VANDERBILT INSTITUTE FOR INTEGRATIVE BIOSCIENCE RESEARCH AND EDUCATION (VIIBRE)

Experimental and Computational Requirements for Post-Genomic Integrative Cellular Physiology

John Wikswo

Instrumenting and Controlling the Single Cell

Topics

- The advantages of micro/nanoscale instruments
- Cellular complexity
- The need for closed-loop control
- How to identify early manifestations of disease
  - Modeling
  - Interactive, dynamical analysis
  - Mining dynamics data

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Reductionism in Science

Thermodynamics

Statistical mechanics

Molecular/atomic dynamics

Electrodynamics

Quantum Chromodynamics

Bulk solids

Devices

Continuum models

Microscopic models

Atomic physics

Anatomy

Physiology

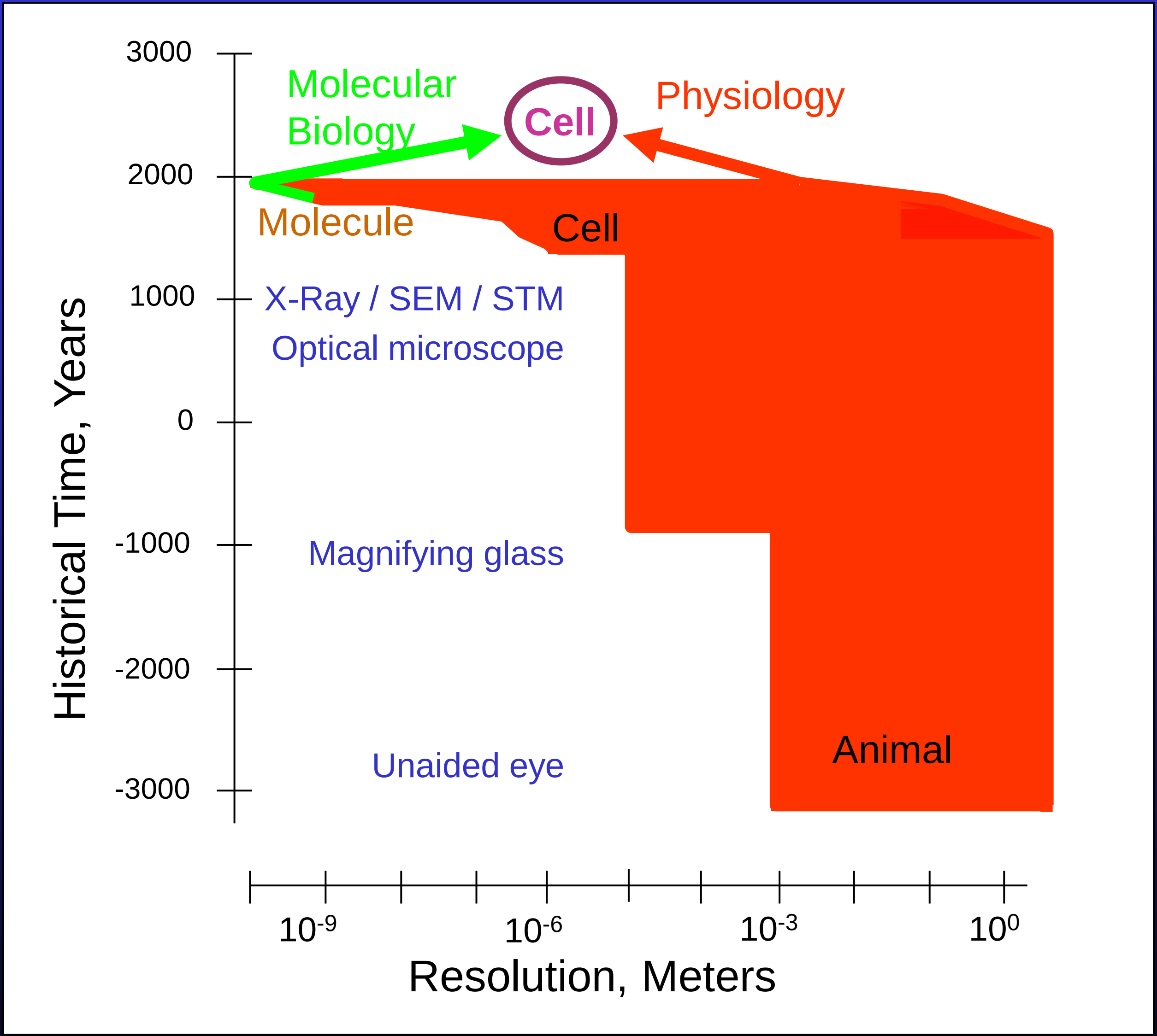
Organ

Cell

Protein

Genome

Historical Evolution of Spatial Resolution in Biology and Physiology

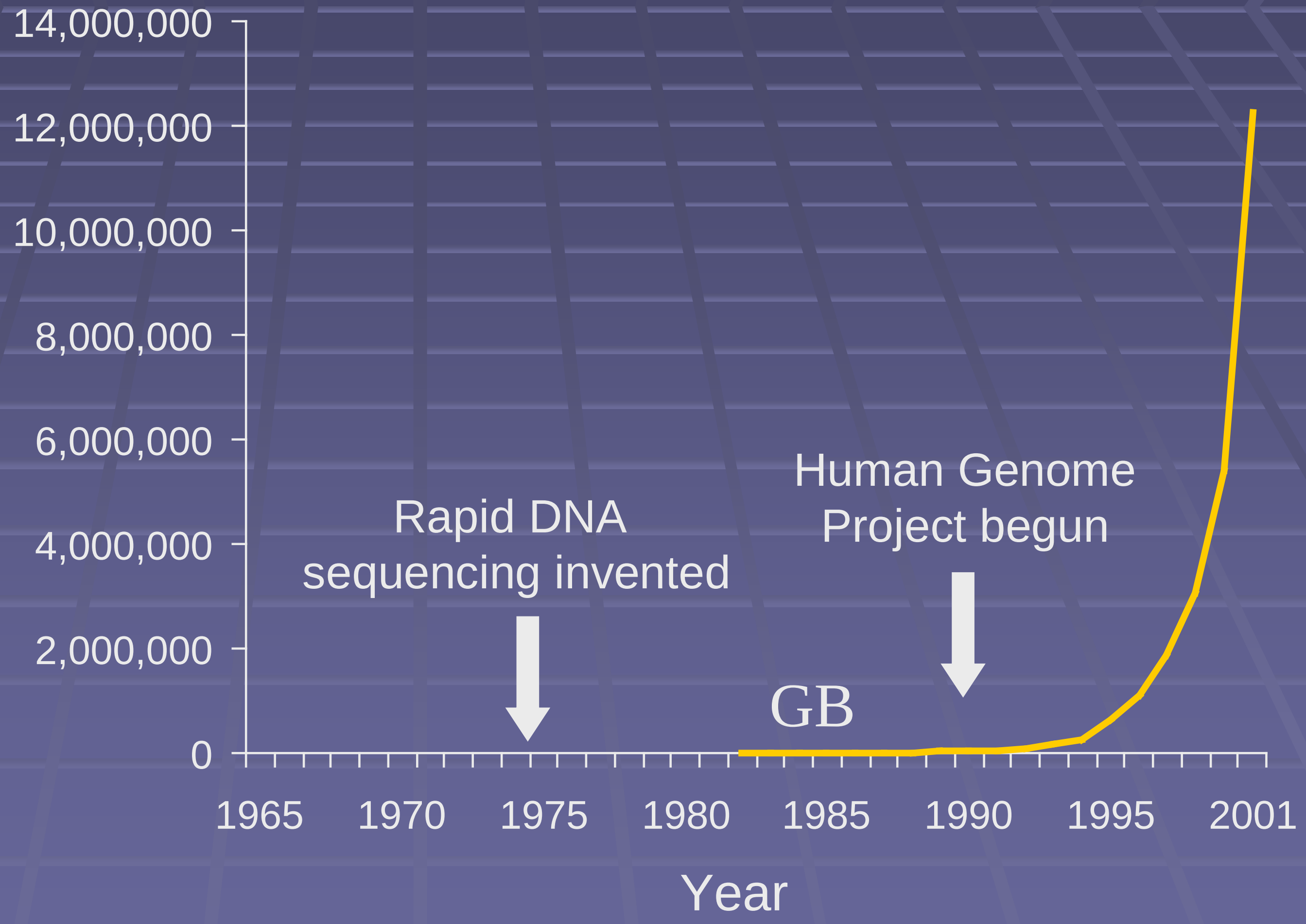


- **Genomics**
  - Structural genomics
  - …
- **Proteomics**
  - Structural proteomics
  - Functional proteomics
  - …
- **What is next?**

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

Hypotheses I and II

I. The explosion in genomic and proteomic knowledge and measurement techniques will revolutionize the early detection of diseases

II. Much of the potential lies in the <u>clinical</u> implementation of the instrumentation and techniques that provided the <u>scientific</u> foundation for genomics and proteomics

Technologies for Early Disease Detection

- **Analysis of biofluids**
  - Molecular profiles that define biological states (Dahl)
  - Disposable plastic lab-on-a-chip devices for point-of-care systems (Luke Lee)
  - Indwelling biosensors and analyzers (Stephen C. Lee)
  - Chemokine and cytokine expression (Barrett Rollins; Philip R. Streeter)
  - Gene expression patterns (Carl W. Cotman; Marti Jett)
  - Detection of mutant alleles (Helmut Zarbl)
  - Protein expression/distribution (Philip R. Streeter; Gordon R. Whiteley)
- **Single-pass analysis of proteins, cells and tissues**
  - Detection of small numbers of molecules (Roger Brent)
  - Multispectral cellular imaging (David Basiji)
  - Protein distribution in tissues (Richard Caprioli)
- **Interactive cellular assays for systems biology**
  - Disease/pathogen-induced changes in cells (Christopher Chen)
  - Nanoscale sensing of single molecule binding (Michael Roukes)
  - Massively Parallel, Multi-Phasic Cellular Biological activity detectors (John Wikswo)

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- **Key Features:**
  - **Low temporal bandwidth sensing**
    - **Slow events, long measurement intervals or single-pass imaging**
  - **Semi-standard, static biochemical analyses**
    - **Feature correlation and pattern recognition**
  - **Will benefit directly from advances in genomics and proteomics**
  - **May involve significant issues in bioinformatics**
  - **Will benefit from Micro/Nano**

Standard Rationale for Micro & Nanoscale Analytical Systems

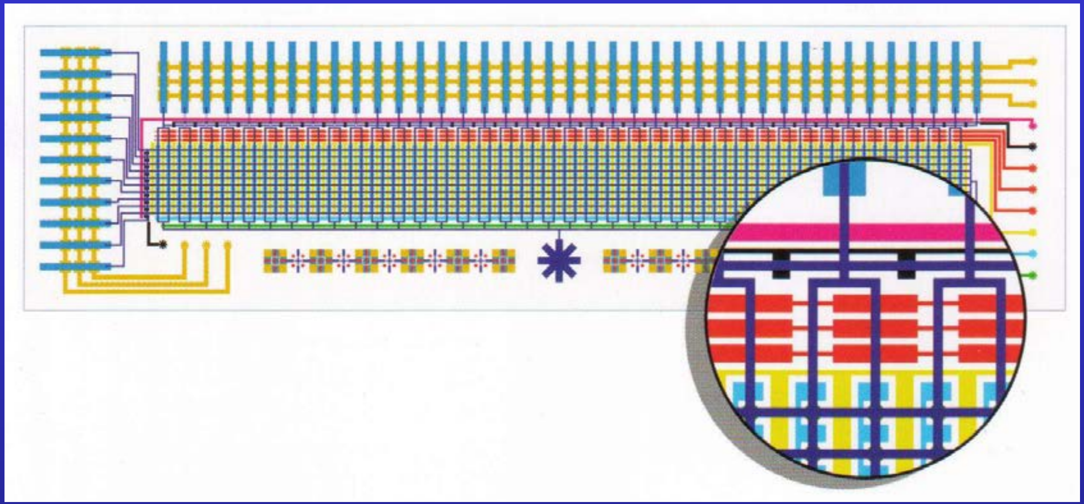
BioMicroElectroMechanical Systems (BioMEMS)

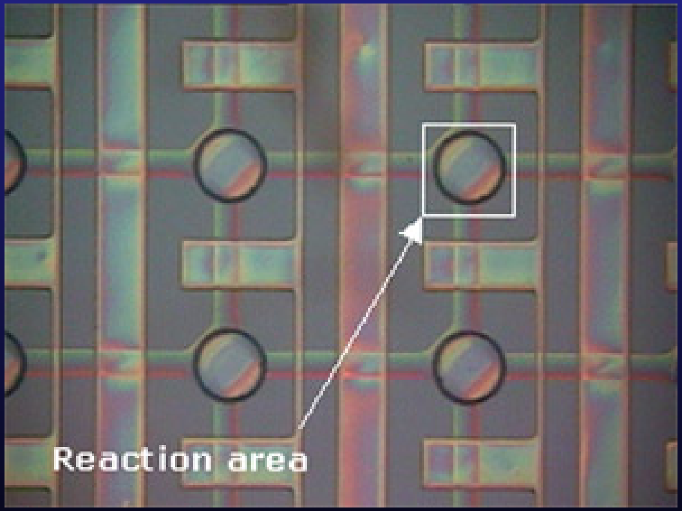
- Low-cost mass production
- Automated analysis
- Reduced instrument footprint
  - Single instruments are very, very small
  - Reduced volumes of analyte and reagents
  - Massively parallel
    - Increased data
    - Lower cost per datum
    - Combinatorics for frontal assault on multivariable systems
- Enabling new physical/chemical properties
  - Single molecule detection
  - Quantum dots

