SQUID Microscopy and Nanophysiometer to monitor cellular dynamics and function

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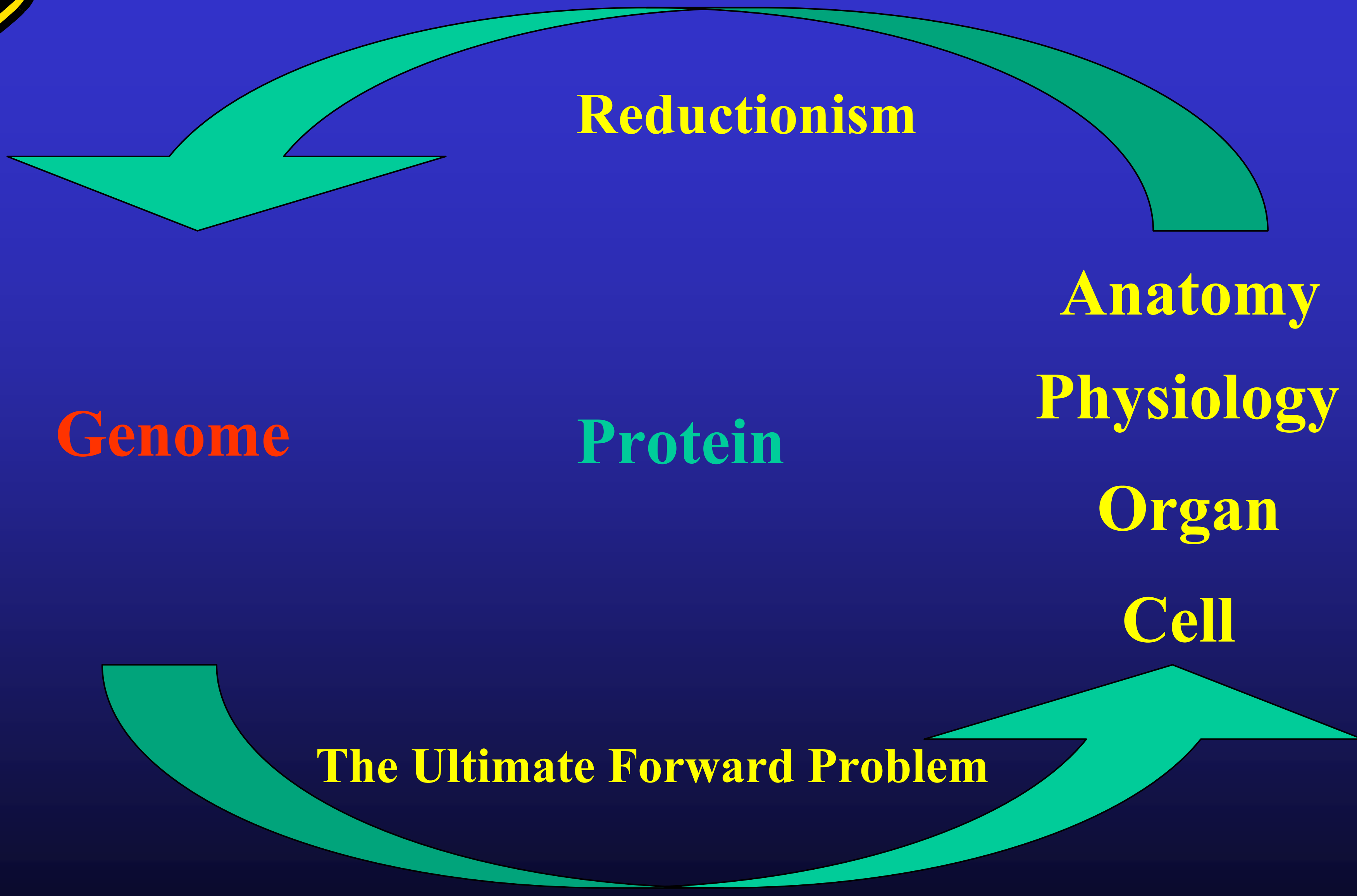
Vanderbilt Institute for Integrative Biosystems Research and Education (VIIBRE)


Reductionism
Genome
Protein
Anatomy
Physiology
Organ
Cell
The Ultimate Forward Problem

LIVING STATE PHYSICS GROUP
DEPARTMENT OF PHYSICS AND ASTRONOMY, VANDERBILT UNIVERSITY

Start with the DNA sequence for a potassium channel…

human Kv1.5

```
GCGGCCGCGCGGCTTTTTGACGTCAGGGCCAAGCGAGGGGATCGCGCCAGCAACCCCAGCTCTCCCCAGAGAGGGGCCGG
CCGACCGCTGGAGCGGAGCCTGACGCCAGGCGCCCGCGGAGCGTGAGTAGGGGGCGCGGGAGCCGGTCAGCTGGGGCGCA
GCATGCCCTCTGCTCCCGCGCCATGGAGATCGCCCTGGTGCCCCTGGAGAACGGCGGTGCCATGACCGTCAGAGGAGGCG
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GTACTTCGACCCCCTGAGGAACGAGTACTTCTTCGACCGCAACCGGCCCAGCTTCGACGGTATCCTCTACTACTACCAGT
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GCCATGGAGCGCTTCCGCGAGGATGAGGGCTTCATTAAAGAAGAGGAGAAGCCCCTGCCCCGCAACGAGTTCCAGCGCCA
GGTGTGGCTTATCTTCGAGTATCCGGAGAGCTCTGGGTCCGCGCGGGCCATCGCCATCGTCTCGGTCTTGGTTATCCTCA
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CGGGAAACAGATTTGTGAAAGGAGATTCAGGCAGACTGGTGGCAGTGGAGTAGGGAATGGGAGGCTTCTGAACATGGATA
TCTACATTATCCGCAGAGTATTTGACTCACTCCTCT
```
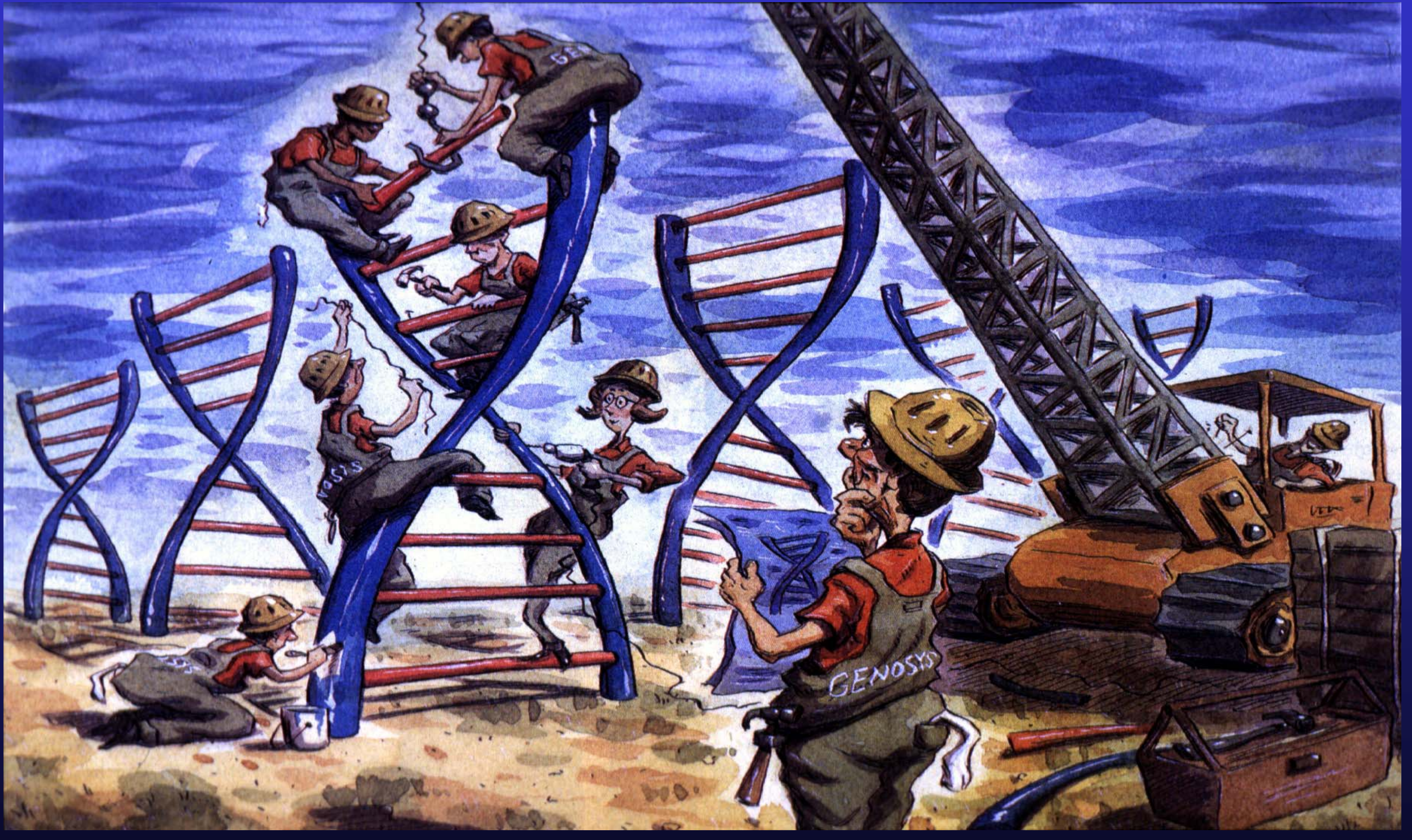
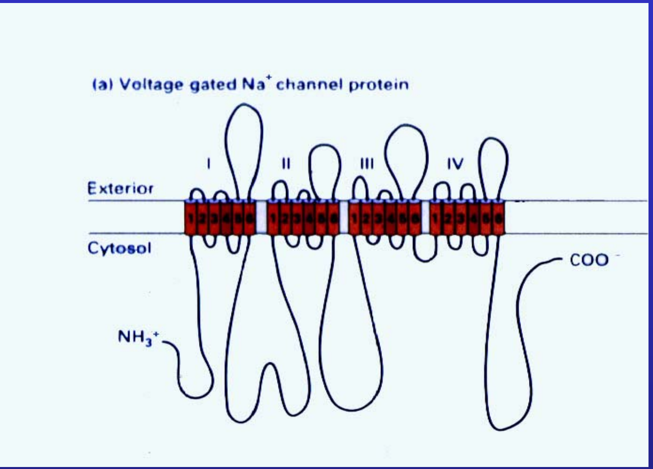

Assemble the proteins

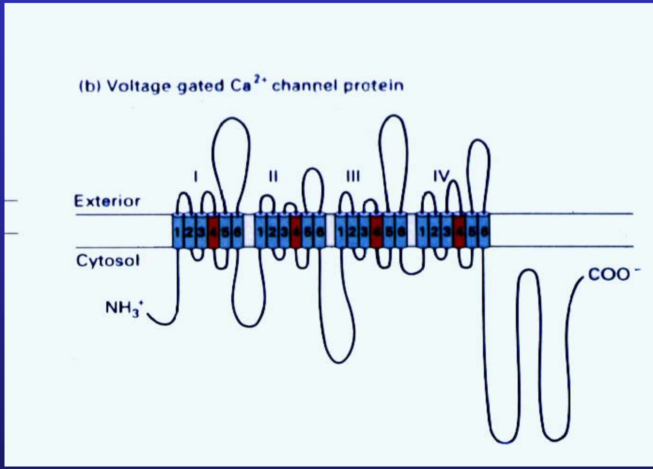


And we solve the protein folding problem…

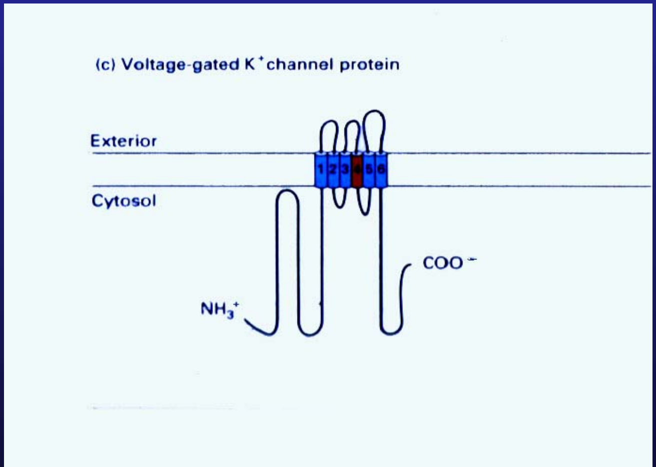
Insert the folded proteins into the membrane



Voltage-gated Na^+ channel



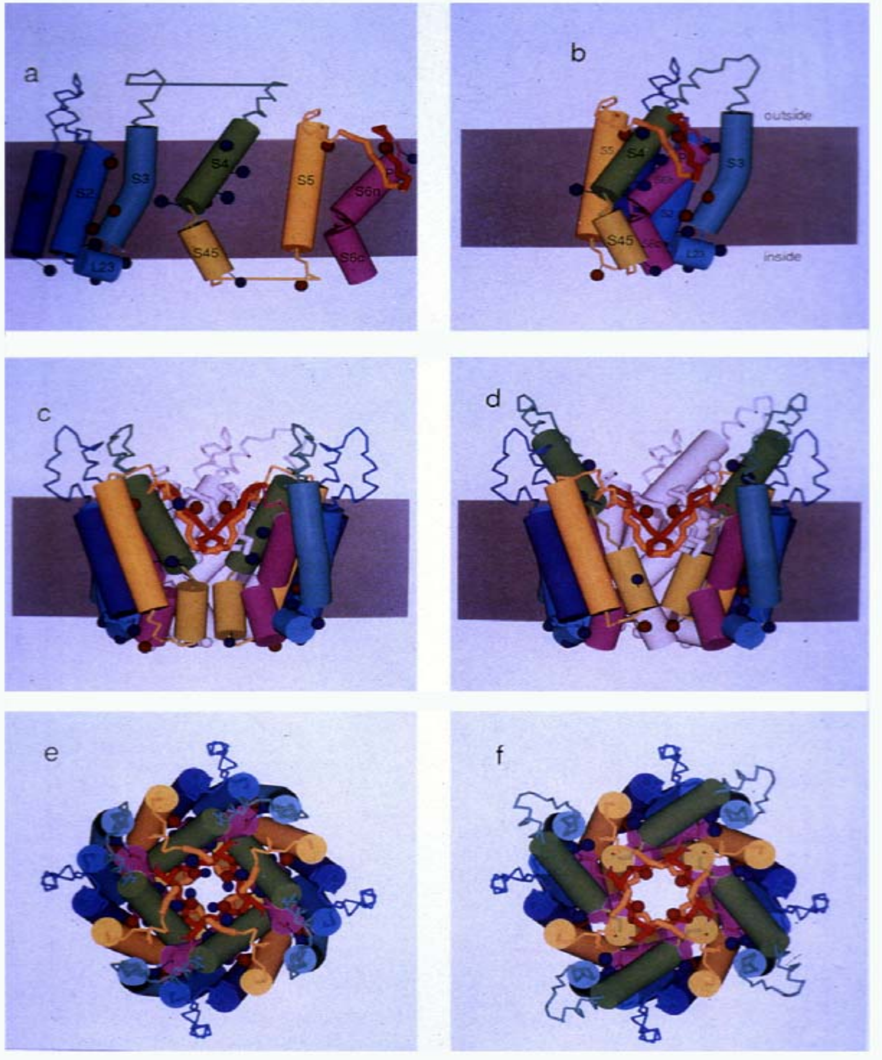
Voltage-gated Ca^{++} channel



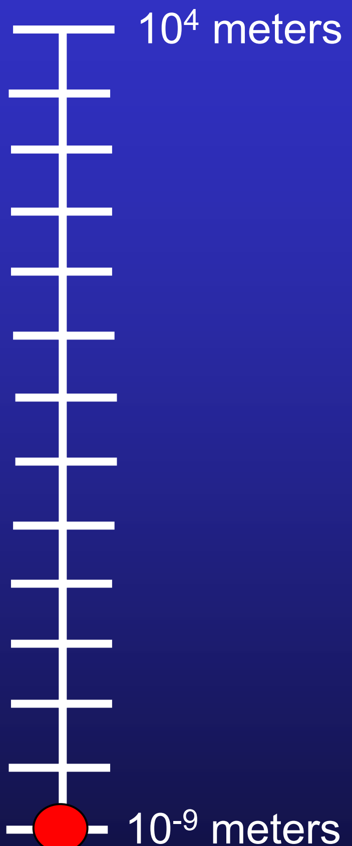
Voltage-gated K^+ channel

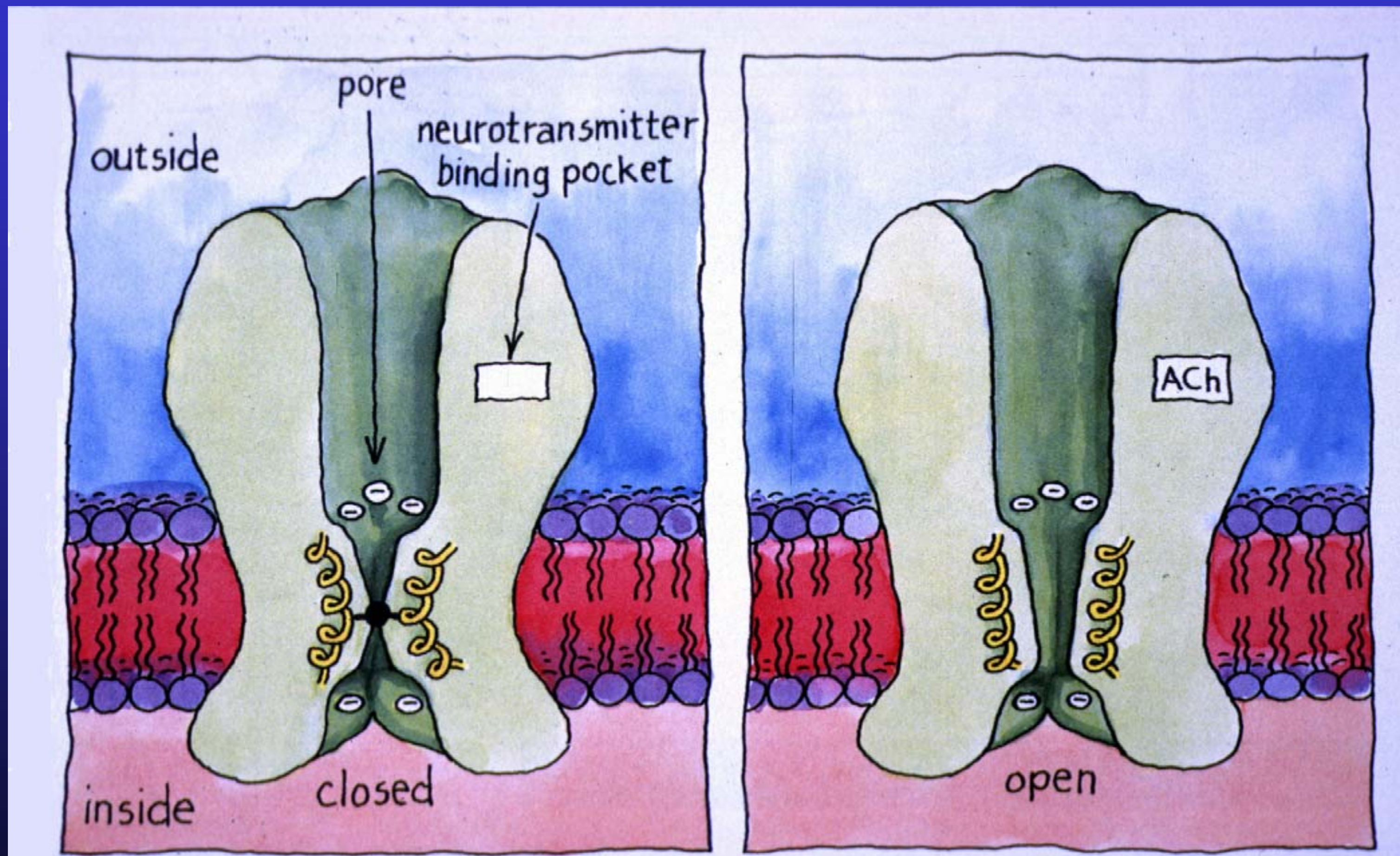
Compute how the protein conformation depends upon voltage or ligand binding

S.R. Durrell and H.R. Guy, *Biophysical Journal*, 62: Discussions 1992 238-250 (1992)

