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Multi-modalities and Non-invasive Imaging In Tissue Engineering : From Microscopy to Macroscopy

PTIBC-IBISA
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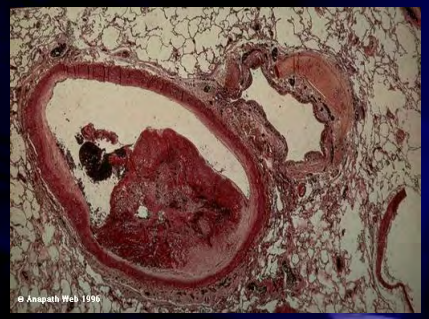
• 3D Imaging : Medical Tissue Engineering (stem cells)

Endothelial Cells
Blood vessel



Occlusion
Atherosclerosis

Hyalin chondrocytes
Cartilage matrix

Degenerative disease
Osteoarthritis

Ligament fibroblasts

LCA Rupture

Thick and opaque specimens

1998 : 3D deconvolution imaging

2002 : CLSM Confocal Laser Scanning Microscopy

2003 : TPE Multiphoton Microscopy (FLIM)

2006 : FCS

2007 : SHG Microscopy

2009 : MP-SHG Macroscopy (prototype)

2010 : Phase Imaging

Future …

Core Facility Service

Industrial Partnership

R&D
Collagen imaging
Phase imaging

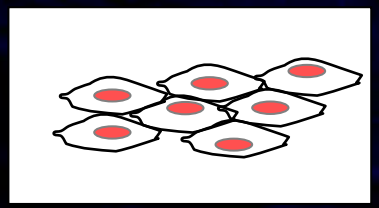
- **Collagen for medical scaffold : Invasiness 3D network Imaging**

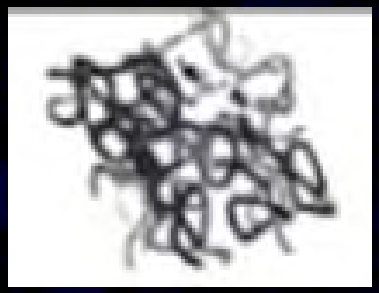
Noninvasive evaluation of MSC –b scaffold for Medical application