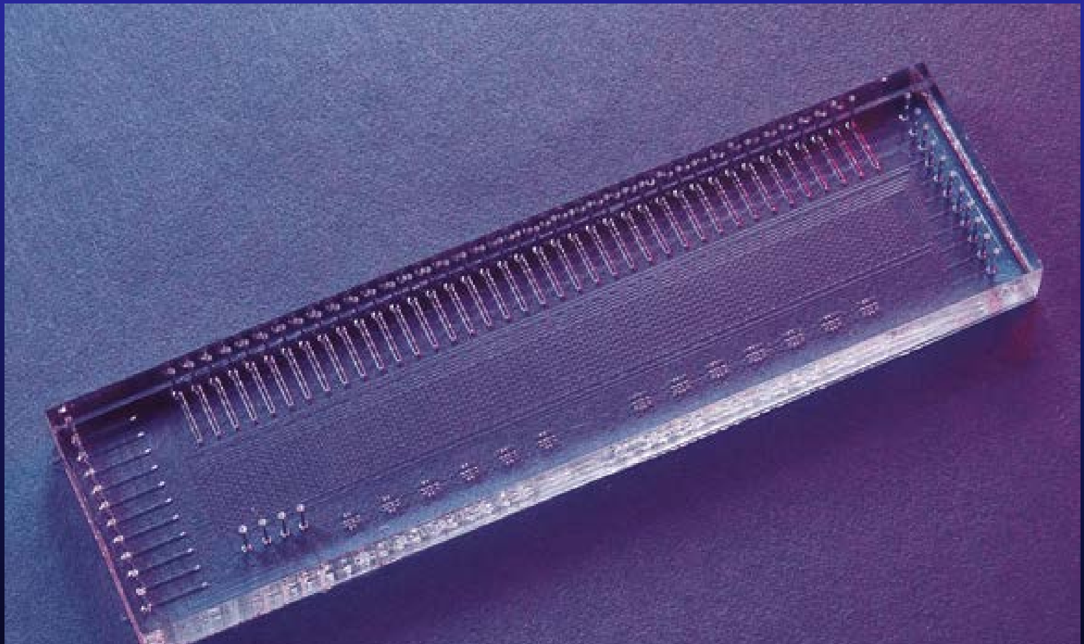
Protein Microprocessor

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

  www.fluidigm.com

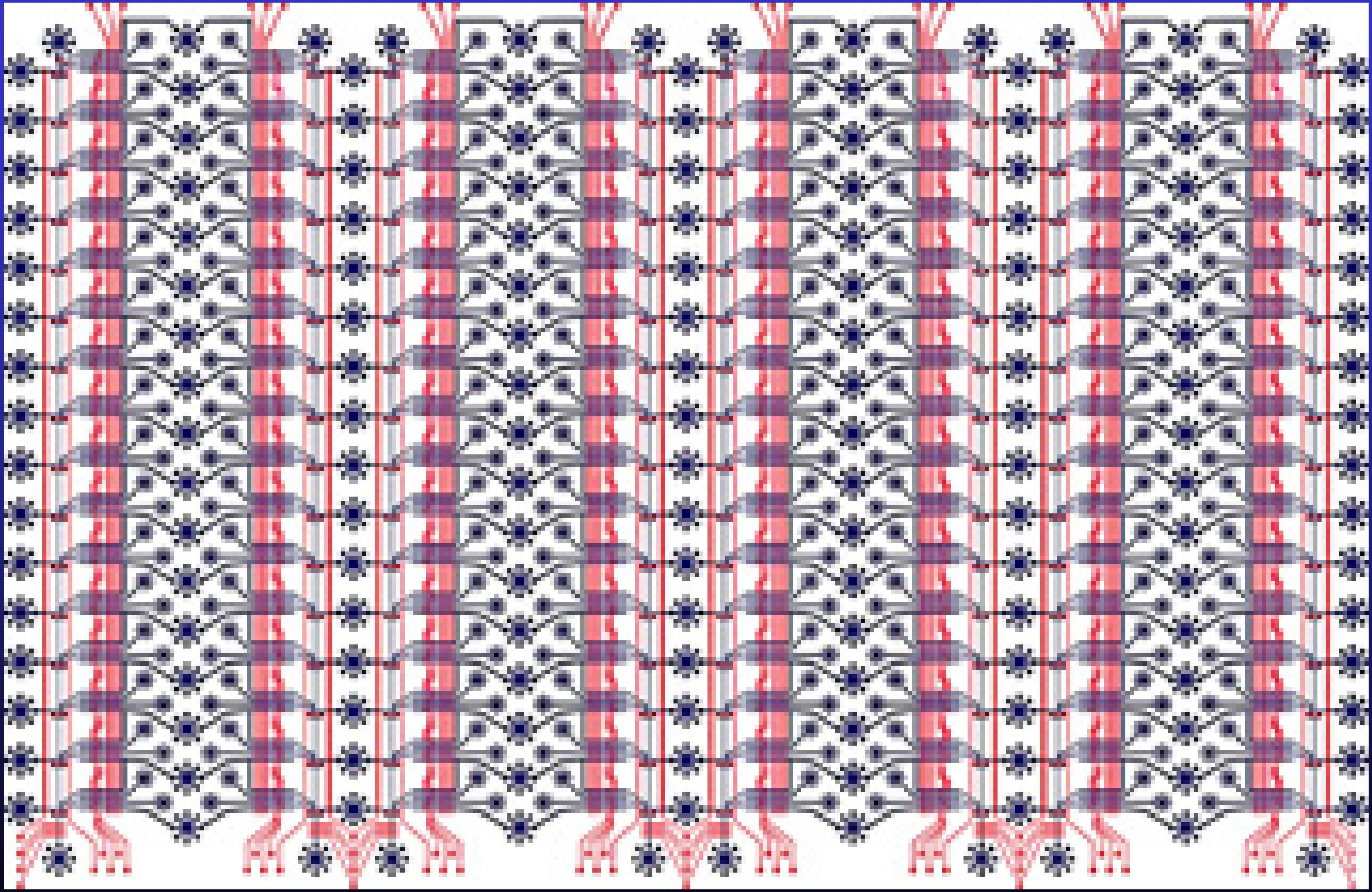






Courtesy of Michael Lee

Live-Cell Microprocessor



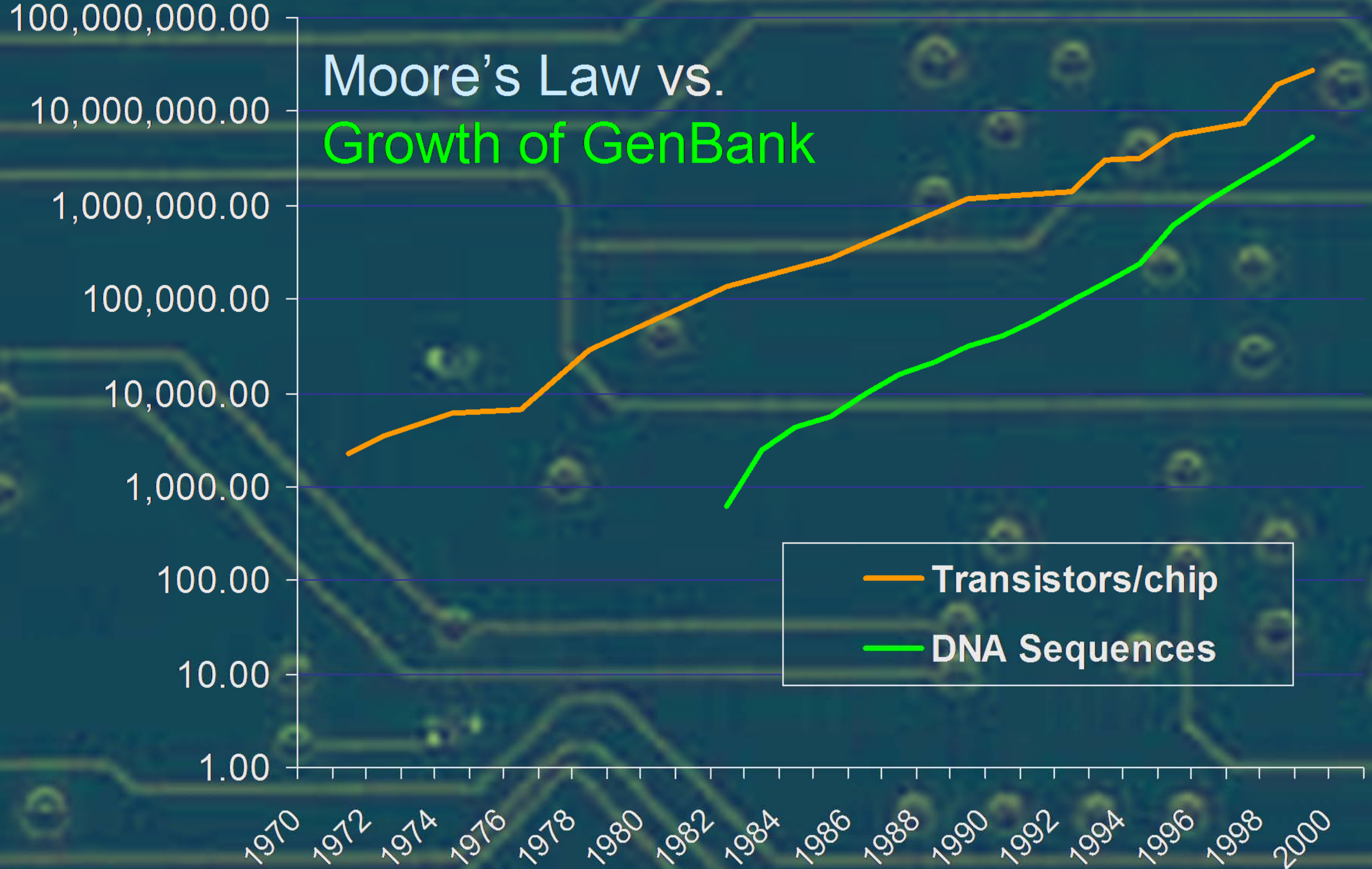
Courtesy of Michael Lee

“Anticipated advances in computer speed will be unable to keep up with the growing [DNA] sequence databases and the demand for homology searches of the data.”

Charles DeLisi, 1988
U.S. Department of Energy

Courtesy of Mark Boguski

Luckily, DeLisi's dire prediction has not (yet) come true



Courtesy of Mark Boguski

Genomics

Proteomics

What is next?

Topics

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- Cellular complexity
- The need for closed-loop control
- How to identify early manifestations of disease
  - Modeling
  - Interactive, dynamical analysis
  - Mining dynamics data

Hypotheses III and IV

III. Historically, dynamical studies of cellular metabolism and signaling pathways have been limited by the bandwidth of laboratory biochemistry

IV. BioMicroElectroMechanical Systems (BioMEMS) offer promise to extend the measurement <u>bandwidth</u> for both research and clinical diagnosis

Technologies for Early Disease Detection

- **Interactive cellular assays for systems biology**
  - Disease/pathogen-induced changes in cells (Christopher Chen)
  - Nanoscale sensing of single molecule binding (Michael Roukes)
  - Massively Parallel, Multi-Phasic Cellular Biological Activity Detectors (John Wikswo)

- **Key Features:**
  - **More closely related to experimental physiology than classical clinical biochemistry**
  - **Can involve rapid sensing of physiological *dynamics***
  - **Measurement bandwidth << physiological bandwidth**
  - **Real-time intervention is REQUIRED to probe the dynamics**
    - **Internal vs. external feedback**
    - **"Bandwidth is everything"**
  - **May require models for interpretation of complex interactions**
    - **There may be significant computational contraints to multiscale dynamical models**

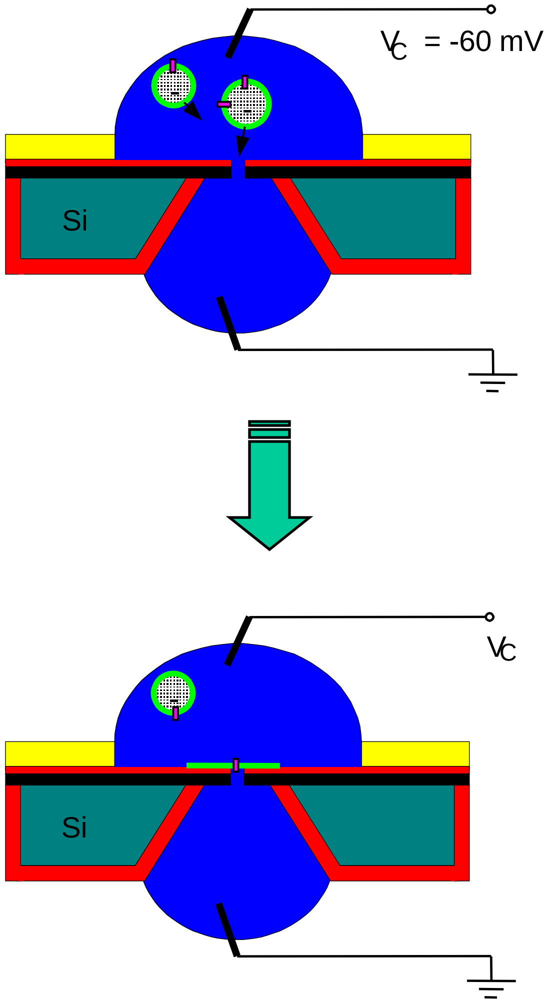
Post-Reductionism

Thermodynamics	Bulk solids	Anatomy
Statistical mechanics	Devices	Physiology
Molecular/atomic dynamics	Continuum models	Organ
Electrodynamics	Microscopic models	Cell
Quantum Chromodynamics	Atomic physics	Protein
		Genome

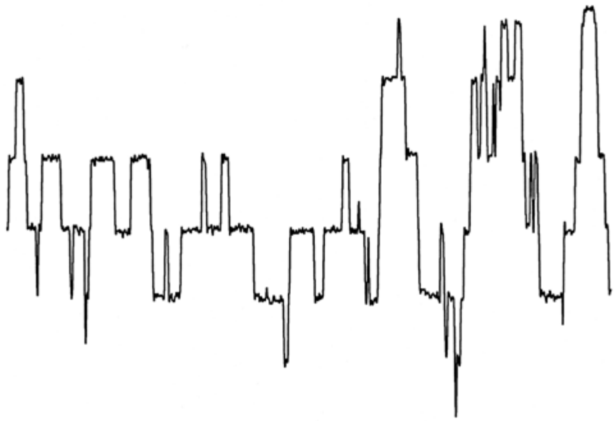
What about dynamic processes?

