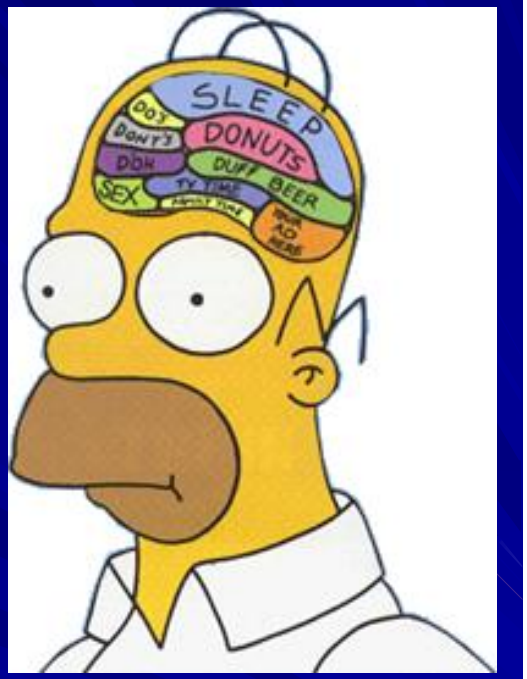
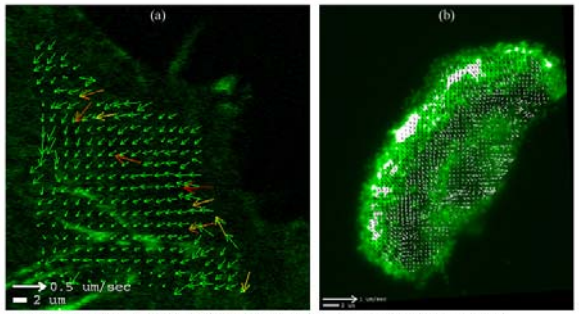
Homer's Physics



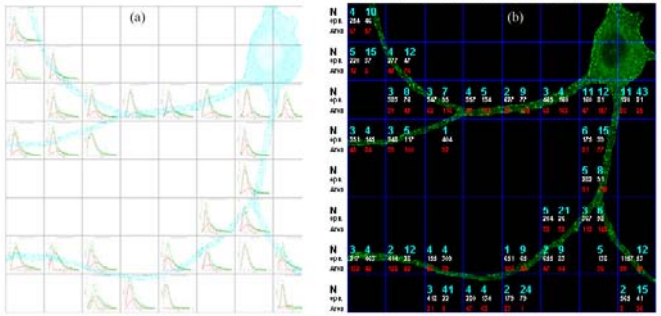
Paul W. Wiseman
McGill University
Dept. of Physics
Dept. of Chemistry
Montreal, Canada

McGill

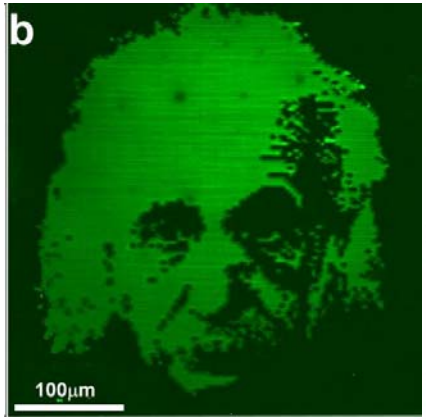
Wiseman Lab Biophysical Research

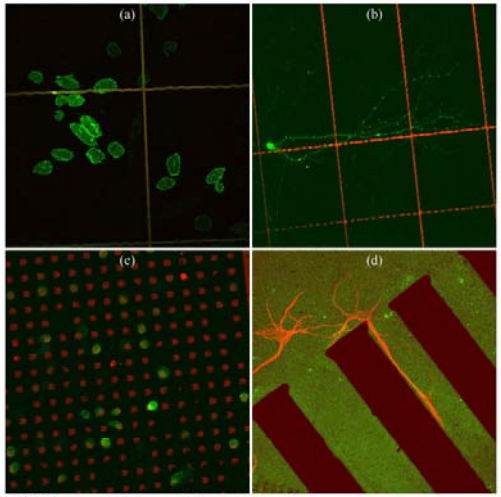


Velocity mapping analysis: (a) adhesion mediating protein alpha-actinin labeled with GFP in a retracting protrusion from a CHO cell; (b) quantum dots in a migrating keratocyte cell (cell movie courtesy of Dr. Julie Theriot Stanford University)

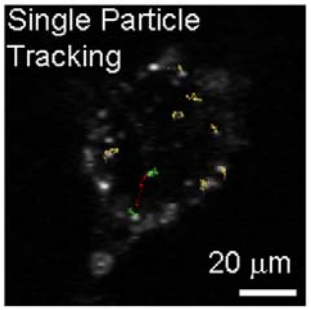


Resolving densities and molecular brightness via image histogram analysis (IHA)

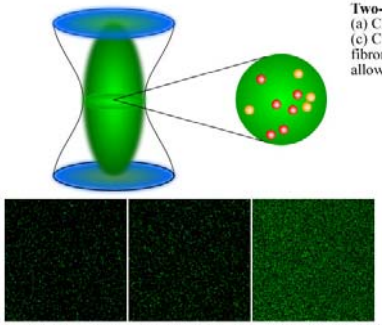




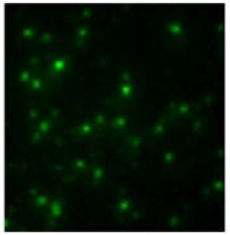
Two-color CLSM images of cells on red fluorescent grids:
(a) CHO cells expressing EGFP/α5-integrin; (b) rat hippocampal neuron (12 DIV) transfected with GFP; (c) CHO cells expressing α5-integrin/EGFP fusion constructs plated on a patterned substrate with a mixture of fibronectin and Alexa633 labeled human fibrinogen; (d) pattern of poly-D-lysine/EGFP on a glass substrate, allowing rat hippocampal neurons (immunostained for MAP2) to grow their neurites in specific corridors.



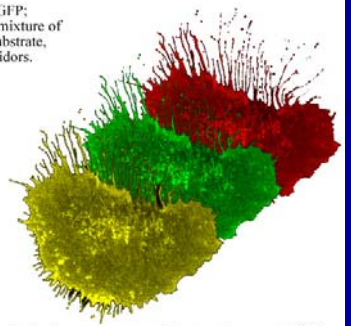
Single particle tracking (SPT) of quantum dots attached to EGF receptors in an NIH 3T3 fibroblast cell



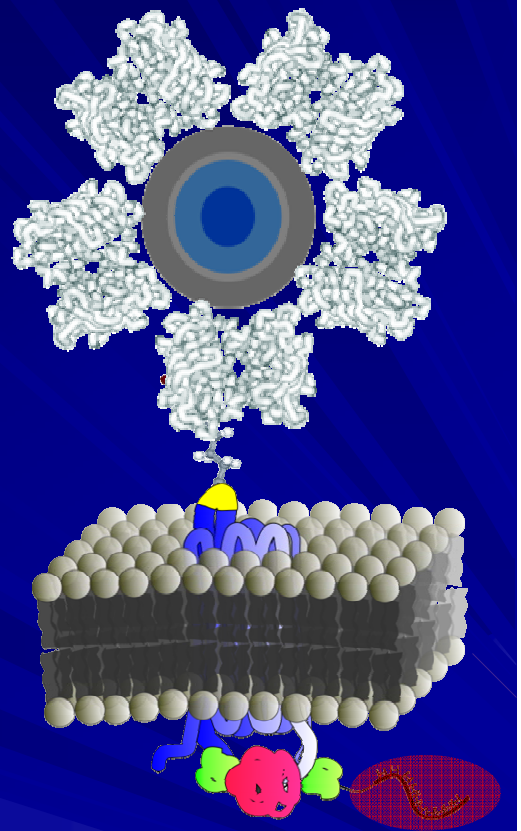
Resolving monomer-dimer distributions of fluorescent particles via high order moment analysis



TIRF image of single CsSe/ZnS quantum dots imobilized on a glass substrate.

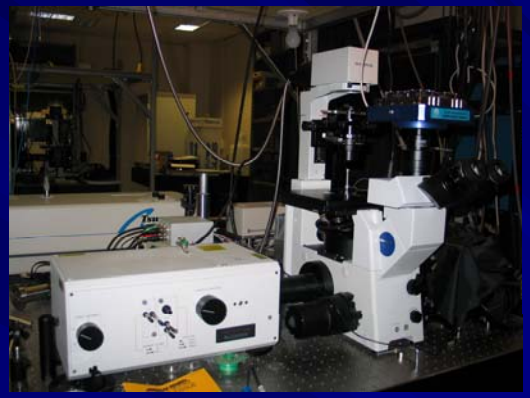


Colocalization studies by image cross correlation spectroscopy(ICCS)



Wiseman Chemistry Lab ~ 9AM

Wiseman Physics Lab…the fun stuff!

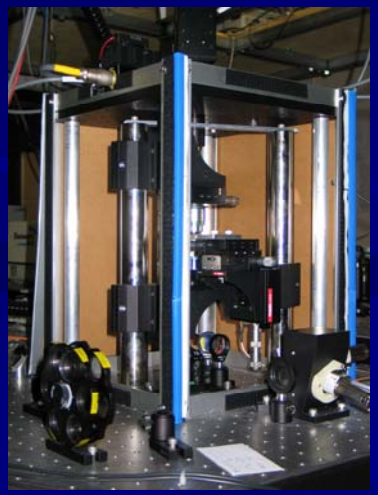


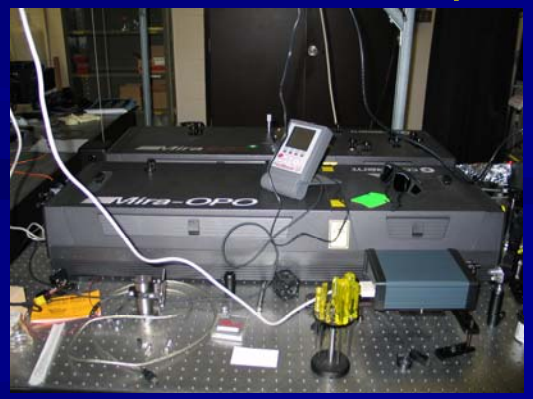
Confocal/2-Photon Microscope

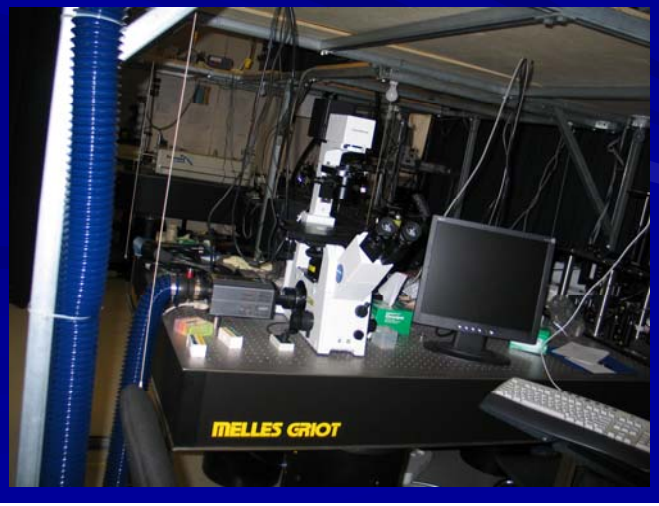
Also AFM/TIRF
(With P. Grütter)
Nonlinear optical Lithography
Laser Tweezers

TIRF Microscopy System with EMCCD Detection

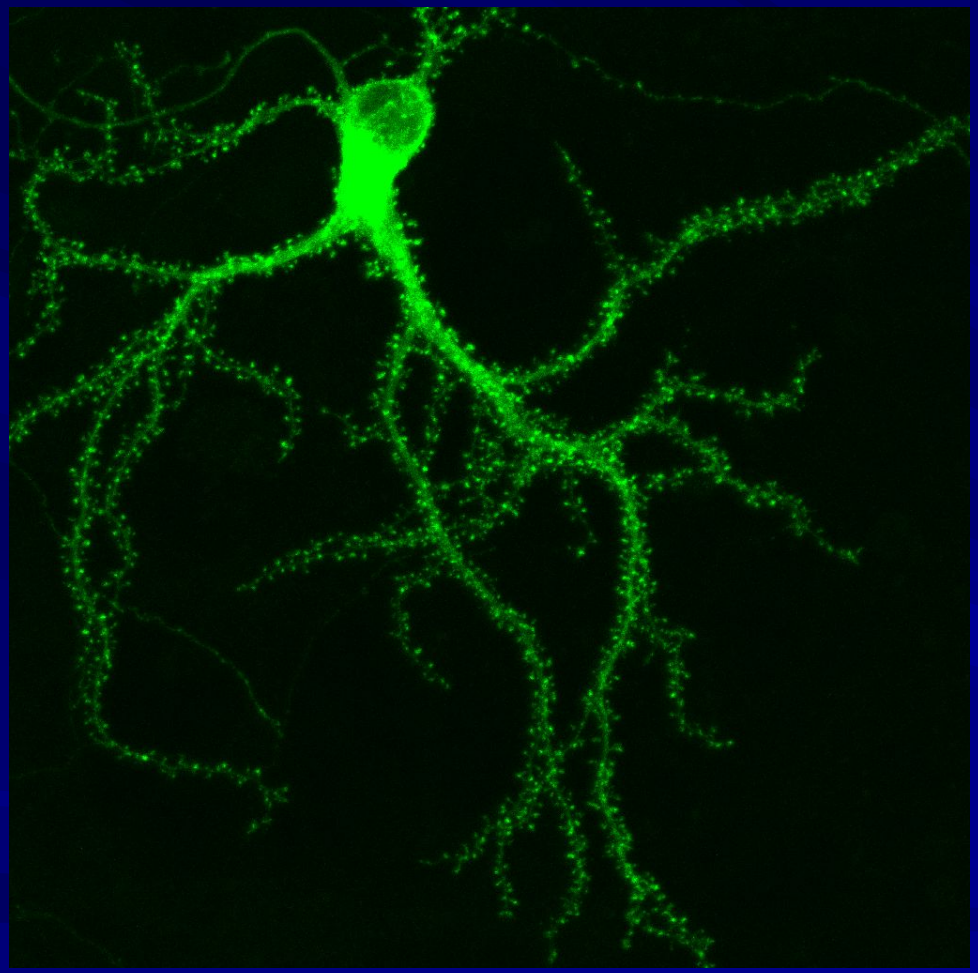
2PF/SHG/THG Nonlinear Microscope



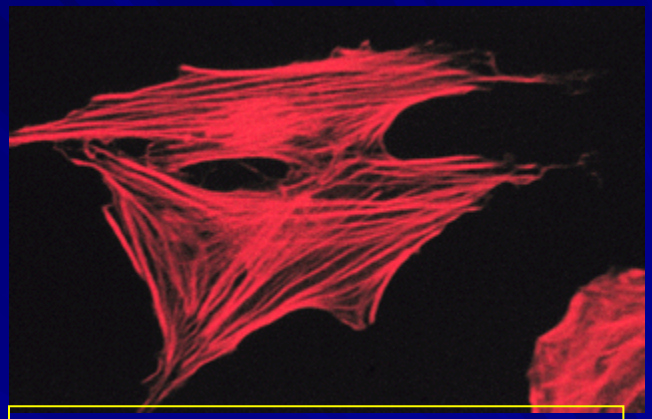




Biophysics in Cells and Neurons



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



Chick Fibroblasts

The Brain

The Brain…A highly Connected Organ

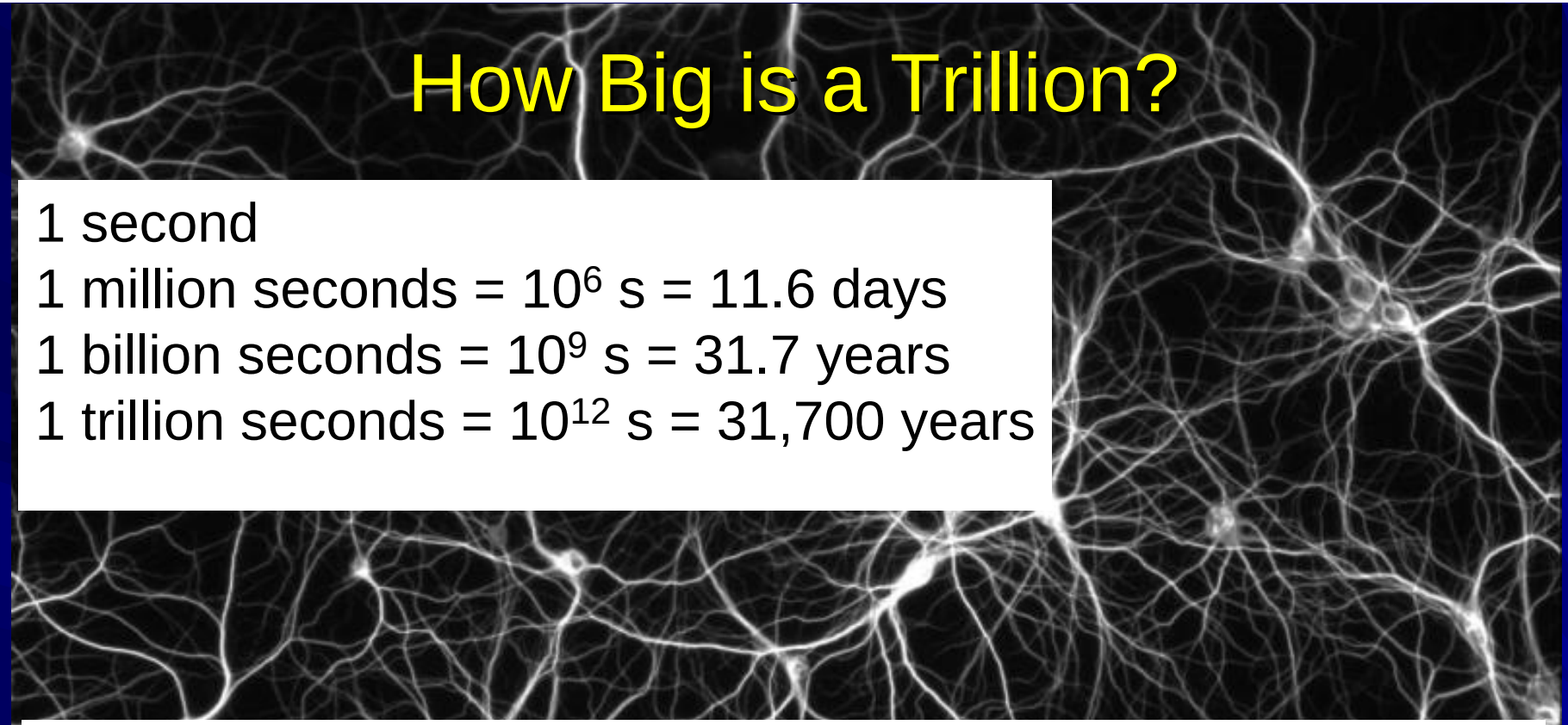
Made of Approximately 10^{12} (trillion) neuron cells

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



The Brain…A highly Connected Organ

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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 = μm = 10^{-6} m One millionth of a metre

1000x smaller than a micrometer…molecular length scales

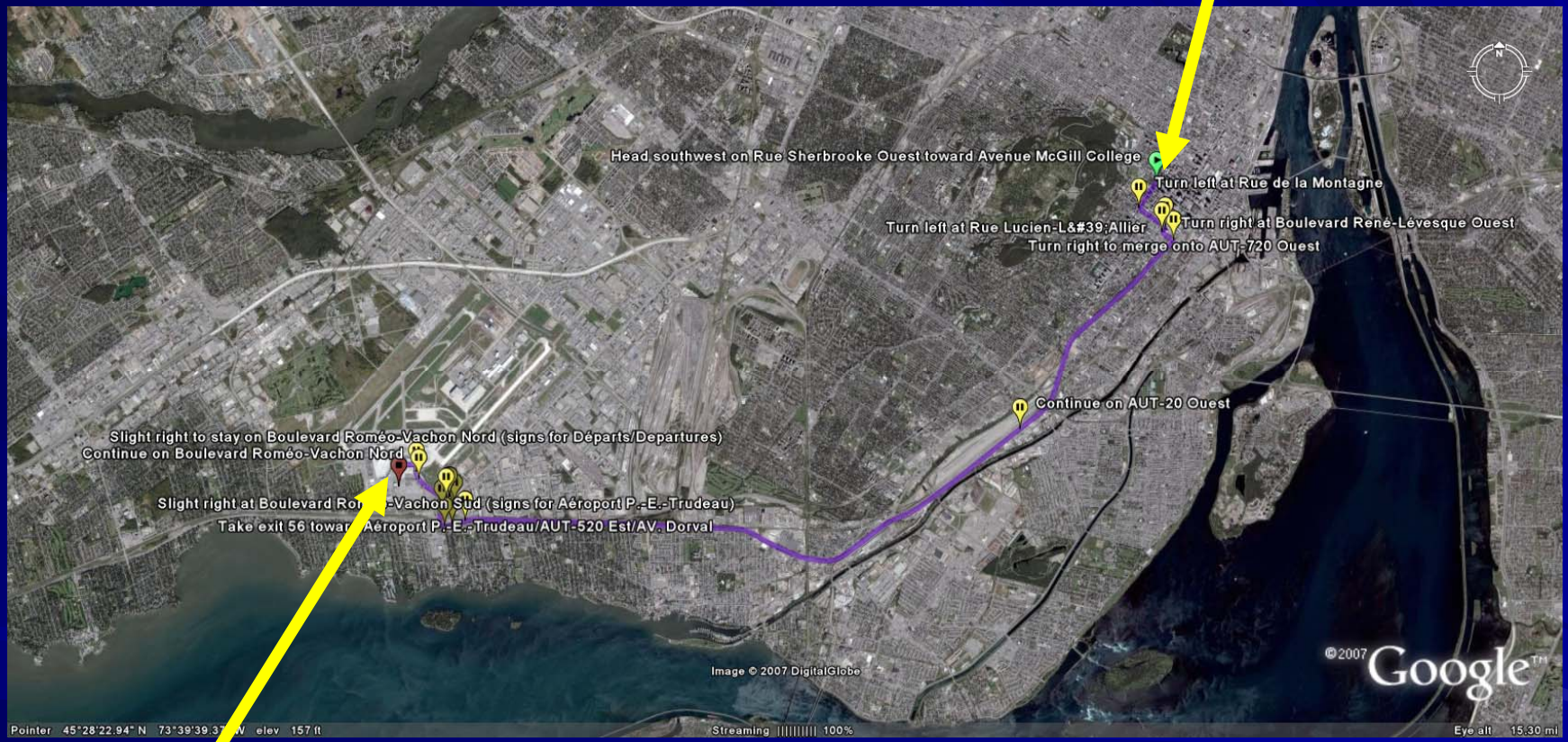
nanometer = nm = 10^{-9} m One billionth of a metre

…But what do these units really mean?
….Let's explore this…

Size Scales…

- Distance from McGill to Dorval Airport 20 km
- Lets Assume that distance equals 2 metres

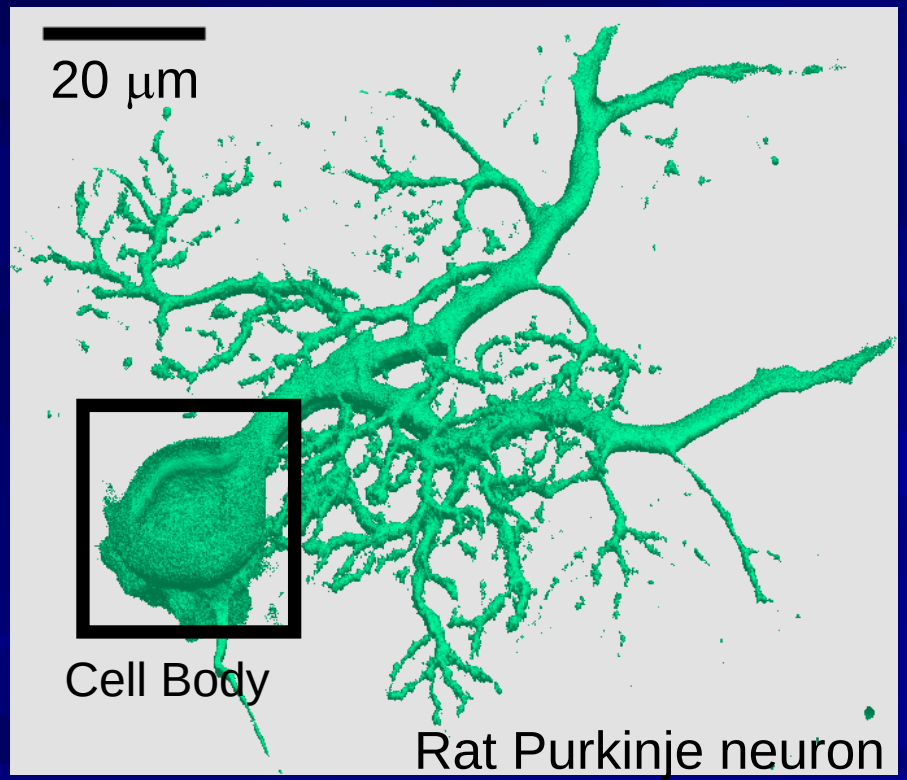
You are here!



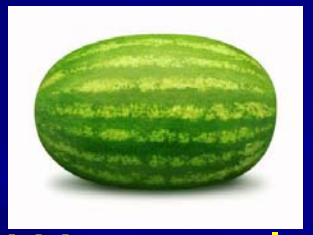
Dorval Airport
Approximately 20 km drive

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!



