VI∫BRE

Vanderbilt Institute for Integrative Biosystems Research and Education and the Cellular Instrumention and Control Project

John Wikswo and Franz Baudenbacher

Vanderbilt Nanodays, 22 October 2002

VIIBRE Mission Statement

This interdisciplinary Institute will have as its mission to:

1) strengthen and broaden our existing foundation of basic research in the biophysical sciences and bioengineering;
2) develop enabling technologies that span these disciplines;
3) provide close articulation of the biophysical sciences and bioengineering with our undergraduate, graduate, and postgraduate educational programs; and
4) foster programs of outreach to industry, government, and other educational institutions. The primary focus of the Institute will be on supporting and enhancing research and both graduate and postgraduate education.

VIIBRE Core Departments

- Physics & Astronomy (Wikswo, Baudenbacher)
- Biomedical Engineering (Harris, Galloway, Gore, and Wikswo)
- Chemistry (Cliffel, Wright, Porter)
- Chemical Engineering (LeVan and Balcarcel)
- Molecular Physiology and Biophysics (Piston, Beth, Cherrington, Wikswo)
- Radiology (Gore)

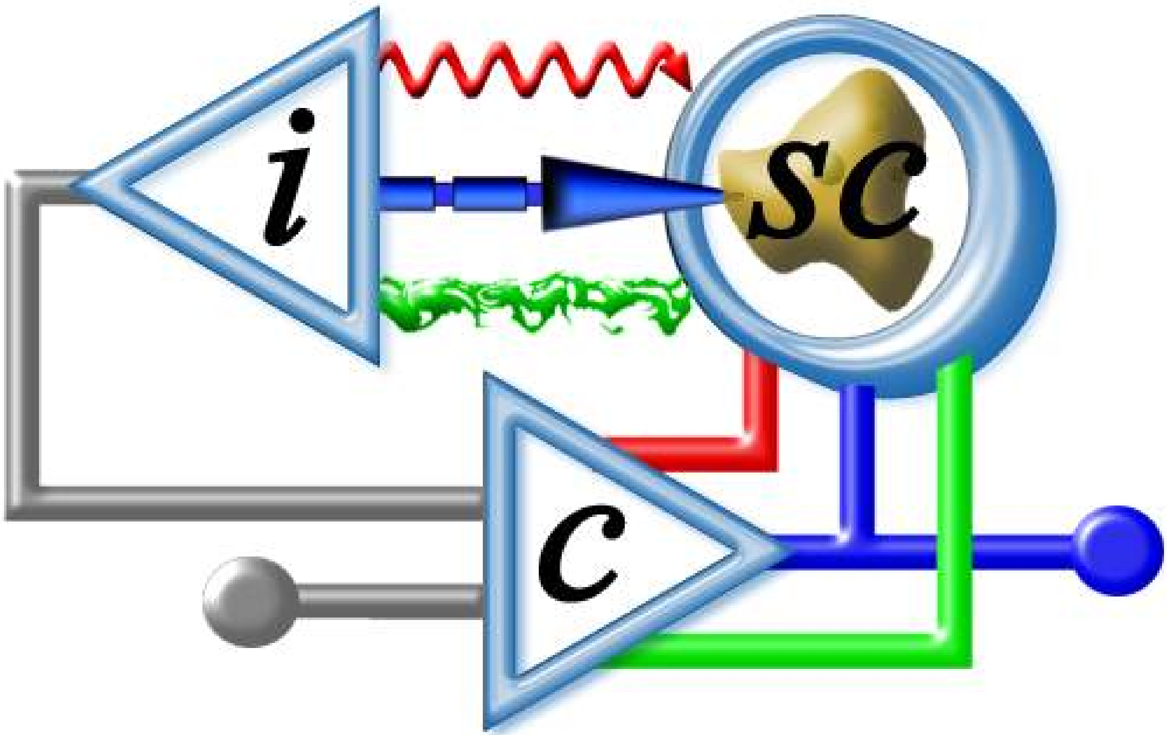
- Structural Biology Program
- Biochemistry
- Biological Sciences
- Pharmacology
- Mathematics
- Mechanical Engineering
- Electrical Engineering
- Civil and Environmental Engineering
- ….

- Other Centers/Institutes
  - Free Electron Laser Center / Biophotonics Institute
  - Vanderbilt Institute for Nanoscale Science and Engineering
  - Cognitive and Integrative Neuroscience
  - Molecular Neuroscience
  - Environmental Risk and Resources Management

MAJOR THEMES AND PROJECTS

- Instrumenting and Controlling the Single Cell will provide new insights into post-genomic and post-proteomic physiology, drug delivery, and toxicology.
- Technology Guided Therapy gathers information about the patient and the disease and directs therapy to maximize the treatment of the disease while minimizing the effect on the patient.
- Biomedical Imaging uses functional MRI and other modalities to learn about human and animal anatomy, physiology, and pathology.
- The VaNTH NSF ERC is aimed at developing the bioengineering educational system of the immediate future. It seeks to integrate learning science, learning technology and the domain areas of bioengineering.
- Predoctoral and Postdoctoral Mentoring of scientists and engineers who want to master interdisciplinary research at the boundary of engineering, medicine, and the natural sciences

