

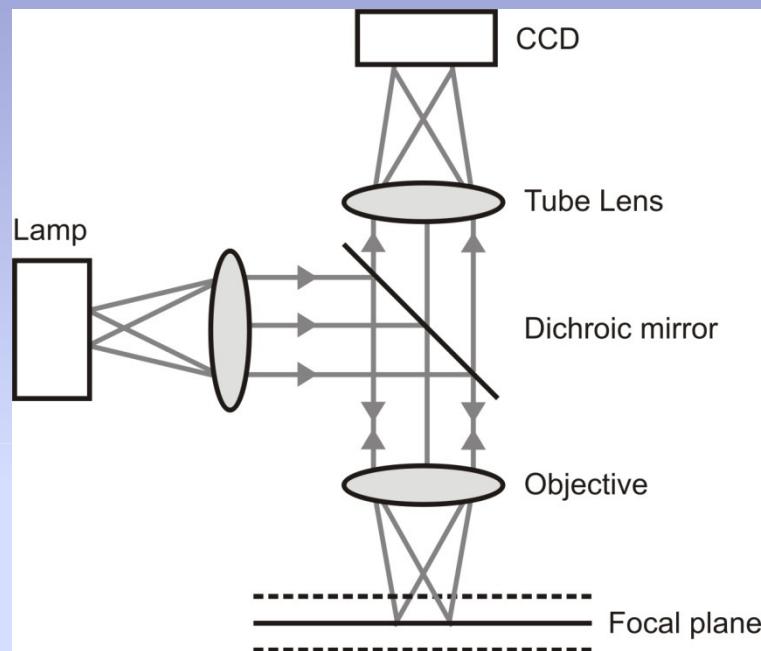
Non linear morpho-functional imaging of tissues

Francesco Pavone

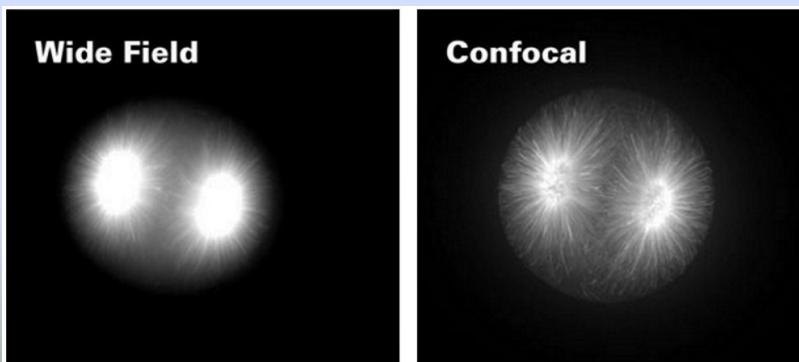
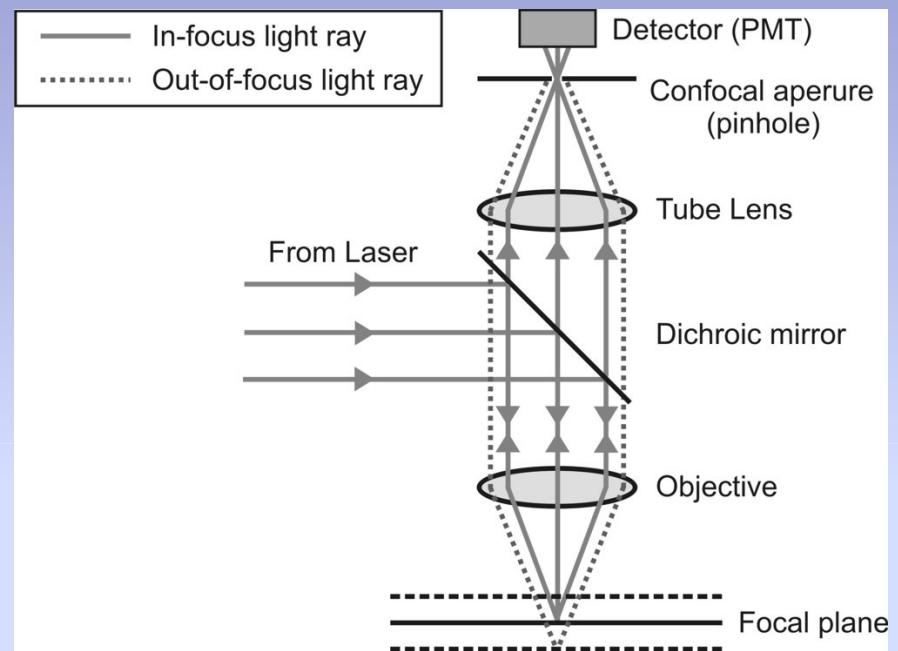
**Lab. N. 30
Dipartimento di Fisica**

Fluorescence microscopy

Wide Field Microscopy



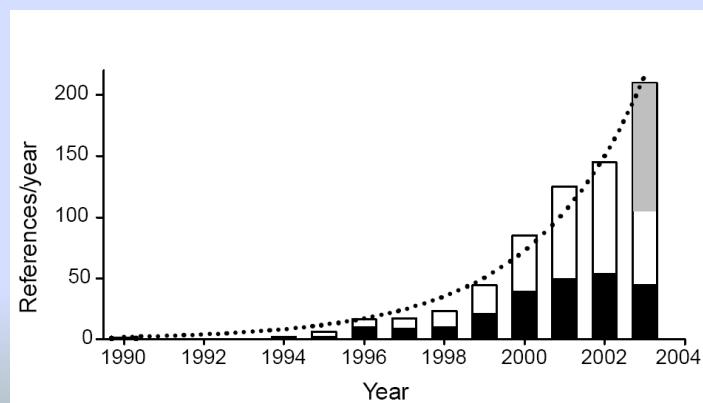
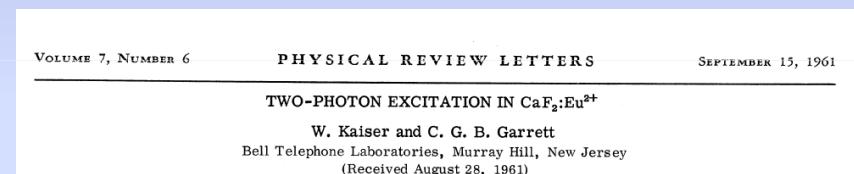
Confocal Microscopy



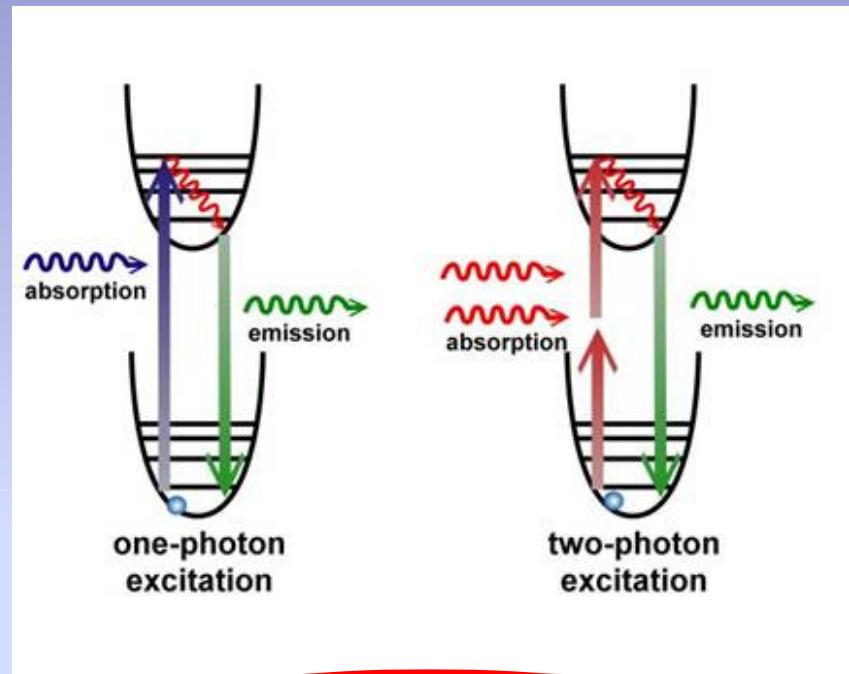
Comparison of a wide filed fluorescence with a confocal image of a sea urchin egg (labeled tubulin)*.

Two-photon excitation

- 1931 First quantum theory for an electronic transition excited by simultaneous multiphoton absorption (Maria Goeppert-Mayer).
- 1961 First experimental observation of two-photon excited fluorescence in a $\text{CaF}_2:\text{Eu}^{2+}$ crystal (Kaiser and Garret).
- 1990 First two-photon laser scanning fluorescence microscope (Denk, Strickler and Webb).
- 2000 Explosion of two-photon microscopy in the biomedical field.

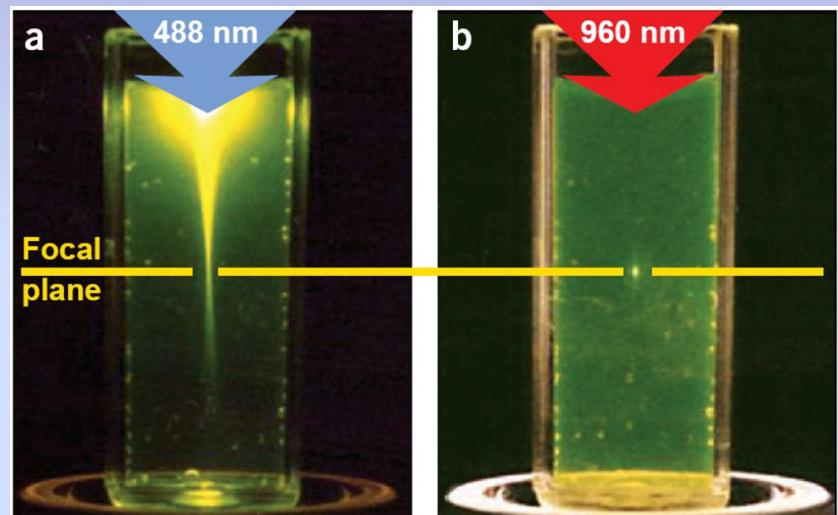


Two-photon fluorescence (TPF) microscopy



Transition probability $\propto I^2$

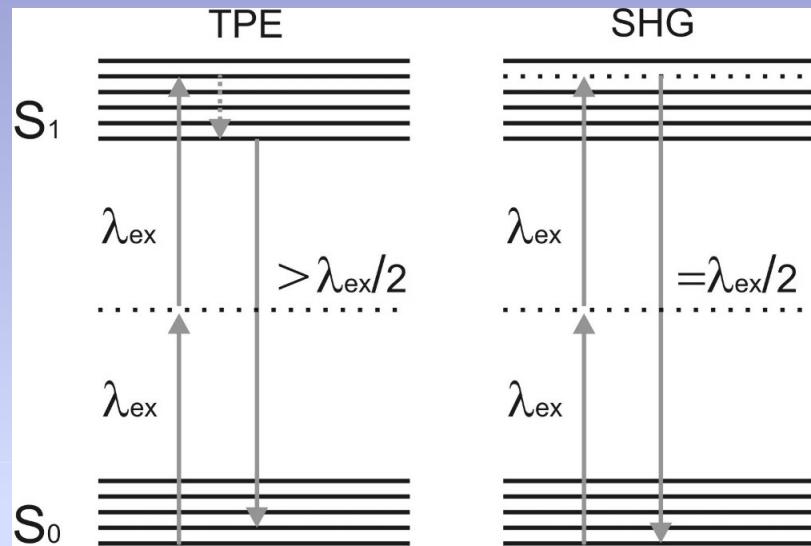
Excitation Volume



(a) Single photon excitation of fluorescein by focused 488 nm light (0.16 NA). (b) Two-photon excitation using focused (0.16 NA) femto-second pulse of 960 nm light*.

Second-harmonic generation microscopy

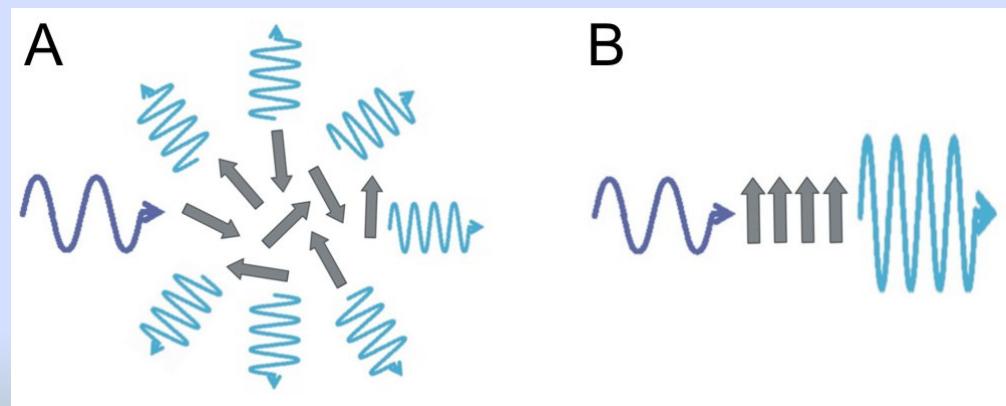
The diagram



The diagram comparing the photo-physical pathways for two-photon excited fluorescence (left side) and resonance enhanced second harmonic generation (right side).

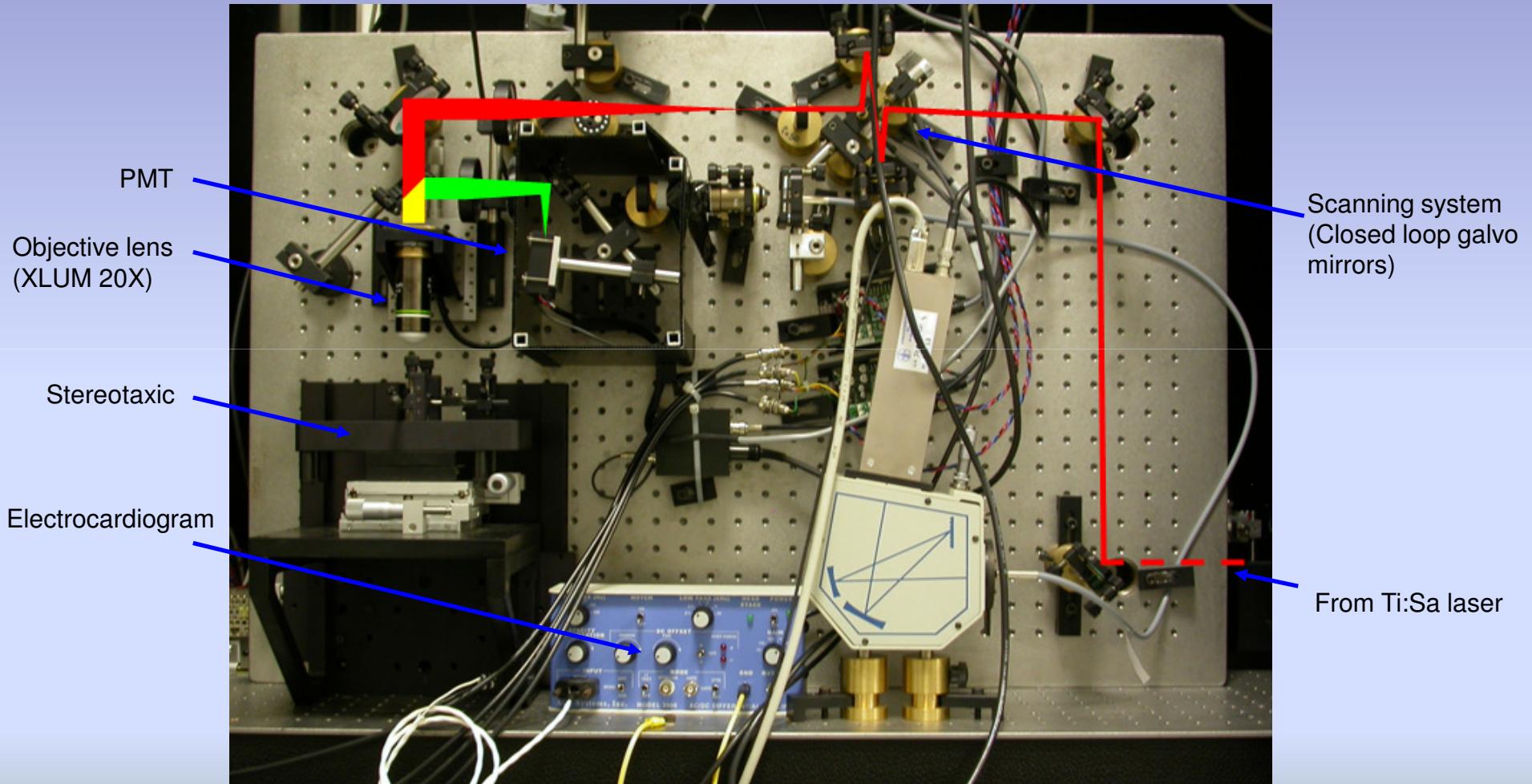
Coherent Summation

- The SHG from a population of N molecules (dash arrows).
- A: Randomly oriented molecules scatter incoherently and the total SHG power scales as N .
- B: Aligned molecules scatter coherently, the power is well collimated and scales as N^2 .



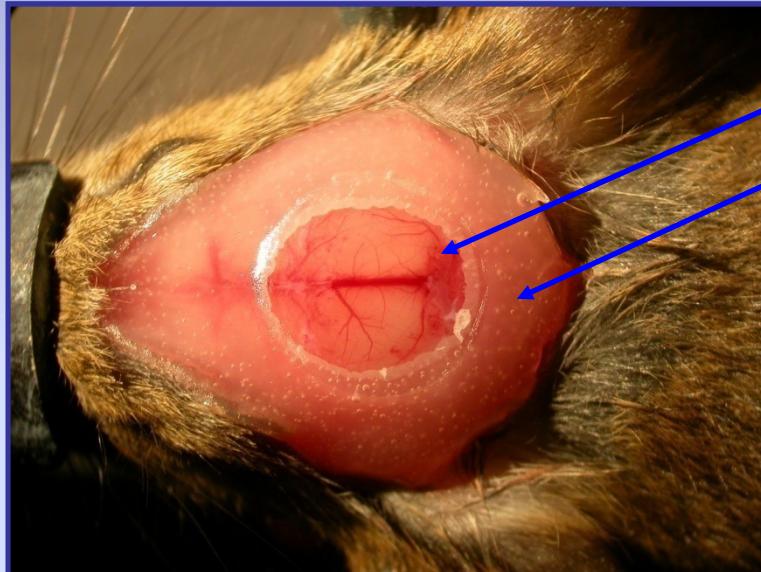
Custom TPE microscope

Custom-made upright two-photon microscope



Permanent window

Mouse was deeply anaesthetized with an intraperitoneal injection of ketamine (0.13 mg per g body weight) and xylazine (0.01 mg g⁻¹). A region of the skull was removed, exposing the brain. An optical chamber was then constructed by covering the intact dura with physiological solution and a cover-glass, sealed with dental acrylic.



Optical glass window (100µm thickness)

Dental acrylic

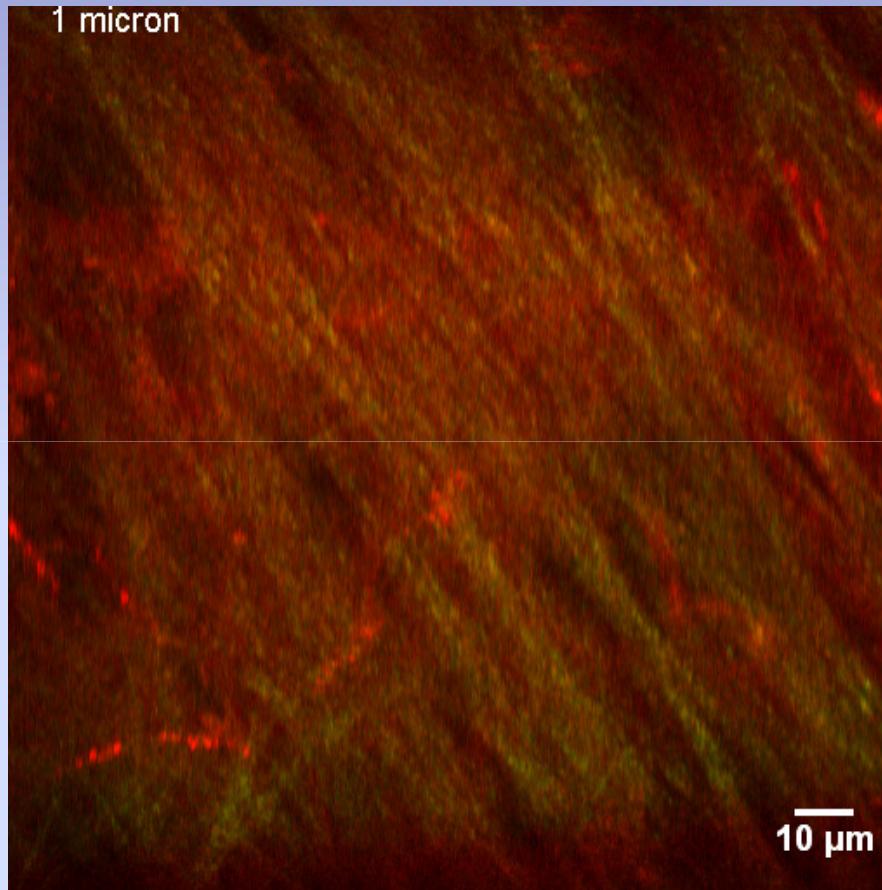


This methodology prevent the motion induced by the
head and allow long-term studies.

Awake mice with the permanent window

All experiments were done in accordance with protocols approved by European Community.

Multiphoton Multicolor *In Vivo* Imaging of the Cortex



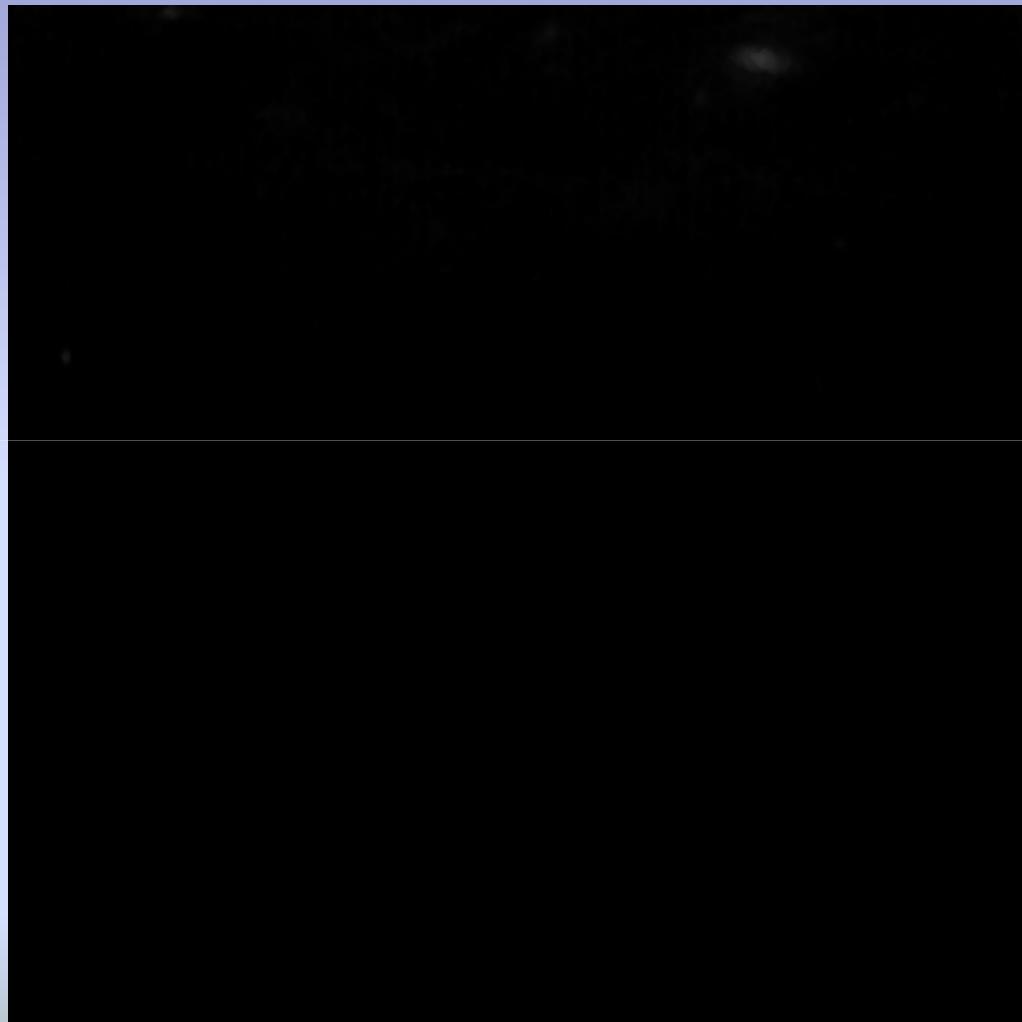
Green fluorescent protein expression in dendrites
Dura visualized by 2nd harmonic generation signal of collagen
Blood vasculature visualized by injection of Texas Red dextran

Plasticity

In vivo imaging

Imaging into brain cortex of a P90 GFP-M transgenic mouse
Developed in Sanes Lab

- Images from 0 to 90 μm depth
- $100 \times 100 \mu\text{m}$ area
- 1 μm z stack
- 512×512 resolution
- Integration time 5 $\mu\text{sec} \times \text{pixel}$
- 50mW @ 935 nm
- 120 fs pulselwidth
- Laser wavelength 935nm

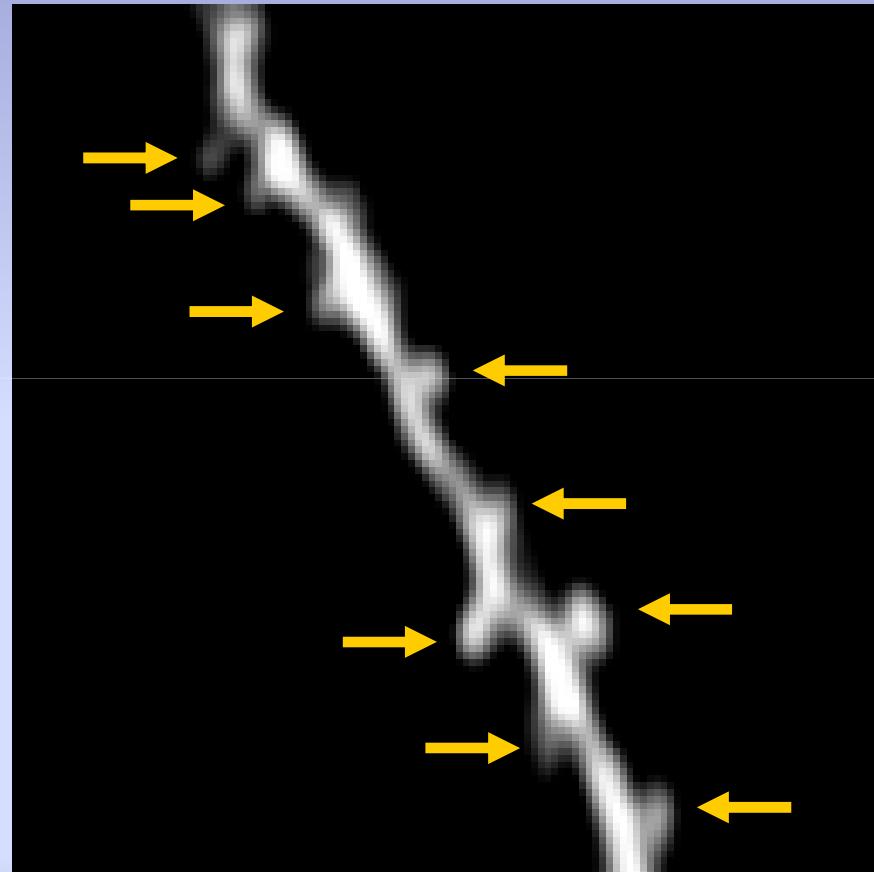


The movie shows a partial neurite network made of the GFP labeled neurons.

