Homer’s Physics

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Velocity mapping analysis: (a) adhesion modifying protein alpha-actinin labeled with GFP in a retracting protrusion from a CHO cell; (b) quantum dots in a migrating keratinocy cell (cell image courtesy of Dr. Julie Thomet Stanford University).

Resolving densities and molecular brightness via image histogram analysis (IHA).

Single Particle Tracking

Single particle tracking (SPT) of quantum dots attached to EGF receptors in an NIH 3T3 fibroblast cell.

Two-color CLSM images of cells on red fluorescent grids:
(a) CHO cells expressing 14-3-3-zeta antibody; (b) rat hippocampal neuron (17 DIV) transfected with GFP;
(c) CHO cells expressing 14-3-3-zeta/EGFP fusion constructs plated on a patterned substrate with a mixture of fibronectin and Alexa633 labeled human fibronectin; (d) pattern of poly-D-lysine/EGFP on a glass substrate; allowing red hippocampal neurons (immunostained for MAP2) to grow their neurites to specific positions.

Resolving transmembrane distribution of fluorescent particles via high order moment analysis

TIRF image of single Ce6-ZnS quantum dots immobilized on a glass substrate.

Colocalization studies by image cross correlation spectroscopy (ICCS).
Wiseman Chemistry Lab ~ 9AM
Wiseman Physics Lab...the fun stuff!

Confocal/2-Photon Microscope

2PF/SHG/THG Nonlinear Microscope

TIRF Microscopy System with EMCCD Detection

Also AFM/TIRF (With P. Grütter) Nonlinear optical Lithography Laser Tweezers
Biophysics in Cells and Neurons

Hippocampal neuron expressing Green Fluorescent Protein (GFP)
Courtesy Prof. Paul De Koninck Laval University

Chick Fibroblasts
The Brain…A highly Connected Organ

Made of Approximately $10^{12}$ (trillion) neuron cells

Each is connected to between 1000 to 10,000 other neurons

The connections between neuron cells are called synapses
How Big is a Trillion?

1 second
1 million seconds = $10^6$ s = 11.6 days
1 billion seconds = $10^9$ s = 31.7 years
1 trillion seconds = $10^{12}$ s = 31,700 years

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Size Scales…

- Units for different size scales
- We are familiar with km, m, cm, mm
- But what about the very small?

1000x smaller than a mm…cellular length scales

Micrometer = $\mu m = 10^{-6} m$   One millionth of a metre

1000x smaller than a micrometer…molecular length scales

nanometer = nm = $10^{-9} m$   One billionth of a metre

…But what do these units really mean?
….Let’s explore this….
Size Scales...

- Distance from McGill to Dorval Airport 20 km
- Let's assume that distance equals 2 metres

You are here!

Dorval Airport
Approximately 20 km drive
How big is a neuron?

- Assume the Distance from Here to Dorval Airport = 2 m
- Then the cell body would be 0.2 m = 20 cm wide
- But the axons for some neurons can reach 2 m!

Watermelon size scale

Distance to the airport

2 metres
How big is a dendritic spine?

Assume the Distance from Here to Dorval Airport = 2 m
Then the spine would be 0.01 m = 1 cm wide

Finger thickness is to the Distance to the airport
How big is a nanoparticle? Small!

- Assume the Distance from Here to Dorval Airport = 2 m
- Then the Nanoparticle would be 0.1 millimetre wide
- Same size scale as proteins and macromolecules

Quantum Dot Nanoparticle

![Diagram of a Nanoparticle with ZnS and CdSe]

Cell Membrane ~ 5 nm thick

Thickness of a strand of hair is to the Distance to the airport
Roadmap to a Neuron


- **Dendrites** (like tree branches)
- **Cell Body** (soma)
- **Axon**
- **Myelin Sheath**
- **Axon Terminal**
- **Nucleus**
- **Node of Ranvier**
- **Schwann cell**

**Signal Flow Direction**

1. Input from Other Neurons (via synapses)
2. Action Potential (Electrochemical Signal)
3. Output to Other Neurons Or Muscle cells (via synapses)
One of the early neuroscientists...