2 metres

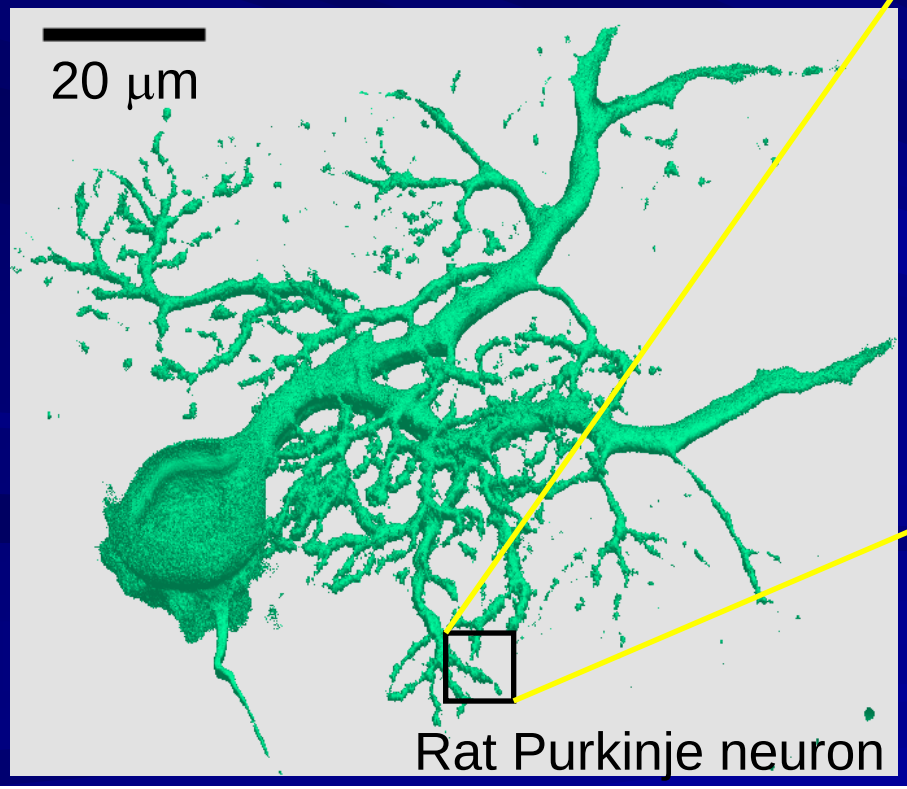


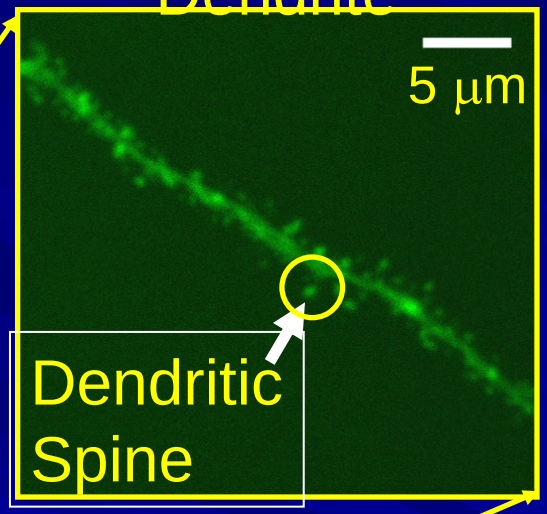
Watermelon size scale

Distance to the airport

How big is a dendritic spine?

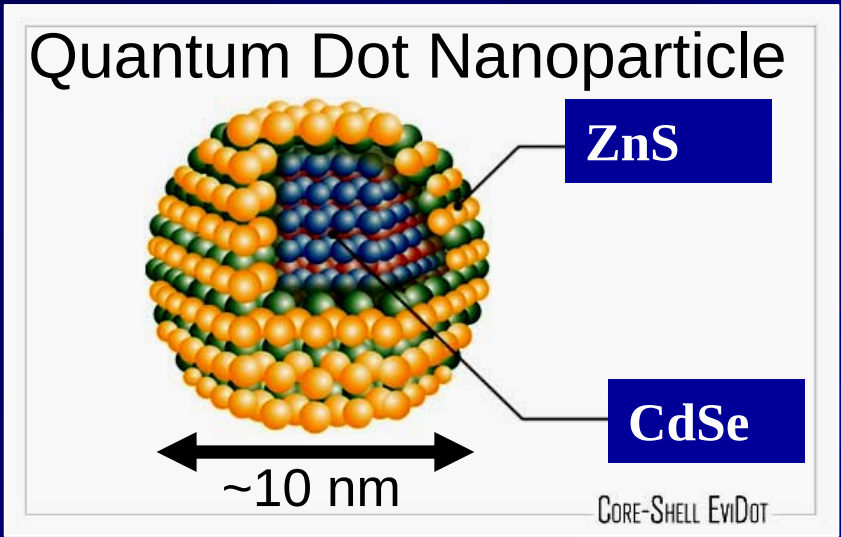
- Assume the Distance from Here to Dorval Airport = 2 m
- Then the spine would be 0.01 m = 1 cm wide

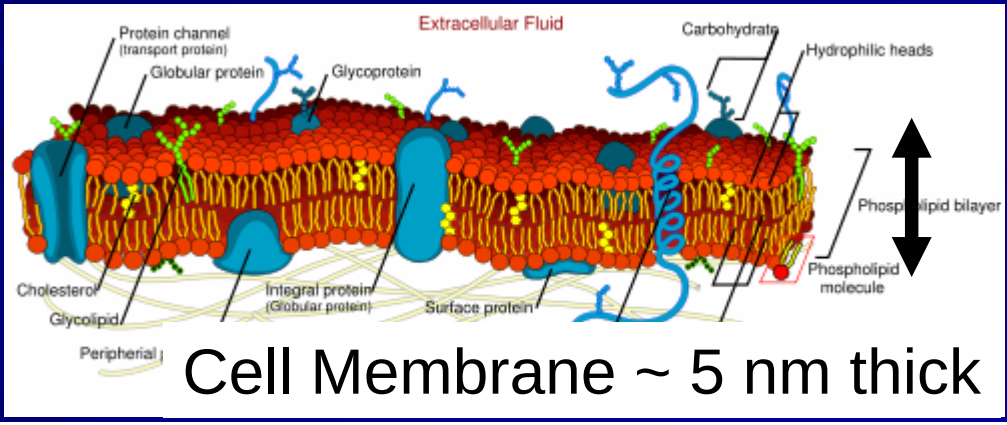




How big is a nanoparticle? Small!

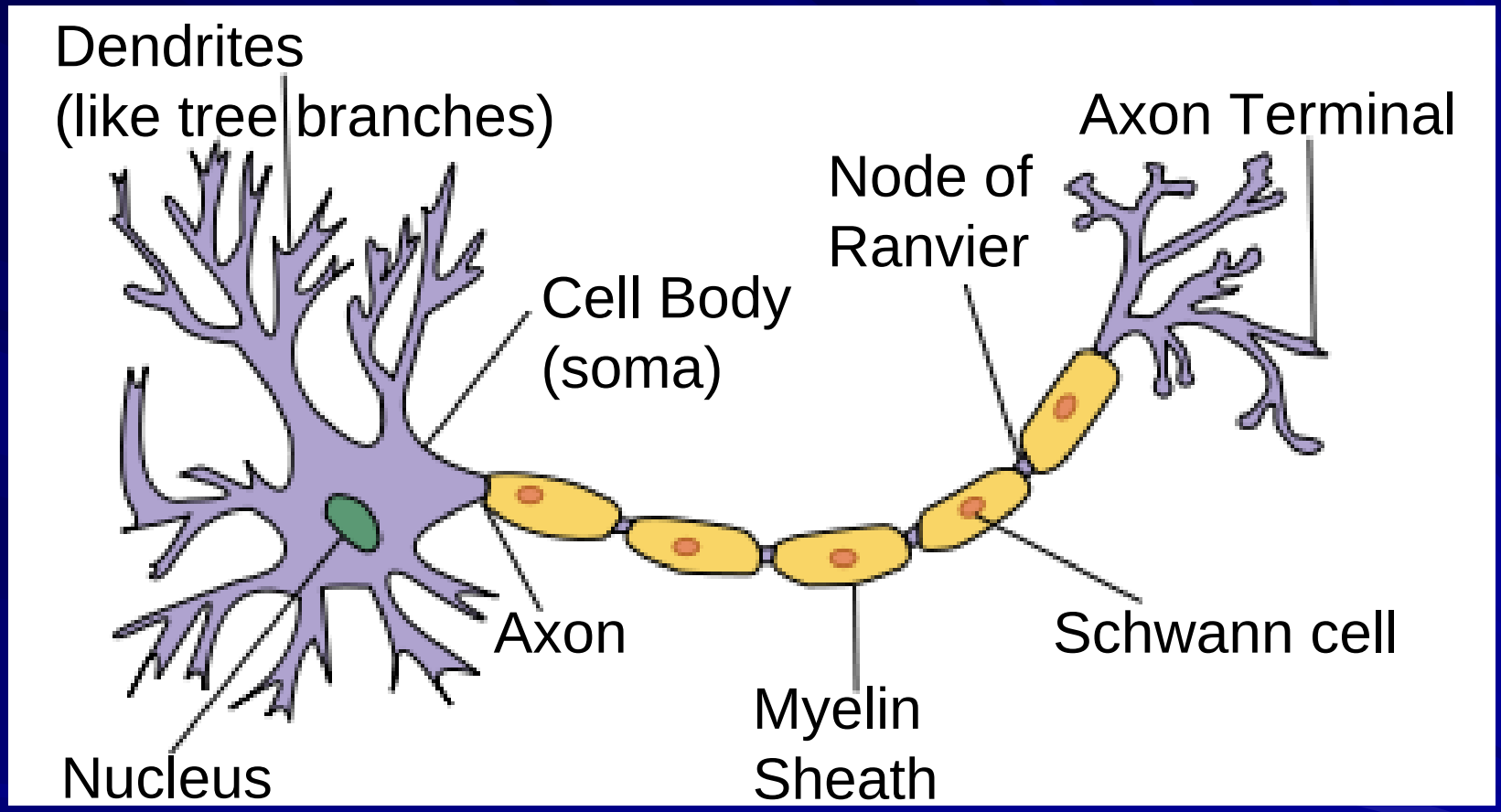
- Assume the Distance from Here to Dorval Airport = 2 m
- Then the Nanoparticle would be 0.1 millimetre wide
- Same size scale as proteins and macromolecules





Roadmap to a Neuron

Basic Reference: http://en.wikipedia.org/wiki/Neuron

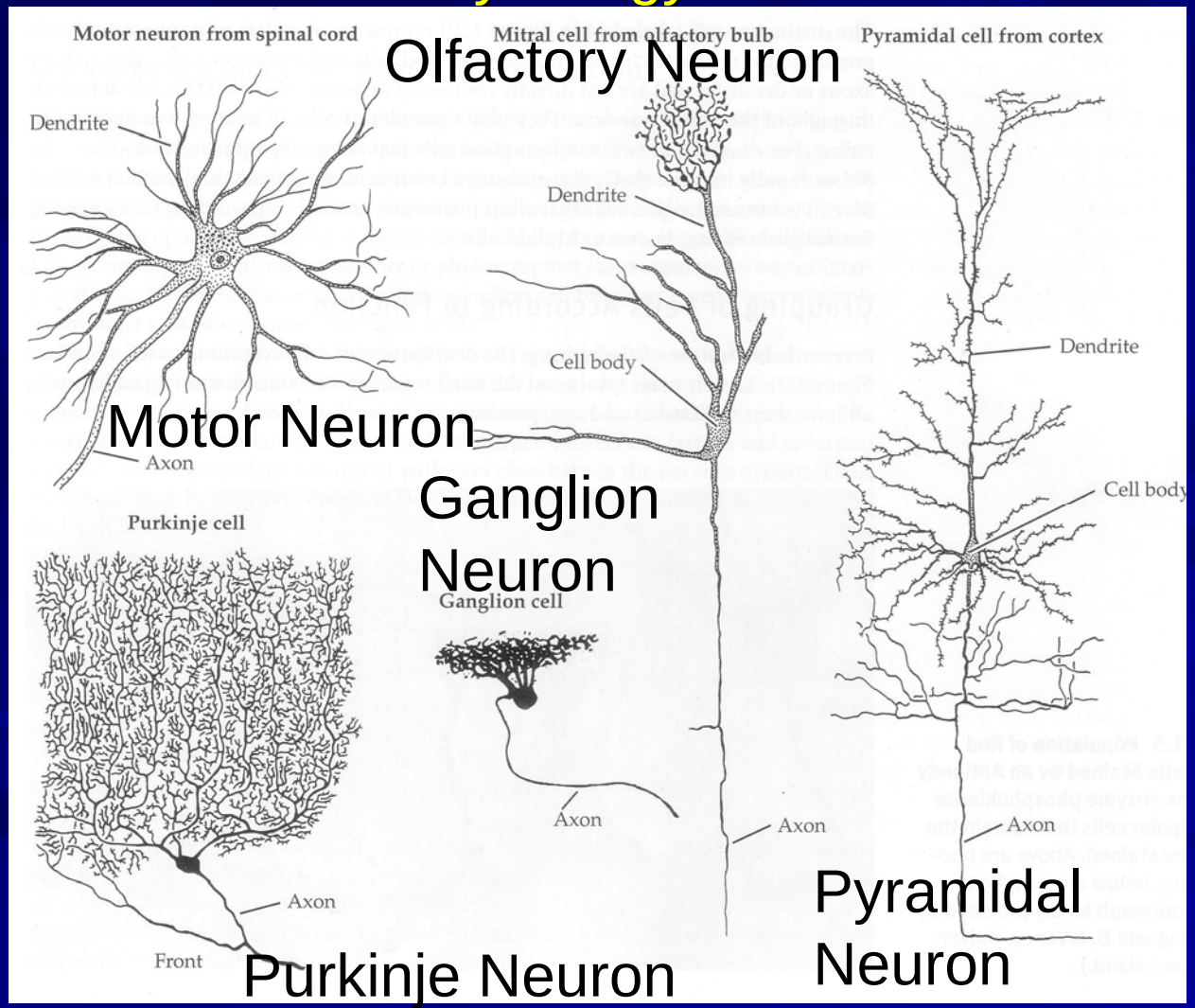


Signal Flow Direction →

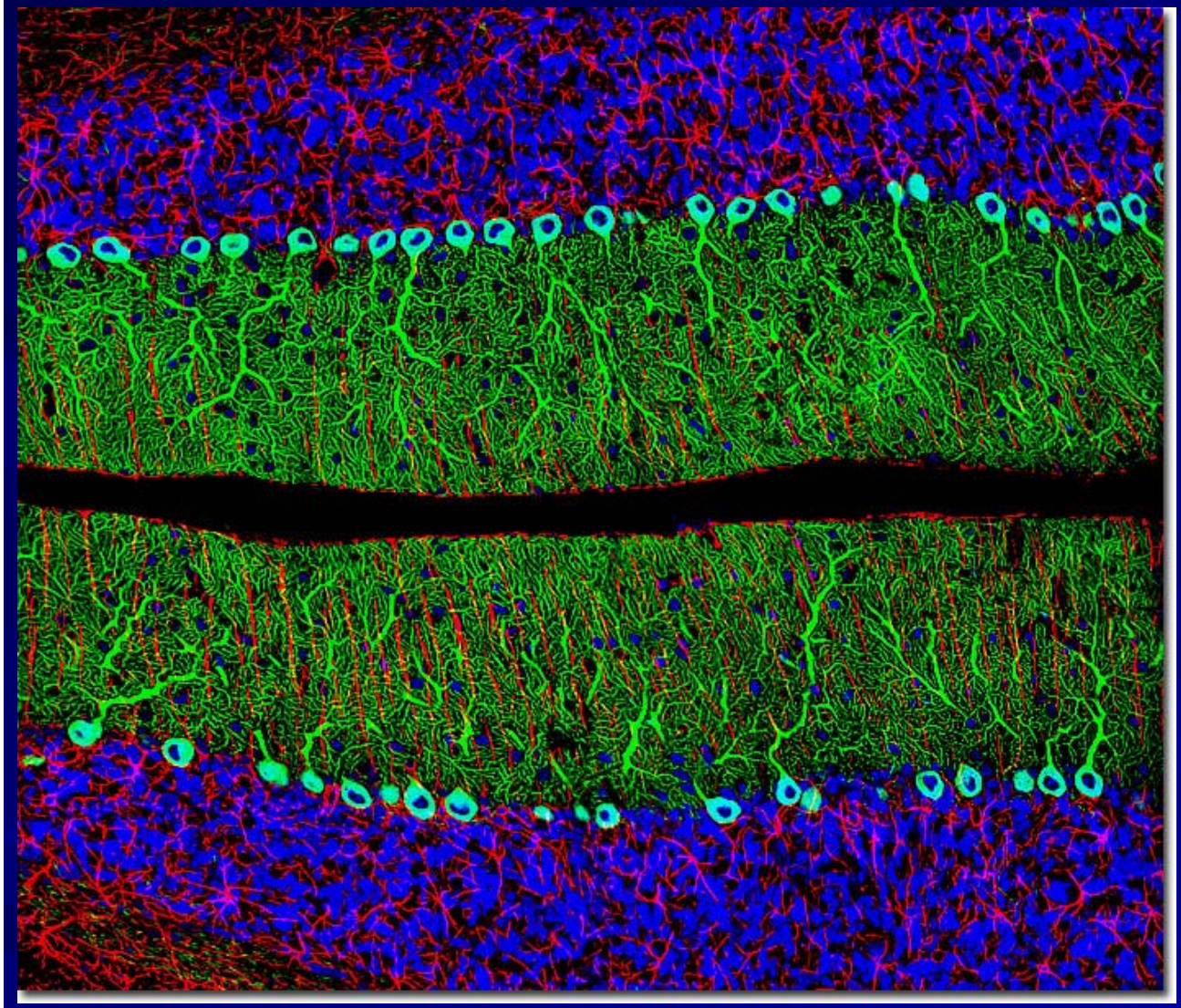
Input from Other Neurons (via synapses) → Action Potential (Electrochemical Signal) → Output to Other Neurons Or Muscle cells (via synapses)

One of the early neuroscientists…

- Santiago Ramón y Cajal 1852-1934 Spanish
- Nobel Prize Physiology or Medicine 1906

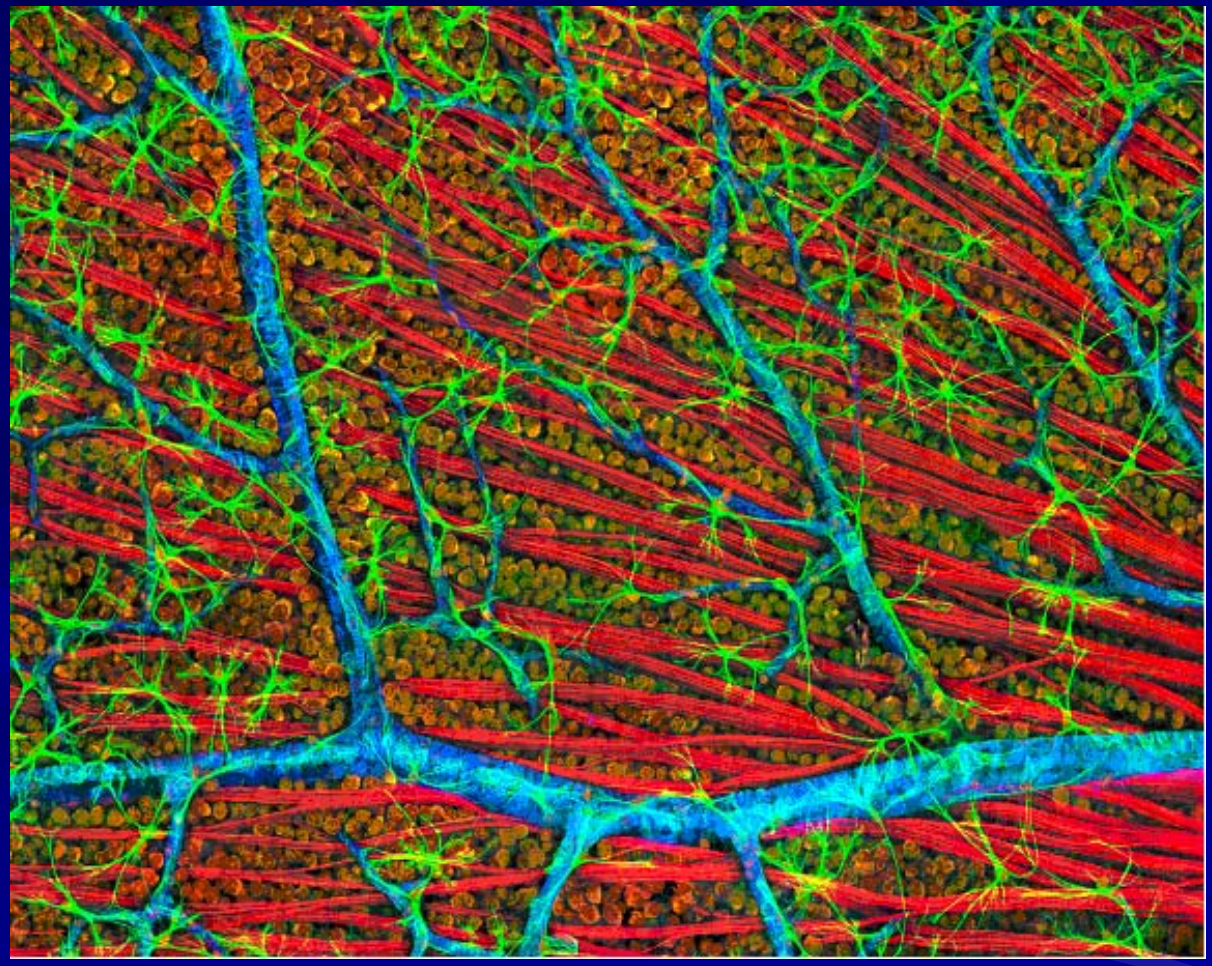


The Brain…Highly Complex…



Mouse Cerebellum
Dr. Thomas Deerinck Prof. Mark Ellisman NCMIR, UCSD

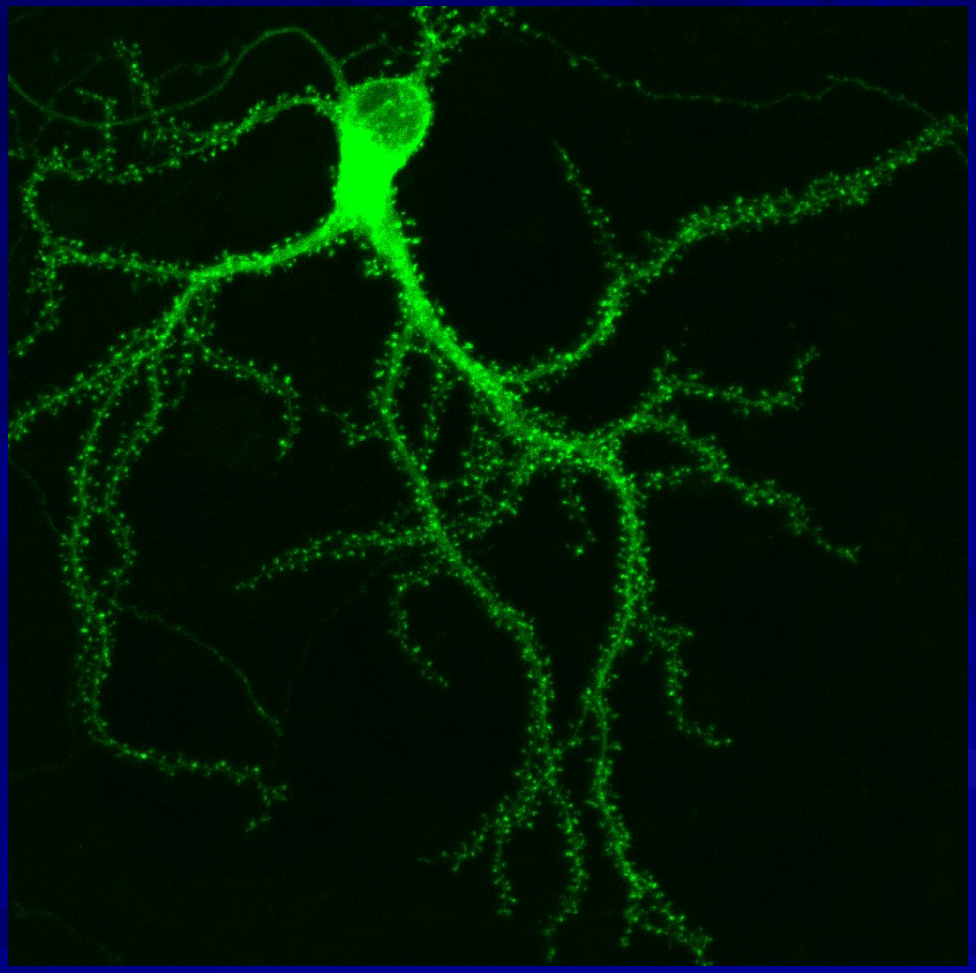
The Nervous System…Interconnected



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



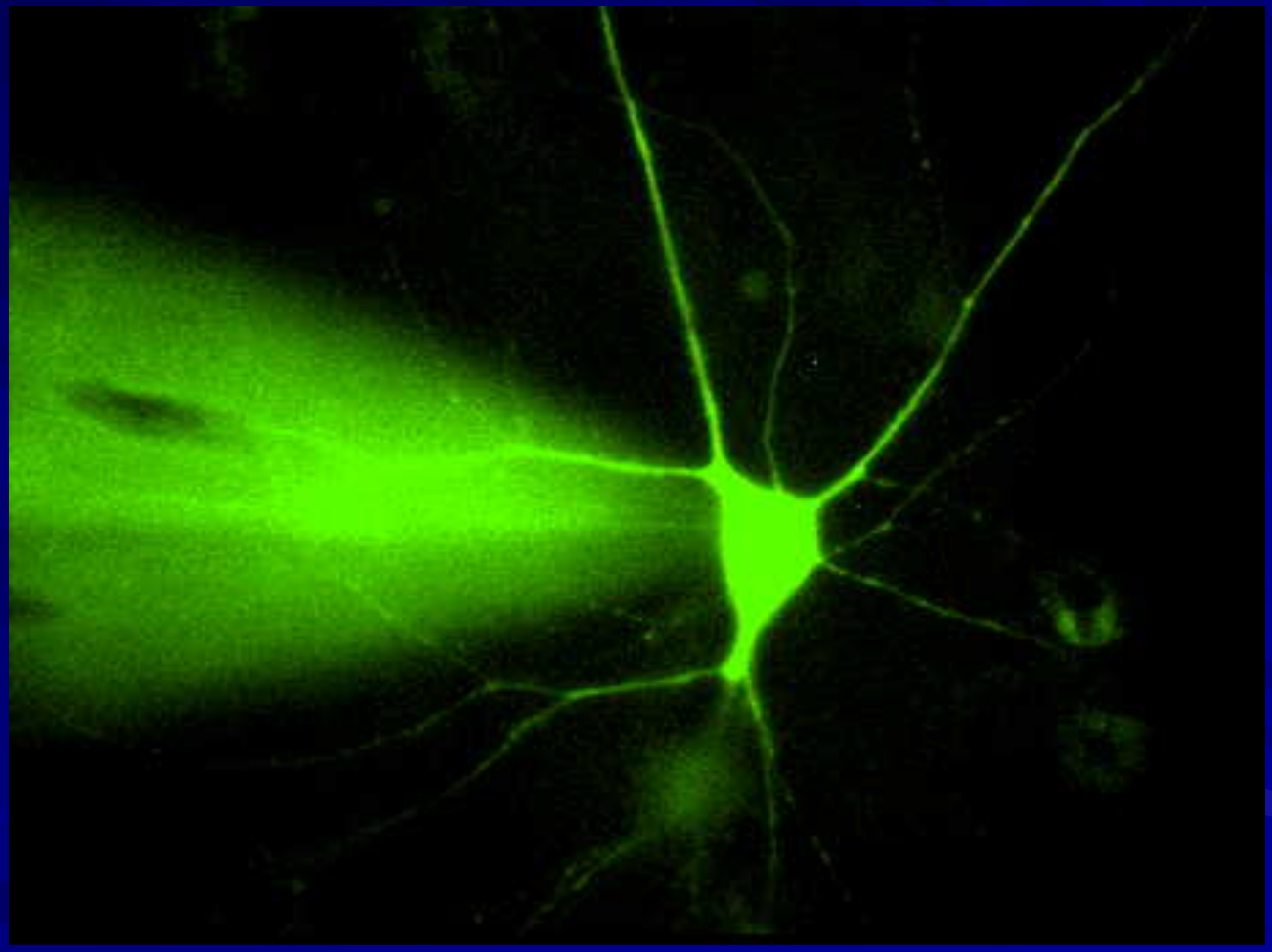
Hippocampal neuron expressing Green Fluorescent Protein (GFP)
Courtesy Prof. Paul De Koninck Laval University