Instrumenting and Controlling



The Single Cell

M. Bray

Reductionism

Thermodynamics

Statistical mechanics

Molecular/atomic dynamics

Electrodynamics

Quantum Chromodynamics

Bulk solids

Devices

Continuum models

Microscopic models

Atomic physics

Anatomy

Physiology

Organ

Cell

Protein

Genome

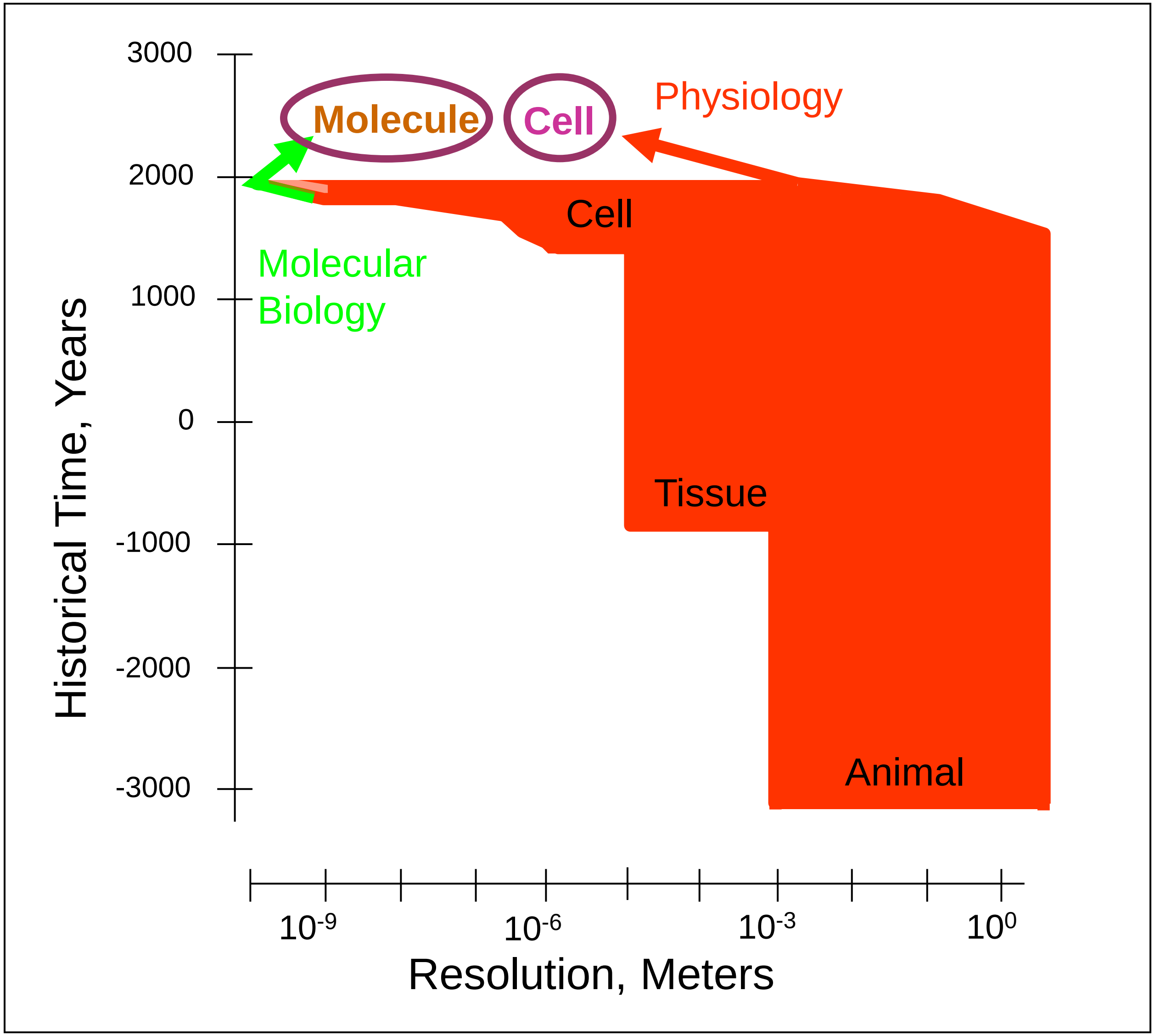
Spatial Resolution in Physiology

X-Ray / SEM / STM

Optical microscope

Magnifying glass

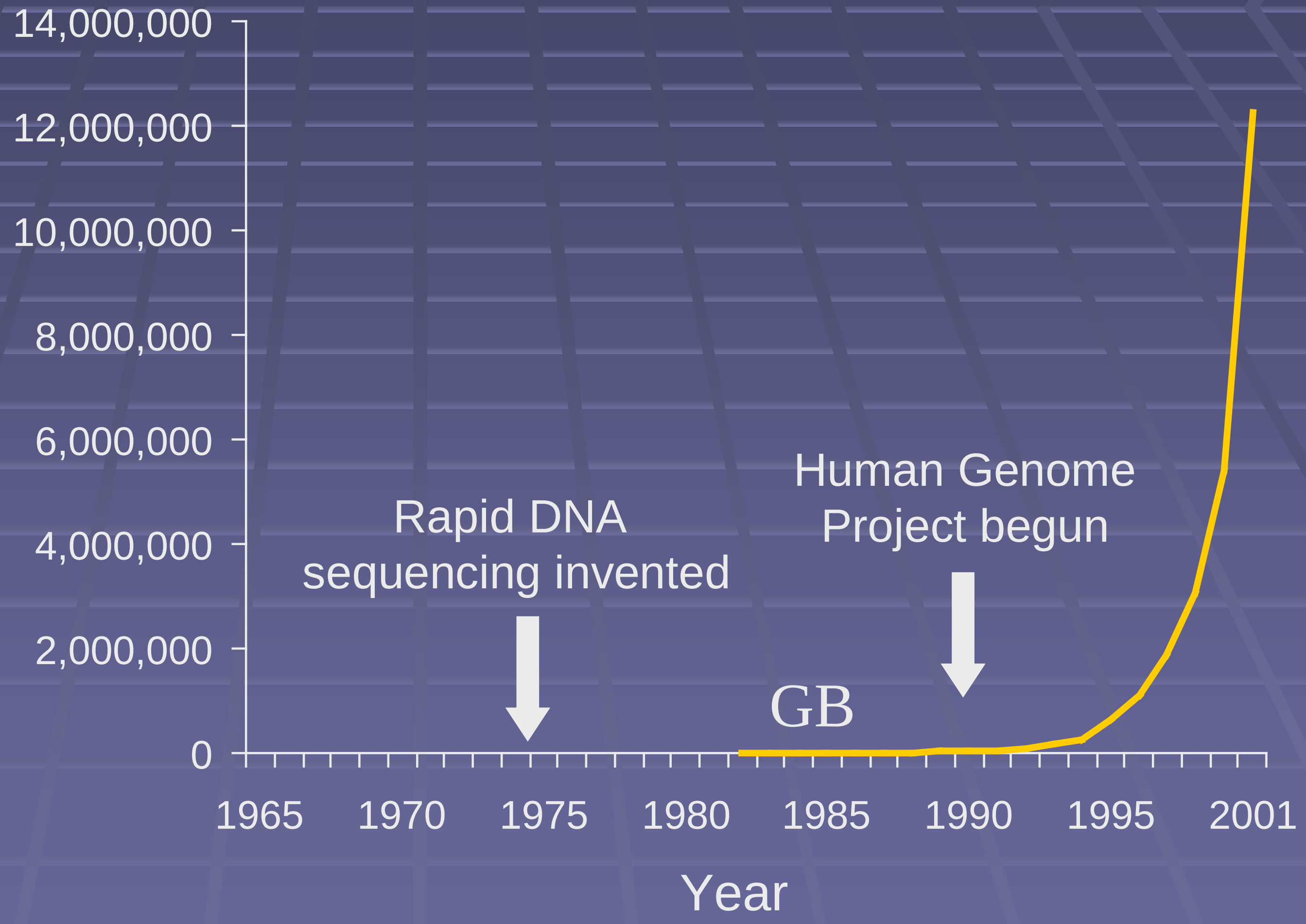
Unaided eye



The rate at which DNA sequences began accumulating was exponential

~13 million sequence entries in GenBank

Nearly 13 billion bases from ~50,000 species



National Library of Medicine

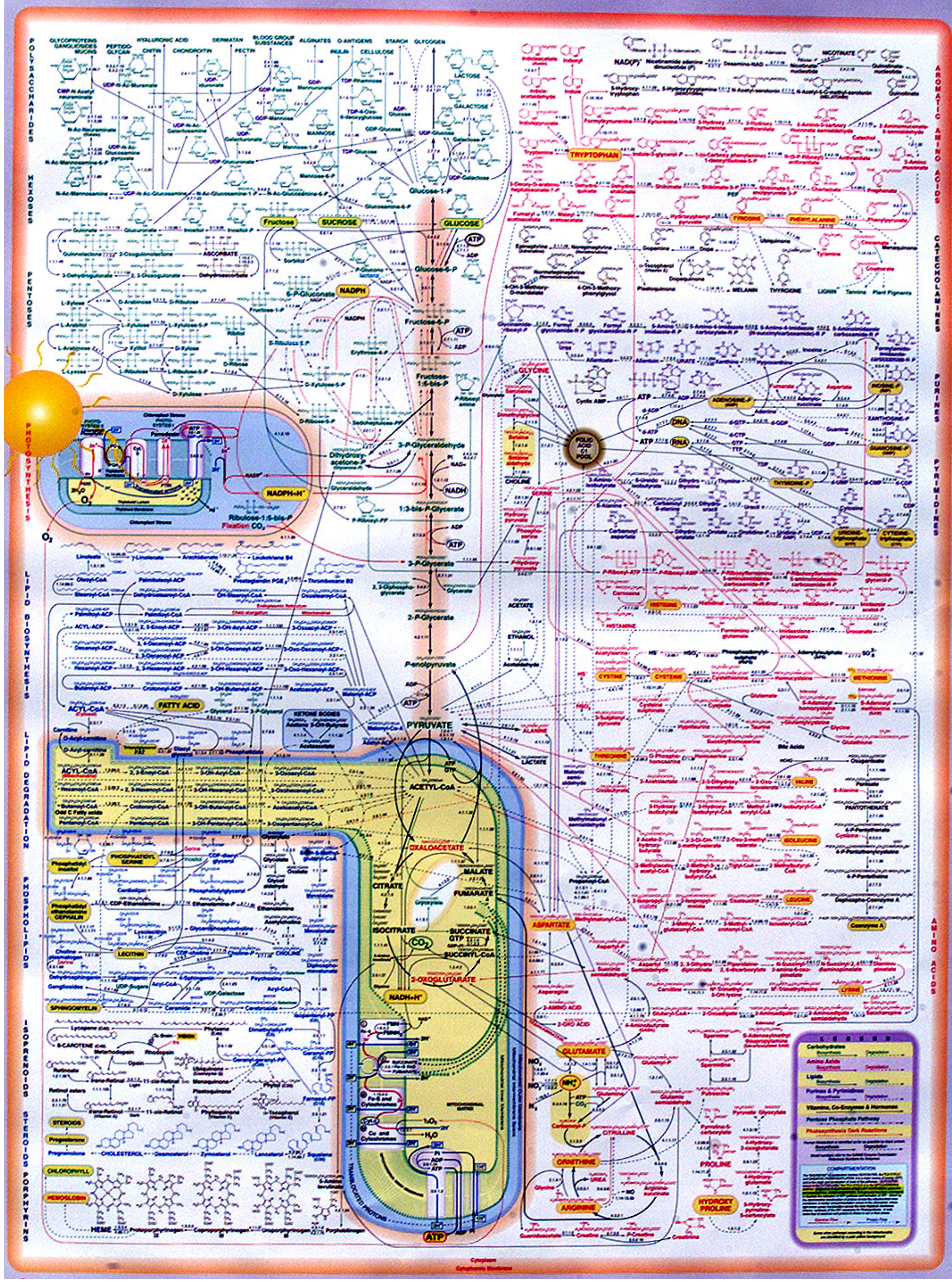
Courtesy of Mark Boguski

Courtesy of Mark Boguski

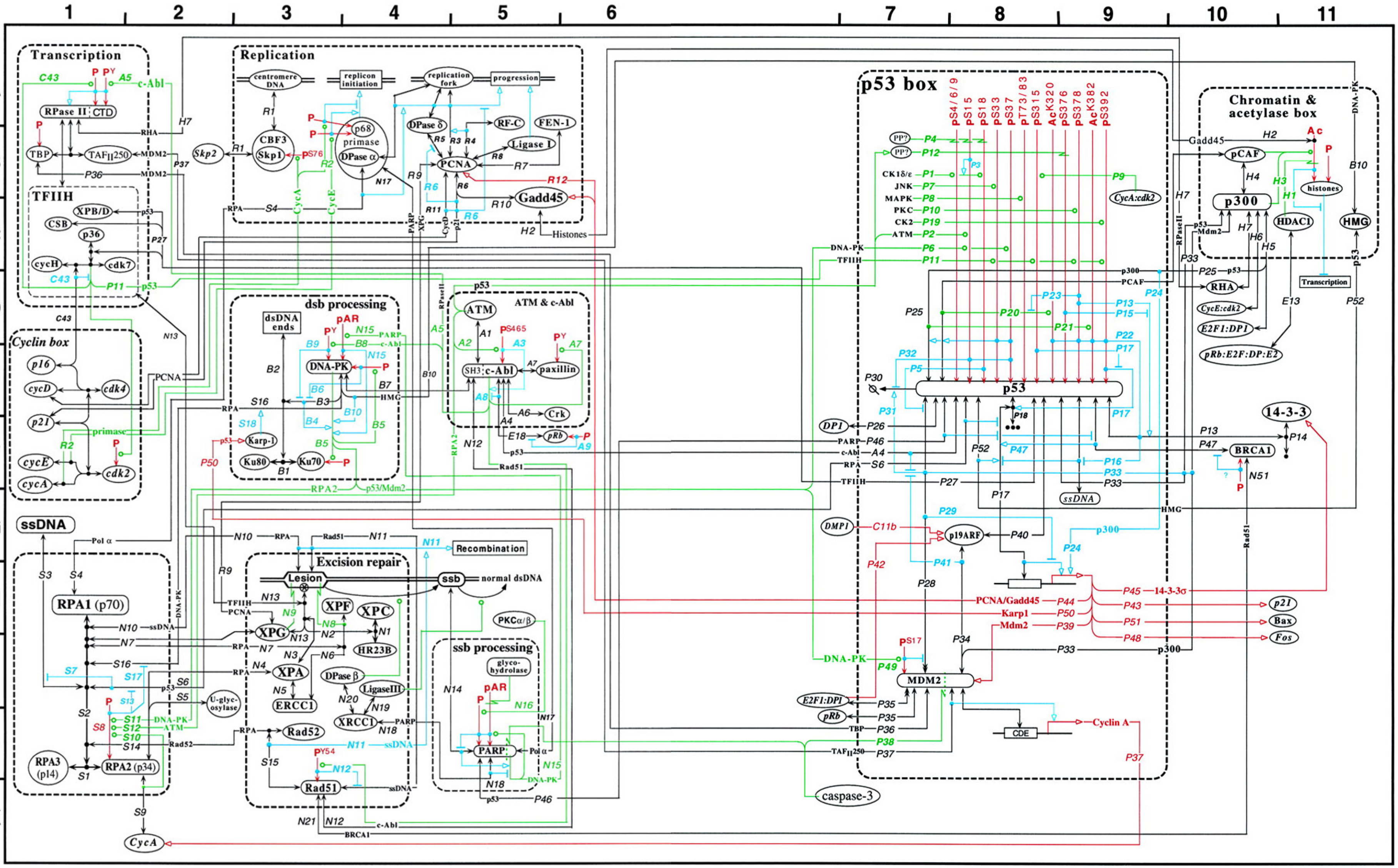
Courtesy of Mark Boguski

Postgenomic Integrative/Systems Physiology/Biology

- Specify concentrations and
- Rate constants
- Add gene expression,
- Protein interactions, and
- Signaling pathways
- Include intracellular spatial distributions, diffusion, and transport
- … and **calculate** how the cell behaves in response to a toxin

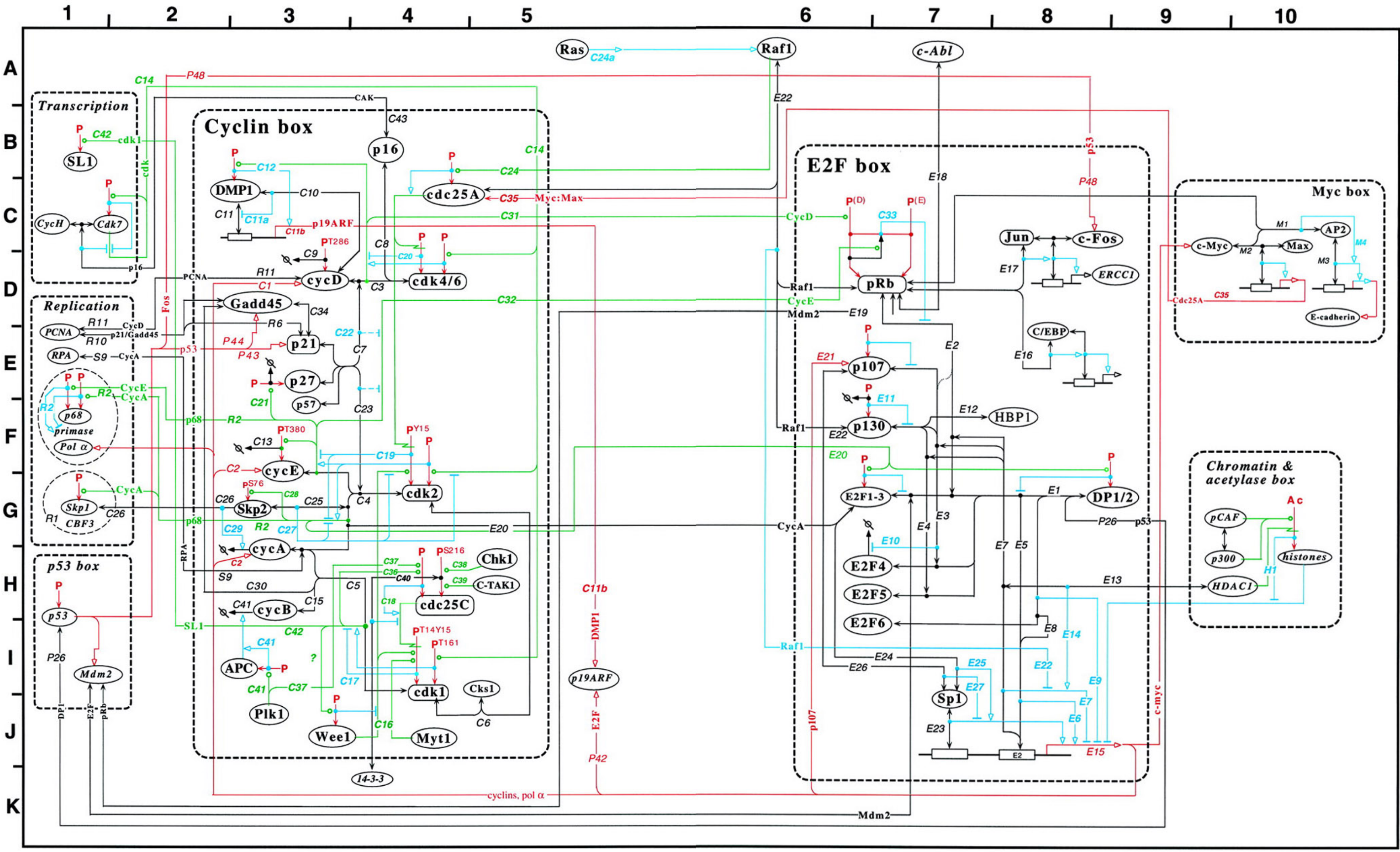


Molecular Interaction Map: DNA Repair



KW Kohn, “Molecular Interaction Map of the Mammalian Cell Cycle Control and DNA Repair Systems,” *Mol. Biol. of the Cell*, 10: 2703-2734 (1999)

Molecular Interaction Map: Cell Cycle



KW Kohn, "Molecular Interaction Map of the Mammalian Cell Cycle Control and DNA Repair Systems," *Mol. Biol. of the Cell*, 10: 2703-2734 (1999)

Post-Reductionism

Thermodynamics

Statistical mechanics

Molecular/atomic dynamics

Electrodynamics

Quantum Chromodynamics

Bulk solids

Devices

Continuum models

Microscopic models

Atomic physics

Anatomy

Physiology

Organ

Cell

Protein

Genome

The Catch

- Modeling of a single mammalian cell may require 100,000 variables and equations
- Cell-cell interactions are critical to system function
- 10^9 interacting cells in some organs
- Many of the interactions are non-linear
- The data don't yet exist to drive the models
- Hence we need to experiment…

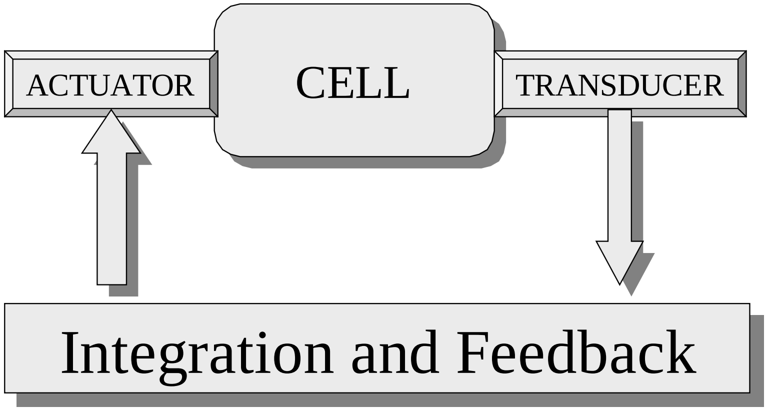
The Challenges

Develop the tools, techniques, and measurements for **Integrative, post-genomic cellular biology**

- Genes (natural and artificial)
- Proteins (natural, artificial, and nanosystems)
- **Metabolic and signaling pathway analysis**
- **Models** (molecular and cellular dynamics)
- **Instruments (micro=cellular; nano=molecular)**
- **Wide-bandwidth dynamic control theory for micro/nano biosystems**
- **Biological applications of nanosystems**

What do we need?