Zooming: dendritic spine observation

- 100 μm below the brain surface
- 20 \times 20 μm area
- 512 \times 512 resolution
- 39nm x pixel
- 800nm radial, 2.4 μm axial resolution
- Integration time 2.5 μsec x pixel
- 50mW @ 935 nm
- 120 fs pulselwidth

Portion of a dendrite with several dendritic spines

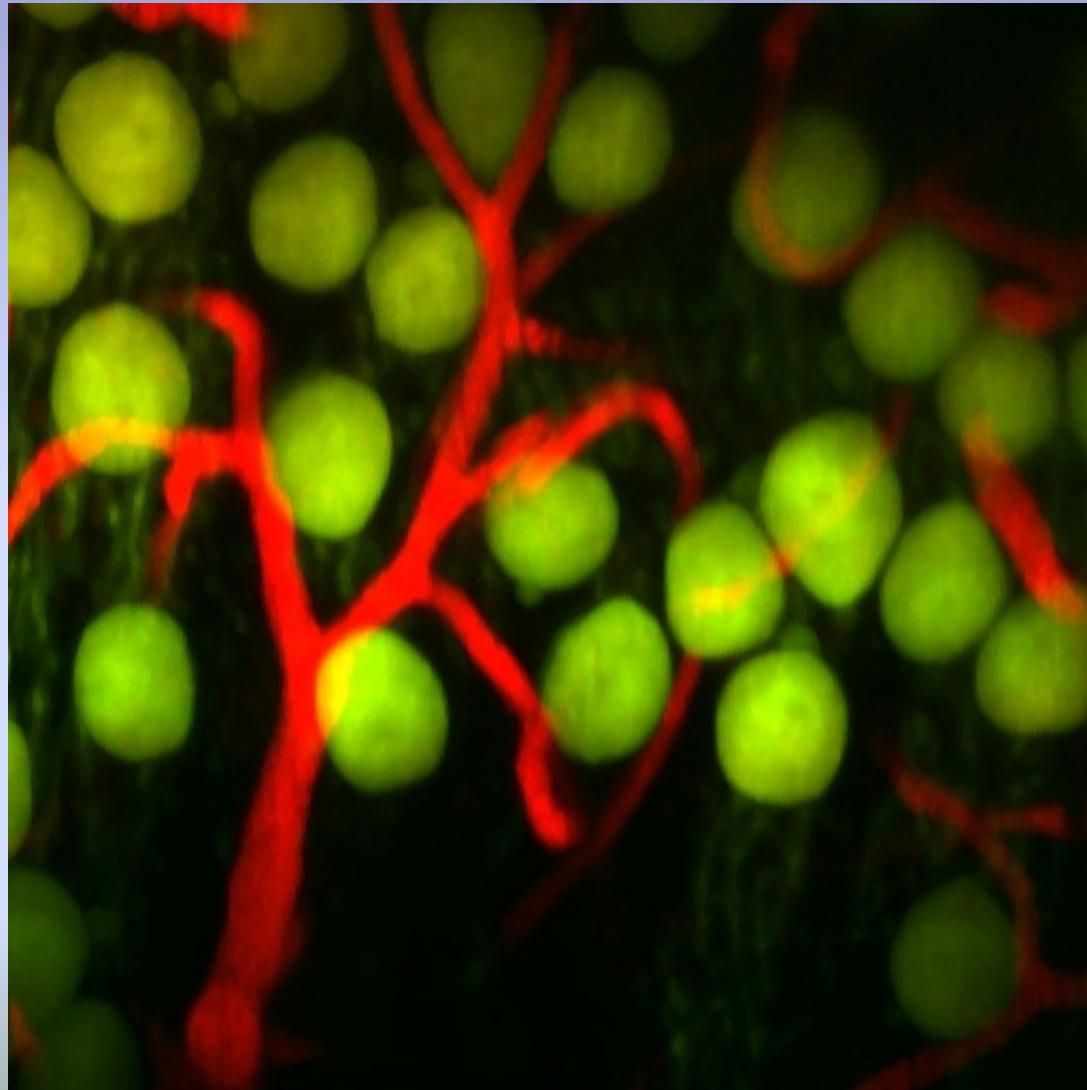


Two-color imaging

Imaging into cerebellum of a P85 GFP-L7 transgenic mouse
Texas red dextran labeling of blood vessel

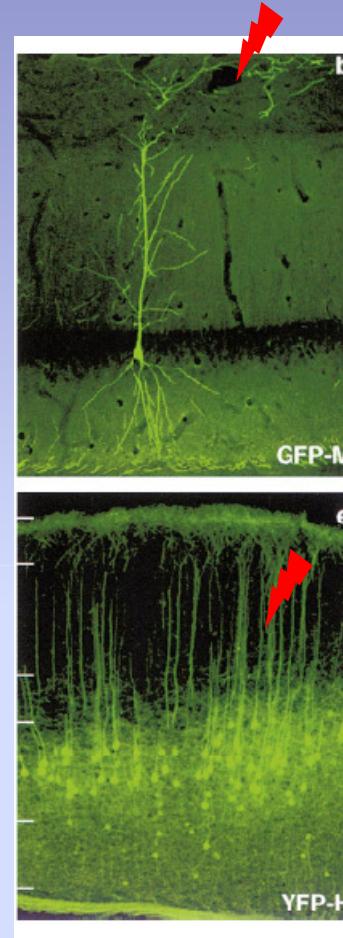
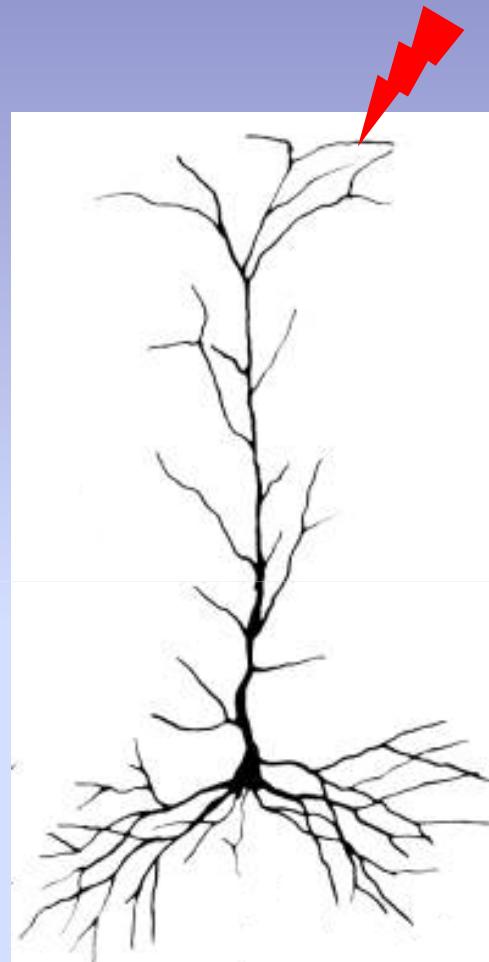
- Maximum-intensity projection (20-120 μ m)
- 150 \times 150 μ m area
- 512 \times 512 resolution
- 290nm per pixel
- 800nm radial, 2.4 μ m axial resolution
- Integration time 2.5 μ sec x pixel
- 50mW @ 935 nm
- 120 fs pulselwidth
- Laser wavelength 935nm

Purkinje neurons
Blood vessel



In Vivo Laser Induced Nanosurgery

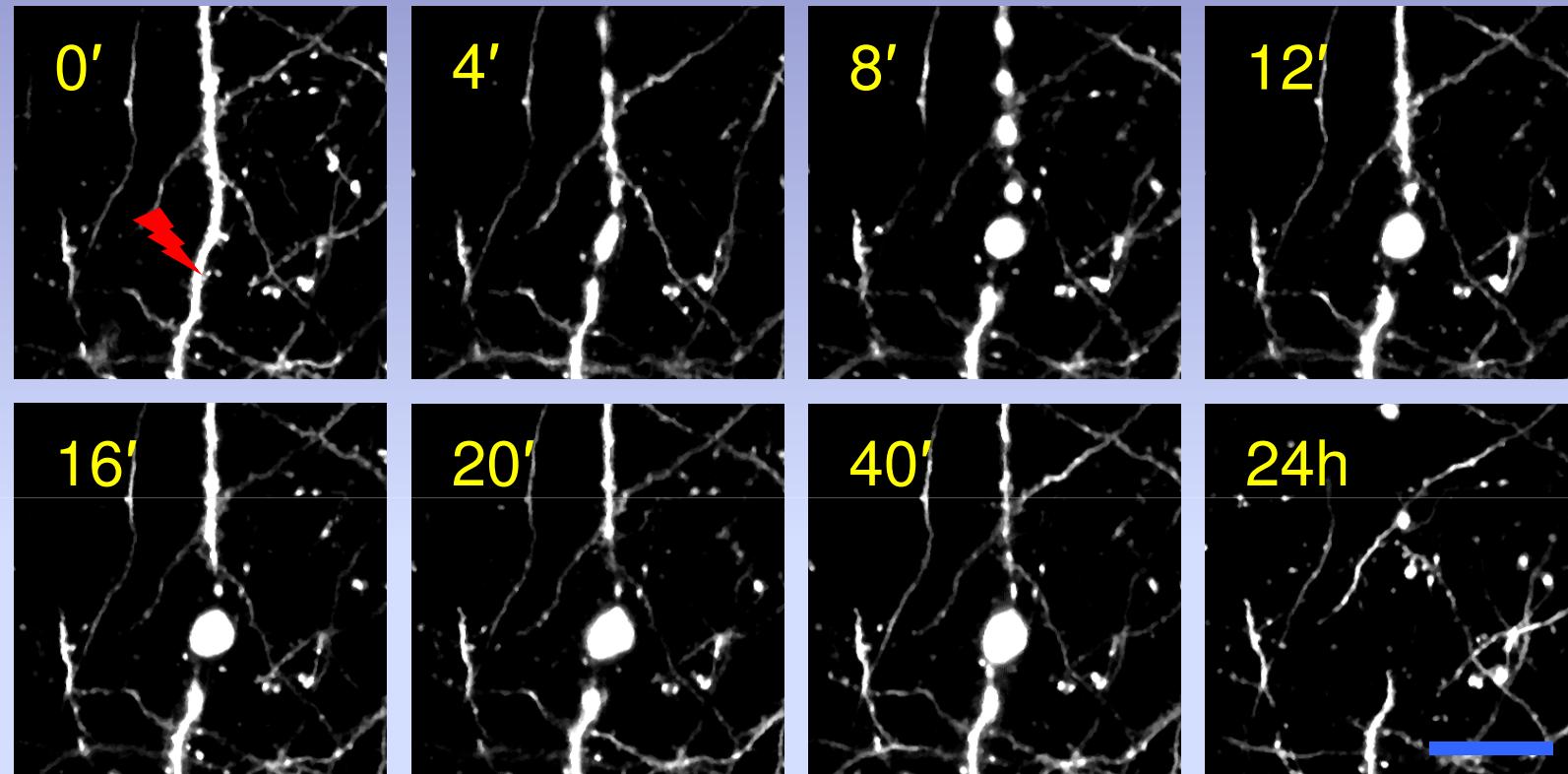
In vivo multiphoton nanosurgery



The morphological consequences of laser irradiating a single dendrite can be grouped into two categories of response:

- **Transient swelling with recovery**
- **Complete dendritic dissection**

Laser-induced lesion of a single dendrite



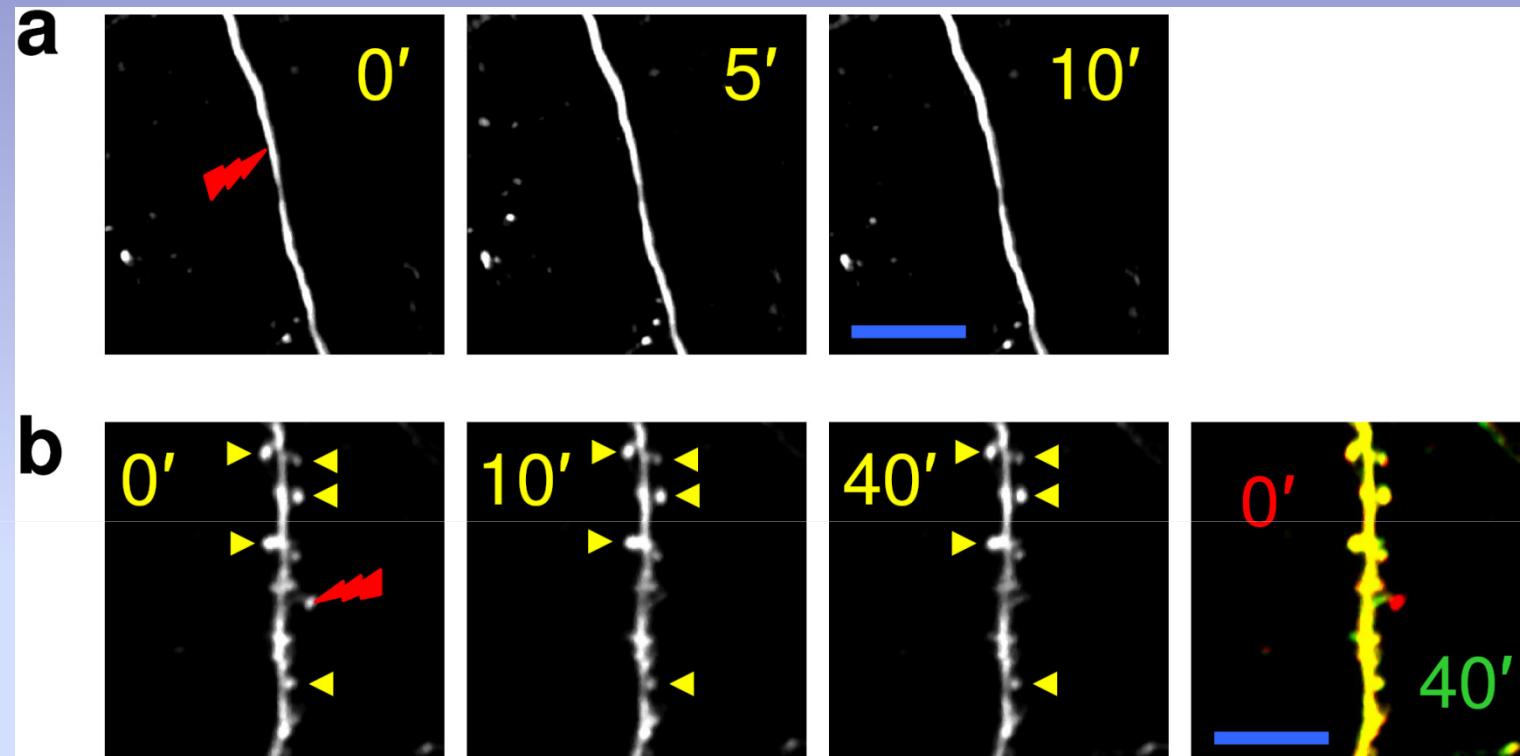
Time-lapse images of an irradiated dendrite (at about 100 μm depth) in a GFP-M transgenic mouse. The dendrite was irradiated just after the acquisition of the first image. Each image is a maximum-intensity projection of a set of optical sections acquired at a 1 μm z-steps.

Scale bar, 25 μm .

As shown in this figure, after laser irradiation the terminal end of the dendrite distal to the dissection point followed a sequence of swelling, degeneration and disappearance.

 = Laser irradiation (~ 25 mJ)

Single dendritic spine ablation



(a) The spatial precision of this method were demonstrated by irradiating an area very near the dendrite of the order of 0.5 μm but with no fluorescent features. As shown in figure, in this case we did not induce any visible alteration of the dendrite. Scale bar, 25 μm .

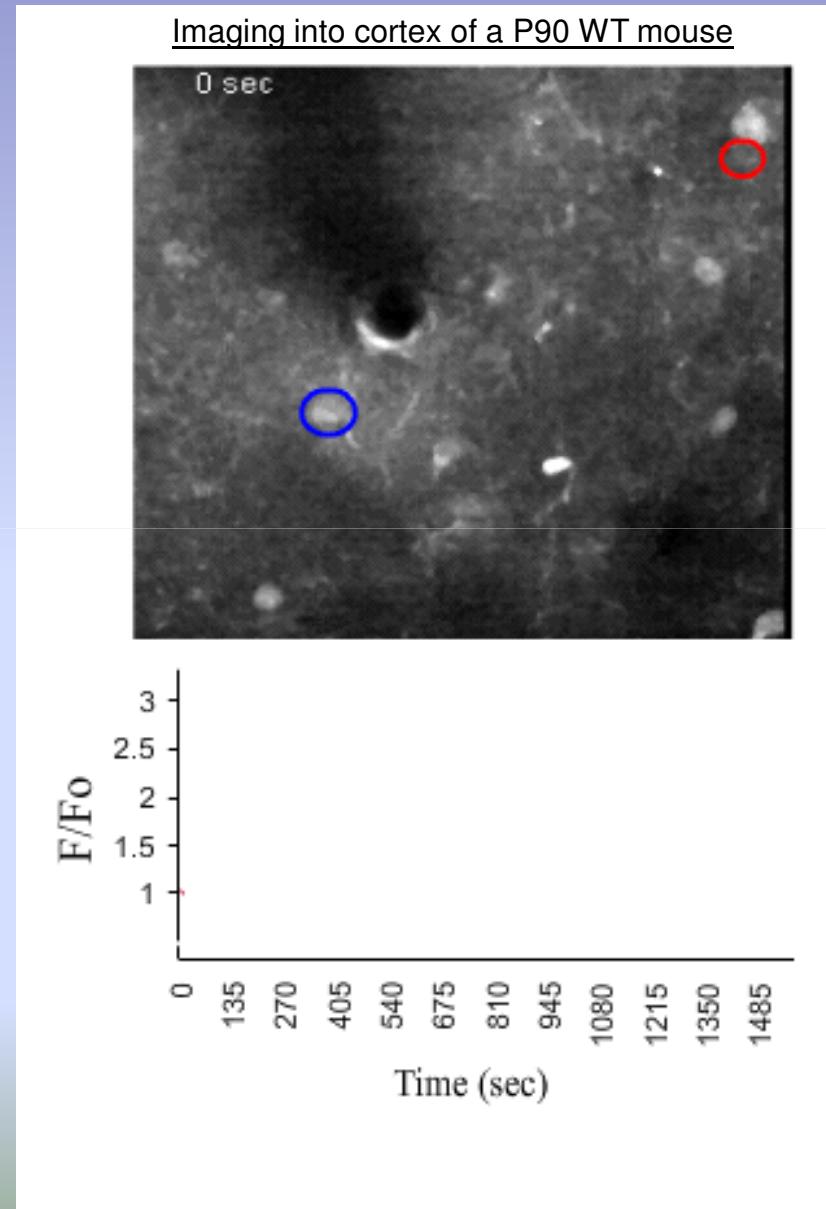
(b) The spatial localization of multi-photon nanosurgery was maximally demonstrated by the ablation of individual dendritic spines. As clearly shown in Figure, we are able to remove a single dendritic spine without causing any visible collateral damage to the adjacent spines or to the parent dendrite. Scale bar, 15 μm .

Functionality

Functional Imaging: In Vivo Calcium Imaging

By staining the brain cortex with calcium dye (Rhod-2) we can visualize calcium signal *in vivo*. This movie shows spontaneous activity in anesthetized mouse.

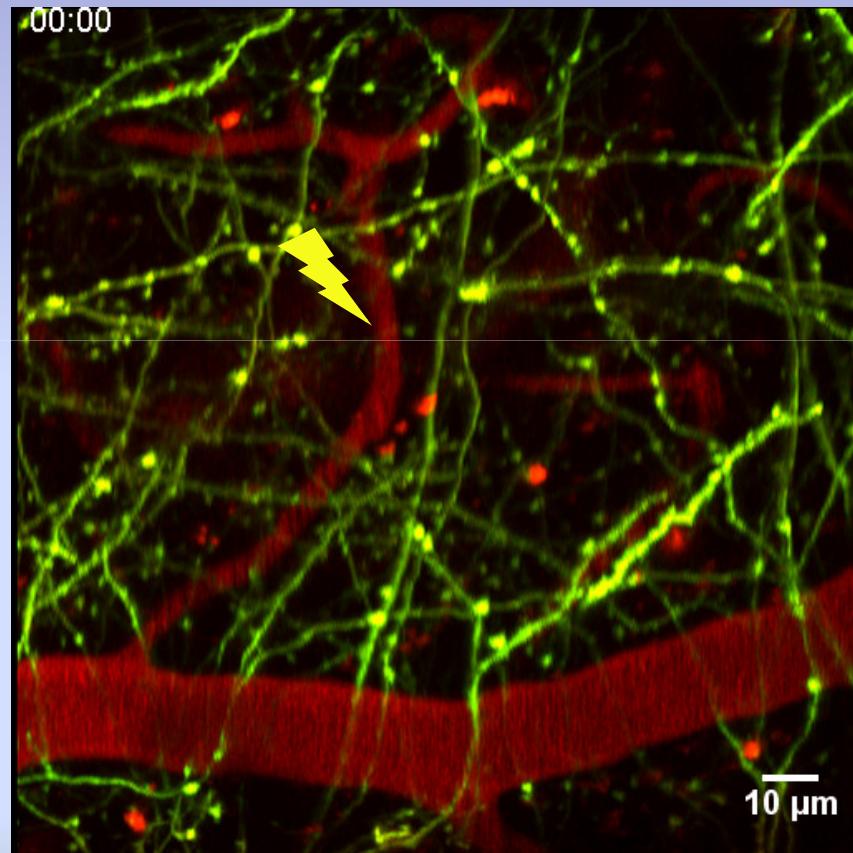
The time course of the calcium oscillation in astrocyte are shown in two different regions of interest the red one and the blue one.