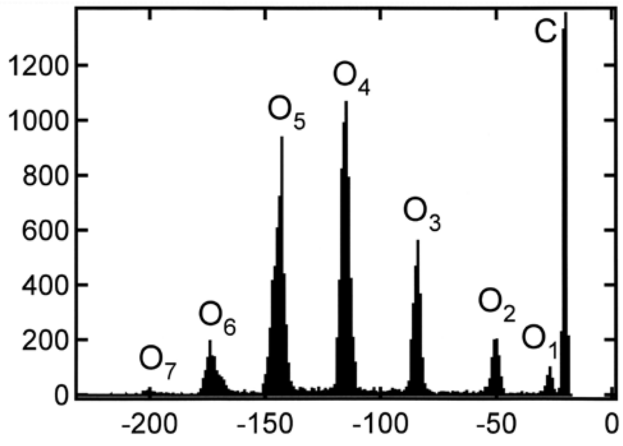
- Physiology is *dynamic*
  - –Cell cycle
  - –Developmental differentiation
  - –Growth
  - –Voltage- and ligand-gates ion channels
    - Propagating waves
    - Signaling cascades
  - –Closed-loop feedback and control

Cytion Planar Patch Clamp



Alamethicin with its typical activation (up to 7 conductance states)

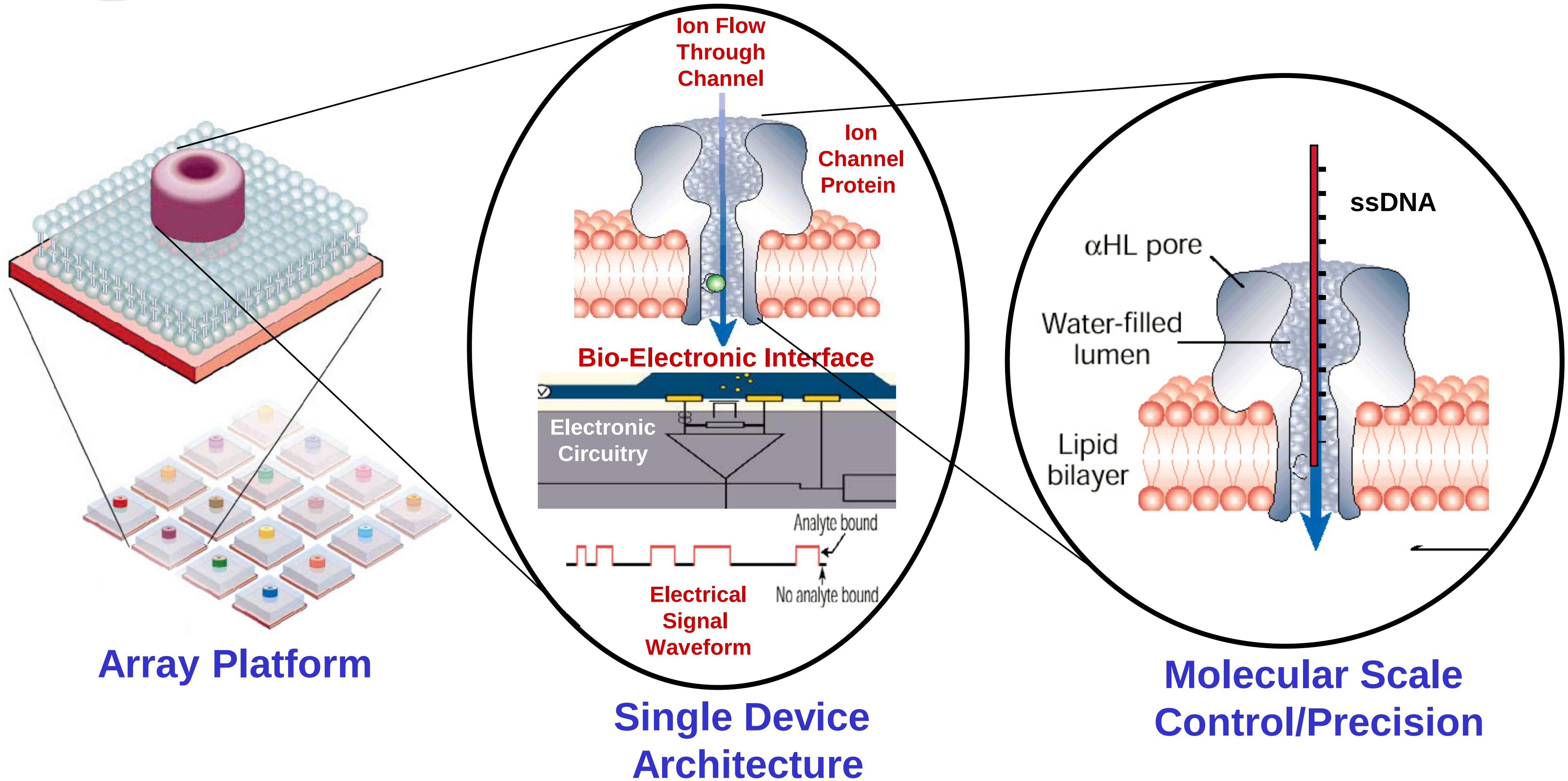




V = +60 mV -> E > 12 000 V/m !!

Courtesy of Christian Schmidt and Will Lachnit

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

Physical and Biological Time Constants, Seconds

Mixing time to homogenize liquid in a large-scale bioreactor (10-100 m^3)	10^4 - 10^8
90% liquid volume exchange in in a continuous reactor	10^5 - 10^6
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Cell proliferation, DNA replication	10^2 - 10^4
Response to environmental changes (temperature, oxygen)	10^3 - 10^4
Messenger RNA synthesis	10^3 - 10^4
Translocation of substances into cells (active transport)	10^1 - 10^3
Protein synthesis	10^1 - 10^2
Allosteric control of enzyme action	1
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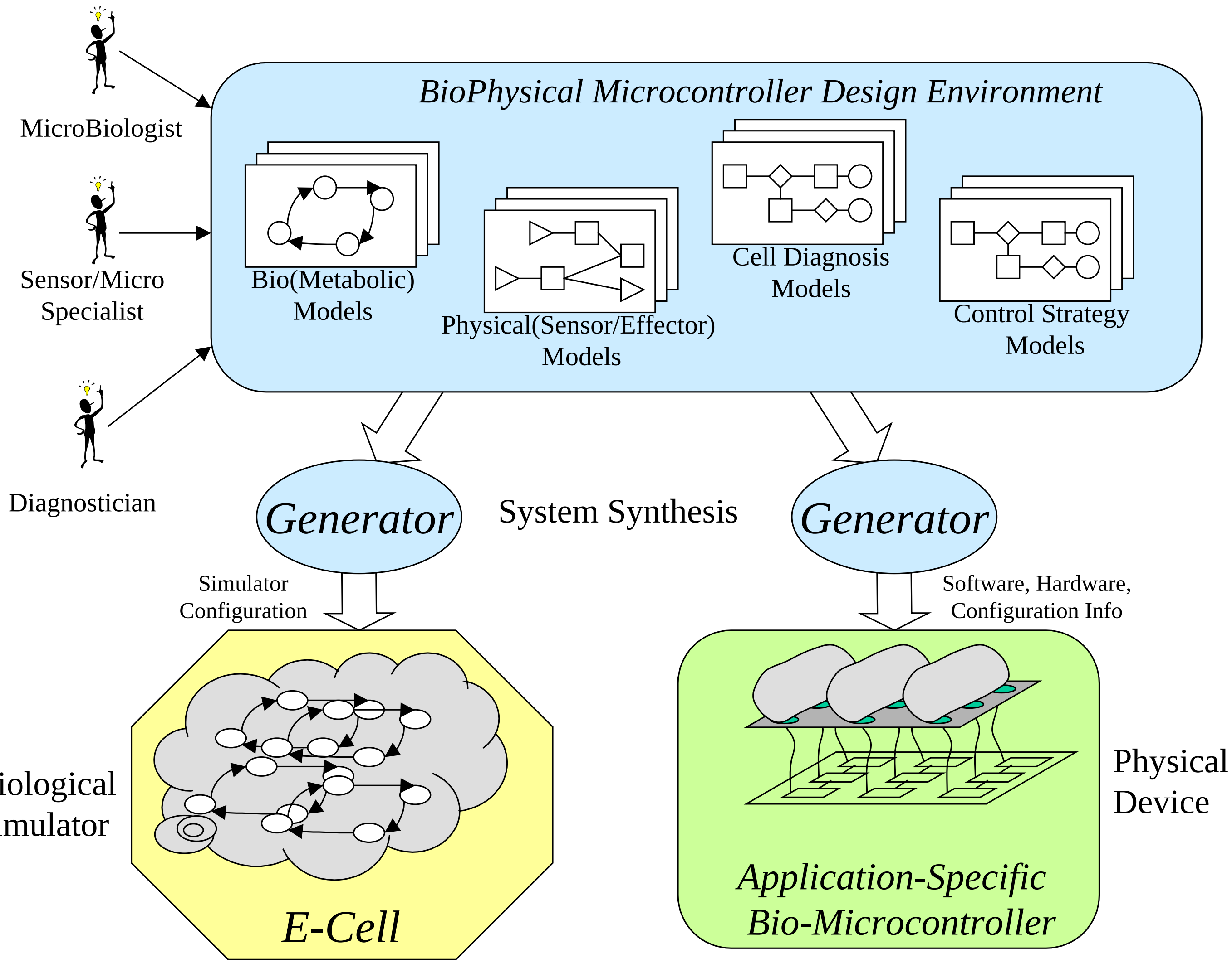
Receptor-ligand interaction	10^{-6}

The Systems Physiology Challenge

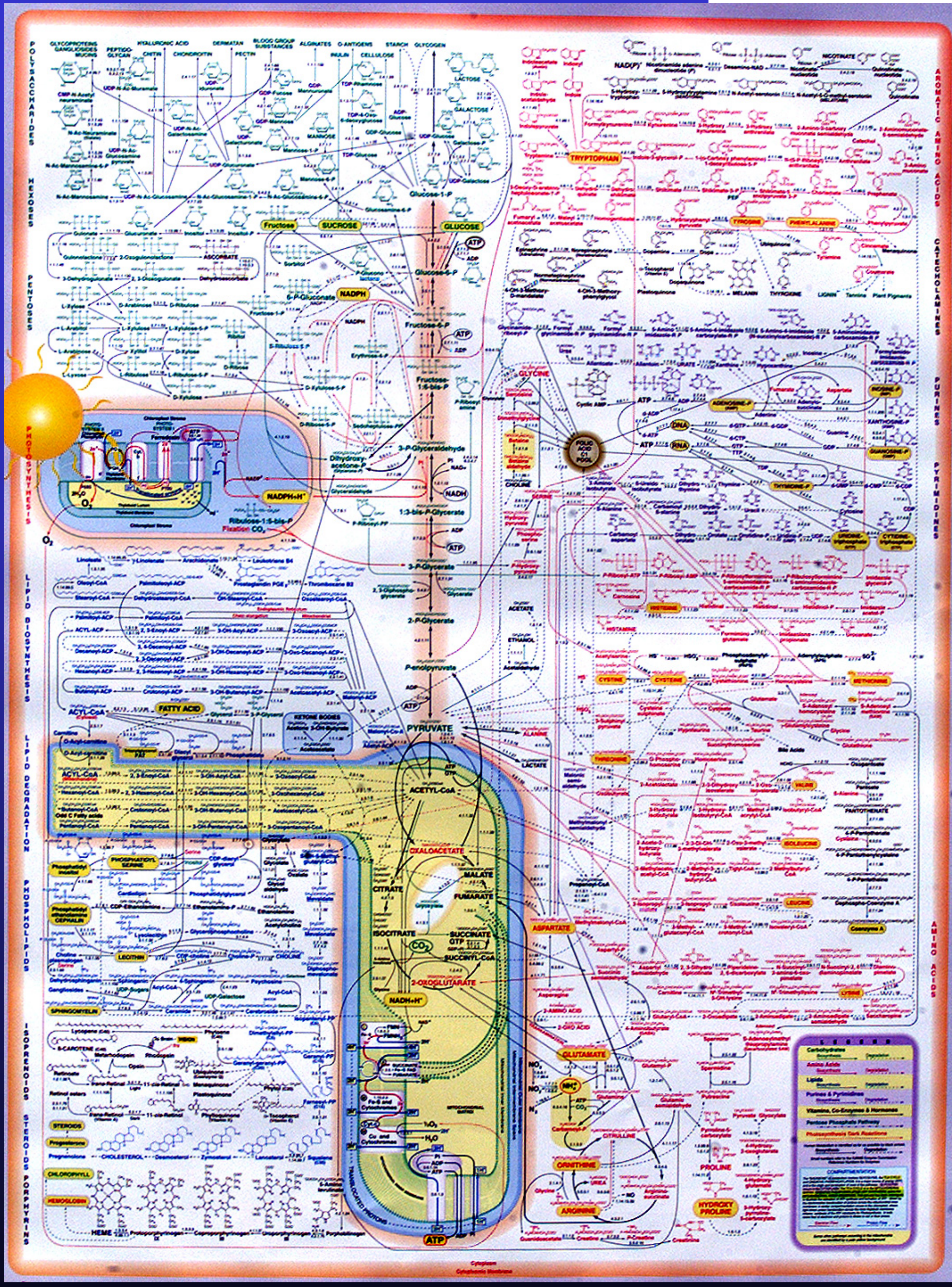
- Use experimental measurements, numerical simulations, and knowledge of the genome and proteome to unravel the complex, multiscale interactions and dynamics in normal physiology, toxic exposures, and disease
  - Metabolic networks
  - Intracellular and extracellular signaling
    - Gene expression
    - Protein interactions
    - Cell-cell interactions
    - Active transport
  - Development, growth, aging, death