- Simultaneous, fast sensors (transducers) that detect a variety of changes within and outside the cell

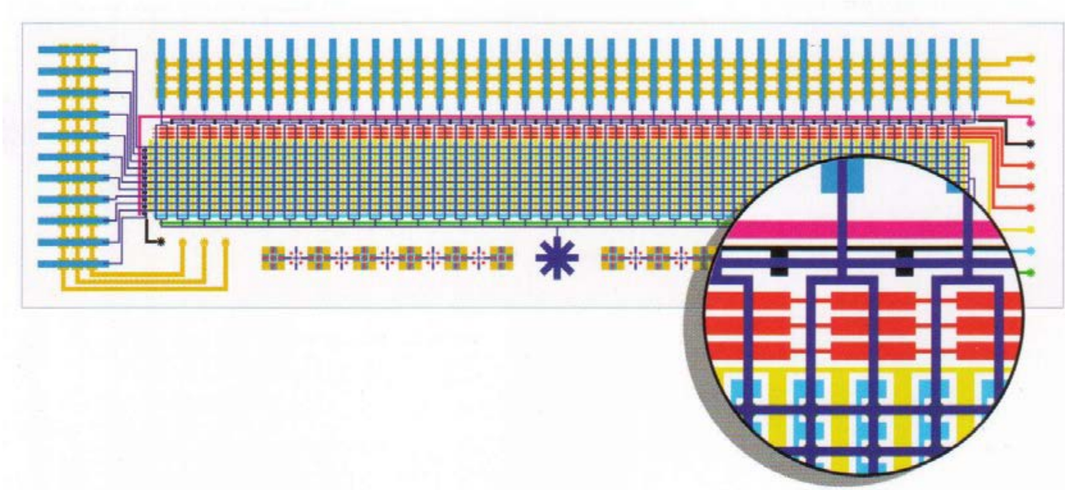


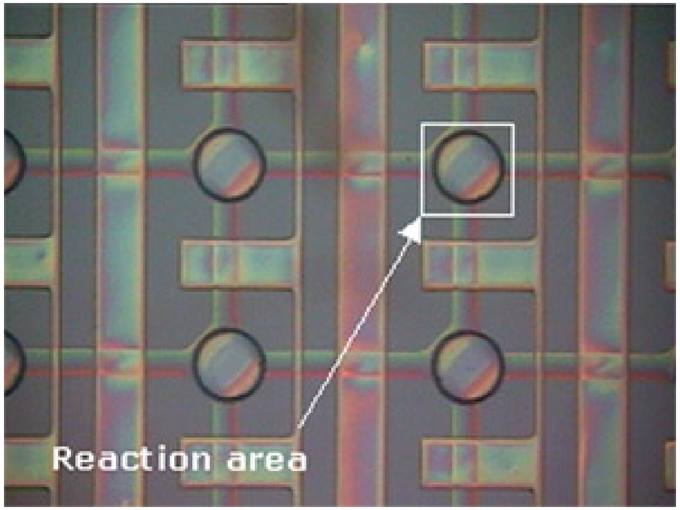
- Actuators that control the microenvironment within and outside the cell
- Openers for the internal feedback loops
- System algorithms and models that allow you to close and **stabilize** the external feedback loop
- …