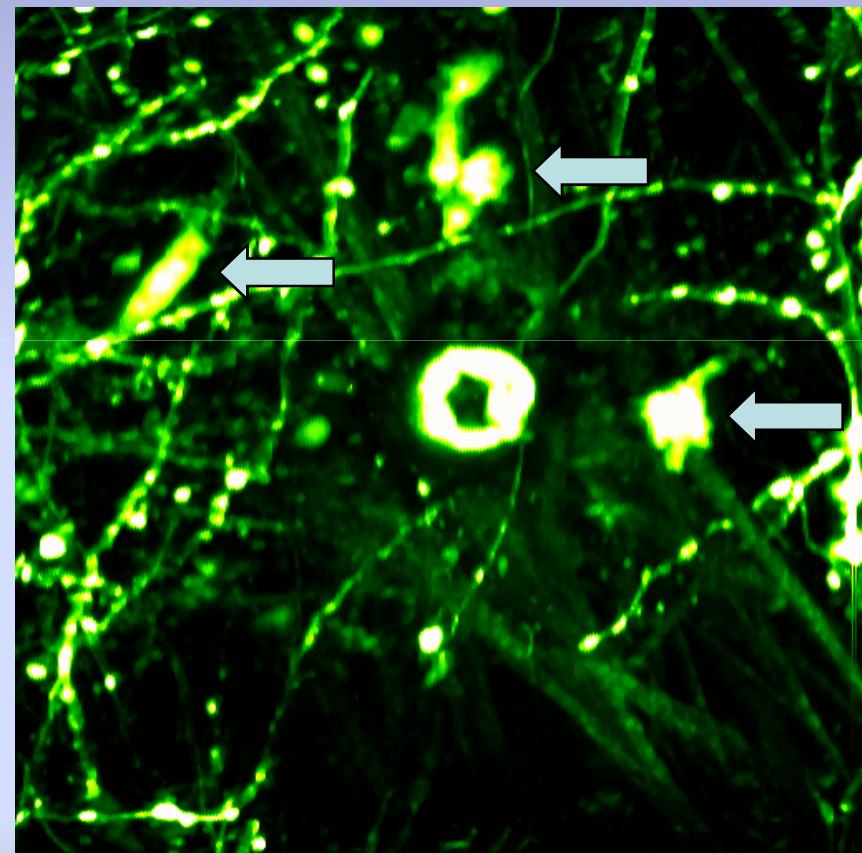
Observing the Response of the Immune System

UFOs Migrate to Laser Induced Vascular Haemorrhage

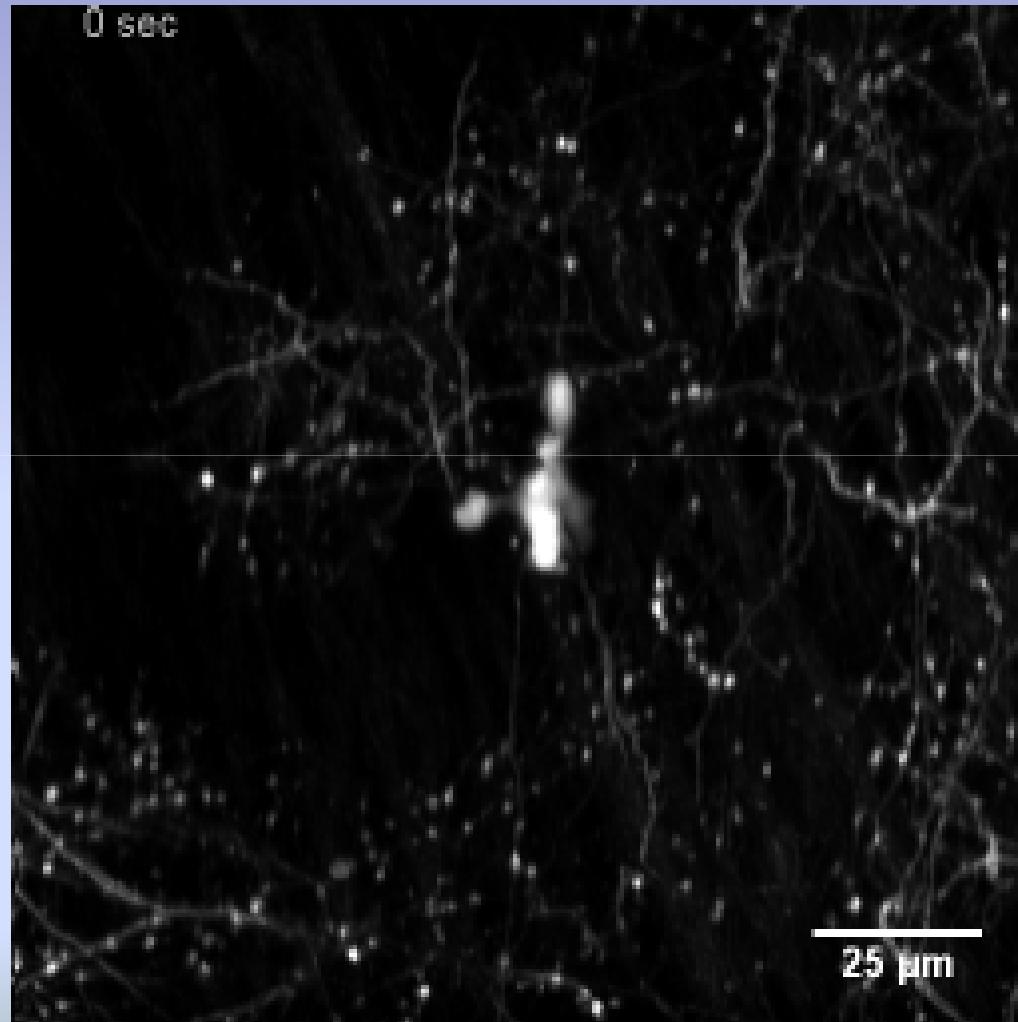
Time lapse Movie of Laser Haemorrhage



Migration of Cells to Laser Site at 24 hours



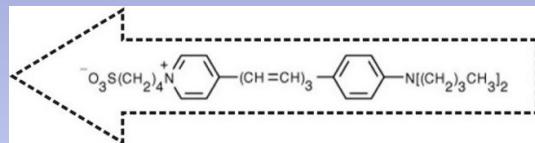
Unexpected Observation of Motile Fluorescent Cells



Optical recording of membrane potential by Second Harmonic Generation (SHG) Microscopy

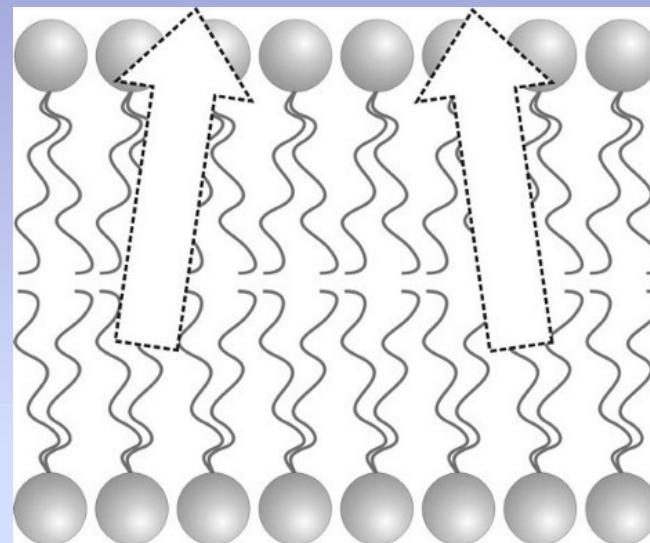
Exogenous labeling: SHG dye in the membrane

The molecule



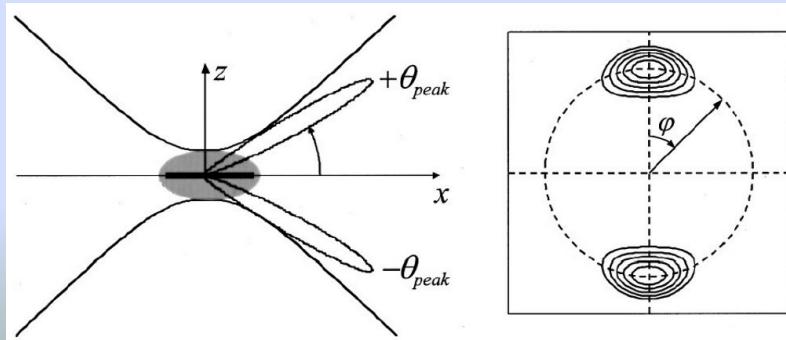
Chemical structure of RH237 dye is show inside the dash arrow.

Inside the membrane...

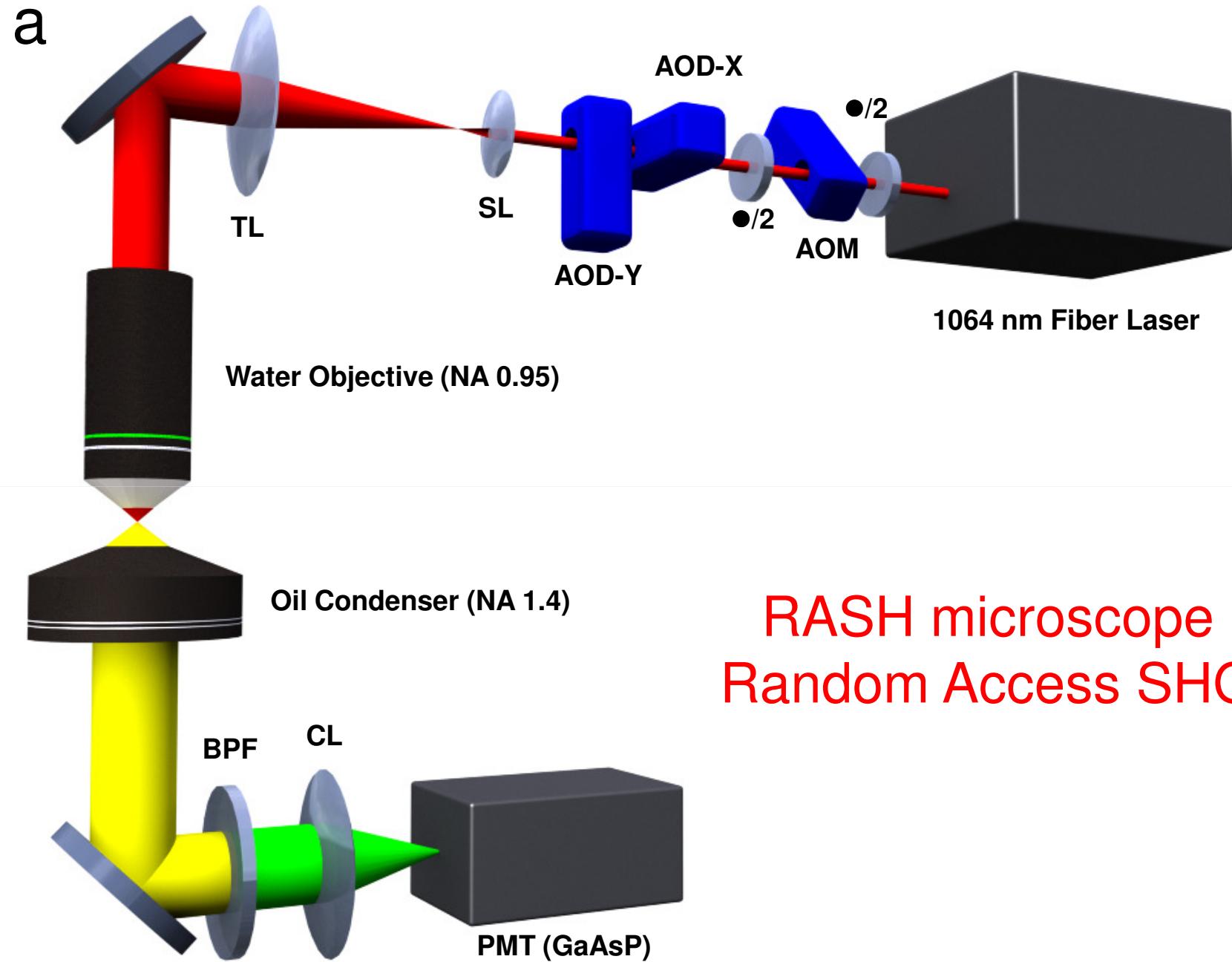


Presumptive insertion geometry of amphiphilic RH237 dye (dash arrow) in a lipid bilayer.

Phase matching condition



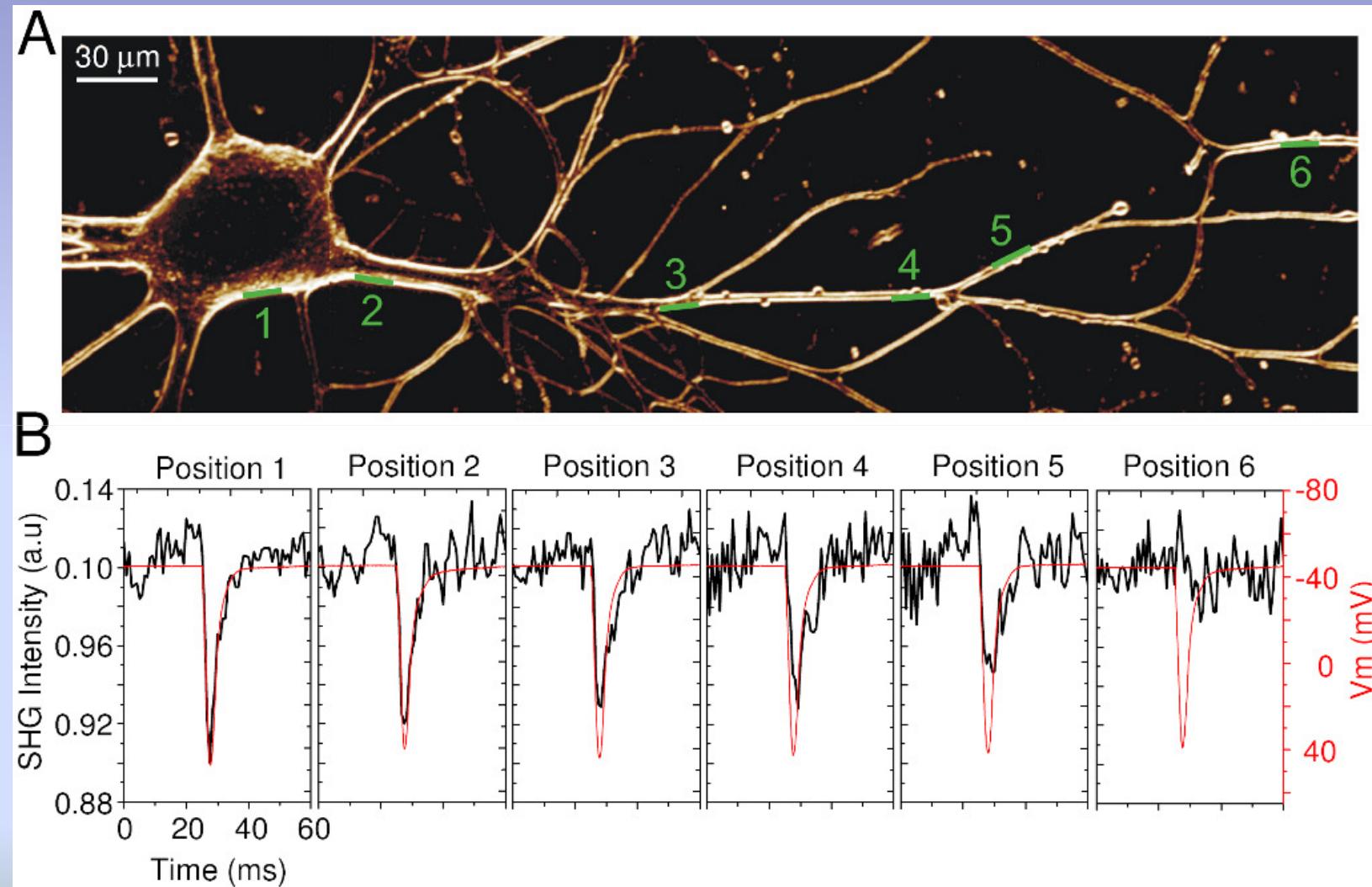
Left, an excitation beam propagation in the x direction and polarized along the z axis is focused onto the membrane (thick segment). Phase matching between the SHG and excitation field cause the SHG radiation to be double peaked in the forward direction. Right side, far-field power distribution of SHG radiation.



RASH microscope
Random Access SHG

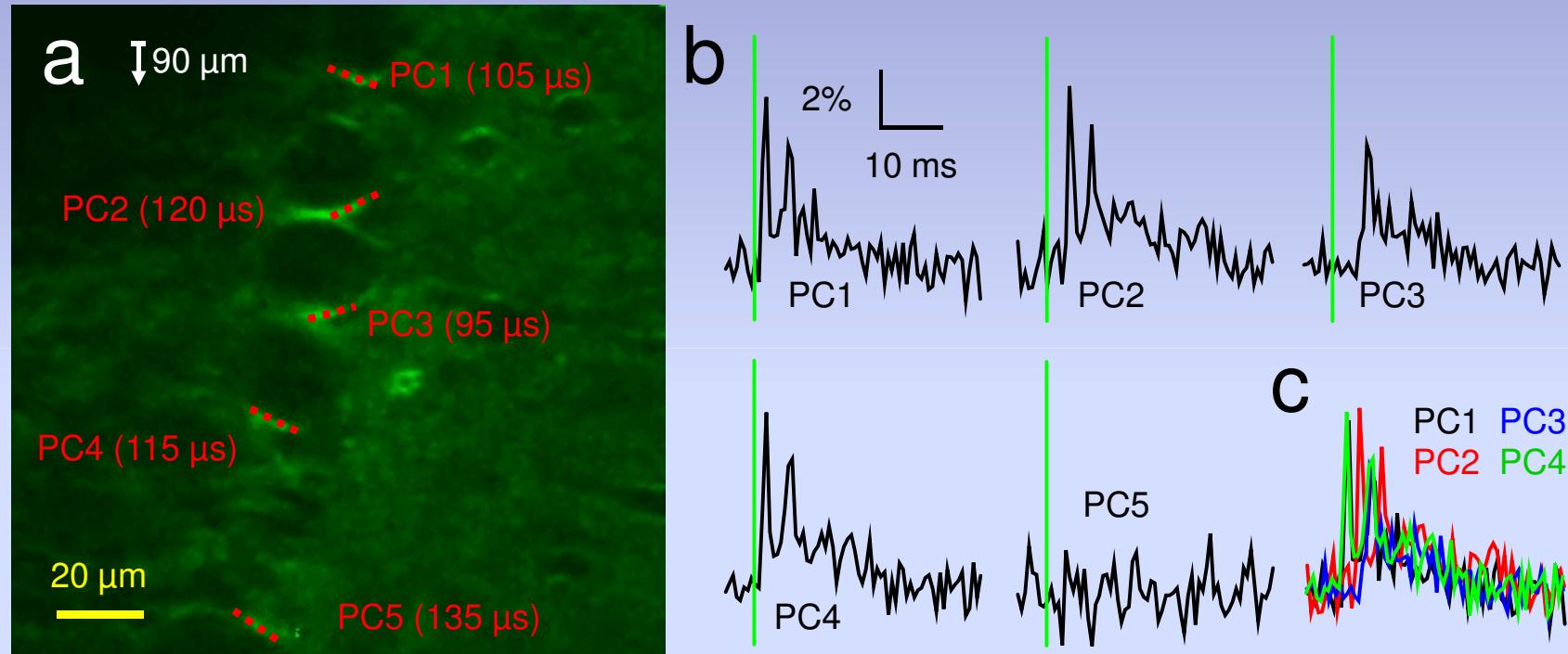
Optical Recording AP along the Neurite

Cultured cell



Multi cell recording

Climbing fiber stimulation
20 avgs on triggered stimulus
spontaneous activity cancelled by avg process



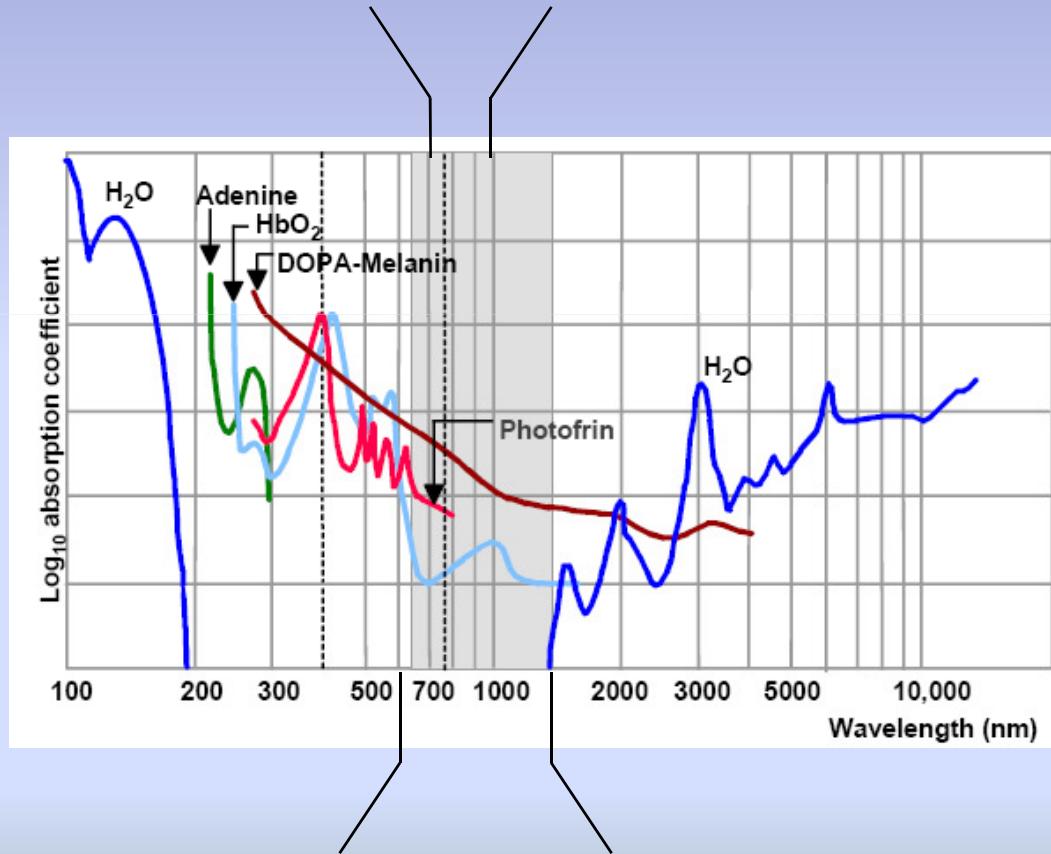
PC1, 3 and 4 are synchronous
PC2 is asynchronous

Skin

Tissue optical properties

Absorption

700-1000 nm : Ti:Sapphire wavelength range



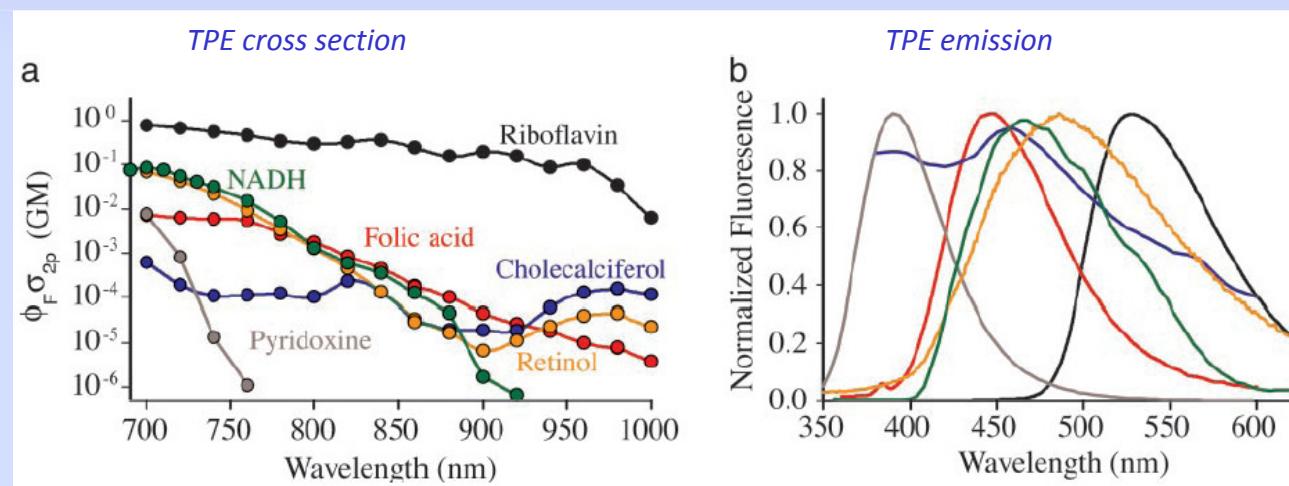
600-1600 nm : Tissue optical window

- Deep imaging capability inside tissues
- Limited out-of-focus absorption
- Reduced scattering with respect to UV light

Tissue intrinsic fluorophores

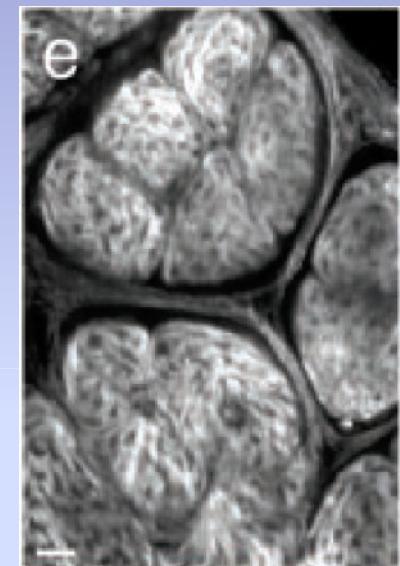
TPE fluorescence

- Living and ex vivo tissues have a large number of fluorescent molecules (NADH, flavins, riboflavins, elastin, retinol, tryptophan, cholecalciferol)
- Their TPE cross section is overlapped (multiple excitation)



Zipfel et al., PNAS, 100, 7075, (2003)

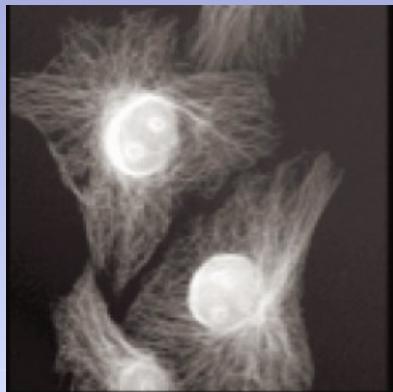
SHG (collagen)



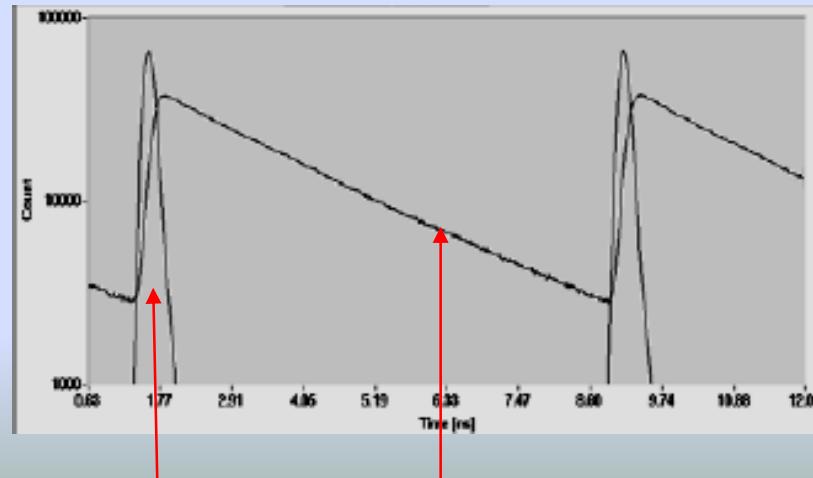
IMAGING WITHOUT ANY EXTERNAL ADDED PROBE !!!