- Santiago Ramón y Cajal 1852-1934 Spanish
- Nobel Prize Physiology or Medicine 1906
The Brain…Highly Complex…

Mouse Cerebellum
Dr. Thomas Deerinck Prof. Mark Ellisman NCMIR, UCSD
The Nervous System…Interconnected

Mouse Retina
Glial cells = green
Retinal ganglion neurons = orange
Optic nerve fibers = red

Dr. Thomas Deerinck Prof. Mark Ellisman NCMIR, UCSD
A Neuron… the Basis of Brain Function

Hippocampal neuron expressing Green Fluorescent Protein (GFP)
Courtesy Prof. Paul De Koninck Laval University
Neurons and Glial Cells

100 x as Many Glial cells As Neurons

Highly Interconnected…they communicate

Hippocampal neurons (green) and glial cells (red)

Courtesy Prof. Paul De Koninck Laval University
Patch Clamp on a neuron...

Patch clamp of rat hippocampal neuron with lucifer yellow fluorescent dye
Courtesy Prof. Paul De Koninck Laval University
Neurons in Tissue Culture...

We are missing the dynamics of what is happening in time...

Dissociated culture of rat hippocampal neurons
Courtesy Prof. Paul De Koninck Laval University
The Brain is a Dynamic Organ
Dynamic Macromolecular Dance

Traditional Approach: Static Microscopic Snap Shots
Try to Understand the Story…The Rules of the Game
Could you Fully Understand Hockey from Pictures?
Snap Shots of the “Game”

What is the meaning…the sequence?
Snap Shots of the “Game”

What are the Key Events in the Game?
Snap Shots of the “Game”

What about capturing Rare or Significant Events?
Snap Shots of the “Game”

Some Events Defy Explanation!
Hockey is Dynamic!
Easier to Figure Out if We Can Watch it Unfold…
In real time

Unravel the Rules of a Dynamic Game…Space & Time Scales

Paxillin-dsRed (red) & α-actinin GFP (green) in CHO Cell
TIRF Microscopy
Total time = 50 min δt = 15 s
The Brain is a dynamic organ
Calcium signalling in Astrocytes...glial cells
Prof. Steve Smith Stanford University
Myelination of an Axon

Schwann cell was transfected with Actin-EGFP (in green) cocultured with DRG neurons 20 hour time loop

Courtesy of Prof. Dave Colman, Dr. Weisong Shan
Montreal Neurological Institute
CAM Kinase II transported into and out of spines

Collaboration with Dr. Paul DeKoninck
Laval University

Rat Neuron with CAM Kinase II

CAM Kinase II: Neuronal Enzyme

~12 nm diameter
The Challenging Arena of the Cell

Cells are Small…Biomolecules are Smaller!

Light Microscopy has Resolution limits ~ 200 nm

Cells & Tissues are Highly Scattering for Photons

Like Seeing Through Milk
Light Microscopy

- Goal...Measure the microscopic world by forming a magnified image of an object
- One of the oldest scientific instruments

The Far Side by Gary Larson
Cajal...with his microscopes
Cajal...Dendritic Spines
Modern Light Microscopy

- Fluorescence Microscopy
- Shine Light on Sample
- Get Fluorescence Emission

Confocal Laser Scanning Microscopy
Modern Light Microscopy

- Fluorescence Microscopy
- Optical Microscopy

Confocal Laser Scanning Microscopy
Multimodal Nonlinear Microscope

Home built Microscope Jon Belisle (Wiseman Group McGill)
Optical Sectioning

Optical sectioning a hard boiled egg
3D imaging...
2D images in height

Collagen (Red)
Elastin (Green)
Optical Sections in z (~1mm)

Human Heart tissue

10 µm
Fluorescence Microscopy

Fluorescence...Shine light on a fluorescent molecule
It will give off light of a different colour...It glows.

Fluorescent Shirts under Ultraviolet Light
Microscopy Imaging of Fluorescence

We Need to “label” proteins of interest

Attach fluorescent probe so we can image it

Hippocampal neuron expressing Green Fluorescent Protein (GFP)
Courtesy Prof. Paul De Koninck Laval University

Fluorescent Label on Protein…Genetically encoded Green Fluorescent Protein (in Jellyfish *Aequorea victoria*)!
(or other colours like blue, cyan, yellow)

GFP ~ 4 x 3 nm

Shine Laser on it…It Glows!
Quantum Dots...Nanoparticles

Different sizes...Different Fluorescence Emission Colour

Small QD...
blue emission

Larger QD...
red emission
Fluctuation Magnitudes & Fluctuation Times

Fluorescence Correlation Spectroscopy (FCS)


Fig. 1 Overview of Fluctuation Spectroscopy
Diffraction Limited Optical Resolution…

Optical Microscopy
Dynamics at the Price of Spatial Resolution

Truly Interacting Species
Dance Partners

Optical Resolution $\sim \lambda/2$ Macromolecules $\sim \lambda/50$

$\lambda = \text{wavelength}$

$\lambda = \text{Wavelength} \sim \text{colour of the light}$
Fluctuation Magnitudes & Fluctuation Times
Define Observation Volume with Beam Focus
Fluctuation Magnitudes