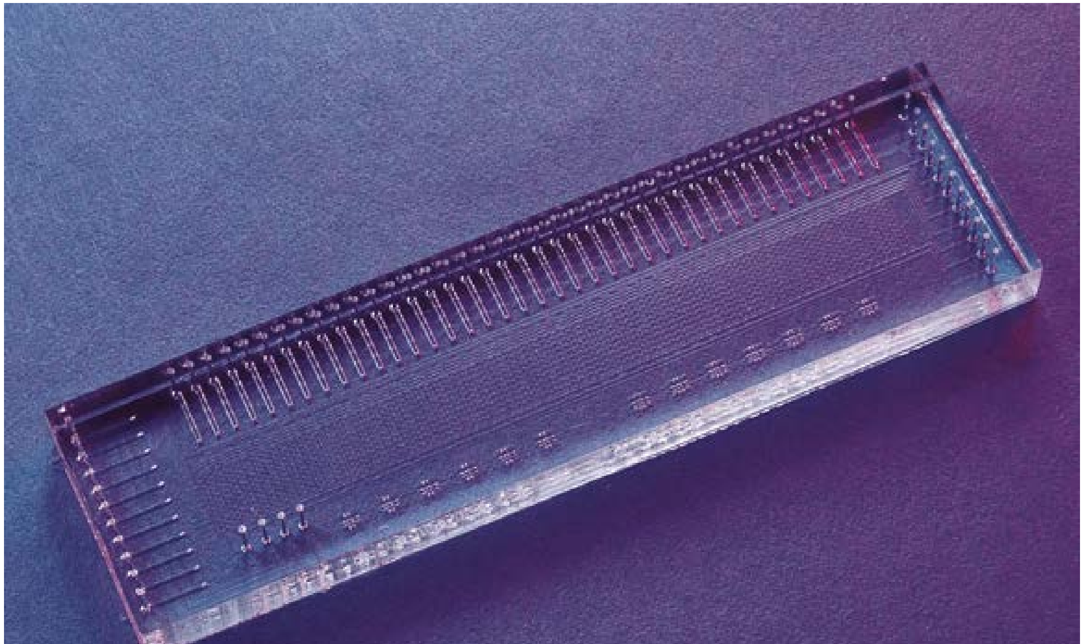
Protein Microprocessor

- ~2,000 valves to control
  - Reagents
  - Samples
  - Wash steps

  www.fluidigm.com

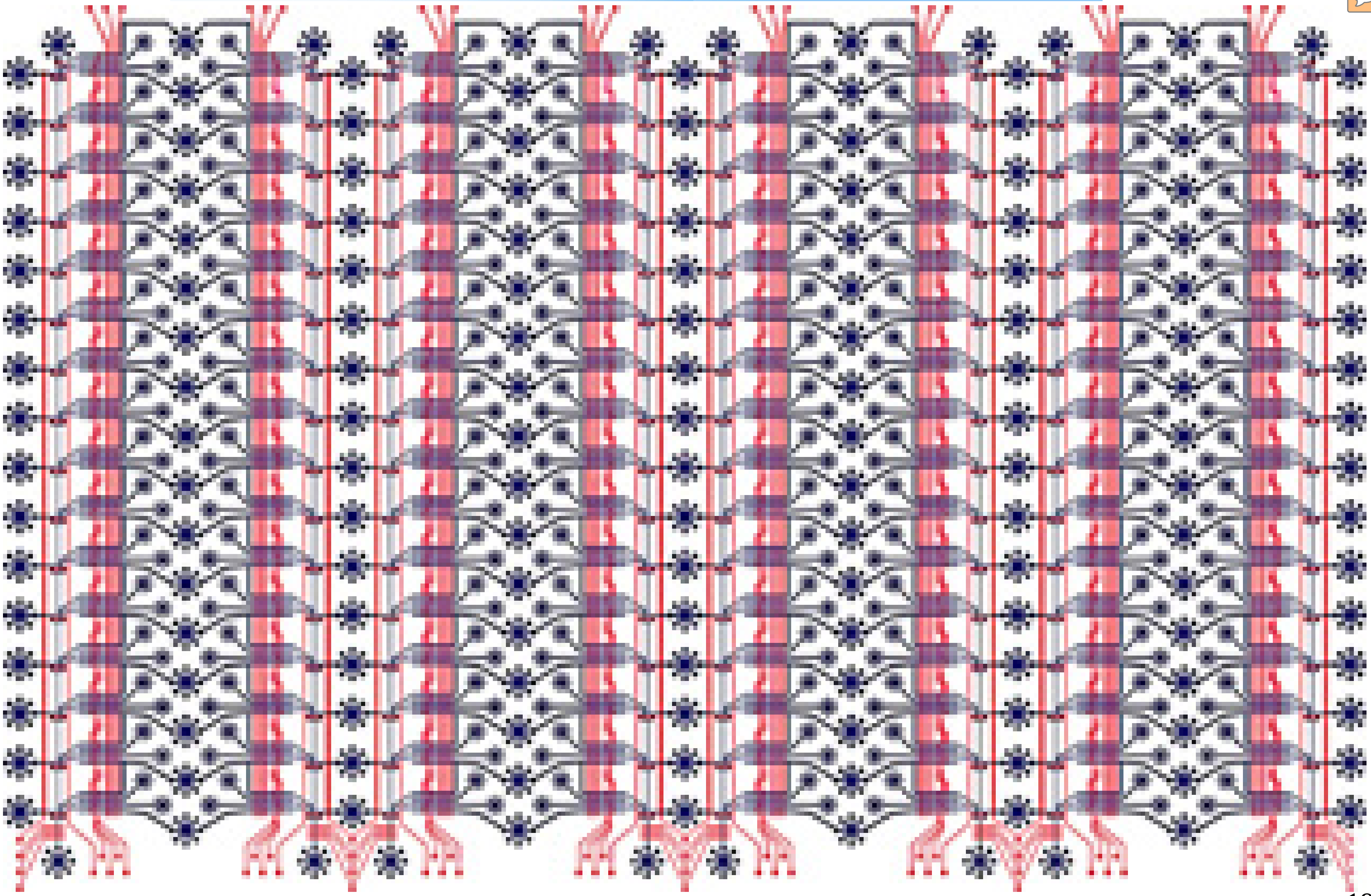






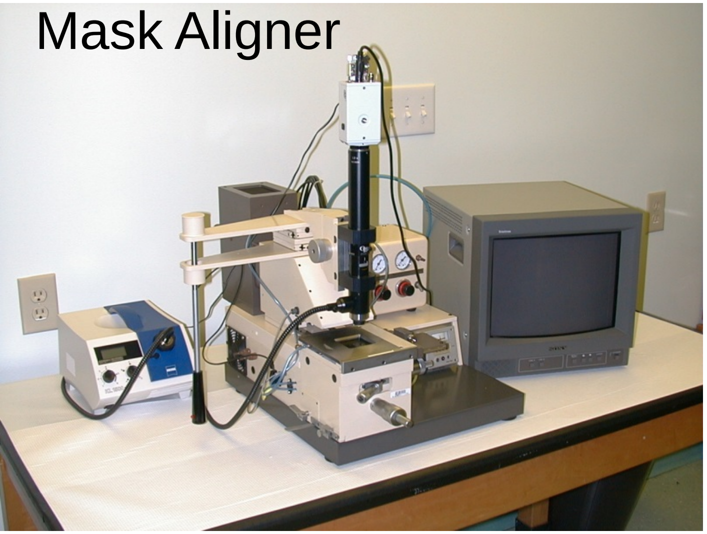
Courtesy of Michael Lee

Live-Cell Microprocessor



Courtesy of Michael Lee

VIIBRE BioMEMS Facility

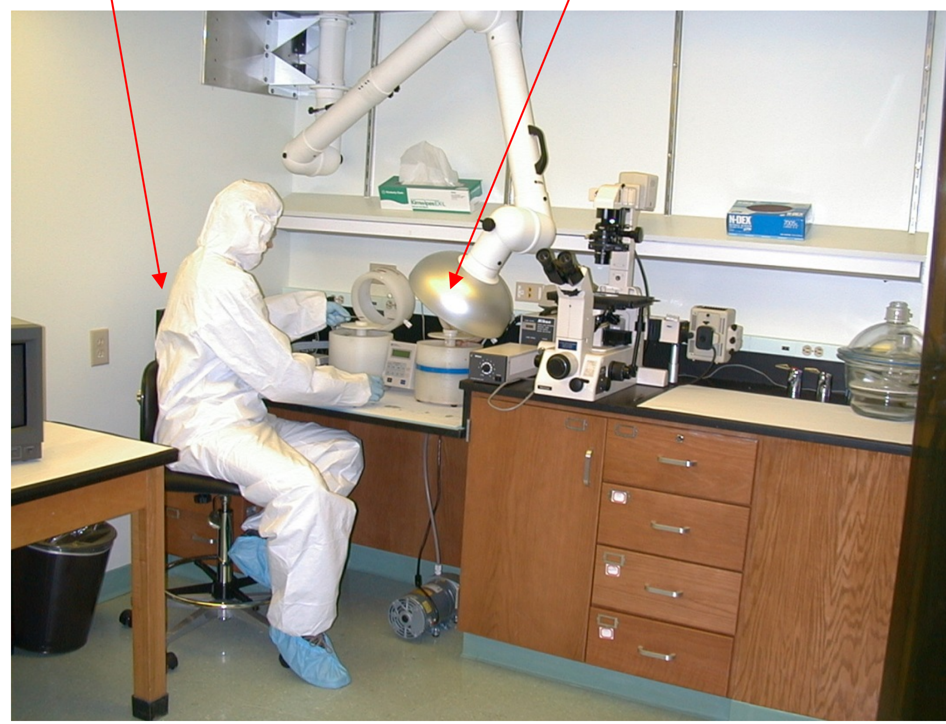


Mask Aligner

E-Beam Evaporator/Ion Etcher

Spin Processor Workstation

Inverted Microscope for photolithography

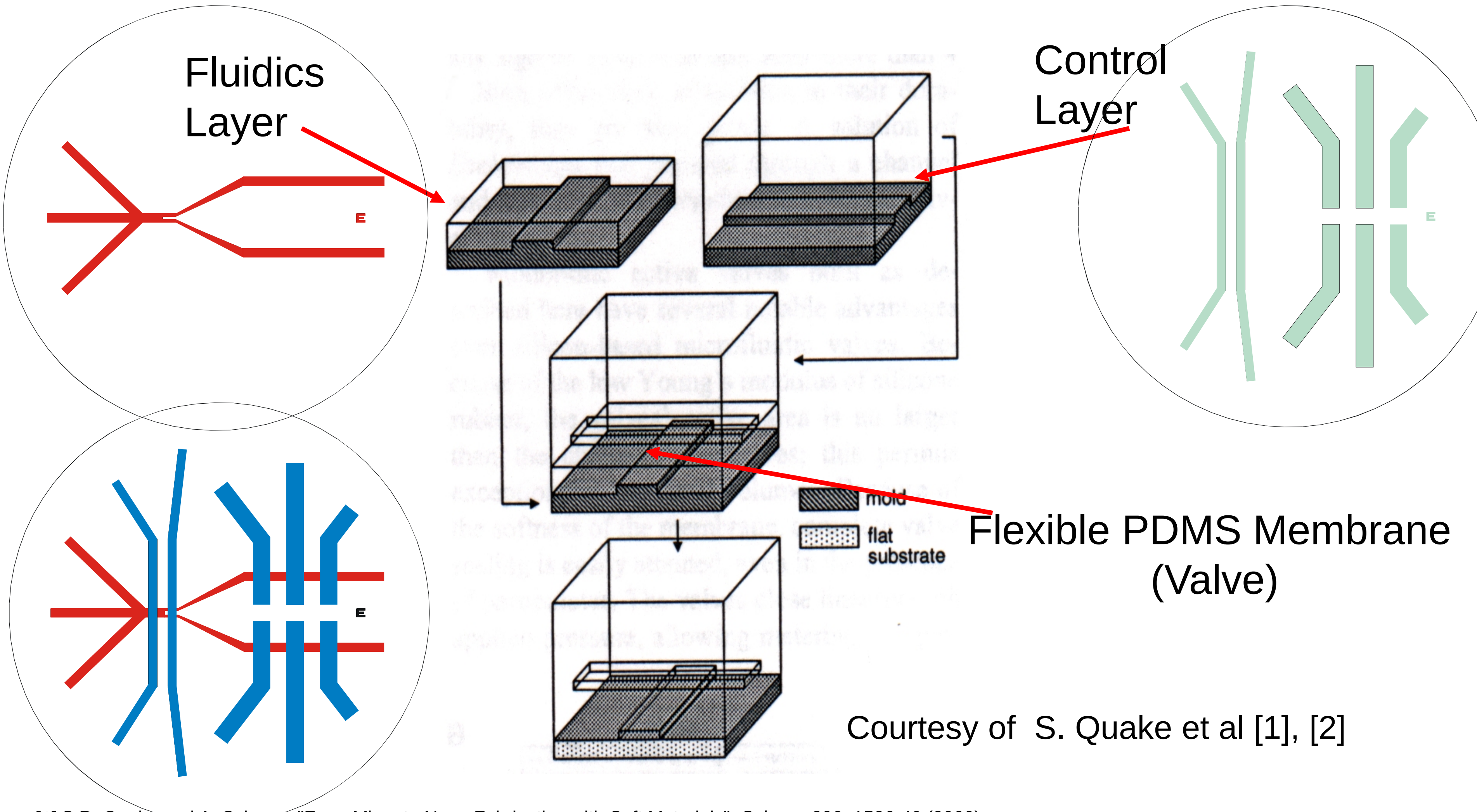


Film Deposition and Photolithography

-- Under Construction --

PDMS Soft Lithography

Werdich, Baudenbacher, Reiserer, Schaefer



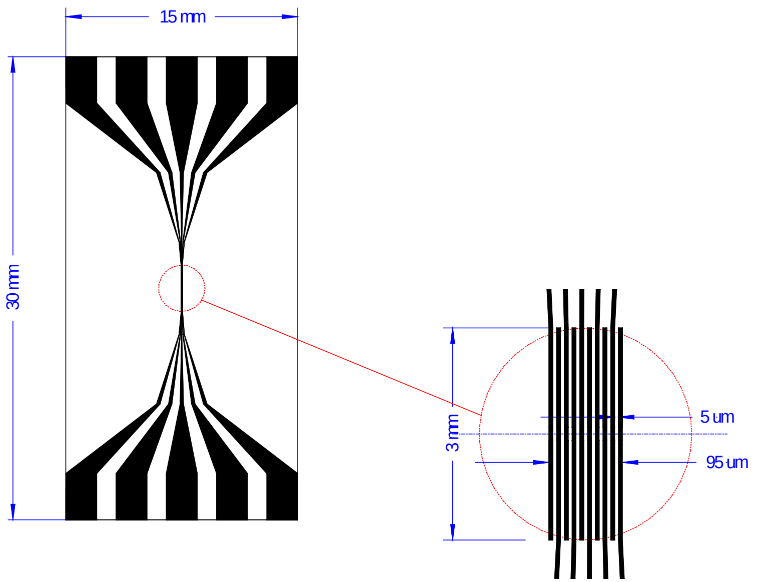
Courtesy of S. Quake et al [1], [2]

[1] S.R. Quake and A. Scherer, "From Micro to Nano Fabrication with Soft Materials", *Science* 290: 1536-40 (2000).

[2] M.A. Unger, H.-P. Chou, T. Thorsen, A. Scherer, and S.R. Quake, "Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography", *Science* 288: 113-