Fluorescence Lifetime Imaging Microscopy (FLIM)

TPE



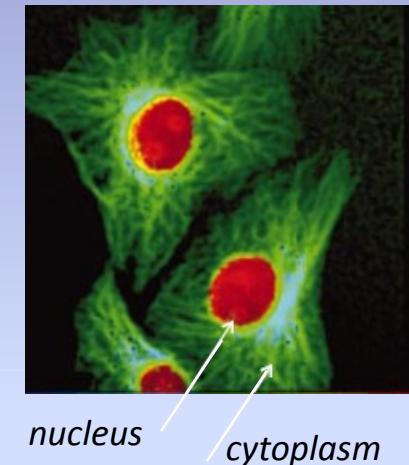
Fluorescence Decay



LASER pulse

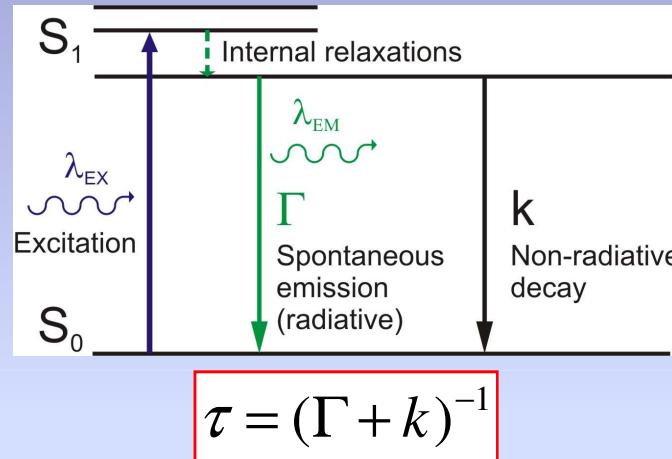
Fluorescence light

FLIM



Fluorescence Light Properties

- Intensity (excitation light, cross section)
- Spectrum (energy band structure)
- Lifetime (relaxation phenomena, collisions, environment)



Layer-by-layer imaging

Horizontal optical sectioning Stacking of sections

- Sample excised during dermatological surgery

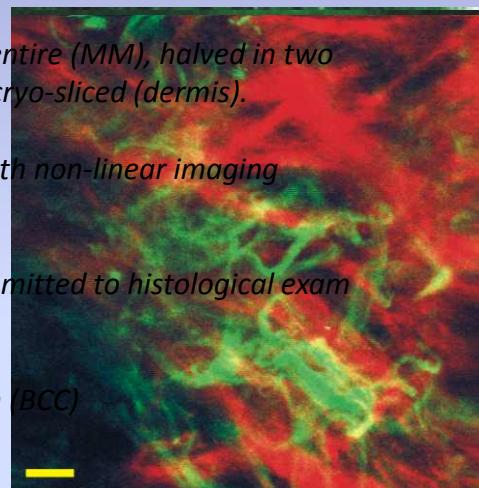
TPETBEG

- Sample collected entire (MM), halved in two parts (BCC,MN), or cryo-sliced (dermis).

- Sample imaged with non-linear imaging within 3 hours

- The other part submitted to histological exam (BCC)

- Image comparison (BCC)



Histology



Scales bars 20 μm

Excitation wavelength: 740 nm

Image dimension: 250 $\mu\text{m} \times 250 \mu\text{m}$

Resolution: 250 \times 250 pixels

Pixel dwell time: 5 μs

Laser power: 28 mW

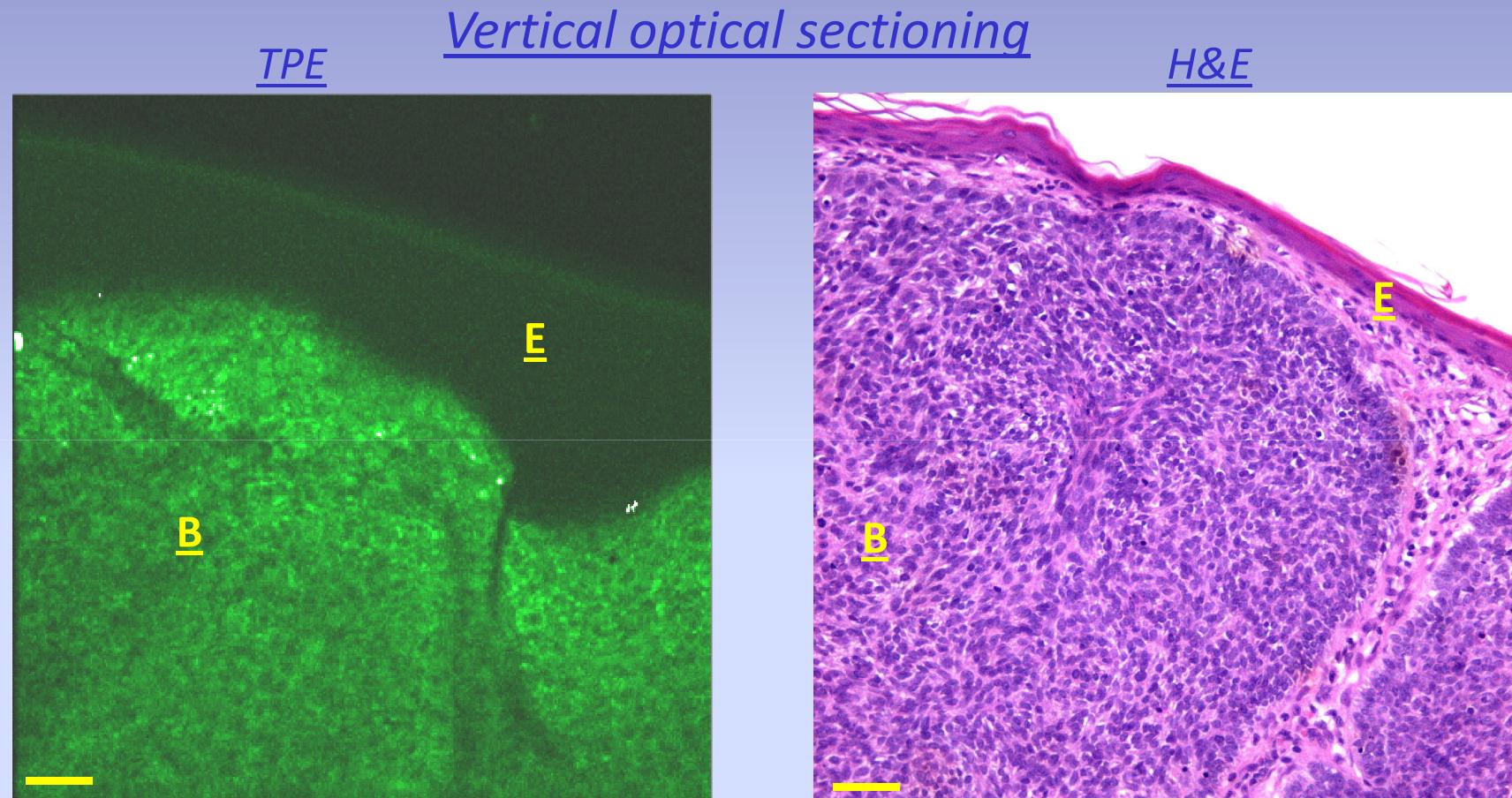
Skin surface perpendicular to the optical axis



Cutaneous BCC



ALA-treated BCC sample (TPE)



Excitation wavelength: 730 nm

Image dimension: 300 $\mu\text{m} \times 300 \mu\text{m}$

Resolution: 1000 \times 1000 pixels

Pixel dwell time: 5 μs

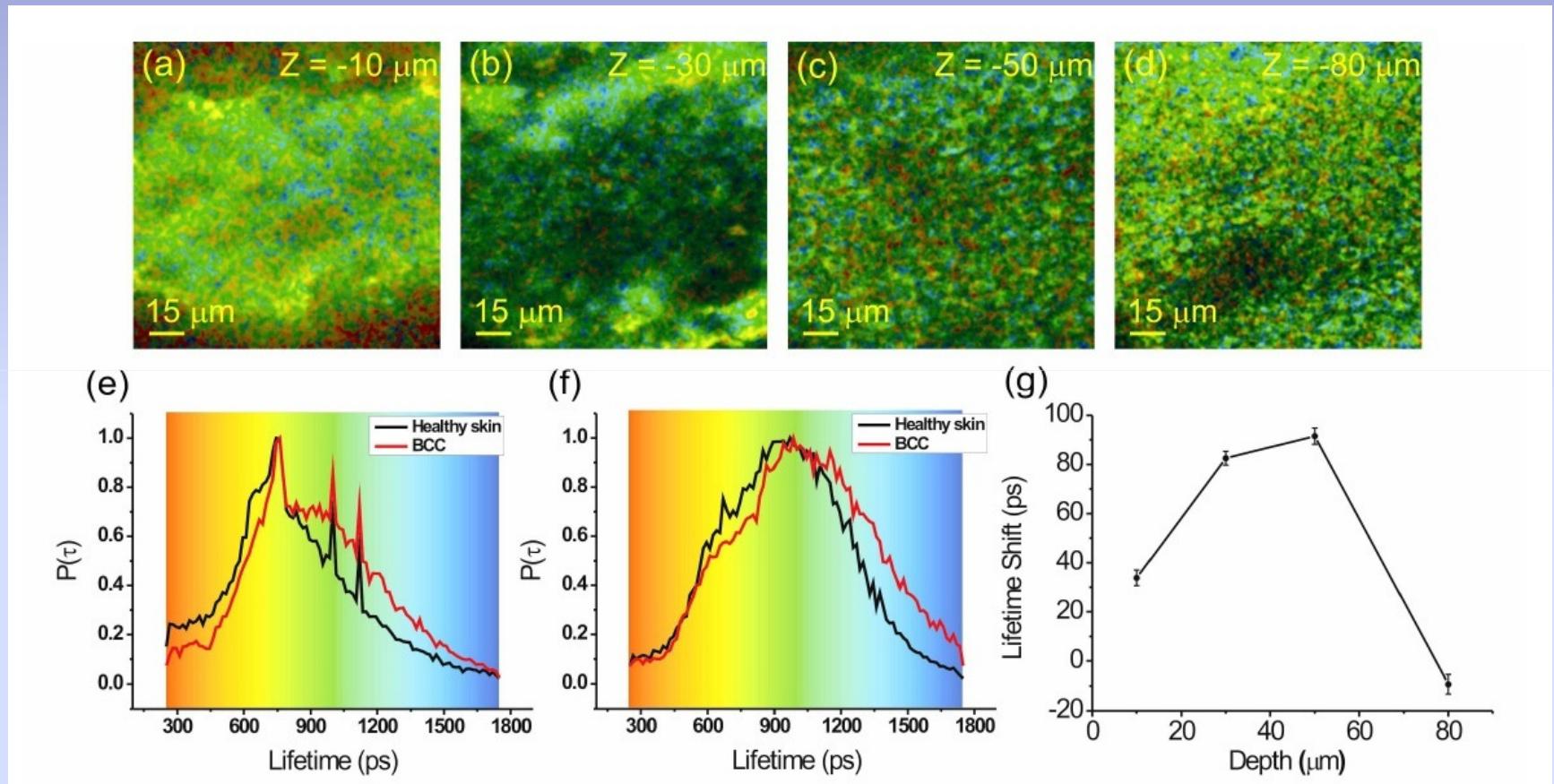
Laser power: 5 mW

Scale bars: 30 μm

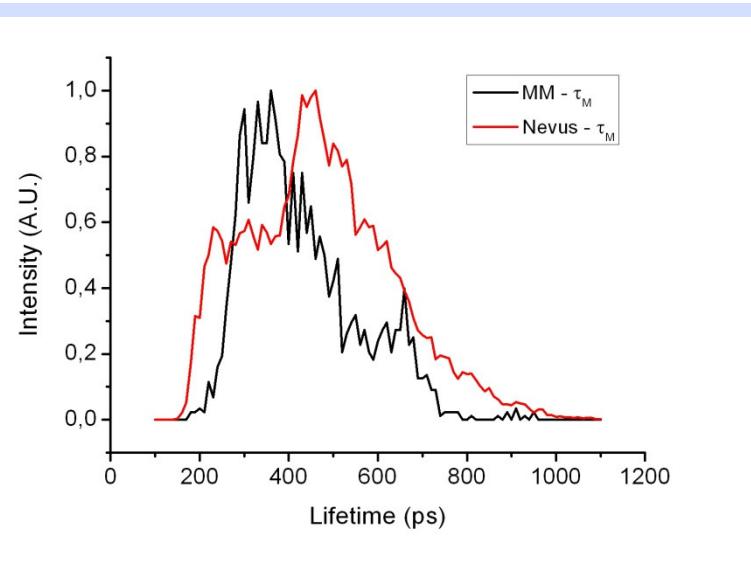
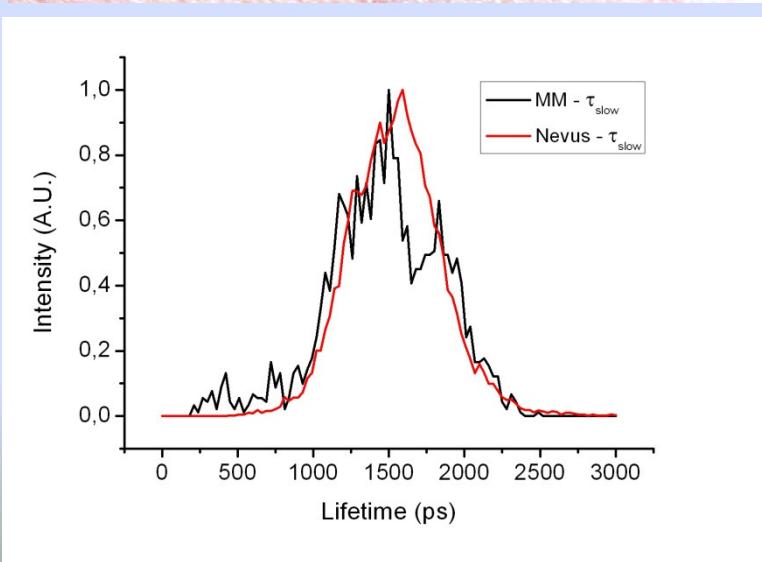
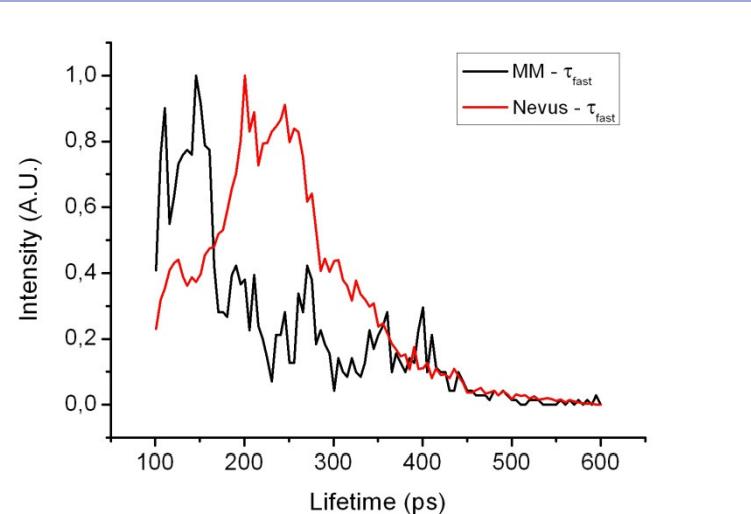
E: healthy epidermis

B: Basal Cell Carcinoma

FLIM in BCC



Melanoma - melanocytic nevi



Bladder

Morphology (combined TPEF-SHG)

Imaging of Carcinoma in situ and surrounding collagenous stroma

Excitation wavelength: 740 nm

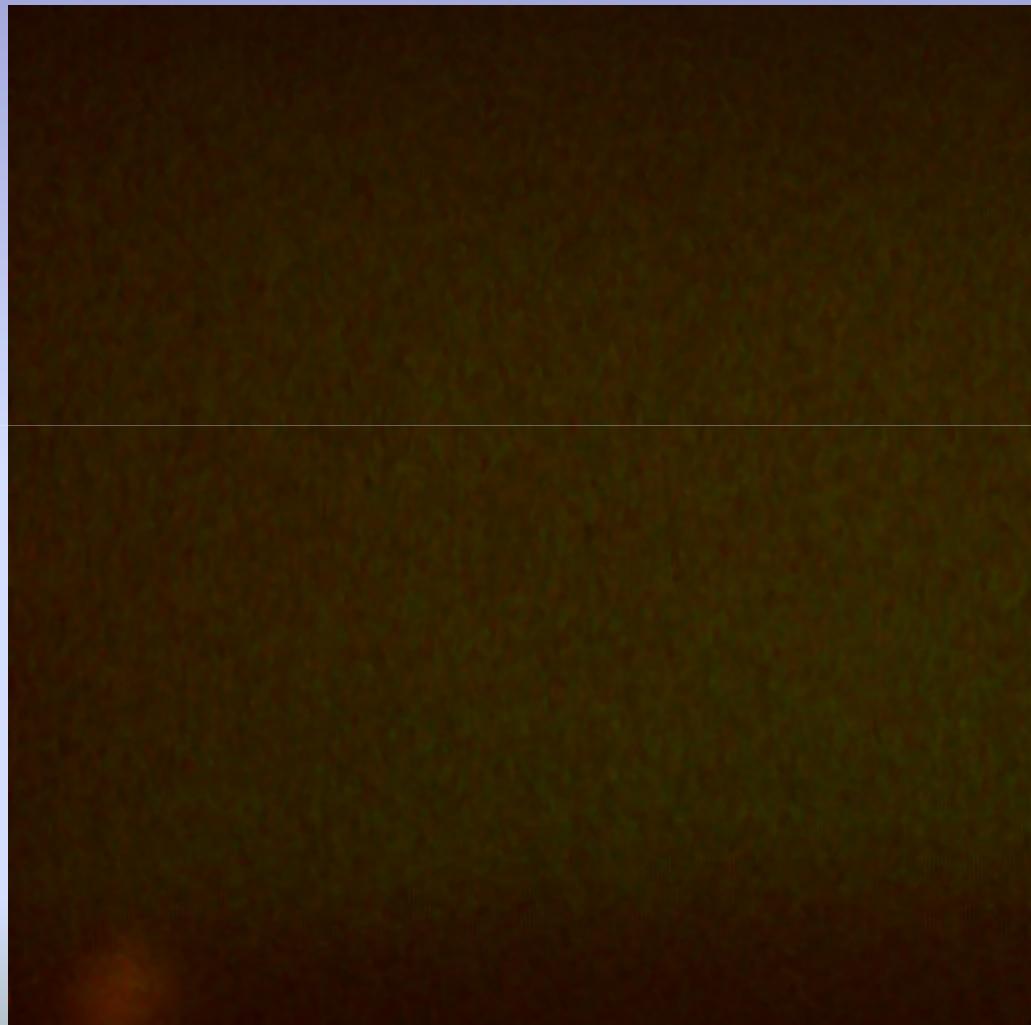
(TPEF) + 840 nm (SHG)

Image dimension: 200 $\mu\text{m} \times 200 \mu\text{m}$

Resolution: 1024 \times 1024 pixels

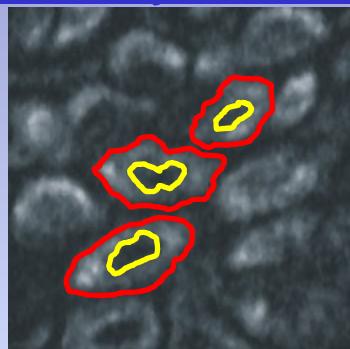
Pixel dwell time: 5 μs

Scale bars: 20 μm



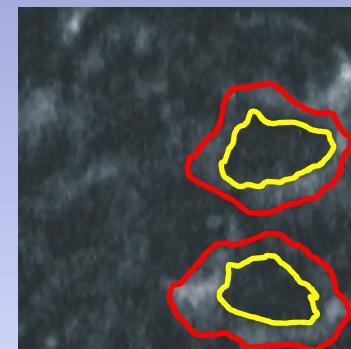
Morphology (nuclear dimension)

Healthy Mucosa

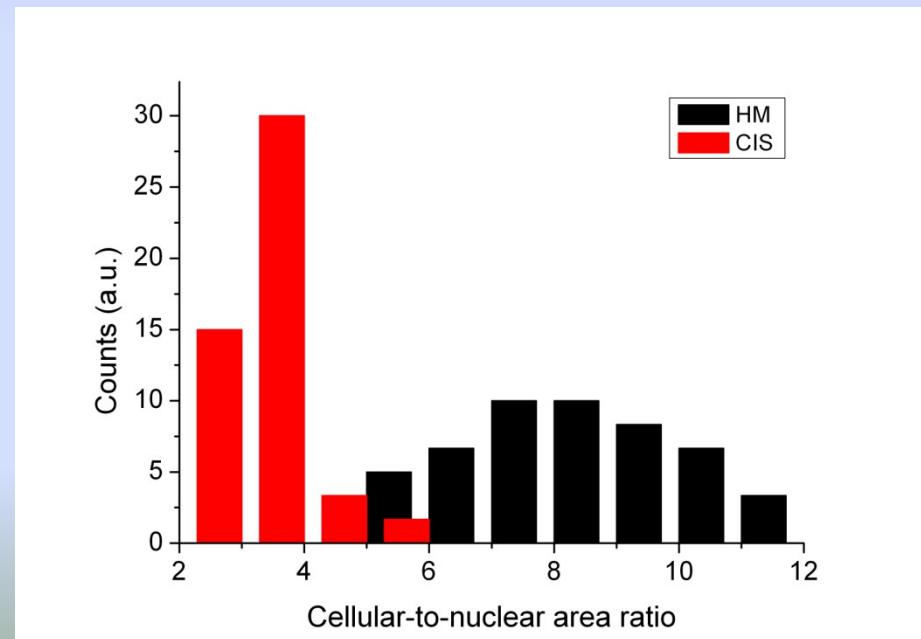


Measuring the **cell area**
and the **nucleus area** on
thresholded images

Carcinoma in situ



- 50 cells (HM)
- 50 cells (CIS)
- measured cellular area
- measured nuclear area
- Cell-to-nucleus area ratio



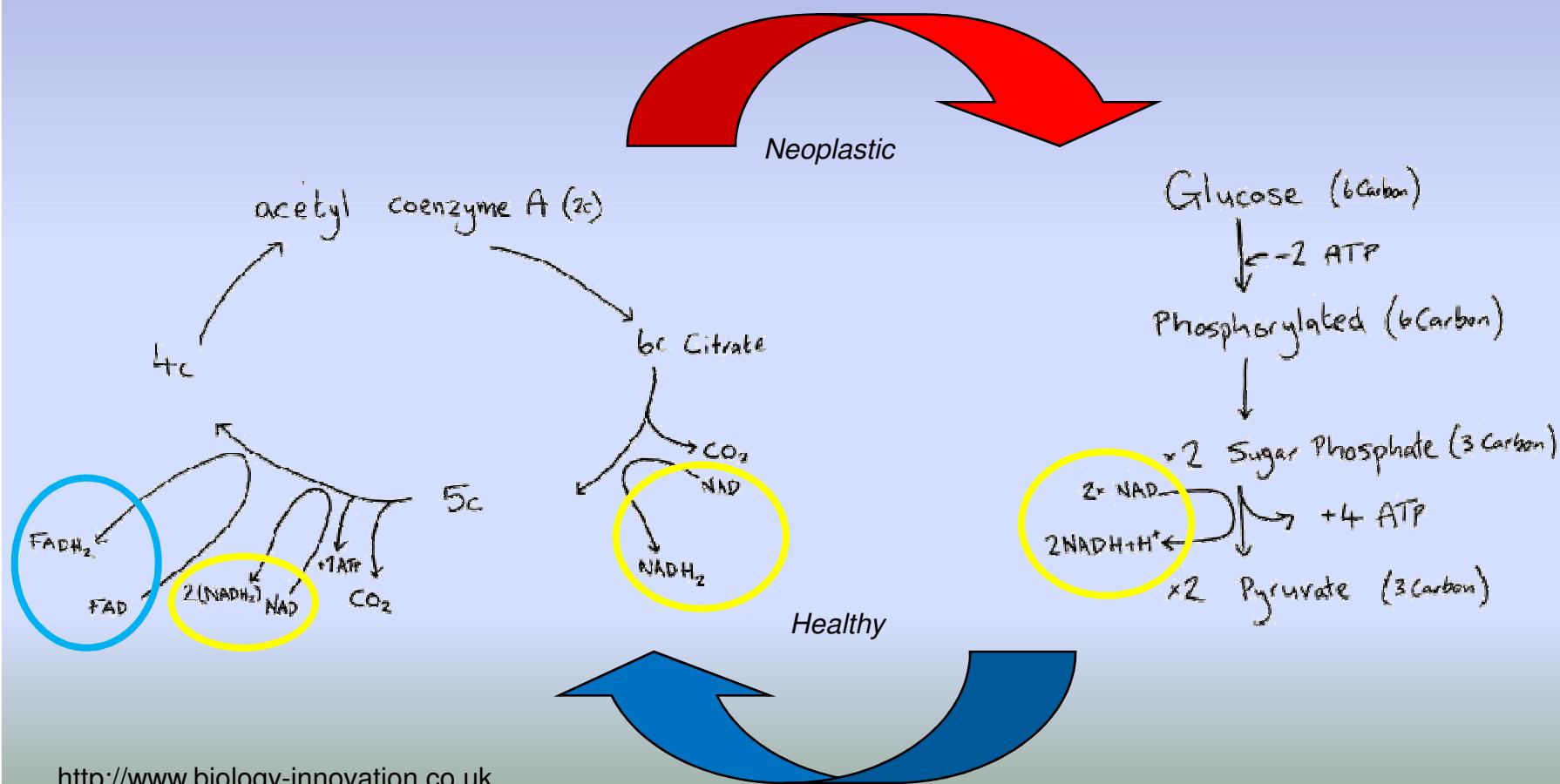
Warburg effect

Oxidative Phosphorylation

- High efficiency
- Requires Oxygen
- NADH & FAD involved

Glycolysis

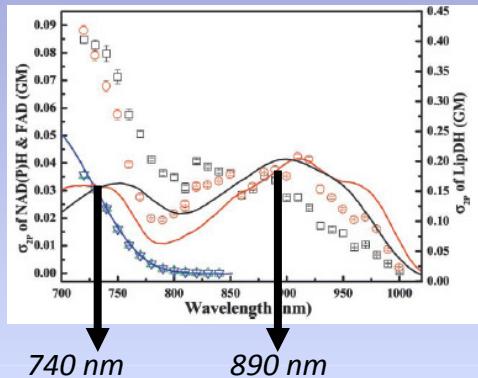
- Low efficiency
- Does not require Oxygen
- Only NADH involved



