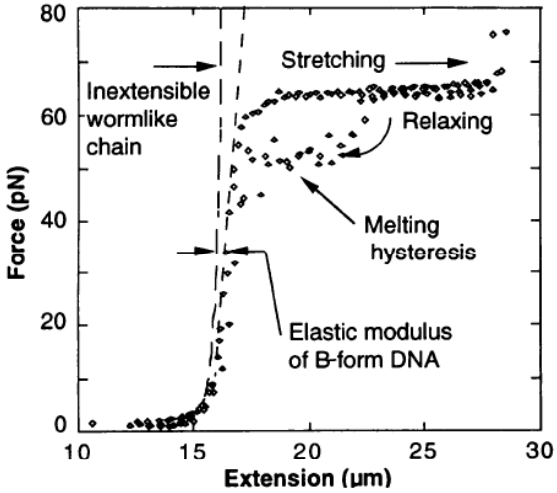
Applications

- ***Biophysics – the most prominent application, it takes advantage of the fact that the force applied can be calibrated, resulting in a capability to measure nanoscale forces well below the levels of mechanical techniques like AFM***
- ***Manipulation – many practical uses of optical tweezers are less celebrated, such as manipulation of nanoparticles and tweezers for various electronic, mechanical, chemical applications. Holographic techniques using spatial light modulators have resulted in highly multiplexed arrays of individually addressable 'tweezers', e.g. Arryx.***

Optical tweezers – premier applications



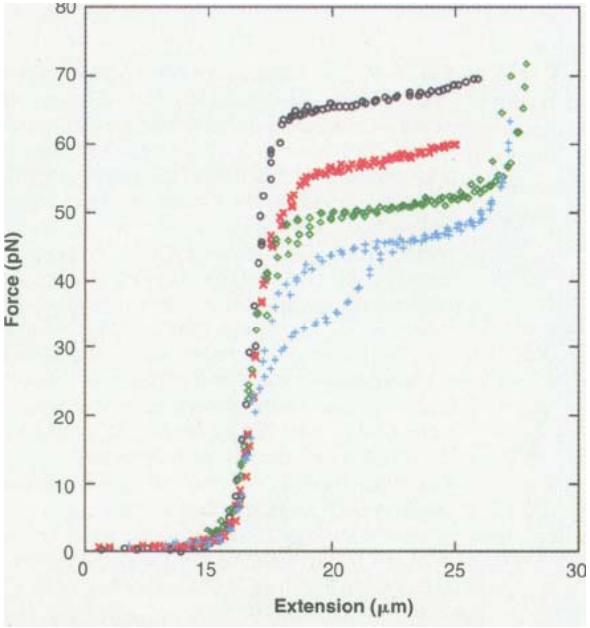
This experiment was published over 10 years ago, and yet this technique is still not widespread…why?



Overstretching B-DNA: The Elastic Response of Individual Double-Stranded and Single-Stranded DNA Molecules

Steven B. Smith, Yujia Cui, Carlos Bustamante*

SCIENCE • VOL. 271 • 9 FEBRUARY 1996



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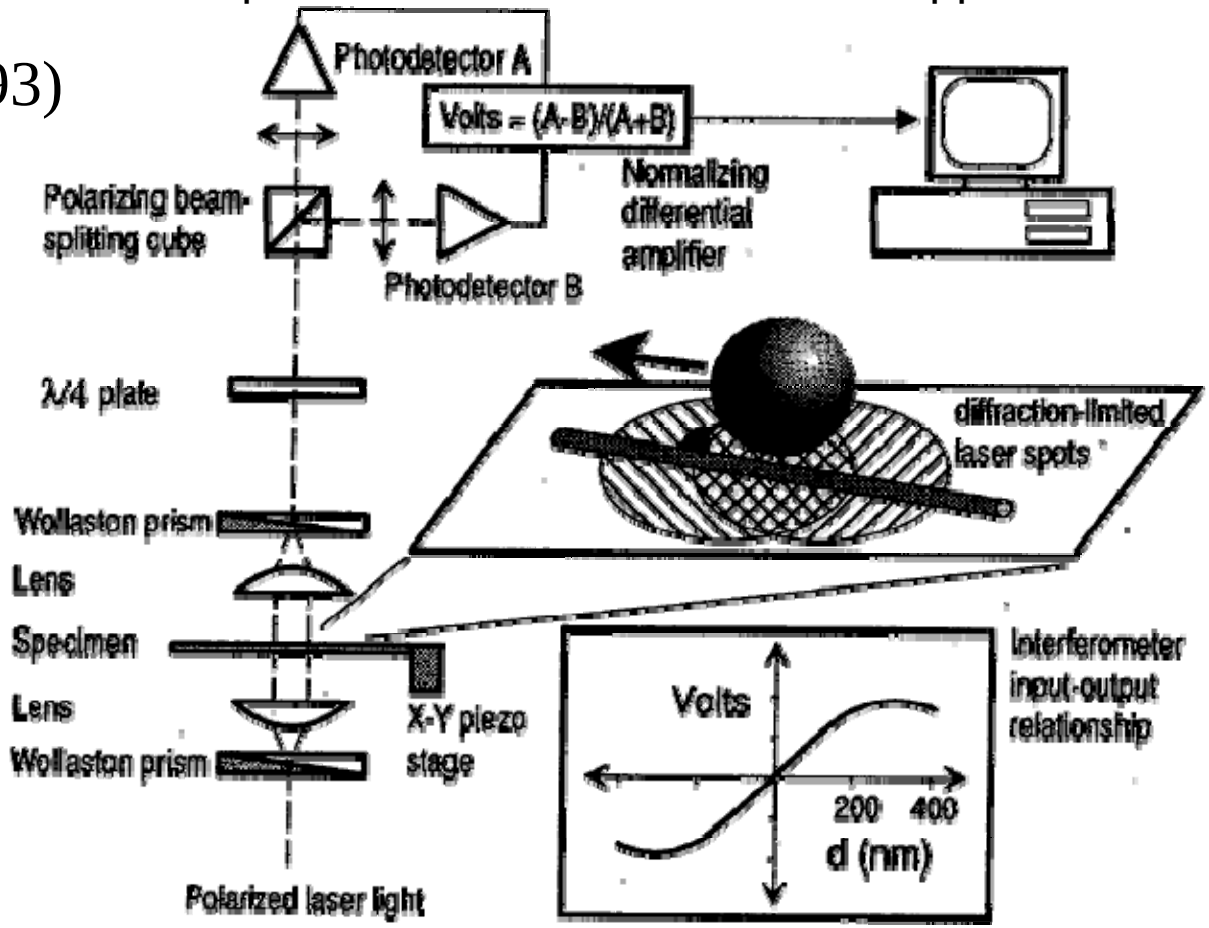
Optical tweezers – premier applications