Neurons and Glial Cells

100 x as Many Glial cells As Neurons

Hippocampal neurons(green) and glial cells(red)
Courtesy Prof. Paul De Koninck Laval University

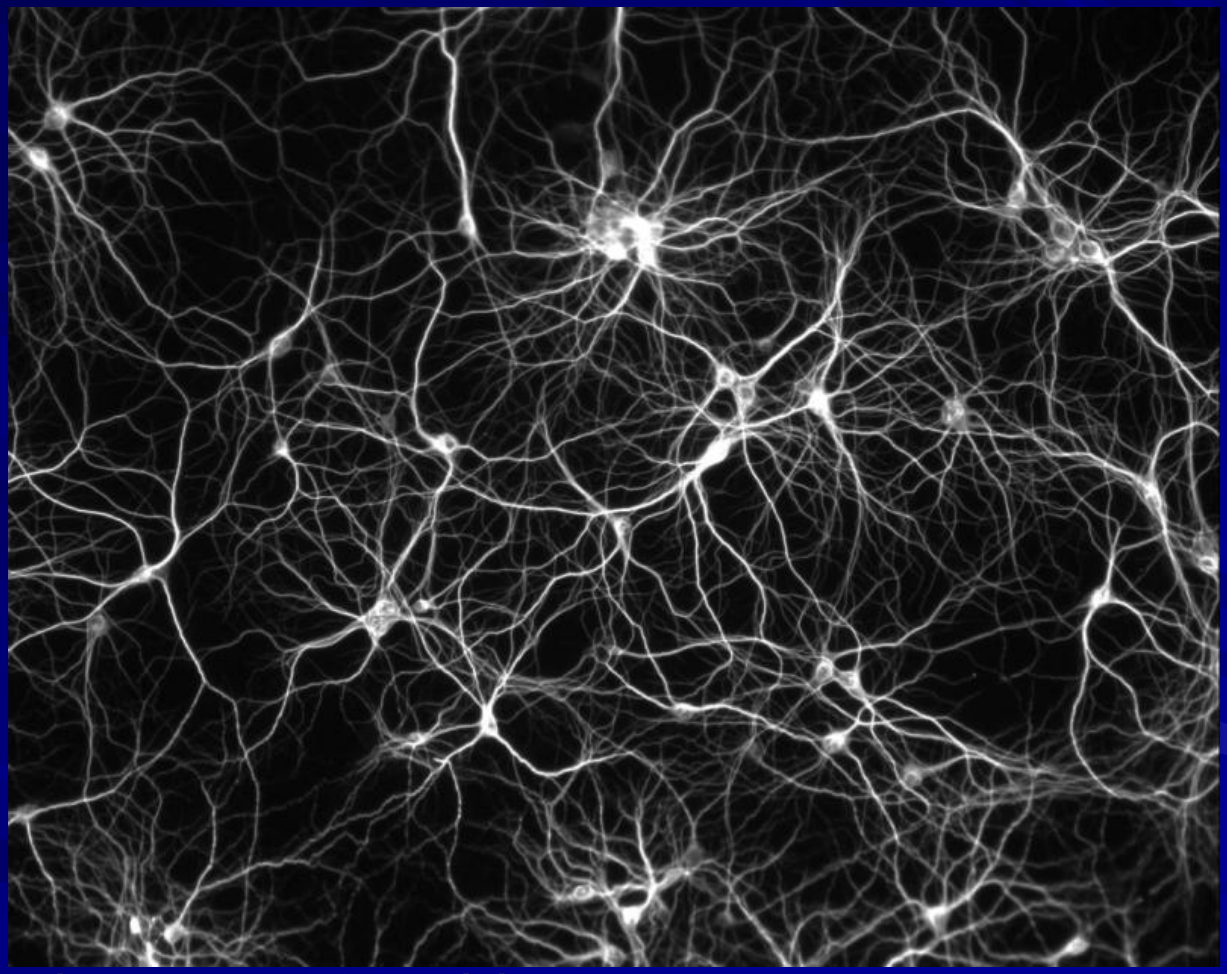
Patch Clamp on a neuron…



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”

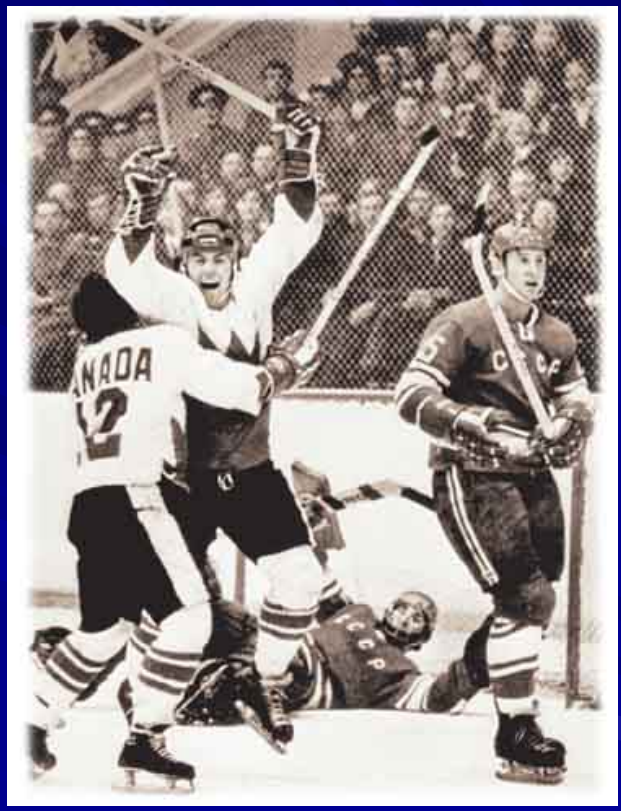
What is the meaning…the sequence?

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!

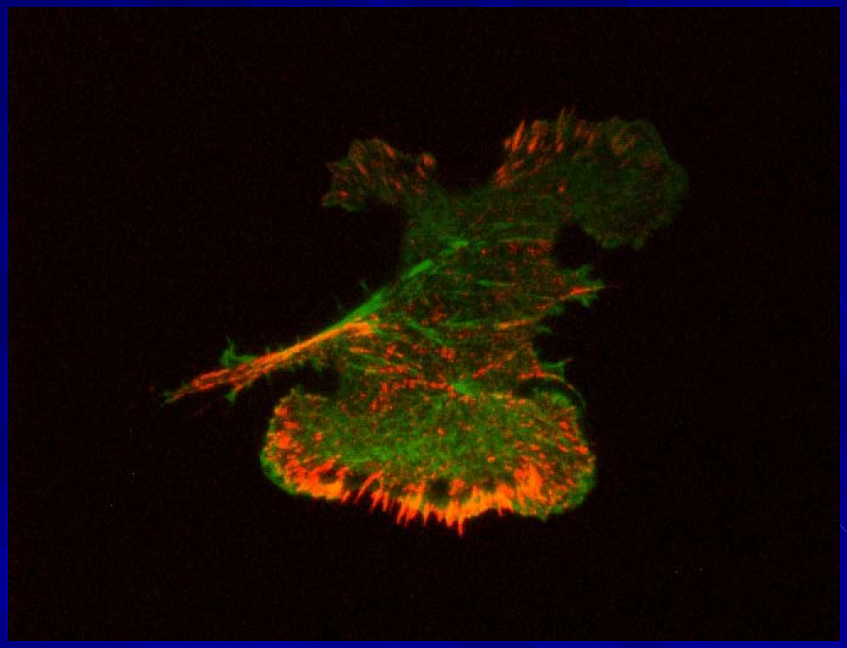
Hockey is Dynamic!

Easier to Figure Out if We Can Watch it Unfold…

In real time

~2 m

170 μm



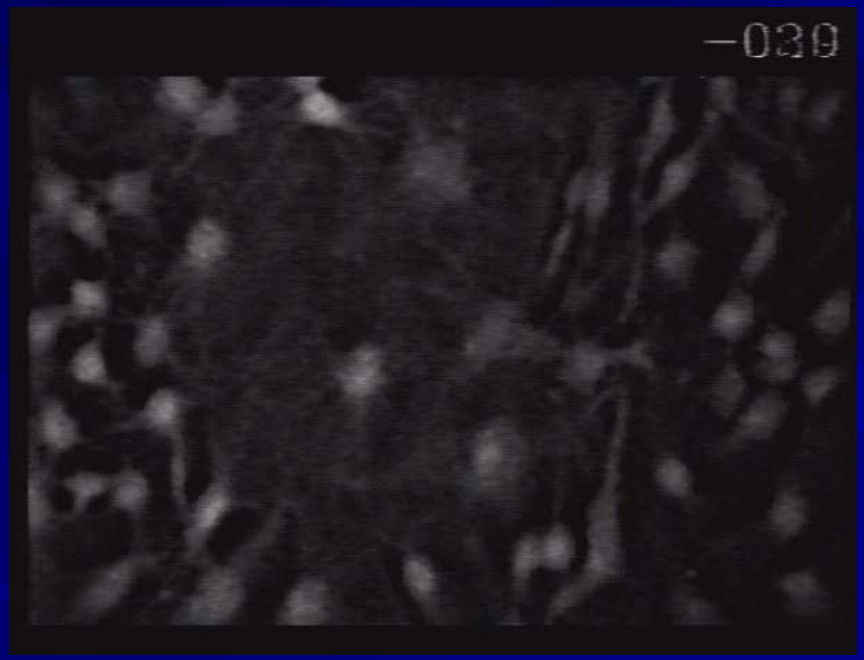
Paxillin-dsRed (red) &
α-actinin GFP (green)
in CHO Cell
TIRF Microscopy
Total time = 50 min δt =15 s

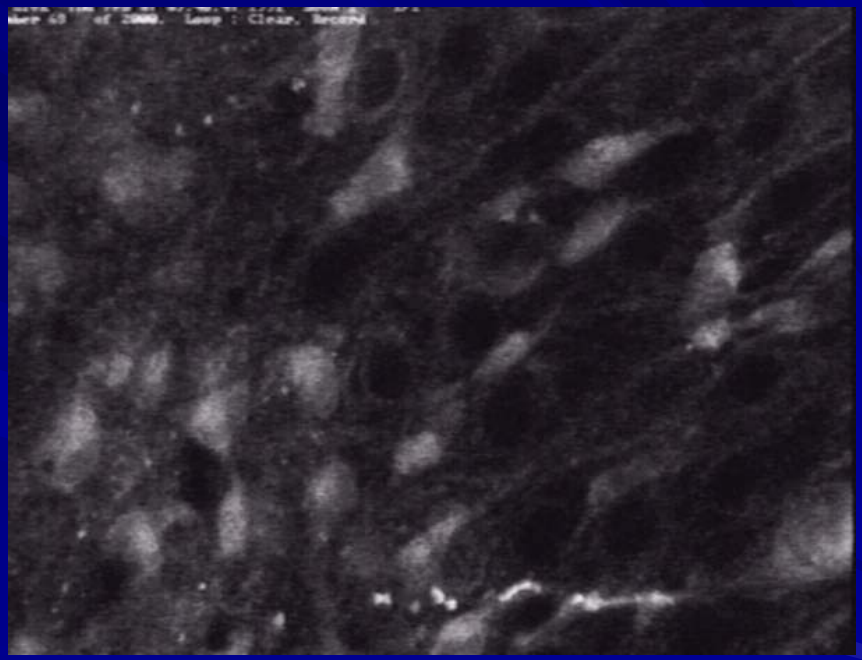
Unravel the Rules of a Dynamic Game…Space & Time Scales

The Brain is a dynamic organ

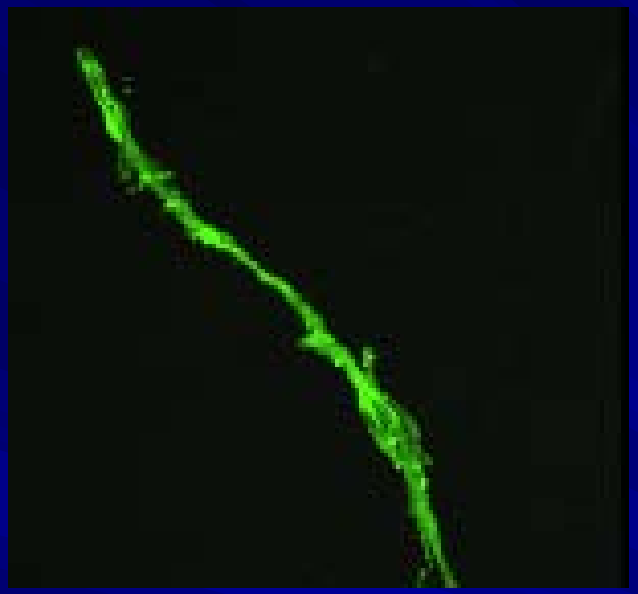
Calcium signalling in Astrocytes…glial cells

Prof. Steve Smith Stanford University





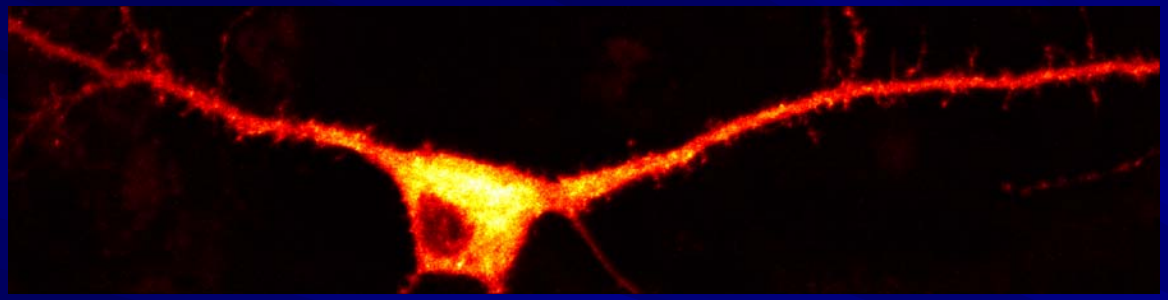
Myelination of an Axon



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

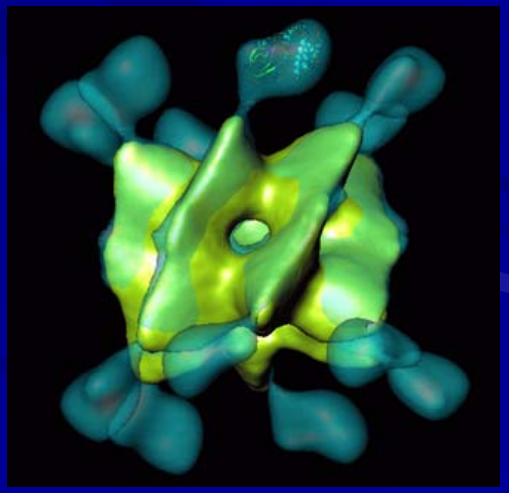
CAM KinaseII transported into and out of spines



Rat Neuron with CAM KinaseII

CAM KinaseII: Neuronal Enzyme

Collaboration with Dr. Paul DeKoninck
Laval University



~12 nm diameter

The Challenging Arena of the Cell

Cells are Small…Biomolecules are Smaller!

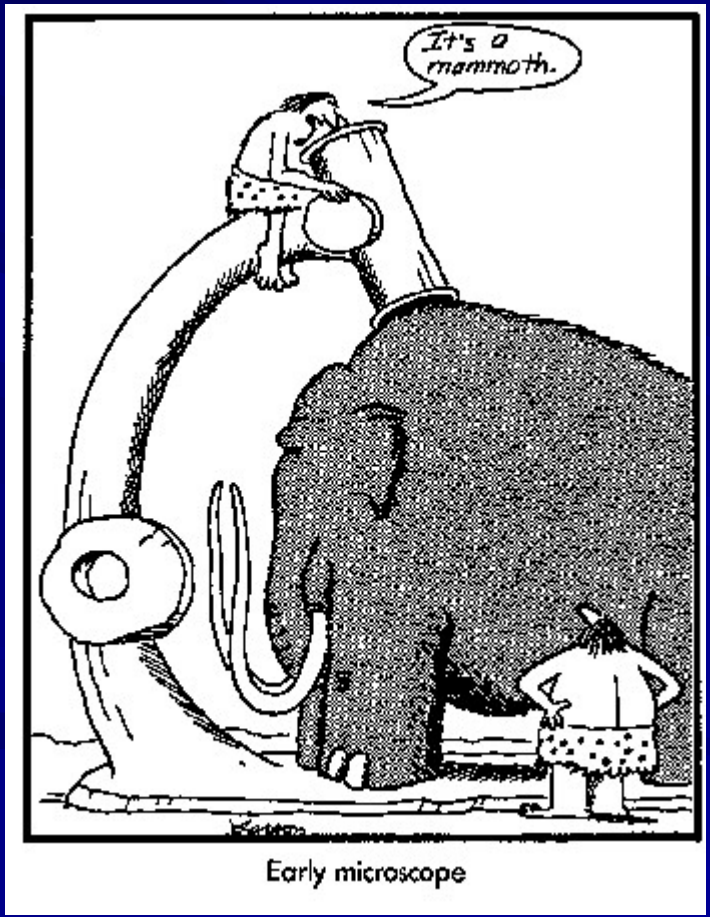
Light Microscopy has Resolution limits ~ 200 nm