Pre-3D culture evaluation

Pre-implantatory evaluation

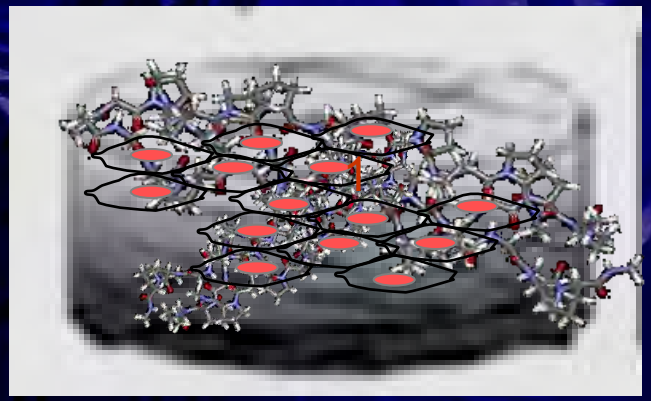
MSC





Scaffold

Expansion
In Vitro

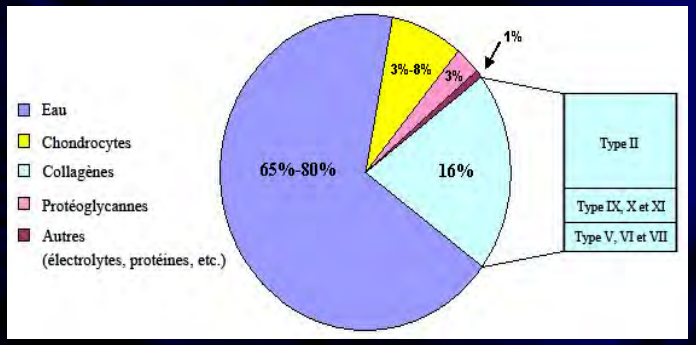


Scaffold + MSC

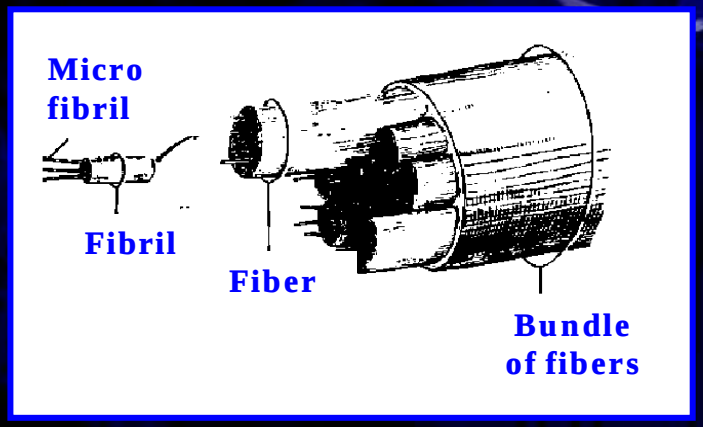
Implantation
In vivo

Multiscaled device (from cell to embedded scaffold)
No contrast agent
Qualitative evaluation of collagen network
Quantitative evaluation : cell proliferation / synthesis

• 3D network : collagen functional



Cartilage Composition



Organization of collagen fibrils into fibers and bundles

	Type	Polymerized form	Representation
Fibrils	**I**	fibrils	Bone, skin, tendon, ligament, cornea, internal organs, fibrous cartilage
	II	fibrils	**cartilage** (elastic and hyaline)
	III	fibrils	hypoderm, vessel, hair
	V	fibrils (with type I)	see type I
	XI	fibrils (with type II)	see type II
Associated with fibrils	IX	Lateral connection with type II fibrils	cartilage
	XII	Lateral connection with type II fibrils	Some other tissues
Network	**IV**	network	Basal lamina
	VII	Anchoring fibrils in basal membranes	epidermis