Direct observation of kinesin stepping by optical trapping interferometry

Karel Svoboda*†, Christoph F. Schmidt*‡, Bruce J. Schnapps & Steven M. Block*

Nature (1993)



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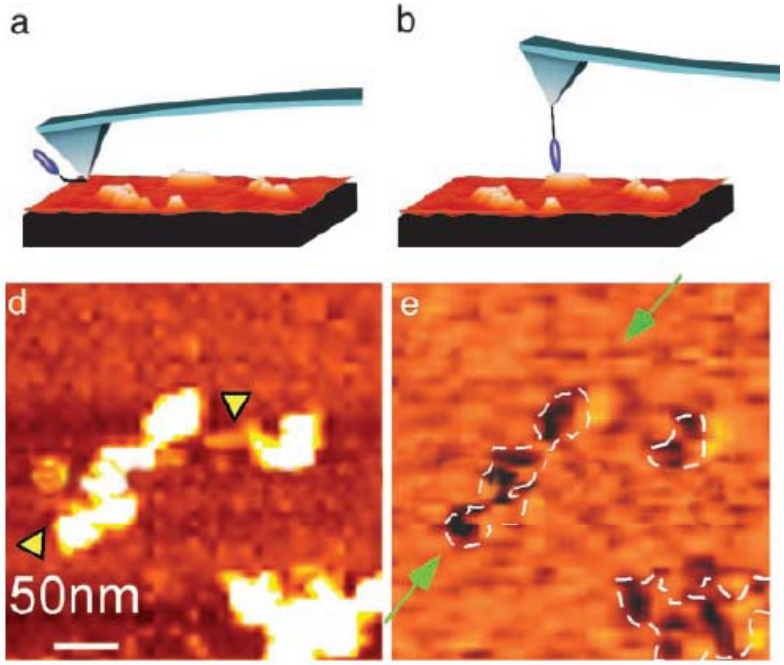
Single-molecule recognition imaging microscopy

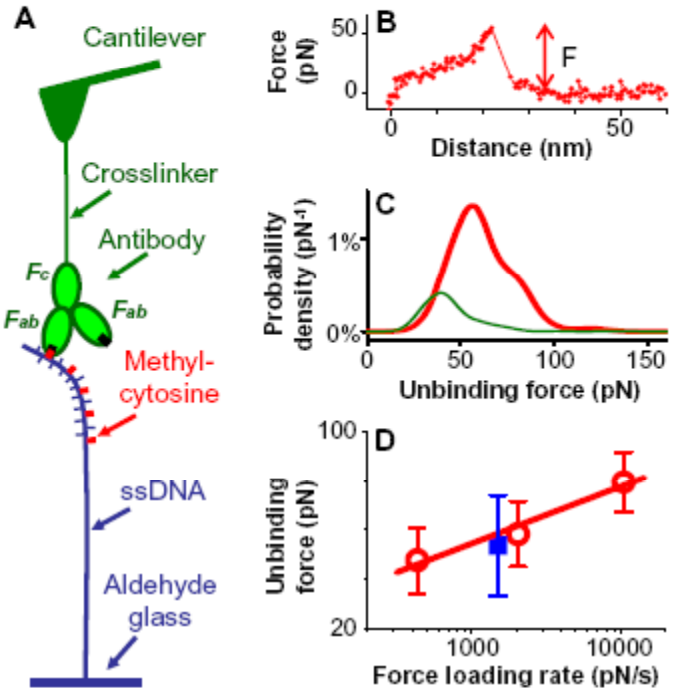
C. Stroh*†, H. Wang†‡, R. Bash‡§, B. Ashcroft‡, J. Nelson¶, H. Gruber*, D. Lohr§, S. M. Lindsay‡§‖**, and P. Hinterdorfer*