Cells & Tissues are Highly Scattering for Photons

Like Seeing Through Milk

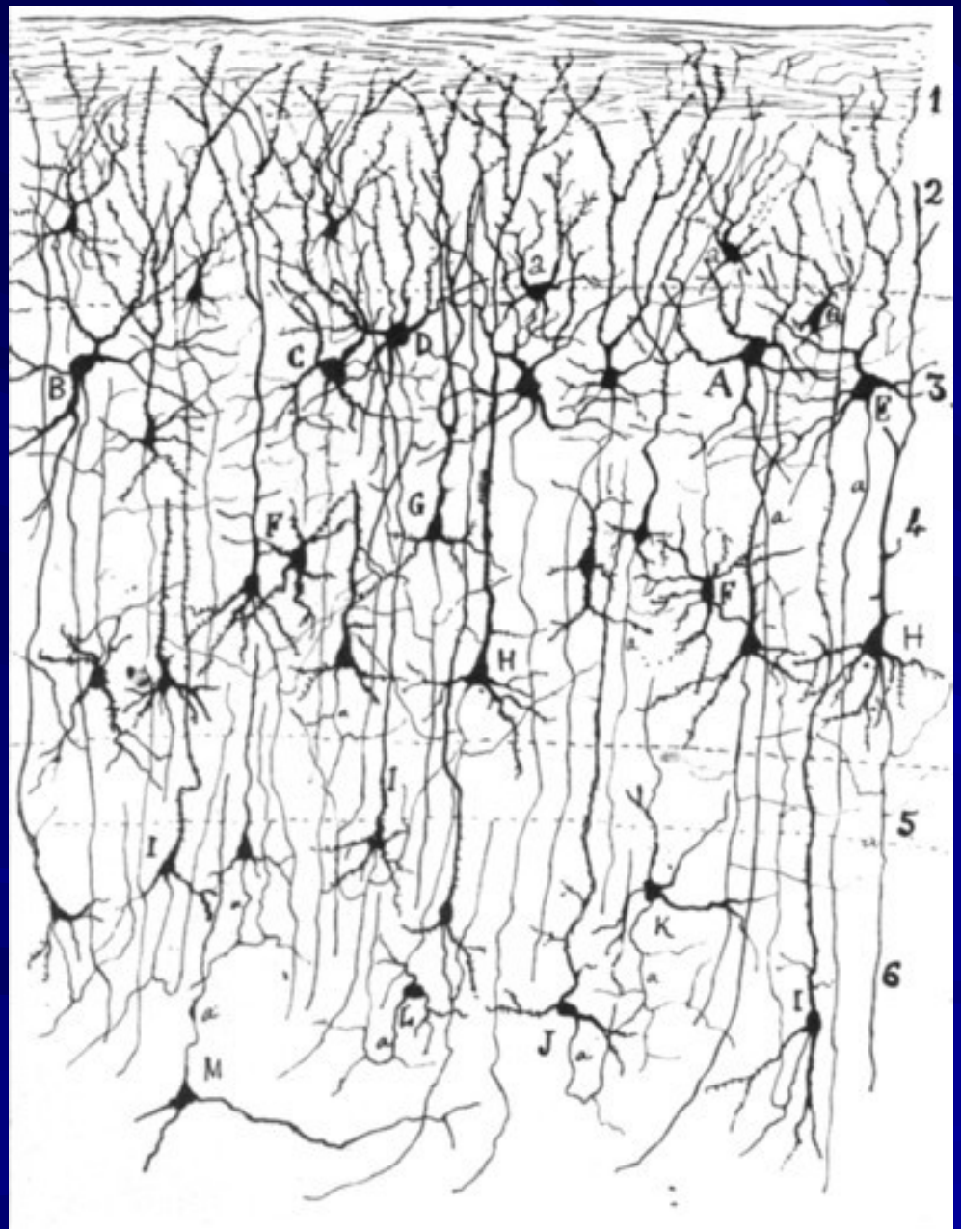
Light Microscopy

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



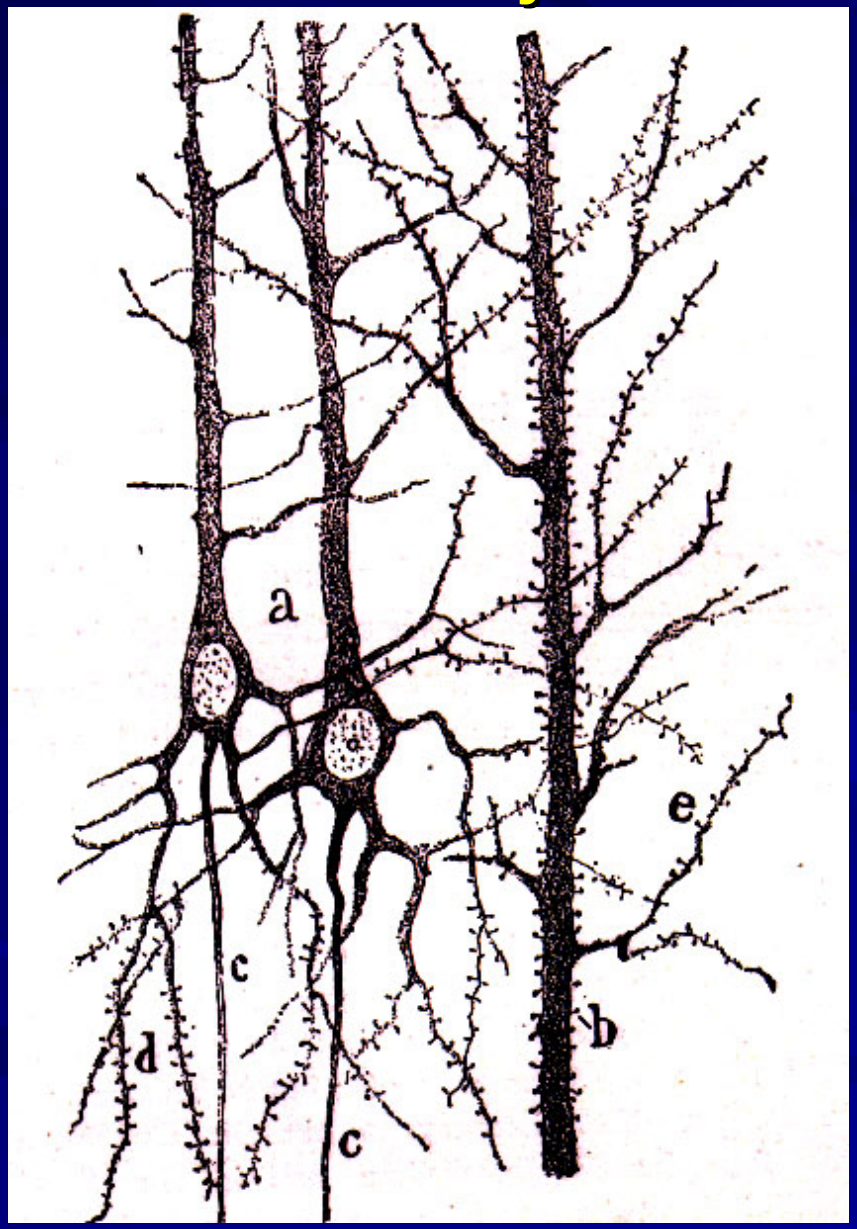
The Far Side by Gary Larson

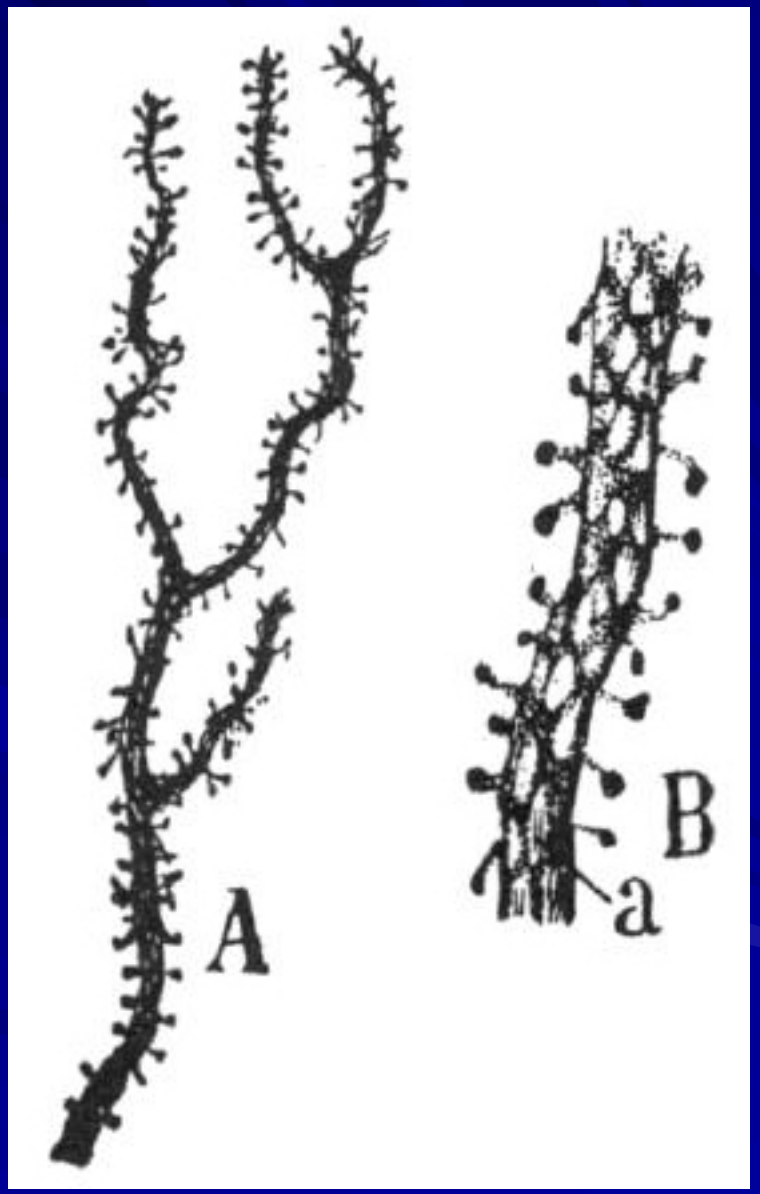
Cajal…with his microscopes



Cajal in his lab…

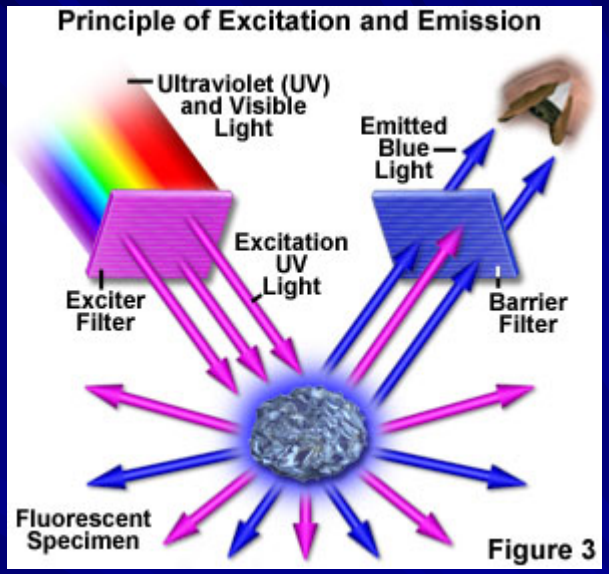
Cajal…Dendritic Spines





Modern Light Microscopy

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

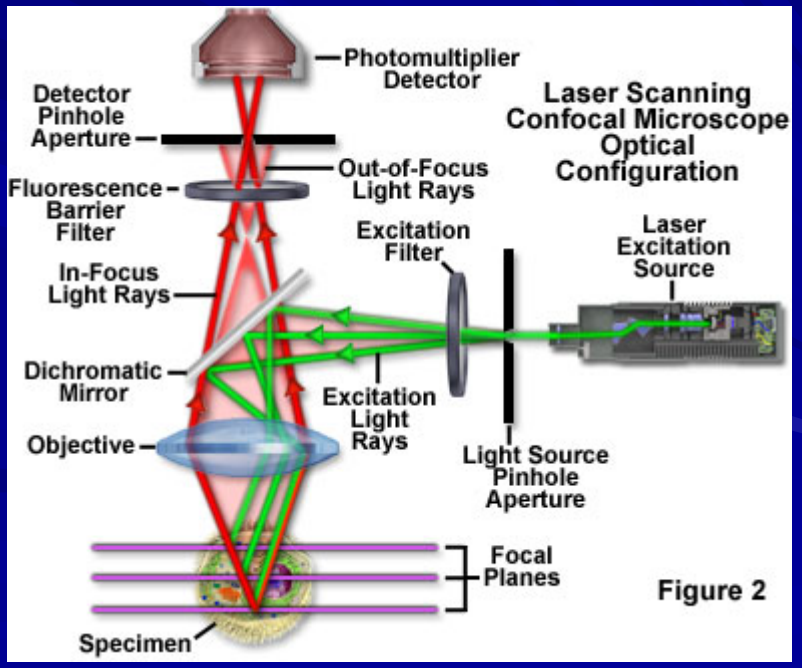


Confocal Laser Scanning Microscopy

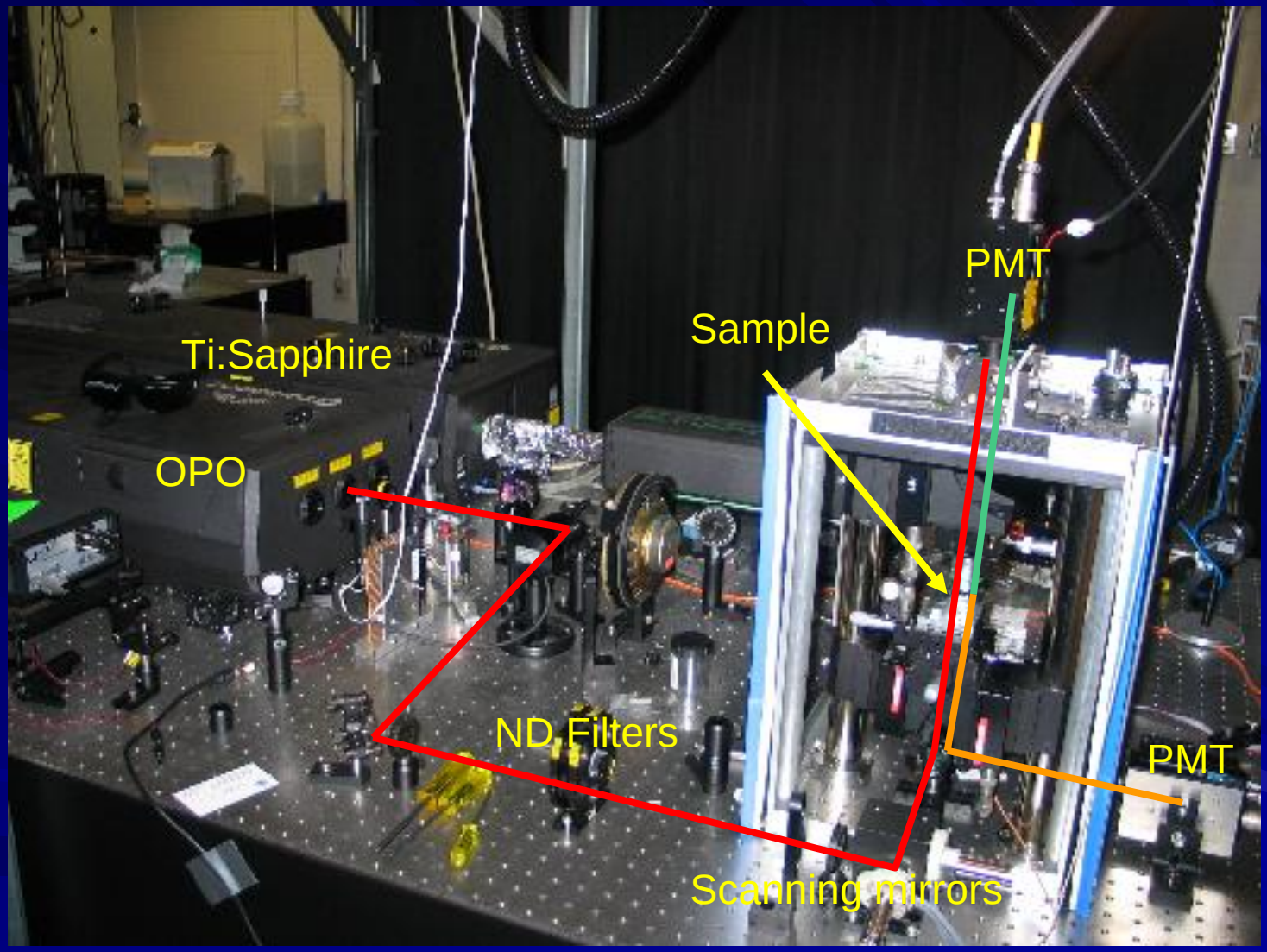
Modern Light Microscopy

- Fluorescence Microscopy
- Optical Microscopy

Confocal Laser Scanning Microscopy



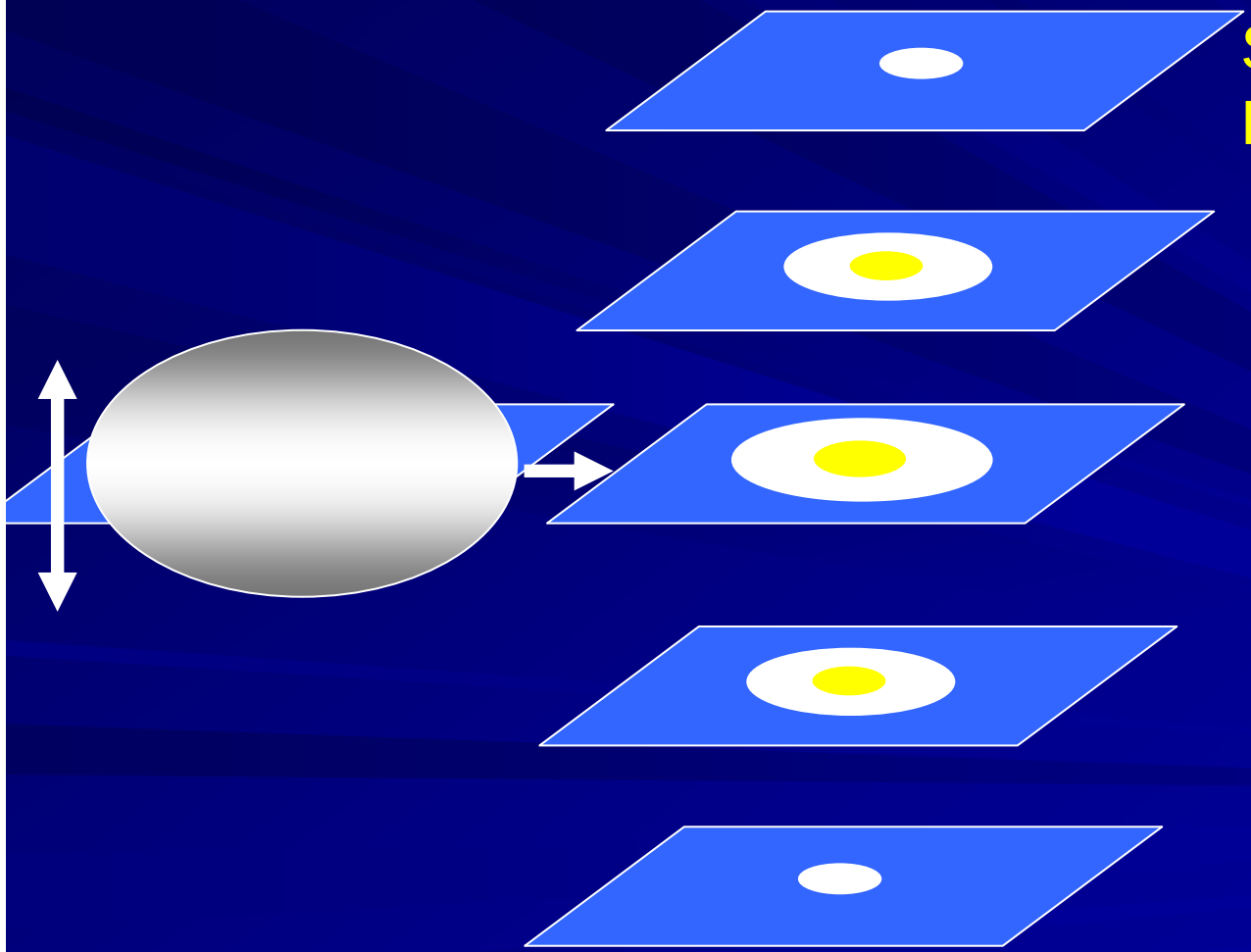
Multimodal Nonlinear Microscope



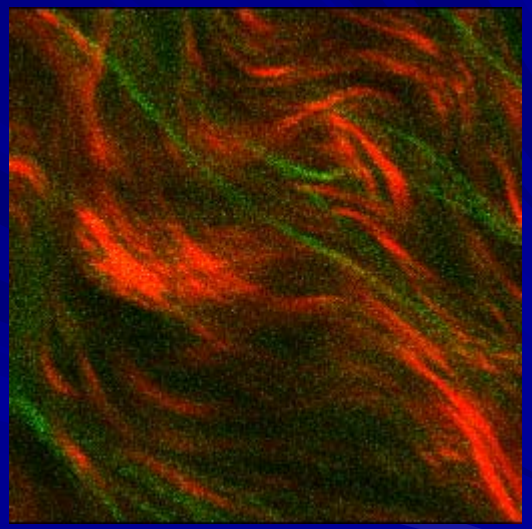
Home built Microscope Jon Belisle (Wiseman Group McGill)

Optical Sectioning

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

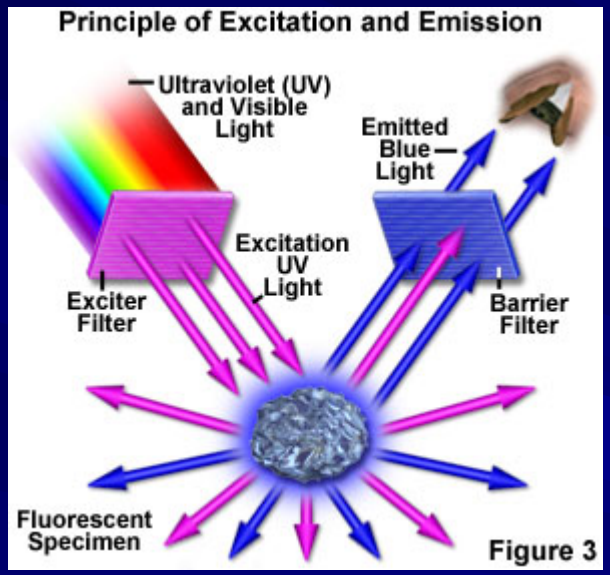


Collagen (Red)
Elastin (Green) Optical Sections in z (~1mm)
Human Heart tissue



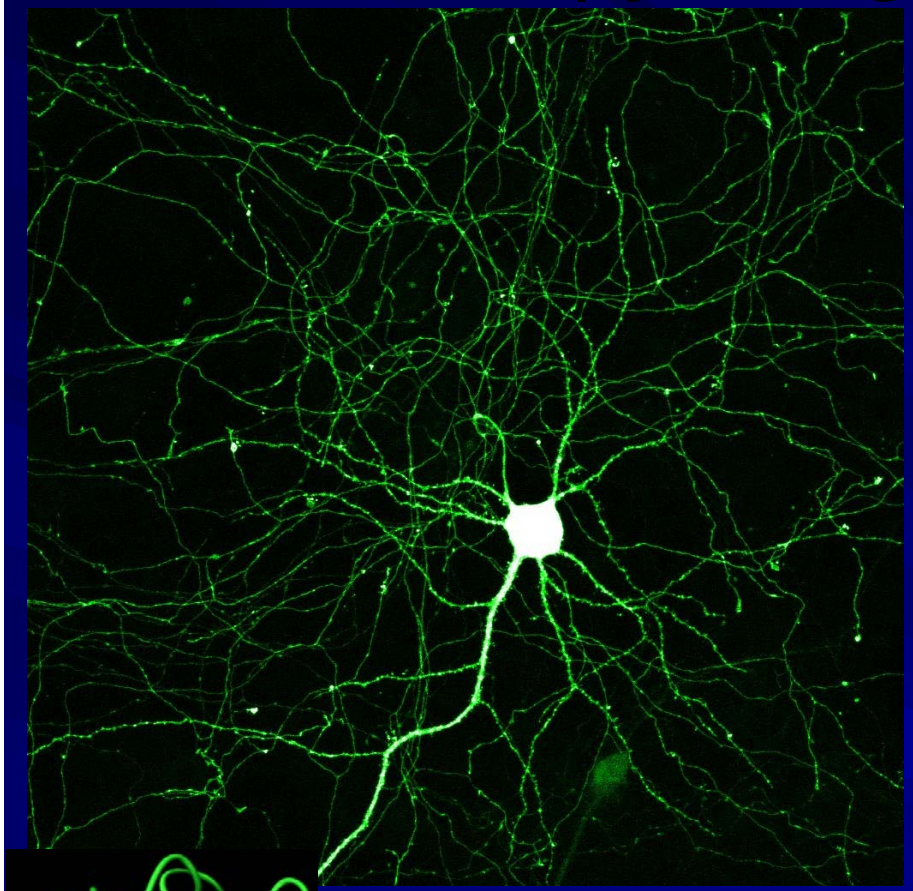
Fluoescence Microscopy

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



Fluorescent Shirts under Ultraviolet Light

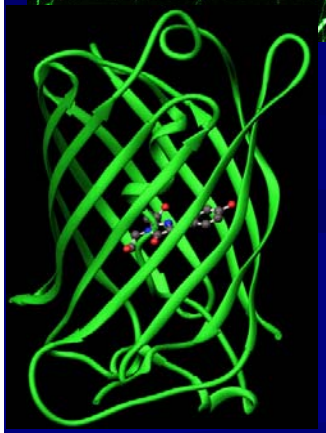
Microscopy Imaging of Fluorescence



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

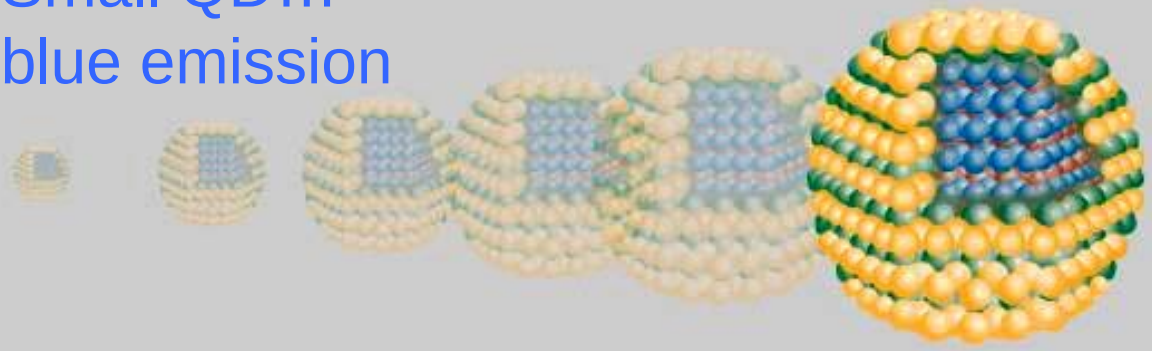


Fluorescent Label on Protein…Genetically encoded Green Fluorescent Protein (in Jellyfish *Aequorea victoria*!)
(or other colours like blue, cyan, yellow
GFP ~ 4 x 3 nm
← Shine Laser on it…It Glows!