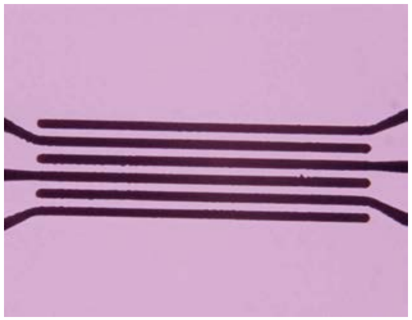
MP²-CBAD Custom-Designed IDE Array

Cliffel, Ecklund, Greene, Ges, Werdich, Baudenbacher

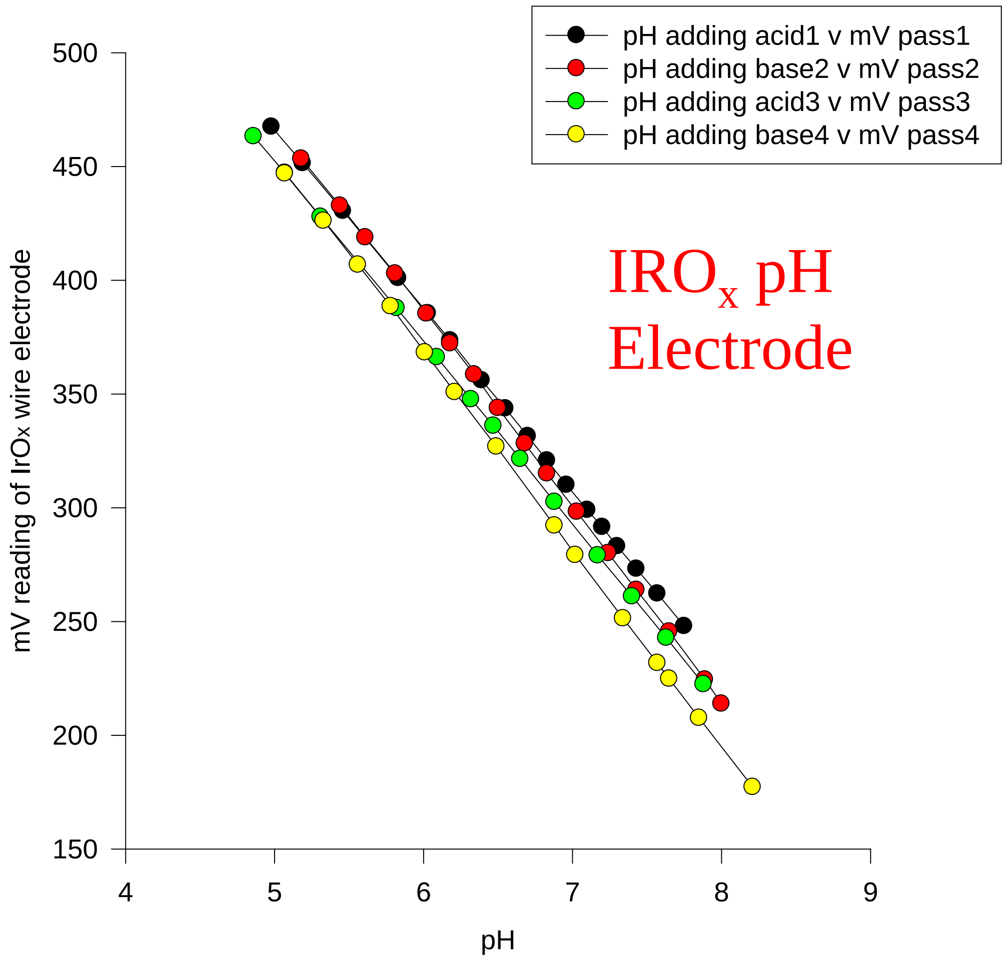


Individually addressable electrodes

D = 5 µm

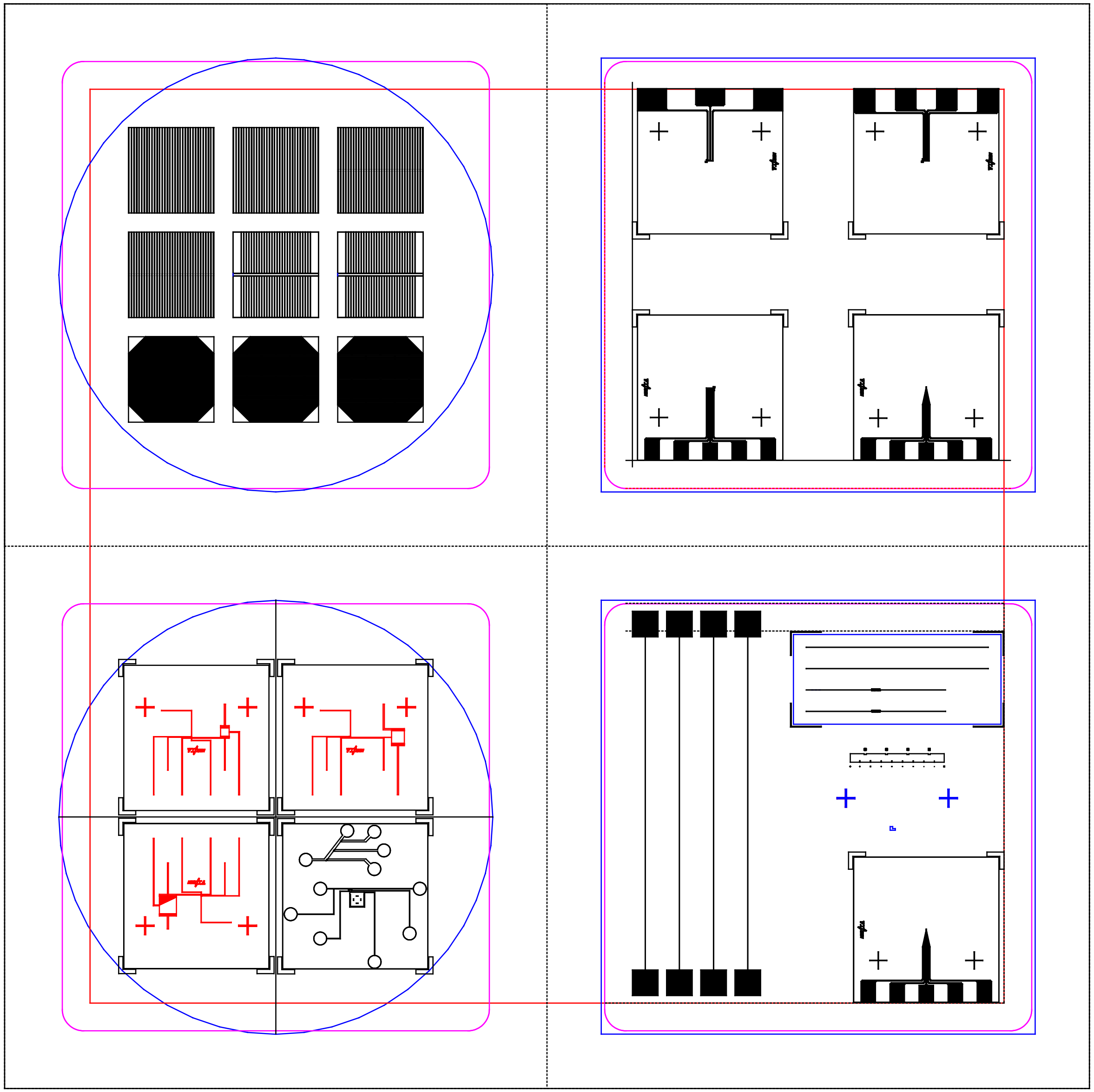


Iridium oxide coated wire electrode response to serial additions of acid and base within physiologically relevant pH range



Mask Designs 2002-10-21

Werdich and Baudenbacher



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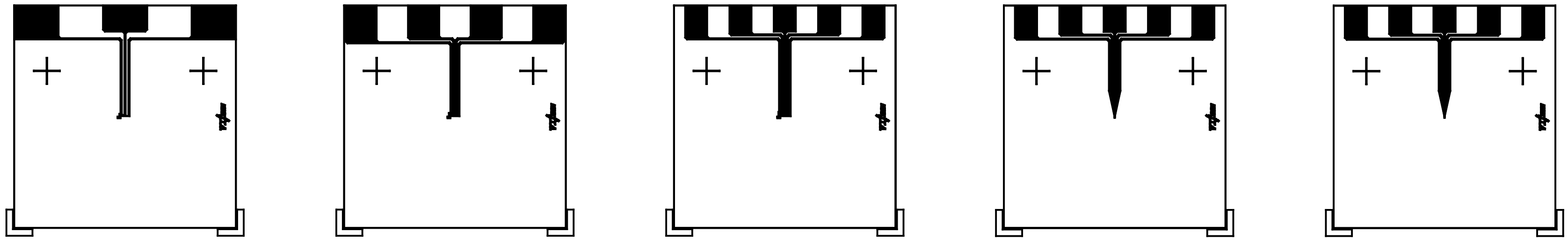
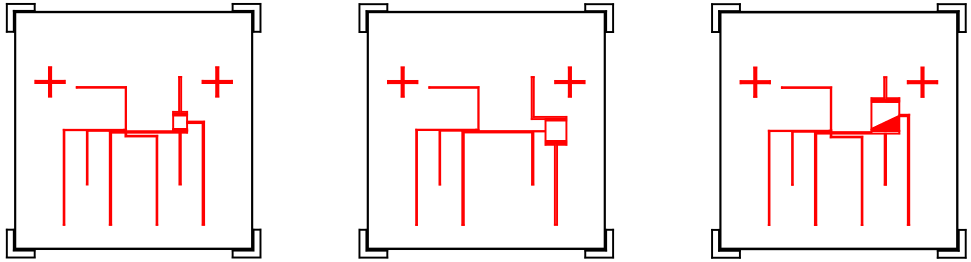
3 x Microband Electrodes for Cell Trap (Scale 2:1)

2 x Universal Microband Electrodes (Scale 2:1)

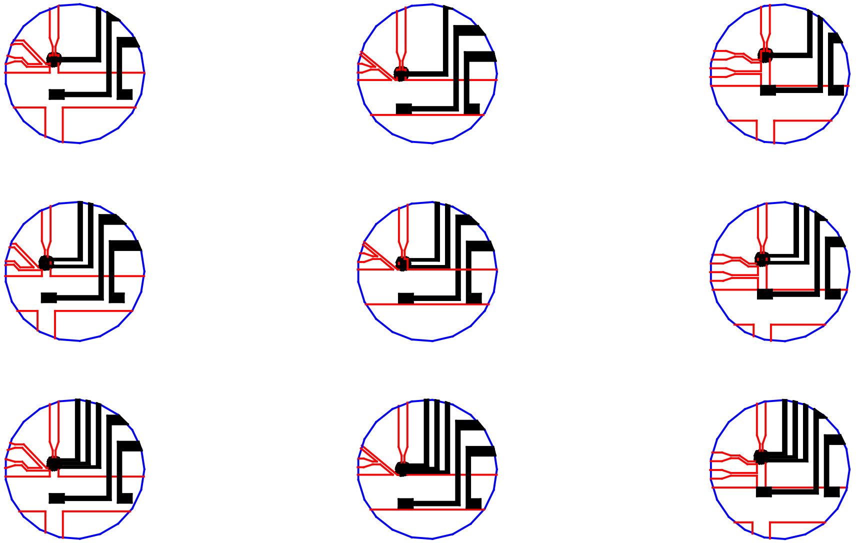


3 x Cell Trap (Scale 2:1)

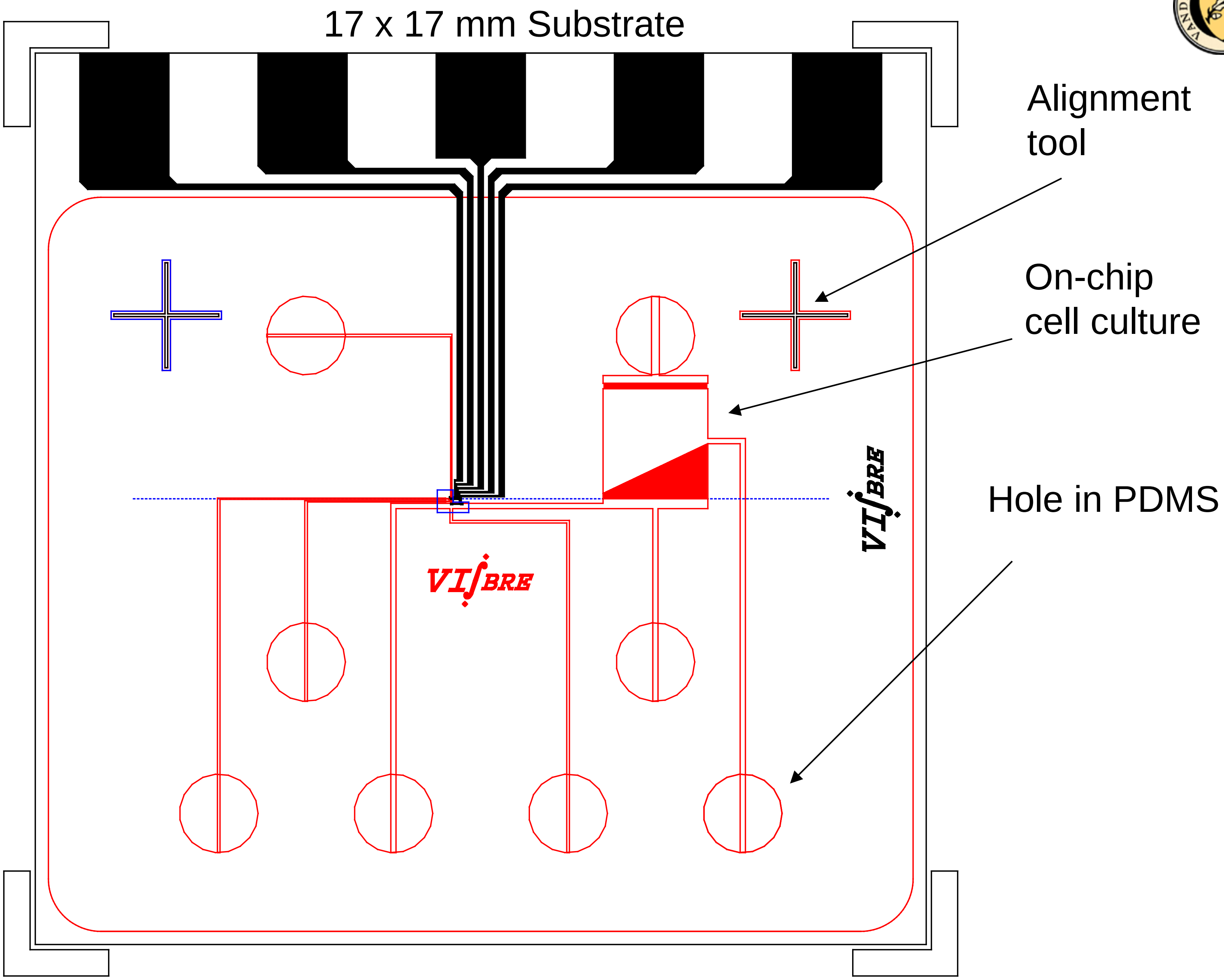


The three microband electrodes can be combined with the three cell traps, giving 9 different combinations.

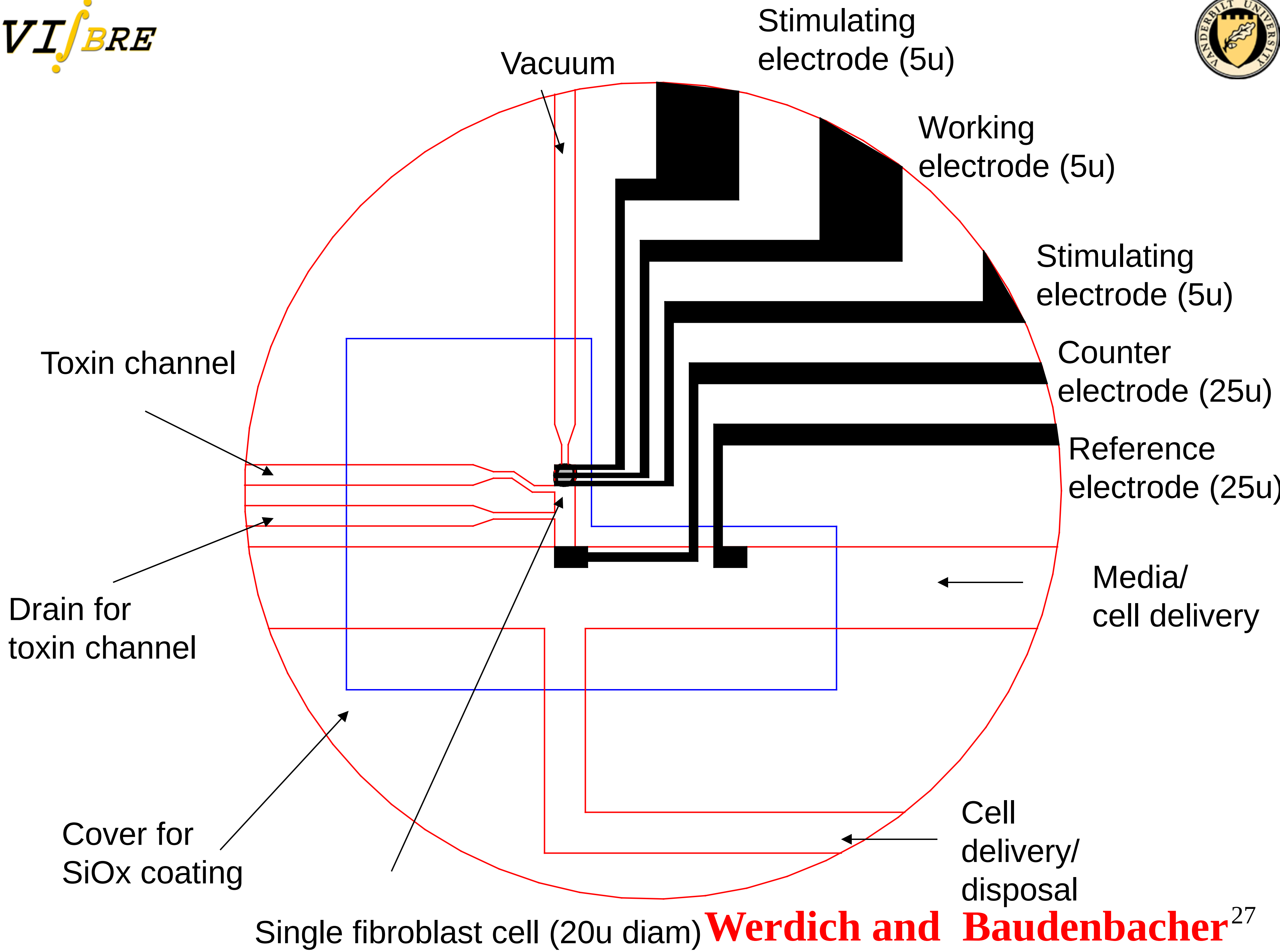
possible combinations (Scale 50:1)