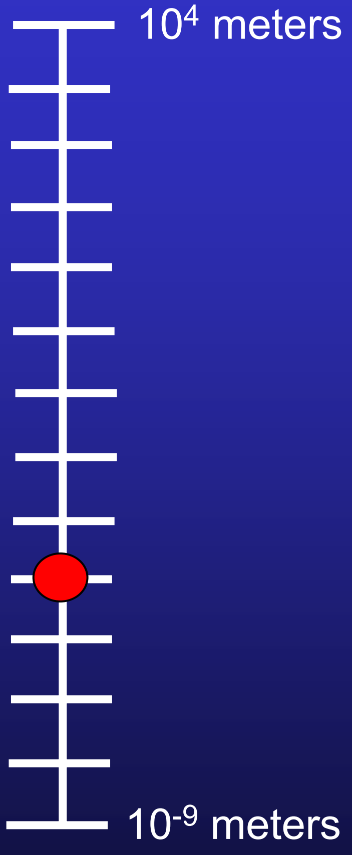


1 nanometer: Pore in a gated ion channel

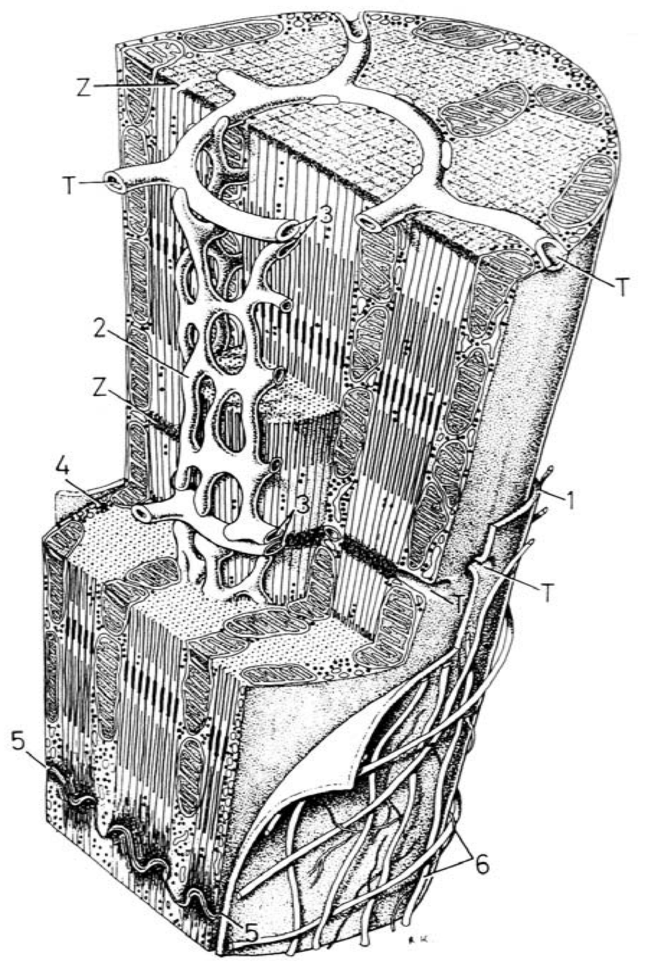




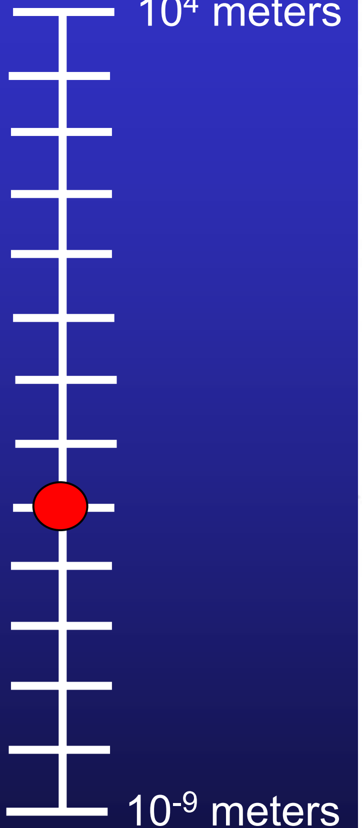
10 micrometers: Cardiac cell diameter

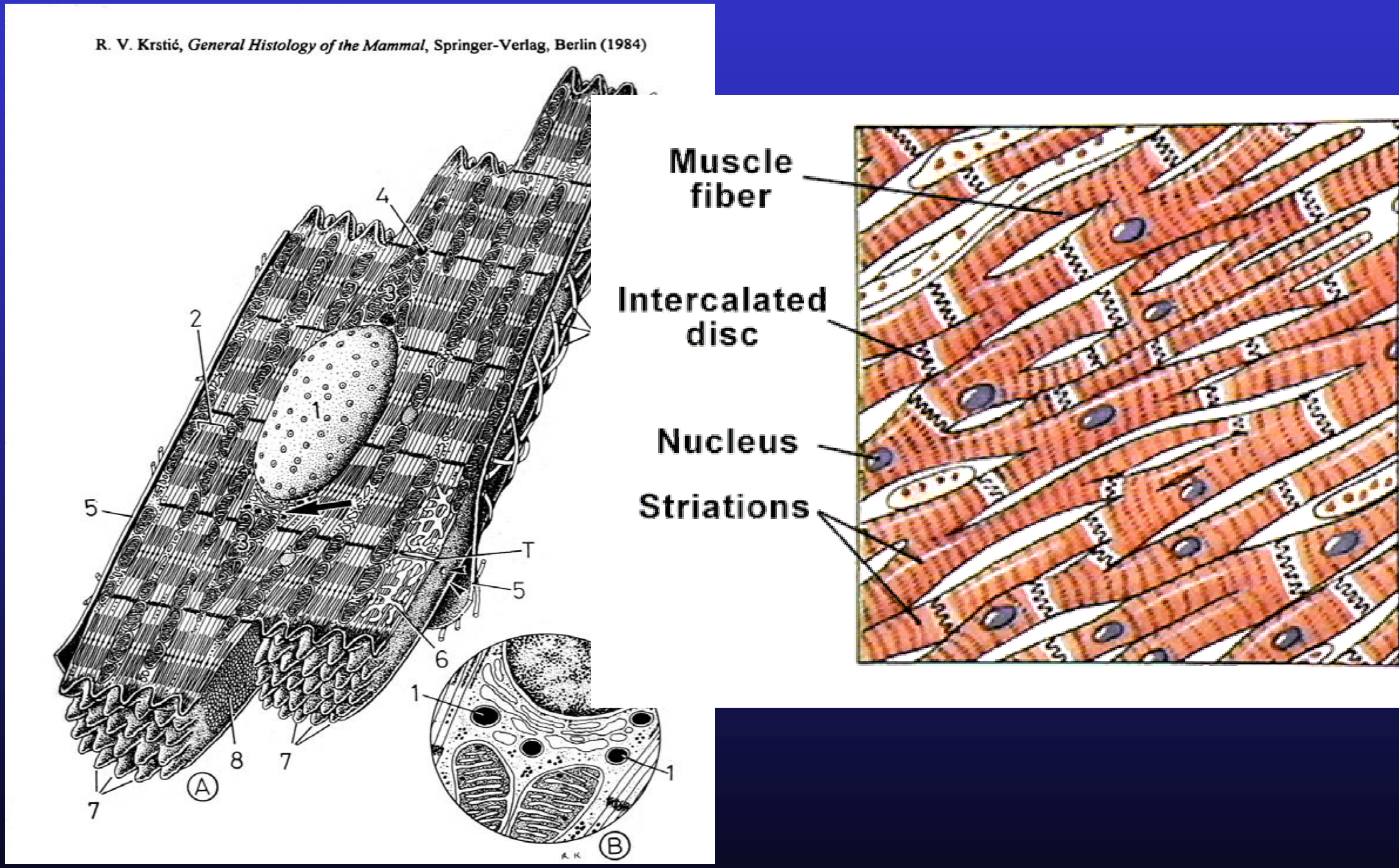


R. V. Krstić, *General Histology of the Mammal*, Springer-Verlag, Berlin (1984)

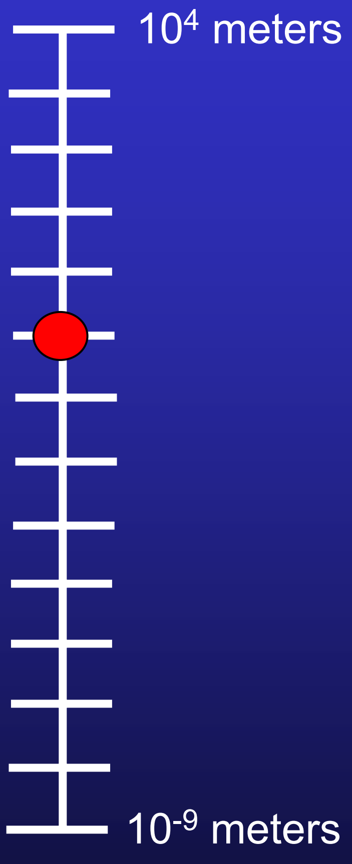


100 micrometers: Cardiac cell length





10 centimeters: The human heart

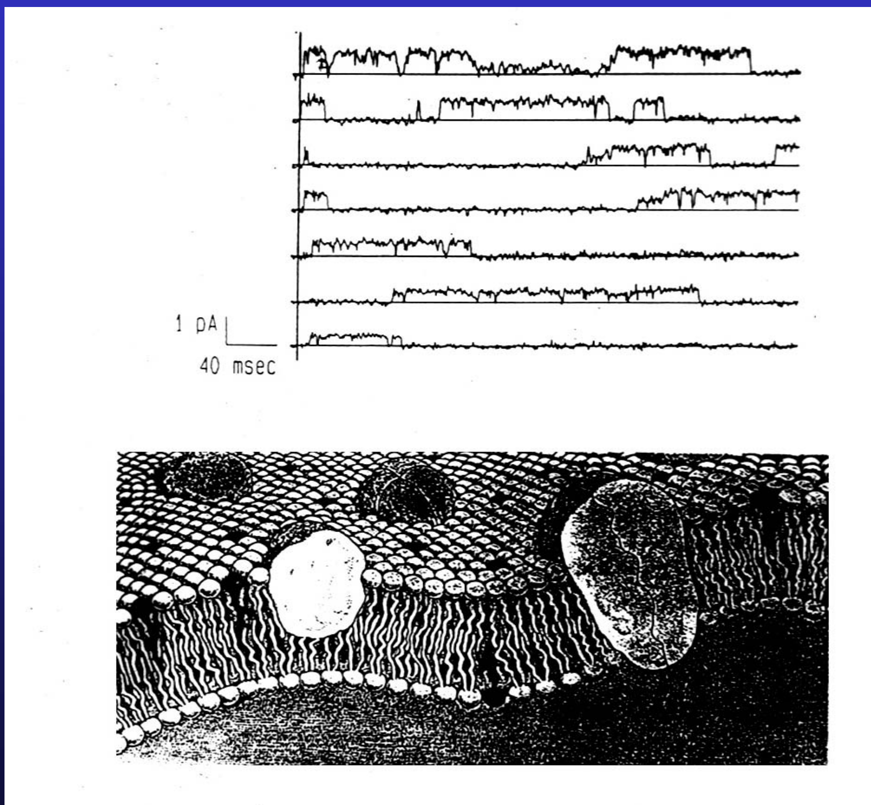


Courtesy Peter Hunter, Aukland University

The Spatial and Temporal Scales of Cardiac Electrodynamics

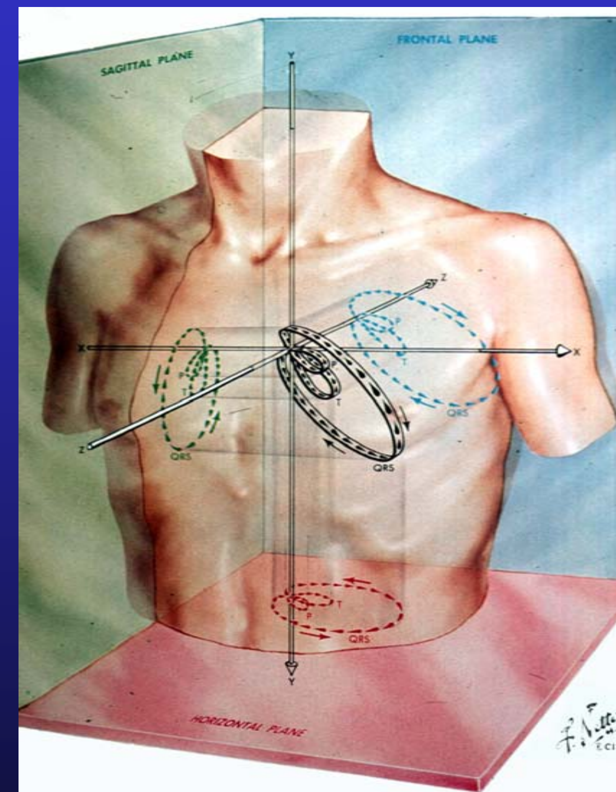
- Spatial Scale: 10^{24} in volume
  - Sequence of the proteins that form those channels
  - Nanometer pore of gated ion channels
  - Ten-centimeter diameter of the entire heart
- Temporal Scale: 10^{9} in time
  - Nanosecond conformational changes of protein channels
  - One-second heart beat
  - Many seconds of a complex arrhythmia

Two Extremes: Models of Cardiac Activity



Einthoven triangle and the cardiac dipole moment

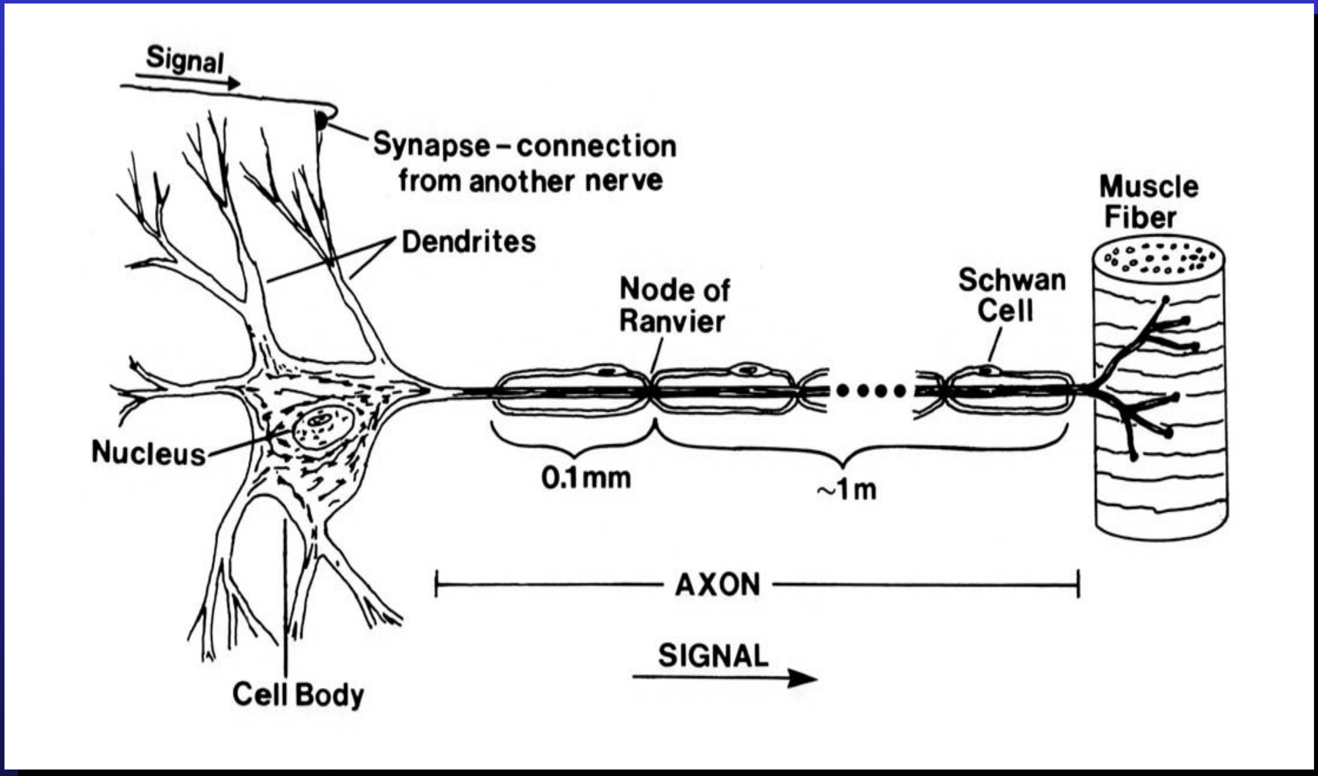
Channel kinetics from patch clamp



Solutions to the Forward Problem

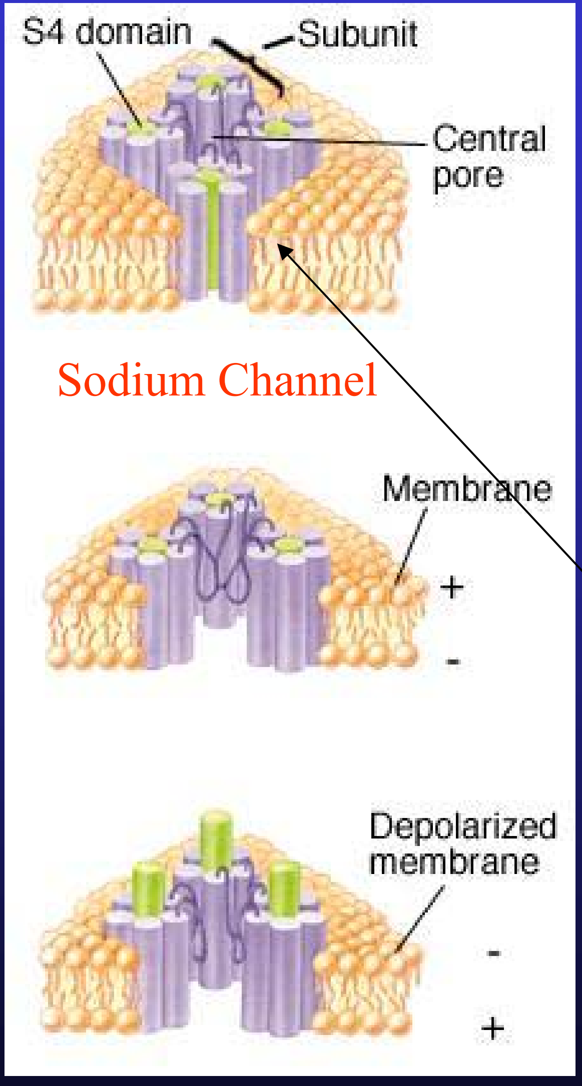
- Develop and refine hierarchical multiscale models to span the full range of space and time
  - Single cell cardiac myocyte models do not extrapolate to the whole heart
- Understand the dynamics, control structures and functions of the smallest living unit
  - "Instrumenting and Controlling the Single Cell"

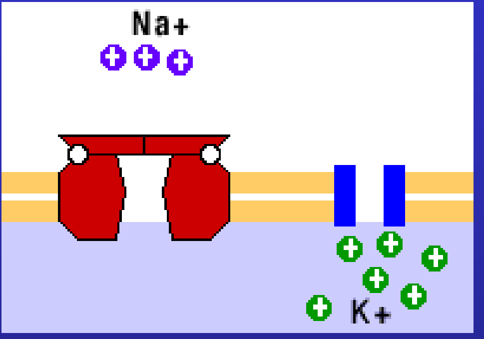
Action Potential Propagation

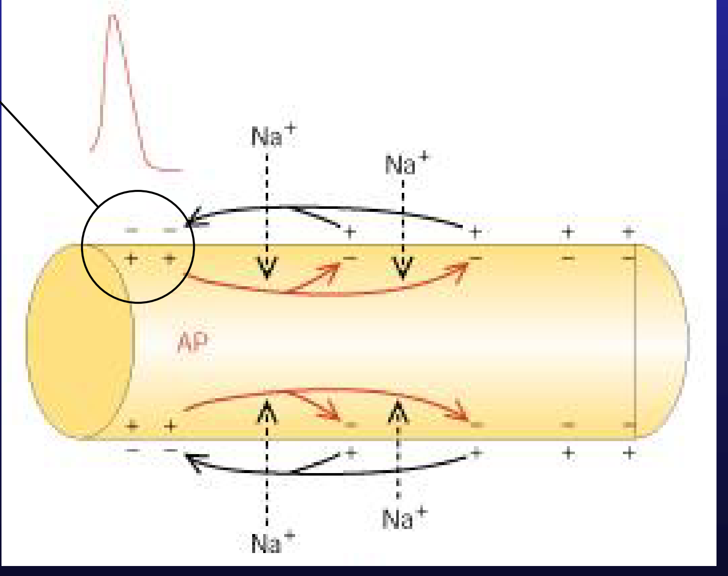


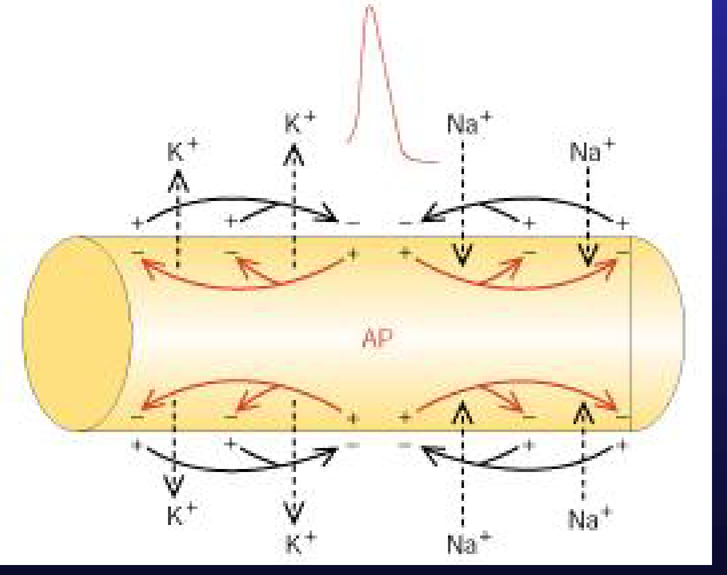
Inside of axon	Extracellular Fluid
$[Na^+]=15$	$[Na^+]=145$
$[K^+]=150$	$[K+]=5$
	$[MISC^+]=5$
$[Cl^-]=9$	$[CL^-]=125$
$[MISC^-]=156$	$[MISC^-]=30$
v=-70mV	v=0V

Action Potential Propagation

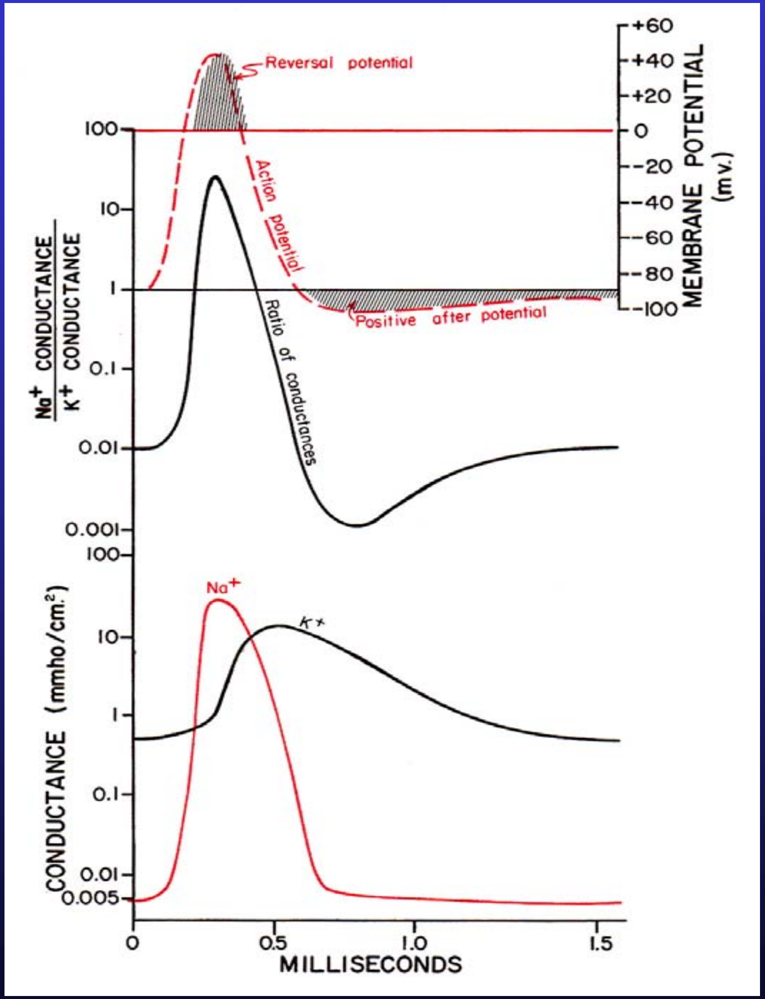


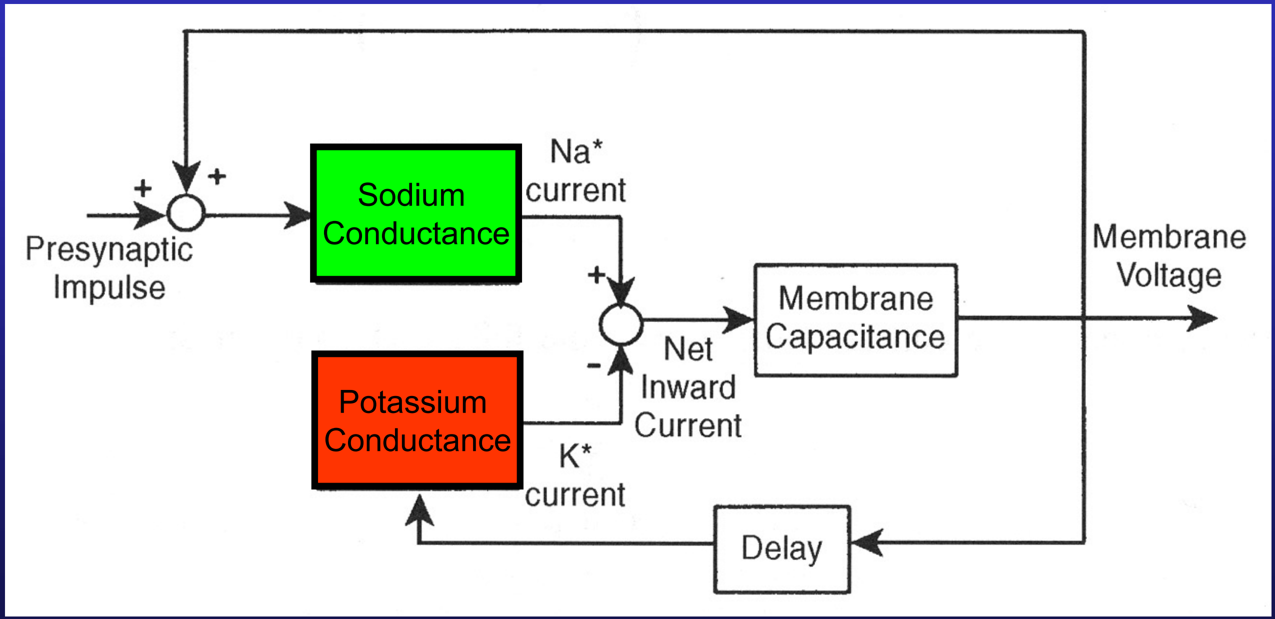






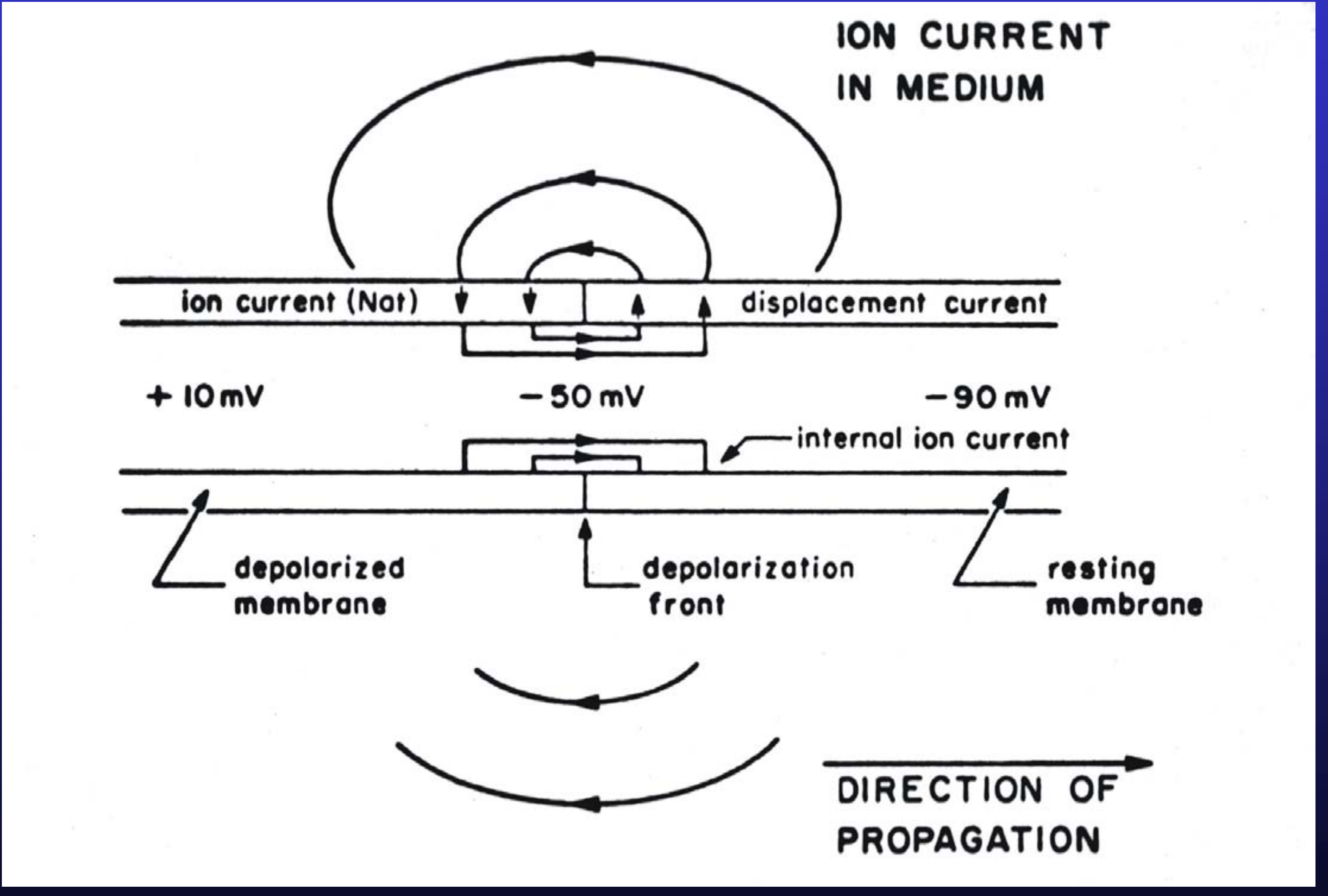
Simplified Hodgkin-Huxley





Guyton, Arthur C.; Textbook of Medical Physiology, 6rd ed.; 1981, W.B. Saunders, p.110

Action Currents Fibers in conducting Media



High Resolution Superconducting Quantum Interference Device (SQUID) magnetometers should allow us to study weak, single-cell action currents by means of their magnetic field.