Departments of ‡Physics and Astronomy, and of §Chemistry and Biochemistry, and ‖Biodesign Institute, Arizona State University, Tempe, AZ 85287; *Institute for Biophysics, University of Linz, 4040 Linz, Austria; and ¶Molecular Imaging Corporation, 4666 South Ash Avenue, Tempe, AZ 85282



PNAS 2004

Science 2005



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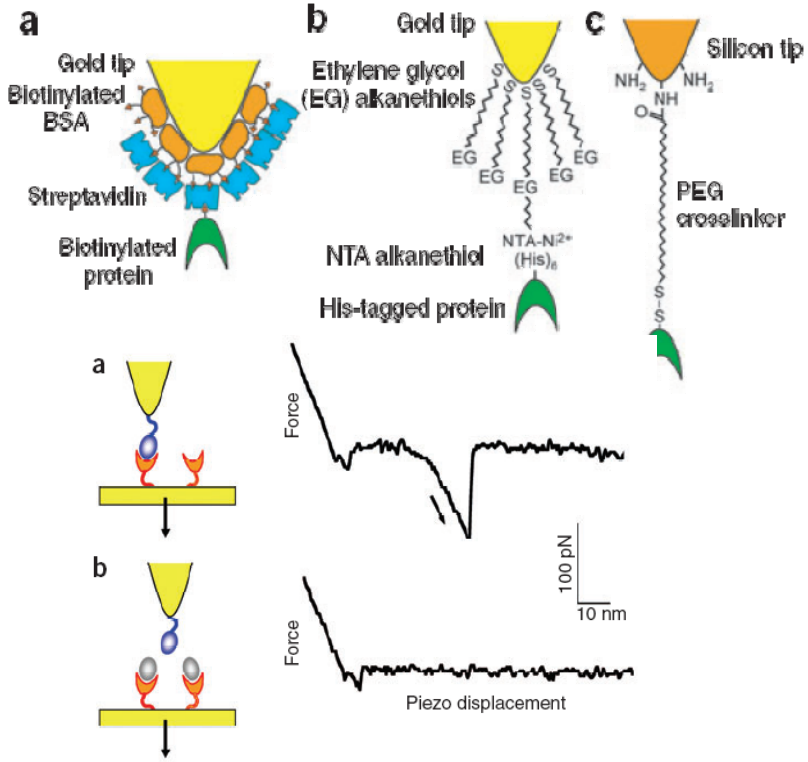
TRec *(simultaneous Topography and Recognition)*

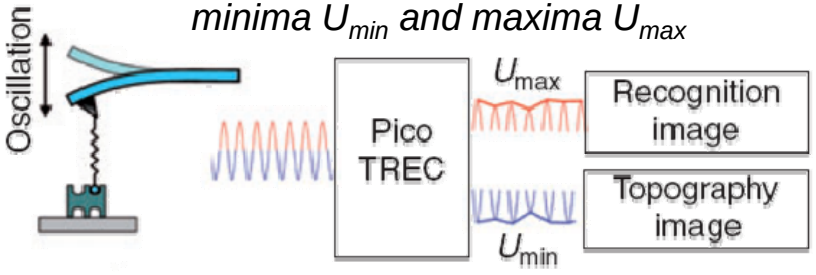
Common surface chemistries used for modifying AFM tips for single-molecule recognition studies.

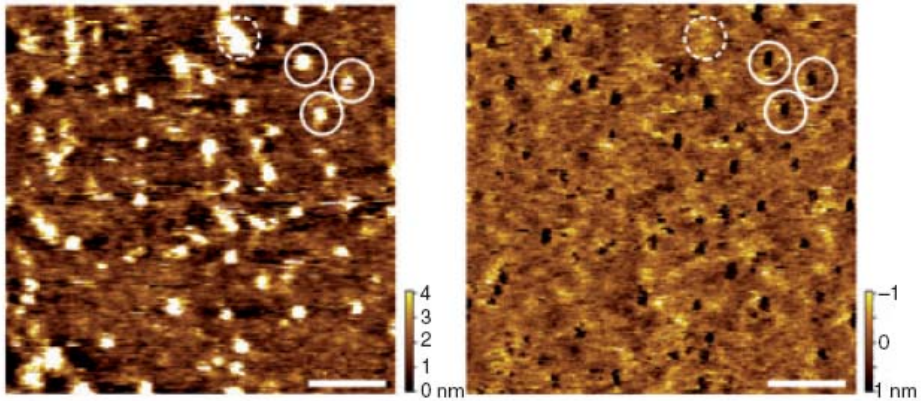


*Measurement of molecular recognition interaction forces. (**a**) Typical force-displacement curve (**b**) Blocking experiment demonstrating that the unbinding force is not observed.*