Werdich and Baudenbacher

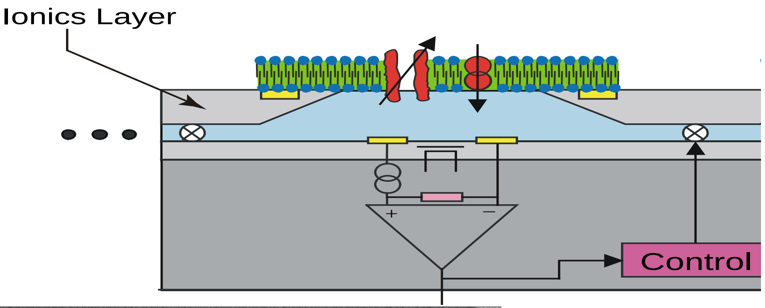


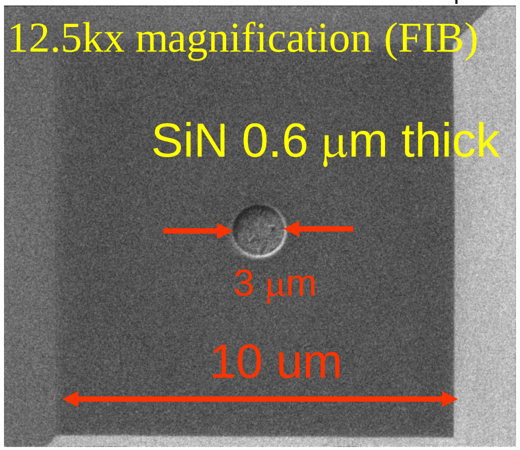
Werdich and Baudenbacher

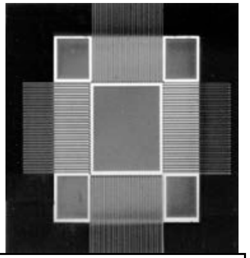


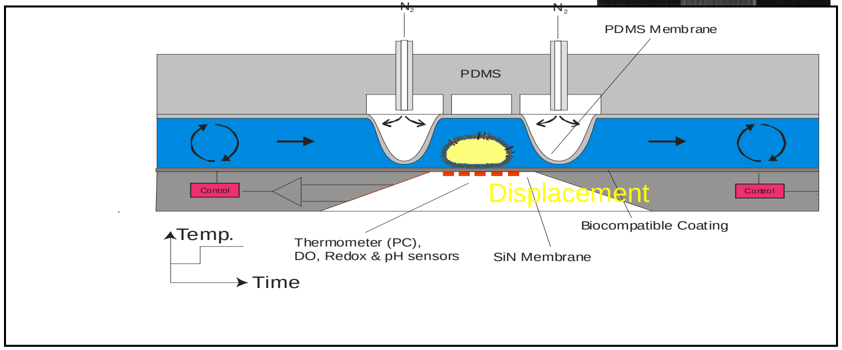
Werdich and Baudenbacher

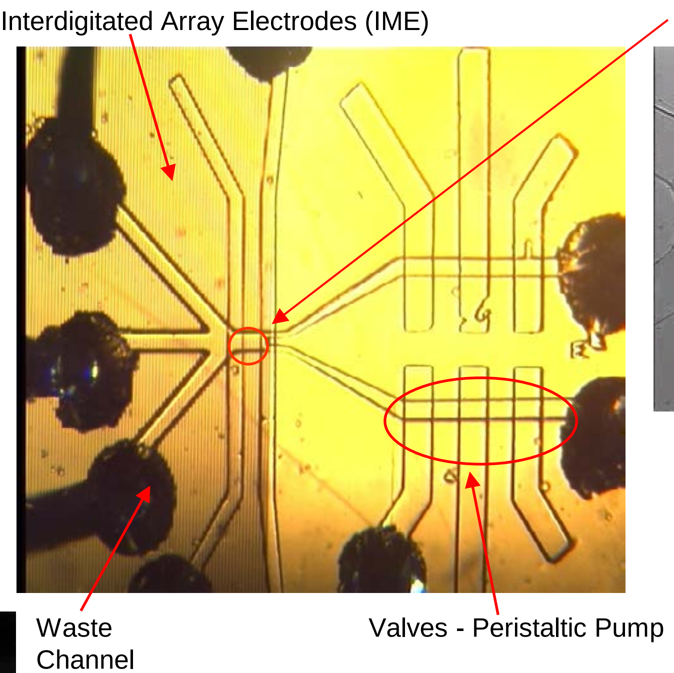
Instrumenting the Single Cell

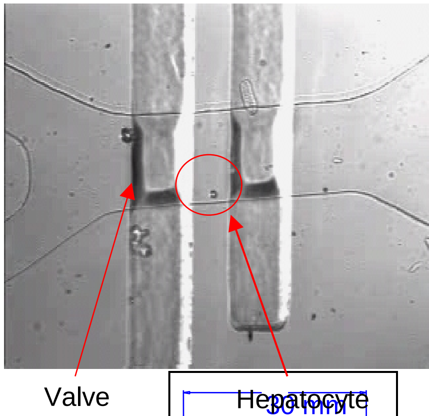


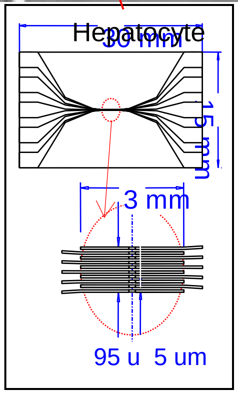


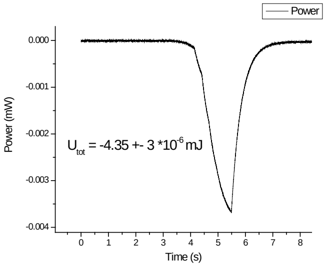


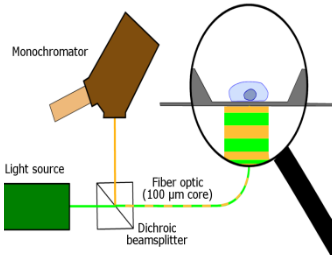


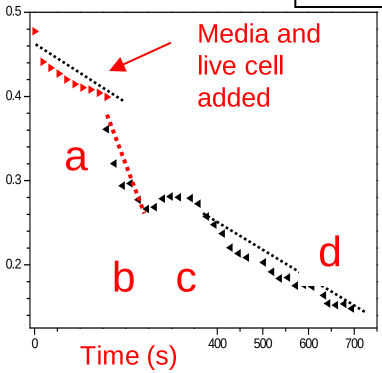










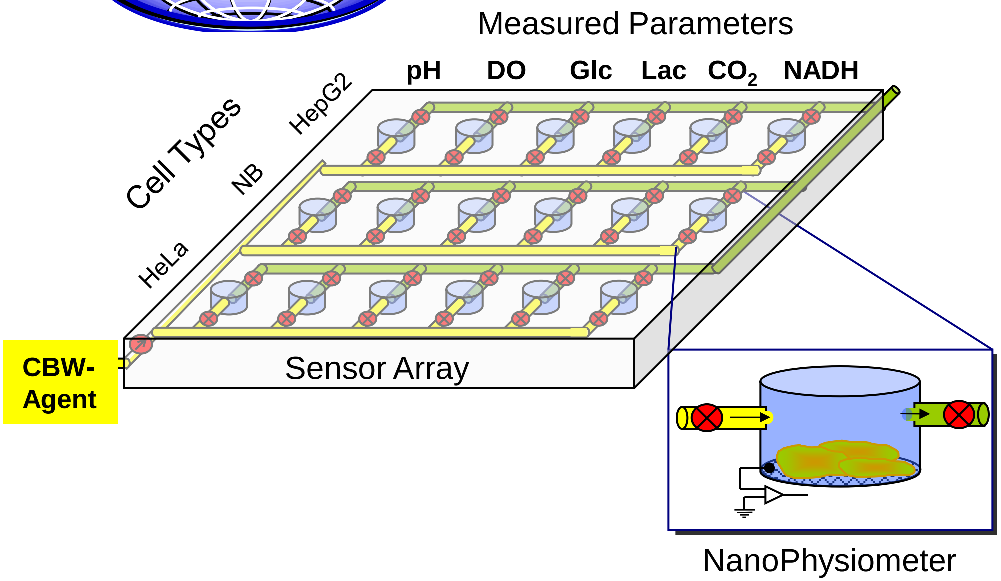


High-Content Toxicology Screening Using Massively Parallel, Multi-Phasic Cellular Biological Activity Detector (MP^2-CBAD)

Vanderbilt University

Departments of Biomedical Engineering, Chemical Engineering, Chemistry, Mechanical Engineering, Molecular Physiology & Biophysics, Physics & Astronomy

Massively Parallel, Multi-Phasic Cellular Biological Activity Detector



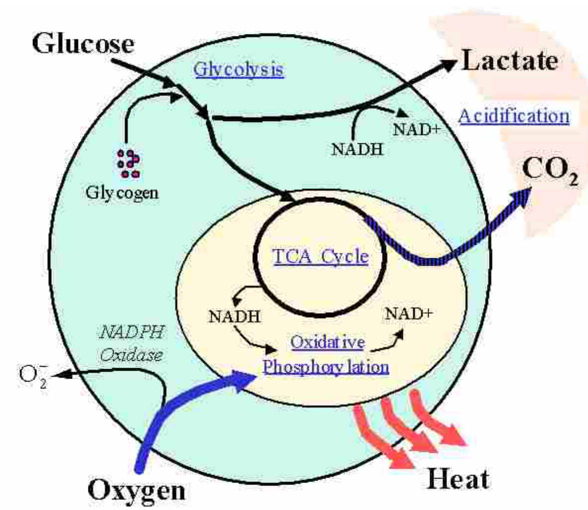
Objective: Develop cell-based, fast-response metabolic sensing arrays for detection and discrimination of unknown CBW agents.

Task: Enhance ECBC MicroPhysiometer to create biosignature library, demonstrate agent discrimination, define cell lines and sensor set

Task: Develop massively parallel NanoPhysiometer arrays with multiple sensors, nanoliter volumes, and ***rapid response***

Hypothesis:Metabolic network pathways are differentially affected by various CBW agents