Biological Modeling and Analysis

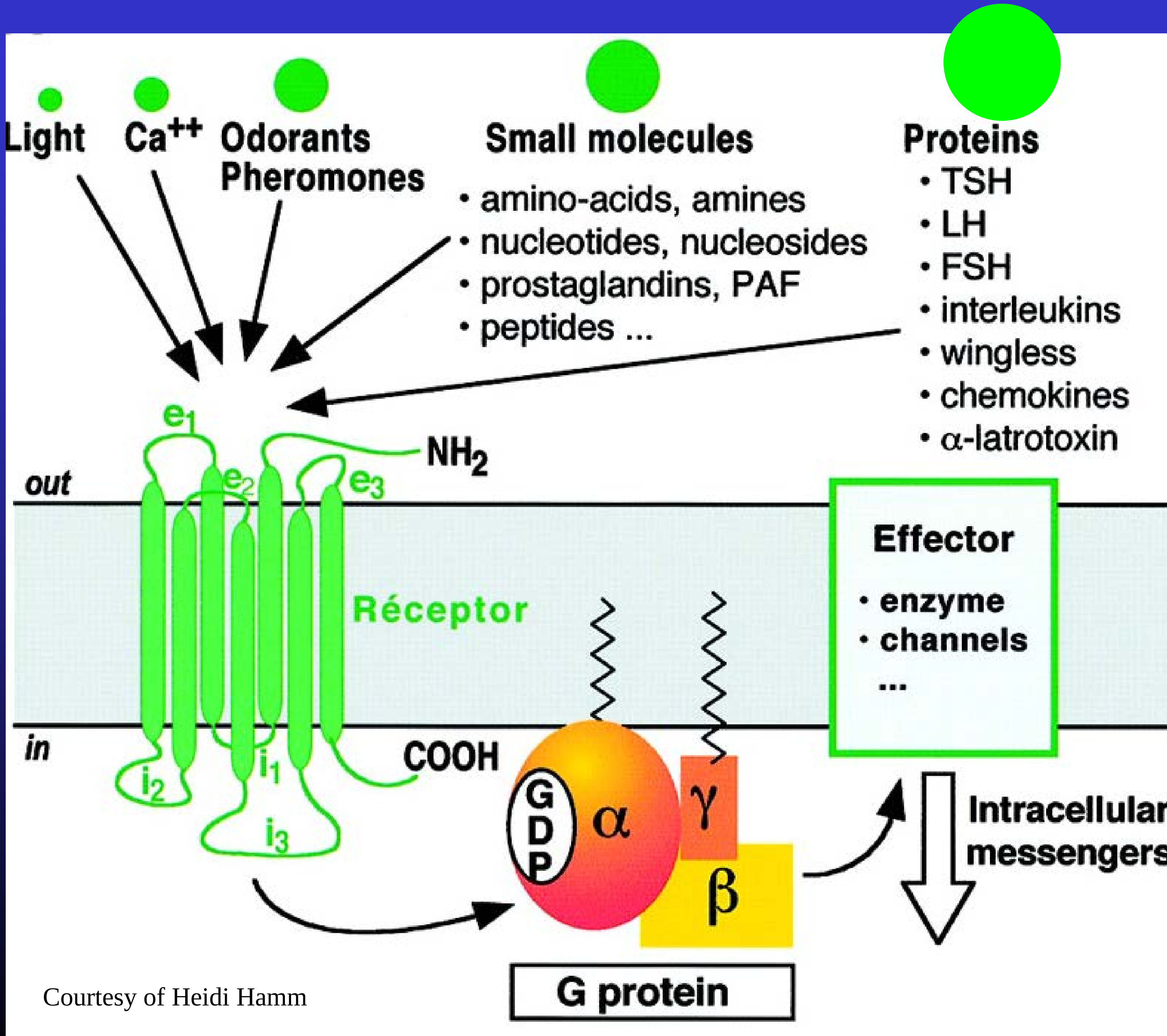


Postgenomic Integrative/Systems Physiology/Biology

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

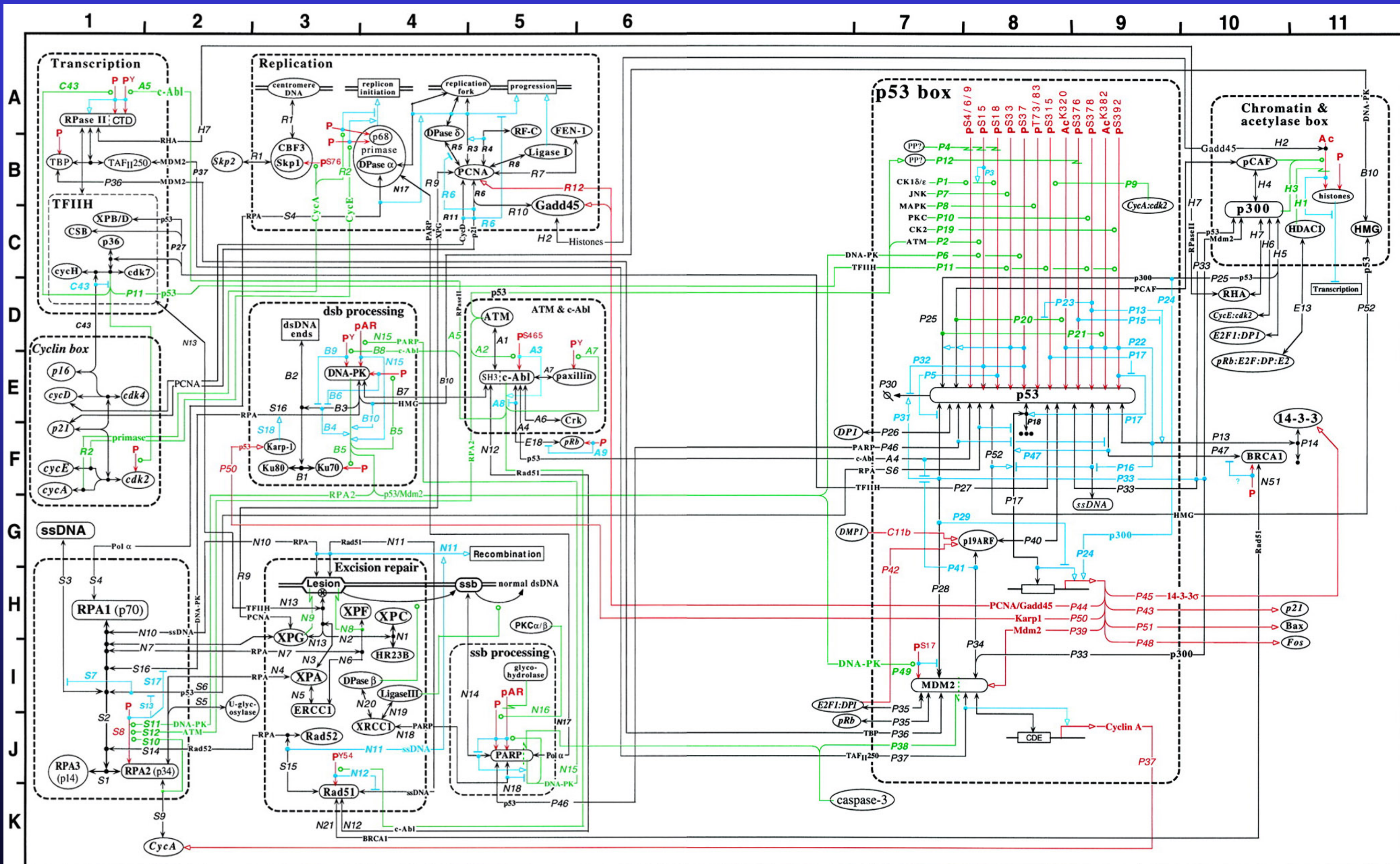


G-Protein Coupled Receptors



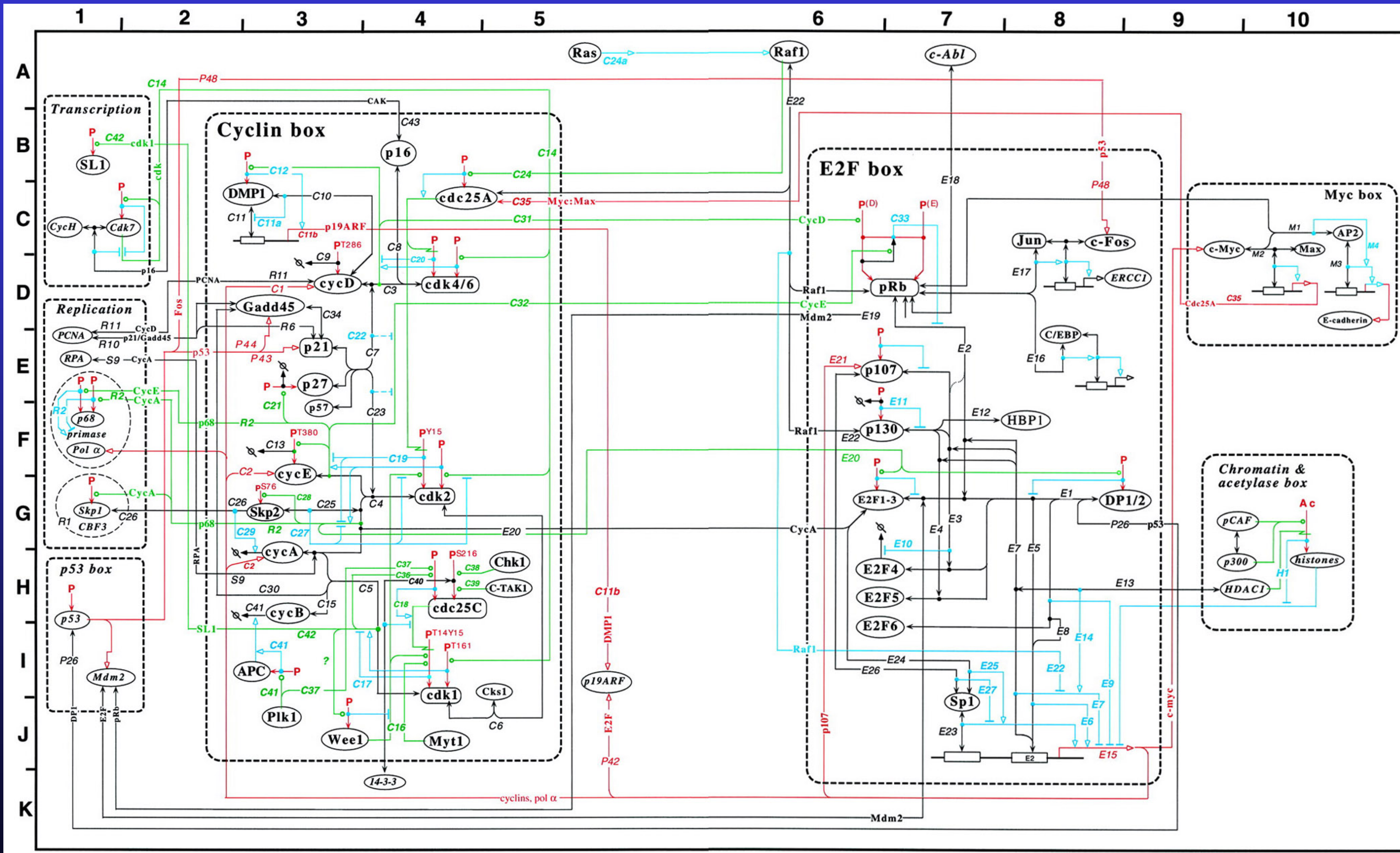
Courtesy of Heidi Hamm

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



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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
- Models may be leibnitz-class
- ***The data don’t yet exist to drive the models!***
- Hence we need to experiment…

*1 leibnitz = 1 mole of PDEs ~ 1 etaFLOPS-year

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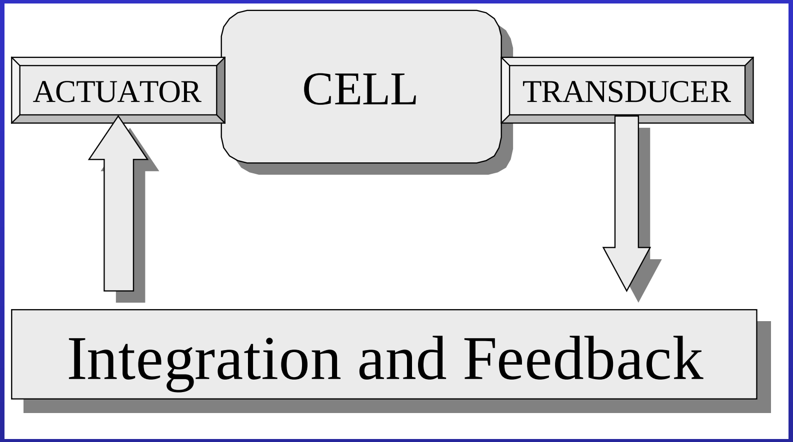
The Experimental Problem

- **Most chemical and metabolic sensors and actuators**
  - Are too slow to track biochemical events at the cellular level
  - Are made one at a time
- **Biological systems contain extensive closed-loop, multilevel, feedback and control**
  - Simple, single-step observations cannot discern how control is distributed through the system.
  - Closed-loop metabolic control is today possible only at the animal and organ level, e.g., glucose clamp
  - Chemical control is limited by diffusion, stirring, uncaging rates, or the time required to move a cell from one medium to another
- **Post-genomic <u>physiology</u> needs multiparameter, wide-bandwidth cellular metabolic and signaling sensing and control**

What do we need?