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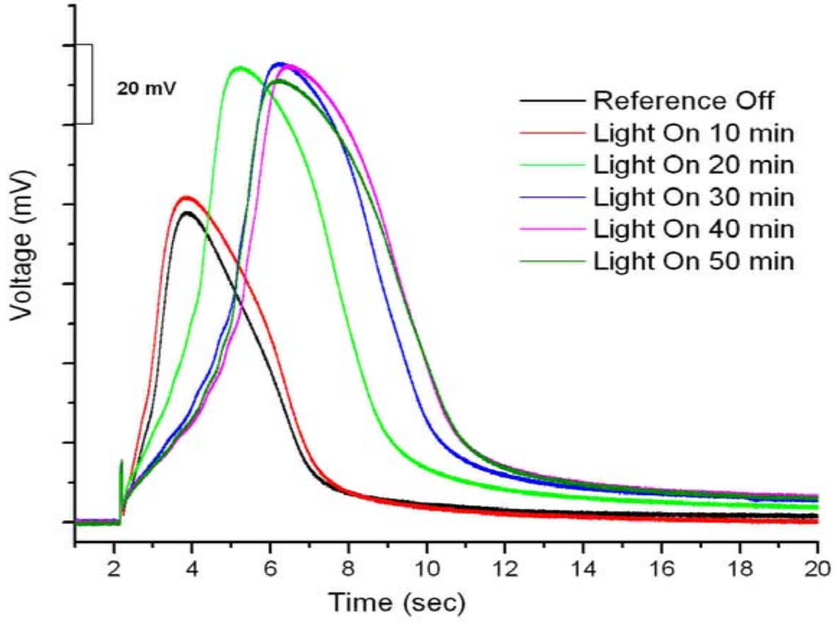
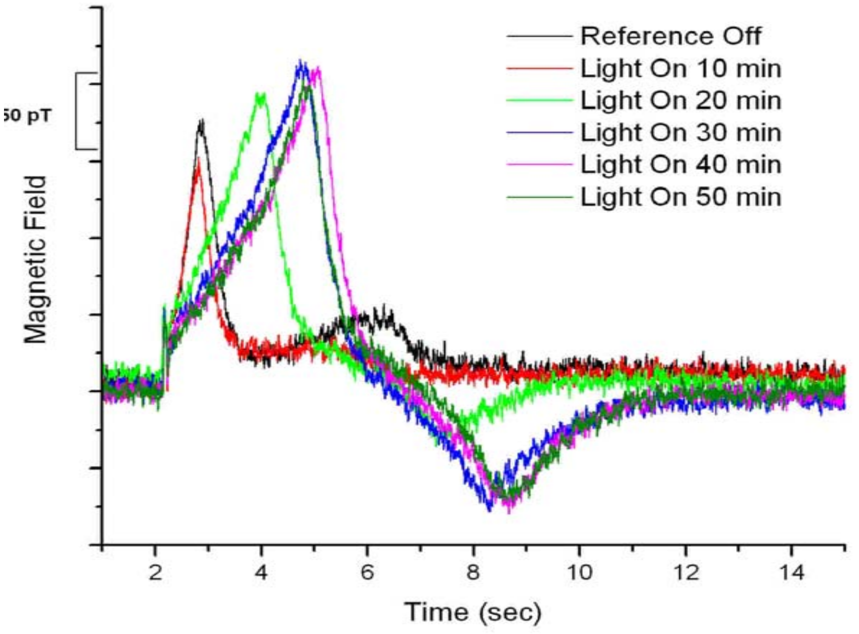
Combined Action Current and Potential Measurements

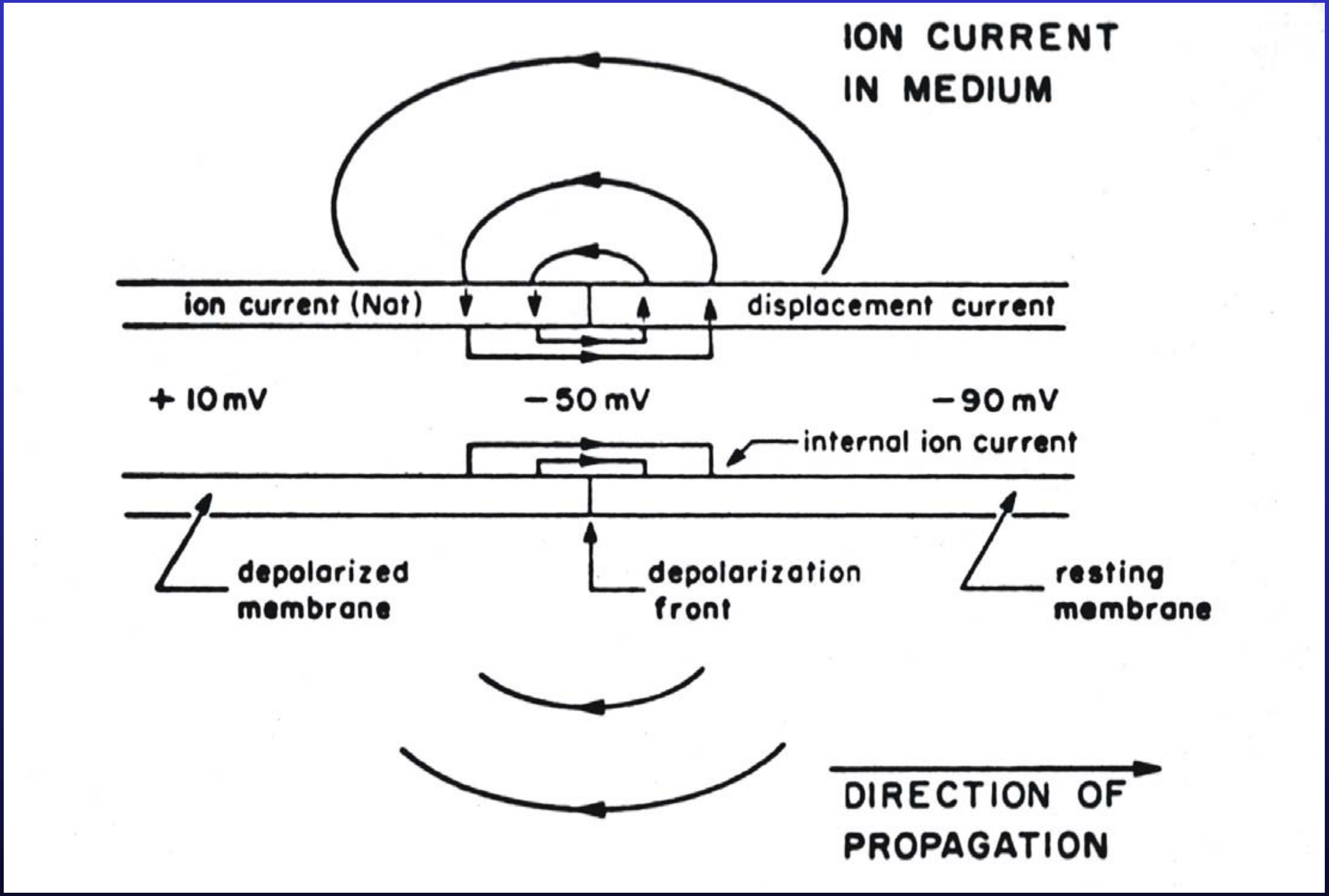
AP Measured Electrically



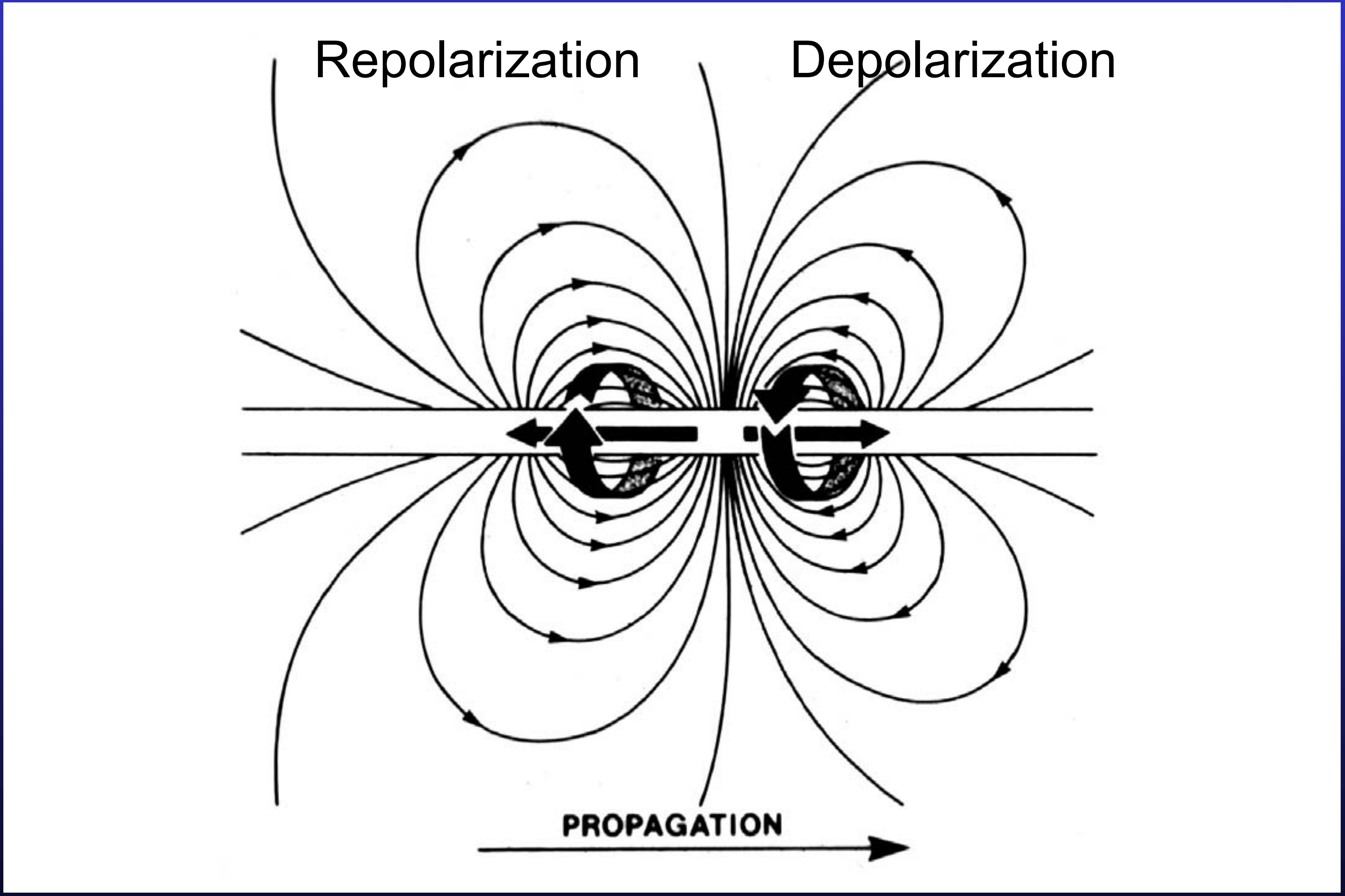
AC Measured Magnetically



Action Currents Fibers in conducting Media



Detectable Action Currents



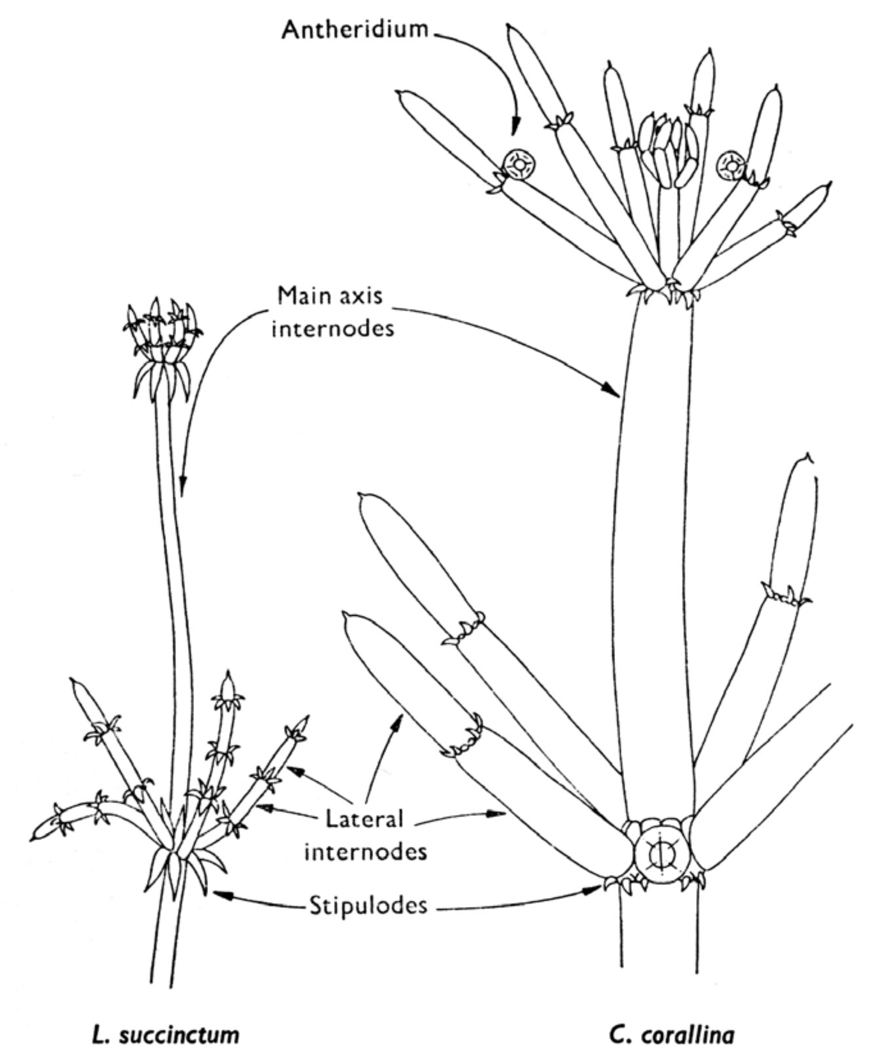


Fig. 1.2 Sketches of growth habit of plants of the algae *C. corallina* and *Lamprothamnium succinctum*.

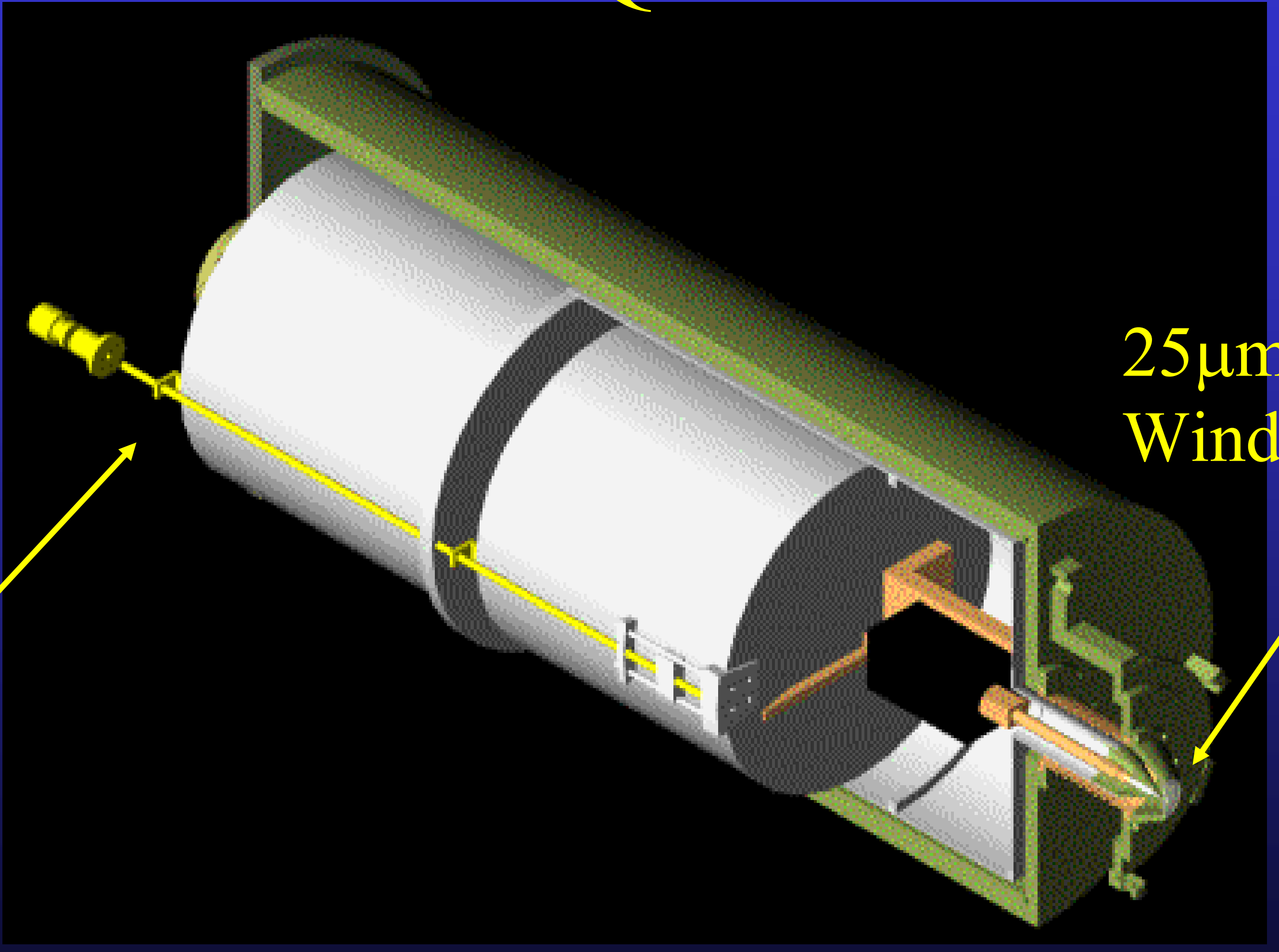
Conclusions: Magnetic Imaging of Algae Action Currents

- The Action potential duration is increased and the propagation velocity is reduce during light exposure.
- The change in ionic transport kinetic could be attributed to a Ca gated Cl channel. The Ca is taken up by PSII and cases a reduction of Ca in the cytoplasm.

Baudenbacher, F., Fong, L.E.*, Thiel, G., Jazbinsek, V., Štampfl, A., Holzer, J. R.+, Trontelj, Z., Intracellular Axial Current in Chara corallina Reflects the Altered Kinetics of Ions in Cytoplasm under the Influence of Light (to be submitted to Biophysical Journal)

The Vanderbilt SQUID Microscope

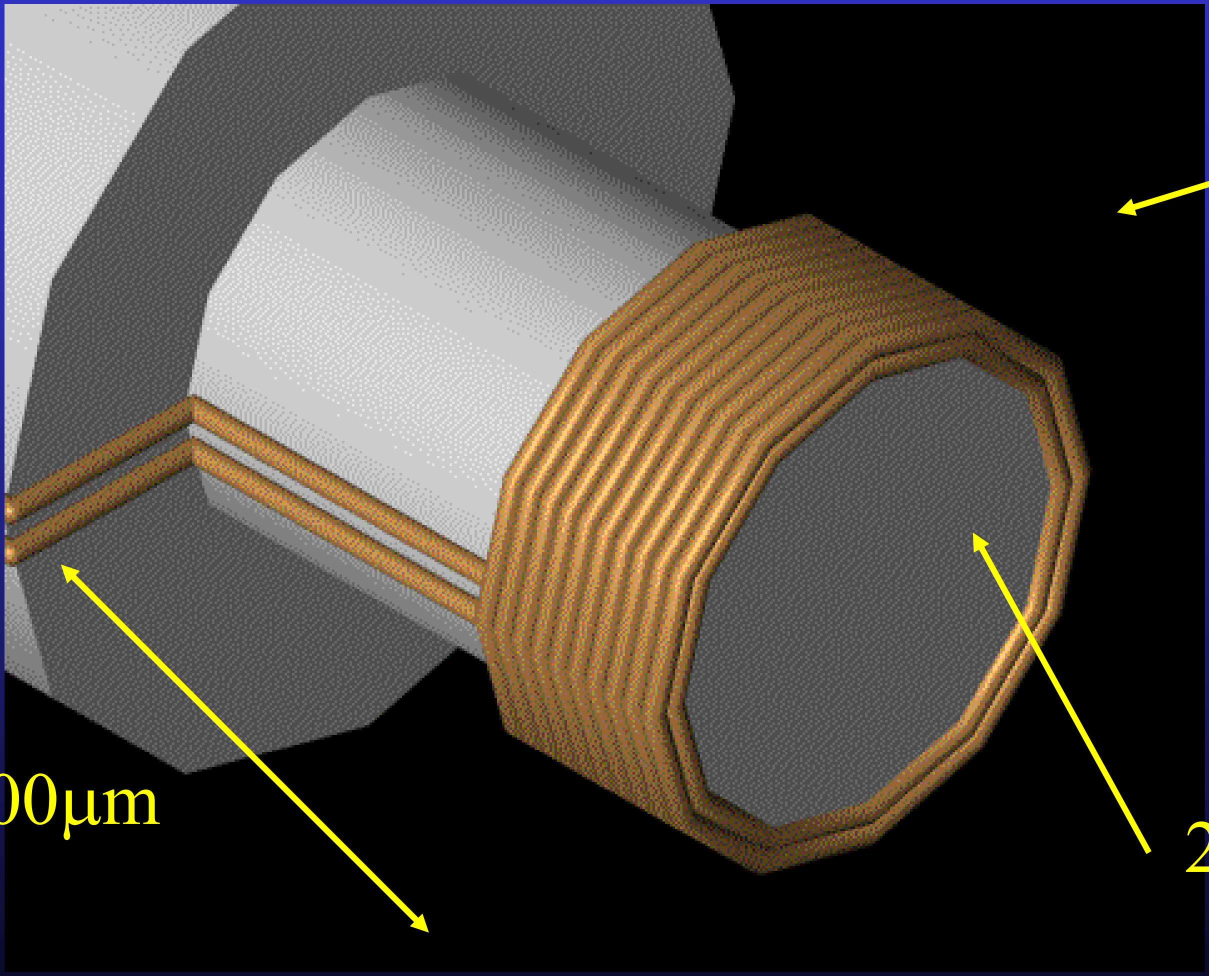
Lever-Mechanism



25μm Sapphire Window

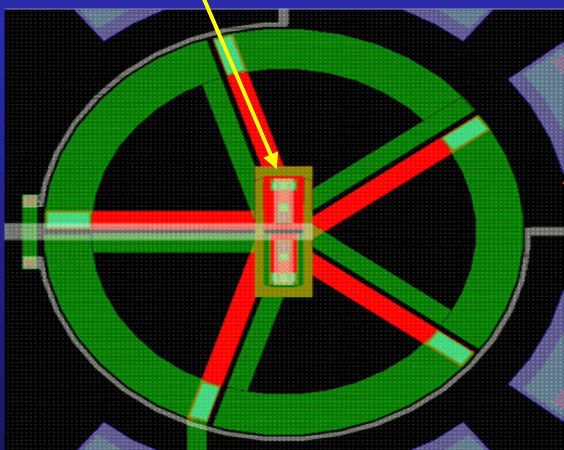
Baudenbacher, F., Peters, N.T. , and Wikswo, J.P., Jr., High Resolution Low-Temperature Superconductivity SQUID Microscopes for Imaging Magnetic Fields of Samples at Room Temperature, Review Scientific Instruments, 73 (3), 1247-1254 (2002).