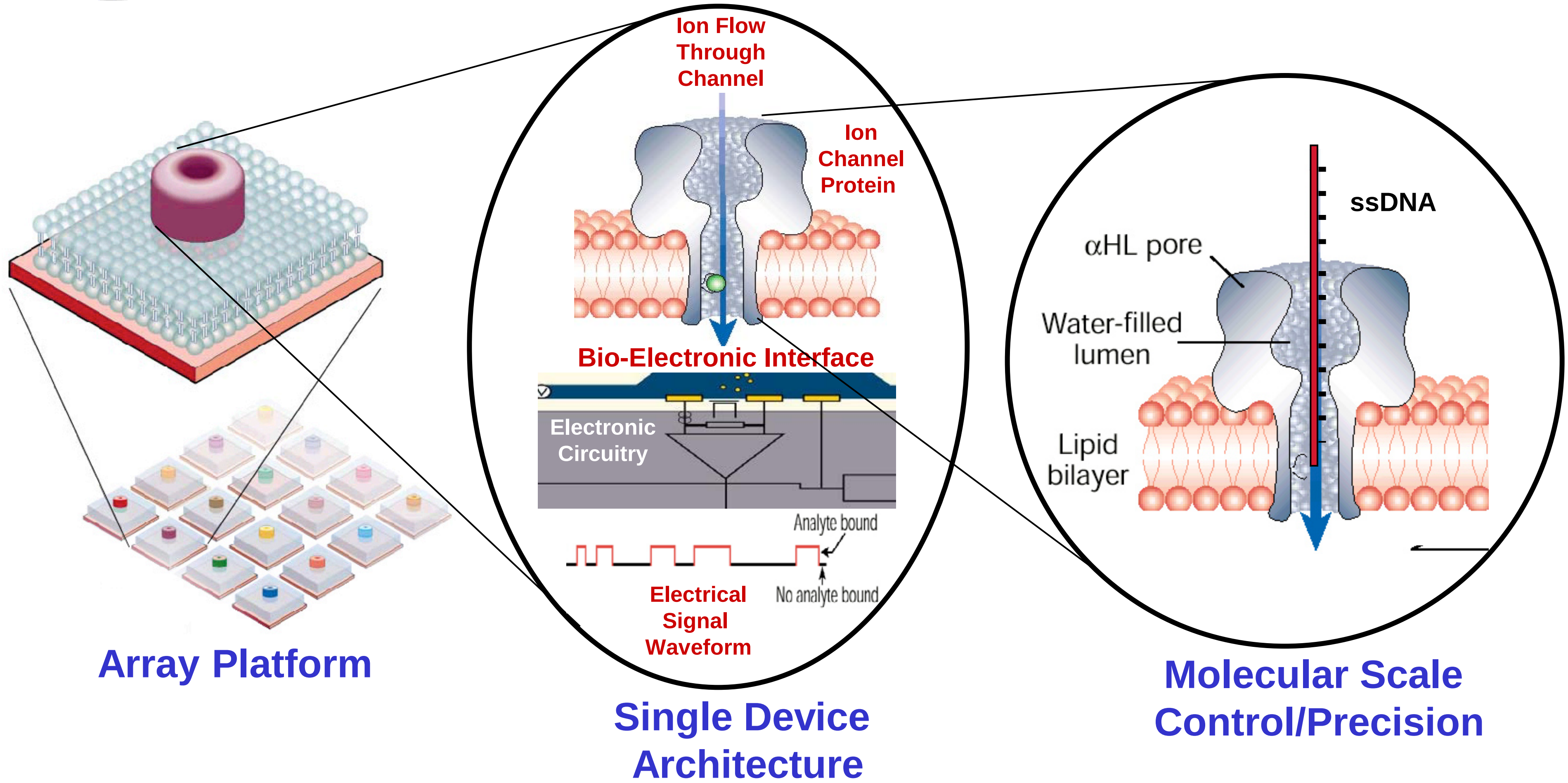
Glucose + 2 ADP + **2** $\mathbf{NAD^+}$	→	**2 Pyruvate** + 2 ATP + **2 NADH**
Pyruvate + **NADH**	→	**Lactate** + $\mathbf{NAD^+}$
Pyruvate + CoA + **FAD** + GDP + **3** $\mathbf{NAD^+}$ + $\mathbf{NAD(P)^+}$	→	**3** $\mathbf{CO_2}$ + **FADH2** + GTP + **3 NADH** + **NAD(P)H**
0.5 $\mathbf{O_2}$ + 3 ADP + **NADH**	→	3 ATP + $\mathbf{NAD^+}$
0.5 $\mathbf{O_2}$ + 2 ADP + $\mathbf{FADH_2}$	→	2 ATP + **FAD**



Balcarcel

The Future, including next week…

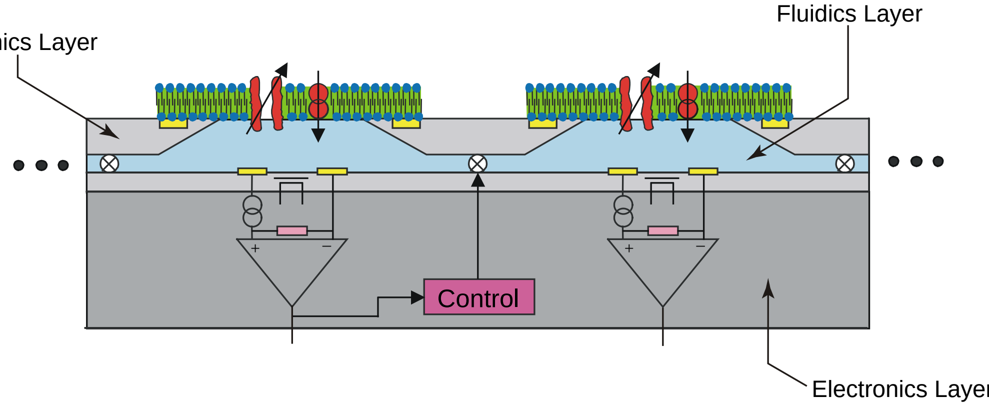
Array of Ion Channels



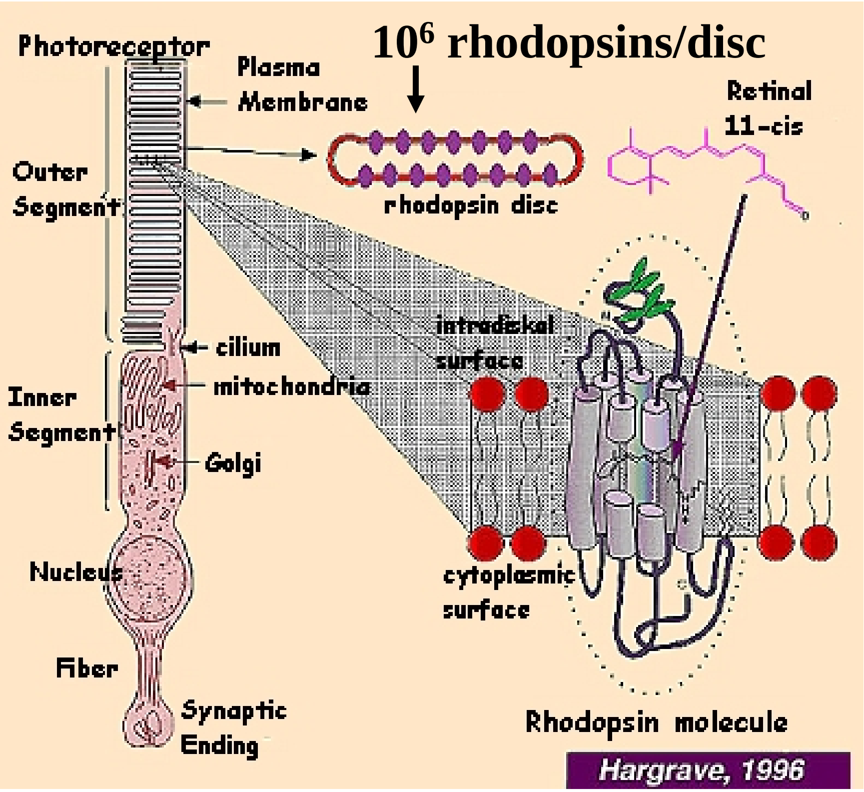
- Direct, Real Time Molecular Sensor/Reader
- Sensors, Switches, Amplifiers, Filters, Power Generators, ….
- Demonstrate High Speed DNA Read-out for Applications such as DNA Computing, Bio-Sensing, …

Courtesy of Ananta Krishnan, DARPA

Ion Channels, The Ultimate NanoDevice

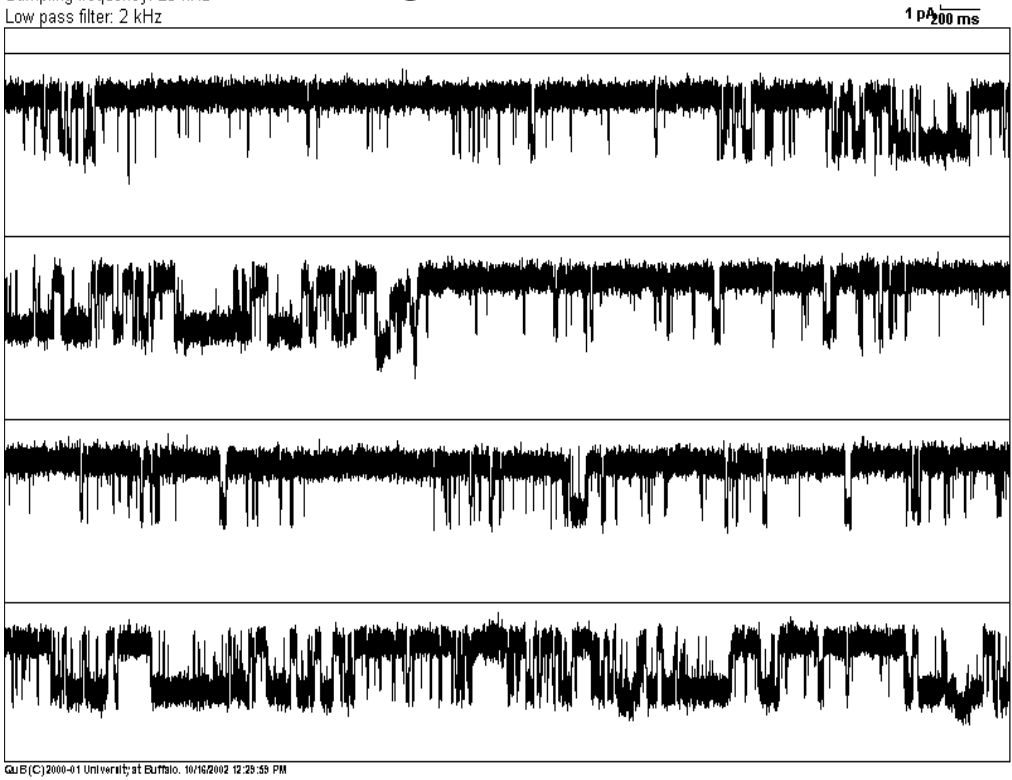


Rod photoreceptor



gluR0

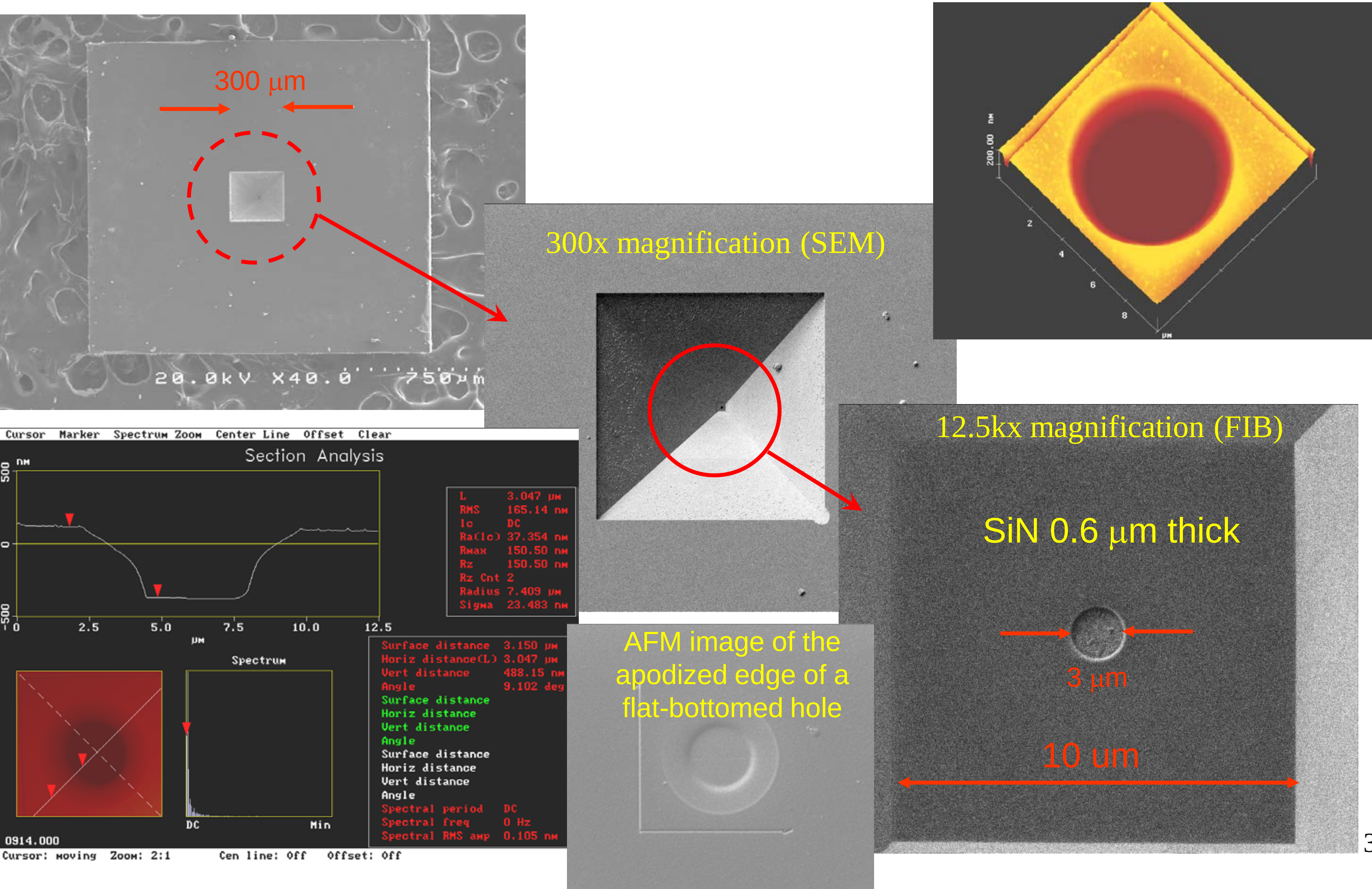
C:\temp\FREDGL~1.LDT
Drawing 0 - 399999, 100000 points/trace
Sampling frequency: 20 kHz
Low pass filter: 2 kHz