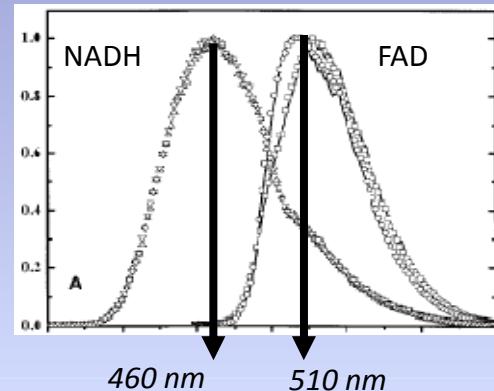
Multispectral Imaging (NADH-FAD)

TPE cross section

Huang et al., Biophys J (2002)



Fluo emission



Excitation wavelengths:

- 740 nm (NADH)
- 890 nm (FAD)

Detection wavelengths:

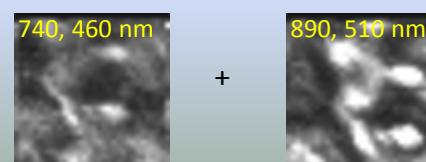
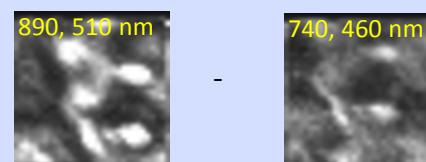
- 460 nm (NADH)
- 510 nm (FAD)

...with images

Red-Ox Ratio index ($-1 < \text{Rox} < 1$)

$$\text{I}_\text{FAD}(890 \text{ nm}, 510 \text{ nm}) - \text{I}_\text{NADH}(740 \text{ nm}, 460 \text{ nm})$$

$$\text{I}_\text{NADH}(740 \text{ nm}, 460 \text{ nm}) + \text{I}_\text{FAD}(890 \text{ nm}, 510 \text{ nm})$$

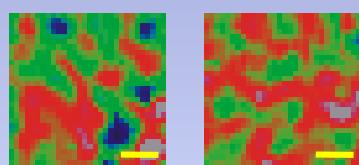


Red-Ox Ratio
Index map

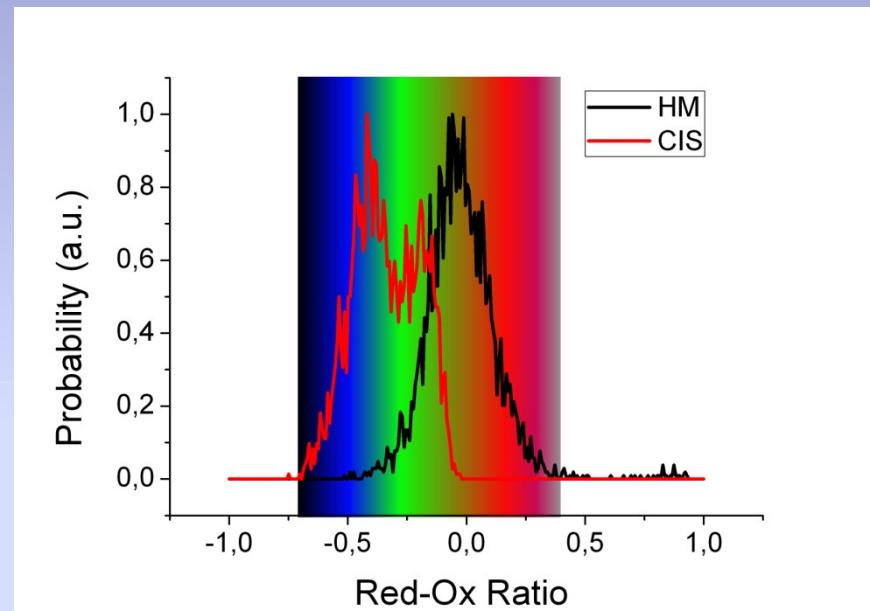
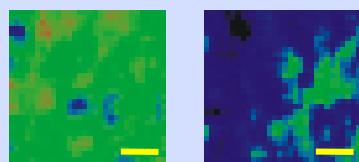


Multispectral Imaging (Red-Ox Ratio)

Healthy Mucosa



Carcinoma in situ



Excitation wavelength: 740 nm (NADH) / 890 nm (FAD)

Image dimension: 20 μm \times 20 μm

Resolution: 32 \times 32 pixels

Detection range: 460 nm (NADH) / 515 nm (FAD)

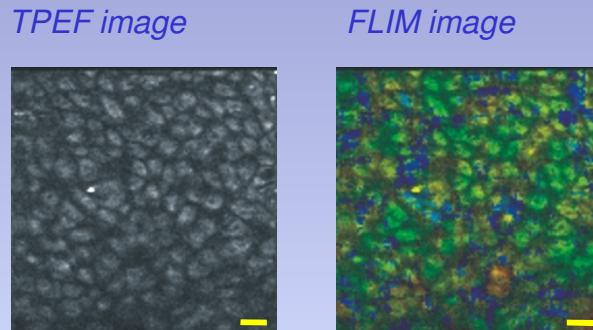
Lambda-resolution: 13 nm/channel

Scale bars: 5 μm

Only NADH involved

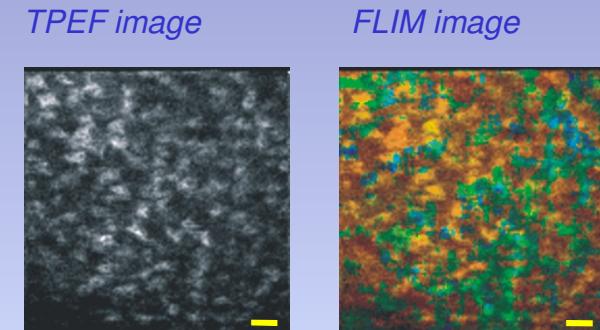
Lifetime components ratio

Healthy Mucosa

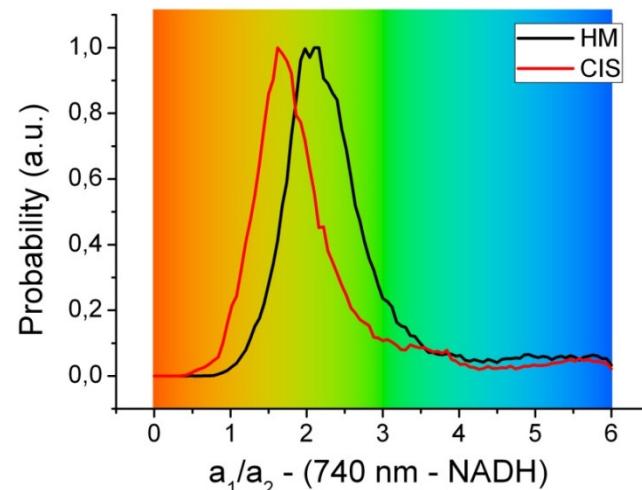


Excitation wav: 740/890 nm
Dim: $100 \mu\text{m} \times 100 \mu\text{m}$
Res: 128×128 pixels
Detection: 415-615 nm
Scale bars: $10 \mu\text{m}$

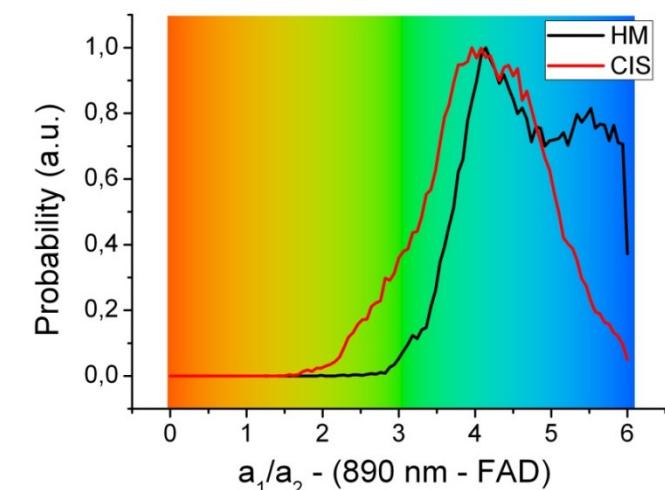
Carcinoma in situ



Free-to-bound NADH



Bound-to-free FAD



Skin collagen disorder

Cutaneous scars

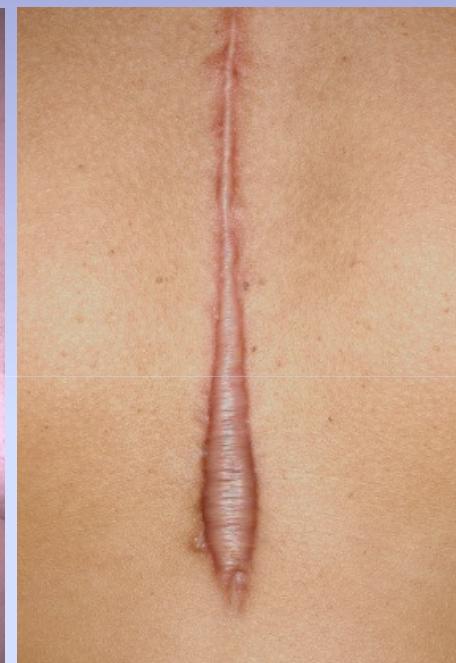
normal scar



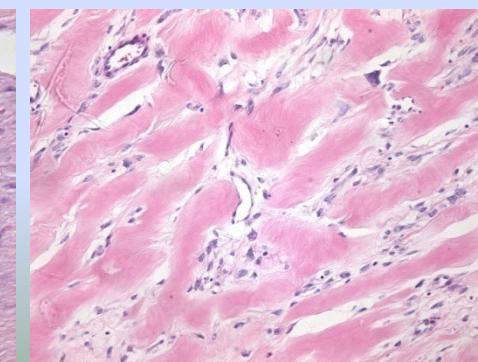
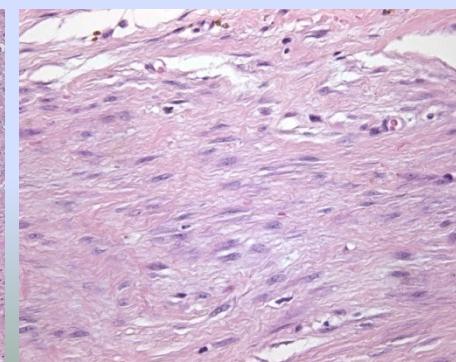
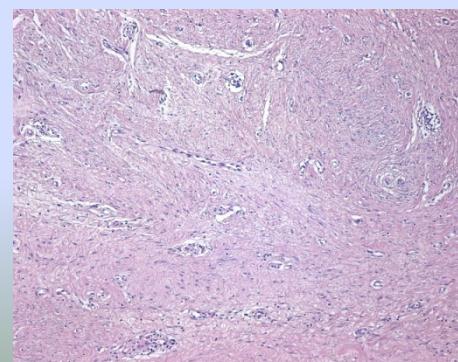
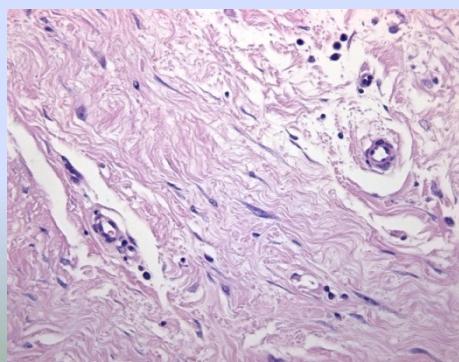
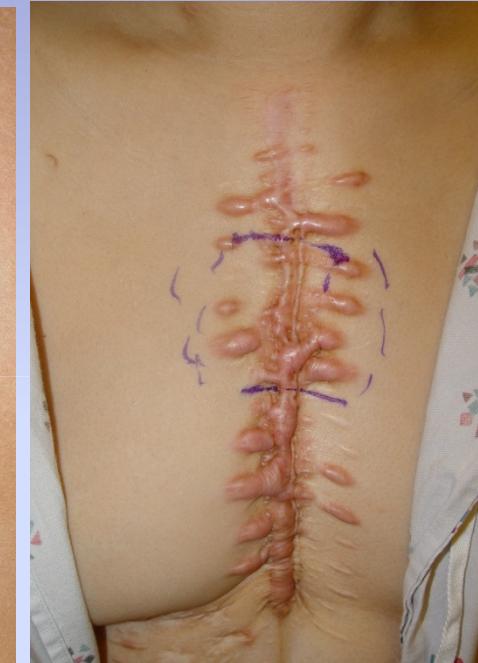
hypertrophic scar



keloid



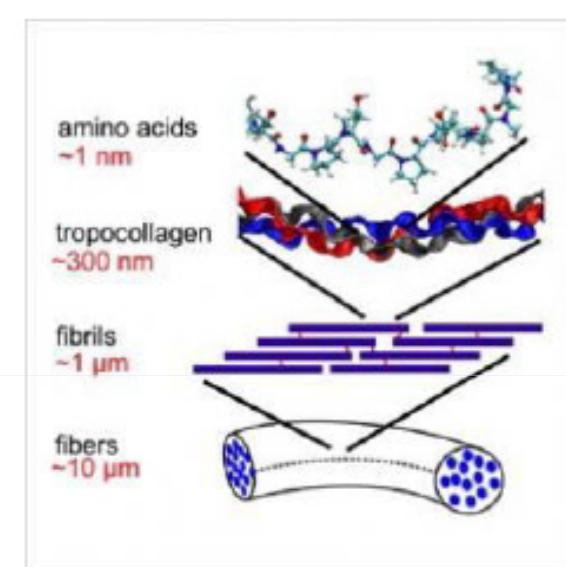
keloid



The structure of Collagen and variations in keloids

1. Biomedical Consideration:
 - Structure of collagen type I
 - Dermis ↔ keloids

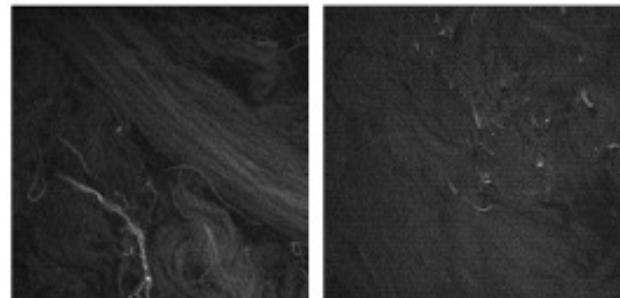
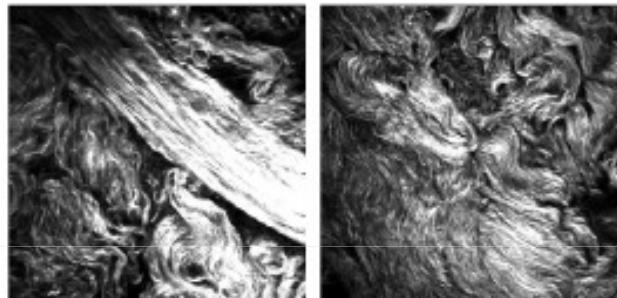
Fibril diameter: 20-30 nm
Fiber diameter: 0.5-3µm



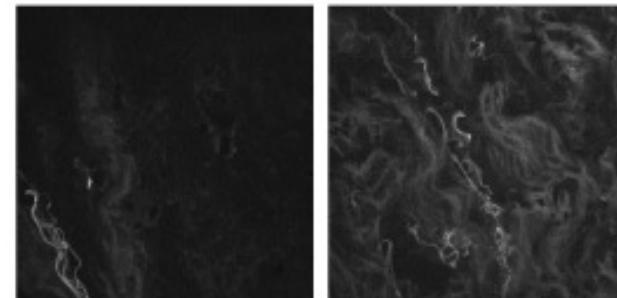
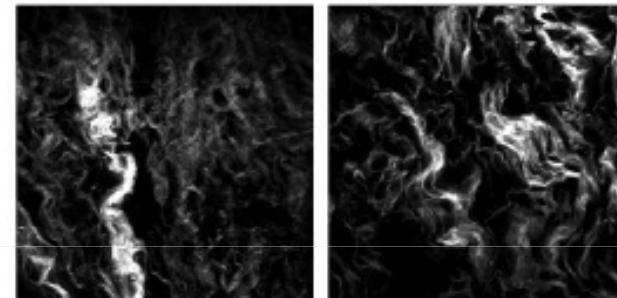
	Dermis	Keloids
Fiber size	diameter between 0,5-3 µm	thicker, larger fibers
Collagen fiber arrangement	parallel and organized	random orientation
Collagen synthesis		20 X dermis

Second Harmonic and Fluorescence Imaging of Collagen

Dermis



Keloids



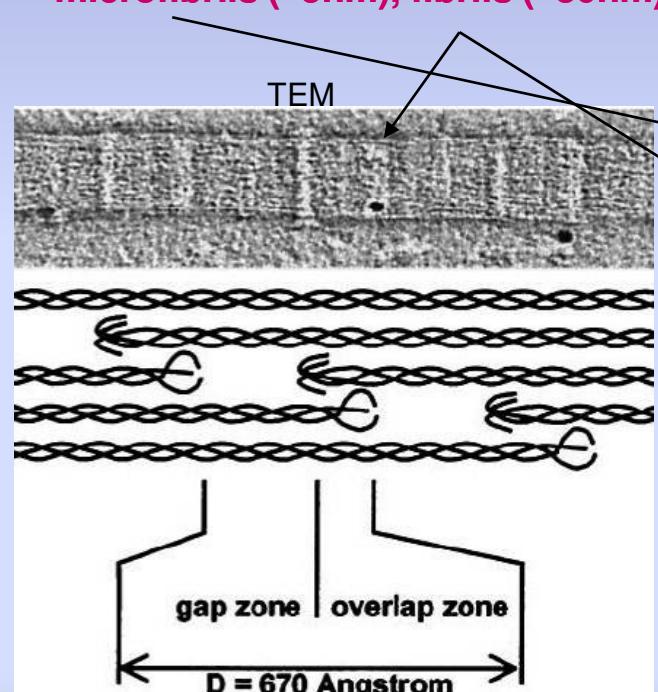
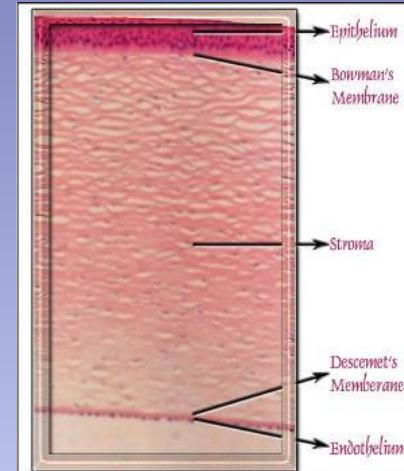
SHG

TPE

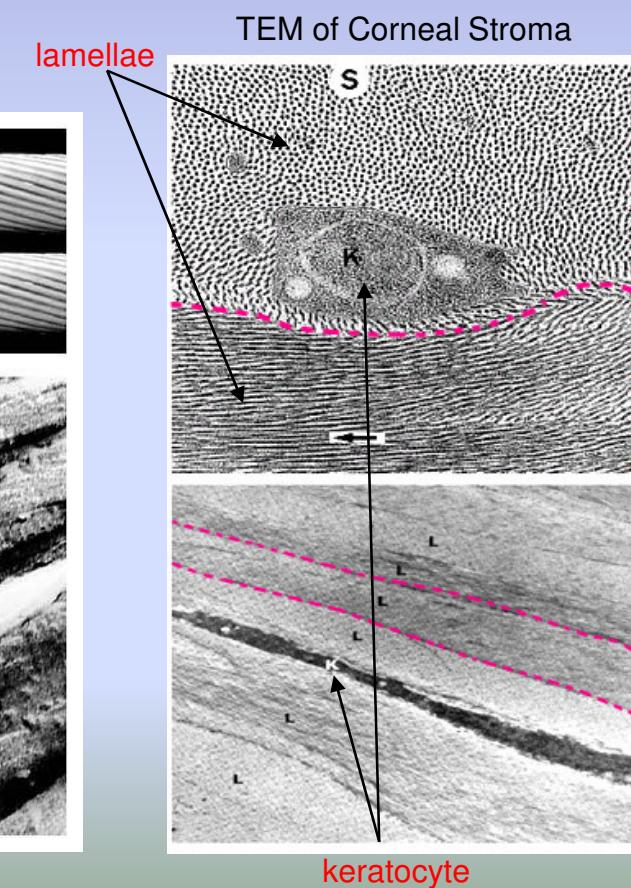
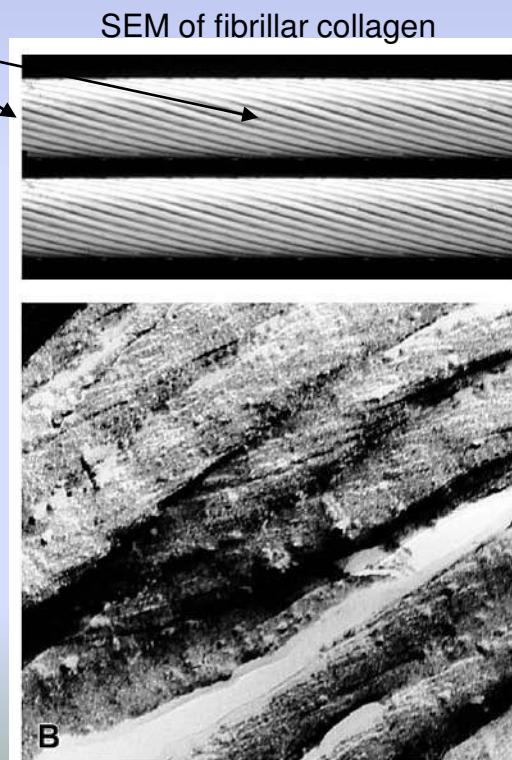
*Cornea
collagen
disorder*

Corneal stroma structure: fibrillar collagen

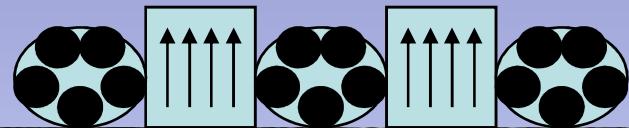
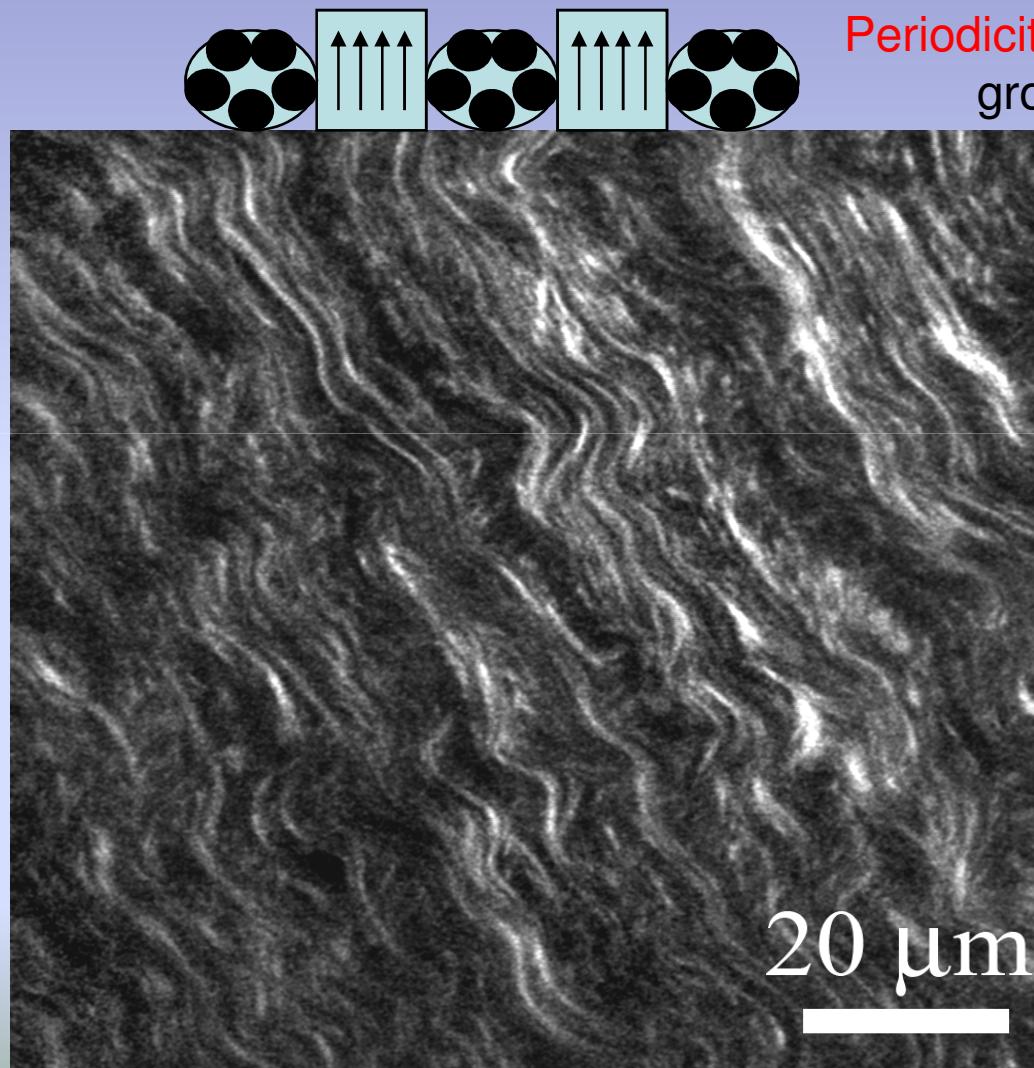
- Corneal stroma constitutes 90 % of the cornea and is mainly composed of fibrillar collagen
- Organization of fibrillar collagen in corneal stroma:
microfibrils (~5nm), fibrils (~30nm), lamellae (0.5-2.5μm)



D-period: 67nm periodicity
of gap and overlap zones



Photothermally-induced disordered patterns of corneal collagen revealed by SHG imaging