Pickup Coil



Cart-Wheel-Design

Resistively Shunted Josephson Junction

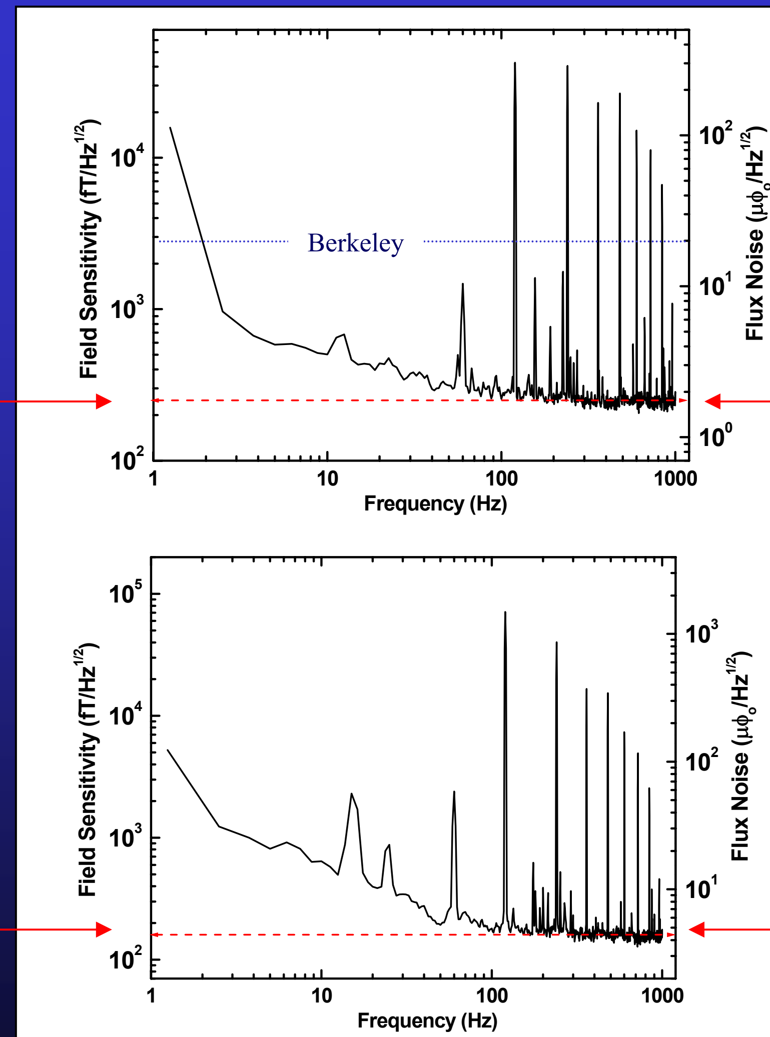


250 μm diameter

500 μm diameter

248 fT/Hz$^{1/2}$

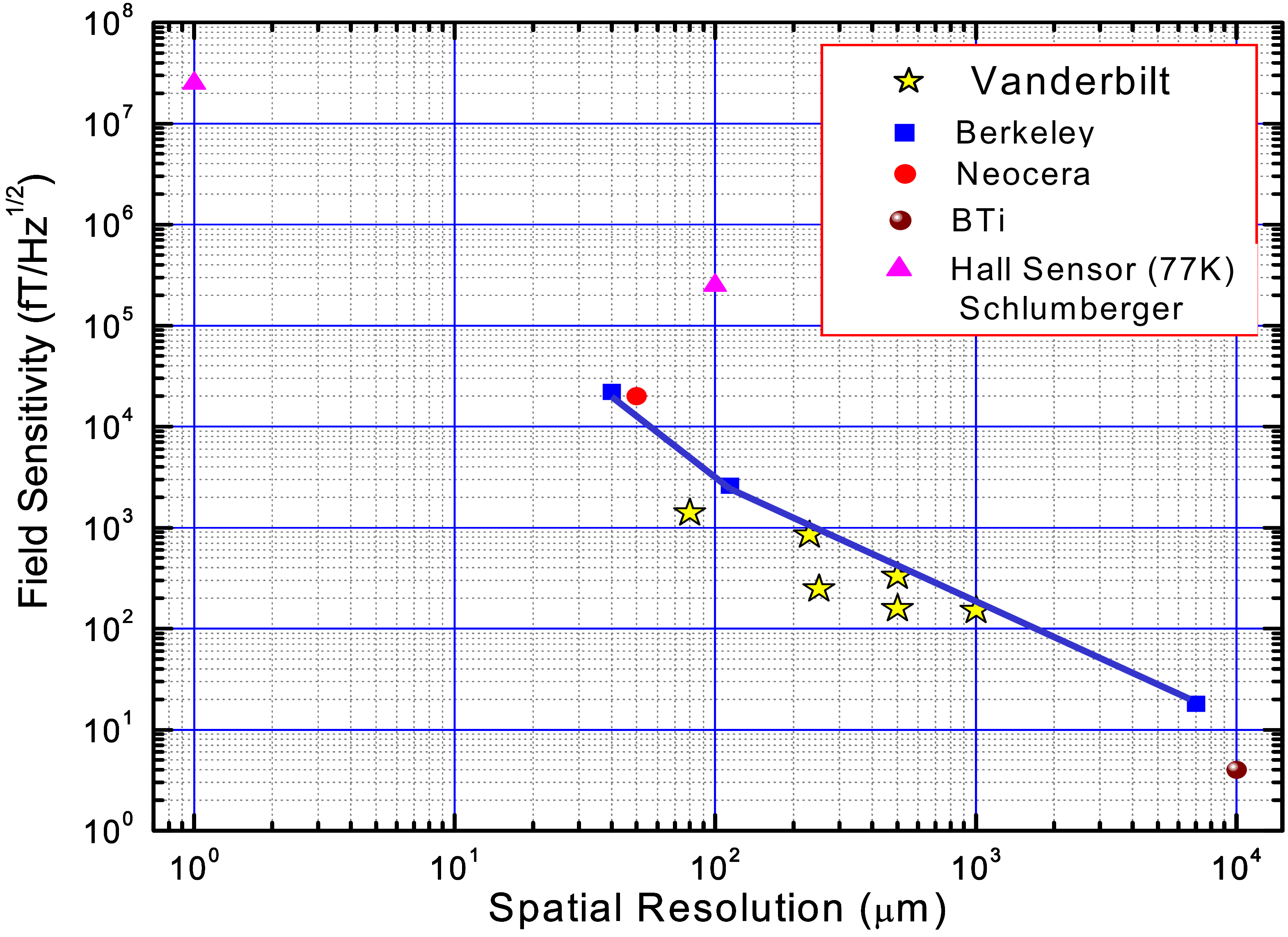
158 fT/Hz$^{1/2}$



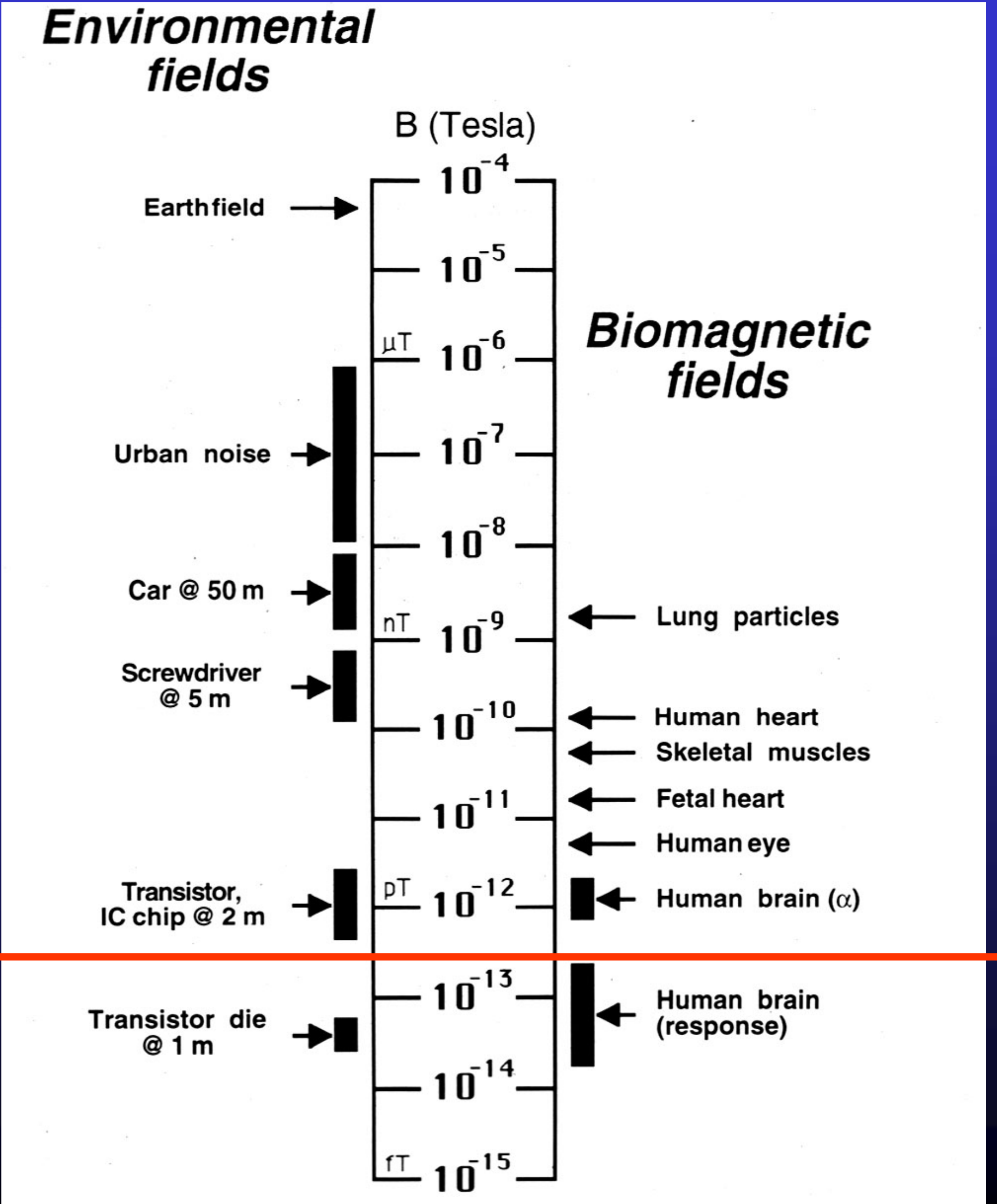
1.7 $\mu\phi_o$/Hz$^{1/2}$

4.4 $\mu\phi_o$/Hz$^{1/2}$

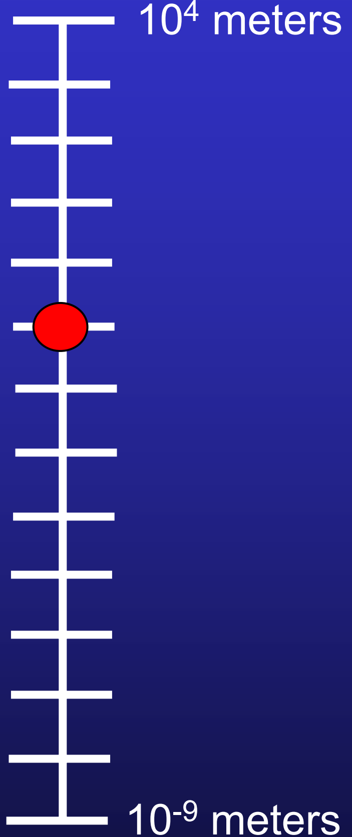
Fong, L. E.*, Holzer, J.R.+, Radparvar M., Baudenbacher F., Multiloop Low-Tc SQUID sensor for imaging biomagnetic fields with submillimeter resolutions (to be submitted to Appl. Phys. Lett.)

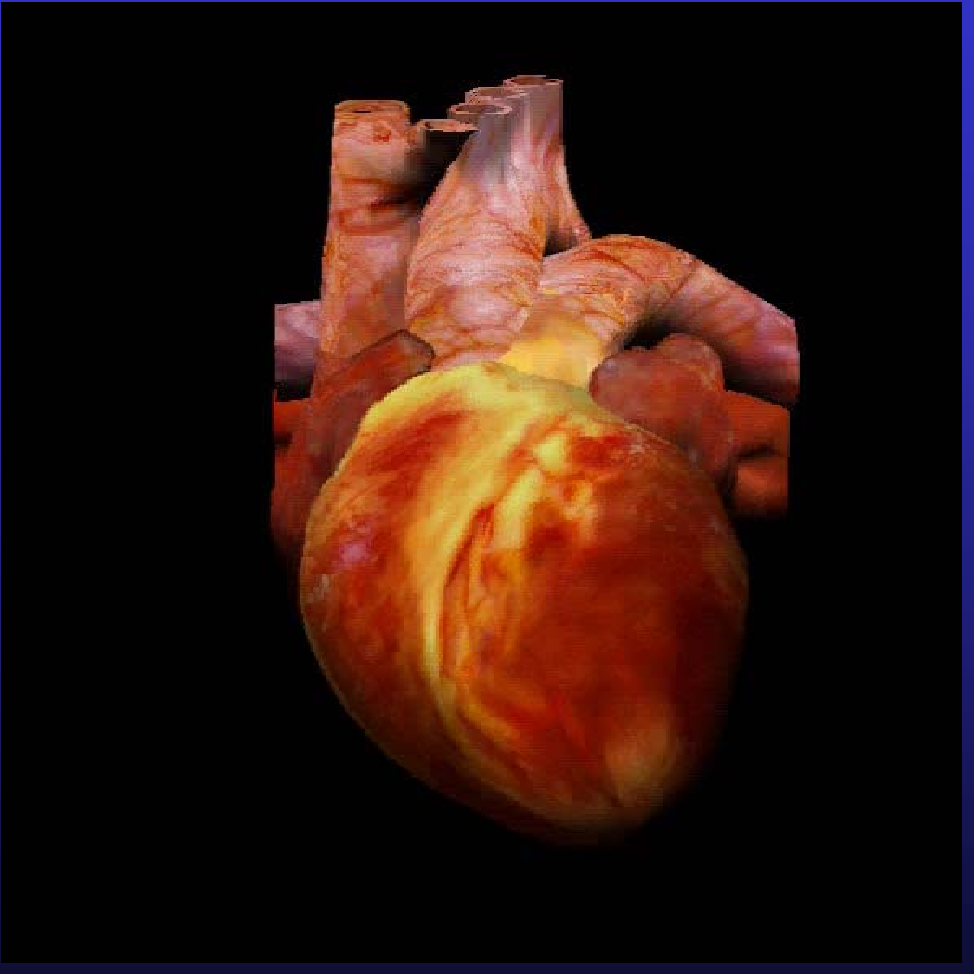

Vanderbilt
Berkeley
Neocera
BTi
Hall Sensor (77K)
Schlumberger
Field Sensitivity (fT/Hz^1/2)
Spatial Resolution (μm)
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
10^8

Vanderbilt SQUID Microscope



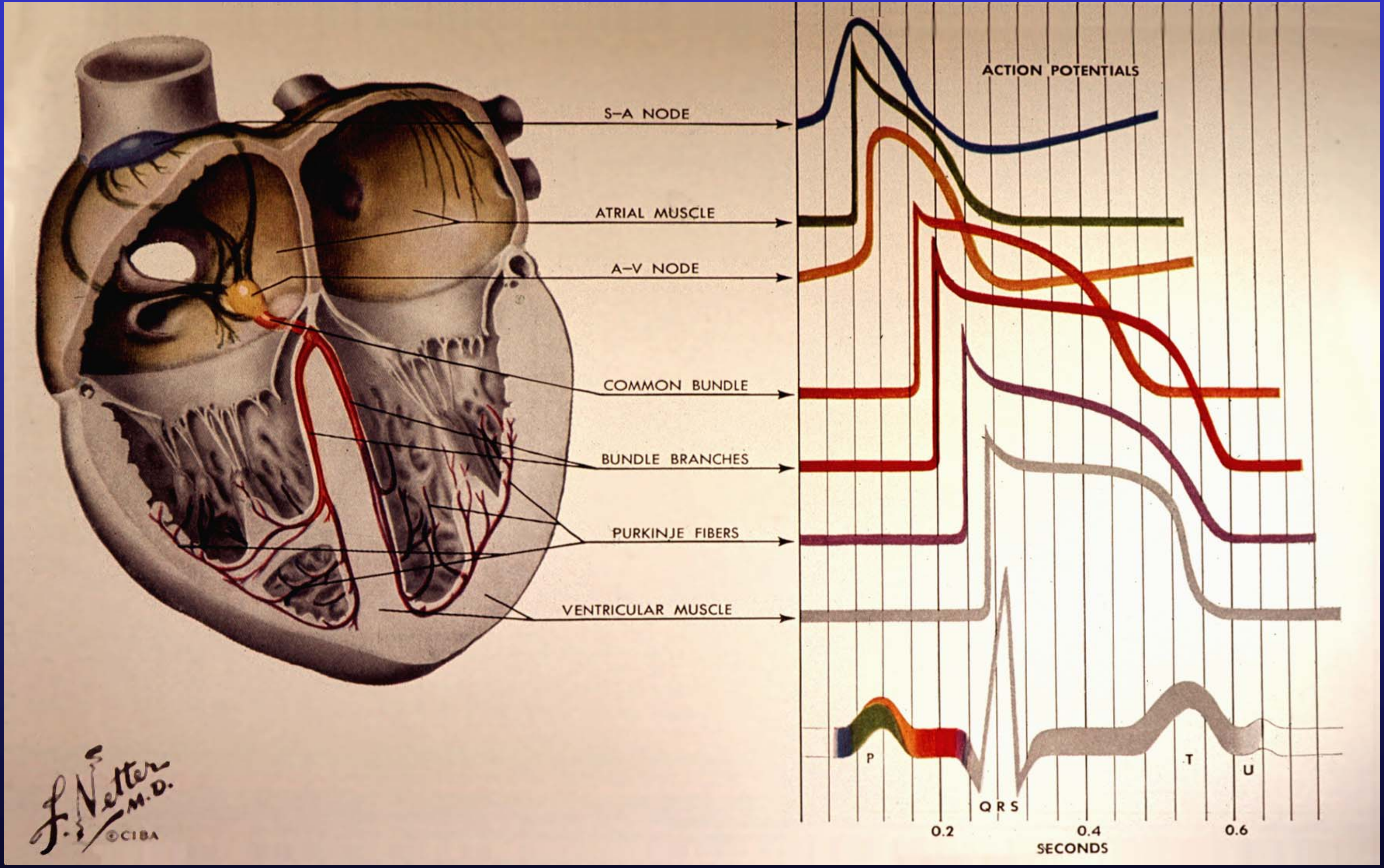
10 centimeters: The human heart



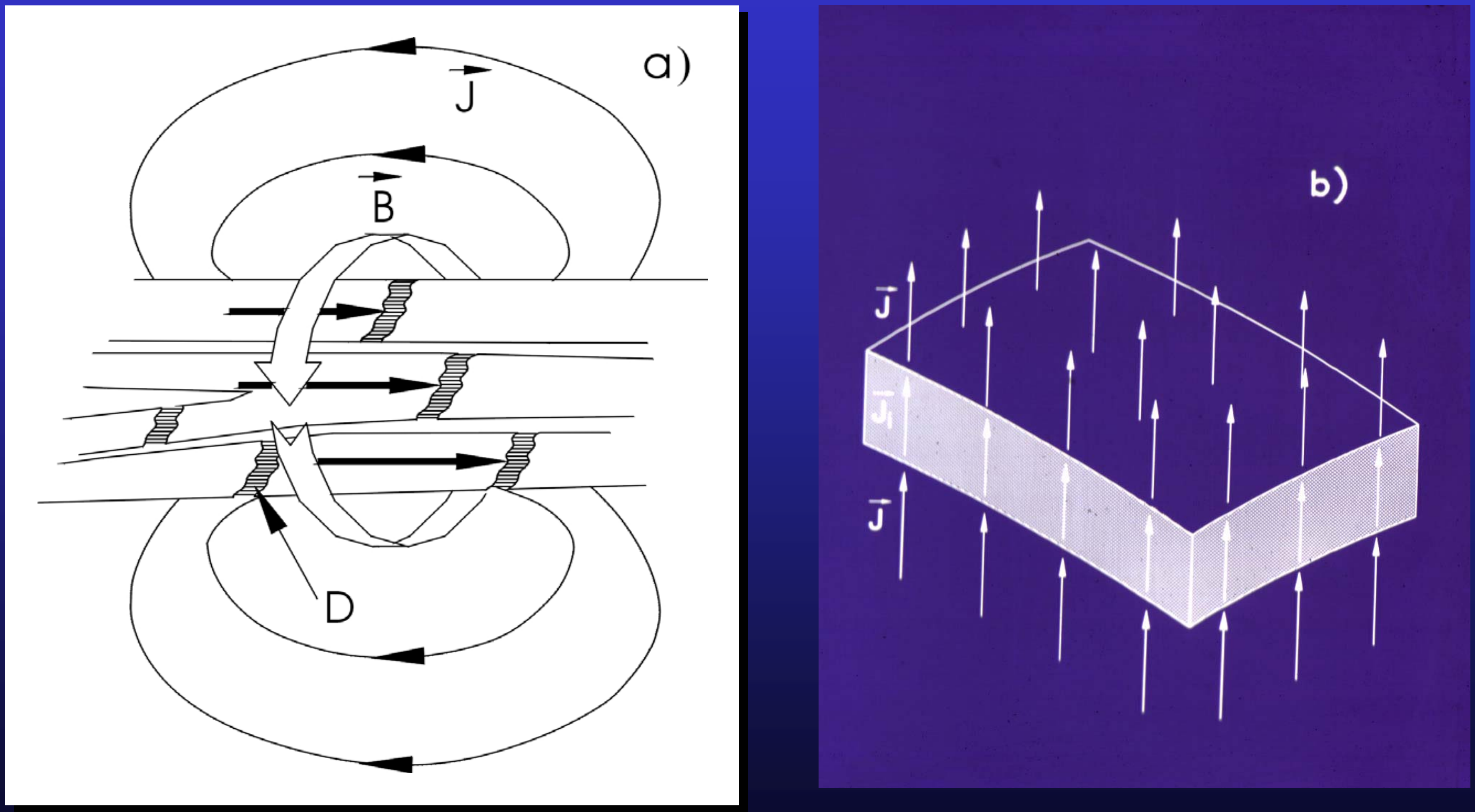


Courtesy Peter Hunter, Aukland University

The Excitatory Sequence

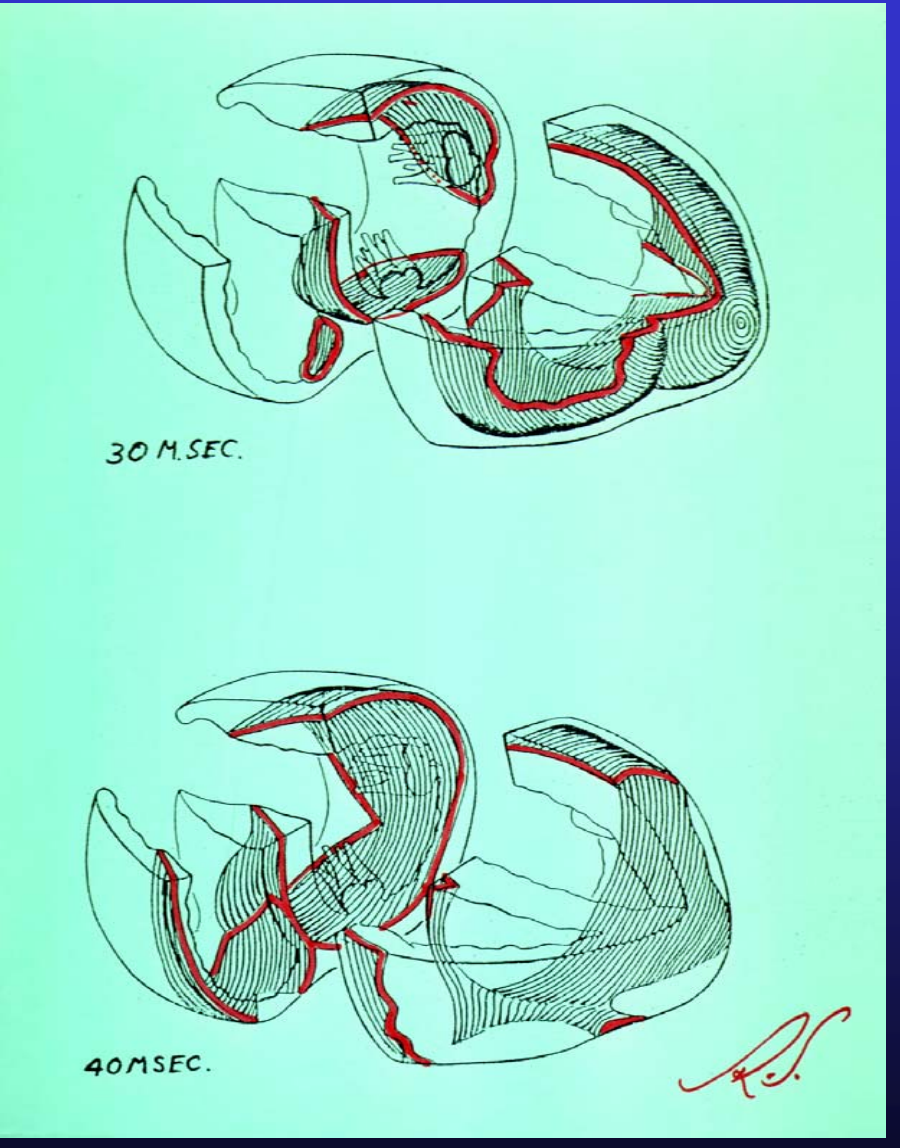


3 Dimensional Tissue



The Cardiac Depolarization Wave Front

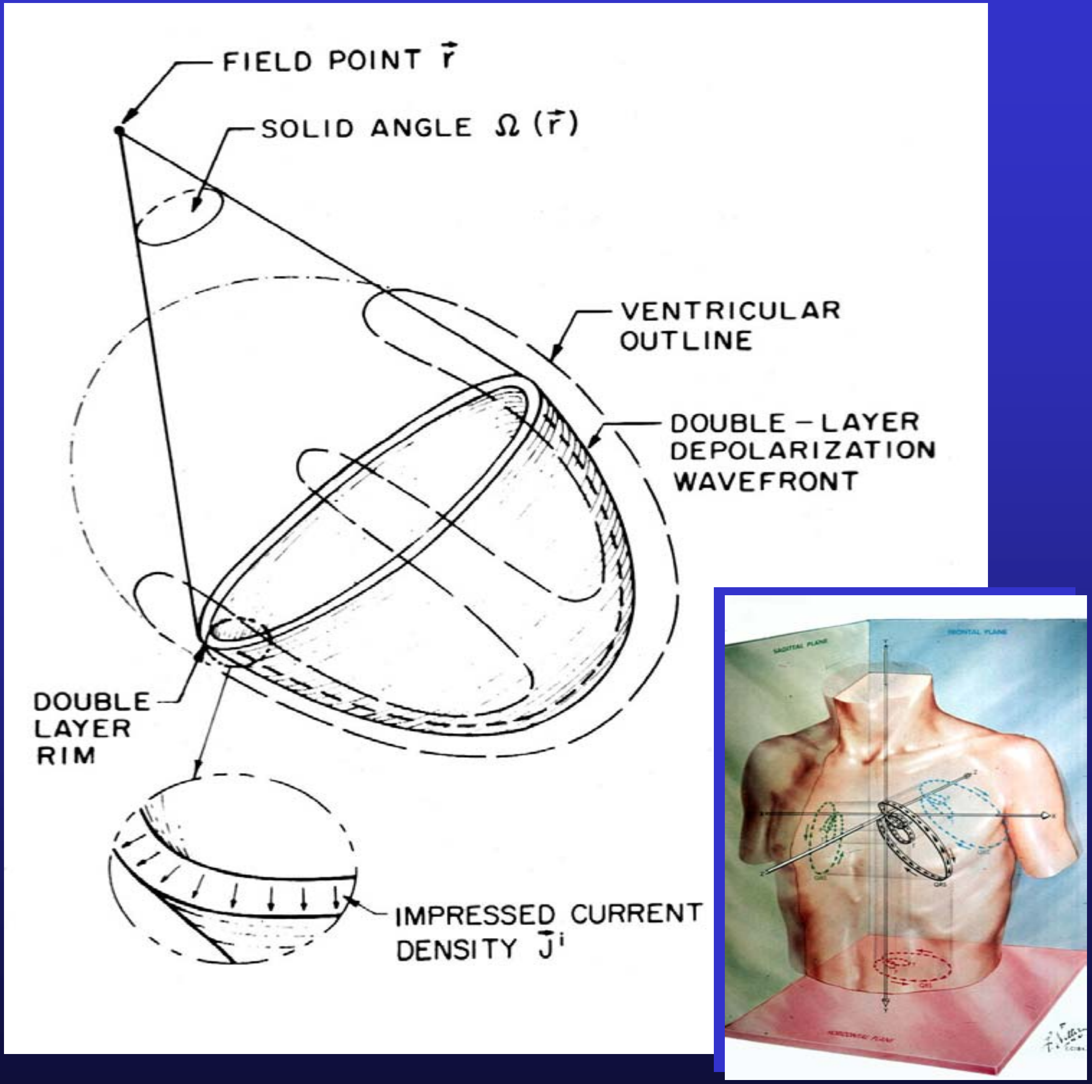
- Activated cells collectively form a sheet that is a moving 3-D battery
- 1 mm thick
- Moving at ~1 m/sec



Courtesy of Ron Selvester

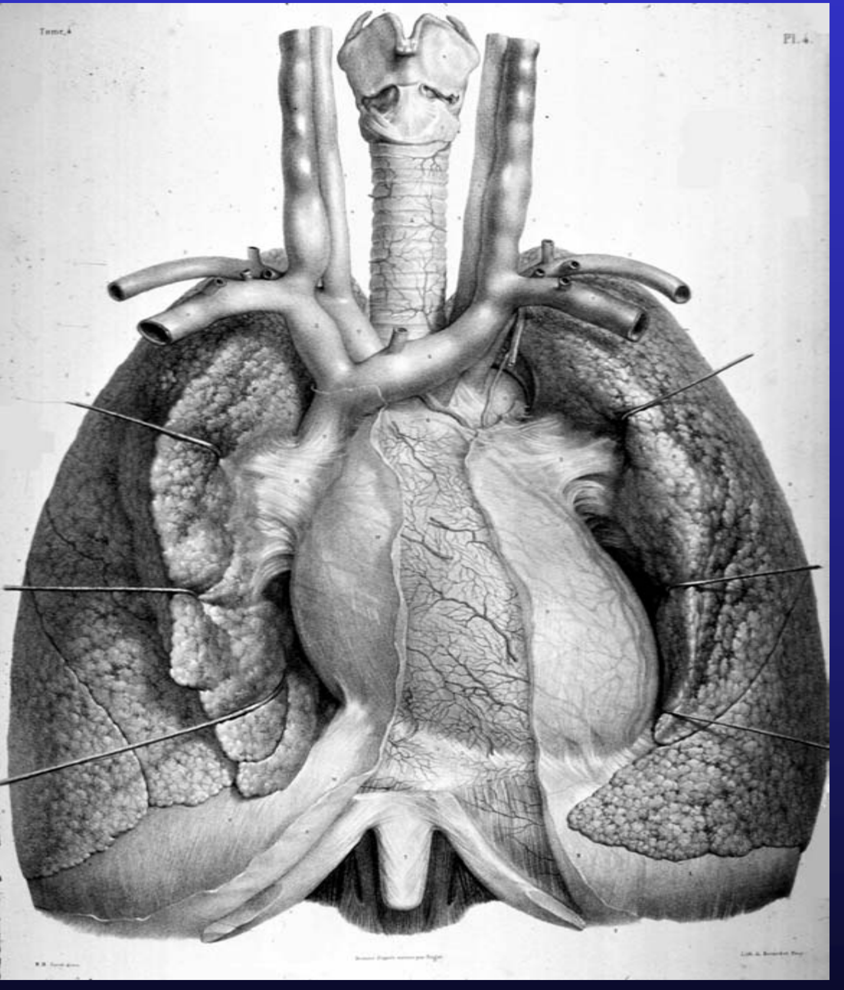
The uniform double-layer model

- Assumes
  - Uniform thickness
  - Uniform strength
  - Current perpendicular to the wave front
- The potential V(r) is determined by the current dipole moment and the solid angle subtended by the double-layer rim