Evaluation of Functional network
Mechanical properties

Standing Position → **0,7 MPa**

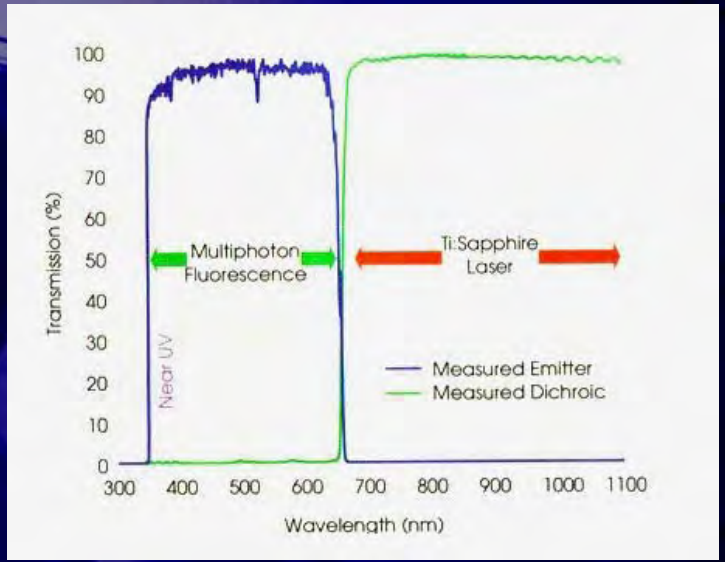
Walk → **5-10 MPa**

Sport → **>18 MPa**

• Interaction light / Matter : IR light for Tissue Imaging

Tissue imaging :
-Cartilage
-Tumors
-Arteries
-Tendon…

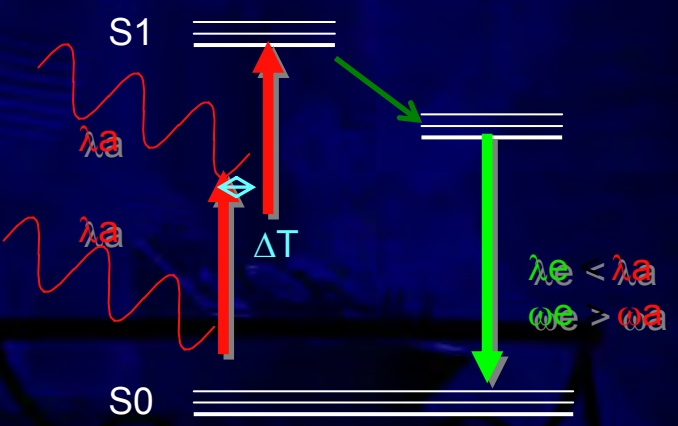
} Thick and opaque specimens



Advantages of multiphoton (IR) excitation

- Less absorption by biological specimens
  → Deeper penetration (than UV-Visible)
- Less photodamage
  → confinement of excitation to the focal plane

TPE (Two Photon Excitation)



Non linear Absorption
Emission in visible

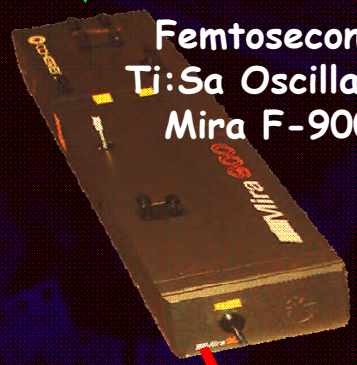
Principle of multiphoton excitation

- Spatio-temporal confinement of photons
- Conditions obtained with short impulsions at high frequency