Occupation Number Fluctuations Scale Inversely with $\langle N \rangle$

Measure the fluctuations by fluorescence intensity
Vector Maps of Protein Transport in Cells

TIRF Microscopy Time 100 s with Images sampled at 0.1 Hz
Dr. Claire Brown and Ben Hebert
Space-Time Correlation of Fluctuations

$\alpha$-actinin/EGFP in MEF Cell

TIRF Microscopy

$r(\xi, \eta, \tau)$
Immobile
Filtered

3.4 µm

Accelerated
40 times faster than
Real-time

$\tau = 0 \text{ s} \rightarrow 200 \text{ s}$
Vector Maps of Protein Transport in Cells

Faculty of 1000 Selection Jan 2007
Roadmap to a Neuron


- Dendrites (like tree branches)
- Cell Body (soma)
- Axon
- Myelin Sheath
- Axon Terminal
- Nucleus
- Node of Ranvier
- Schwann cell

Signal Flow Direction

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Signal Flow Direction:

Input from Other Neurons (via synapses) → Action Potential (Electrochemical Signal) → Output to Other Neurons or Muscle cells (via synapses)
Dendritic Spines

Dendritic spines of rat hippocampal neurons
Courtesy Prof. Paul De Koninck Laval University
The Synapse...Action Potential ends becomes chemical signal

Axon Terminus
Presynaptic Neuron 1

Vesicle (40 nm size “bag”)
Containing
Neurotransmitter
Chemicals

Synapse (just a Gap)

Dendritic Spine
Postsynaptic
Neuron 2

http://www.univ-orleans.fr/neurobiologie/
ENGLISH/images_recherche.htm
3D Reconstruction of Spines...
Pruning of Synaptic Connections

Young Rat 2 months
Young adult

Mature Rat 32 months
= 80 years old

More Spines
Fewer Spines

Courtesy of Prof. Yves De Koninck, CRULRG Laval Univ.
Synaptogenesis... New Connections

Once again...
Santiago Ramón y Cajal
Was the pioneer...
Dendritic Spines are Dynamic!

CA1 pyramidal cell  GFP
3 week old hippocampal tissue culture

Courtesy of Prof. Anne McKinney McGill Univ. Pharmacology

Membrane-targeted GFP spines (postsynaptic)

FM 4-64-labeled bouton (presynaptic)

15 min

Synaptogenesis... Formation of New Synapses

The Brain and Nervous System are “plastic”

New connections being made

Old ones connections disassembled

Rewiring of the Brain...
Neuroplasticity

Mounting Evidence that using the brain is Important in keeping function...
“Use it or lose it!”
Sometimes it wasn’t meant to be!

Calcium signalling in Astrocytes
Prof. Steve Smith Stanford University

Synaptogenesis...New Synapses

Receptors for neurotransmitters Redistribute into spines...

They are dynamic...
How do they move?
Track receptors with Nanotechnology

- Article by Alivisatos on Quantum Dots
- Very small particles made out of semiconductor materials
Quantum Dots...Nanoparticles

Cadmium selenide/Zinc sulfide Quantum Dots in solution

Green Fluorescence

Under Room Light

Under ultraviolet Light
Quantum Dot Marker

Attach QD to Protein in Cell Membrane

Video Microscopy
Single Particle Tracking

Track Trajectories of QD Protein Complexes In Time

Like Observing Skiers with Torches at Night
Tracking Protein Receptor Motions
With Quantum Dots

Green = Synapse Marker

Red = QD Glutamate Receptor in the Dendrite of Living neuron

Wiseman Group McGill with Prof. Paul DeKoninck Laval University
Tracking Protein Receptor Motions With Quantum Dots

Rat Purkinje neuron

Richard Naud (Wiseman Group) with Prof. Paul DeKoninck Laval University
Interdisciplinary Science...

http://www.neurophysics.ca/
Laval and McGill Universities

"Setting new frontiers in neuroscience" –
"Repousser les frontières des neurosciences"

CIHR Training Grant
Training Physical Scientists
To Tackle Problems in Neuroscience
Interdisciplinary Science... 

Training Future Scientists Within Canada to Tackle such complex Problems
Imaging Paul Wiseman’s Brain...
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The Brain is a dynamic organ…
We are only beginning to scratch the surface…

So much still to learn!
Lifelong learning!
Special Thanks to the Group!
Those who really do the work!