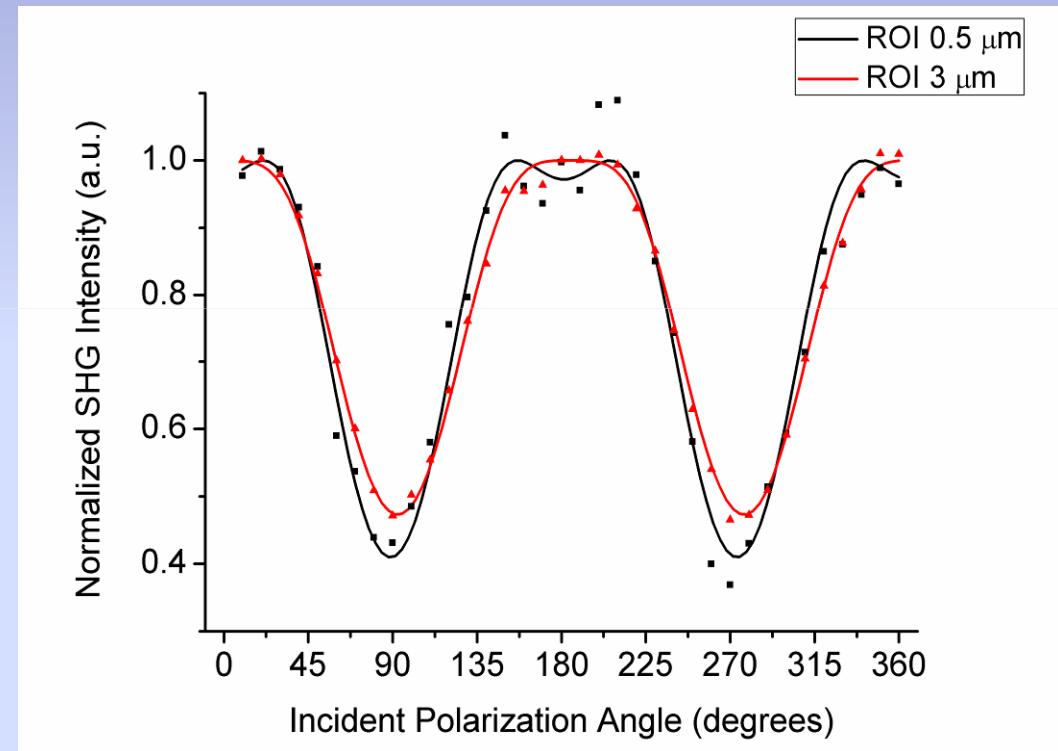
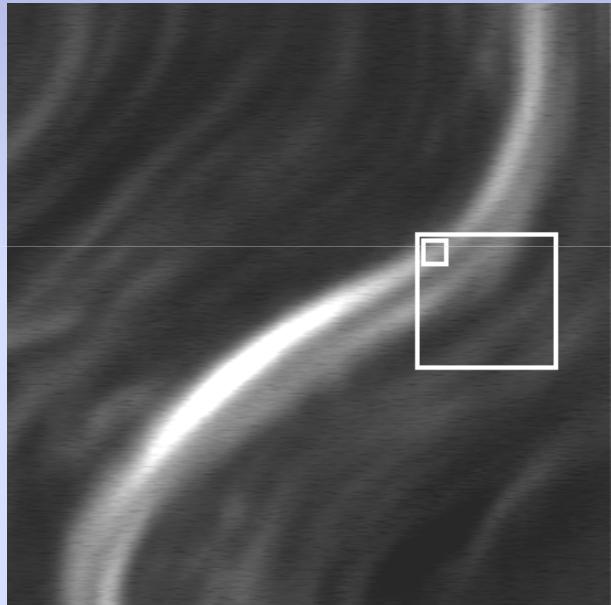
Periodicity of structured collagen bundles
groups of very ordered fibrils

Circularly-polarized SHG images of corneal stroma revealed ~0.5 μm thick fiber-like structures, which actually consisted of many collagen fibrils (only 30 nm thick), organized in lamellar domains. The size of these structures is comparable with the minimum resolvable distance for our microscope (~400 nm)

Polarization Scanning Imaging

Alignment of harmonofores at both scale levels (0.5 and 3 μm).

$20 \mu\text{m} \times 20 \mu\text{m}$



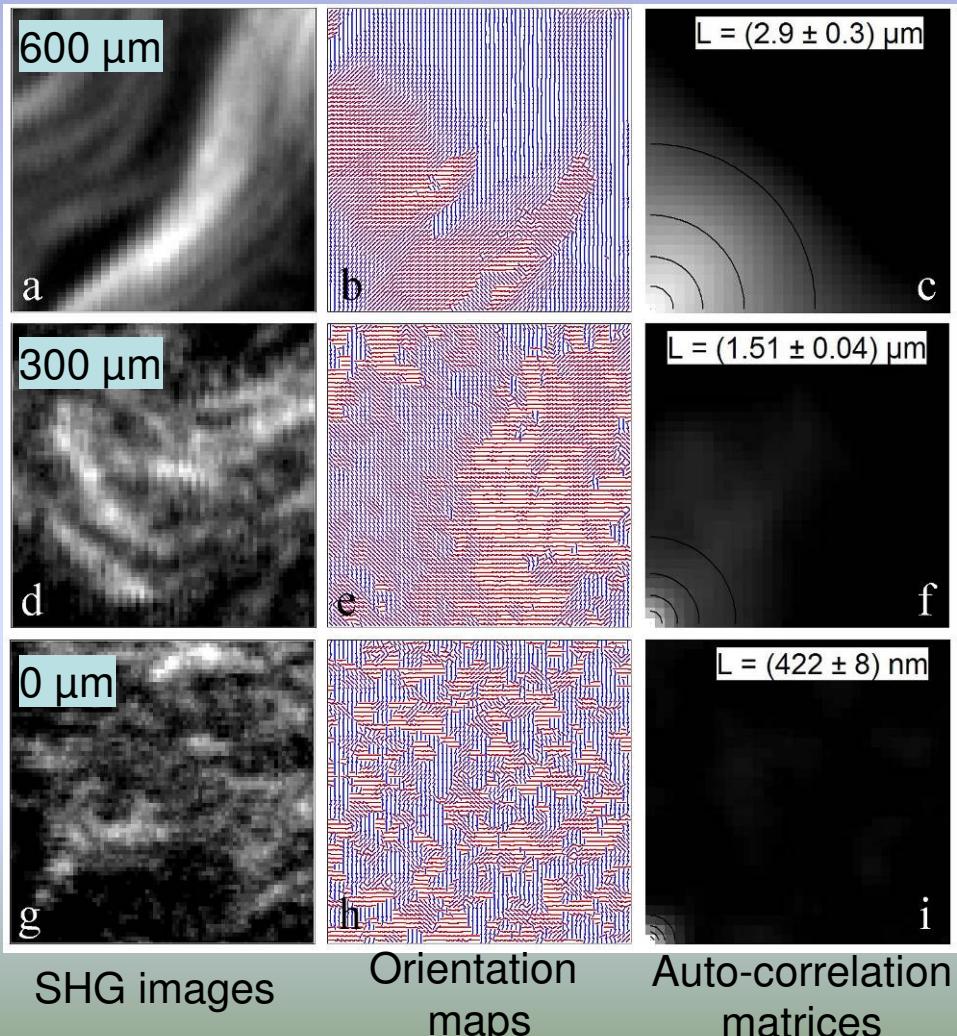
ROI 0.5 μm : corresponding to a collagen fiber bundle.

ROI 3.0 μm : corresponding to a single lamellar domain.

Photothermally-induced disordered patterns of corneal collagen revealed by SHG imaging

Image analysis of sub-lamellar anisotropy: Zooming to $8 \times 8 \mu\text{m}$

The Order Length Parameter



Laterally-resolved maps of the local mean orientation of the collagen fibrils within each pixel were extracted.

The lateral correlation between the mutual orientations of the collagen fibrils was calculated through an auto-correlation analysis.

The Order length L is taken to represent the typical distance over which the regular sub-lamellar arrangement is preserved.

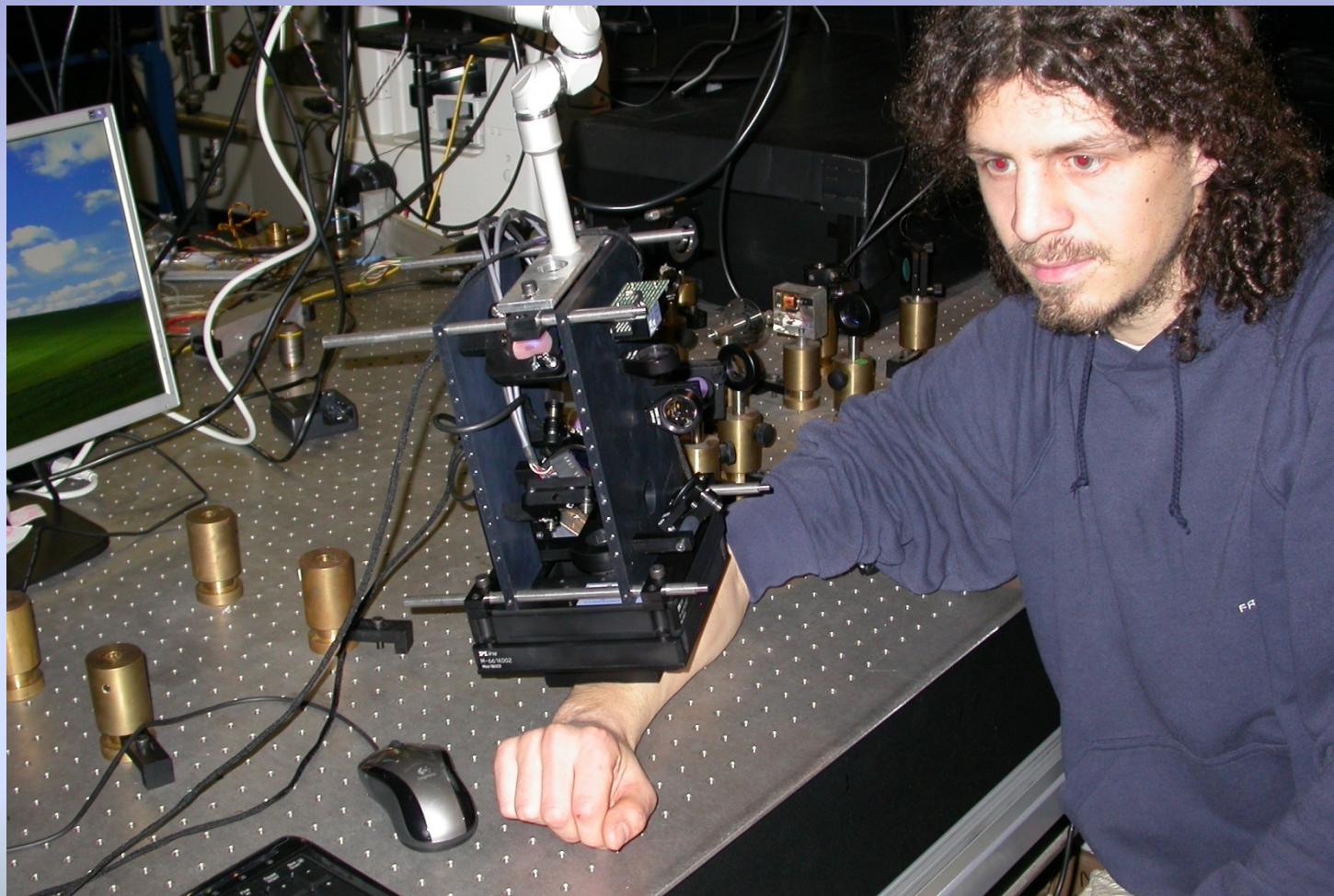
2.9 μm is comparable to a regular lamellar thickness (between 0.5-2.5 μm)

0.422 μm is comparable to our lateral resolution

Mobile Multiphoton Microscope

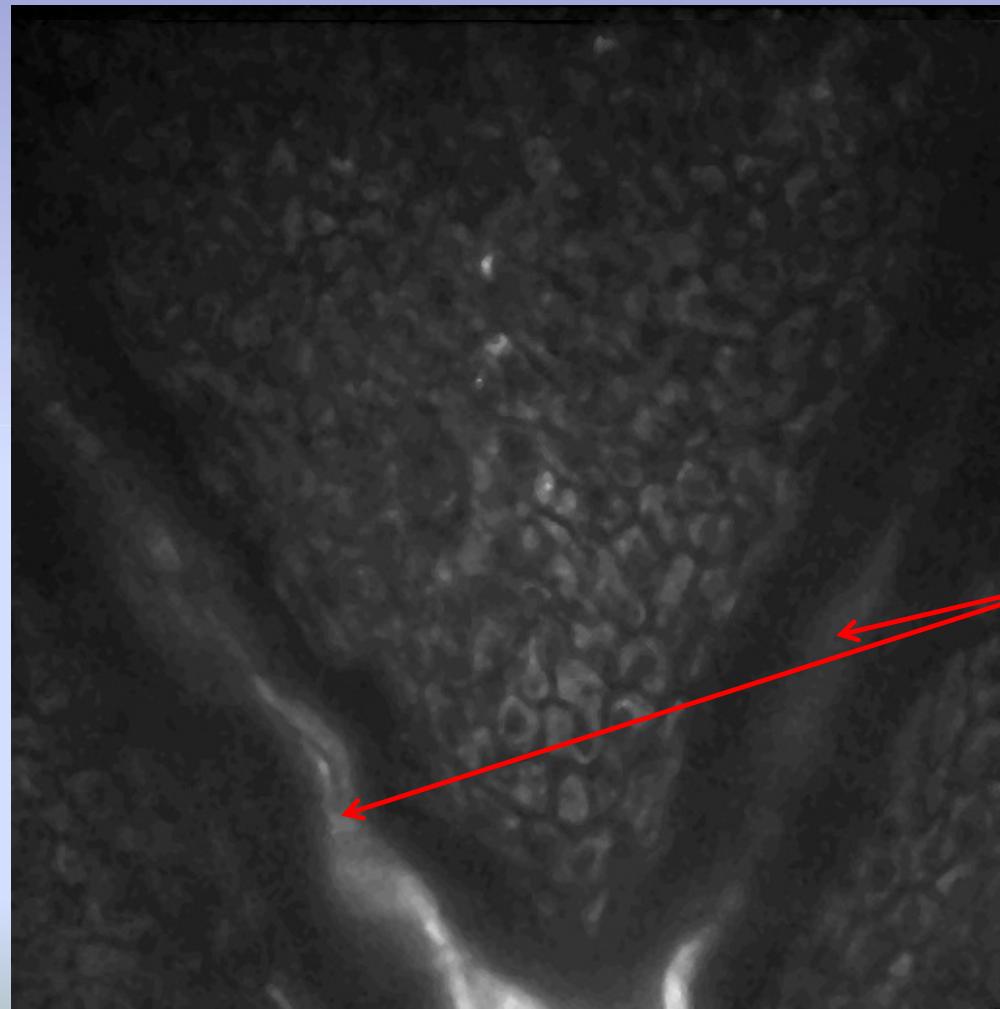
In-Vivo Imaging

Compact, portable, home-made Non-Linear Multimodal Microscope



In-Vivo Imaging

Forearm; 40 μm field – 740 nm excitation

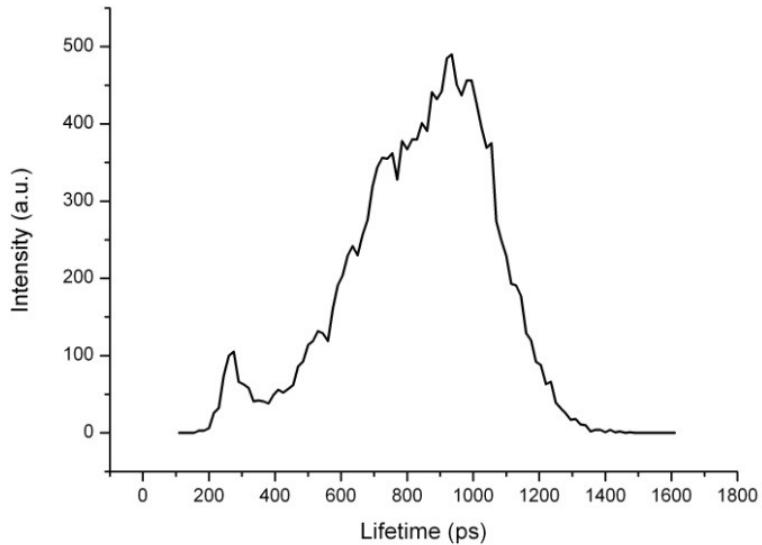


Scalability: 6400 nm depth

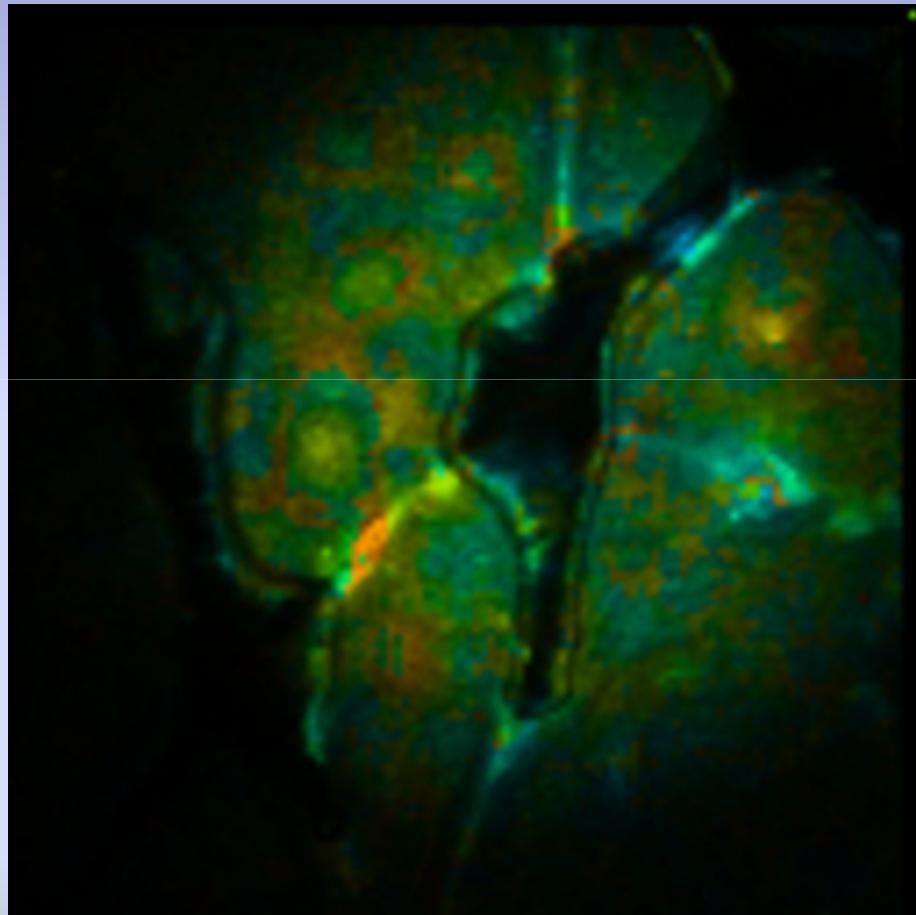
wrinkles

Lifetime In-Vivo Imaging

100 um field – 740 nm excitation

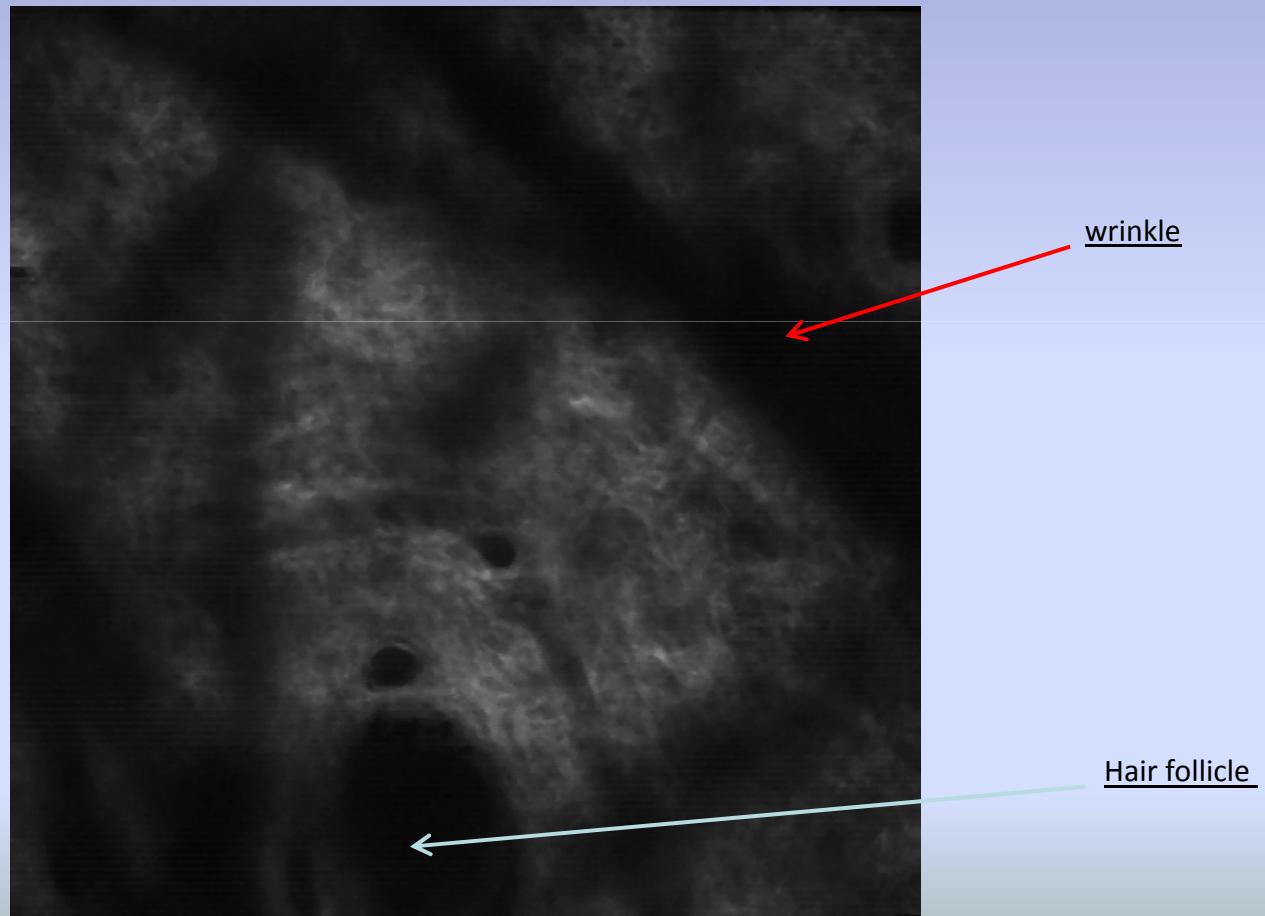


Epidermis – 30-40 um depth



In Vivo Collagen Imaging

100 um field – 740 nm excitation

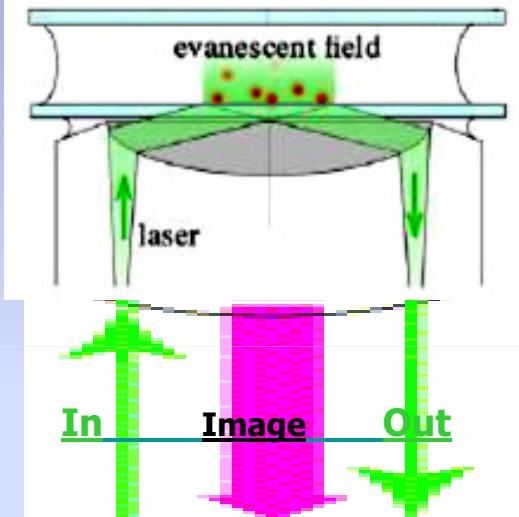


Collagen – 130 um depth

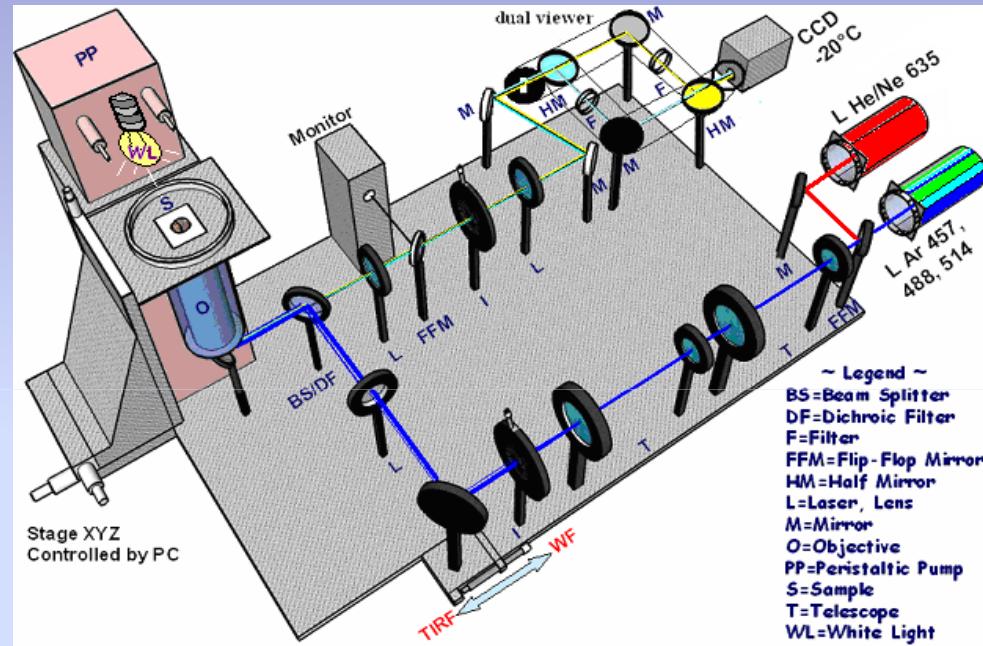
**Altre linee di ricerca presso il
dipartimento di Fisica**