- Simultaneous, fast sensors (transducers) that detect a of changes within 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
- …

The Challenge: Instrument and Control the Cell

- Develop the tools and techniques for integrative, post-genomic **cellular** biology
  - Genes
  - Proteins
  - Metabolic and signaling pathways
  - **Instruments**
  - **Models**
  - **Wide-bandwidth dynamic control theory for cellular systems**
- **How do normal and diseased cells *function*?**

Instrumenting the Single Cell

- **Arrays of instrumented single or multiple cells:** Rapid, sensitive, and accurate differential diagnosis of cellular pathophysiology
- **Cellular dynamics:** Discrimination between causal and secondary events
- **Functional biopsy:** Determine the state of specific physiological pathways and mechanisms affected by an as-yet undetected disease, and thus define a prophylaxis or therapy.
- **Artificial, minimal cells:** engineered to serve as dedicated, configurable, robust on-chip biosensors.

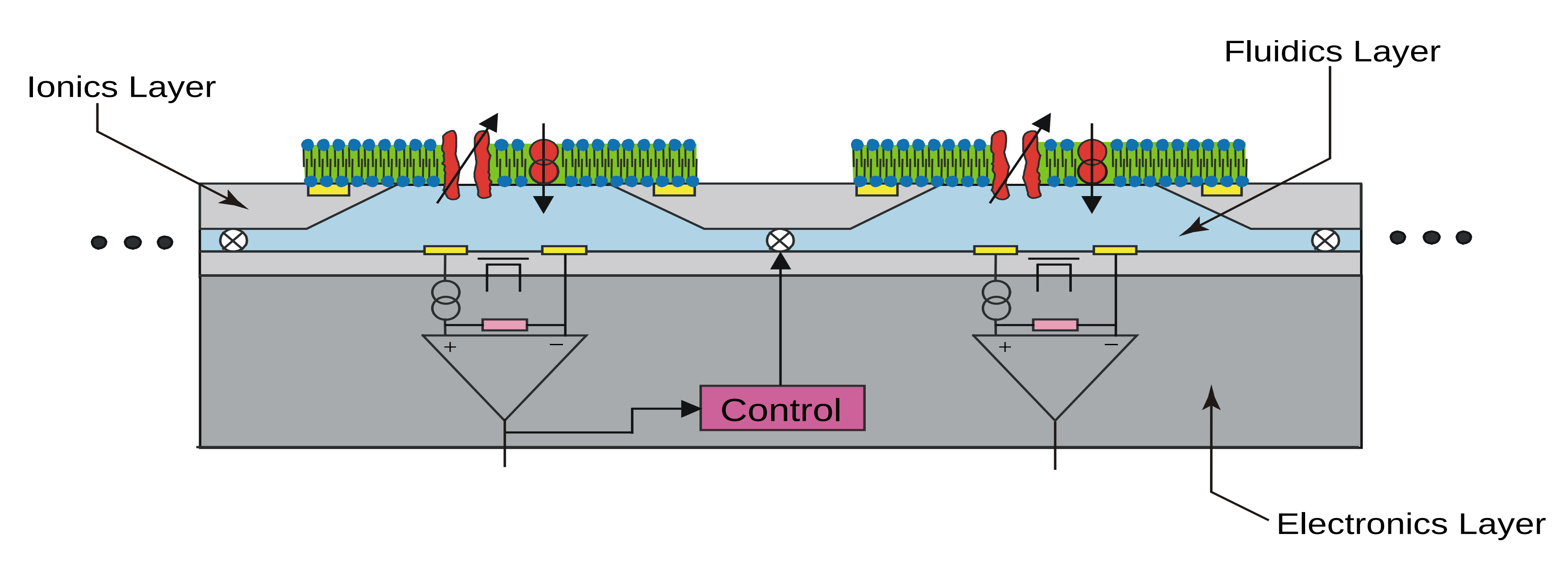
"Quantitative physiology at the speed of life," *C. Kovac*

Possible Approaches

- A biological cell or molecule inserted into a microinstrument, e.g., a single-cell spectrophotometer or a whole-cell patch clamp
- A nanoinstrument inserted into the cell/molecule, e.g., caged ATP
- Combine the two approaches to form an FAST integrated, closed-loop bio/nano/micro system

MicroBottle

- Goal – utilize proven gigaOhm seals to biological membranes
- Result – high-speed microfluidics and silicon microelectronics can be placed “inside” a living cell



Hypotheses V and VI

V. Great advances in physiology have been made through opening physiological feedback loops and applying external control

- Frank-Starling cardiovascular regulation
- Glucose/insulin regulation
- Hodgkin-Huxley model of the nerve action potential

VI. There will exist a class of diseases or susceptibilities to drugs or toxins that can be diagnosed through altered cellular dynamics

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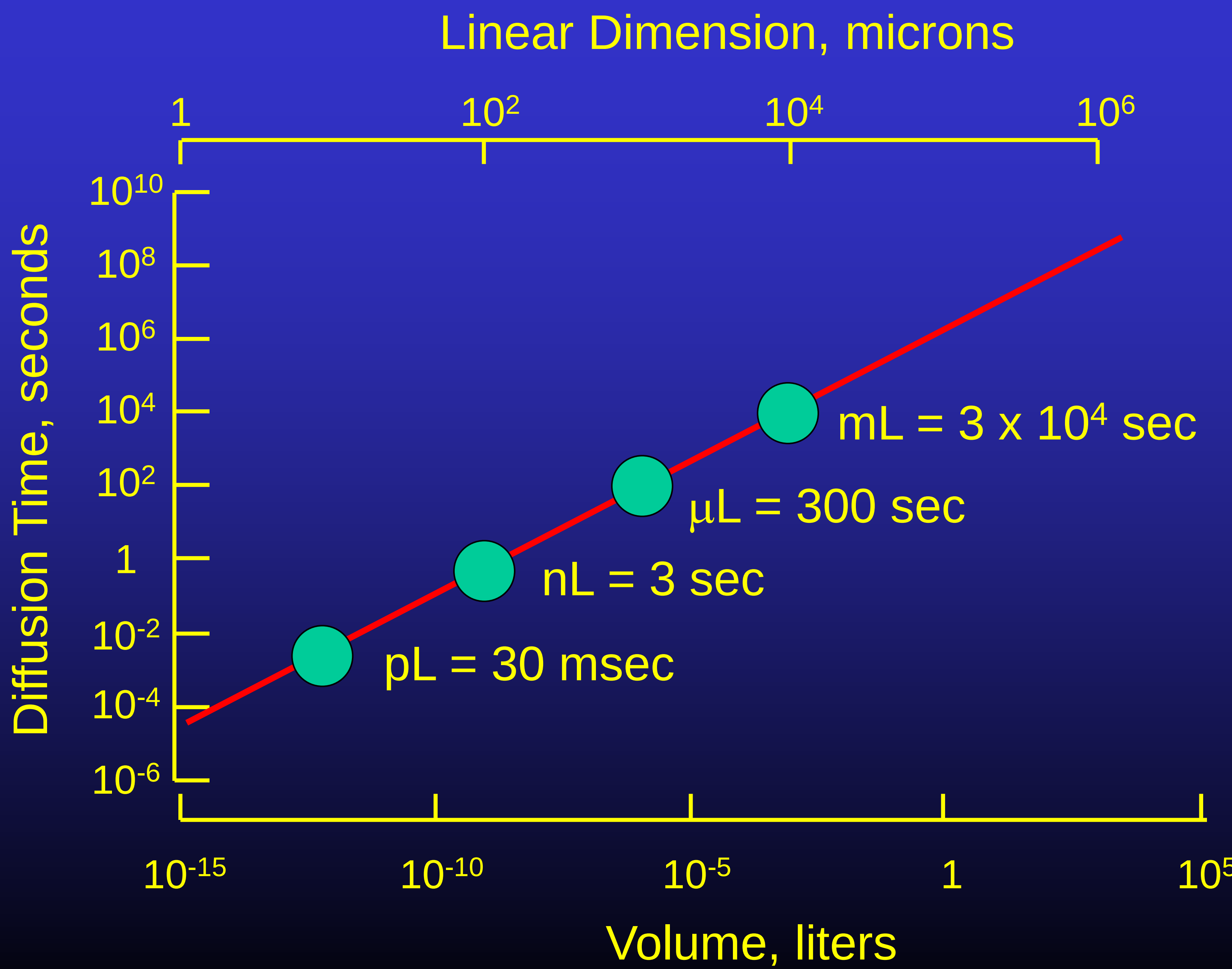
A Key Rationale for Micro & Nanoscale Analytical Systems

- Wide measurement bandwidth, *i.e.*, good response to high frequencies, is required to track *fast* cellular events
- Stable control of fast systems requires high bandwidth
- Small is the best way to beat the time for diffusional mixing in large-scale assays
- Small lets one look at individual cellular events rather than ensemble averages

Lactate Diffusion Times

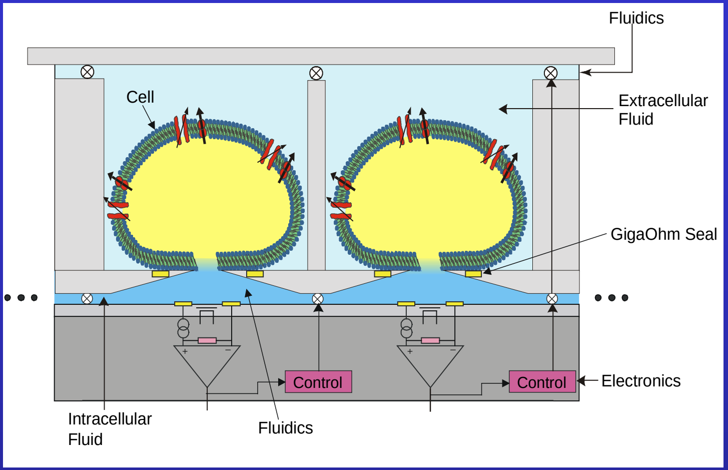


What do we gain by small and fast?

- Electrochemical sensitivity scale-invarient; frequency response improves as size is decreased
- Decreased mixing times for mass and heat transfer
- Reduced reagent volumes for rapid injection
- Many nanocultures within a single device
- Monitor known, small (N=1?) number of cells in each nanoculture
- Array of NanoBioReactors, in parallel, in series, and with redundancy for high-content screening

Fast, small, and many by moving from milliliters and microliters to nano and picoliters!

Chemical Clamp



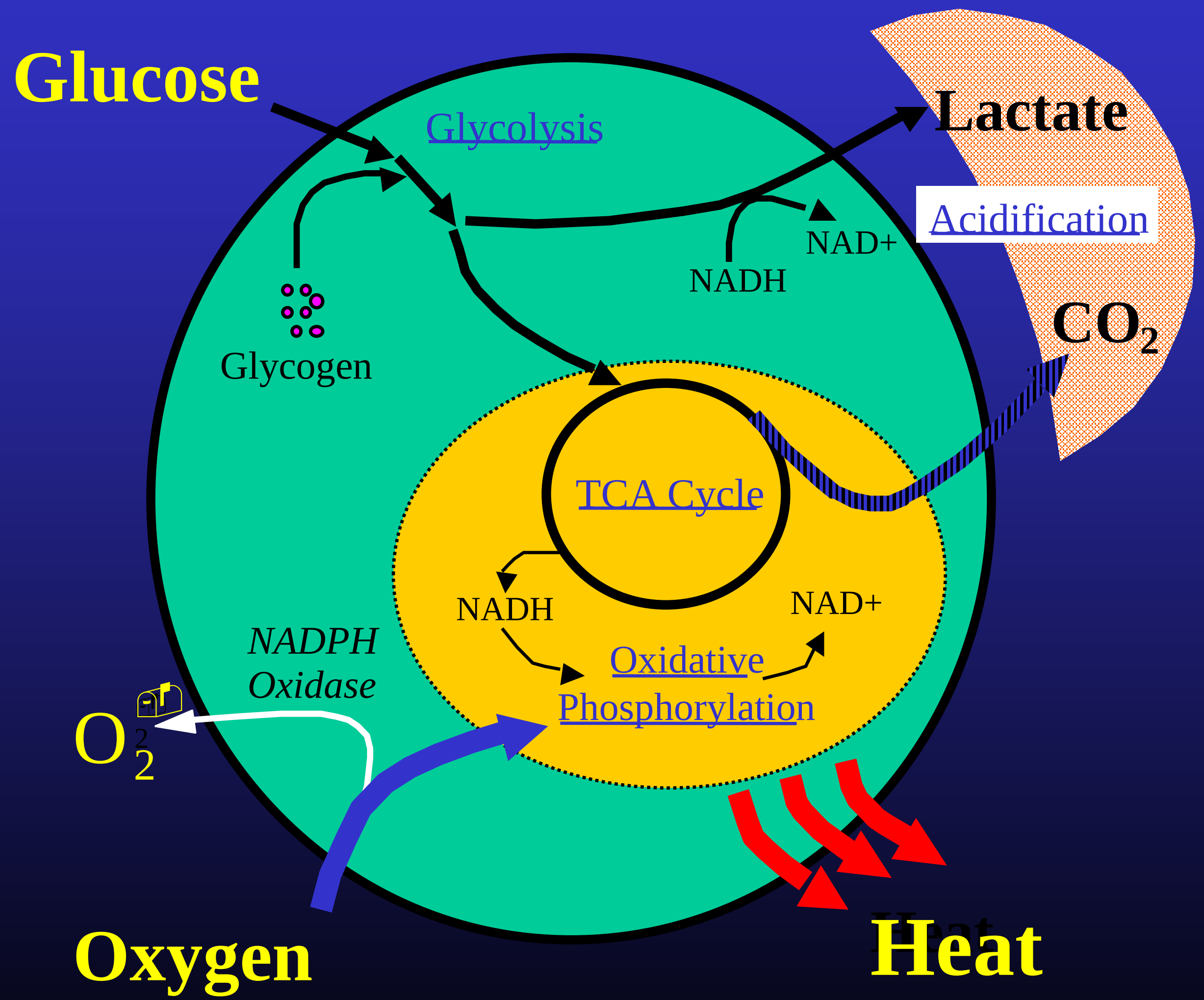
- **Sensors:** Advanced micro and nanosensors can quantify the extra-cellular and intra-cellular environments with unprecedented temporal resolution
- **Actuators:** Microfluidics can control extracellular and intracellular concentrations of key chemicals with millisecond-response picoliter pumps
- **Openers:** RNAi, genetic knockouts, and blockers will allow opening of the internal feedback loop
- **Controllers:** It will be possible to create high-speed extracellular and intracellular chemical clamps functionally equivalent to voltage clamp for V_m