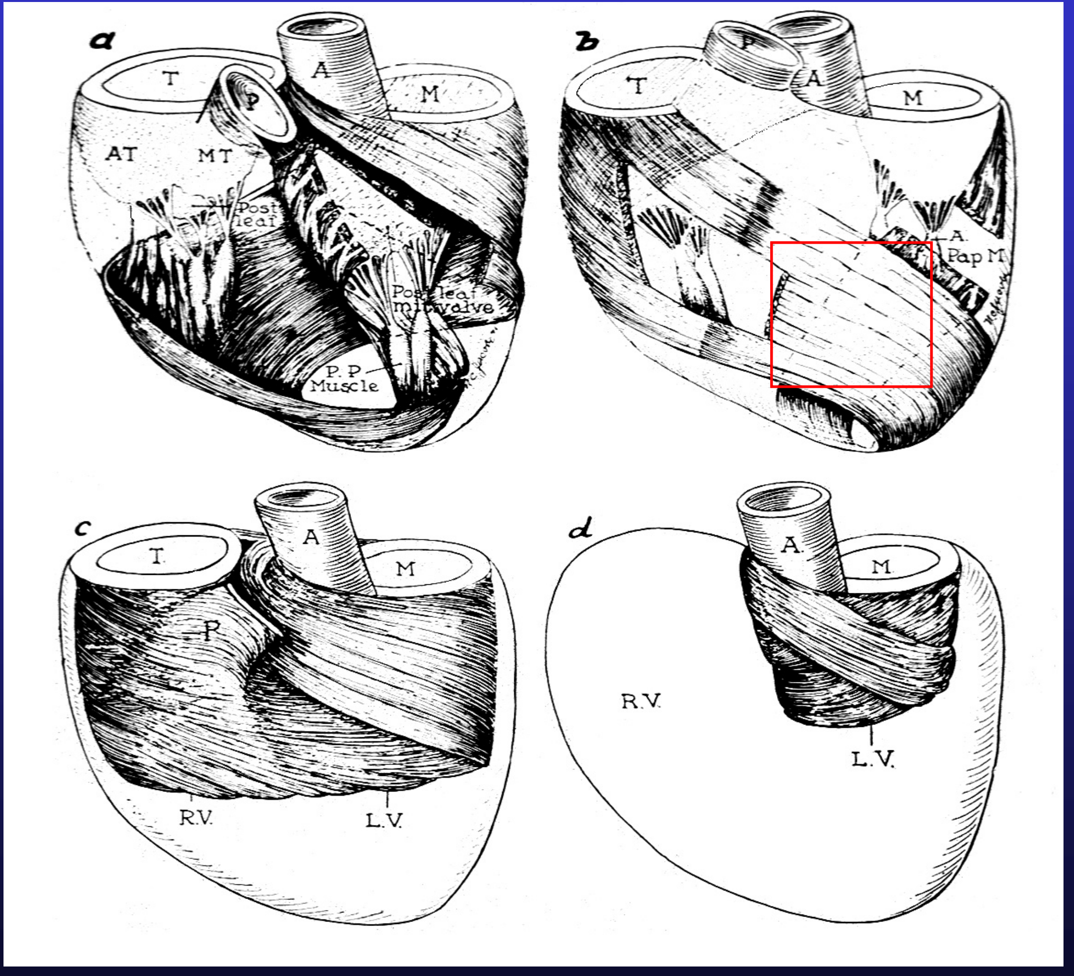


Heart vector or dipole moment versus time

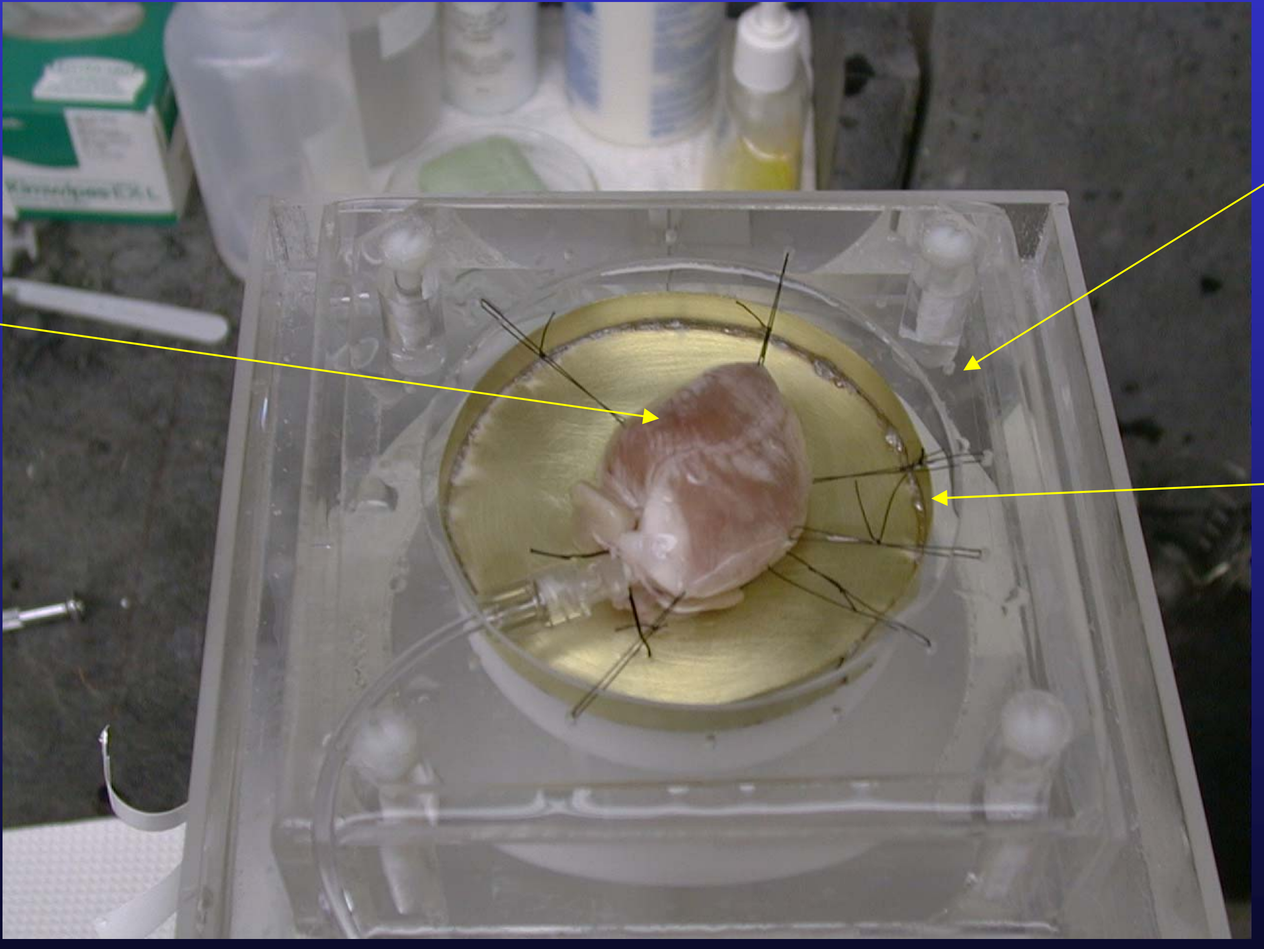
Getting closer ...



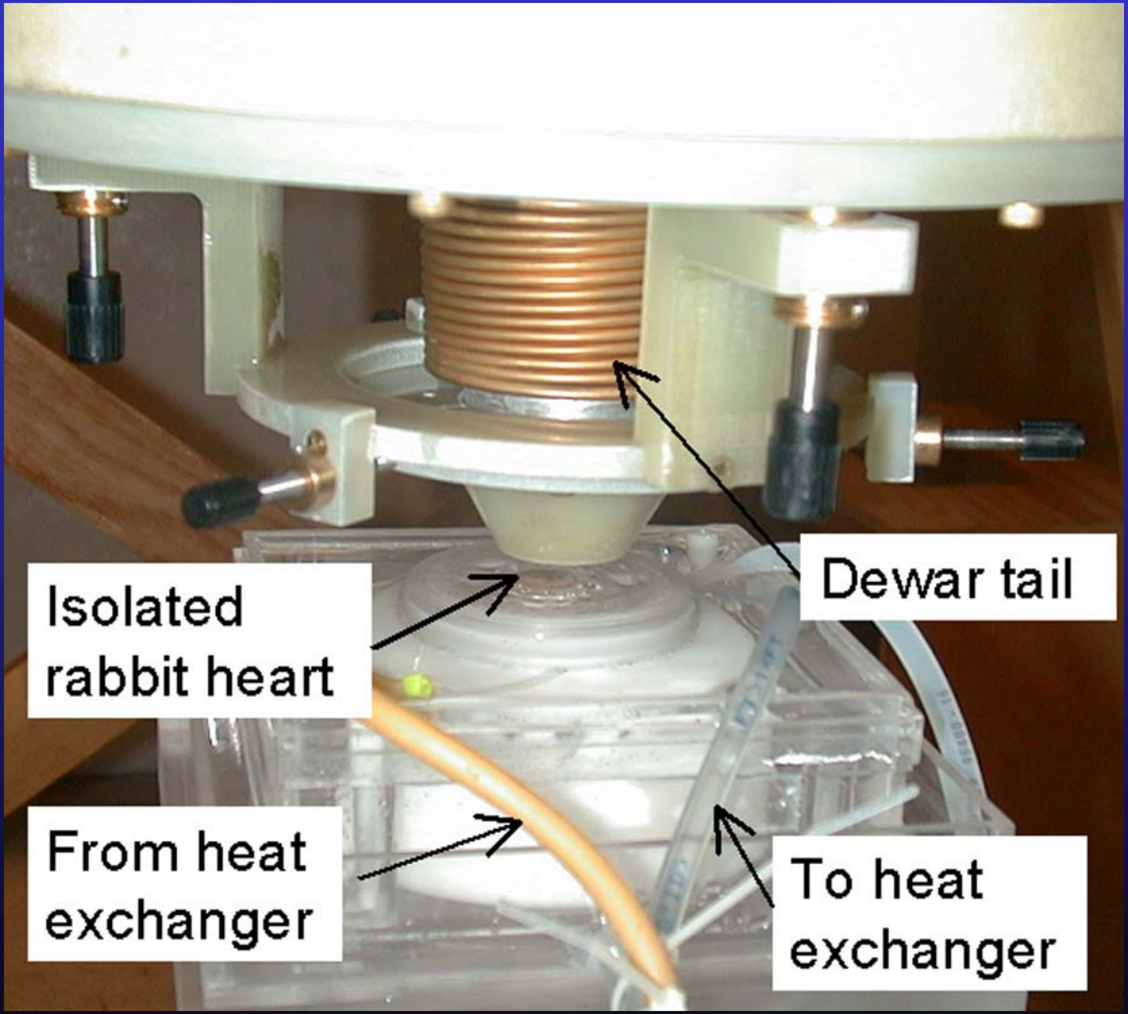
Simplify the Problem ...



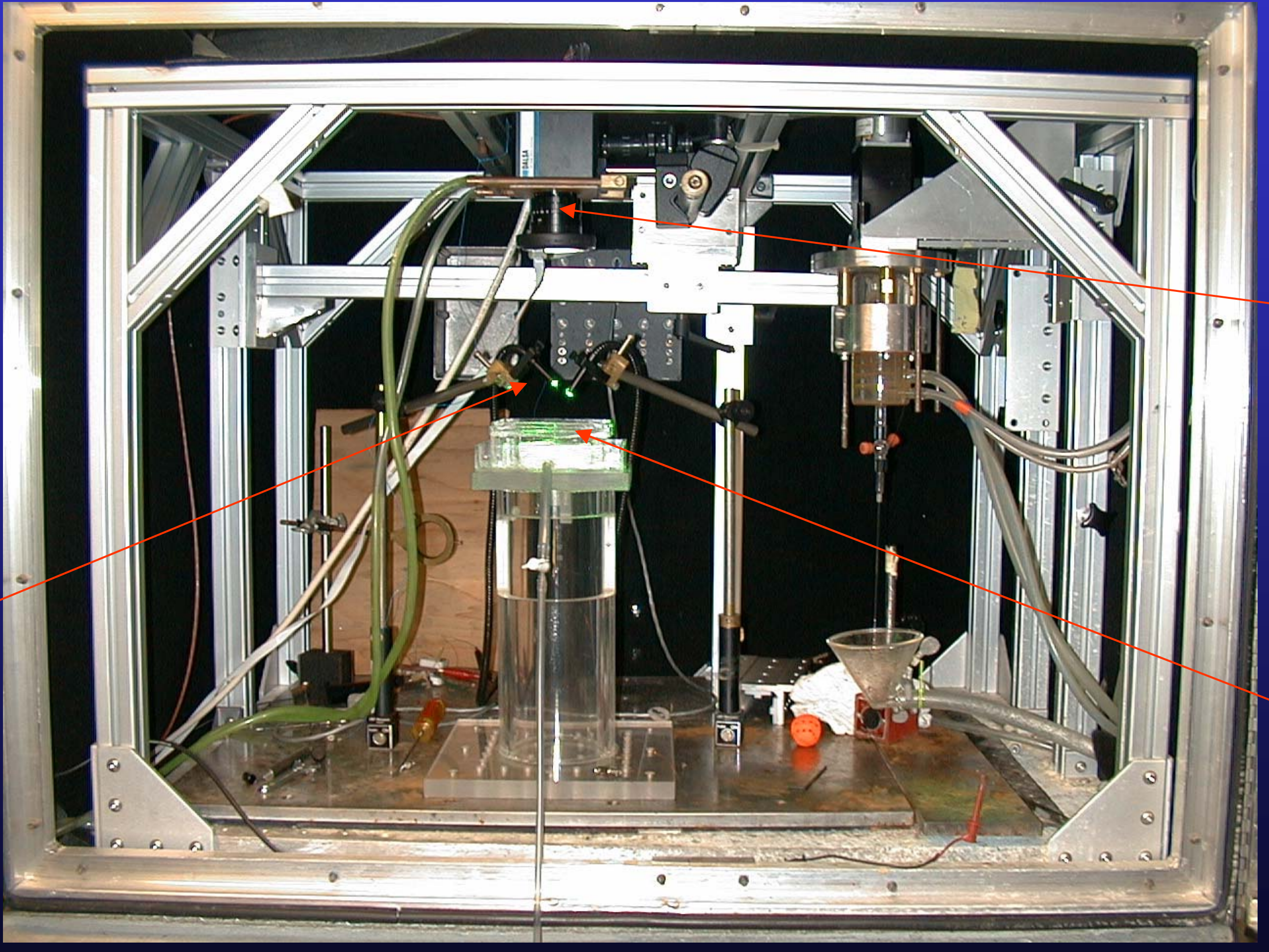
Langendorff perfused Isolated Rabbit Heart



Measure the magnetic field !



Experimental Setup - Optical Imaging



Laser Light

CCD-Camera
1200
Frames/sec

Heart

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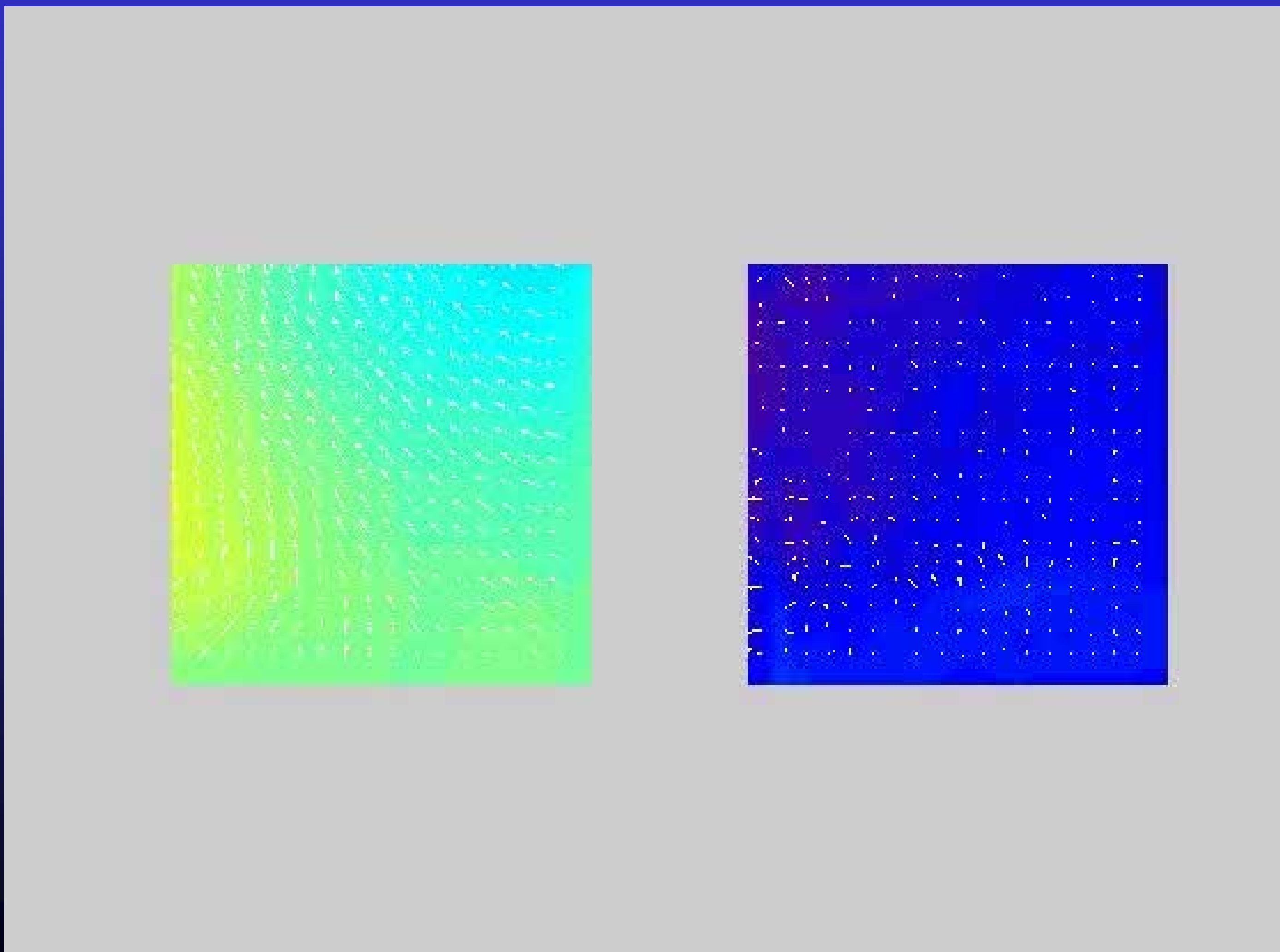
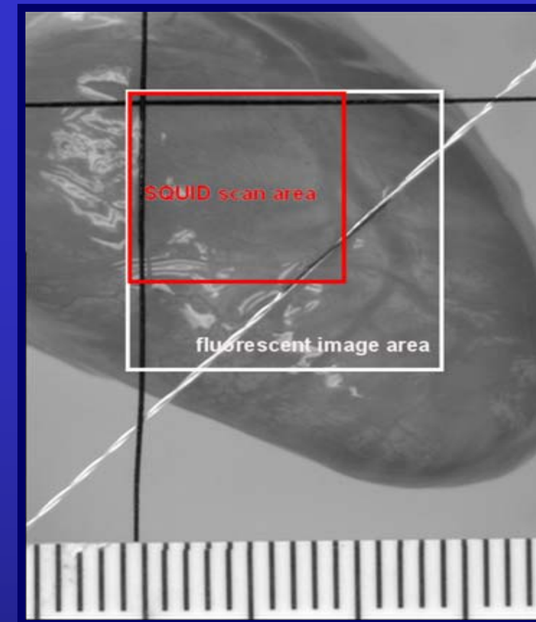
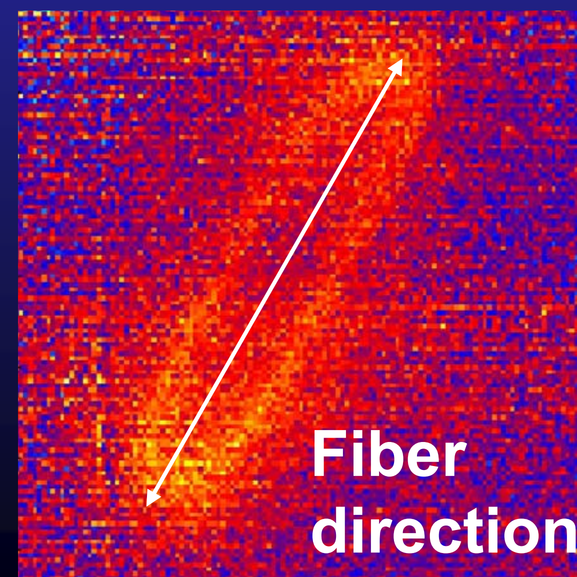
Courtesy of J Holzer

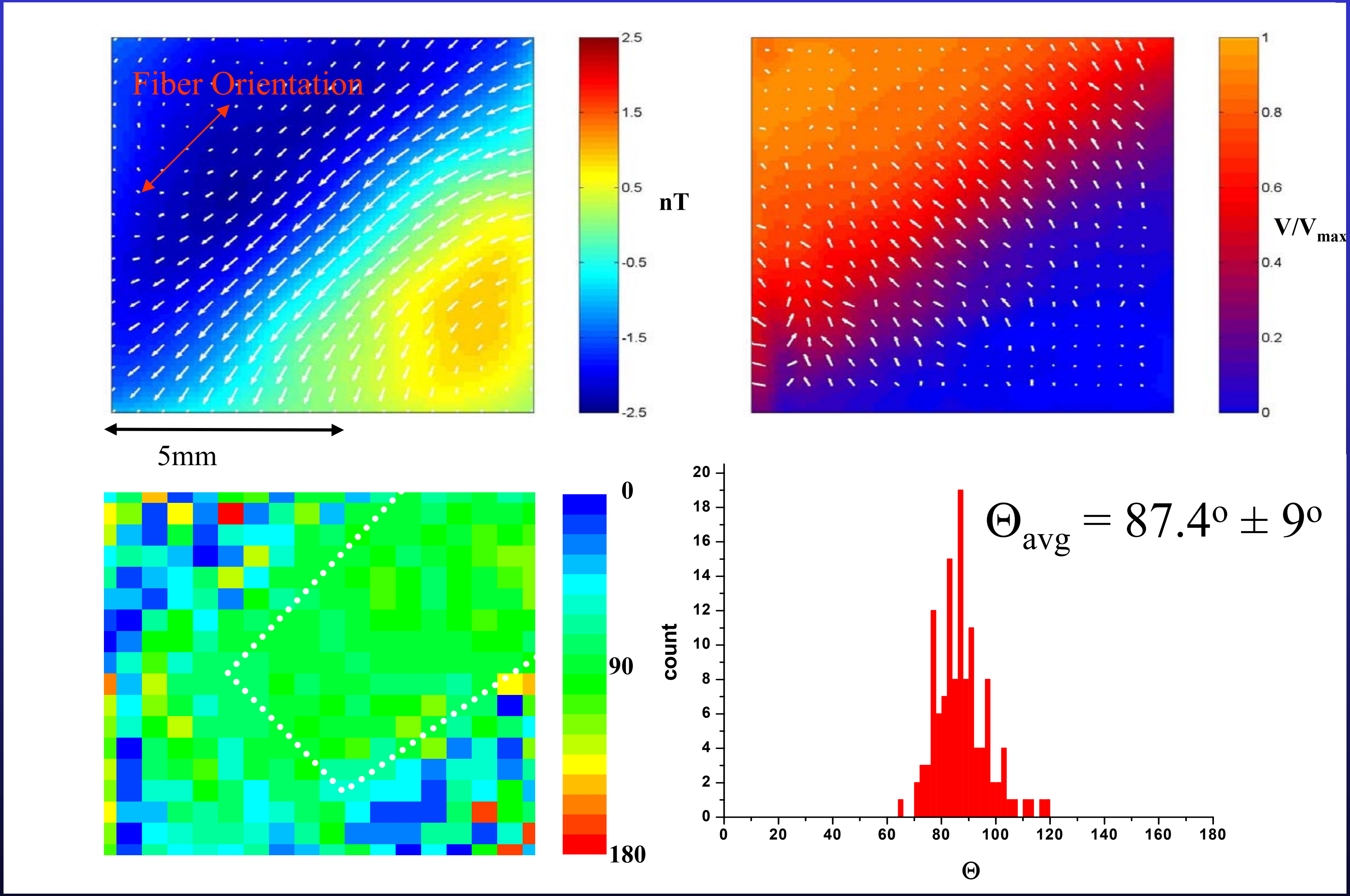
Combine V_m and net current measurements:



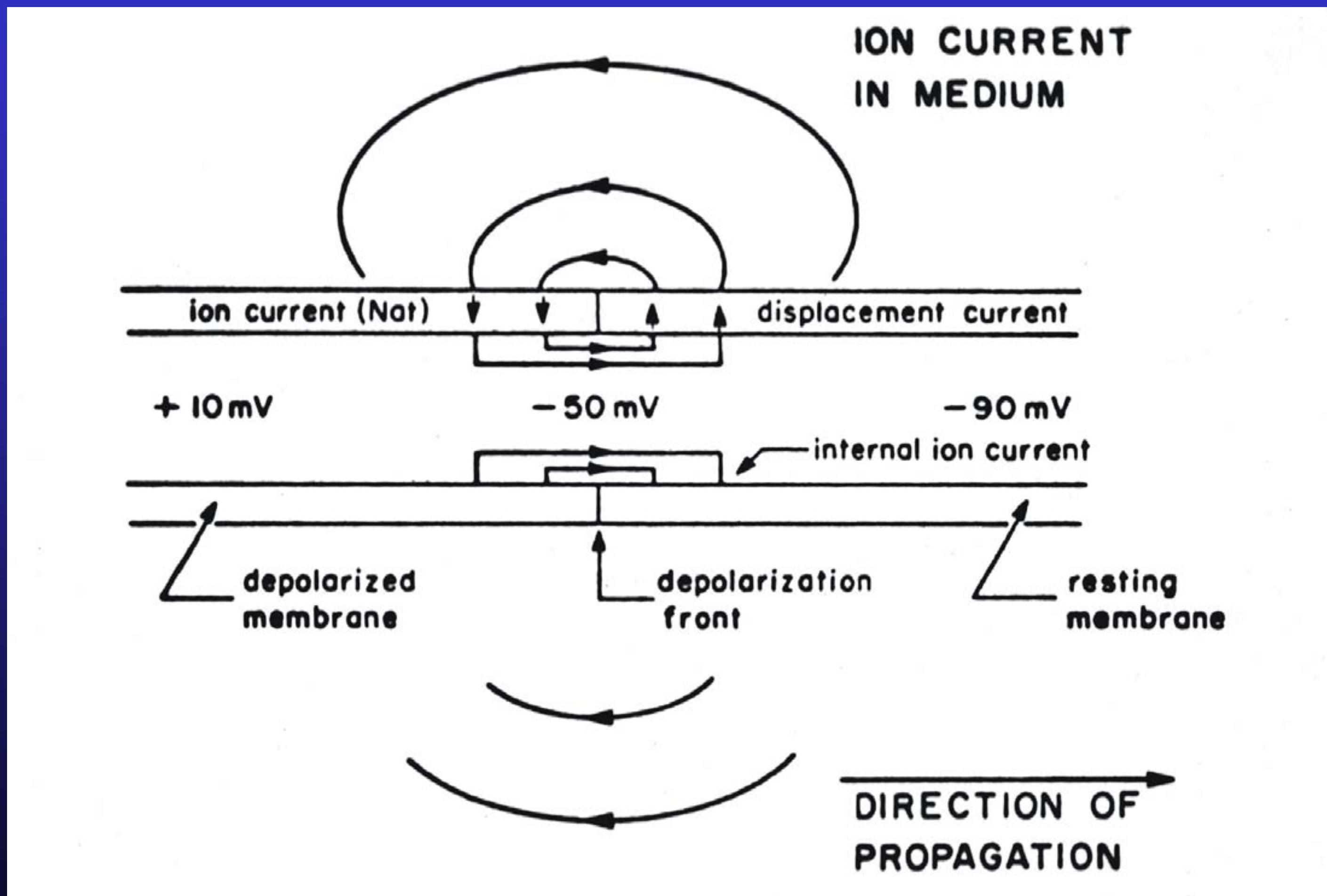


Point electrode stimulation

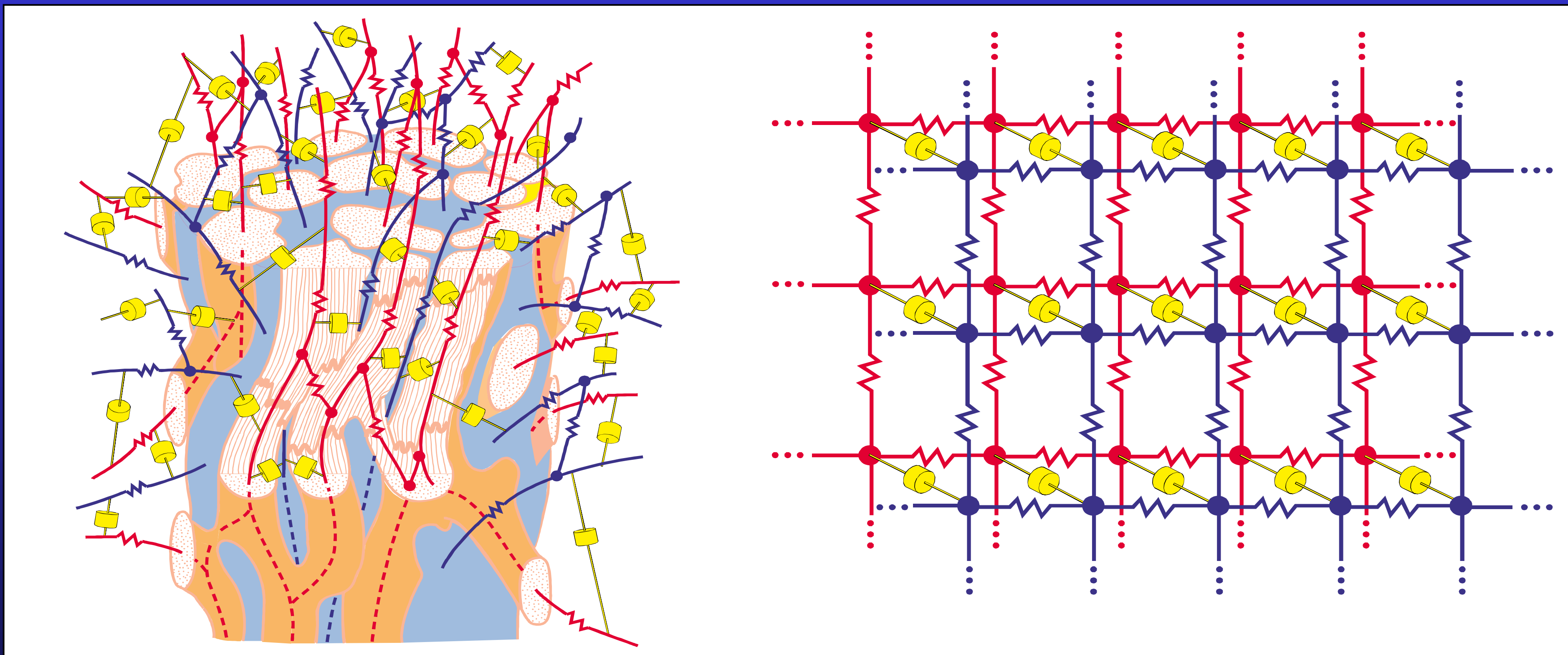


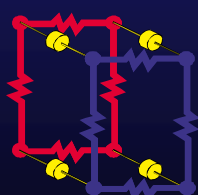

Fiber Orientation
2.5
1.5
0.5
-0.5
-1.5
-2.5
nT
1
0.8
0.6
0.4
0.2
0
V/Vmax
5mm
0
90
180
count
Θ
Θavg = 87.4° ± 9°

Action Currents Fibers in conducting Media



Cardiac Bidomain





Unit Block

Intracellular

Extracellular

Non-linear Membrane

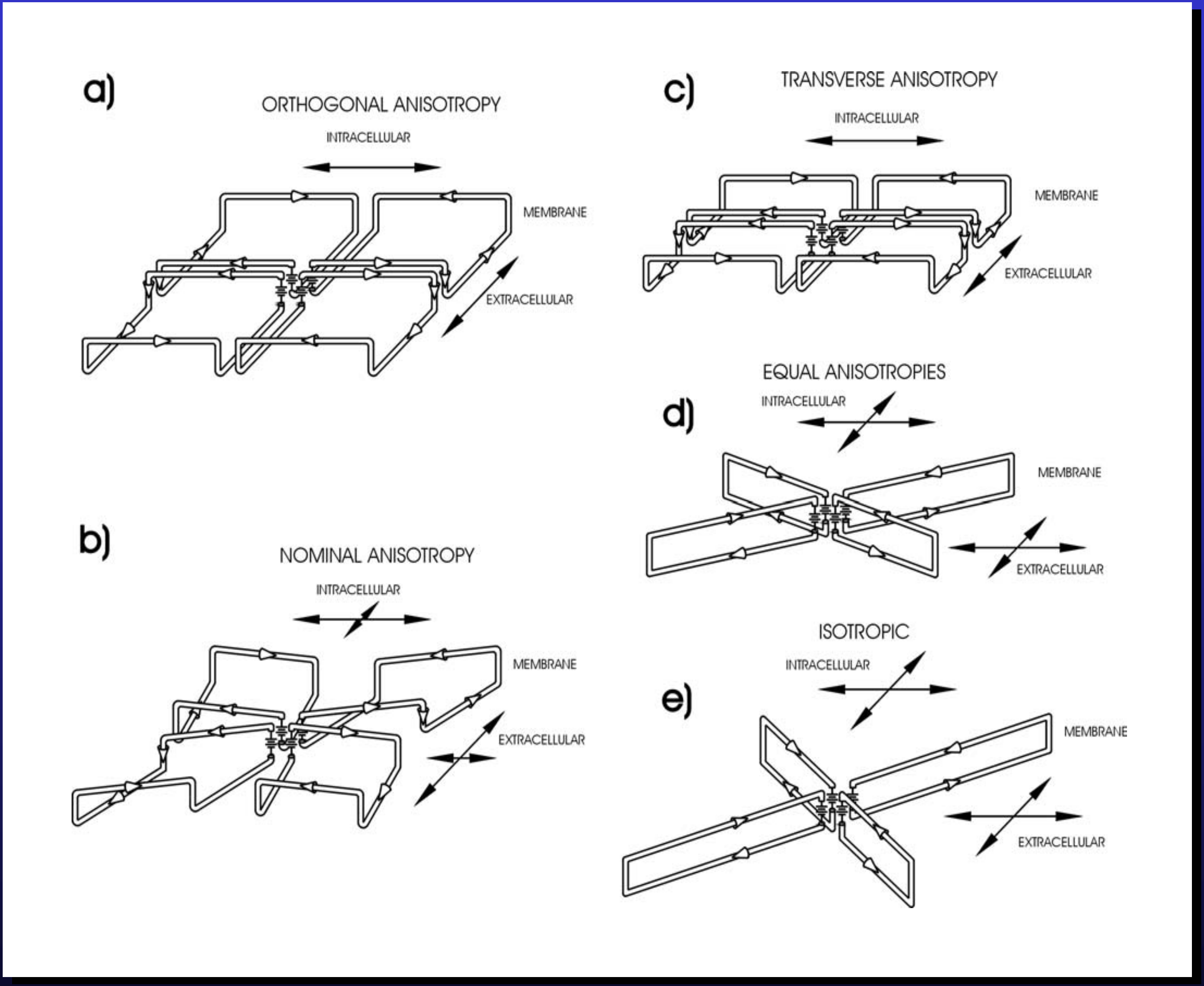
2-D Bidomain Equations

- Homogenized
- Coupled V_i & V_e
- Two nonlinear reaction-diffusion equations

$$S_{ix}\frac{d^2V_i}{dx^2} + S_{iy}\frac{d^2V_i}{dy^2} = \beta * [C_m\frac{dVm}{dt} + J_{ion}(V_m)] - I_i$$

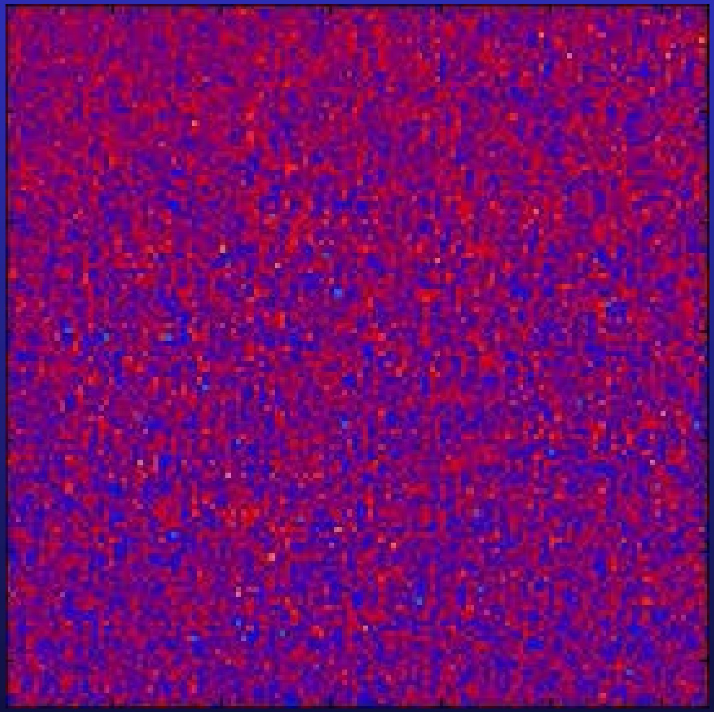
$$S_{ex}\frac{d^2V_e}{dx^2} + S_{ey}\frac{d^2V_e}{dy^2} = -\beta * [C_m\frac{dV_m}{dt} + J_{ion}(V_m)] - I_e$$

where x is along the fibers and y is transverse; the anisotropy ratios for the tissue conductivities in S/m are $S_{ix} = 0.2$, $S_{iy} = 0.02$, $S_{ex} = 0.8$, $S_{ey} = 0.2$; the membrane capacitance is $C_m = 0.01$ F/m^2; and the ratio of cell surface area to volume is $\beta = 0.2 \times 10^6$ m^{-1}

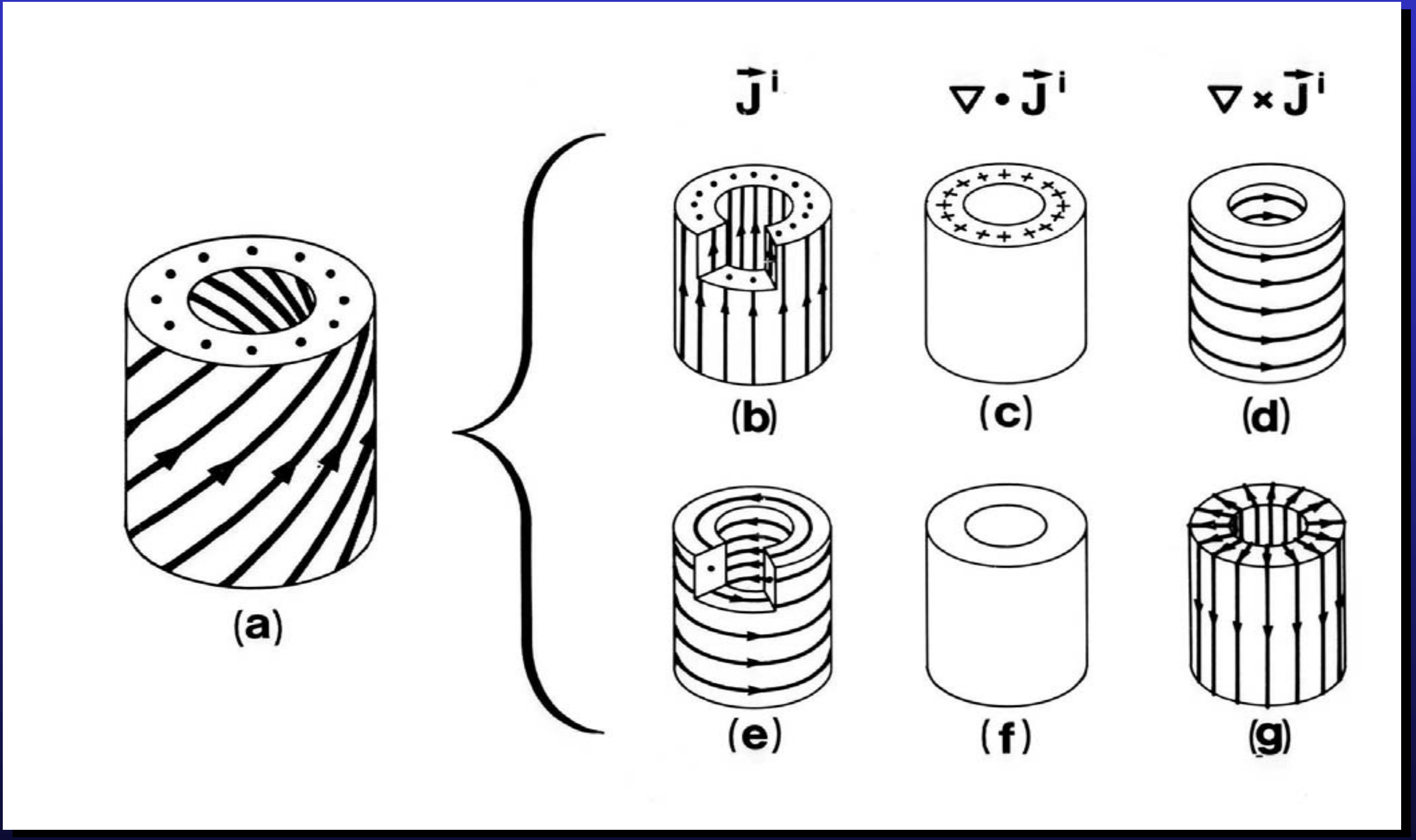

a)
ORTHOGONAL ANISOTROPY
INTRACELLULAR
MEMBRANE
EXTRACELLULAR
b)
NOMINAL ANISOTROPY
INTRACELLULAR
MEMBRANE
EXTRACELLULAR
c)
TRANSVERSE ANISOTROPY
INTRACELLULAR
MEMBRANE
EXTRACELLULAR
d)
EQUAL ANISOTROPIES
INTRACELLULAR
MEMBRANE
EXTRACELLULAR
e)
ISOTROPIC
INTRACELLULAR
MEMBRANE
EXTRACELLULAR

Information Content of the Magnetocardiogram

The Apex



Electrically and Magnetically Silent Current Sources



Wikswo, J. P. Barach, J. P. J. Theo. Bio. 95, 721-729

Findings from SQUID Microscopy

- Magnetic mapping can provide high resolution images of net action current in cardiac tissue.

Baudenbacher, F., Peters, N. T. *, Baudenbacher, P., Wikswo, J. P., High Resolution Imaging of Biomagnetic Fields Generated by Action Currents in Cardiac Tissue using a LTS-SQUID microscope. Physica C 368, 24-31 (2002).

- We have found electrically silent currents.
- The MCG appears to arise from sheets of current || wave front, not current dipoles ⊥ wave front.

J.R. Holzer+, V. Y. Sidorov, L. Fong*, F. Baudenbacher, Magnetic and Fluorescent Imaging of Wave Front Propagation in Cardiac Tissue reveal currents parallel to the wave front, (to be submitted to Biophysical Journal)

?

- What fraction of cardiac electrical activity results in electrically silent current patterns that do not contribute to the electrocardiogram?
- What role do steady currents that are difficult to detect electrically play during ischemia?

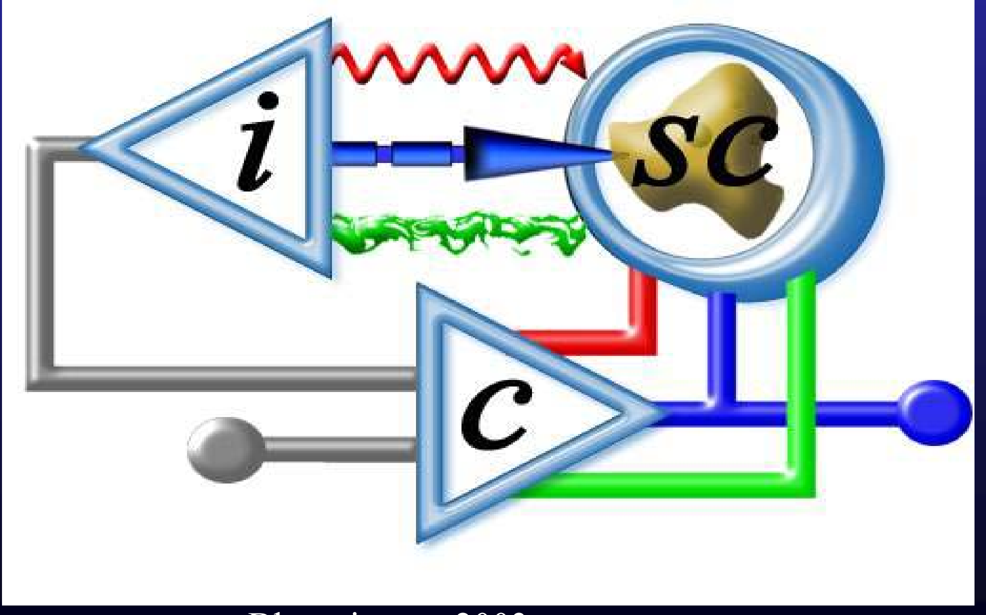
Solutions to the Forward Problem

- Develop and refine hierarchical multiscale models to span the full range of space and time
  - Single cell cardiac myocyte models do not extrapolate to the whole heart
- Understand the dynamics, control structures and functions of the smallest living unit
  - "Instrumenting and Controlling the Single Cell"

How can we understand the dynamics associated with cellular function ?

→ Quantitative integrative physiology on the single cell level

BioMEMS for Instrumenting and Controlling



The Single Cell

High-Content Toxicology Screening Using Massively Parallel, Multi-Phasic Cellular Biological Activity Detectors

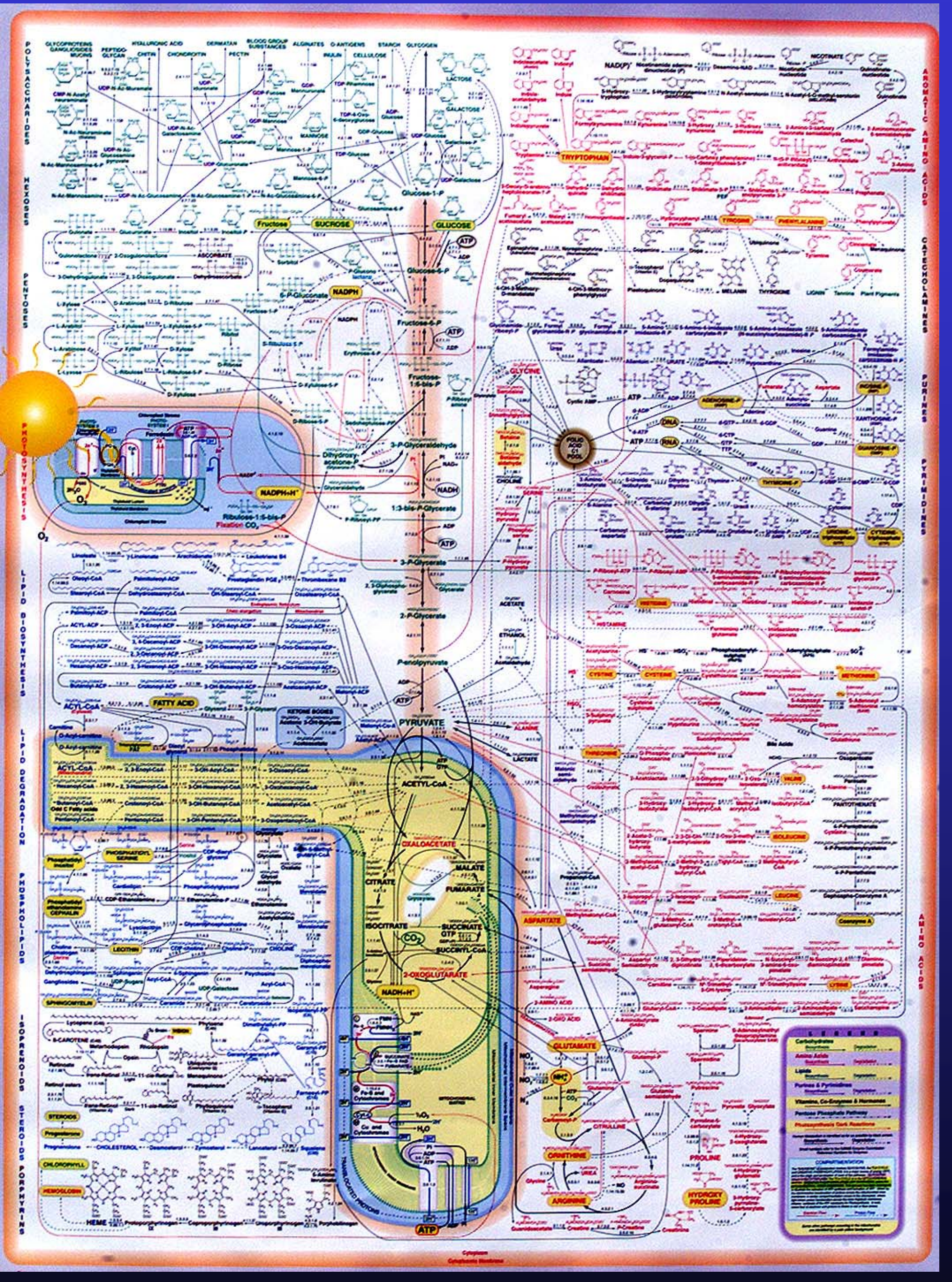
Can we use single cell as toxin detectors by probing cellular function and dynamics?

Can we develop High throughput High Content Devices for Pharmaceutical Drug Screening?

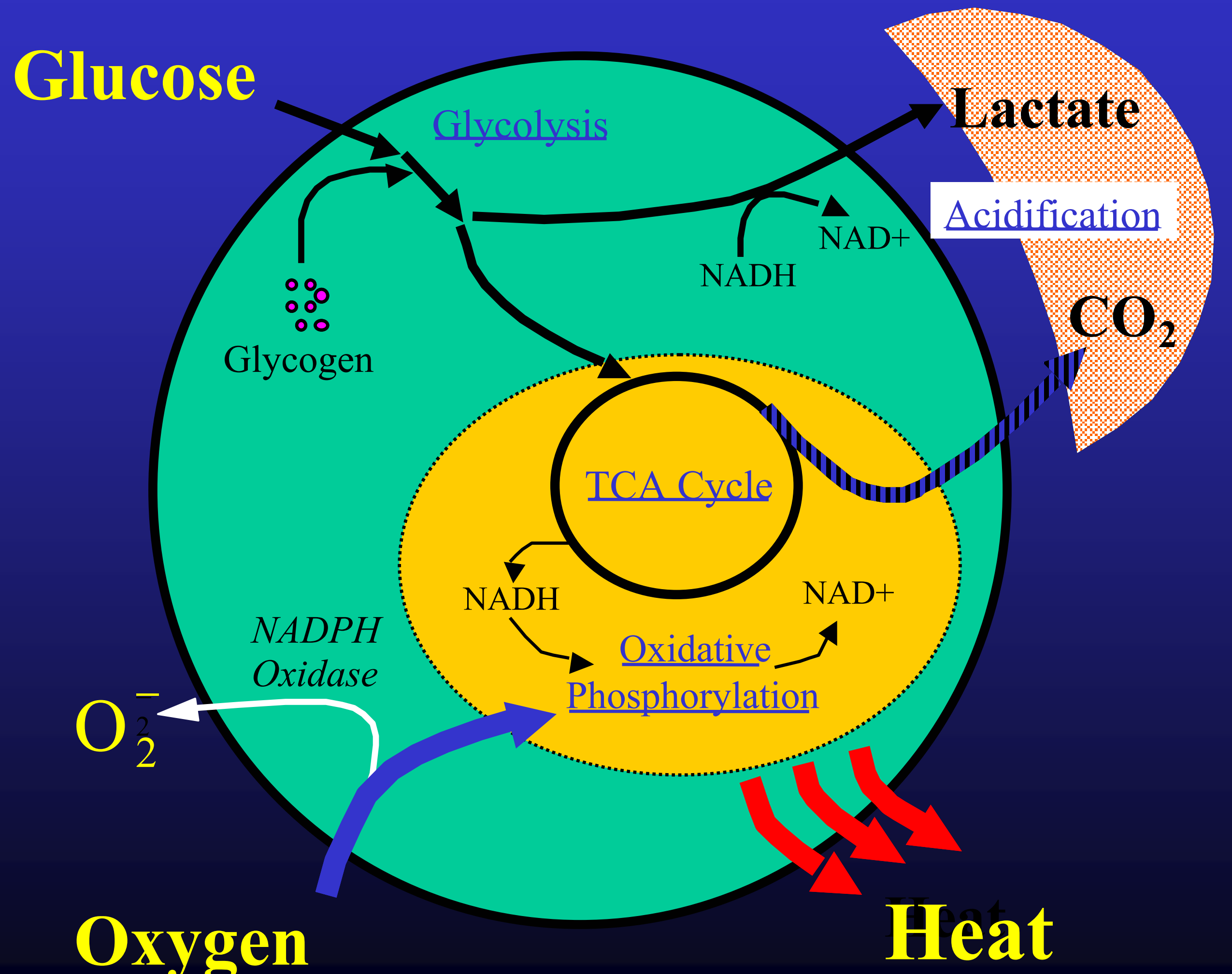
Can we develop hand held devices for environmental monitoring?

Can we develop devices for point of care diagnosis?

Cell Physiology as a linked Network of Metabolic and Signaling Pathways



Cell Metabolism



Cell Based Biosensor as Generalized Toxicity Sensor

- We do not measure the toxin itself. We are measuring the impact of the toxin on cell physiology by probing cell functions!
  - Metabolic pathways
  - Electrical Excitability
  - Cell to Cell Communication ……..