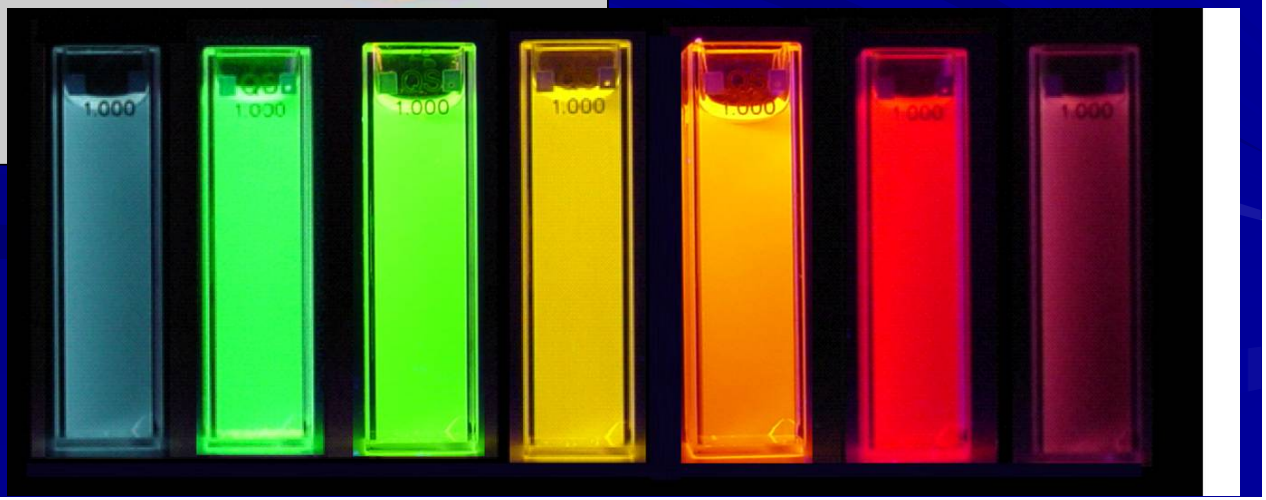
Quantum Dots…Nanoparticles

- Different sizes…Different Fluorescence Emission Colour

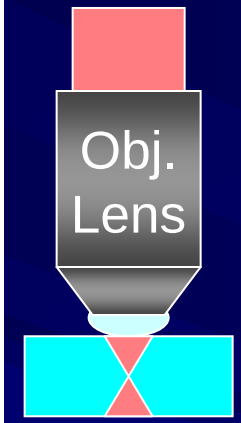
Small QD…
blue emission



Larger QD…
red emission

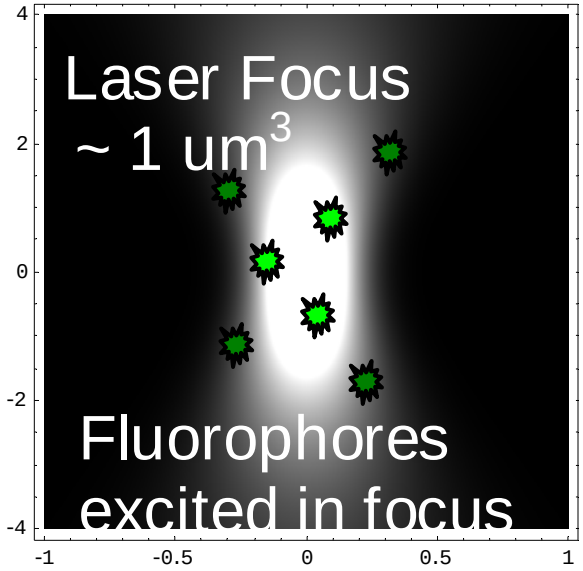


Fluctuation Magnitudes & Fluctuation Times

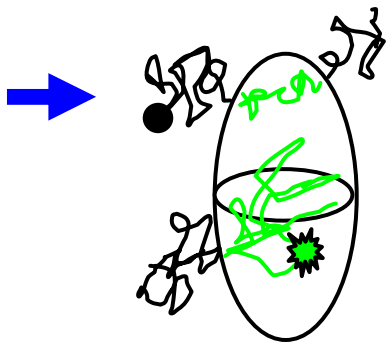


Fluorescence Correlation Spectroscopy (FCS)

Elson and Magde ; Magde, *et al. Biopolymers* (1974) 13, 1-27 ; 29-61



Molecular Dynamics



Number in the
Focus fluctuates

i(t)

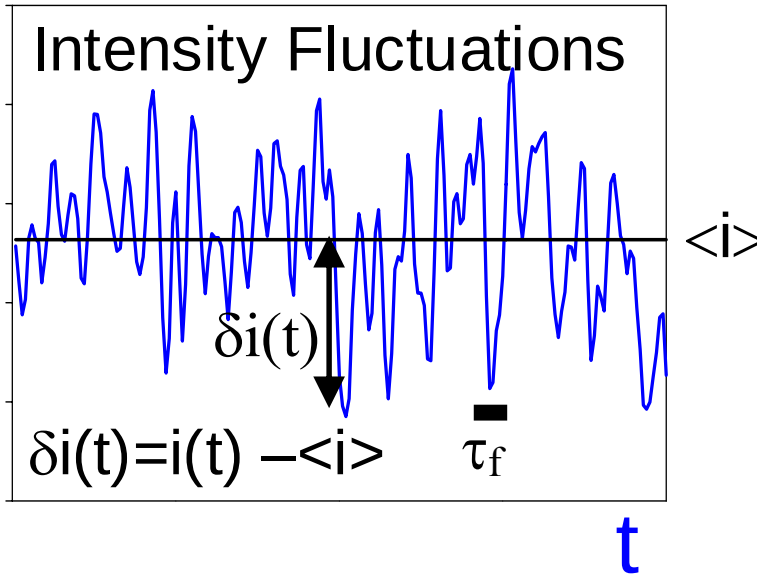
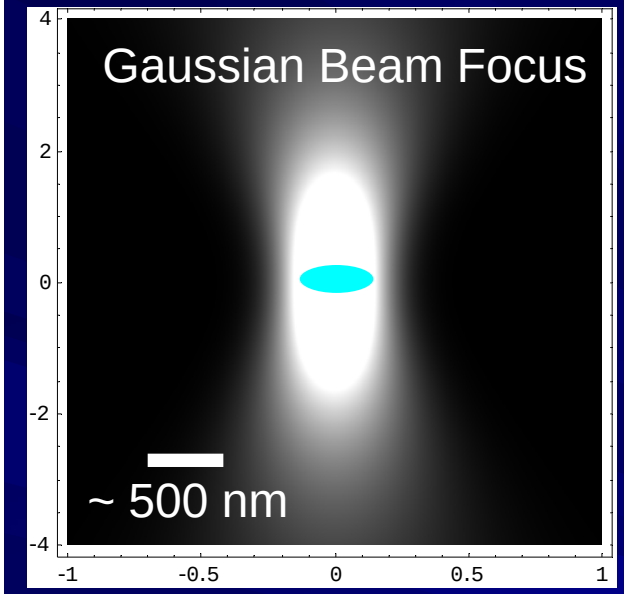


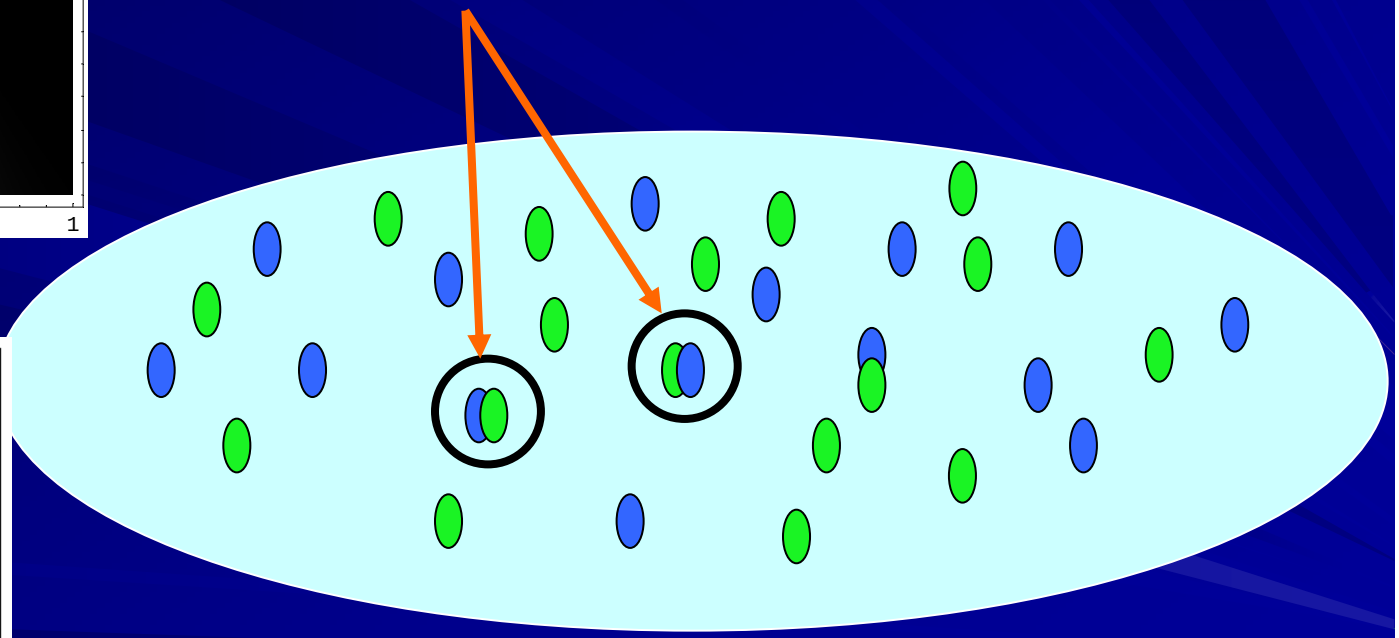
Fig. 1 Overview of Fluctuation Spectroscopy

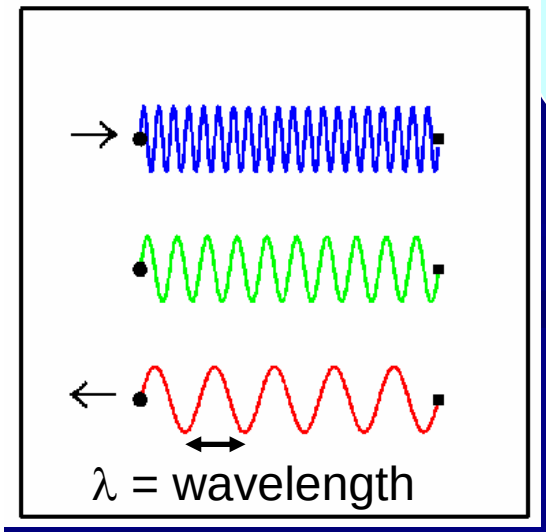
Diffraction Limited Optical Resolution…



Optical Microscopy
Dynamics at the Price of Spatial Resolution

Truly Interacting Species
Dance Partners

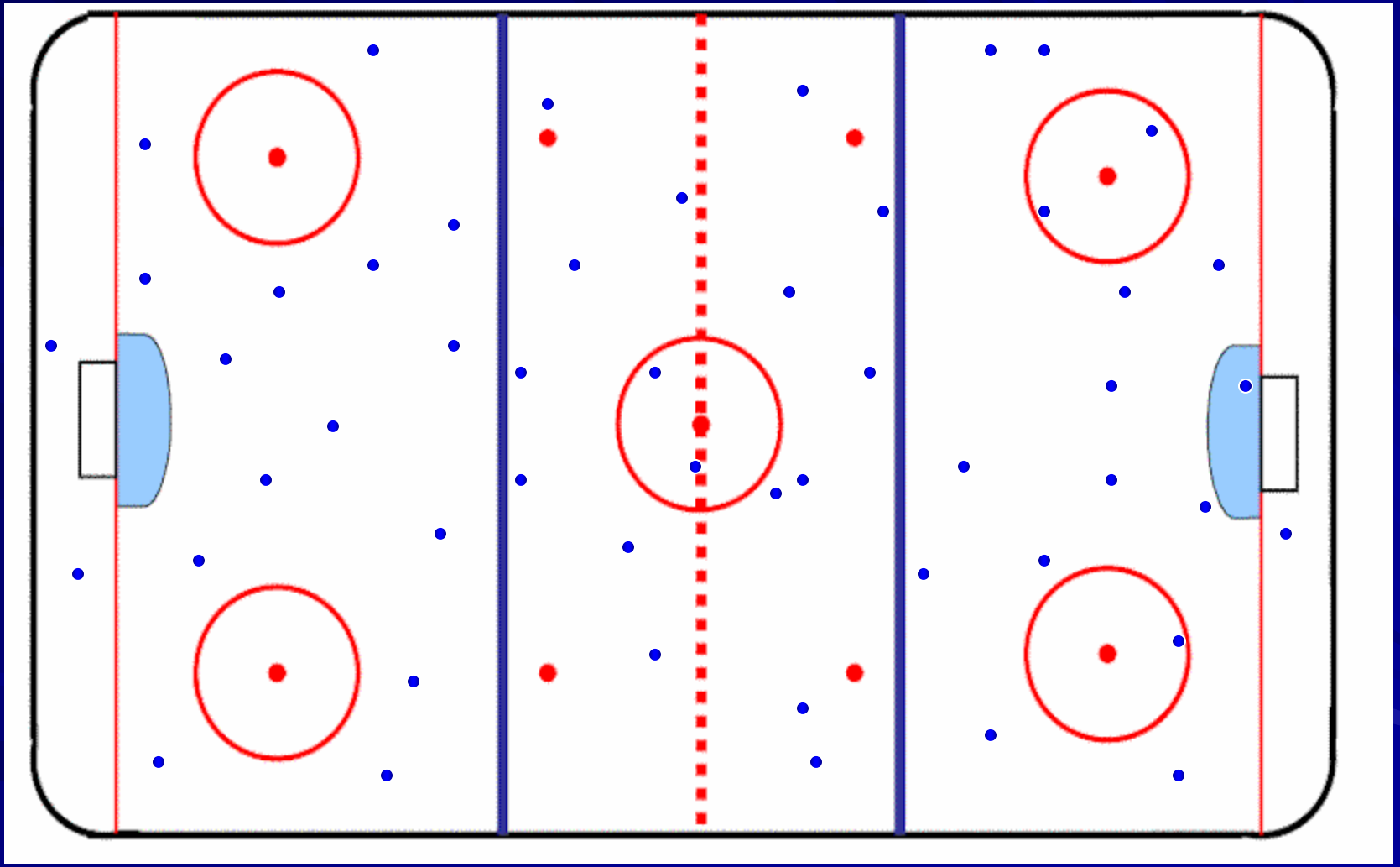




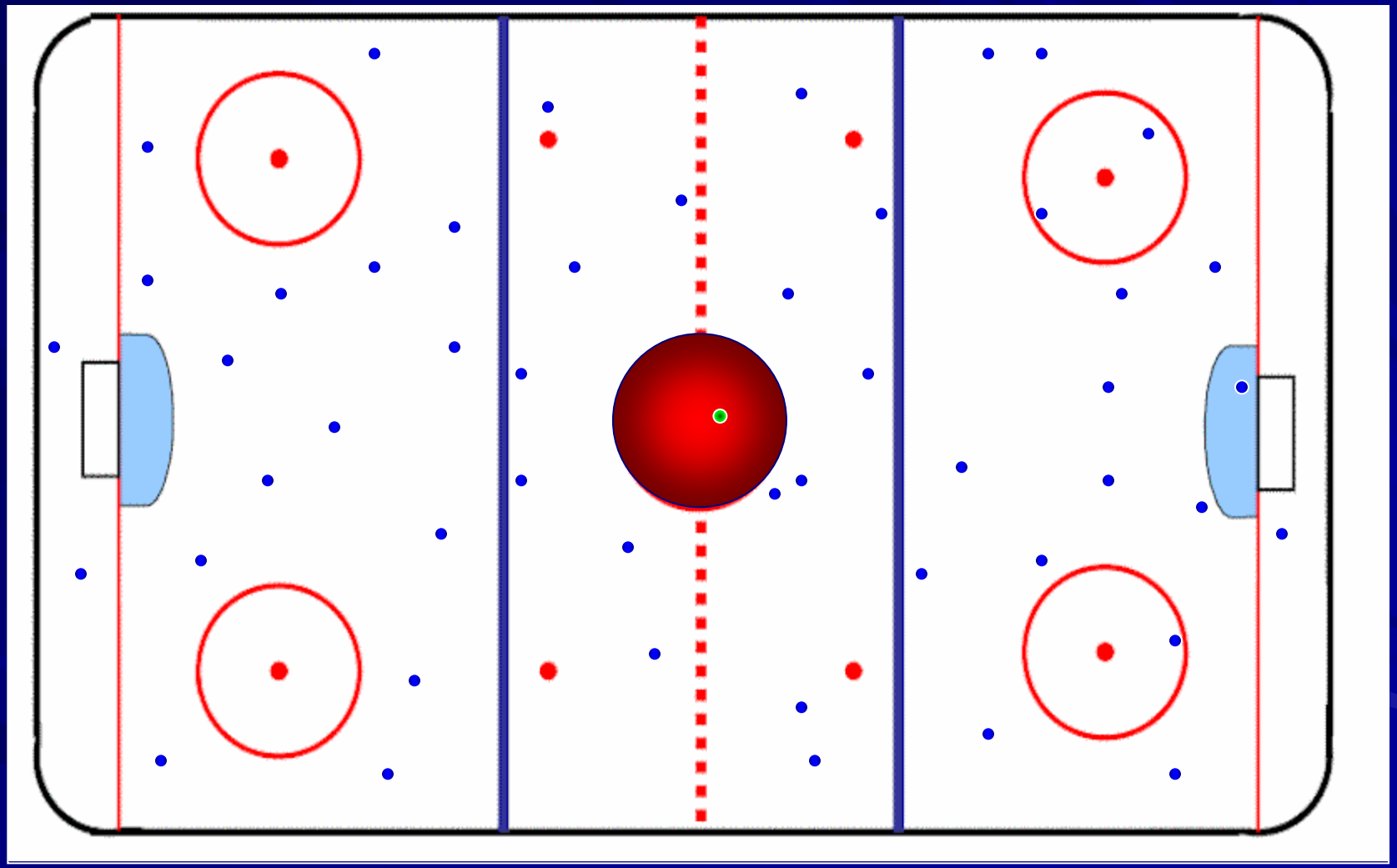
Optical Resolution ~ λ/2 Macromolecules ~ λ/50

λ = Wavelength ~ colour of the light

Fluctuation Magnitudes & Fluctuation Times

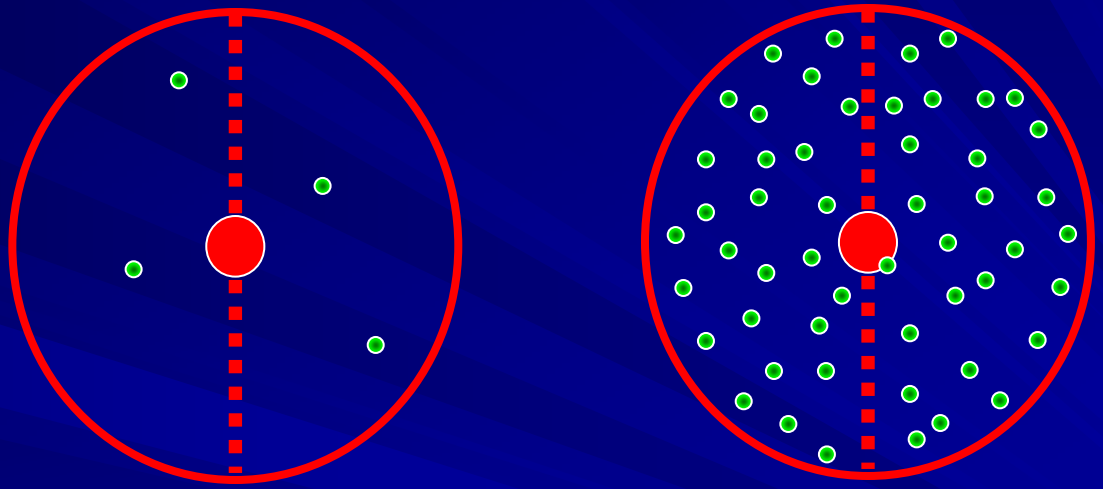


Define Observation Volume with Beam Focus



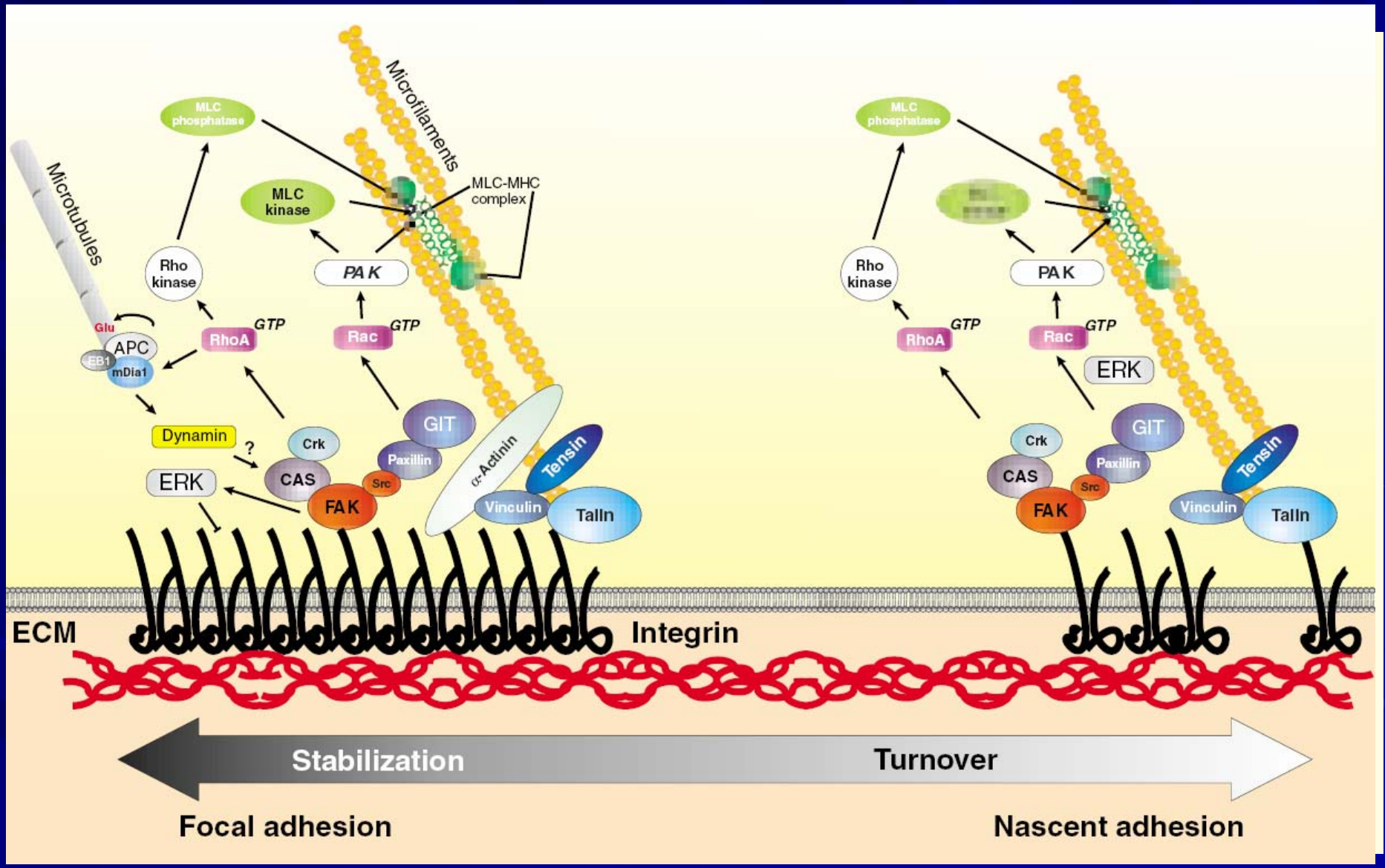
Fluctuation Magnitudes

- Occupation Number Fluctuations Scale Inversely with <N>



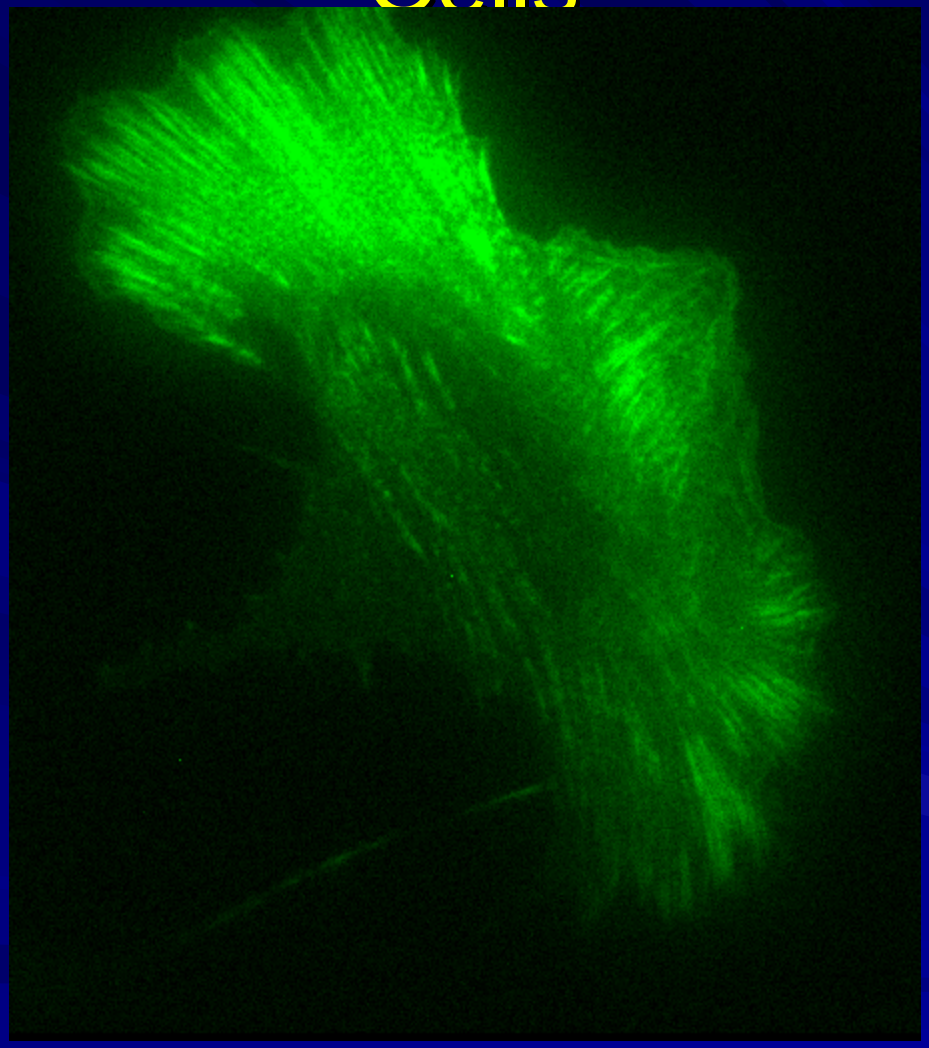
Measure the fluctuations by fluorescence intensity