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

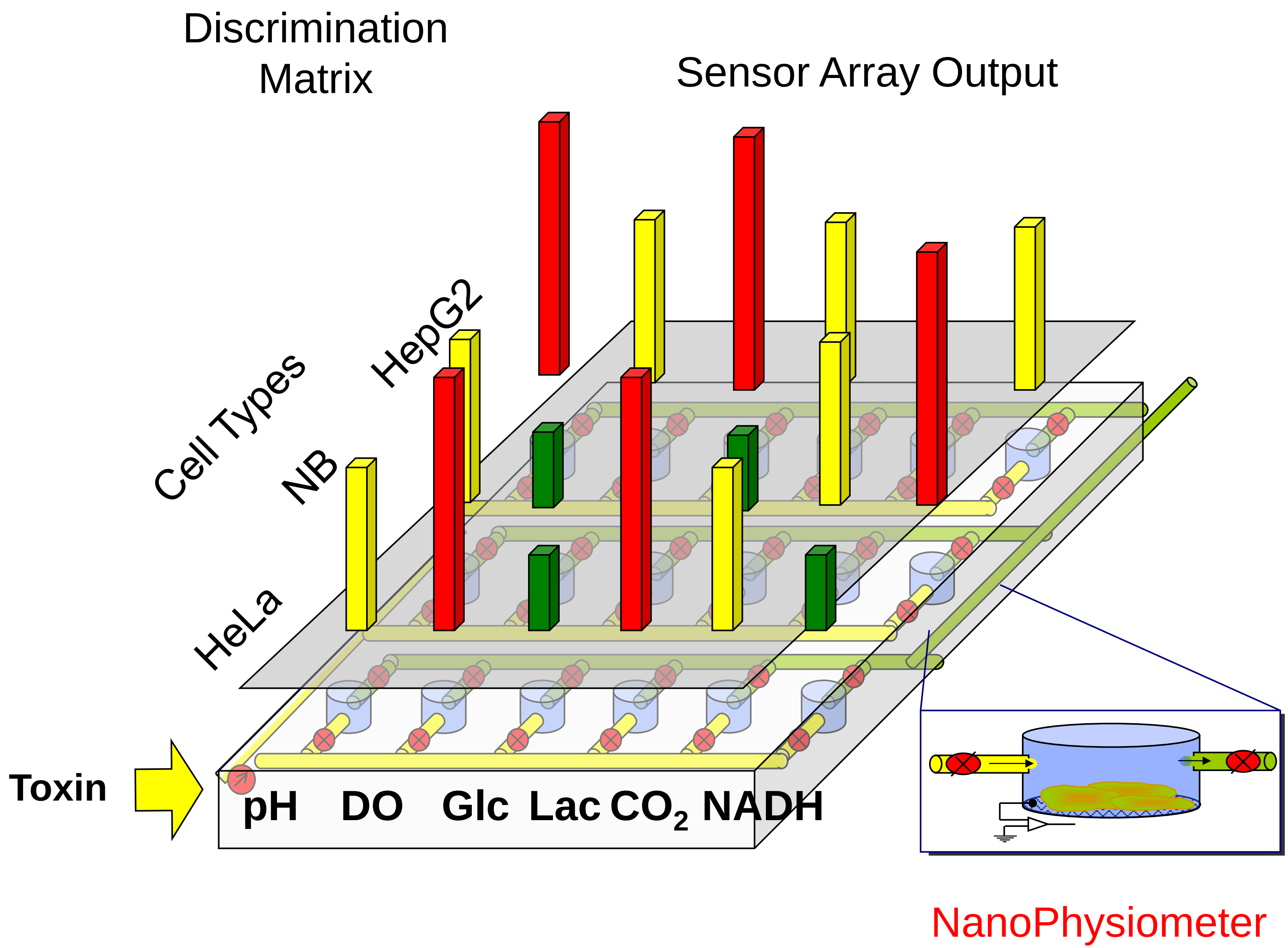
Vanderbilt University

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

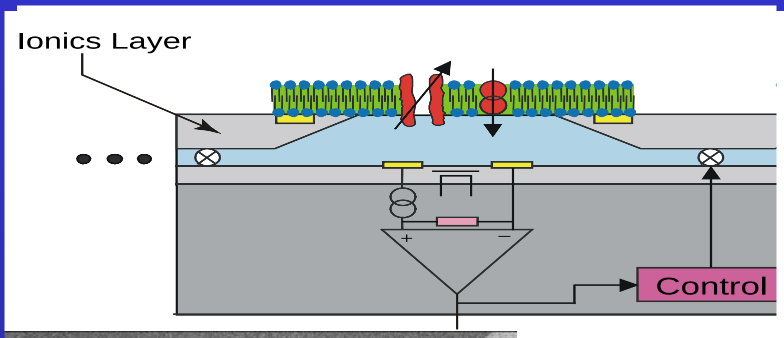
Cell Metabolism

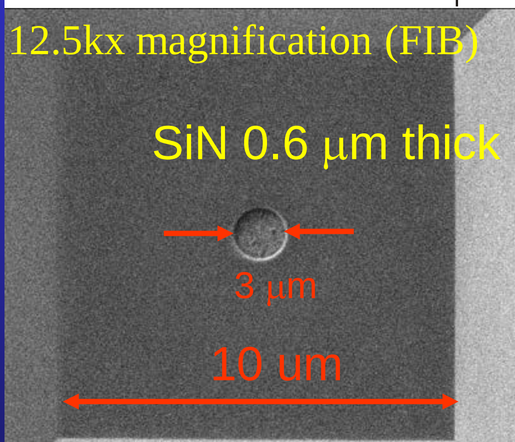


Discrimination



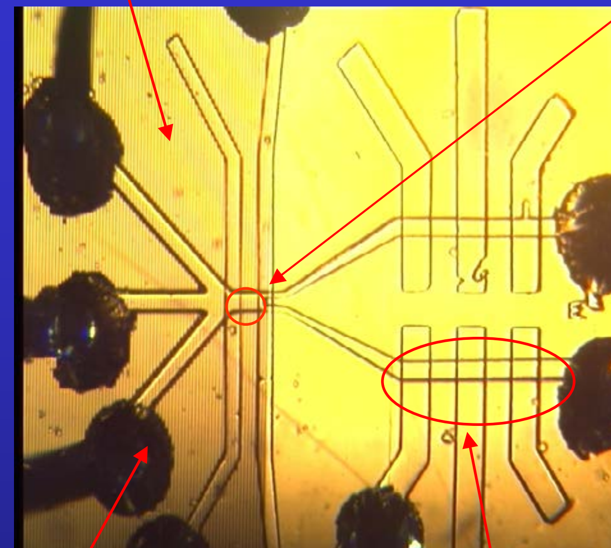
Vanderbilt Instrumenting the Single Cell

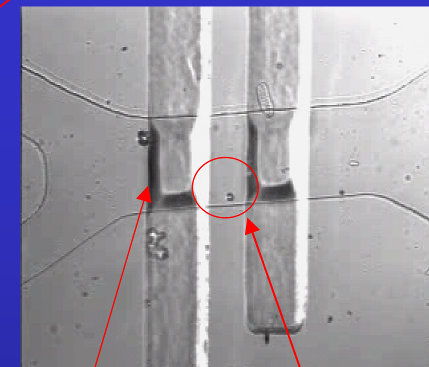




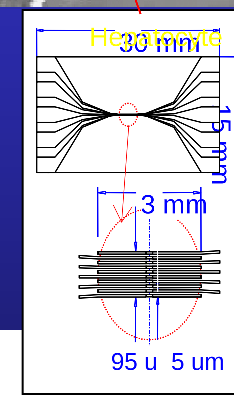
Interdigitated Array Electrodes (IME)

Sensing volume (0.25 nL)



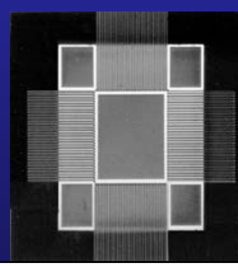


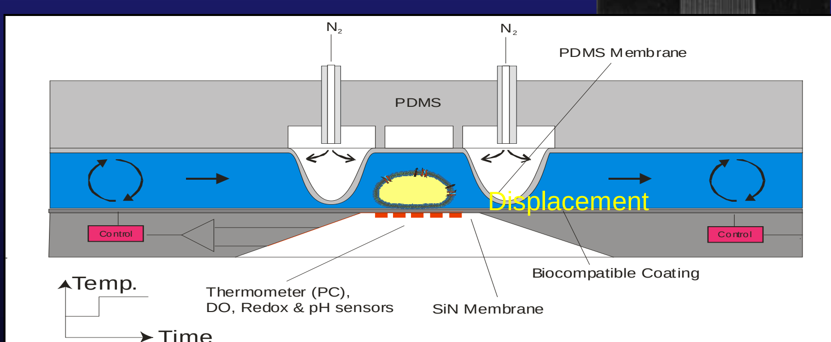
Valve

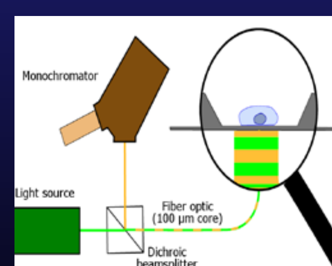


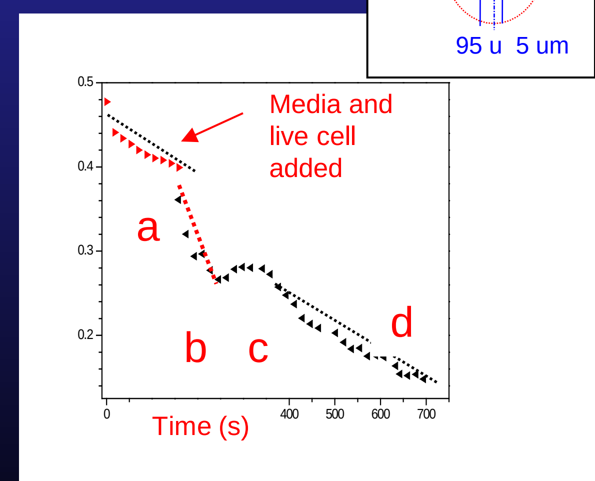
Waste Channel

Valves - Peristaltic Pump









Advanced Technology for next generation CBW Biosensors

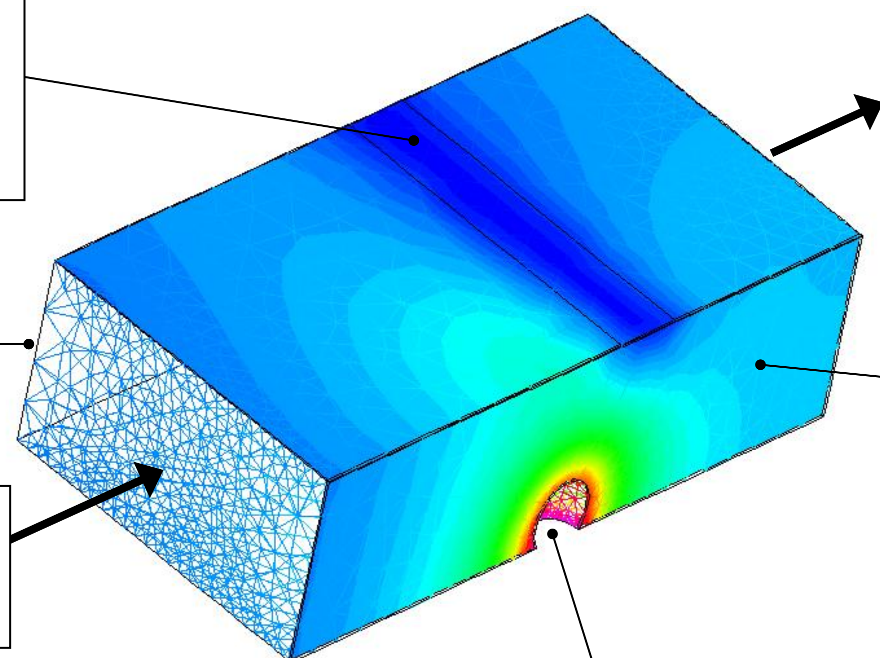
Coupled Modeling of Cell and Environment

- Convective-diffusive transport of analytes by a 3D time-dependent flow
- Cell boundary conditions controlled by dynamic metabolism model
- Computational model built with CFD-ACE+ (CFDRC)