Hinterdorfer, Dufrene, Nature Methods, May 2006

Simultaneous topography and recognition imaging (TREC). The cantilever oscillation signal is split into minima U_{min} and maxima U_{max}





Singly distributed avidin molecules imaged with a biotin-tethered tip. The bright dots 2 to 3 nm in height and 15 to 20 nm in diameter visible in the topography image (left, solid circles) are single avidin molecules, and the black dots of the recognition image (right) arise from a decrease of the oscillation maxima that result from the physical avidin-biotin connection during recognition.

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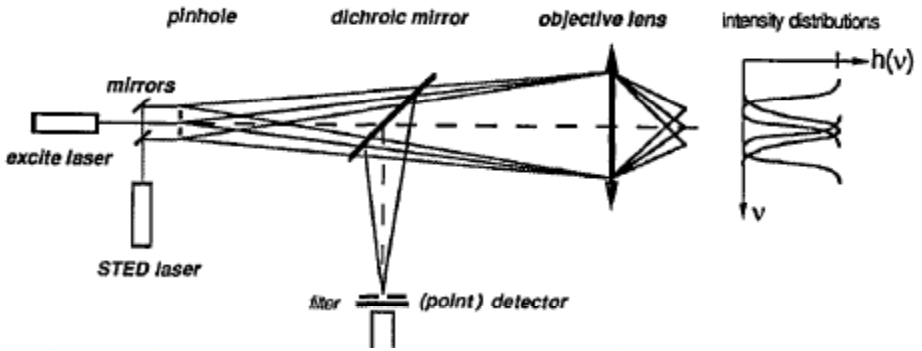
Super-resolution optical microscopy

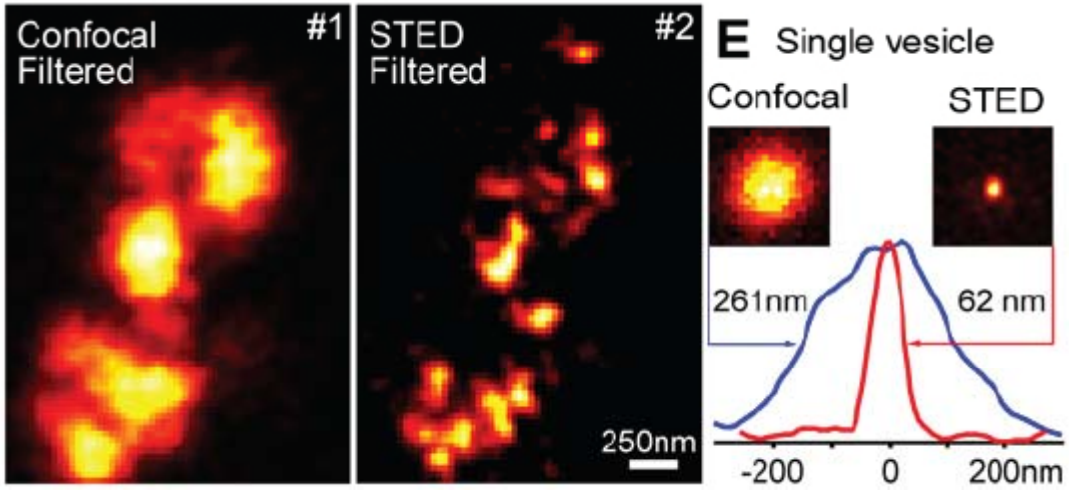
OPTICS LETTERS / Vol. 19, No. 11 / June 1, 1994

Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy

Stefan W. Hell and Jan Wichmann

Department of Medical Physics, University of Turku, Tykistökatu 6, 20521 Turku, Finland