Focal Adhesions



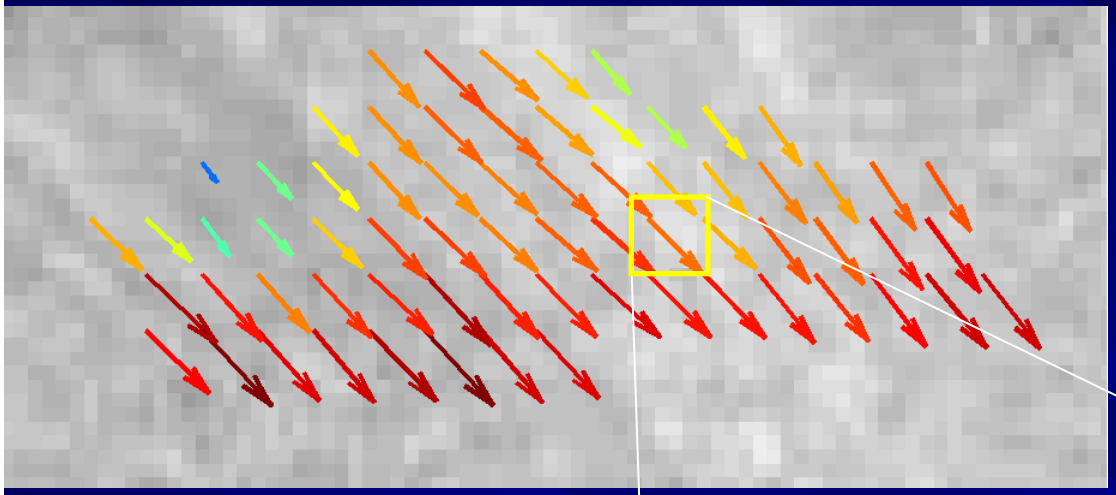
Vicente-Manzanares, M. et al. JCS (2005)

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

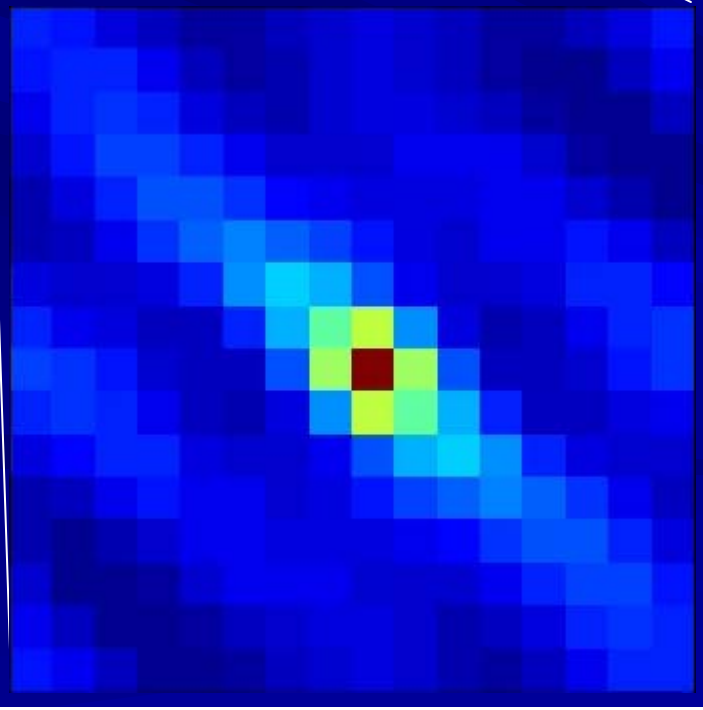
Space-Time Correlation of Fluctuations



α-actinin/EGFP in MEF Cell
TIRF Microscopy

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

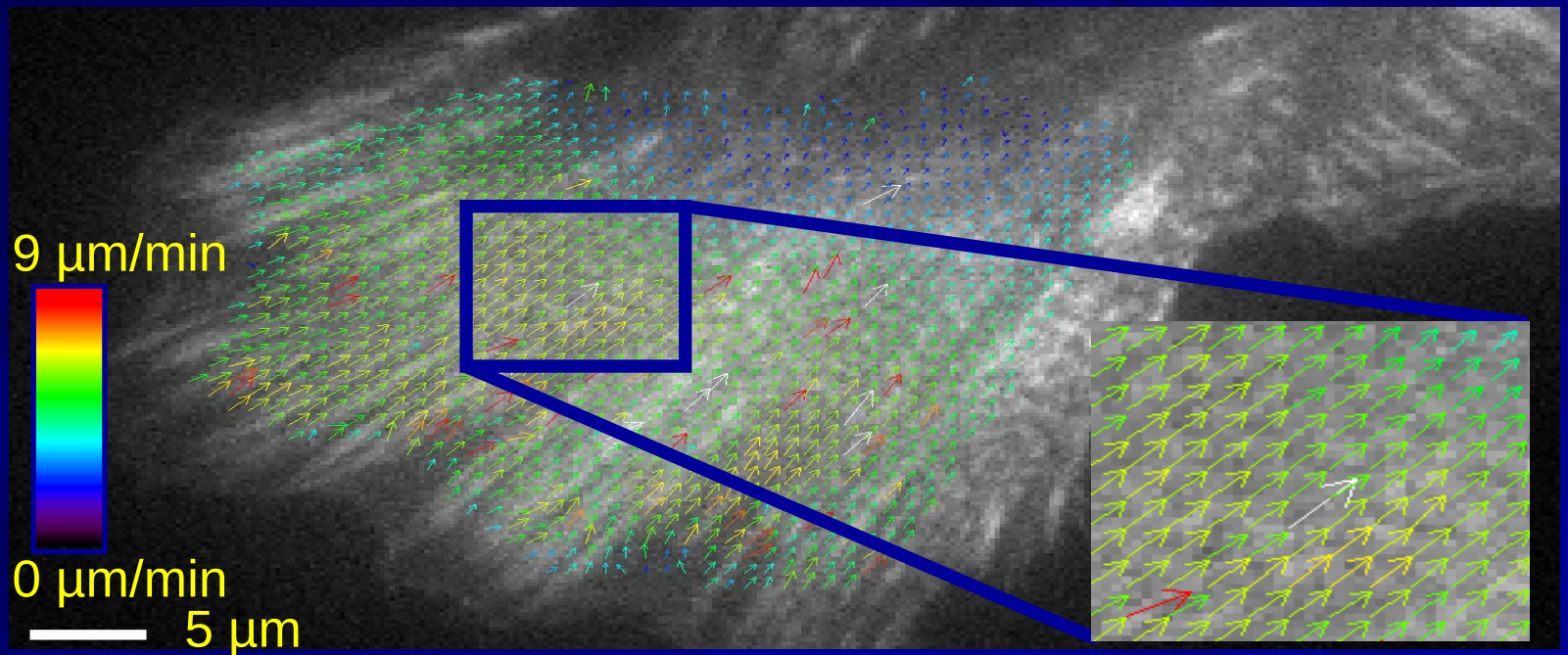
3.4 μm

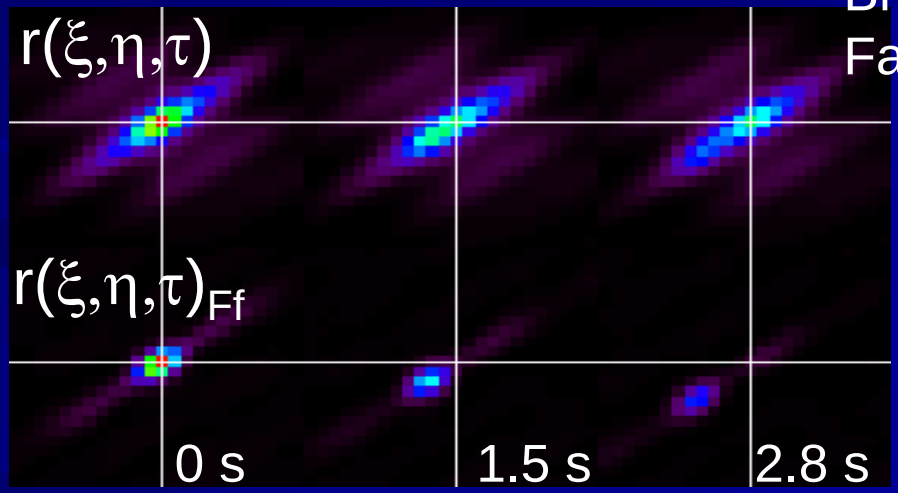


Accelerated
40 times faster
than
Real-time

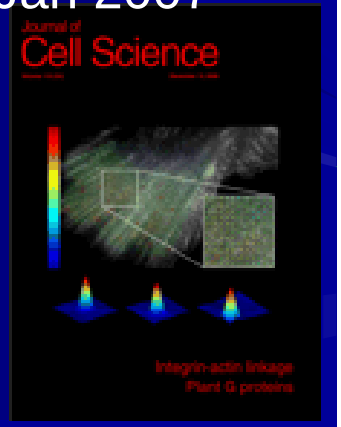
$\tau = 0$ s ➔ 200 s

Vector Maps of Protein Transport in Cells



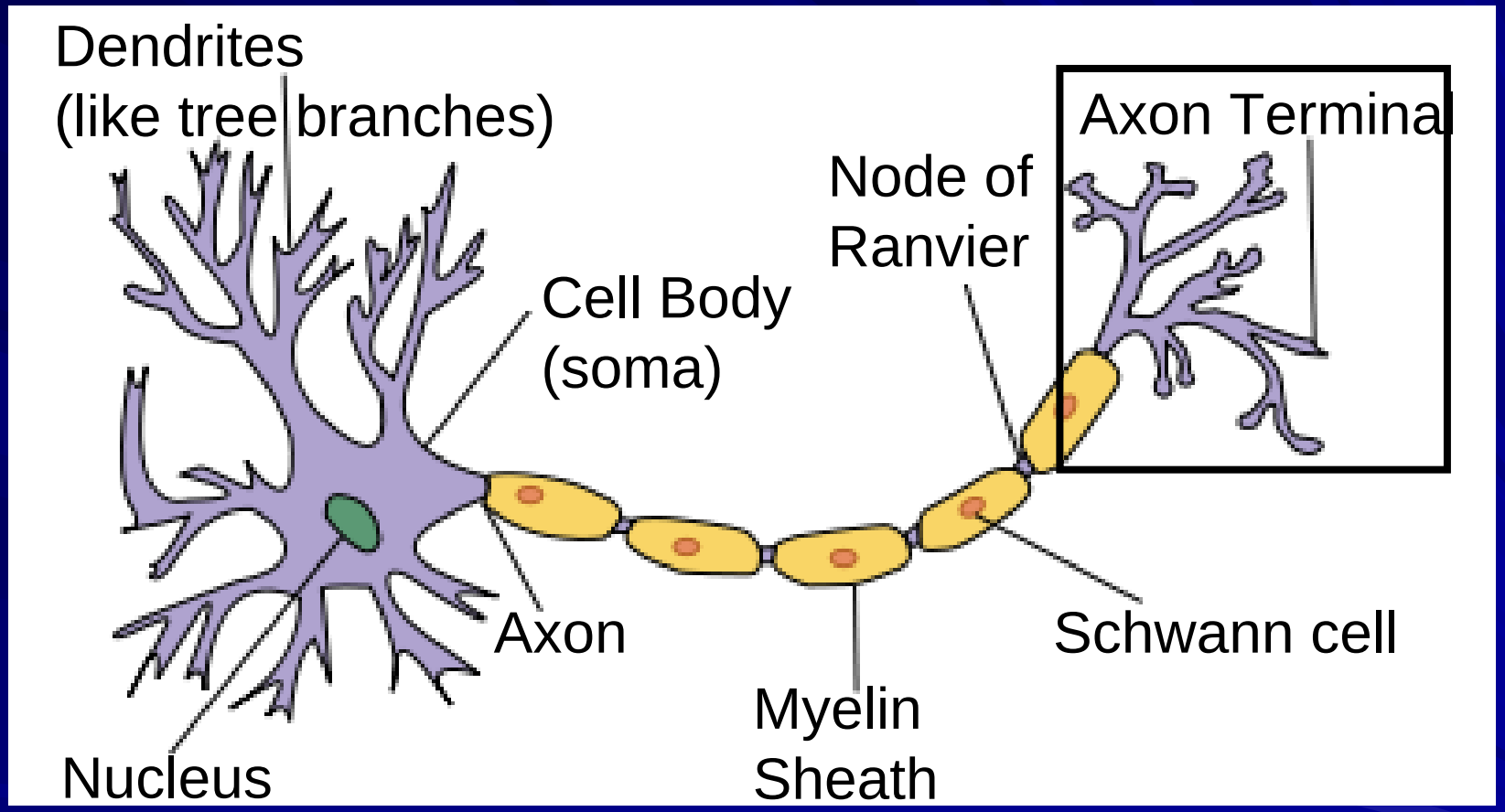


Brown et al. JCS (2006) 119: 5204-5214
Faculty of 1000 Selection Jan 2007



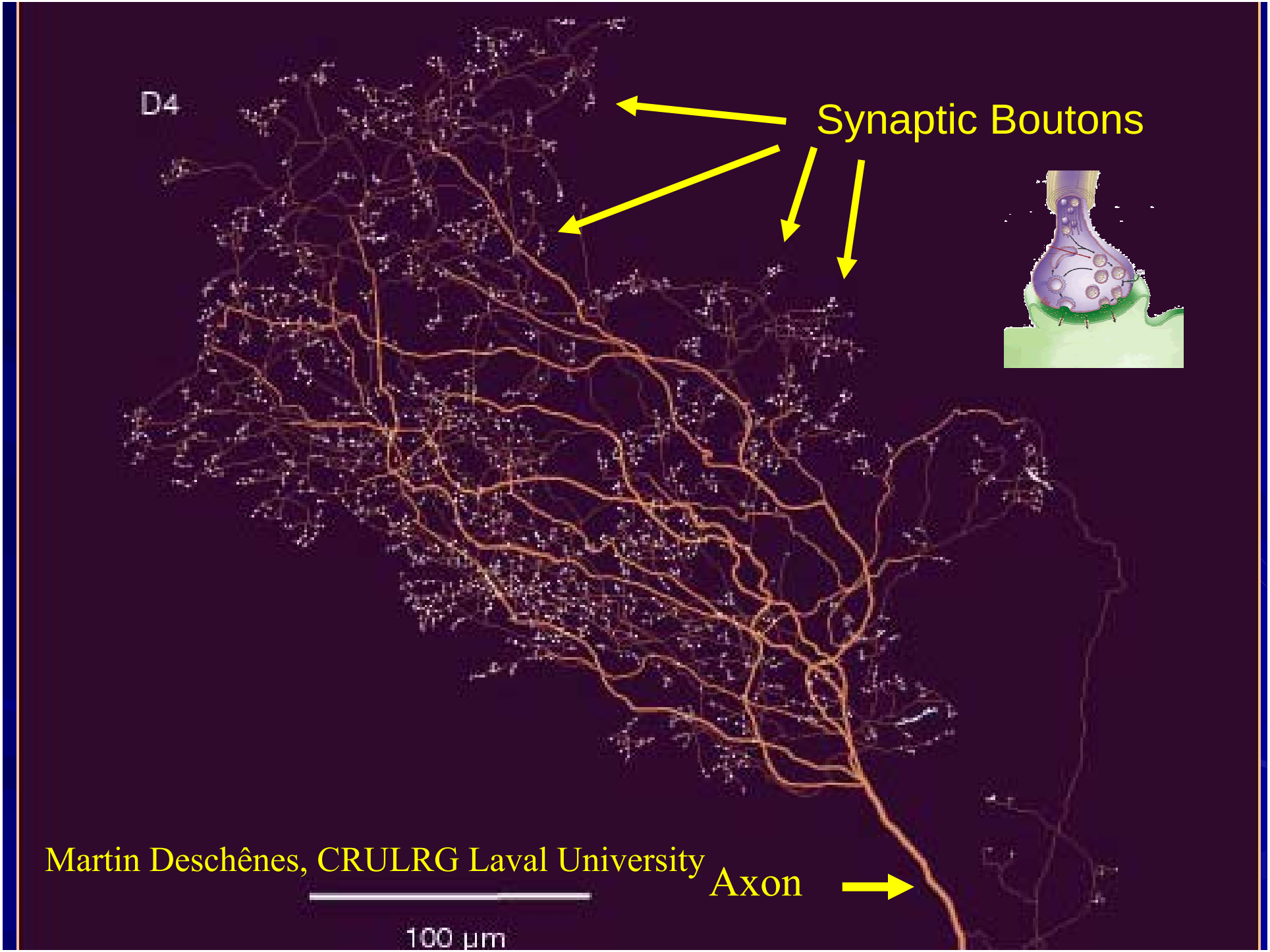
Roadmap to a Neuron

Basic Reference: http://en.wikipedia.org/wiki/Neuron



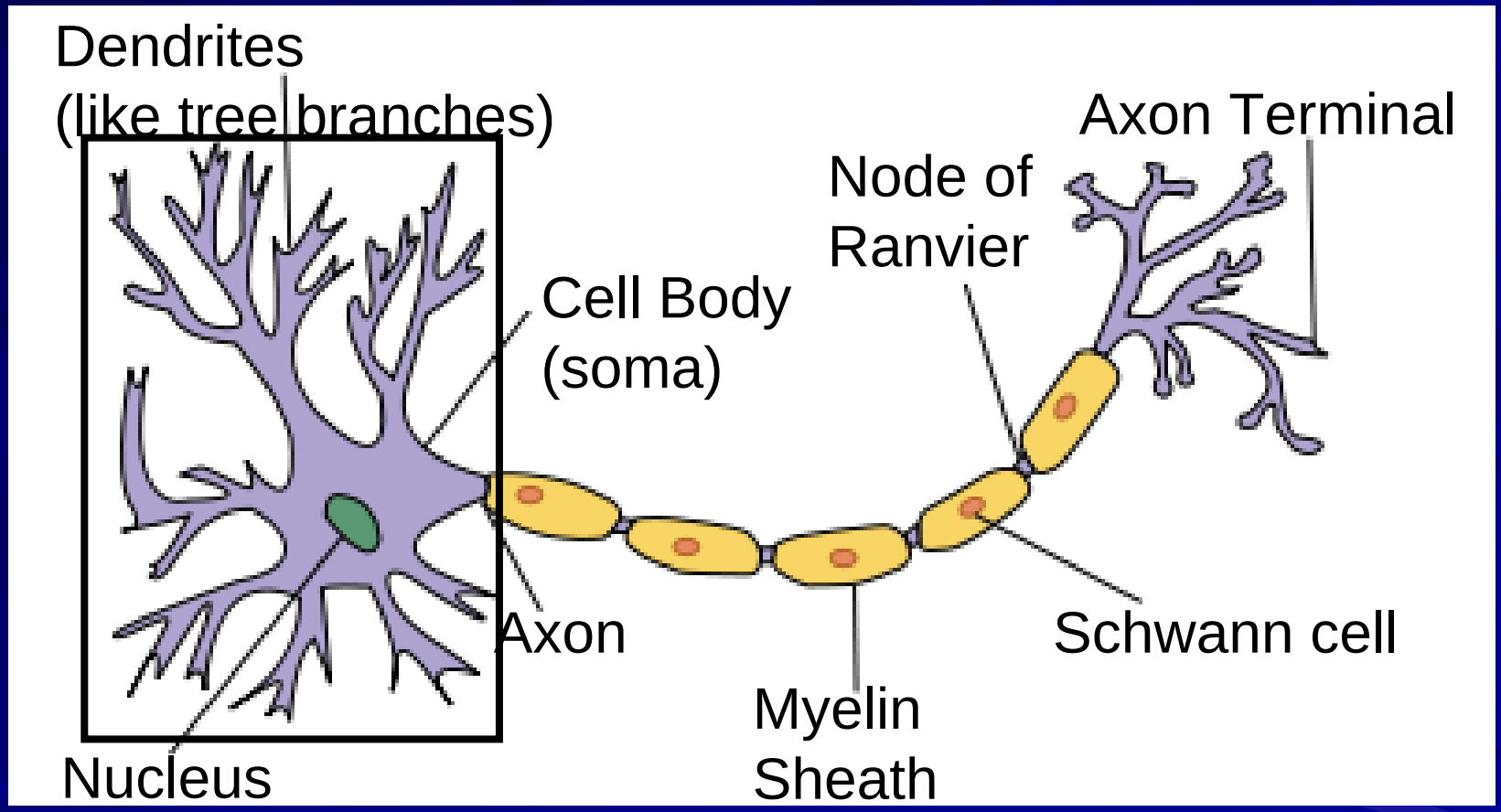
Signal Flow Direction →

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


D4
Synaptic Boutons
Martin Deschênes, CRULRG Laval University
Axon
100 µm

Roadmap to a Neuron

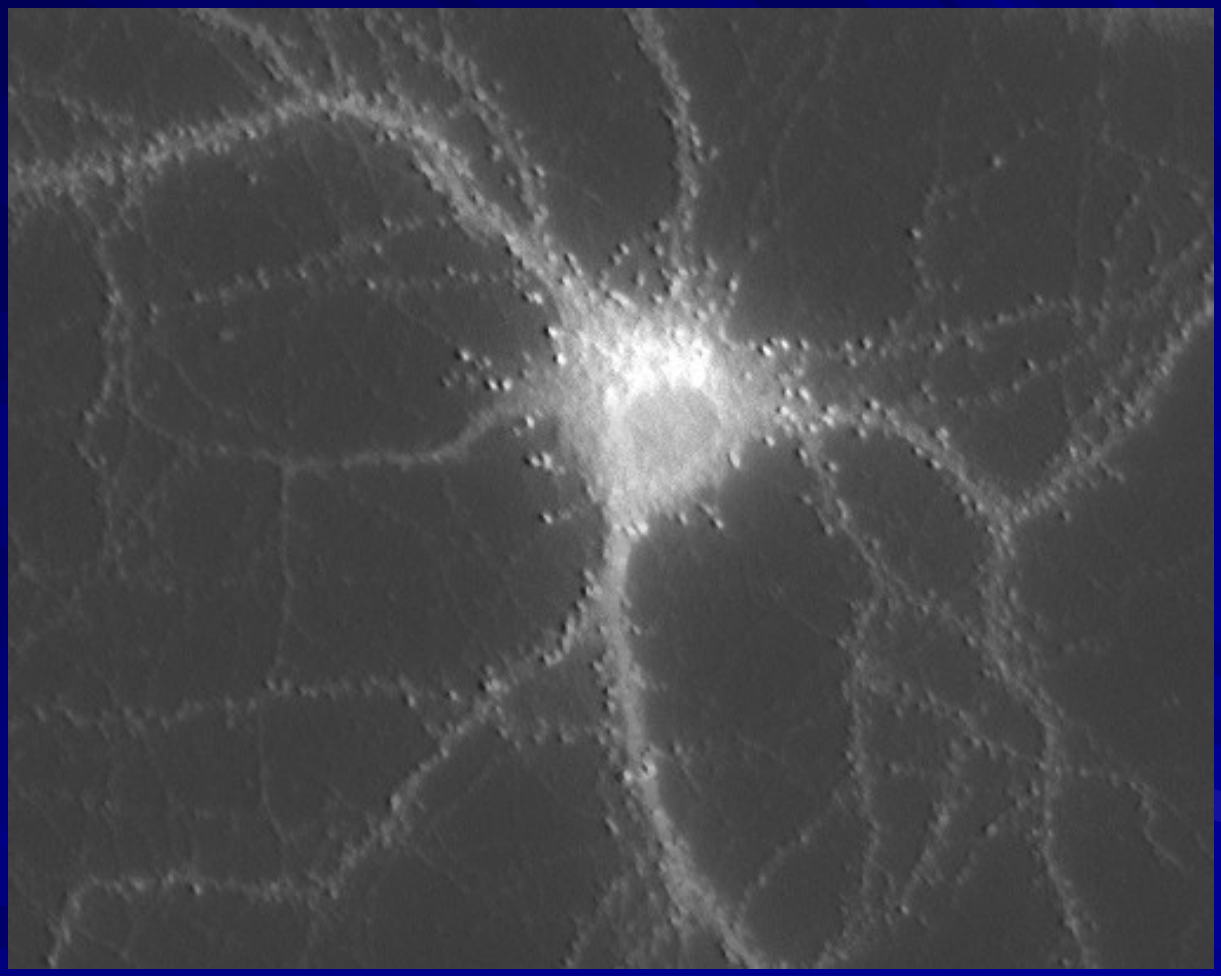
Basic Reference: http://en.wikipedia.org/wiki/Neuron



Signal Flow Direction →

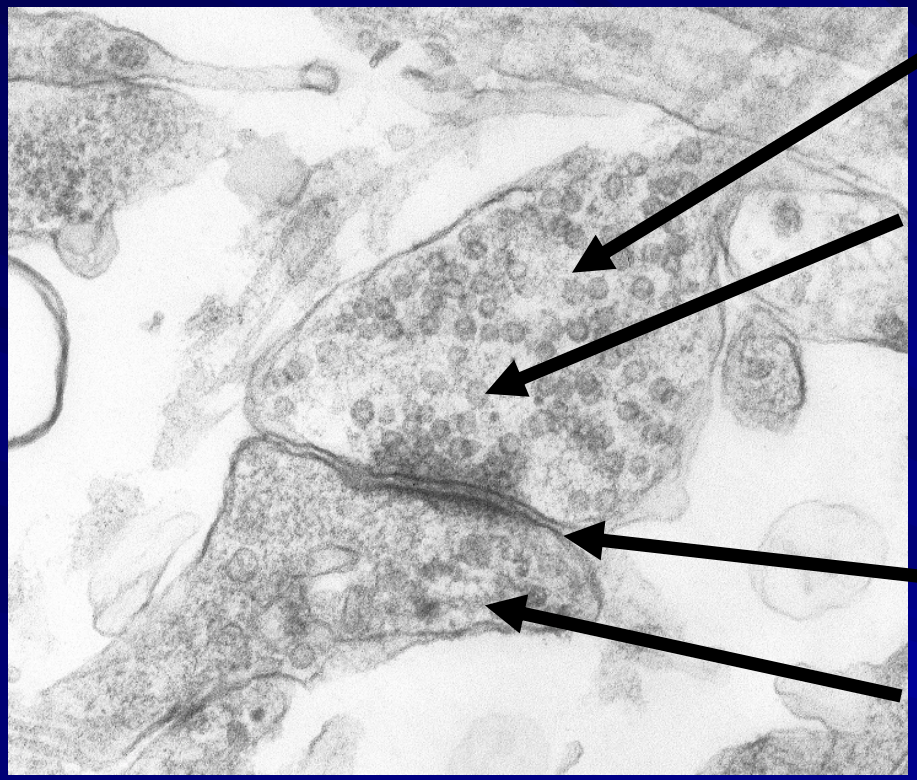
Input from Other Neurons (via synapses) → Action Potential (Electrochemical Signal) → Output to Other Neurons Or Muscle cells (via synapses)