A. Auerbach, SUNY-B

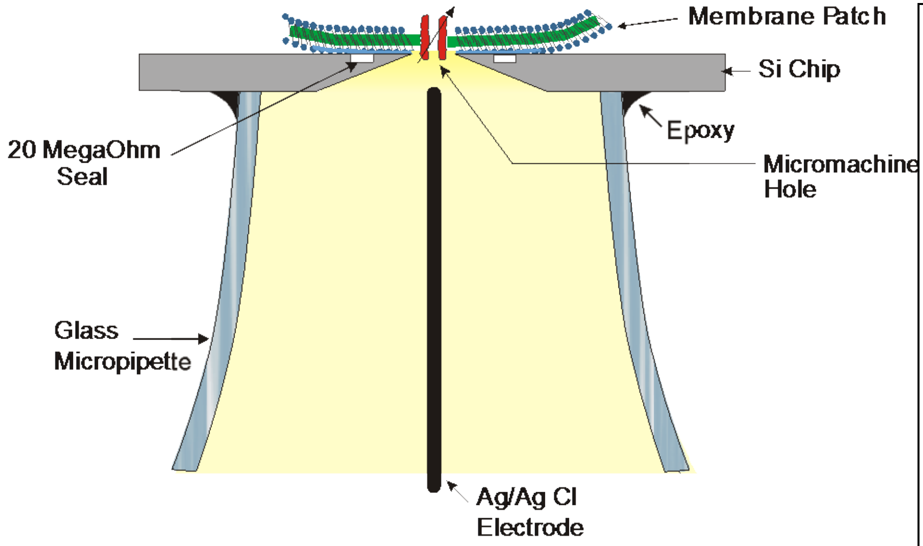
Planar Patch Clamp to Silicon

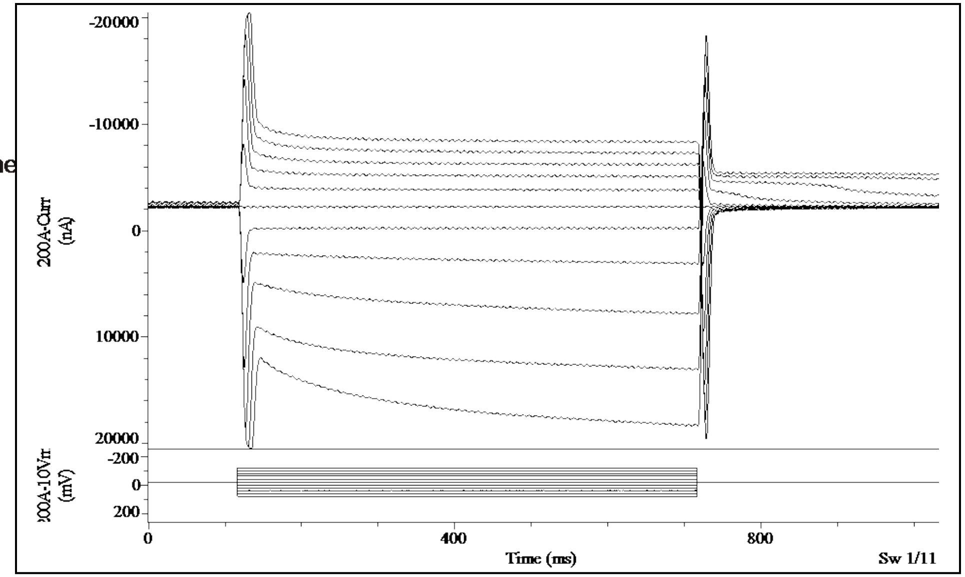
Weller, Baudenbacher, Renkes

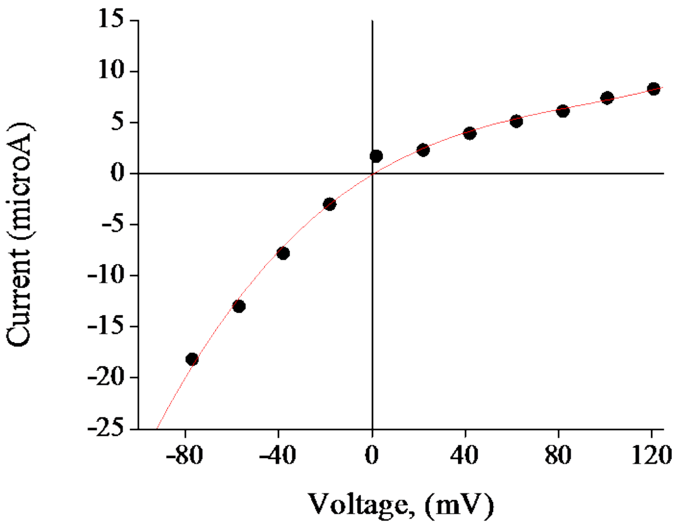


Whole Oocyte clamp – Cl^- Channel

Baudenbacher, Weller, Renkes, DeFelice







The Future
The Nano/Cellular Interface

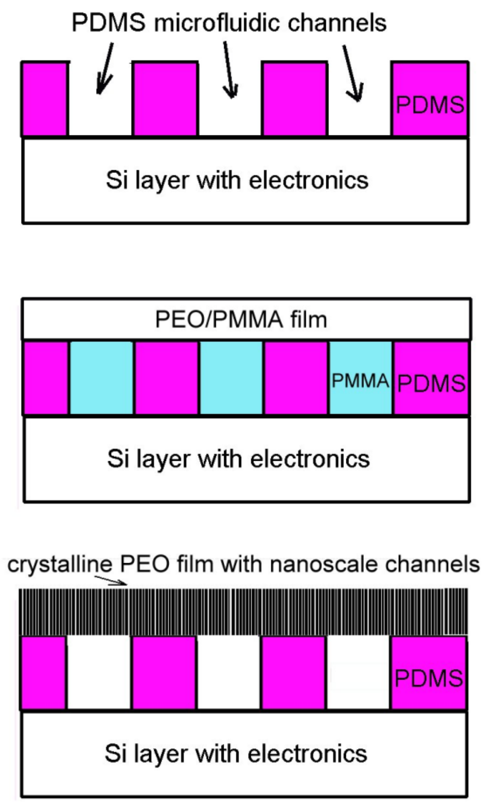
- Bob Weller

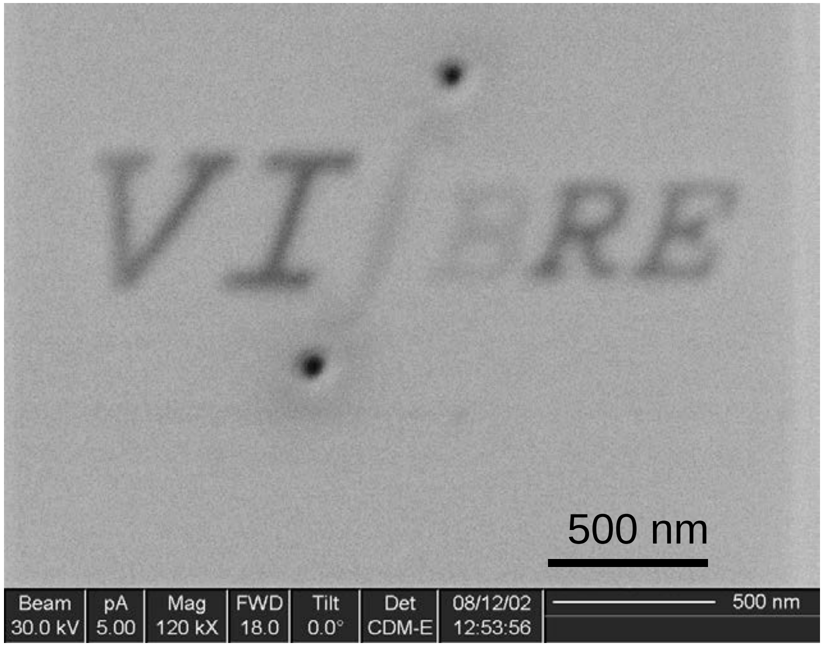
  <50 nm hole ~ ½ virus diameter

- Bill Hofmeister

  PEO/PMMA

  membranes





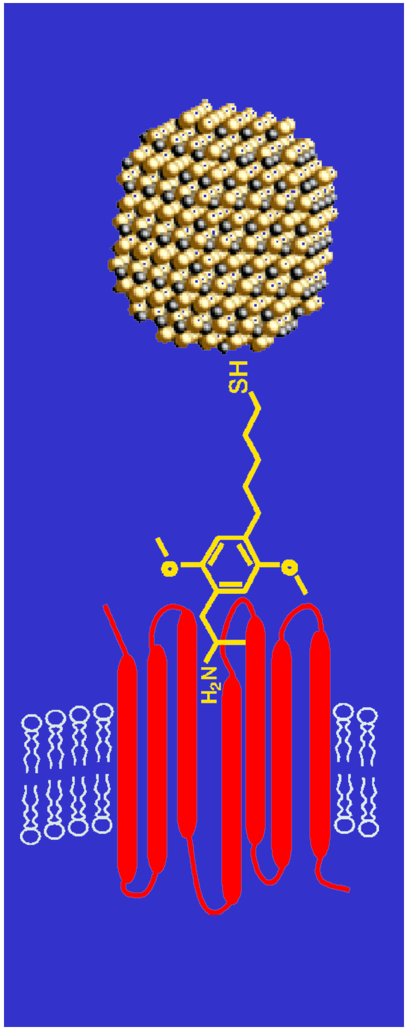
The Future - Nanocontrol

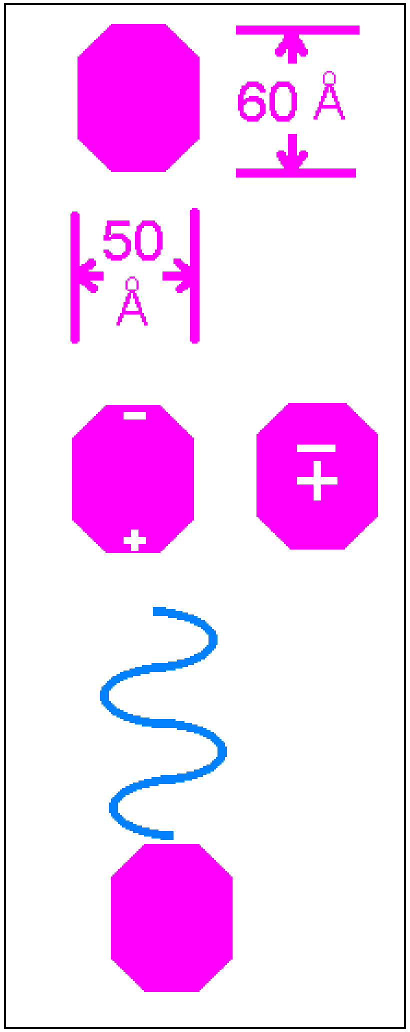
- Optical control of transmembrane potential (SCNC)
- Optically-addressable intracellular nanoheater (PRNS)
- Optically-addressable intracellular nanothermometer (PRNS)
- Nanoforces (FeNC)

Future – Talking to Cells with Light

- Fluorescent tags
  Rosenthal & DeFelice

- Intrinsic shielded dipole moment of 70-80 debye for a 60 A nP
  - +/- 0.3 e at ends, or
  - +/- e separated by 17 A
  - 0.25 volt drop between ends of nP
  - 10^7-10^8 V/m internal field
  - nP dipole moment reduced by light





Metallic NanoShells (Halas@Rice)

- 10^{12} Raman enhancement
  - Can we resolve the Stokes/AntiStokes lines or an adsorbed molecule and construct an optically-addressable intracellular nanothermometer?
- Infrared heating by bioconjugate nanoshells
  - Local control of enzymatic reactions
  - Selected destruction of tagged organalles

Magnetic Nanoparticles - Baudenbacher

- Translational and rotational forces
  - Viscosity -- Nanorheometry
  - Molecular motor characterization
- Magnetic separation
- Magnetic identification (Quake/CalTech)
  - Magnetobacteria
  - Determination of mechanisms of biomagnetic sensing
  - Tagged cells and molecules

Where is the Nano*Bio*Physics?

- What happens when the spatiotemporal scale of a physical, solid-state system approaches that of a biological, living-state system? What are the equations that govern nano-bio hybrid systems? How do you control them?
  - Bio-silicon interface phenomena
  - Nano-bio device physics
  - e^-, ionic, and chemical diffusion and transport in nano-bio hybrid systems
  - Membrane channel and transporter dynamics
  - Artificial membrane proteins
  - Self-organizing systems for bio-nano fabrication
  - Non-linear dynamics and control
  - Complexity
  - Reductionist descriptions of complex non-linear systems and hierarchical models
  - Bio-silicon hybrid computers