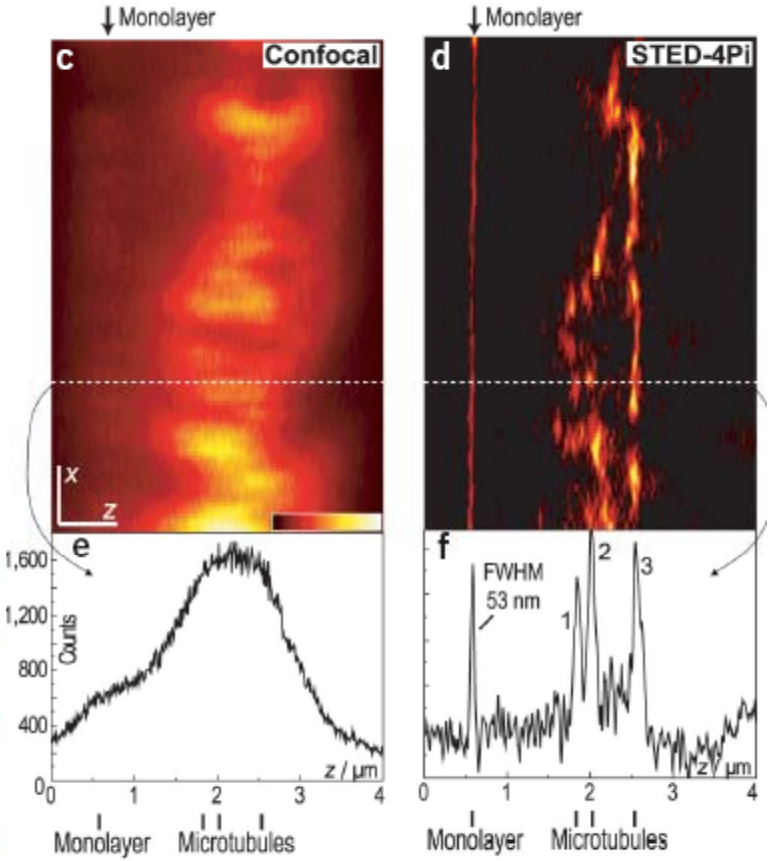


Sciencexpress / www.sciencexpress.org / 21 February 2008

nature biotechnology October 2003

Immunofluorescence stimulated emission depletion microscopy

Marcus Dyba, Stefan Jakobs & Stefan W Hell



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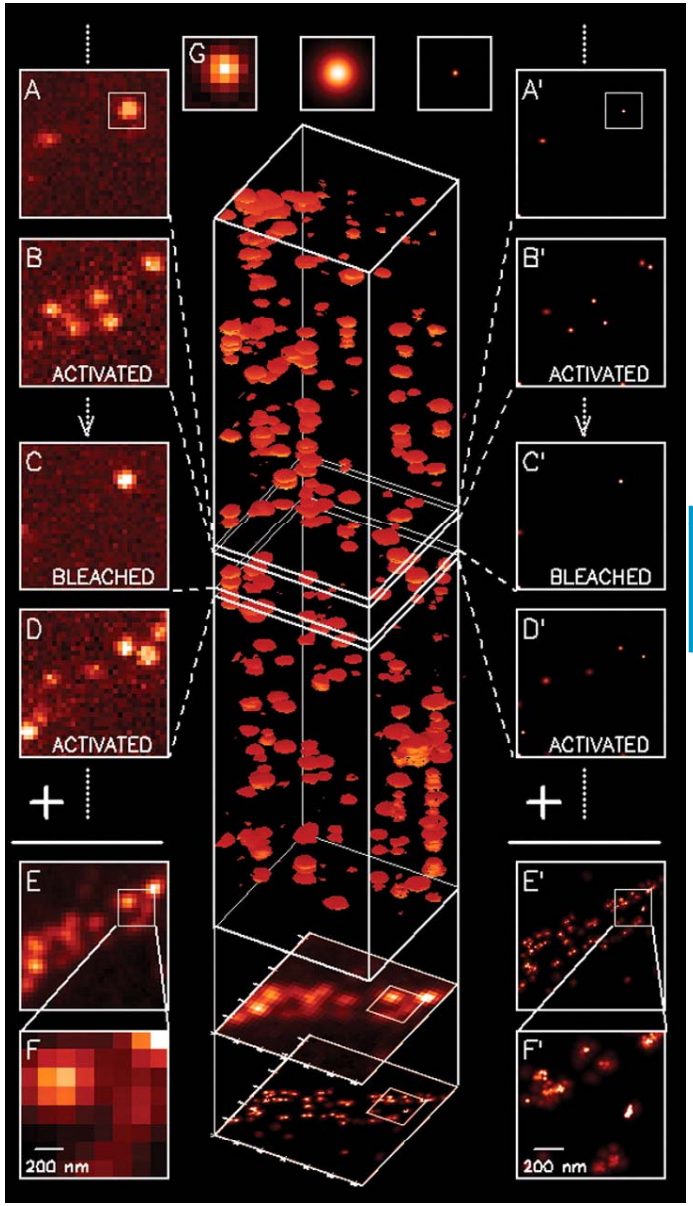
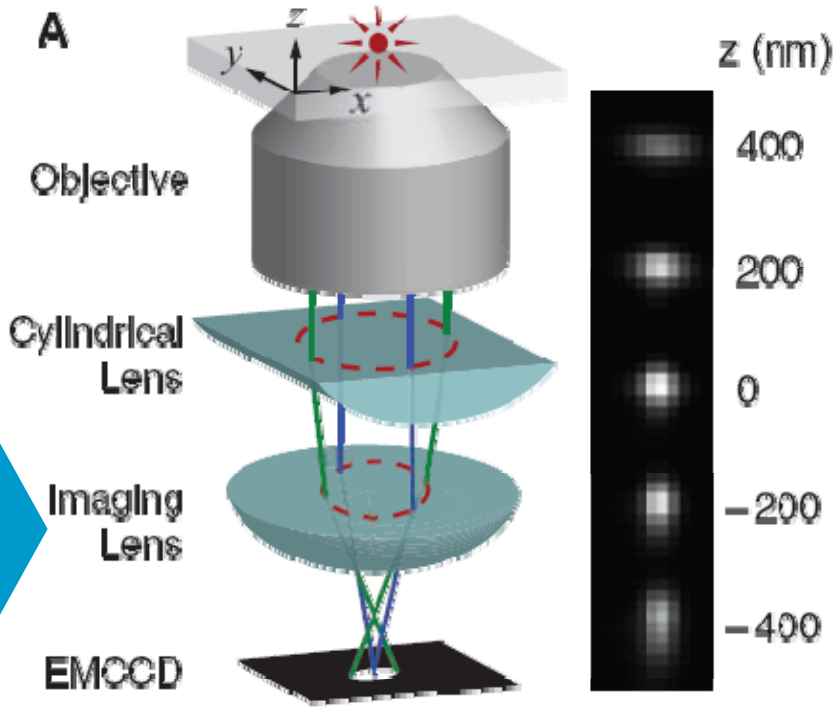
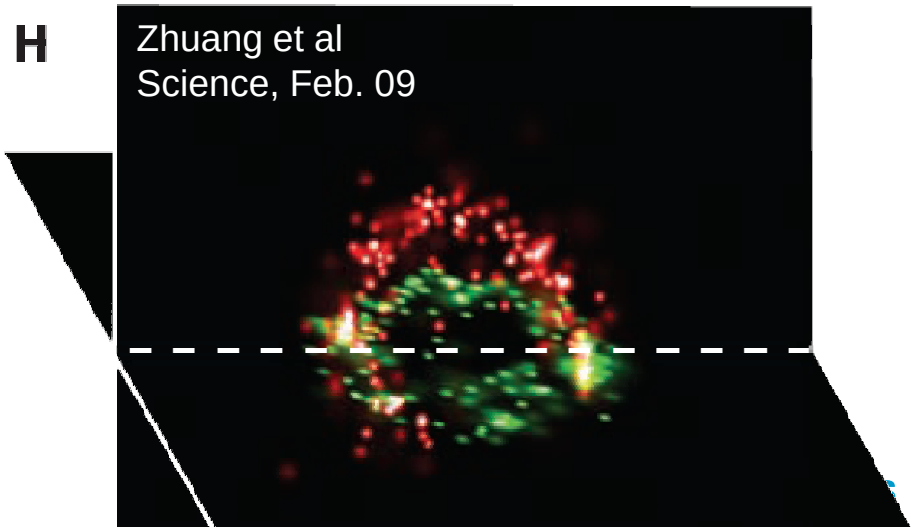
Super-resolution localization microscopy