Dendritic Spines



Dendritic spines of rat hippocampal neurons
Courtesy Prof. Paul De Koninck Laval University

The Synapse…Action Potential ends becomes chemical signal



Electron Microscopy Image

Axon Terminus
Presynaptic Neuron 1

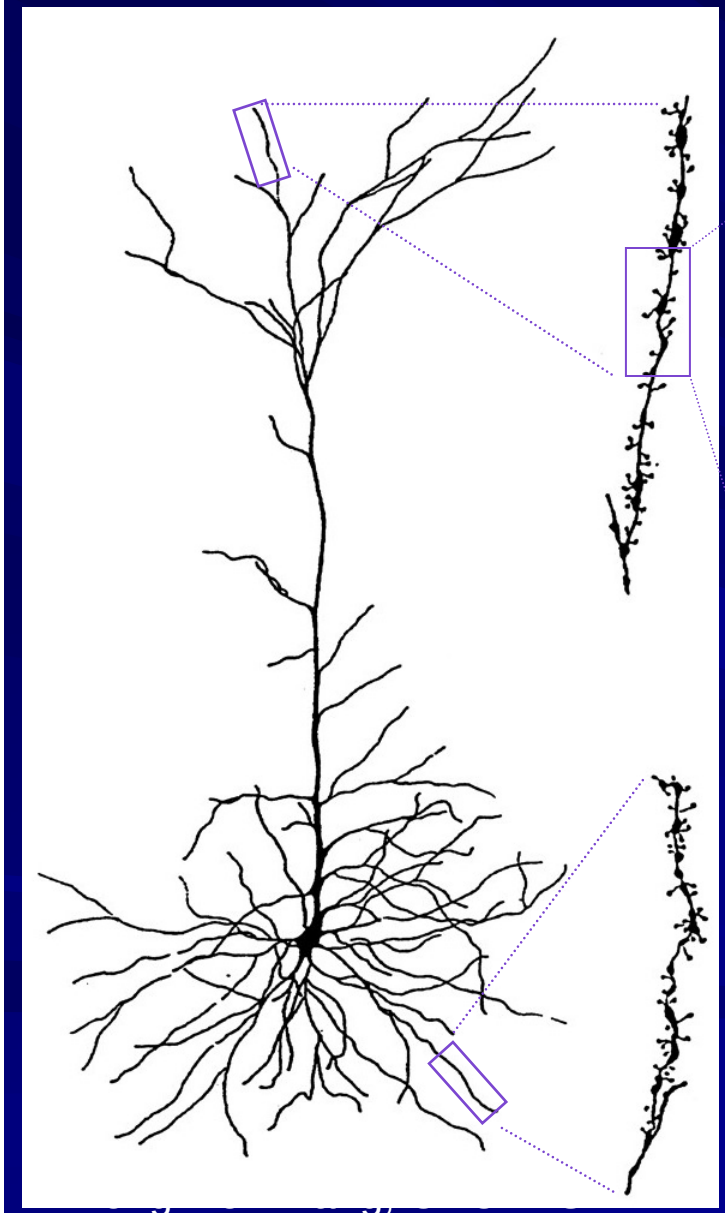
Vesicle (40 nm size “bag”)
Containing
Neurotranmitter
Chemicals

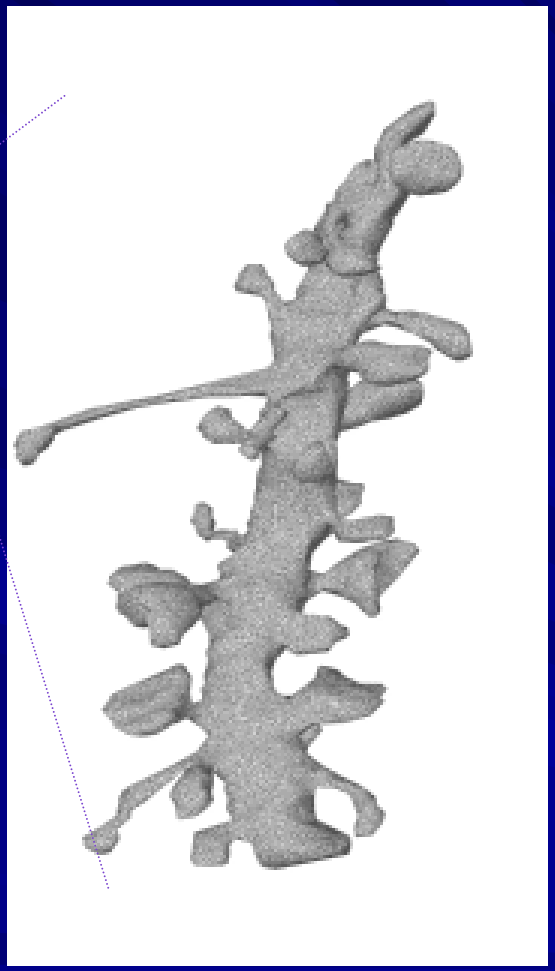
Synapse (just a Gap)

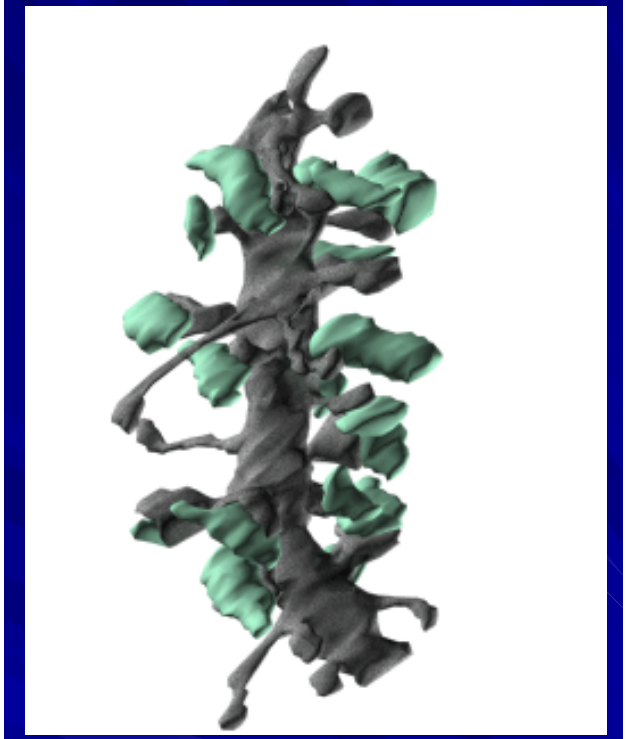
Dendritic Spine
Postsynaptic
Neuron2

http://www.univ-orleans.fr/neurobiologie/ENGLISH/images_recherche.htm

3D Reconstruction of Spines…





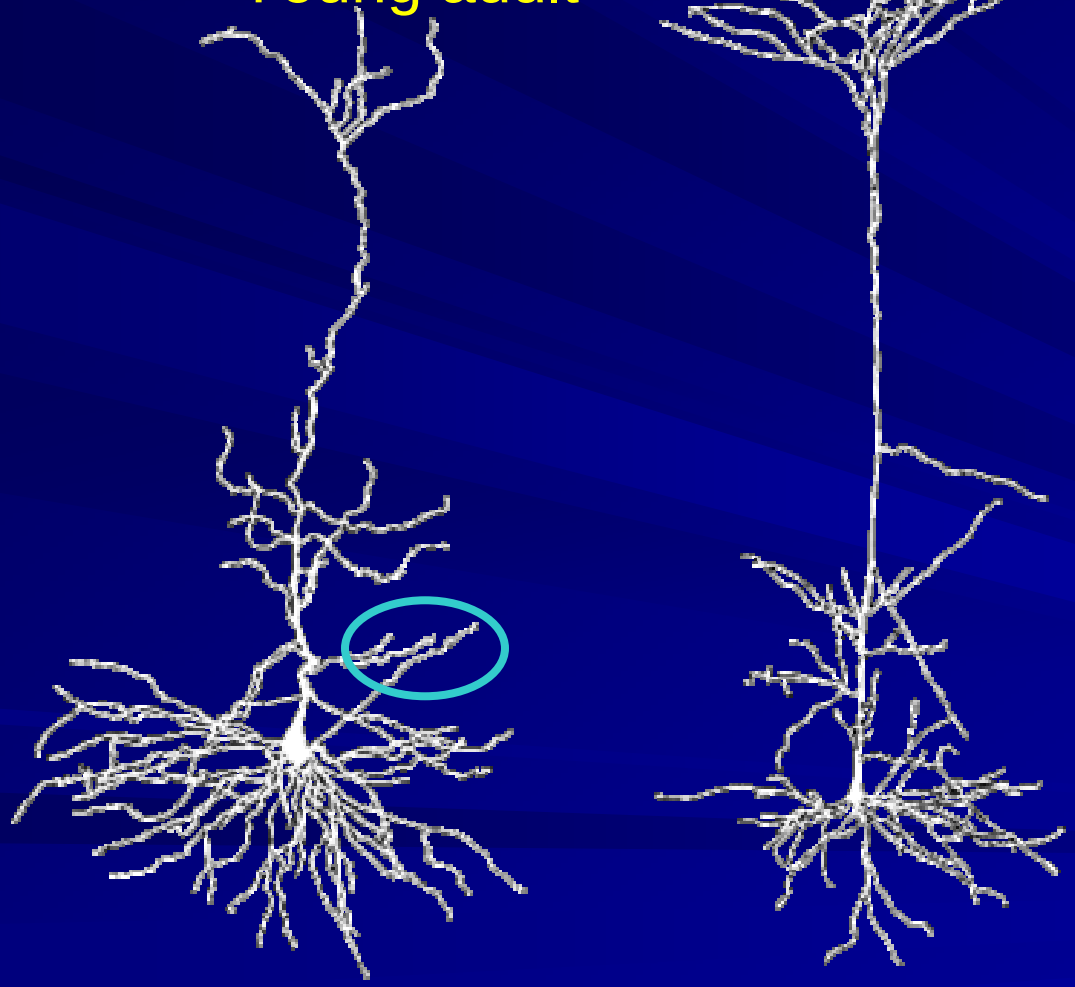


http://synapse-web.org/

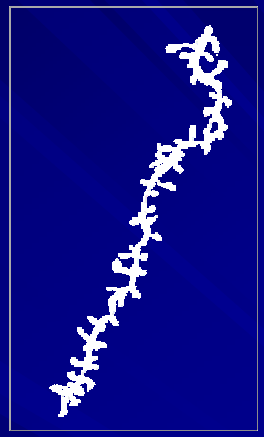
Pruning of Synaptic Connections

Young
Rat 2 months
Young adult

Mature
Rat 32 months
= 80 years old

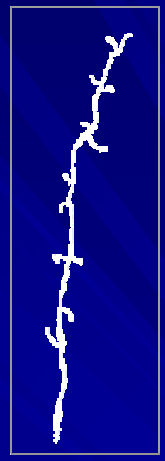


Young



More Spines

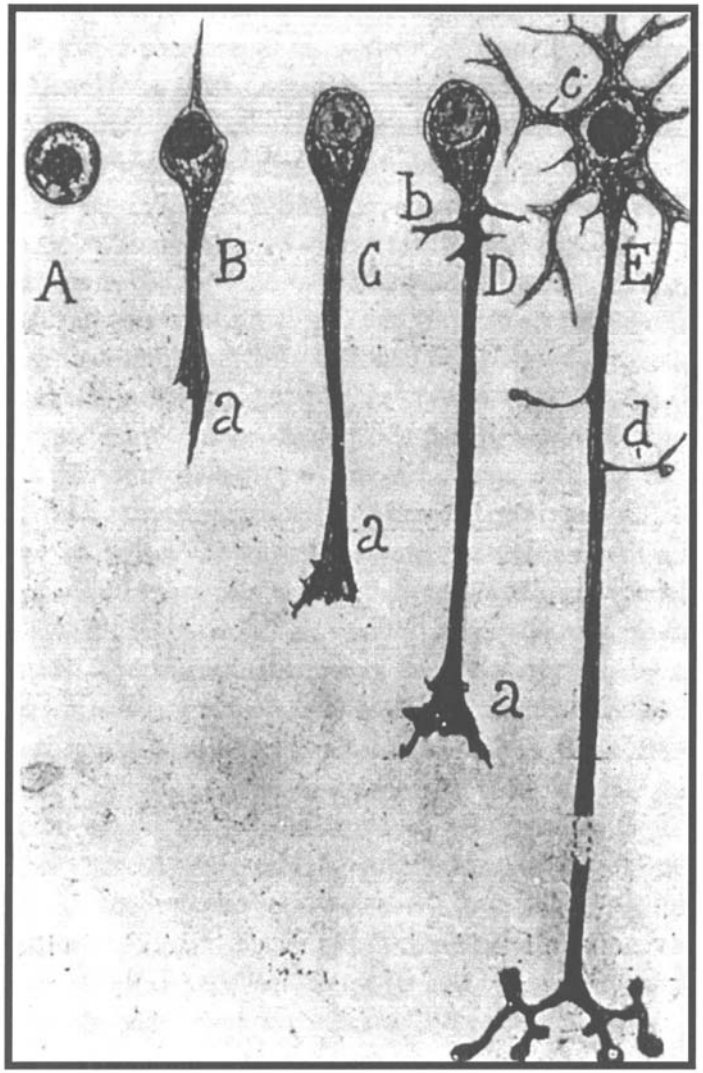
Mature



Fewer Spines

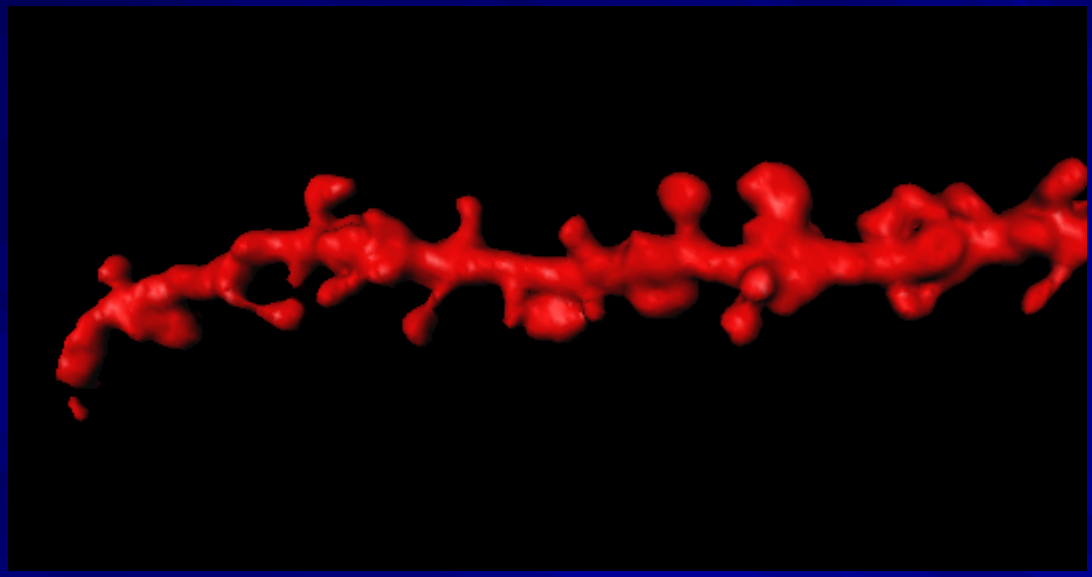
Courtesy of Prof. Yves De Koninck, CRULRG Laval Univ.

Synaptogenesis…New Connections



Once again…
Santiago Ramón y Cajal
Was the pioneer…

Dendritic Spines are Dynamic!



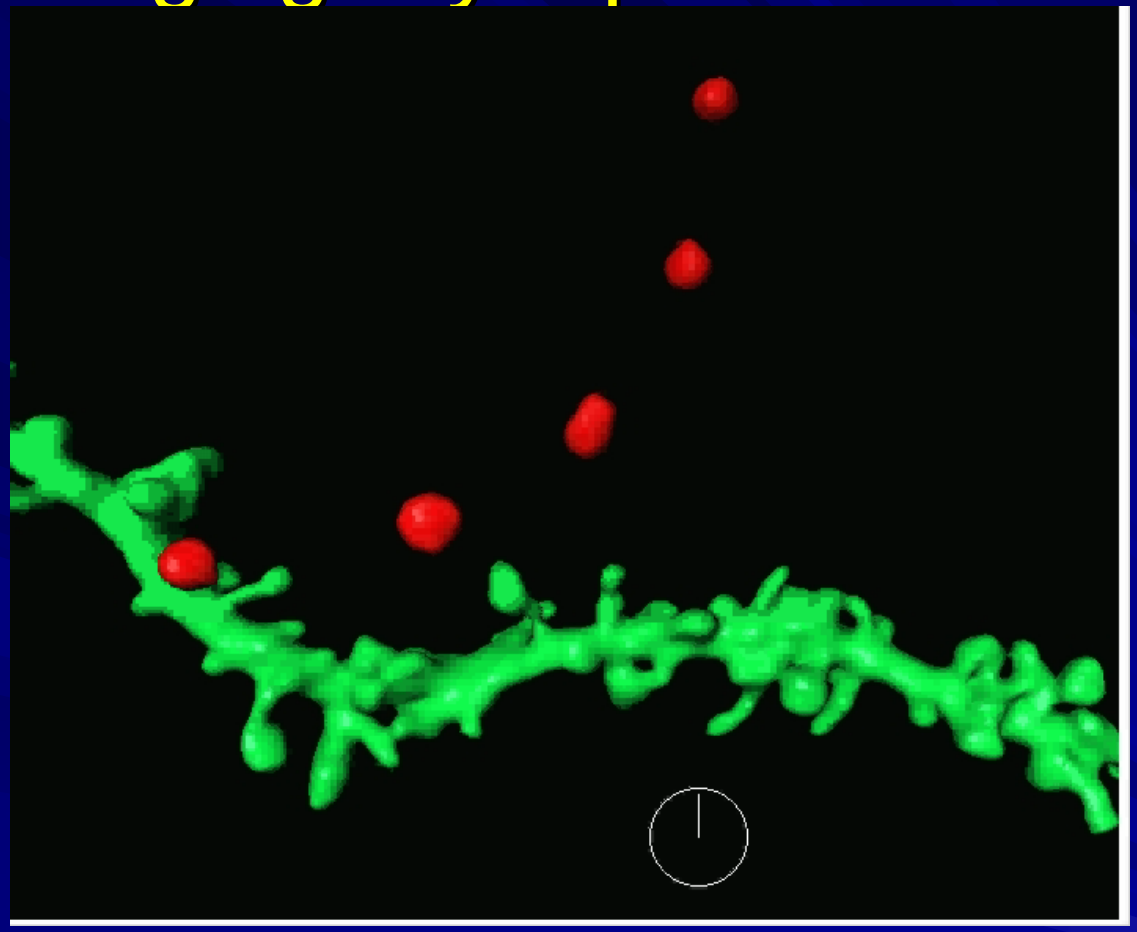
15 min loop

CA1 pyramidal cell GFP

3 week old hippocampal tissue culture

Courtesy of Prof. Anne McKinney McGill Univ. Pharmacology

3D Imaging: Synapse Formation?



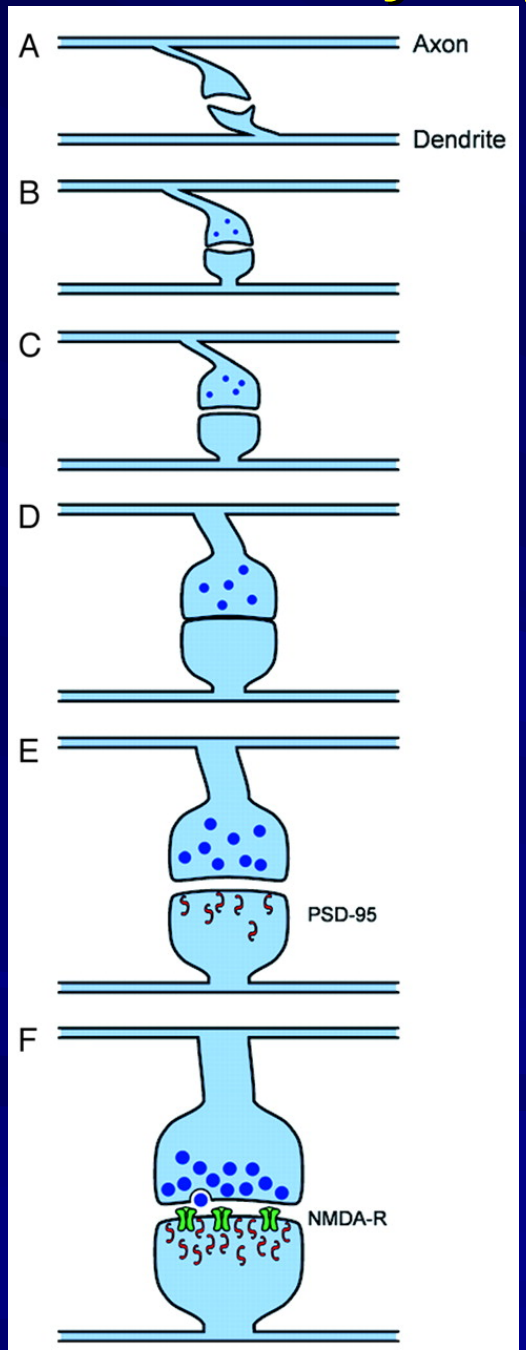
15 min

Membrane-targeted GFP spines (postsynaptic)

FM 4-64-labeled bouton (presynaptic)

Prof. Anne McKinney McGill Univ. Proc Natl Acad Sci U S A. 2005 Apr 26;102(17):6166-71.

Synaptogenesis… Formation of New Synapses



The Brain and Nervous System are “plastic”

New connections being made

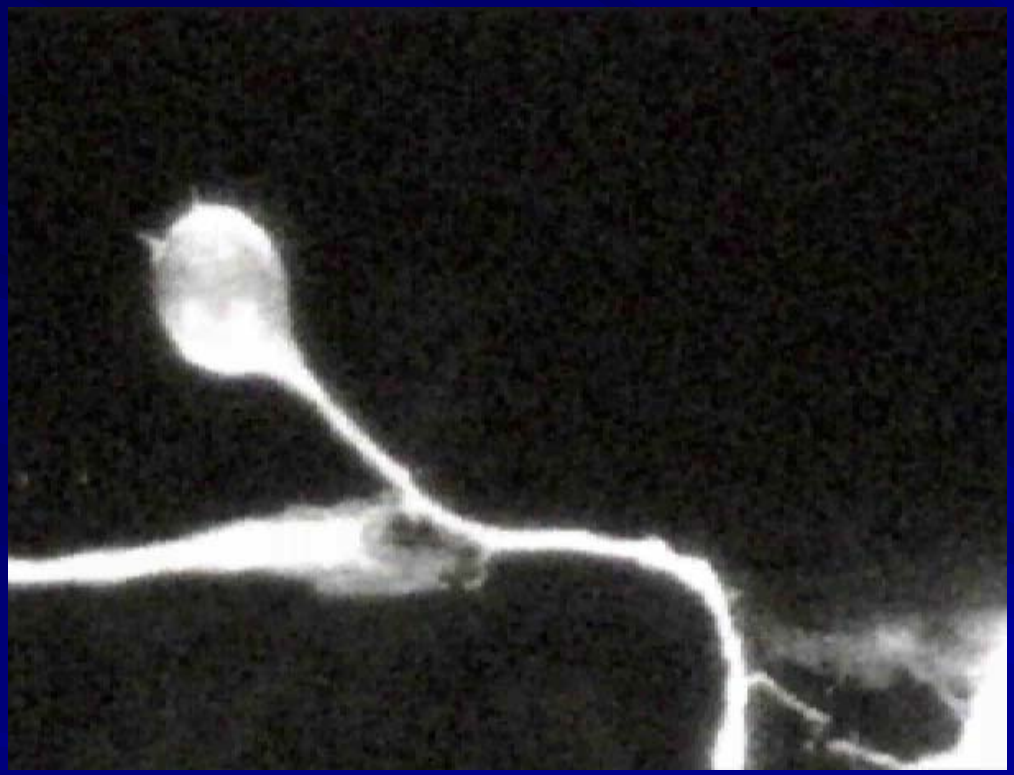
Old ones connections disassembled

Rewiring of the Brain…
Neuroplasticity

Mounting Evidence that using the brain is Important in keeping function…
“Use it or lose it!”