Biosensore di singola molecola

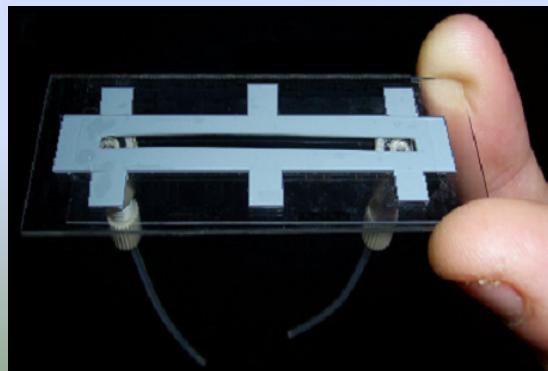
Objective-TIR



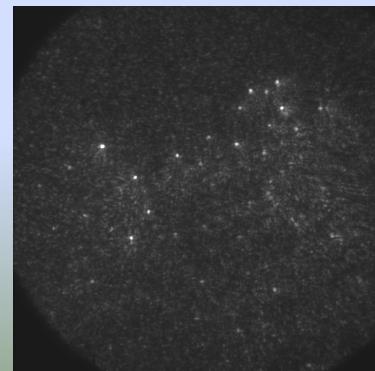
laboratory prototype



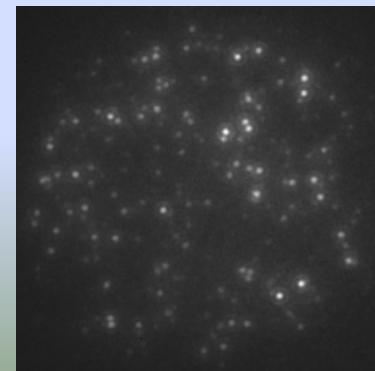
Silica flow cell



Gold NPs

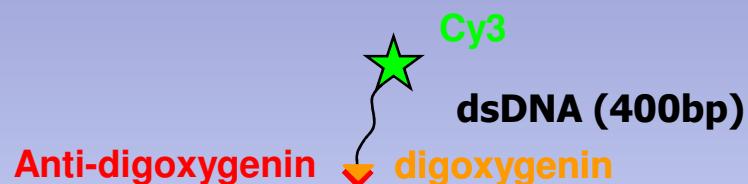


Quantum dots



Scattering
or
Fluorescence
detection

DNA_Cy3 single molecule detection

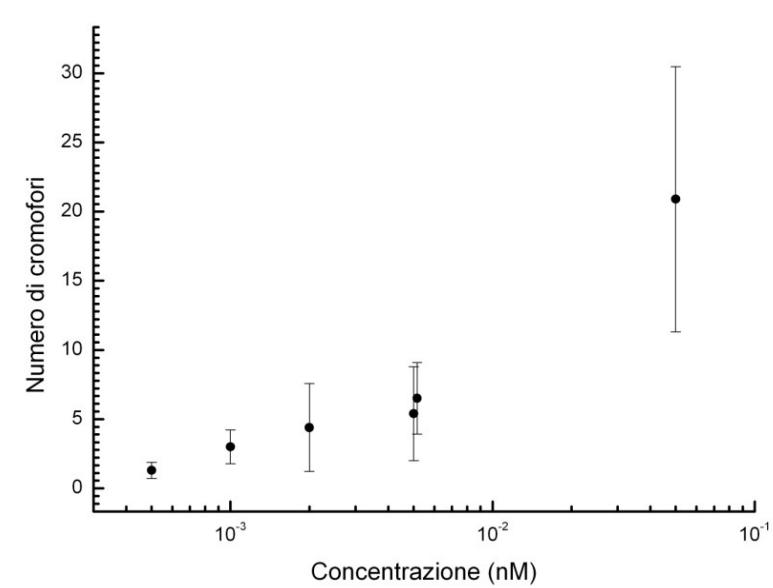
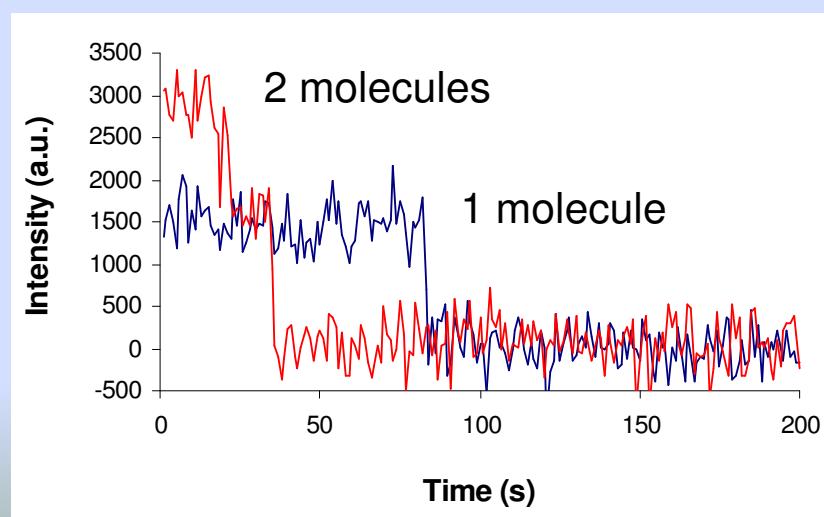
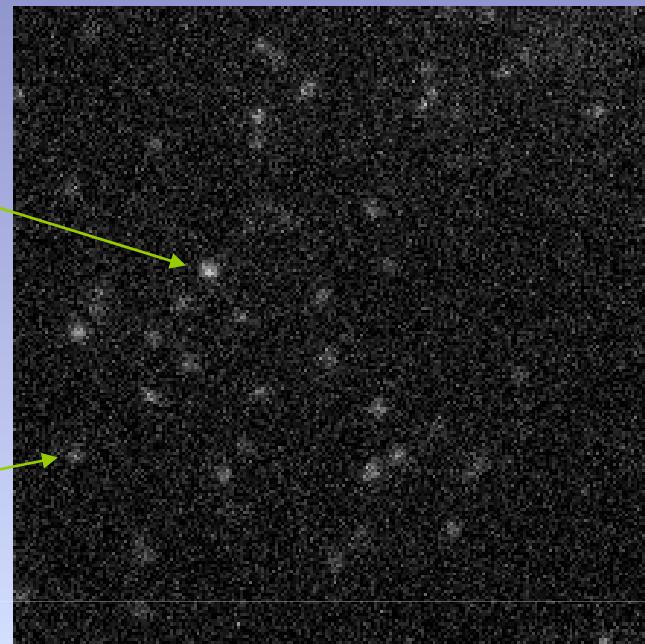


Microscope slide

2 molecules

1 molecule

Bleaching of Cy3



Tracking di singola molecola in cellule viventi

- Morbo di Alzheimer più diffusa malattia neurodegenerativa del cervello
- Disfunzione neuronale correntemente attribuita all'interazione di oligomeri di A-beta con la plasmamembrana
- Meccanismi non chiari
- Finora studi basati solo sulle caratteristiche medie di un insieme di molecole

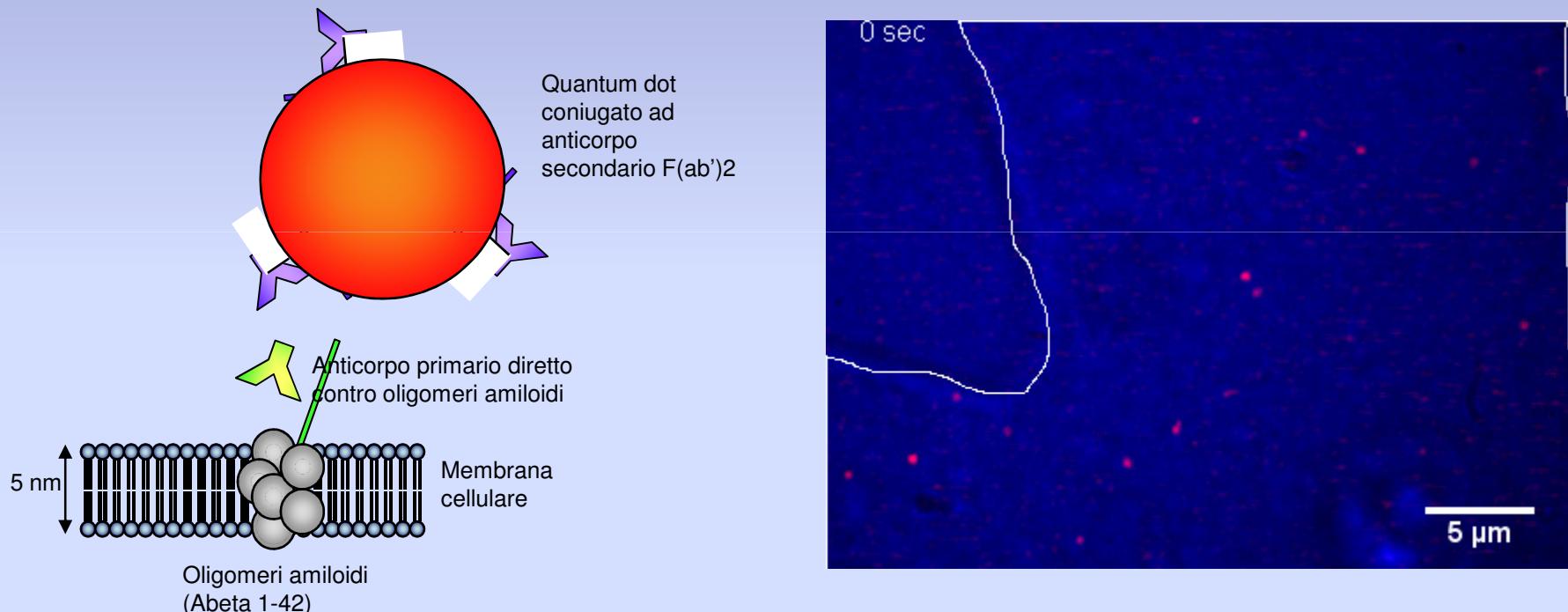
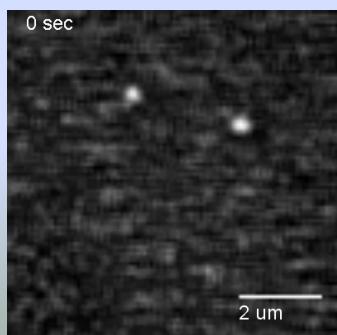
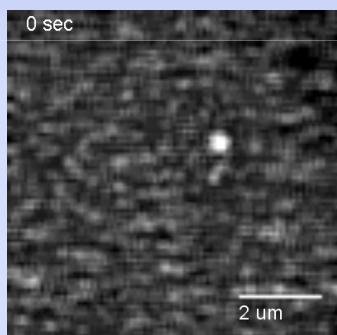
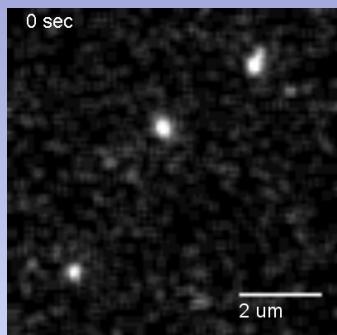


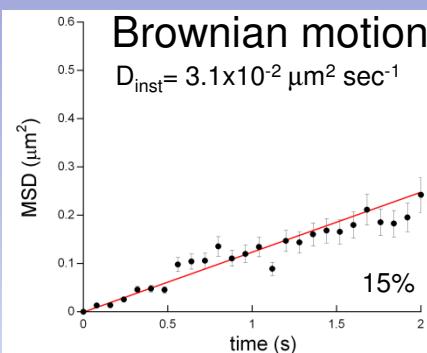
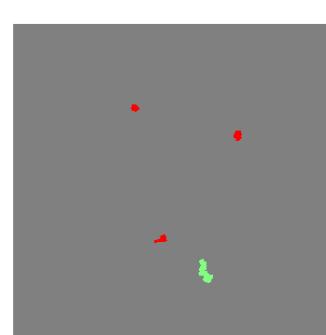
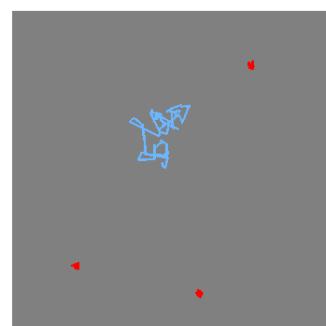
Immagine in onda evanescenze

Distinzione di differenti tipi di moto

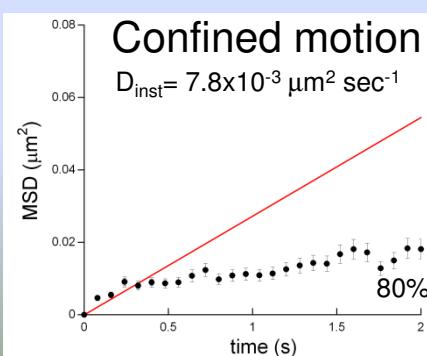
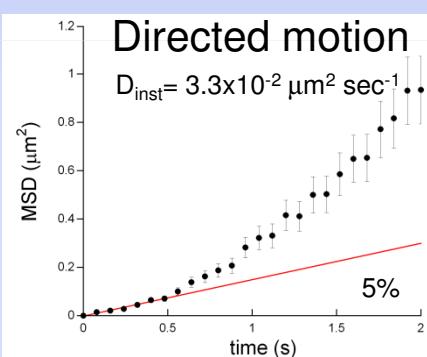
Filmati real time



Ricostruzione delle traiettorie



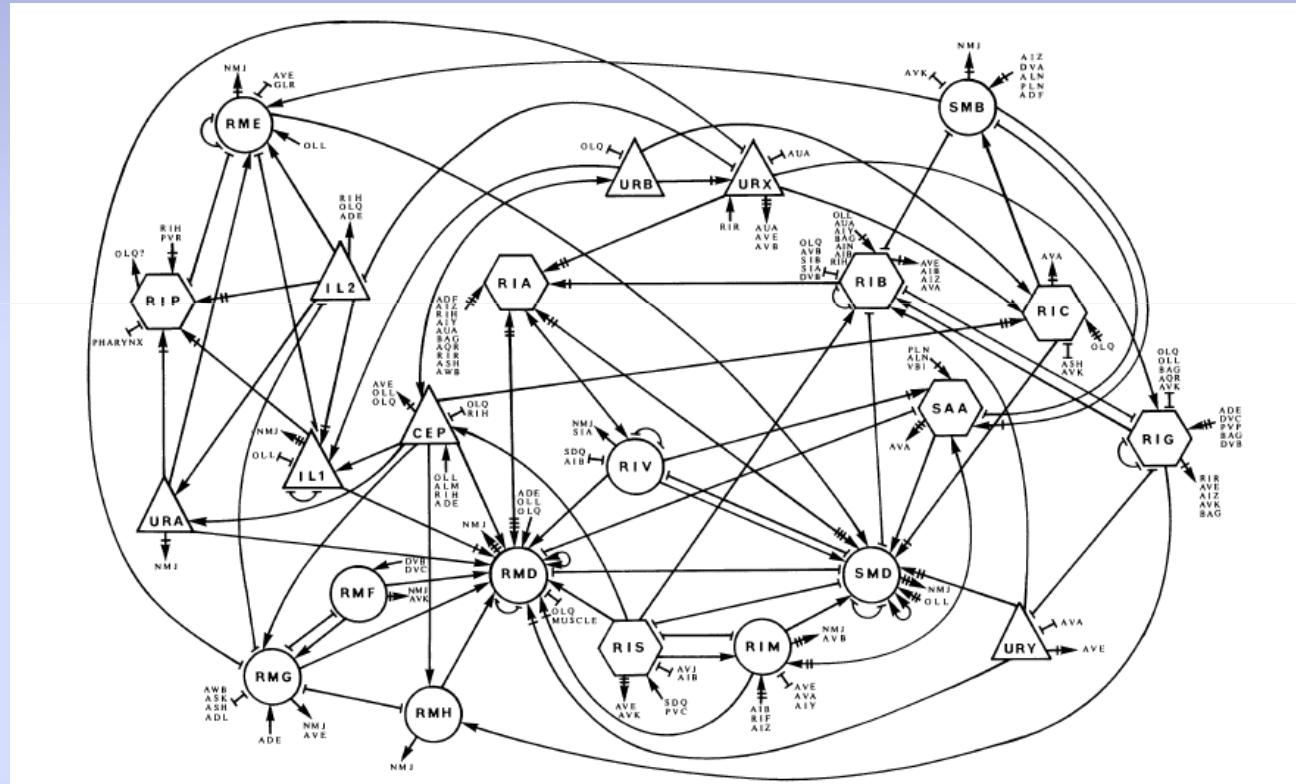
$$\text{MSD}(\tau) = \langle [x(t)-x(t+\tau)]^2 + [y(t)-y(t+\tau)]^2 \rangle$$
$$\text{MSD}(\tau) = 4D\tau$$



Il progetto Connettoma

Cos'è il connettoma?

Il connettoma è la mappatura completa di tutte le connessioni celebrali



Sino ad ora è stata ricostruita solo per un verme, *C. Elegans*, ed è costituita da 302 neuroni

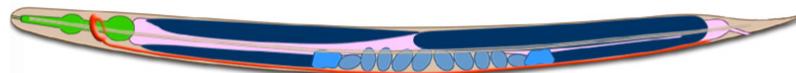
¹ White, J.G. et al., Phil. Trans. R. Soc. Lond. B **314**, 1-340 (1986)

Si può navigare in rete nel CNS del *C. Elegans*

<http://www.wormatlas.org>

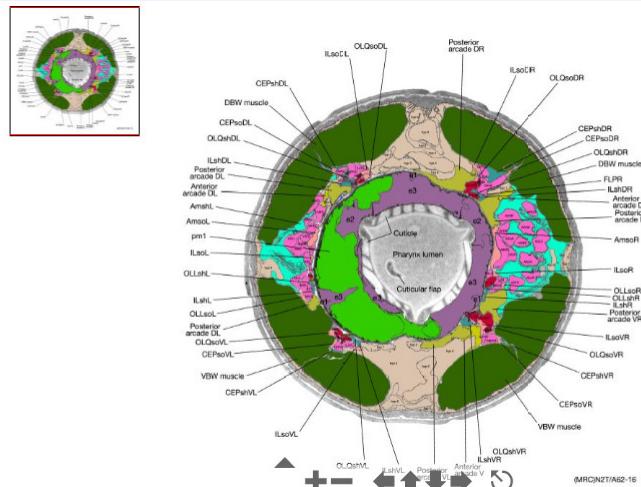
Slidable Worm - WormViewer

Currently available slices: [1](#), [2](#), [3](#), [4](#), [5](#), [6](#), [7](#), [8](#), [9](#), [10](#), [11](#), [12](#), [13](#), [14](#), [15](#), [16](#), [17](#), [18](#), [30](#), [50](#), [83](#), [273](#), [315](#), [716](#), [720](#), [726](#), [730](#), [740](#), [754](#), [771](#), [772](#), [773](#), [775](#), [779](#), [783](#), [784](#), [789](#), [798](#)



Slice #: 17

Label Options: Labels Off Opaque Transparent show



Altri progetti simili

IIC Member login

Initiative in Innovative Computing at Harvard

home > research

the connectome

40,000x40,000 pixels
1.6 GB
120x120 nm (3 nm/pixel)
Here shown 40x undersampled or 1/1600th of the data in a single section. The goal: 10,000 such sections making up a 3D volume

Reconstruct the big axons first: the "wires"

Then find the big synapses: the connections

Then zoom in

1.5 μm

Lead investigators

Hanspeter Pfister (SEAS/IIC), Jeff Lichtman (FAS/Molecular & Cellular Biology, Center for Brain Science) and Clay Reid (HMS/Neurobiology, Center for Brain Science)

Description

The overall goal of the Connectome project is to map, store, analyze and visualize the actual neural circuitry of the peripheral and central nervous systems in experimental organisms, based on a very large number of images from high-resolution microscopy. The proposing team from the Center for Brain Sciences has already demonstrated its capacity for, and expertise in, high-throughput imaging. The critical challenges are computational, as the total number of voxels needed to establish the Connectome is $\sim 10^{14}$. The principal challenges are to develop: (a) algorithms for efficient 3D segmentation, circuit identification (b) the ability to transfer, store and analyze 3D images in multi 100GB range; and (c) scalable database techniques to store, manage and query multi-TB, multi-modal datasets.

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[IIC Basecamp login](#)

Altri progetti simili

The screenshot shows the homepage of PLOS BIOLOGY, a peer-reviewed open-access journal published by the Public Library of Science. The top navigation bar includes links for Home, Browse Articles, About, For Readers, and For Authors and Reviewers. On the right, there are options to PROFILE, SEARCH, and LOG IN. A search bar is also present. Below the navigation, a blue banner indicates the article is a RESEARCH ARTICLE and OPEN ACCESS. The main title of the article is "Serial Block-Face Scanning Electron Microscopy to Reconstruct Three-Dimensional Tissue Nanostructure". The lead authors are Winfried Denk^{1*} and Heinz Horstmann¹. The article is associated with Max Planck Institute for Medical Research, Heidelberg, Germany. The abstract discusses the challenges of obtaining 3D structural information at various length scales and how automated block-face imaging combined with serial sectioning can overcome these challenges, particularly in the nervous system.

PLOS BIOLOGY
a peer-reviewed open-access journal published by the Public Library of Science

RESEARCH ARTICLE OPEN ACCESS

Serial Block-Face Scanning Electron Microscopy to Reconstruct Three-Dimensional Tissue Nanostructure

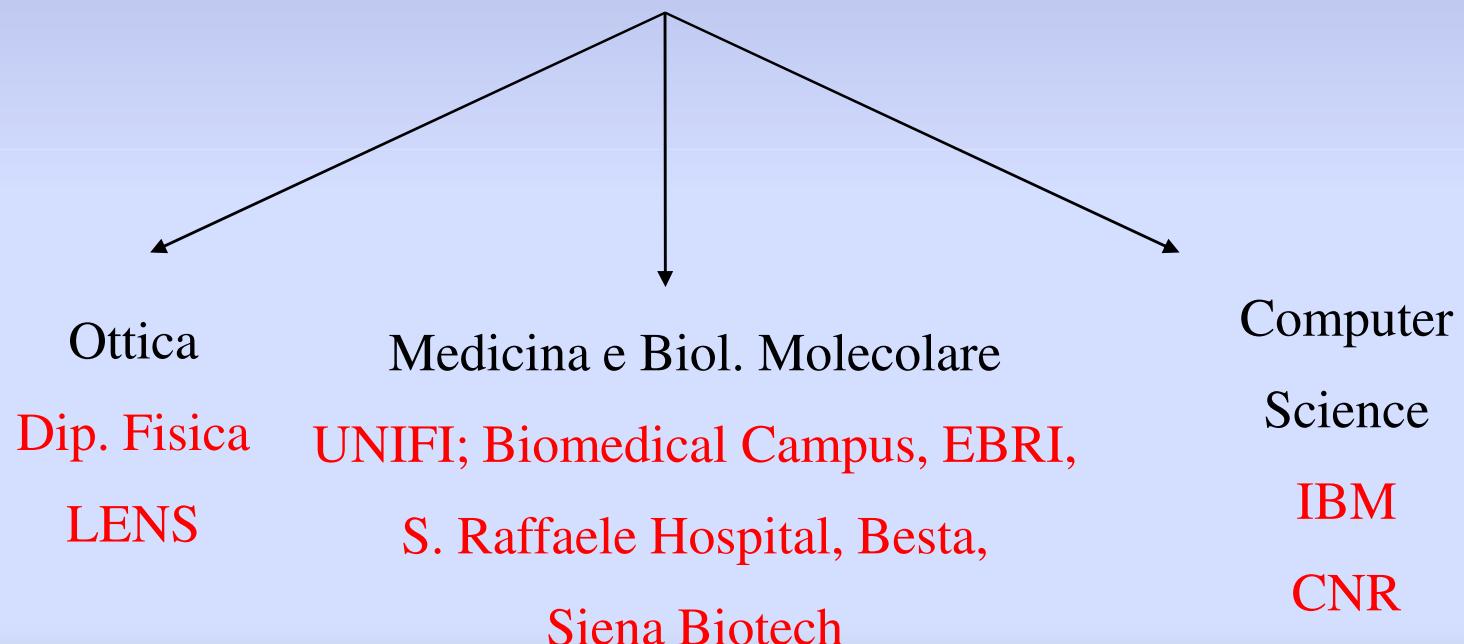
Winfried Denk^{1*}, Heinz Horstmann¹

¹ Max Planck Institute for Medical Research, Heidelberg, Germany

Three-dimensional (3D) structural information on many length scales is of central importance in biological research. Excellent methods exist to obtain structures of molecules at atomic, organelles at electron microscopic, and tissue at light-microscopic resolution. A gap exists, however, when 3D tissue structure needs to be reconstructed over hundreds of micrometers with a resolution sufficient to follow the thinnest cellular processes and to identify small organelles such as synaptic vesicles. Such 3D data are, however, essential to understand cellular networks that, particularly in the nervous system, need to be completely reconstructed throughout a substantial spatial volume. Here we demonstrate that datasets meeting these requirements can be obtained by automated block-face imaging combined with serial sectioning inside the chamber of a scanning electron microscope. Backscattering contrast is used to visualize the heavy-metal staining of tissue prepared using techniques that are routine for transmission electron microscopy. Low-vacuum (20–60 Pa H₂O) conditions prevent charging of the uncoated block face. The resolution is sufficient to trace even the thinnest axons and to identify synapses. Stacks of several hundred sections, 50–70 nm thick, have been obtained at a lateral position jitter of typically under 10 nm. This opens the possibility of automatically obtaining the electron-microscope-level 3D datasets needed to completely reconstruct the connectivity of neuronal circuits.