Betzig/Hess et al
Science, Sept. 06

Increased content





Mechanical properties of cells

Nanomechanical analysis of cells from cancer patients

nature nanotechnology |

SARAH E. CROSS[1,2†], YU-SHENG JIN[3†], JIANYU RAO[3*†] AND JAMES K. GIMZEWSKI[1,2*†]

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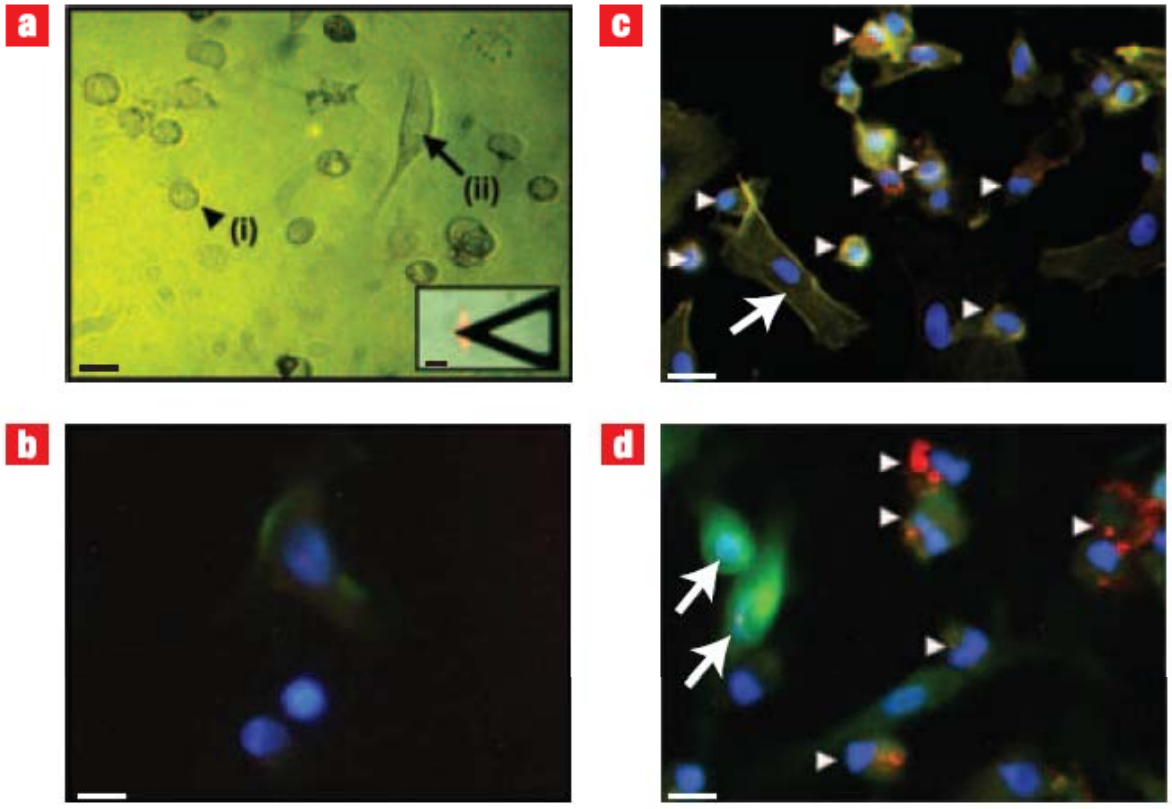
VOL 2 | DECEMBER 2007 |