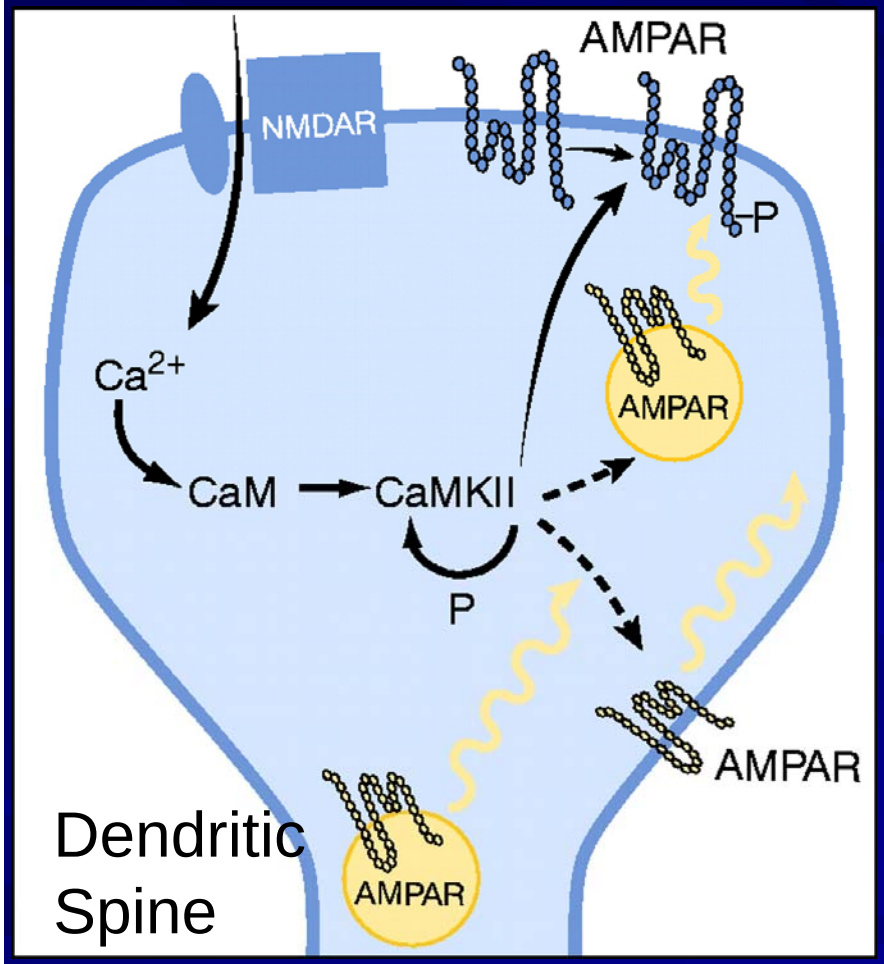
Sometimes it wasn't meant to be!

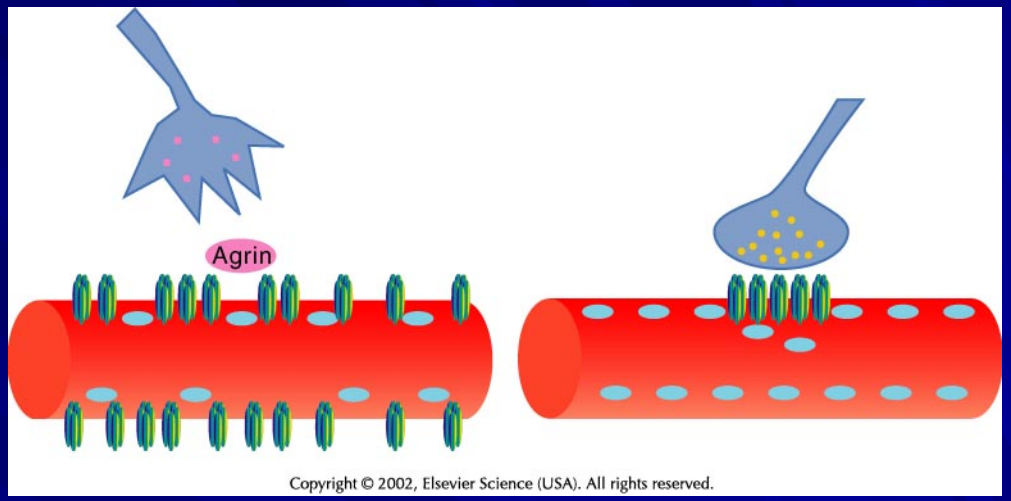
Calcium signalling in Astrocytes
Prof. Steve Smith Stanford University



Jontes, J.D., Buchanan, J. and Smith, S.J (2000)
Growth cone and dendrite dynamics in zebrafish embryos: in vivo imaging of early events in synaptogenesis.
Nature Neuroscience 3: 231-237

Synaptogenesis…New Synapses



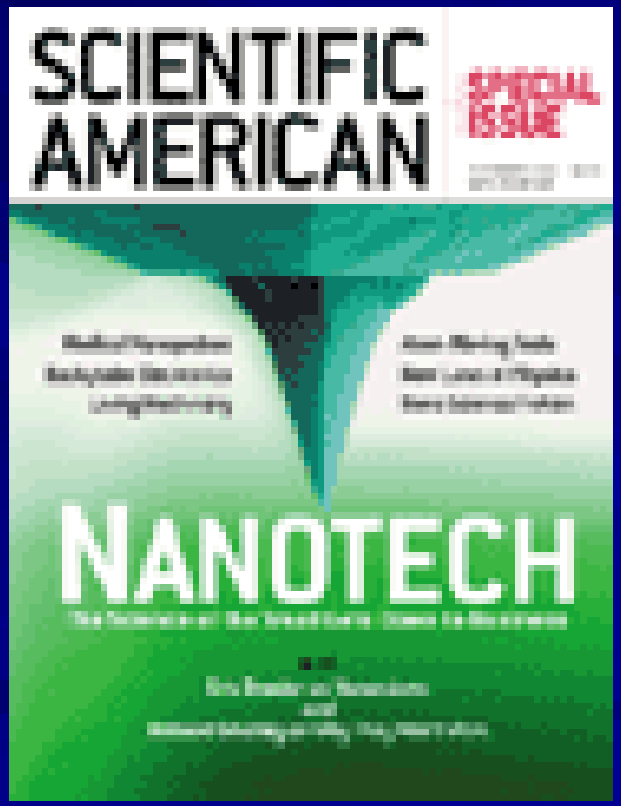


Receptors for neurotransmitters
Redistribute into spines…

They are dynamic…
How do they move?

Track receptors with Nanotechnology

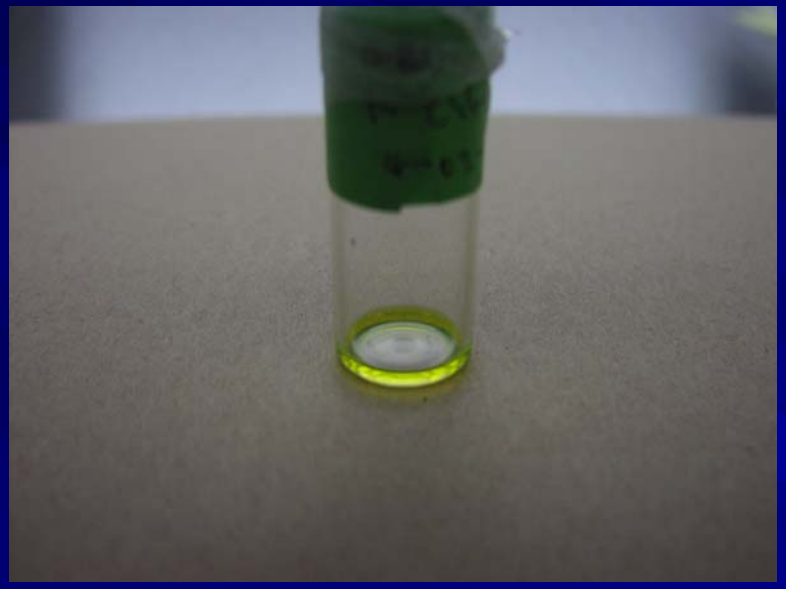
- See Sept. 2001 Sci. American: Nanotech
- Article by Alivisatos on Quantum Dots
- Very small particles made out of semiconductor materials



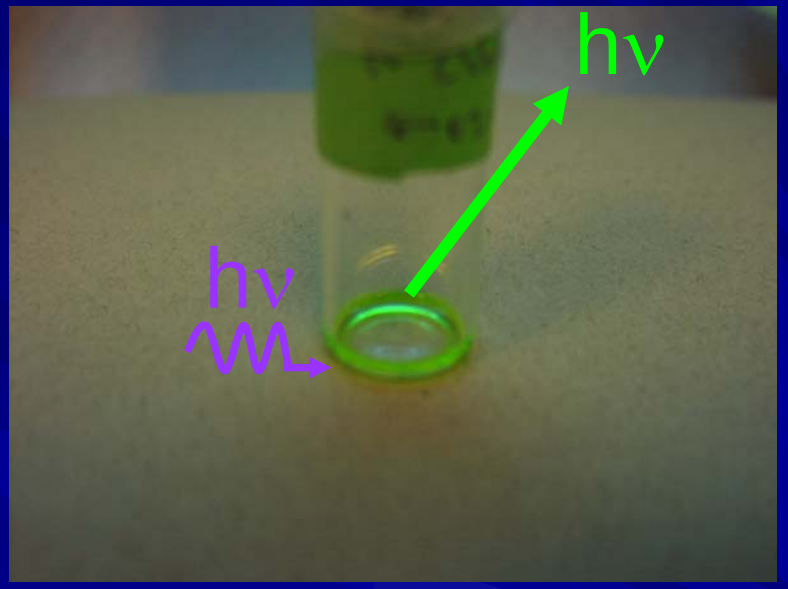
Quantum Dots…Nanoparticles

- Cadmium selenide/Zinc sulfide Quantum Dots in solution

Green Fluorescence

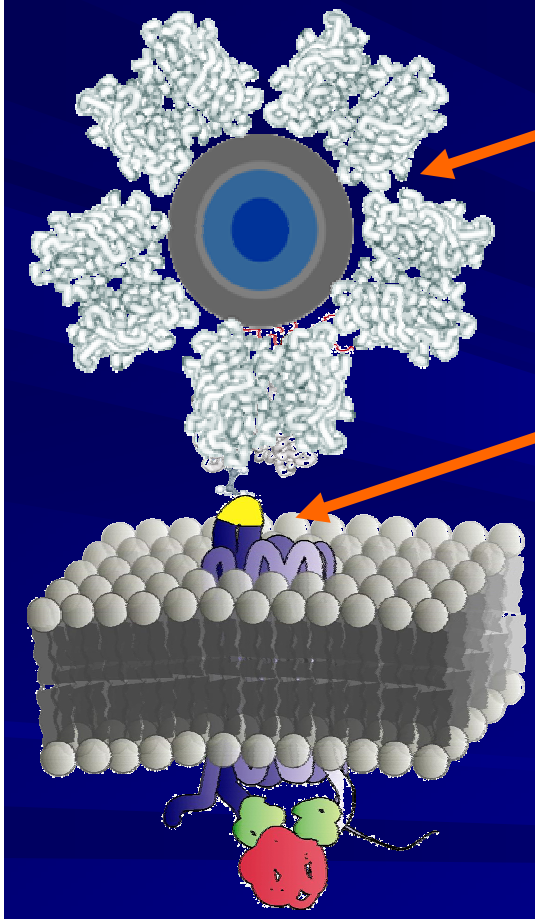


Under Room Light



Under ultraviolet Light

Tracking Protein Receptor Motions With Quantum Dots



Quantum Dot Marker

Attach QD to Protein in Cell Membrane

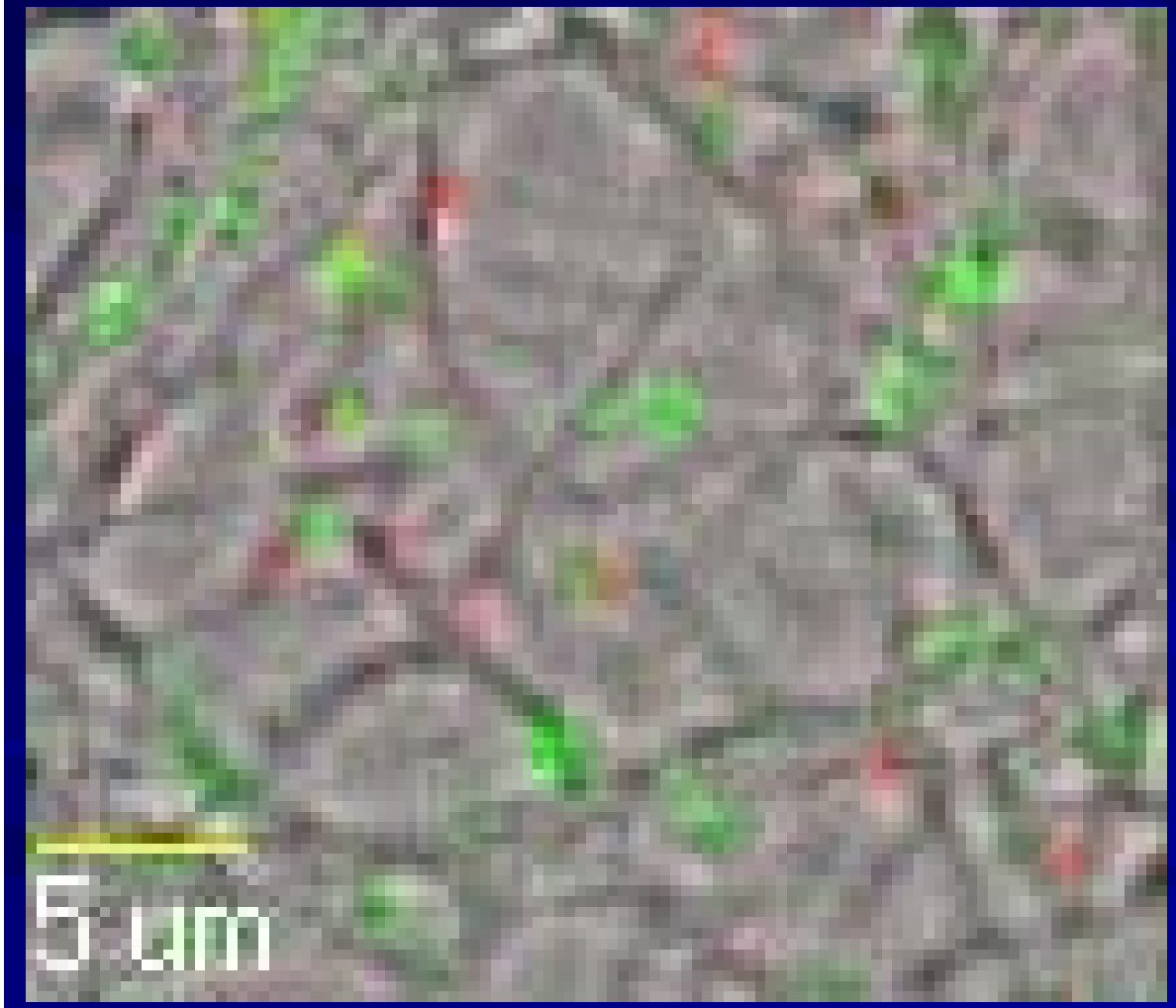
Video Microscopy Single Particle Tracking

Track Trajectories of QD Protein Complexes In Time

Like Observing Skiers with Torches at Night

Tracking Protein Receptor Motions With Quantum Dots

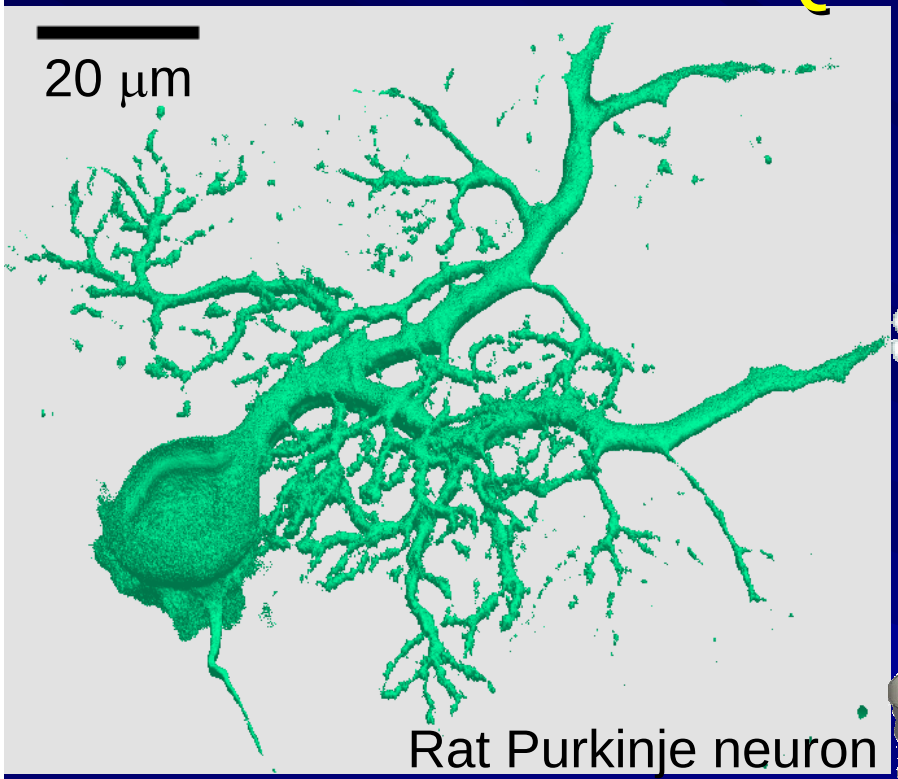
Green = Synapse Marker

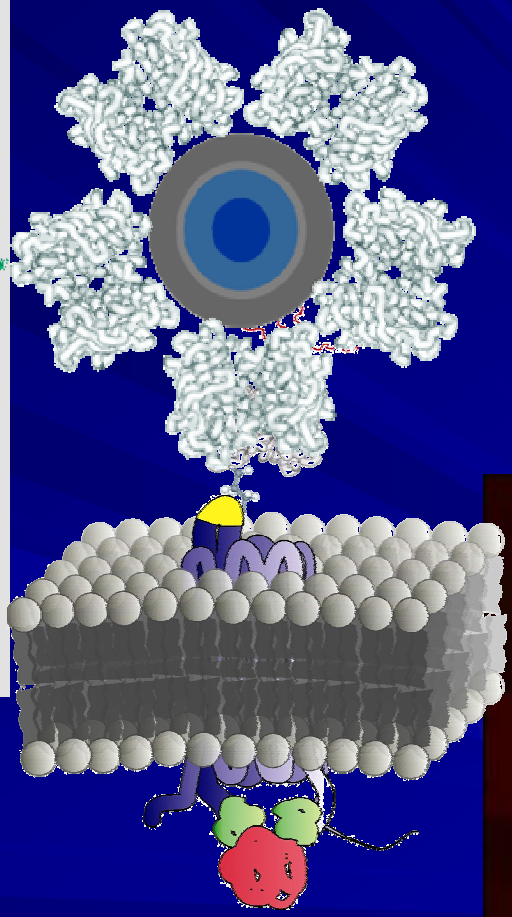


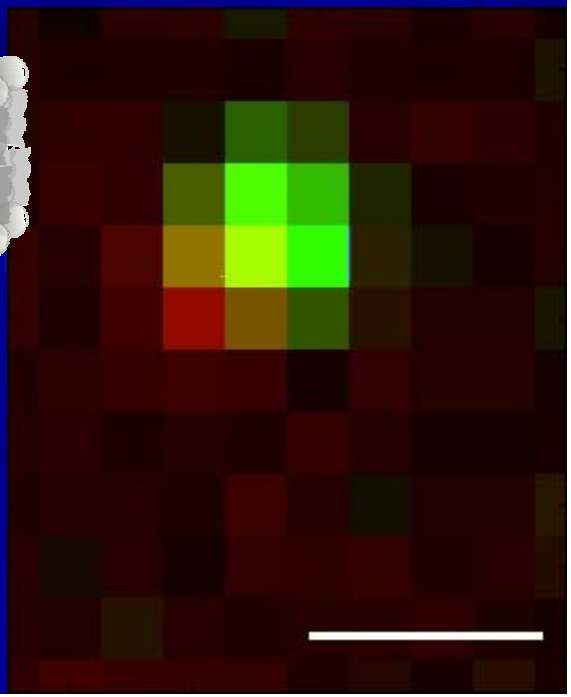
Red = QD
Glutamate
Rector in the
Dendrite of
Living neuron

Wiseman Group McGill with Prof. Paul DeKoninck Laval University

Tracking Protein Receptor Motions With Quantum Dots







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

Interdisciplinary Science…

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

NeuroPhysics

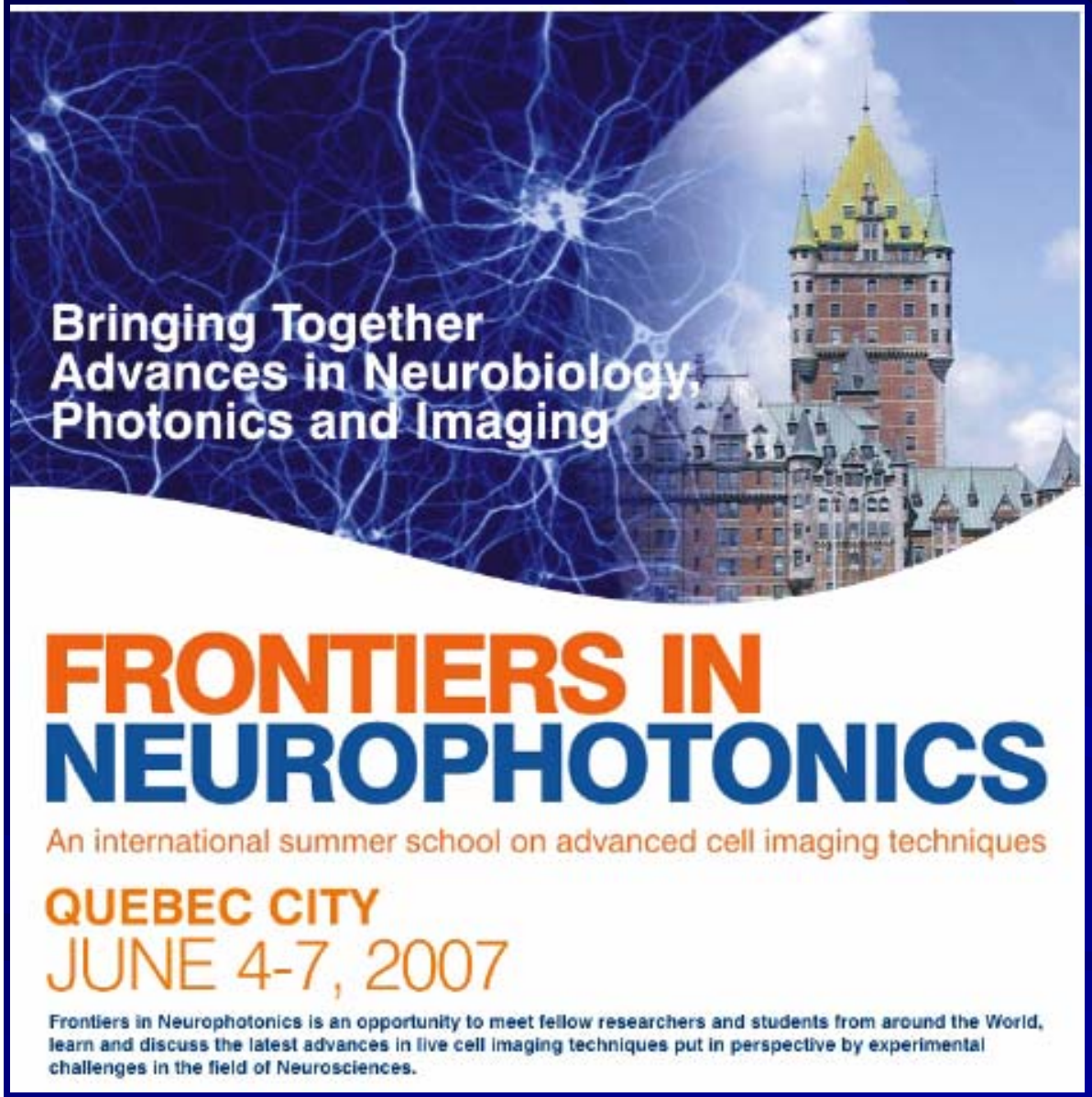
"Setting new frontiers in neuroscience" –
"Repousser les frontières des neurosciences"

UNIVERSITÉ LAVAL

CIHR IRSC

CIHR Training Grant
Training Physical Scientists
To Tackle Problems in Neuroscience

Interdisciplinary Science…



Training Future Scientists Within Canada to Tackle such complex Problems

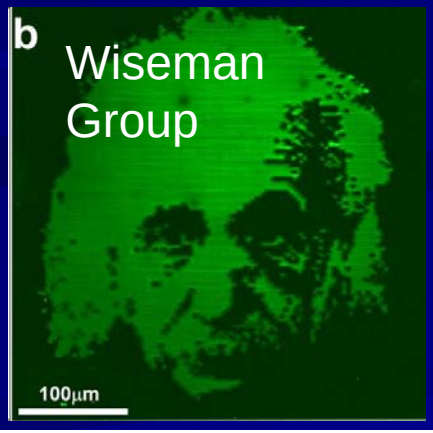
Imaging Paul Wiseman's Brain…

Acknowledgements





- Prof. Paul De Koninck Laval University
- Prof. Anne McKinney McGill University
- Prof Yves De Koninck Laval University
- Prof. Dave Colman MNI McGill University
- Prof. Tim Kennedy MNI McGill University



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!