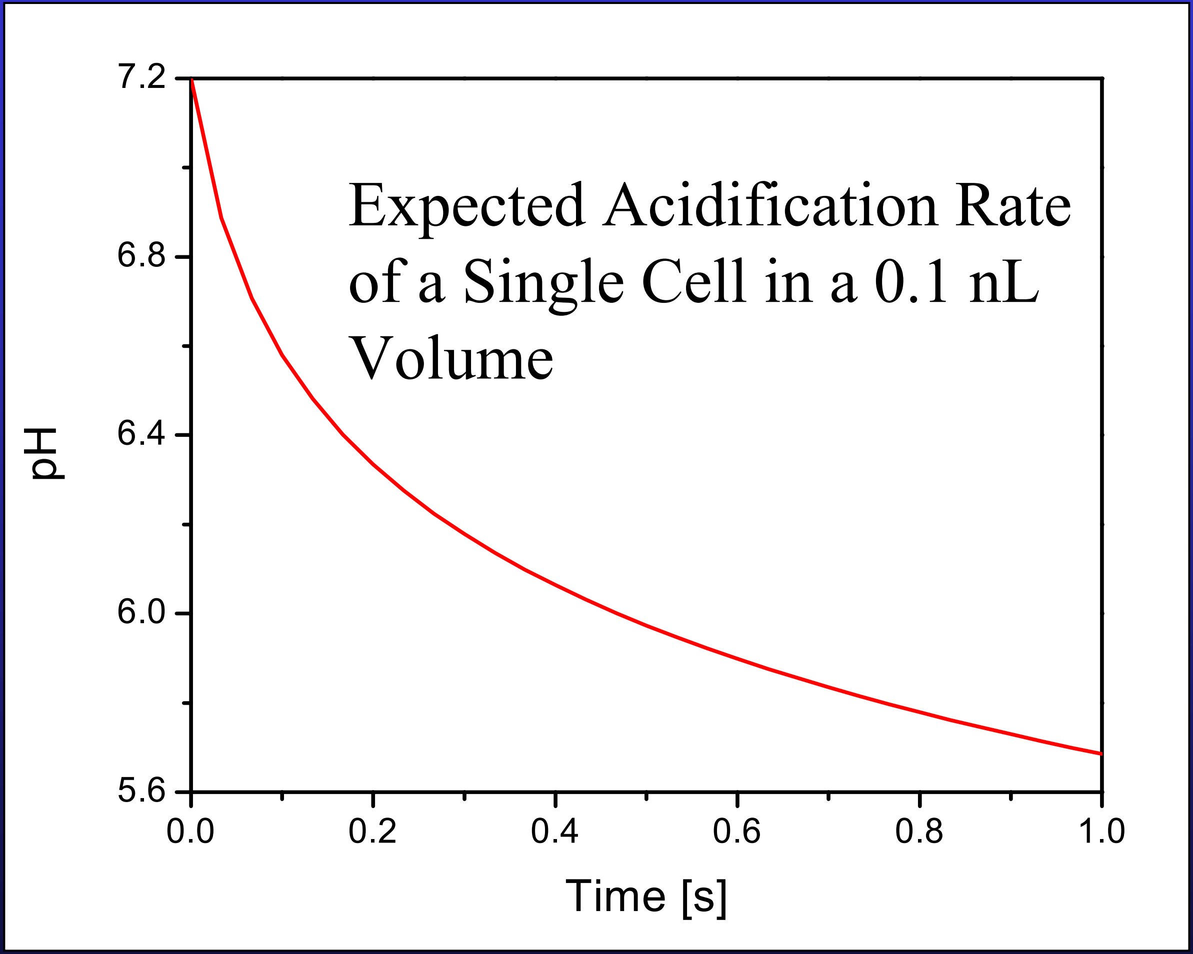
→ Intrinsic amplification

How to can we build a High Gain Amplifier?

Size does Matter!

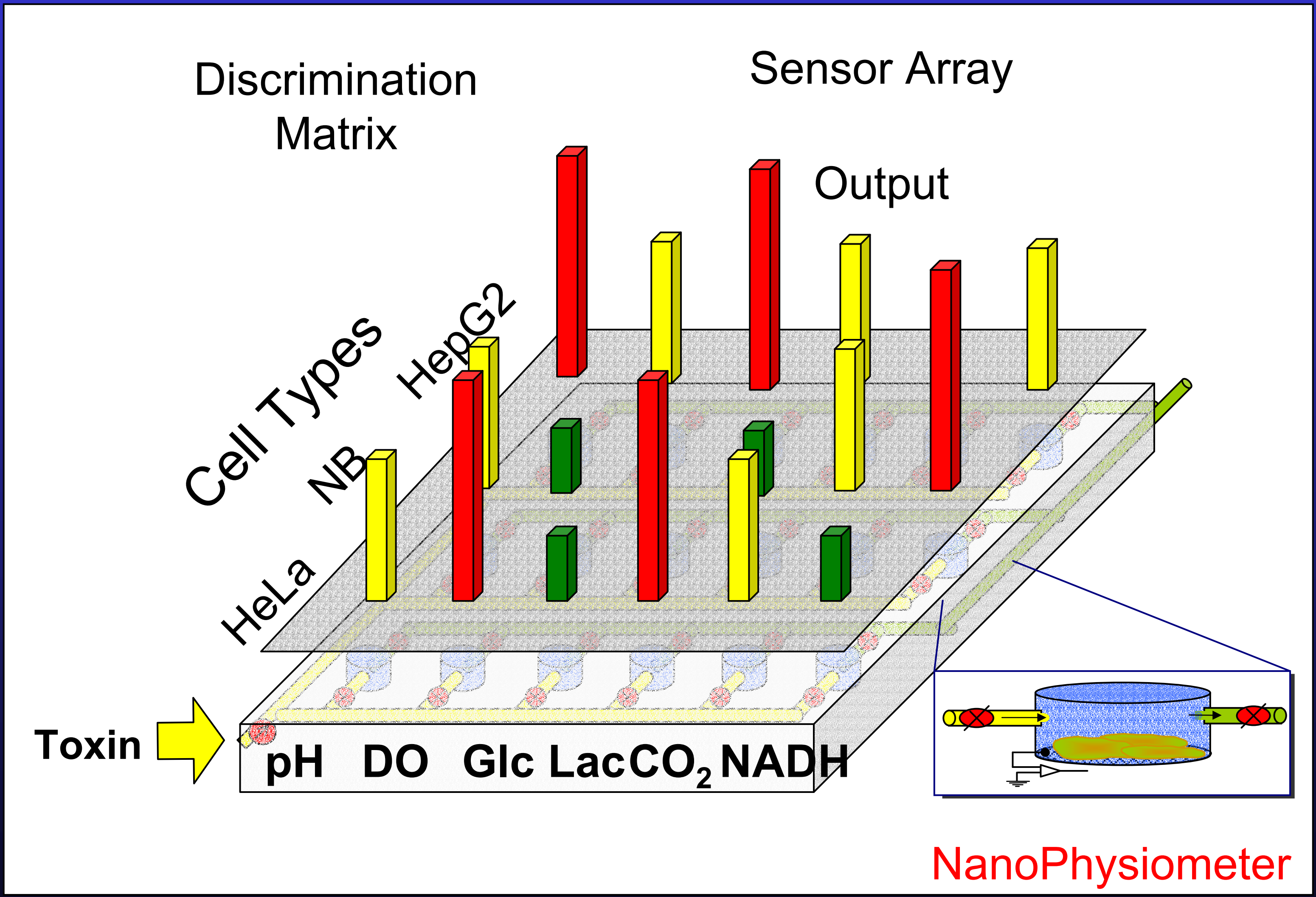
- Single Cell Experiments
- Confined Extracellular Space

→ Monitor physiological events in real time



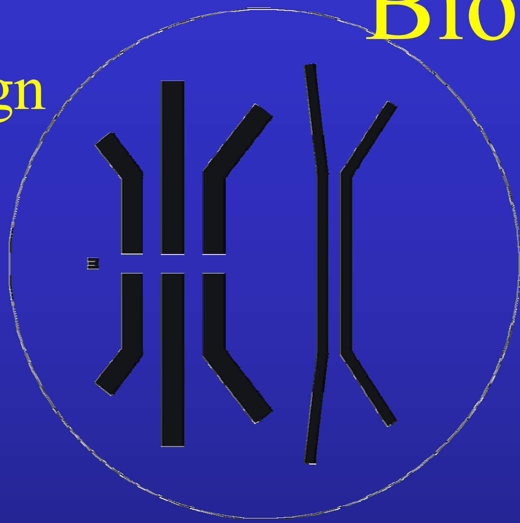
Feedback – Closed Loop Control

Discrimination



BioMEMS Device Fabrication

Mask Design

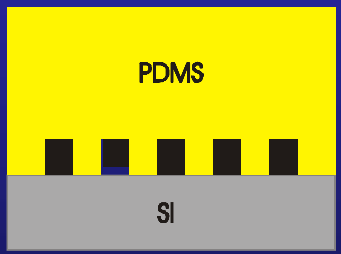


Positve Photoresist

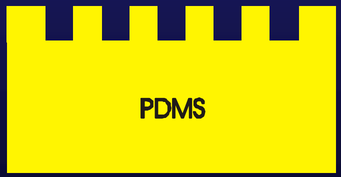
Substrate

Exposed and Developed
Photo Resist

PDMS Casting



Cure and Release



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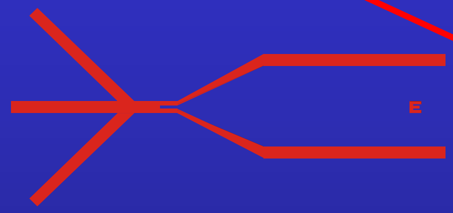
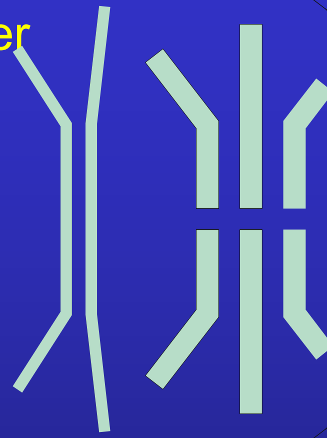
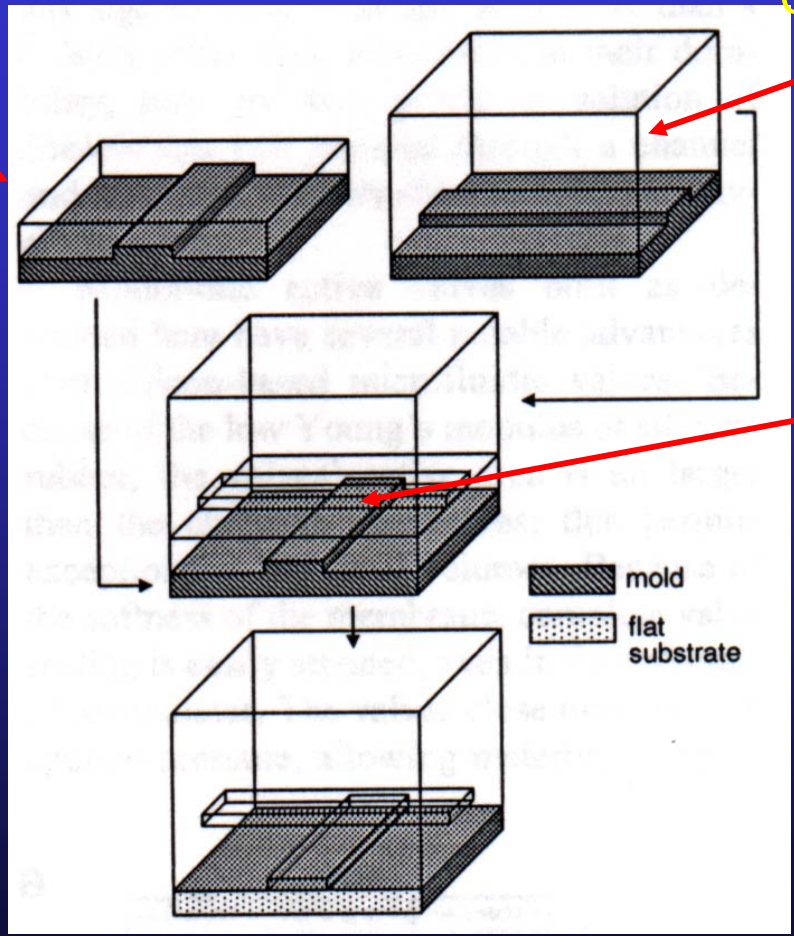
Device Design and Fabrication

Fluidics Layer

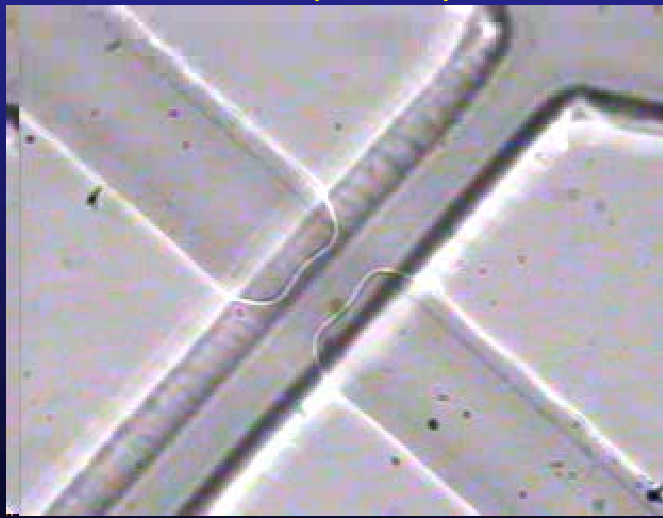


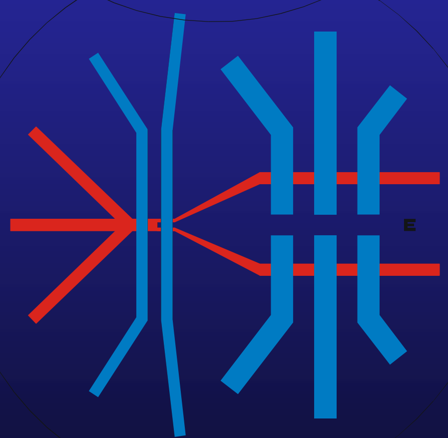
Control Layer





Flexible PDMS Membrane (Valve)





Courtesy of S. Quake et al [1]

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

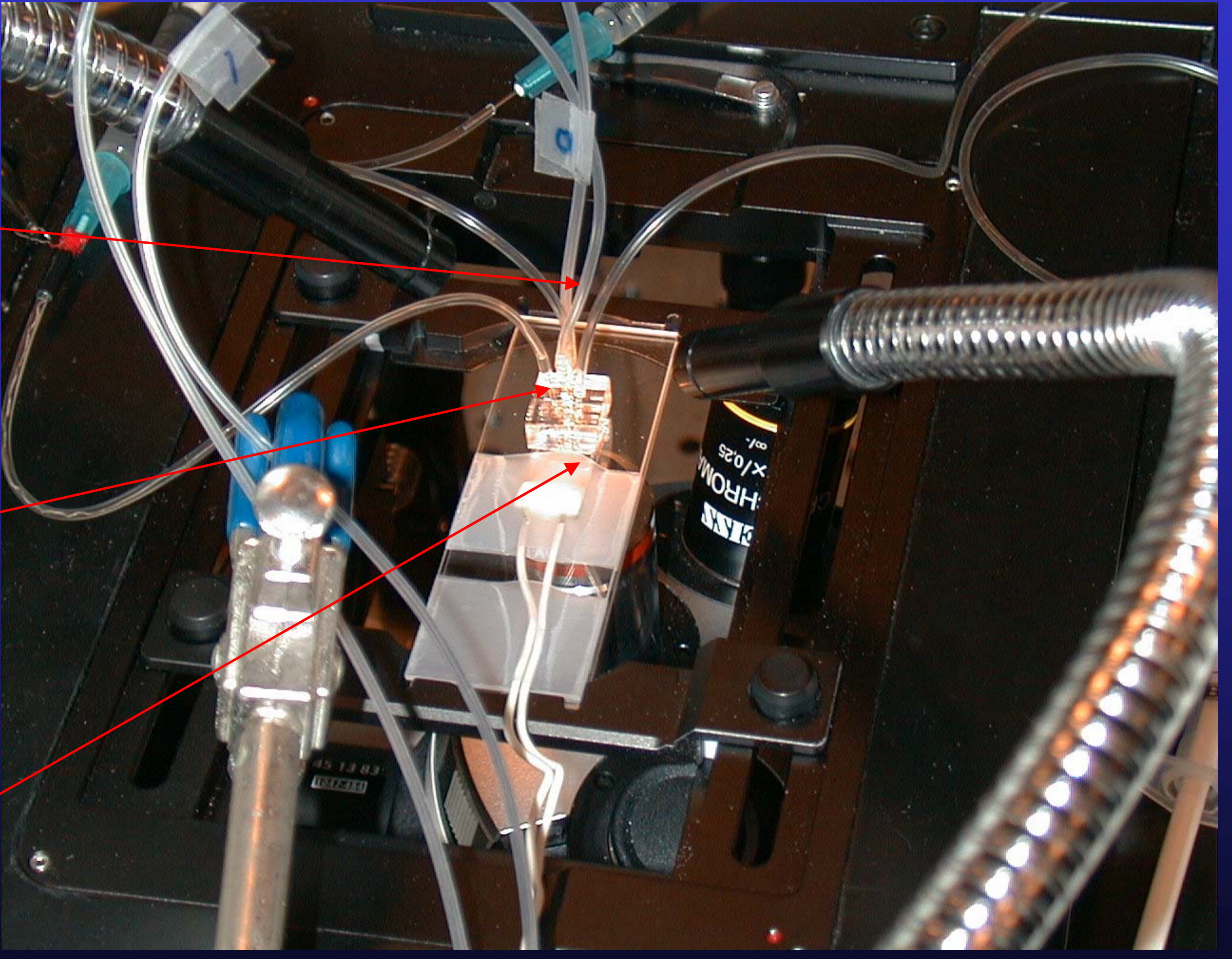
[2] M.A. Unger, H.-P. Chou, T. Thorsen, A. Scherer, and S.R. Quake, "Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography", *Science* 288: 113-116 (2000).