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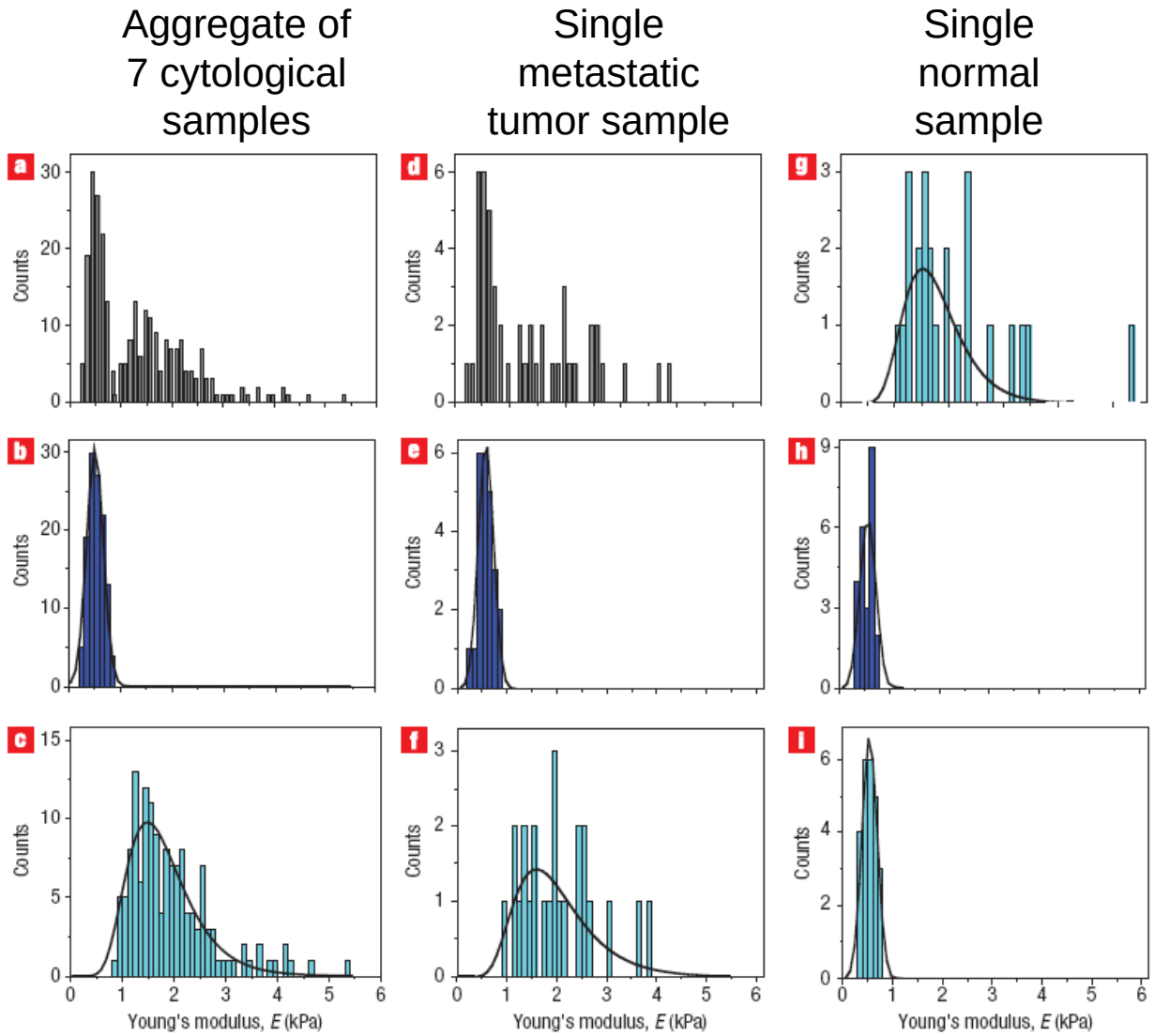
Mechanical properties of cells