Electrochemical Sensors:
- Zero concentration at surface
- Sensor signal proportional to concentration gradient at surface
- Customizable location, geometry

Channel Walls:
- Impermeable or permeable

Inlet Flow:
- Pressure driven or electrokinetic
- Specified analyte concentrations



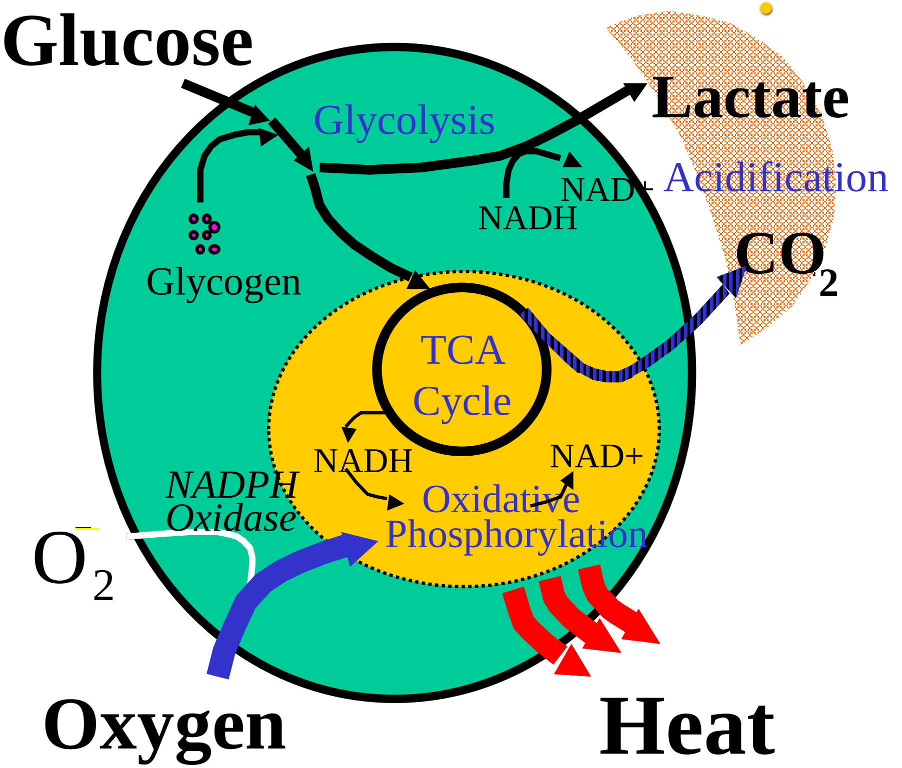
Outlet

Symmetry plane

Single Cell Model:
- Membrane shape coupled to flow
- Membrane fluxes specified according to environment and dynamic metabolism model

Mark Stremler and Steven Yu,
Mechanical Engineering

The Challenge: Convert Steady-State Metabolic Flux Balances to Dynamic Metabolic Network Responses

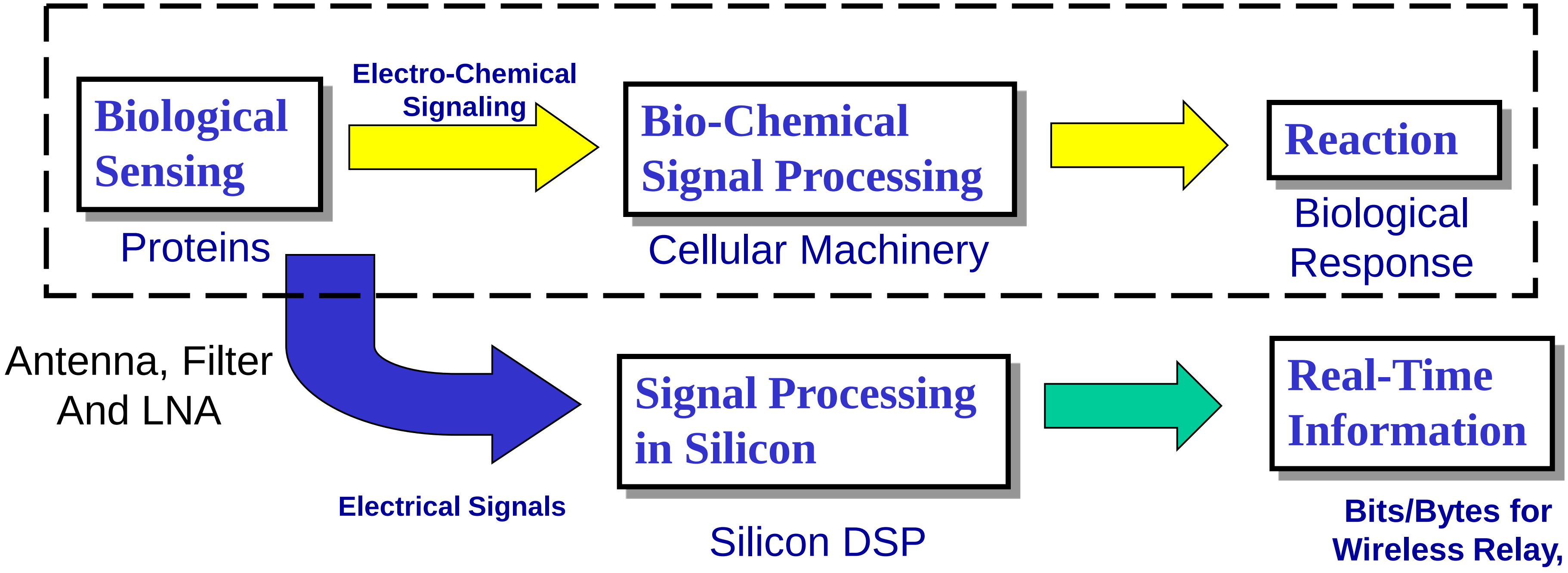


Glucose + 2 ADP + **2 NAD^+**	→	**2 Pyruvate** + 2 ATP + **2 NADH**
Pyruvate + **NADH**	→	**Lactate** + **NAD^+**
Pyruvate + CoA + **FAD** + GDP + **3 NAD^+** + **$NAD(P)^+$**	→	**3 CO_2** + **FADH2** + GTP + **3 NADH** + **NAD(P)H**
0.5 O_2 + 3 ADP + **NADH**	→	3 ATP + **NAD^+**
0.5 O_2 + 2 ADP + **$FADH_2$**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

Devices/Systems

Exploit Proteins as High Performance Nanoscale Signal Processing Devices

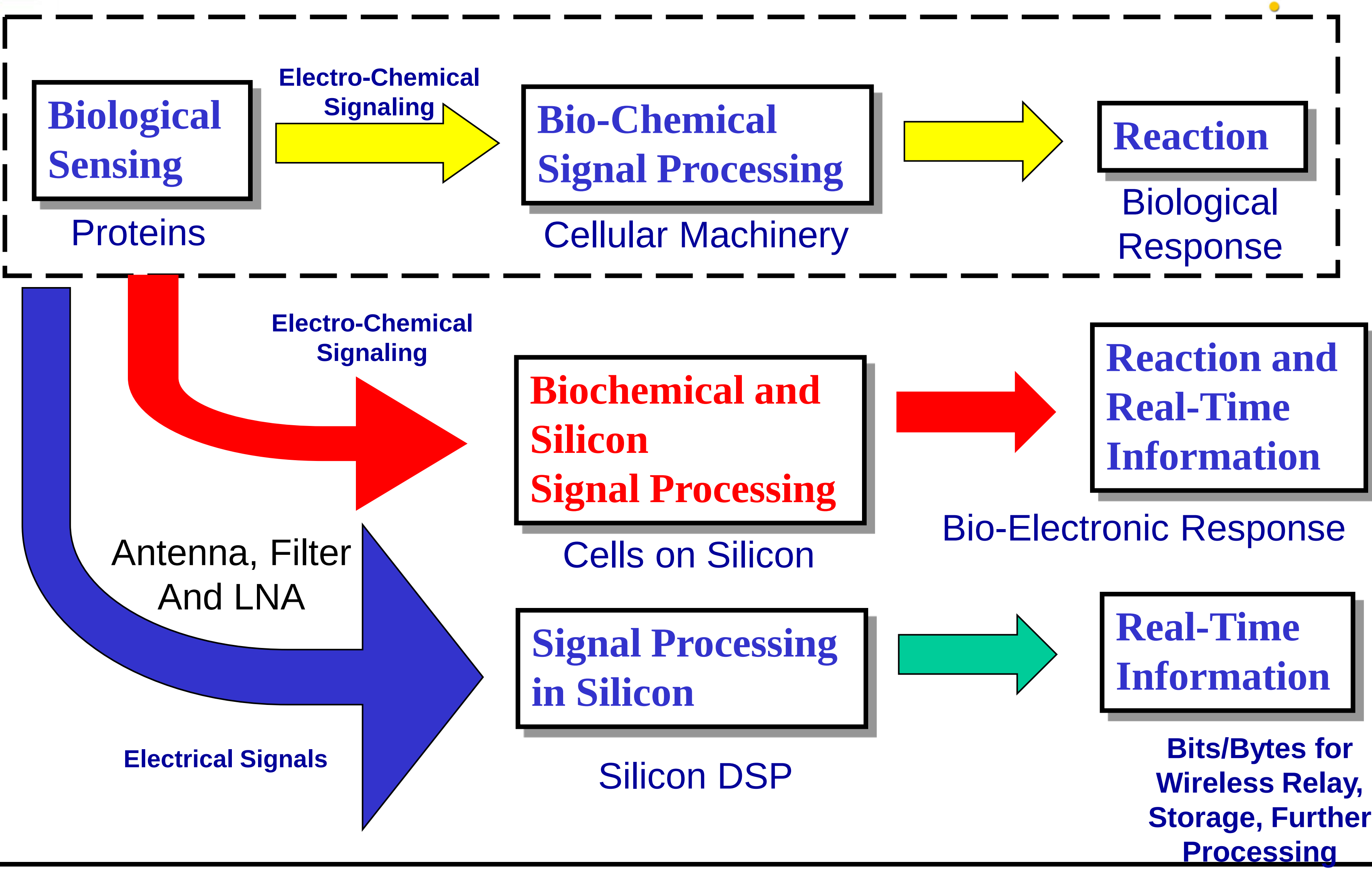
➢ **Create Technologies to Assemble, Integrate and Interconnect Protein Devices with Silicon Circuitry (Biology-to-Digital Converter)**



Direct, Real Time Conversion of Bio-Molecular Signals into Electronic Information

Courtesy of Ananta Krishnan, DARPA

Bio-Molecular Devices/Systems

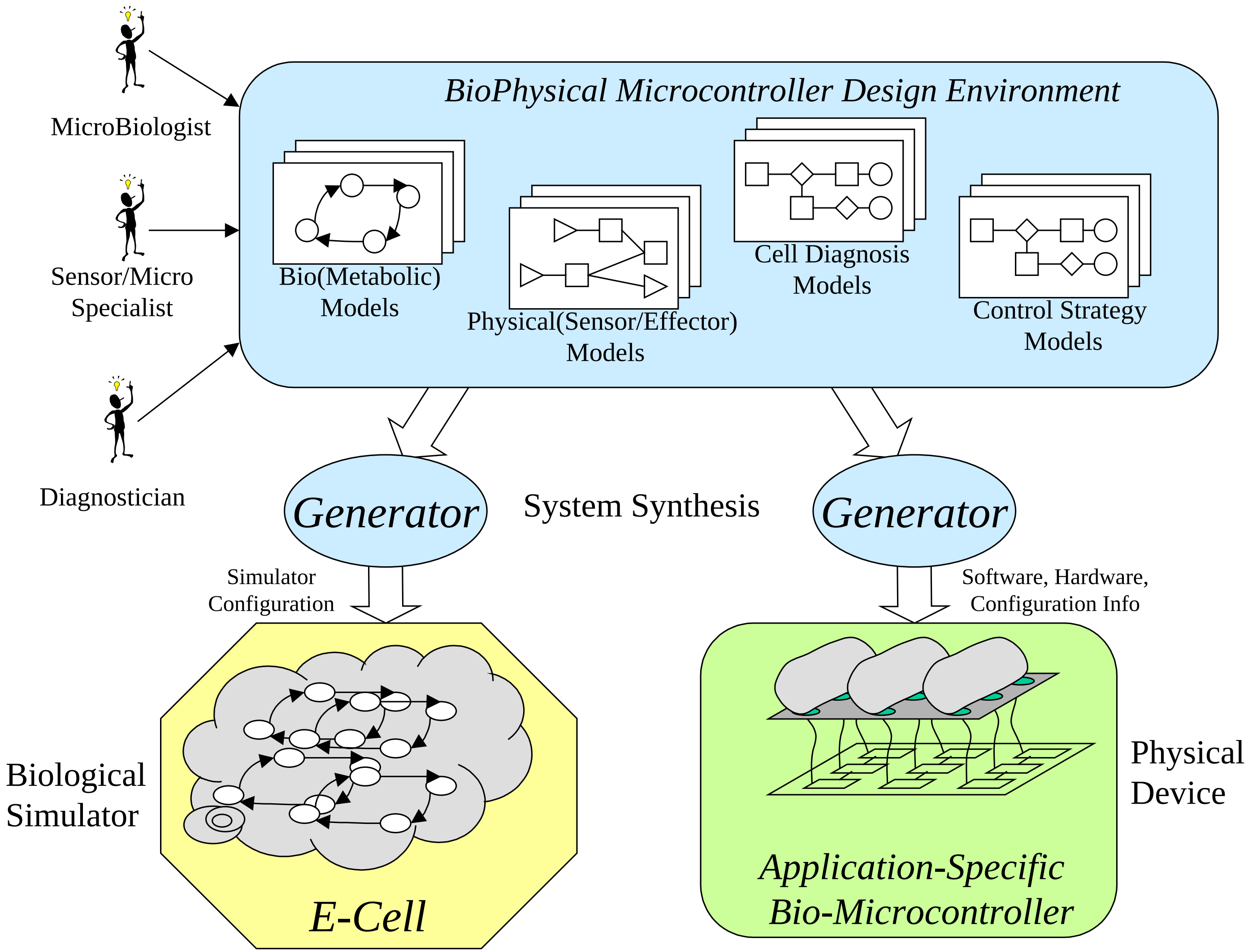


Biological and Biochemical <u>Pre</u>amplifiers to Biological Amplifiers and Detectors