Experimental Setup

Fluid and Valve Control Lines

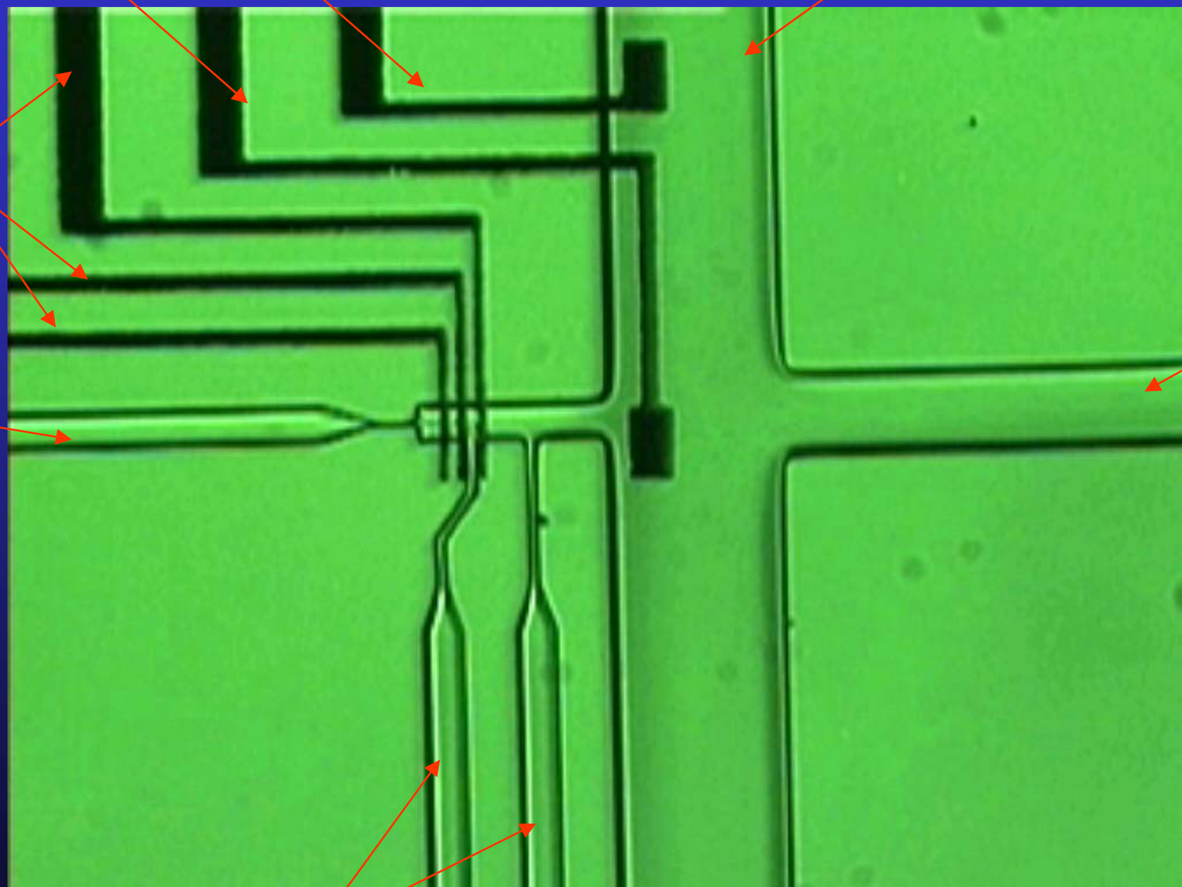
PDMS Device

Interdigitated MicroElectrode (IME) Array

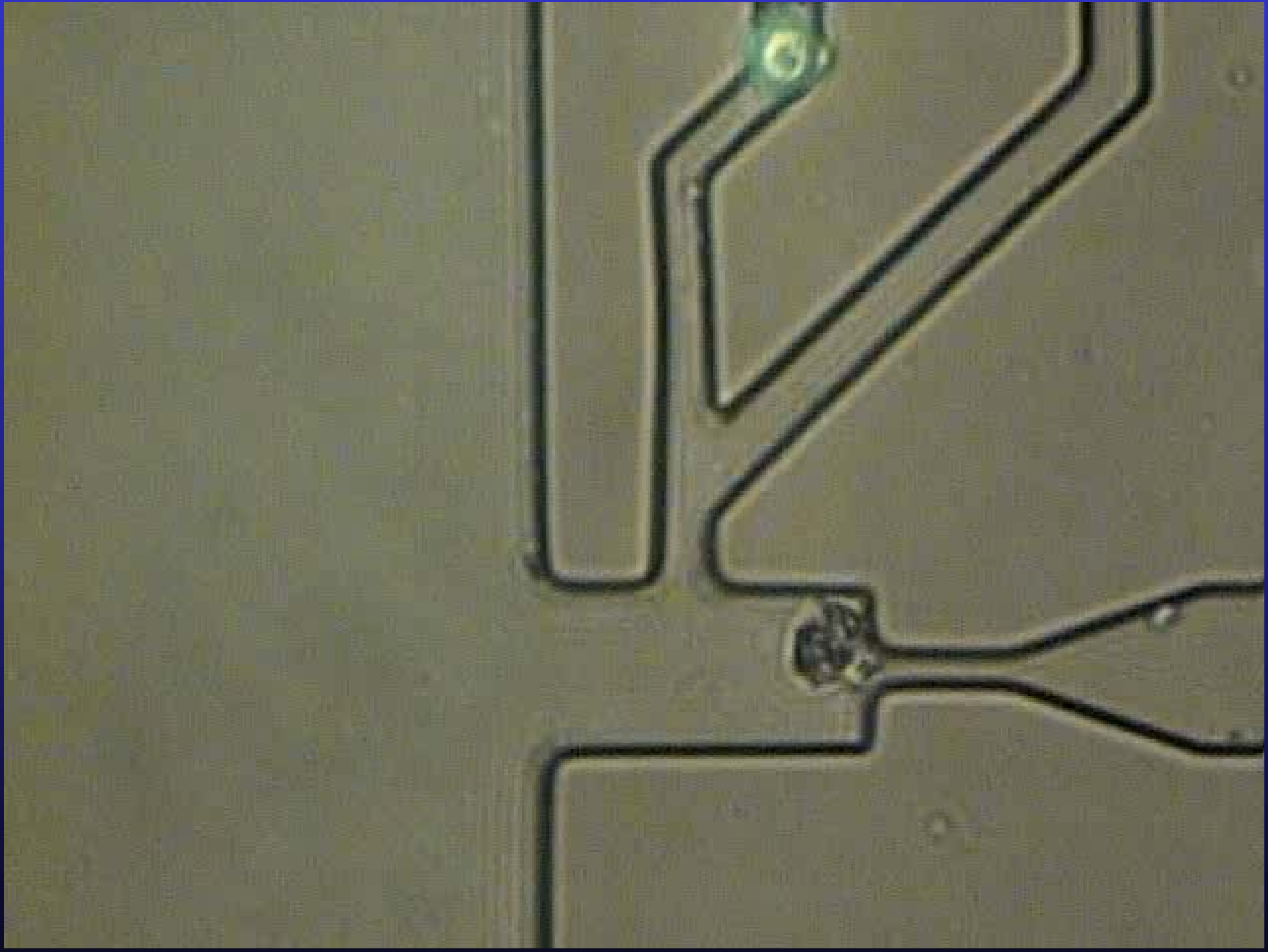


Courtesy of A Werdich & R. Reisserer

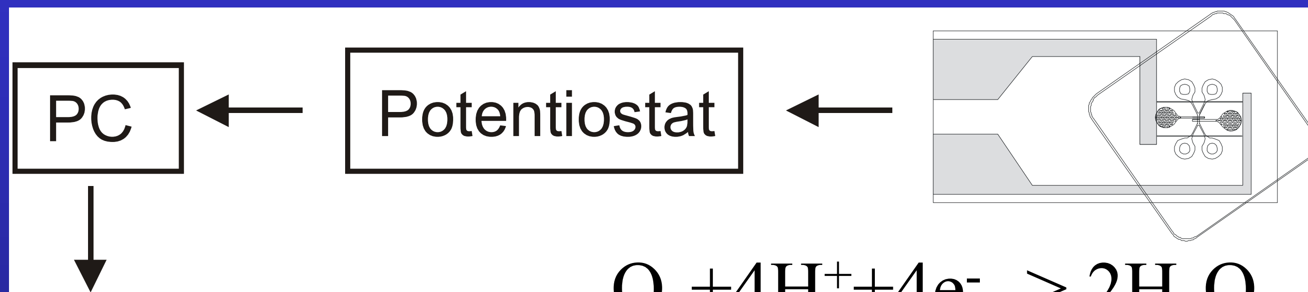
Third Generation of NanoPhysiometers



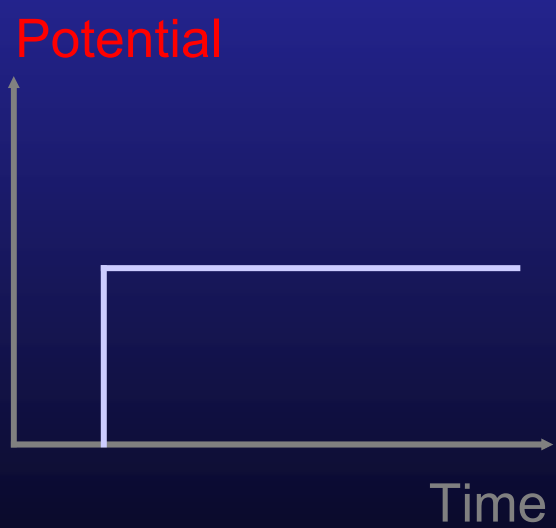
Changing the Extracellular Volume…

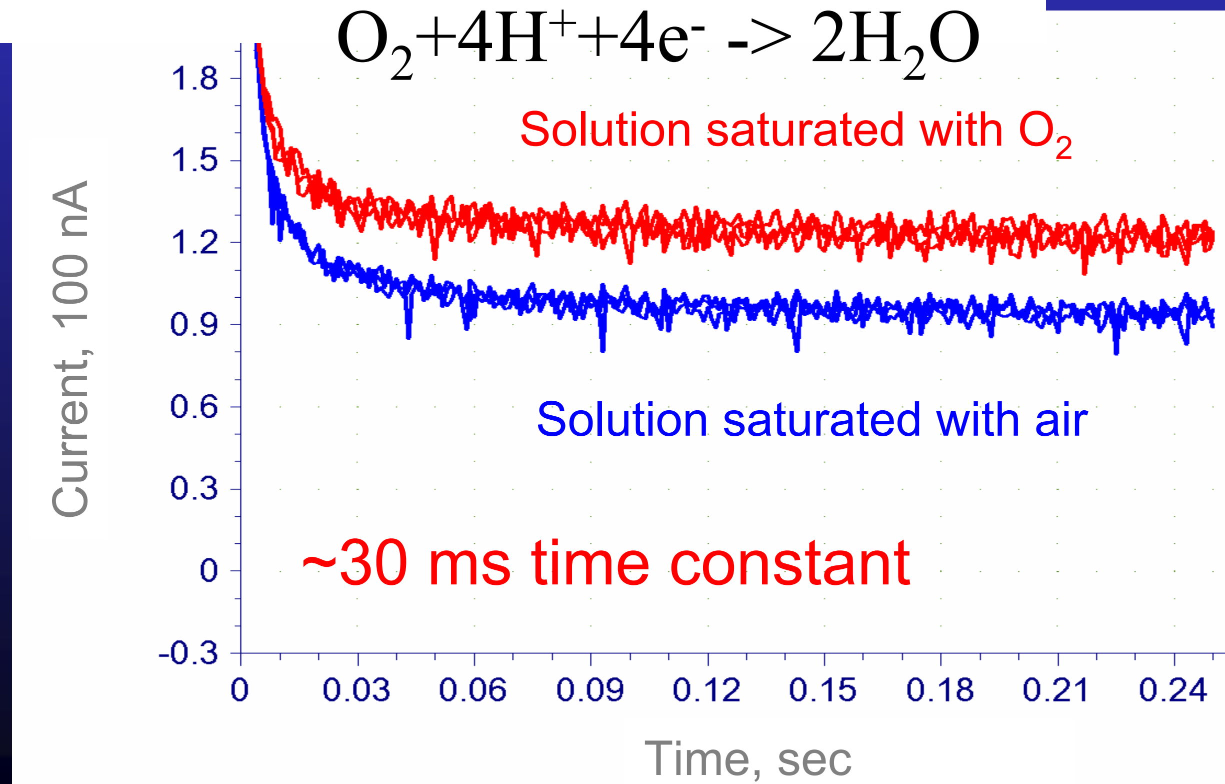


Electrochemical Detection

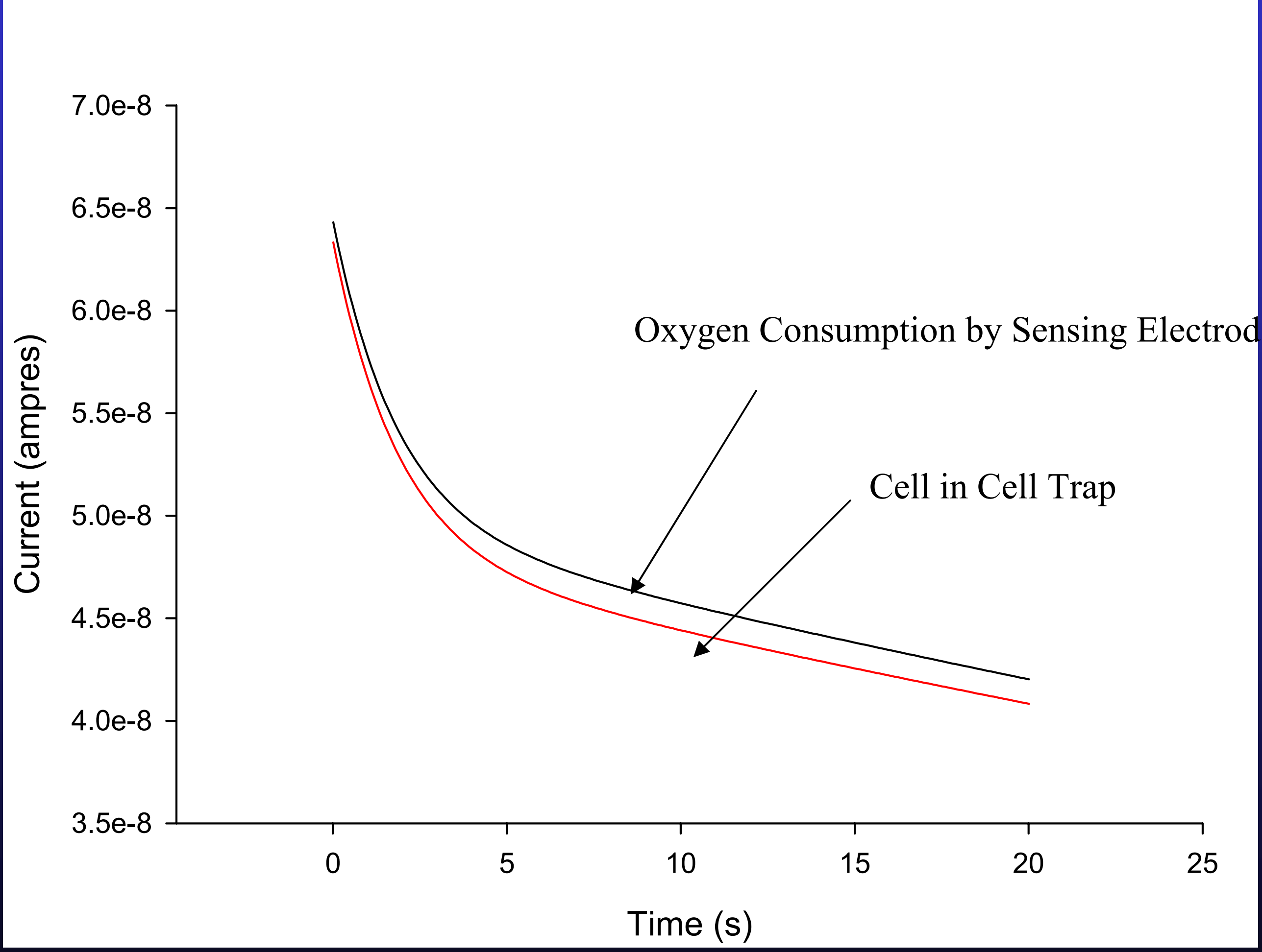


Chronoamperogram



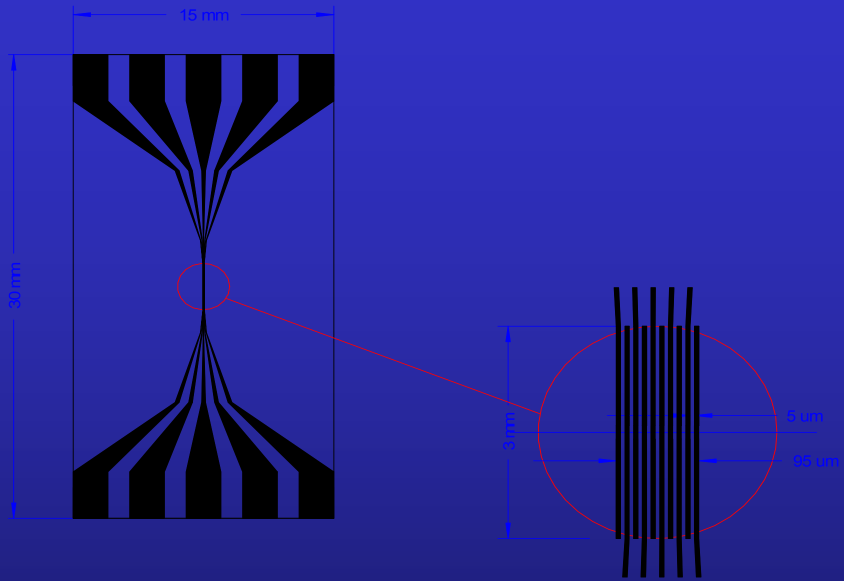


Oxygen Respiration of a Single Cell in the Cell Trap



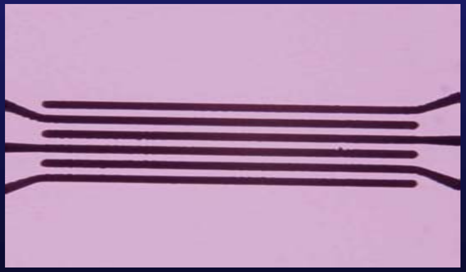
Courtesy R. Reisserer

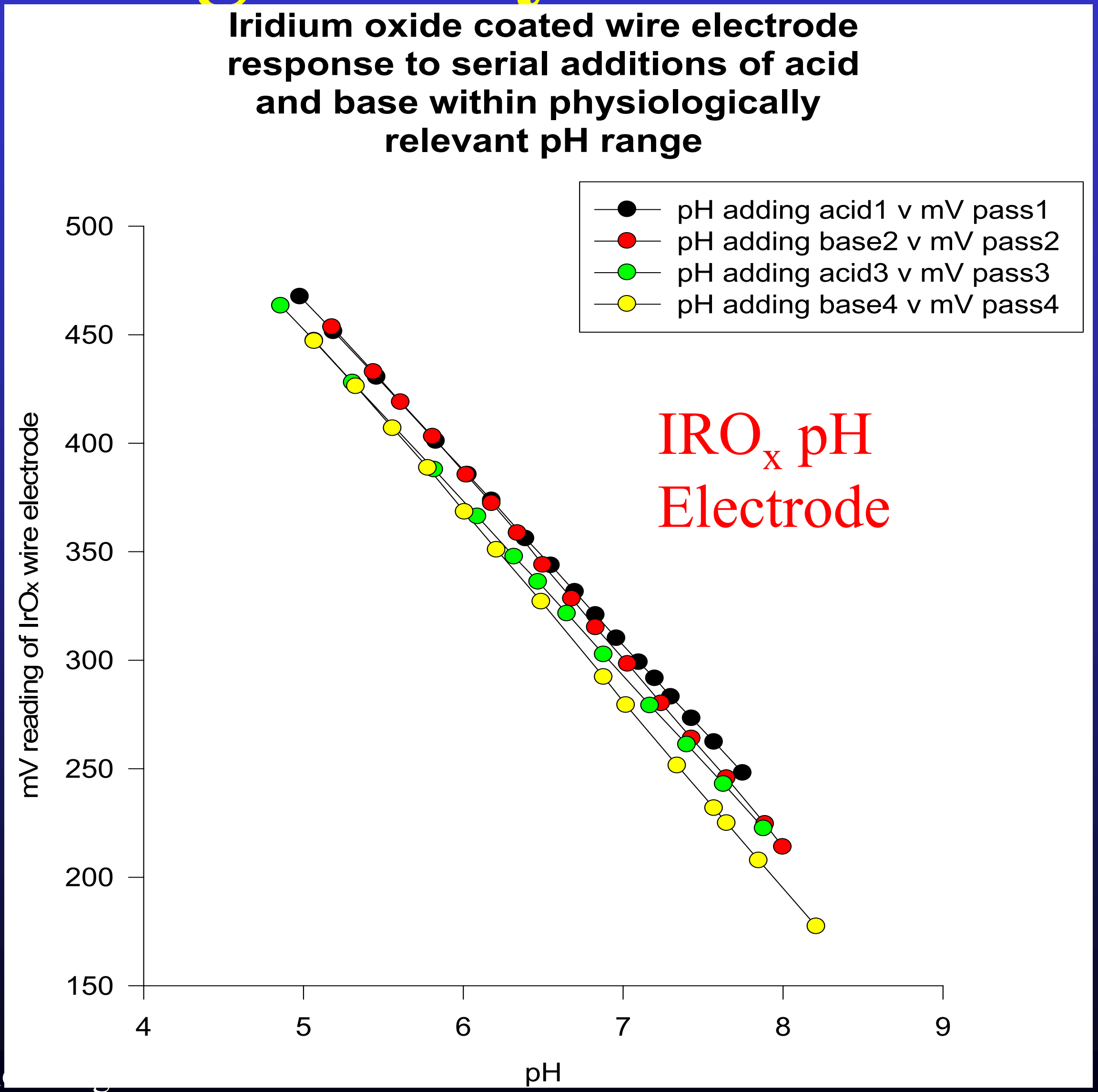
IDE Sensing Arrays



Individually addressable electrodes

D = 5 µm





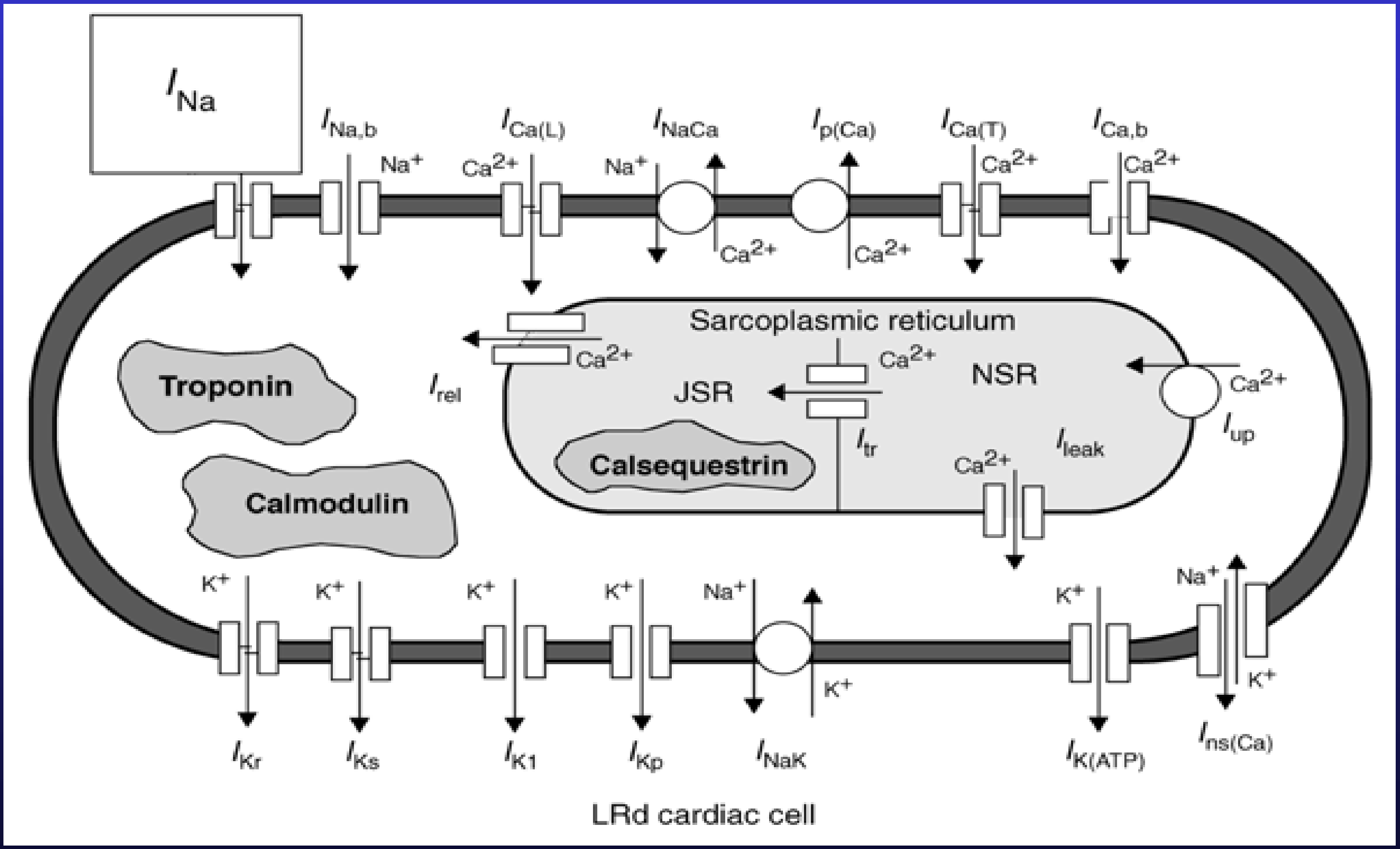
Courtesy J. Ges & J. Greene

Current Status - NanoPhysiometer

- Micro- and Nanosensors can be incorporated into small sensing volumes
- Single cell oxygen respiration in sub-nanoliter volumes demonstrated in NanoPhysiometer.
- High sensitivity: ~0.1-1 pmol of analyte detected electrochemically.
- Fast response time: 30 ms (a few ms possible)
- Arrays of NanoPhysiometers will operate in parallel.

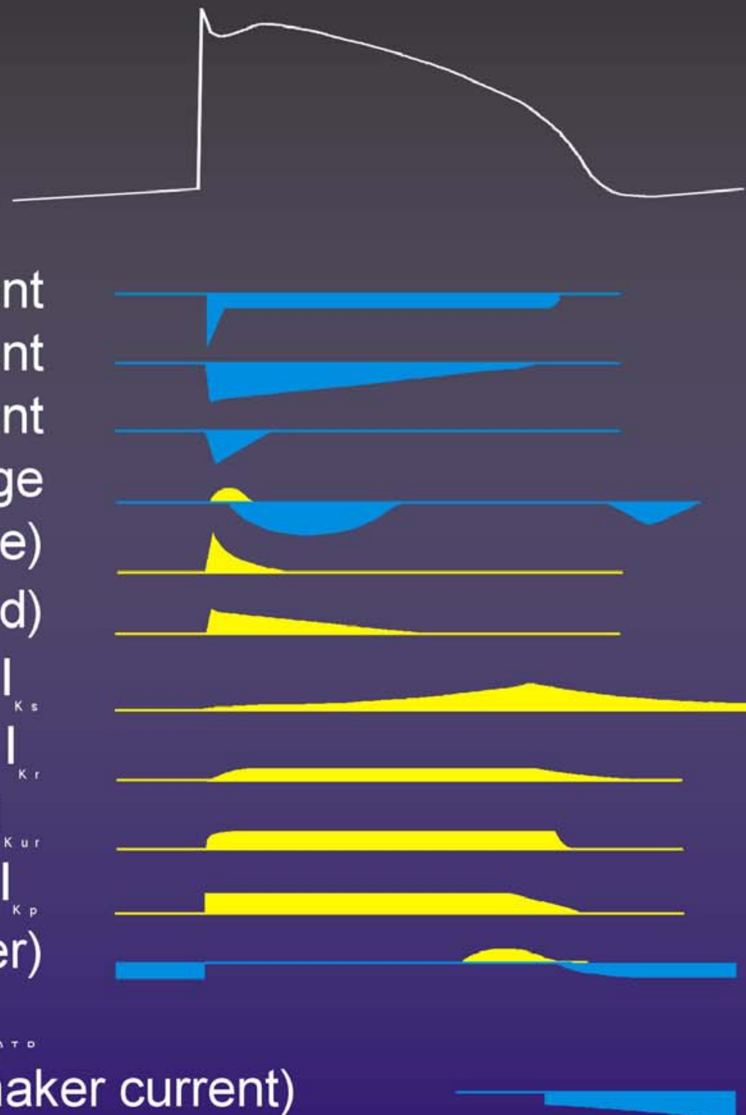
How are metabolic and signaling pathways coupled?

Cardiac Cell Model



Clancy, C. E. and Y. Rudy. Nature 400 (6744) 566-569, 1999.

Ion currents and ion channel clones

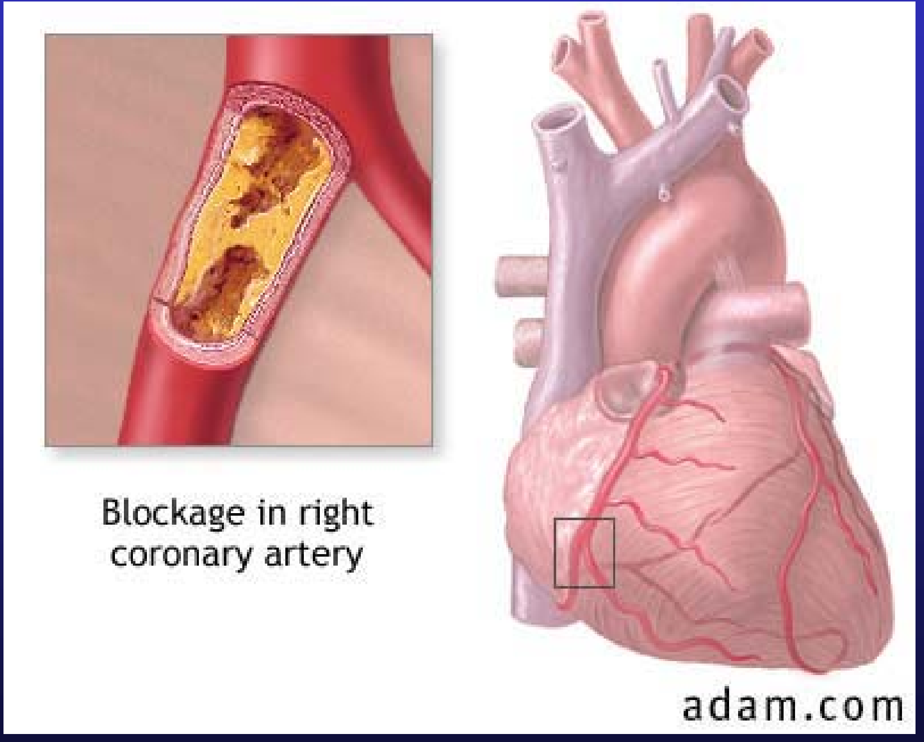


Current	Probable clone
sodium current	H1, SCN5A*
L-type calcium current	✓*
T-type calcium current	✓
Na-Ca exchange	Na-Ca exchanger
I_{TO1} (4-AP-sensitive)	Kv4.3 (?1.2, 1.4, 1.5, 2.1, 4.2)*?
I_{TO2} (Ca-activated)	--
I_{Ks}	KvLQT1 + minK (IsK)
I_{Kr}	HERG + MiRP1
I_{Kur}	Kv1.5
I_{Cl} or I_{Kp}	CFTR, TWIK (?others)
I_{K1} (inward rectifier)	Kir2.x
$I_{K\text{-}ACh}$; $I_{K\text{-}ATP}$	Kir3.1/3.4; Kir6.x/SUR
I_f (pacemaker current)	hCNG

*+sub-units

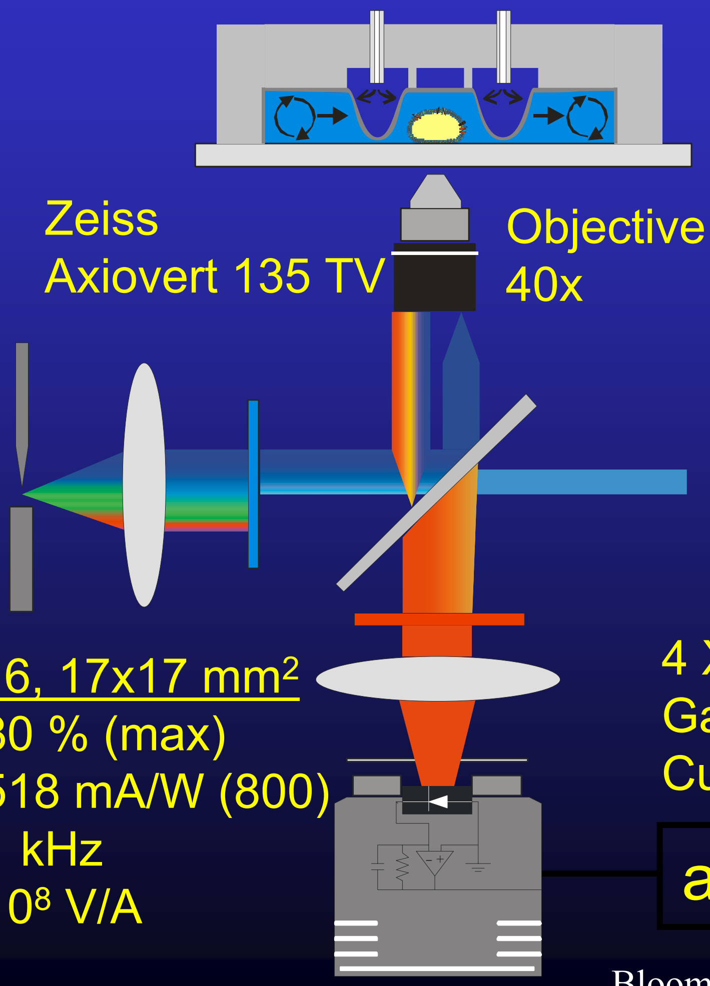
Courtesy of Dan Roden

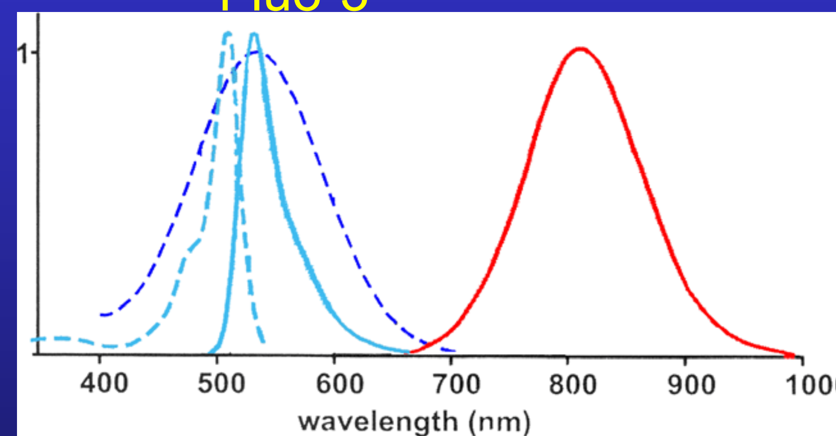
Myocardial Ischemia



- Manifestation
  - Anoxia: inhibits aerobic respiration and reduces intracellular ATP
  - Acidosis: pH 7.5 to 6.5, reduces Na, Ca conductivity by 25%, reduces upstroke velocity, Vrest reduction by 3-5 mV
  - Hyperkalemia: Reduces rest potential from 85 – 60 mV and causes a shortening of the Action Potential Duration

Combine NanoPhysiometers with fluorescence imaging!





PDA 16x16, 17x17 mm²

QE	80 % (max)
Res	518 mA/W (800)
SampR	1 kHz
Gain	10^8 V/A

4 X 64 ch amplifier
Gain 10, 50, 100
Cutoff 0.5 – 10 kHz

amplifier — ADC — PC LabView

Myocardial Ischemia

- NanoPhysiometers will allow us to measure the dynamics associated with anoxia in cardiac myocytes.
- Develop a model, which couples membrane potential to intracellular ATP level

Plans for the future

- High Resolution Imaging of Magnetic Fields
  - SQUID Microscopy
  - Information content and origin of biomagnetic fields in the brain for a future brain machine interface.
  - The integration of nanoscale magnetics with biology as a novel technique for the detection, manipulation, and functional control of single cells.
- Quantitative integrative physiology on the single cell level
  - Metabolism in cardica myocytes with a particular focus on ATP depended transport across cell membranes and compartmentalization
  - PicoCalorimeter to monitor phase transitions and heat generation of single cells

Acknowledgements

L. E. Fong, SQUID Microscopy
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R. Reisserer, NanoPhysiometer - BioSensor
A. Werdich, NanoPhysiometer – Cardiac Myocytes
B. Weiss, Rockmagnetism, CALTECH
J. P. Wikswo

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Instrumenting the Single Cell