Il Nostro Approccio sul Modello Animale

Le competenze in gioco



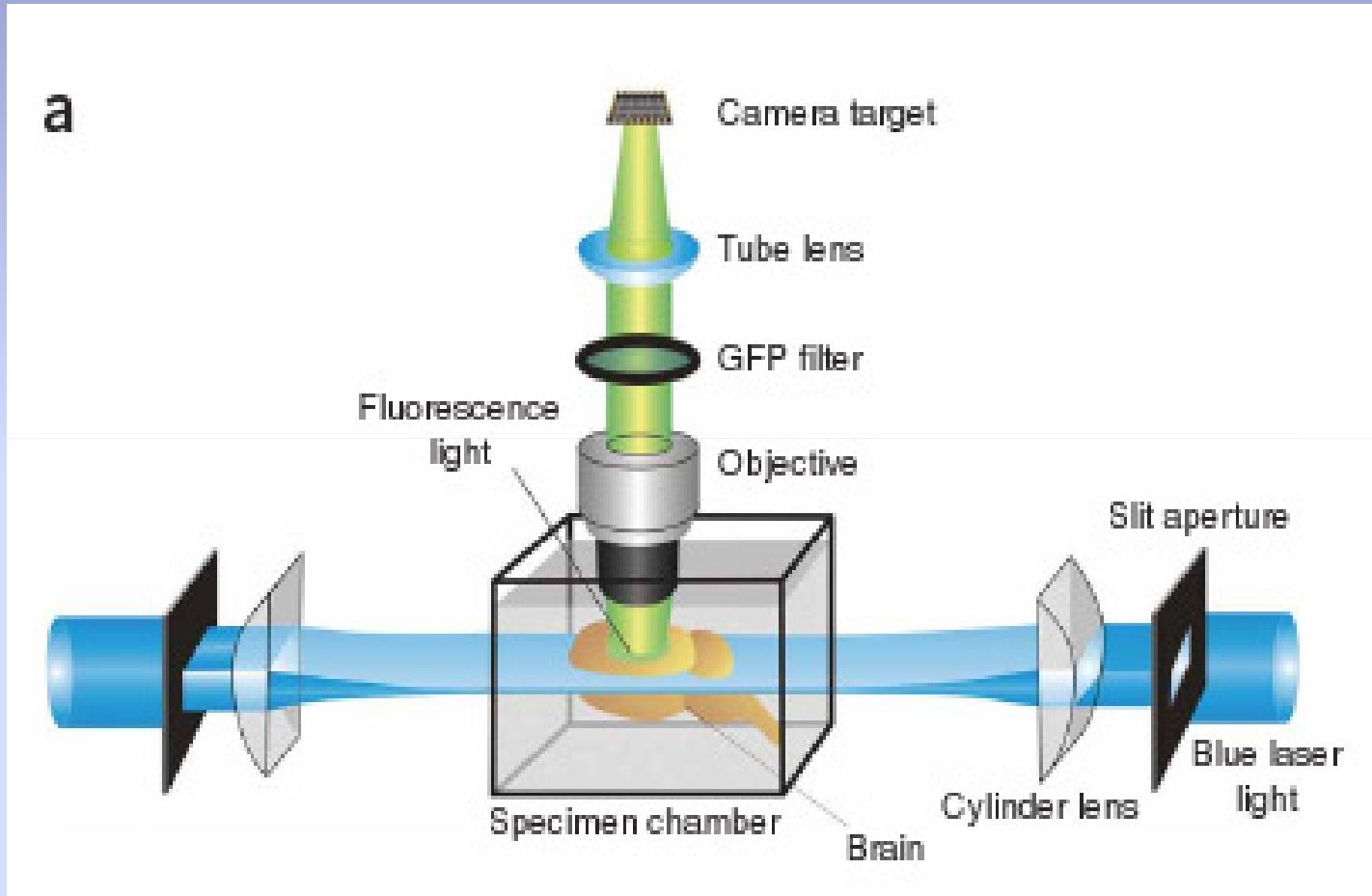
Vantaggi

**Penetrazione Profonda nei tessuti
Imaging Funzionale (fMRI, PET)**

Svantaggi

**Bassa capacità di distinguere particolari
(risoluzione del millimetro)**

La Tomografia ottica sviluppata al dip. di Fisica

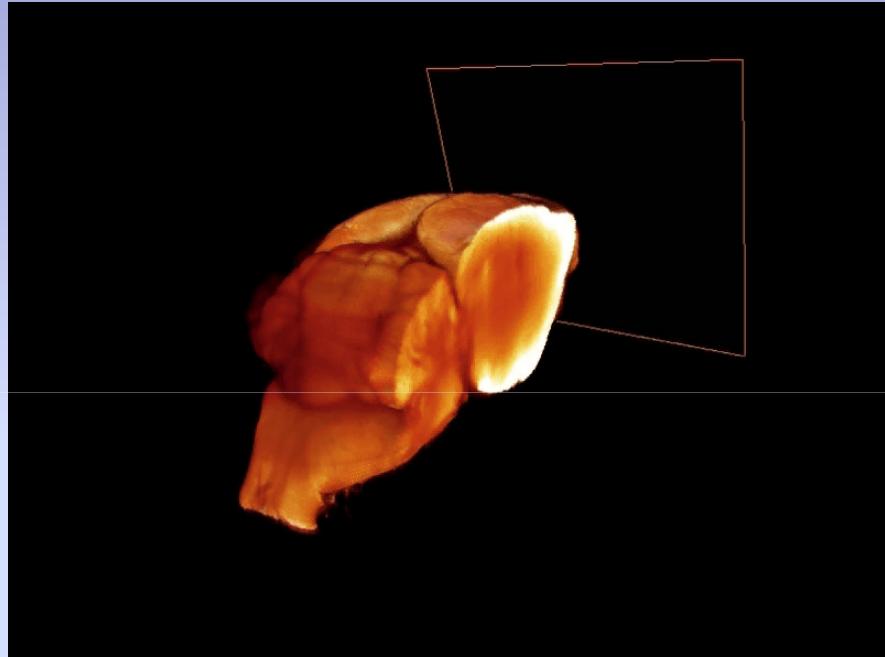


¹ Siedentopf, H. and Zsigmondy, R., Annalen der Physik **10**, 1-39 (1903)

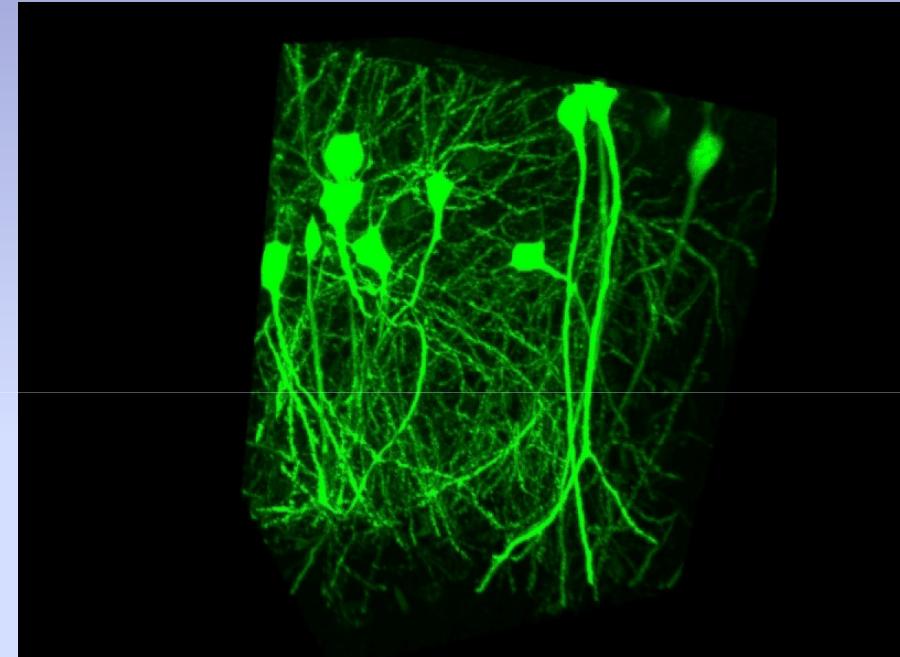
² Dödt, H.-U. *et al.*, Nature Methods **4**, 331-336 (2007)

La Tomografia Ottica

Topolini transgenetici con neuroni fluorescenti



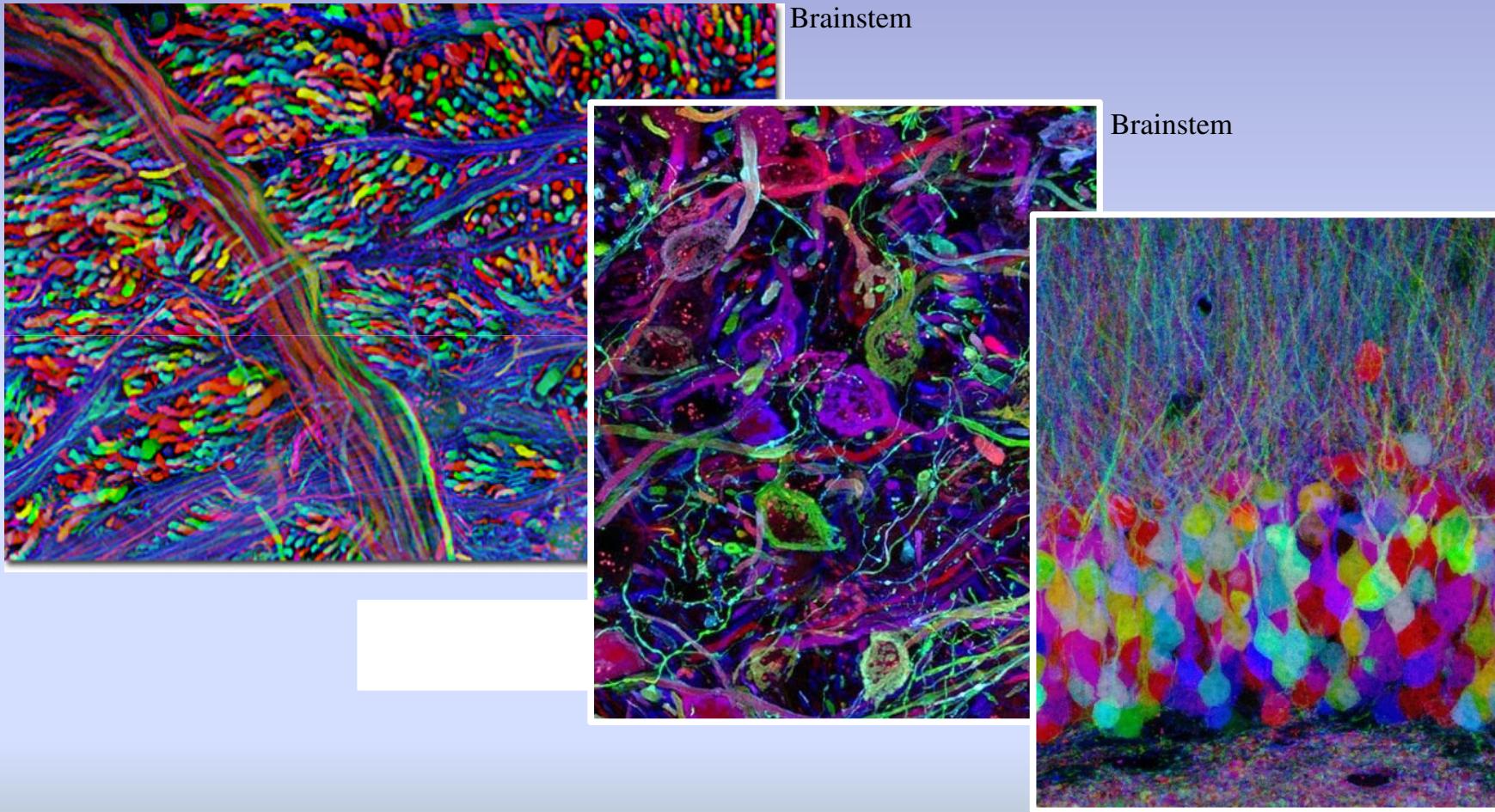
Tomografia Ottica



Ricostruzione tridimensionale dei neuroni
piramidali nell'ippocampo

¹ Dodt, H.-U. *et al.*, Nature Methods **4**, 331-336 (2007)

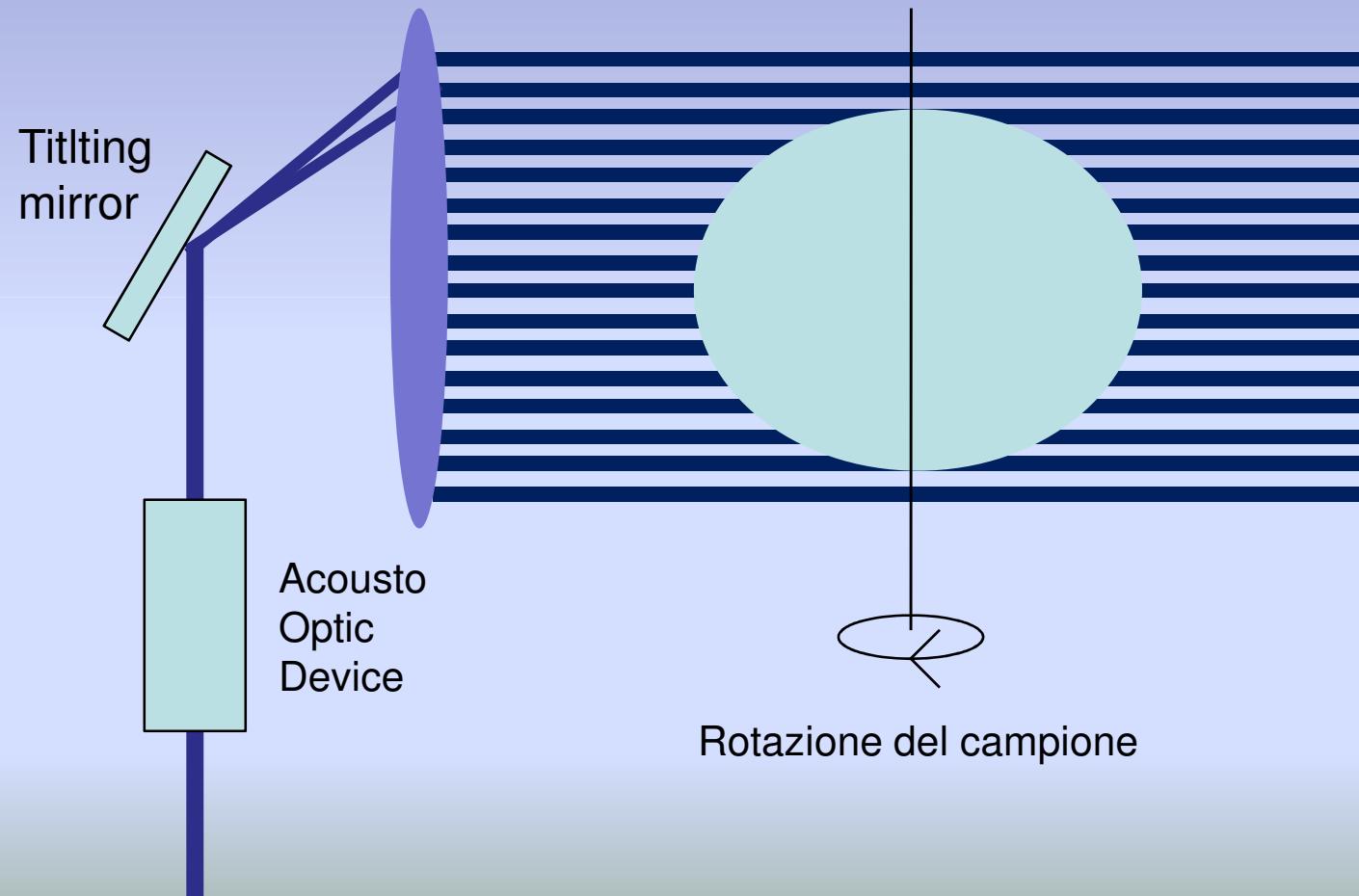
I Topolini “Arcobaleno”



Livet, J., *et al.*, Nature **450**, 56-62 (2007)

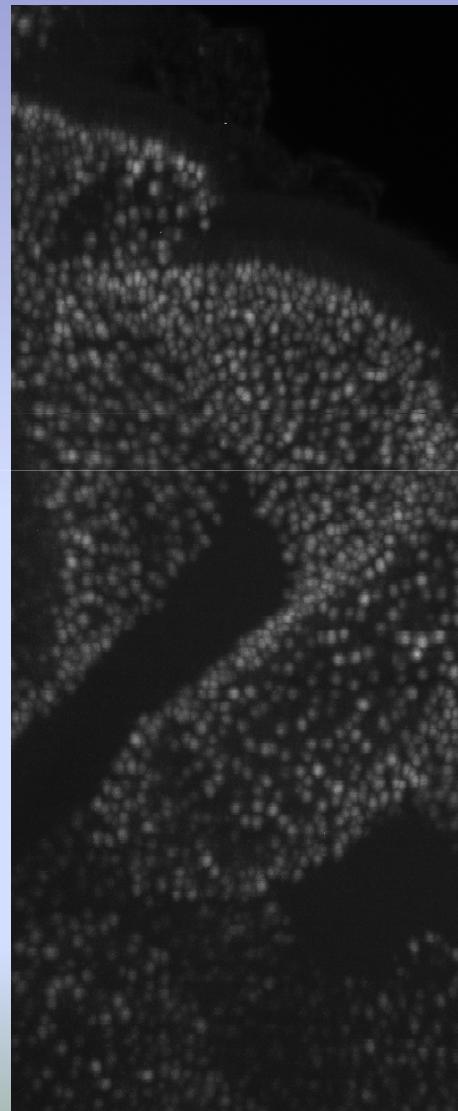
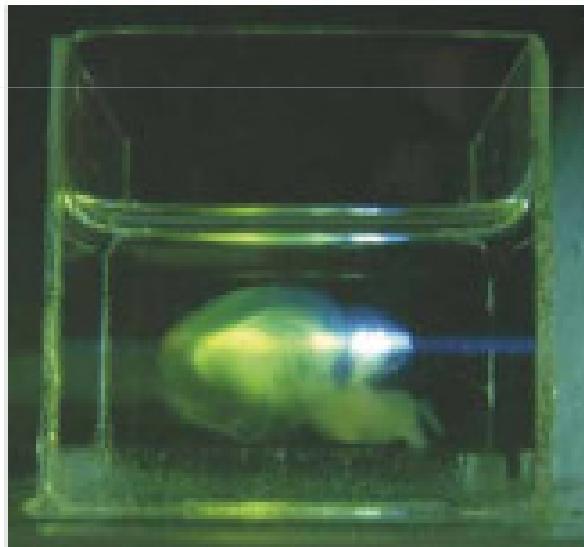
Risultati preliminari con Ultramicroscopio

Creazione di una fetta di luce strutturata
Singolo beam : fascio di Laguerre Gauss



Risultati preliminari con SPIM

Cervello intero di topo
L7 GFP
Bleaching Completo



- Proiezione 2D di Cervelletto
- 500microns- 1000microns
- Stack su 1mm
- eccitazione a 488 nm ---
- potenza 5-7 mW
- larghezza a metà altezza del fascio circa 7.2 micron
- esposizione di 100 ms.
- NA 0.35
- emissione con picco a 515 nm, filtro 500-540 nm.
- Luce strutturata con duty cycle 1:2 (il singolo beam è largo circa 15 micron)

Prospettive mediche: Verso un'anatomia di rete

- Ci sono differenze strutturali tra maschi e femmine?
- Quali sono le dipendenze dei comportamenti dalla struttura di rete del cervello? Schizofrenico, autistico, compulsivo, killer
- Ci sono sostanze chimiche che possono modificare la struttura del cervello?
- Alcune malattie neurodegenerative, Alzheimer, dipendono dalla struttura della rete?

Collaborazioni con altri gruppi italiani

Urologists

G. Nesi
A.Crisci
B.M. Carini



Cornea

R. Pini (CNR)
F. Ratto
P. Matteini
F. Rossi

Coherent

M. Arrigoni
D. Armstrong
C. Dorman

Dept. of dermatology

D. Massi
V. De Giorgi
T. Lotti

Neurologists

P. Strata (EBRI)
F. Keller (Biomed. Campus)
E. d'Angelo (Univ. Pavia)

Persone coinvolte nelle ricerche presso il lab 30 del Dipartimento

Letizia Allegra Mascaro (Chimico)
Paride Antonucci (Biologo)
Martino Calamai (Biologo)
Marco Capitanio (Fisico)
Riccardo Cicchi (Fisico)
Alessandro Cosci (Fisico)
Dimitris Kapsokalivas (Fisico)
Jacopo Lotti (Biotecnologo)
Leonardo Sacconi (Fisico)
Ludovico Silvestri (Fisico)
Francesco Vanzi (Biologo)

Persone e finanziamenti di progetti svolti presso il dipartimento di Fisica

R. Cicchi (senior post doc) : Development of a compact, low cost and easy to use device based on LED technology for non-invasive selective haemostasis to benefit the people suffering from coagulation problems: **EU Collaborative Project** Grant agreement no.: 232397

P. Antonucci (thesis student), L. Sacconi (senior post doc): Identification and therapeutic targeting of common arrhythmia trigger mechanisms **EU Coll. Proj.** Grant agreement no.: 241526

L. Sacconi (senior post doc), J. Lotti (PhD) :

Neural Transmission of Electrical Signals in developmental embryos. **Human Science Frontiers Project.**

Misura simultanea di attività di singoli neuroni nel cervelletto. **Ente cassa di risparmio di Firenze**

Misura simultanea di attività elettrica di singoli neuroni in rete intatta **PRIN 2007**

F. Vanzi (senior post doc): Enhanced sensitivity Nanotechnology-based Multiplexed Bioassay Platform for diagnostic applications **EU Coop. Proj.** Contract NMP4-SL2008-211383

M. Calamai (post doc) and M. Capitanio (senior post doc) SingleMolAlzheimer Dissecting Alzheimer's disease at a single molecule level. **EU Marie Curie project** FP7-PEOPLE-2009-IEF

D. Kapsokalyvas, PhD, A. Cosci , PhD, Morphochemistry of skin disease, **photronics4life project (NOE)**

L. Silvestri PhD, L. Allegra Mascaro PhD, ultramicroscopy of neural connections, **ente cassa di risparmio di firenze**

Articoli 2007 ad oggi

Author(s): Cicchi, R (Cicchi, Riccardo); Crisci, A (Crisci, Alfonso); Cosci, A (Cosci, Alessandro); Nesi, G (Nesi, Gabriella); Kapsokalyvas, D (Kapsokalyvas, Dimitrios); Giancane, S (Giancane, Saverio); Carini, M (Carini, Marco); Pavone, FS (Pavone, Francesco S.)

Title: Time- and Spectral-resolved two-photon imaging of healthy bladder mucosa and carcinoma in situ

Source: OPTICS EXPRESS, 18 (4): 3840-3849 FEB 15 2010

ISSN: 1094-4087

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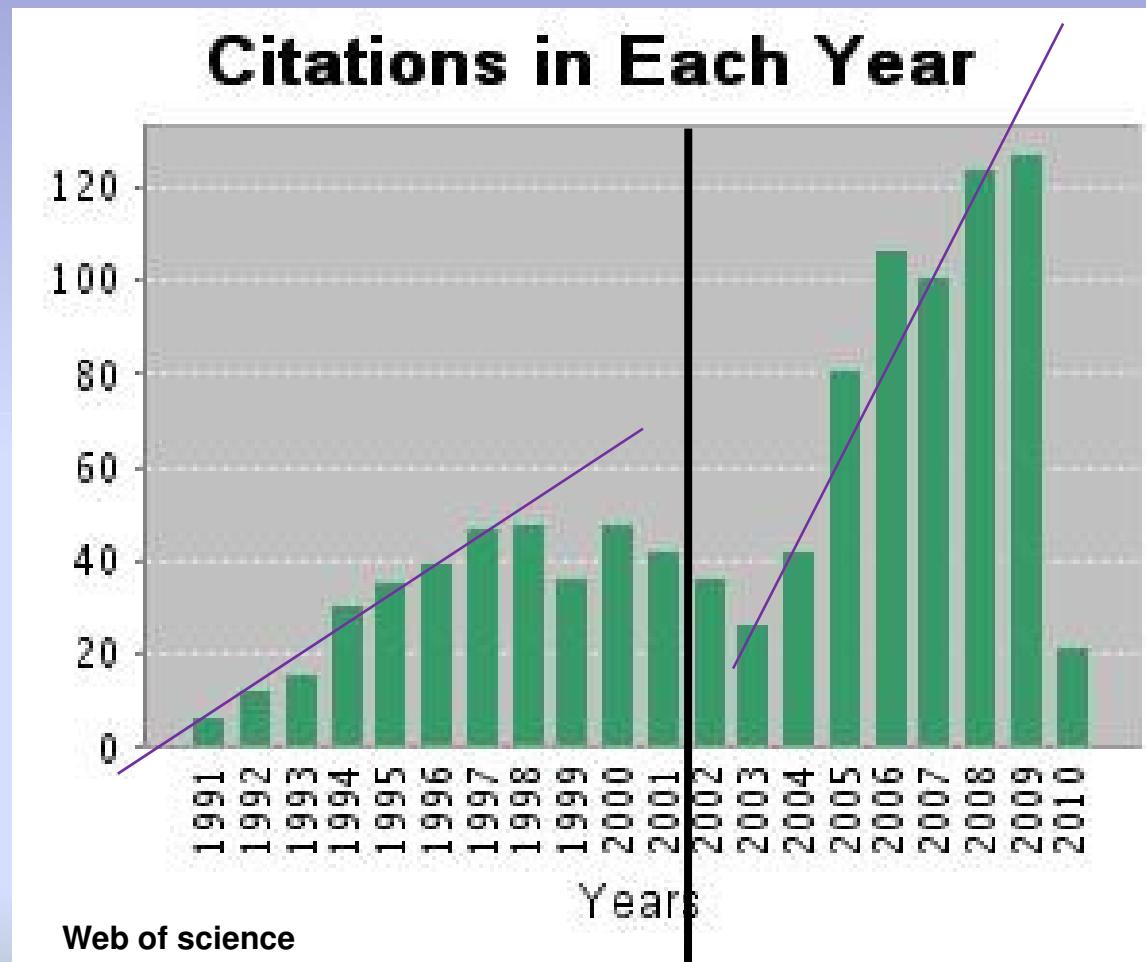
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