Topics

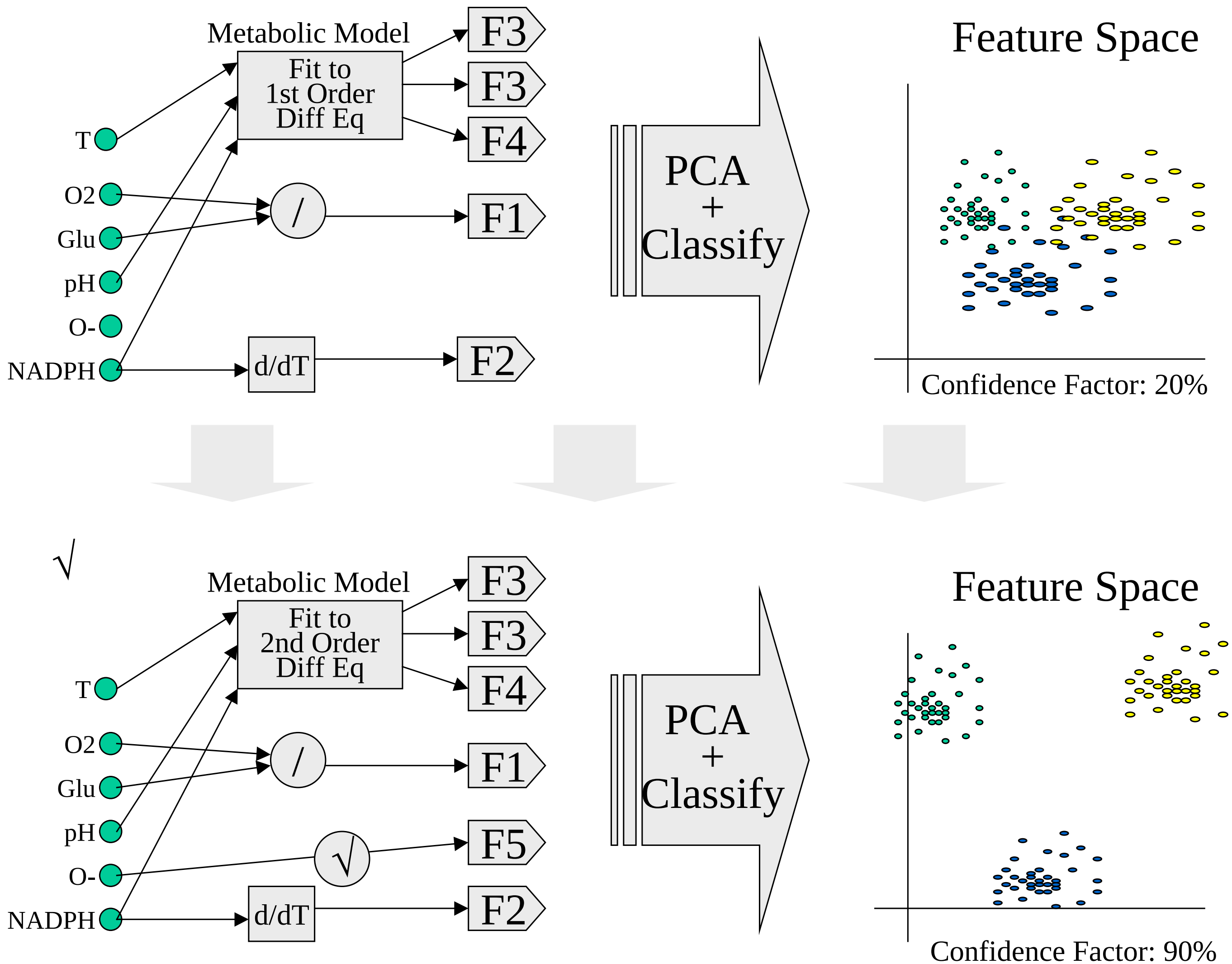
- The advantages of micro/nanoscale instruments
- Cellular complexity
- The need for closed-loop control
- How to identify early manifestations of disease
  - Modeling
  - Interactive, dynamical analysis
  - Mining dynamics data

Biological Modeling and Analysis

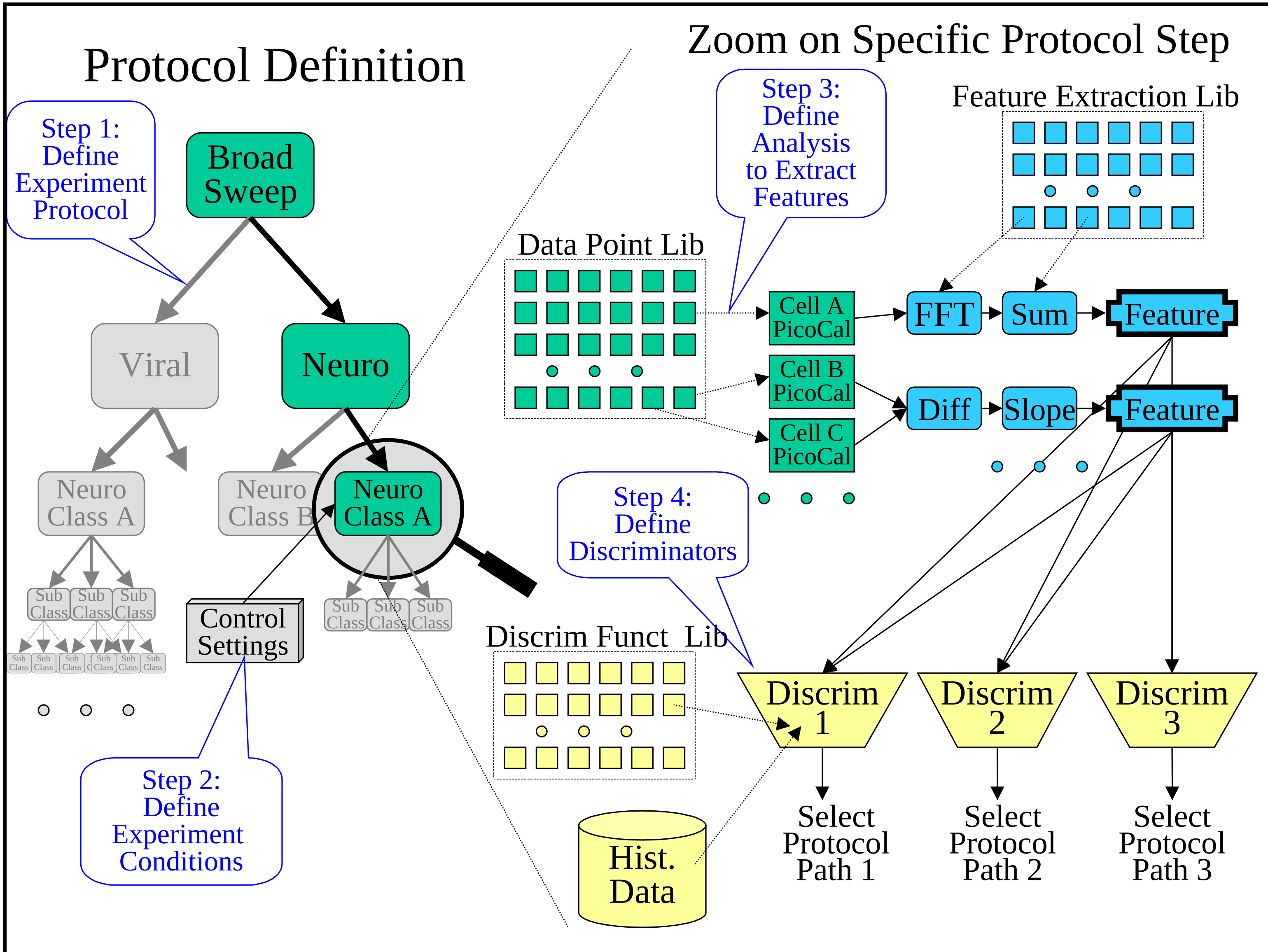


Courtesy of Ted Bapty

Signal Classification: Feature Extraction



Agent Discrimination Algorithms



Courtesy of Ted Bapty

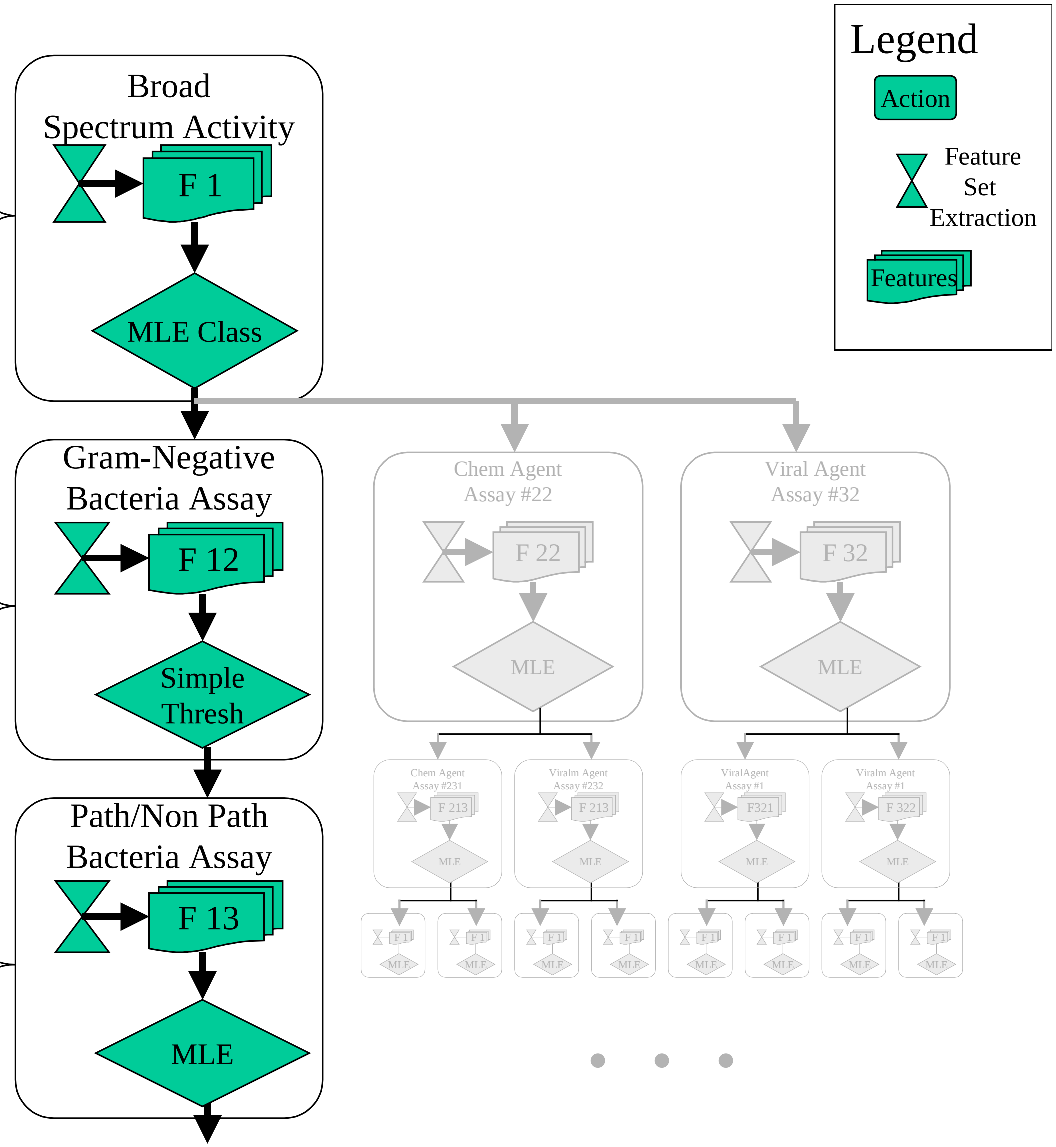
J0265-d05036_Protocol Definition

Data Mining/Exploration

Cell Line: Macrophage +Liver
Sensors: Heat, Oxygen, Free Radical NADPH, Glukconegenisis
Feat Extraction: Slope and AUC,
Expected: Both Cell Lines Activated NADPH Divergent Response
REF: TABLE xxx
Computed Robustness: 10%

Cell Line: Macrophage(-CD14)
Sensors: Heat, Oxygen, Free Radical NADPH
Feat Extraction: Slope and AUC,
Expected: Discrim on Activation
Robustness Factor: 40%

Cell Line: Intestinal Cell Line
Sensors: Heat, Oxygen, Free Radical NADPH
Feat Extraction: Slope and AUC
Expected: Discrim on Met Levels
Robustness Factor: 80%



Conclusions

- Micro/Nano will "increase throughput and automation, reducing cost per analysis, and enabling entirely new applications." *C. Dahl*
- Understanding cellular dynamics is key to understanding cellular physiology
- Micro/Nano will enable closed-loop control of certain cellular functions
- Biology and biochemistry can serve as preamplifiers for biological, biochemical, and biophysical detectors
  - PCR of course, but what else?
- Cell harvesting may be a problem for many tissues
  - Physiological biopsy
  - Pretransplant certification (pancreatic beta cells in islet transplants)
  - Well suited for probing drug interactions for particular phenotypes
- Dynamic model complexity is a major challenge
  - Specification
  - Verification