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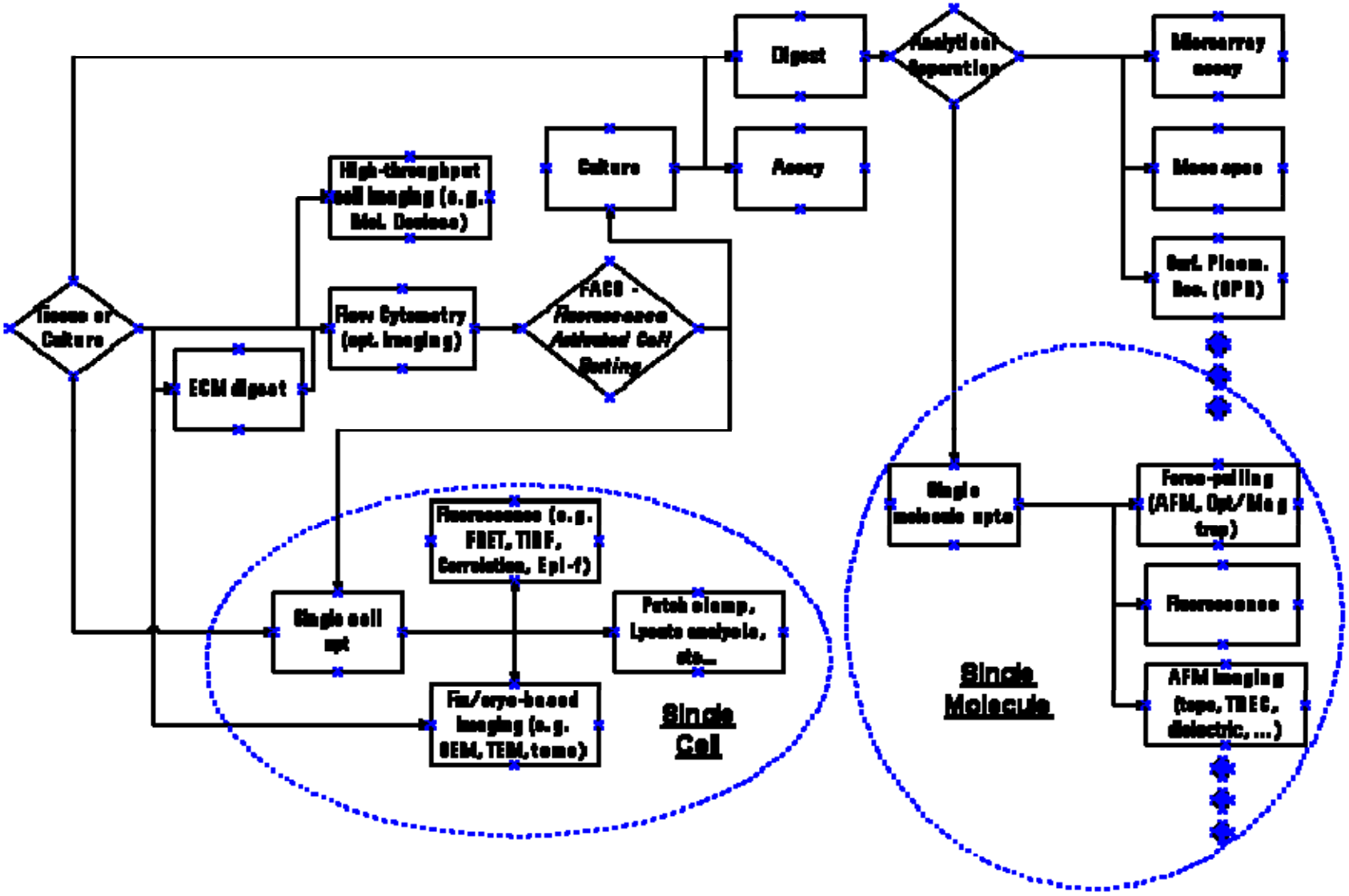
Biophysics workflow of the *(?near?)* future



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Real question:

What is the biologist's measurement paradigm of the future?

- Just like all high variance situations…

  more measurements → more confidence

  How can biophysicists increase impact?

- Need to query reaction kinetics, cell phenotypes, cell genotypes across heterogeneous populations
  - How do we increase the throughput of biophysical measurements beyond simple fluorescence?
  - How do we automate pathway studies? What are the correct tools?
    - AFM
    - Optical tweezers
    - Surface plasmon resonance
    - 'Workstation' combinations of several capabilities

…something else that we haven't though of yet???

Biophysics should embrace the concept of scalable solutions to increase relevance to experimental biologists in the future

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