• IR pulsed laser : modalities imaging techniques

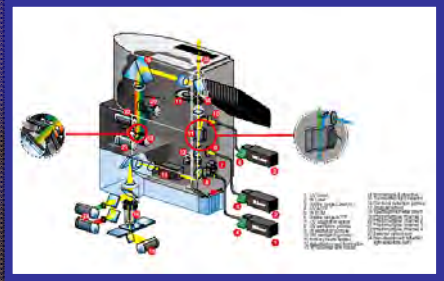
e

Femtosecond
Ti:Sa Oscillator
Mira F-900

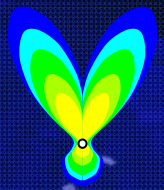


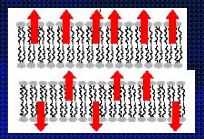
Galvano
metric
mirrors

Spectral



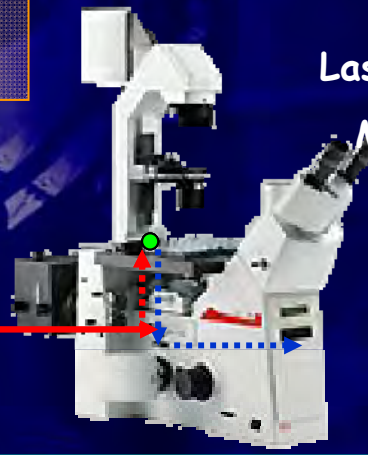
SHG





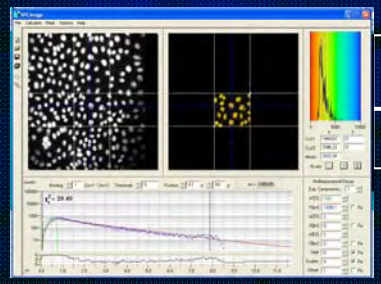
SHG

SHG



Laser Scanning
Microscope

FLIM



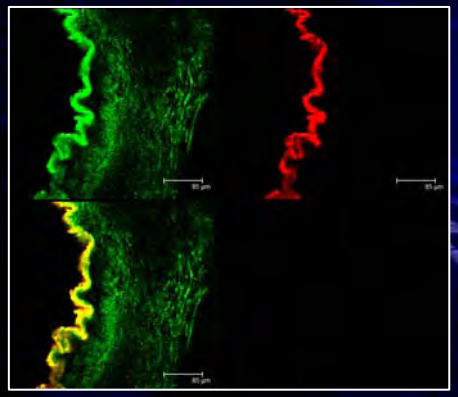
Module SPC730

Time Correlated Single
Photon Counting

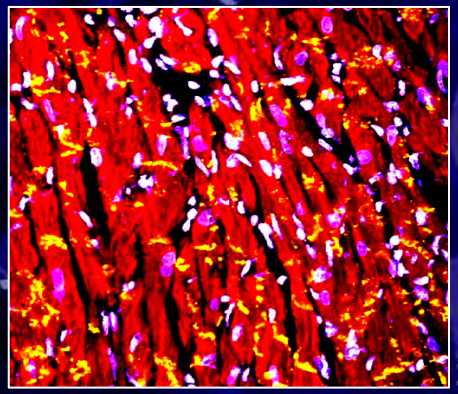
Multiphoton Excitation

• MP imaging applied to tissue : fluorescent probe

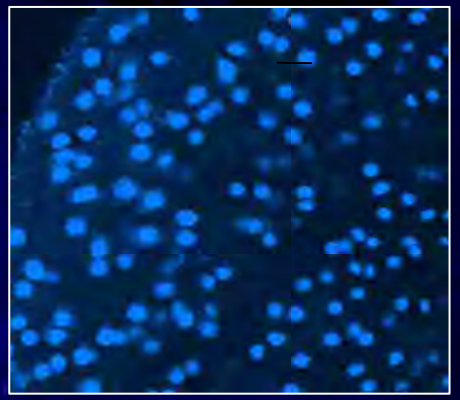
Femoral artery

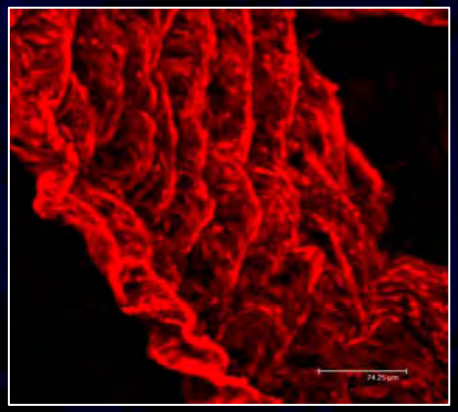


Graft Cardiomyocyte

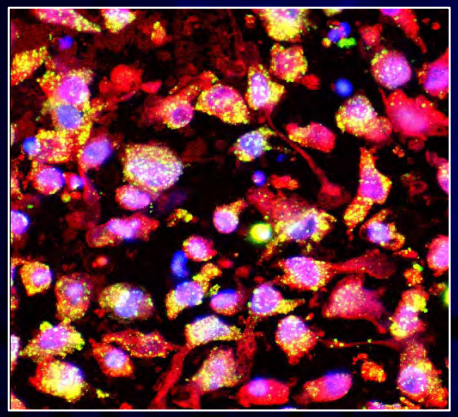


Rat articular cartilage
Chondrocyte (nucleus in blue)
Depth : 219 µm

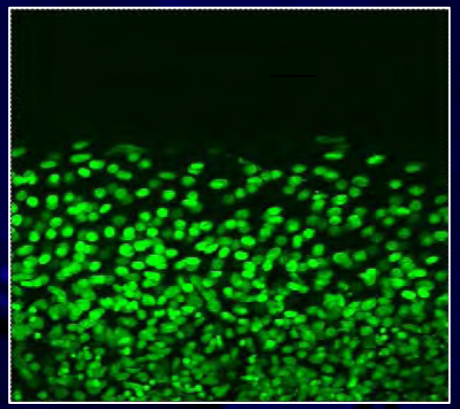




Femoral artery
+ film PAH- Rhodamine

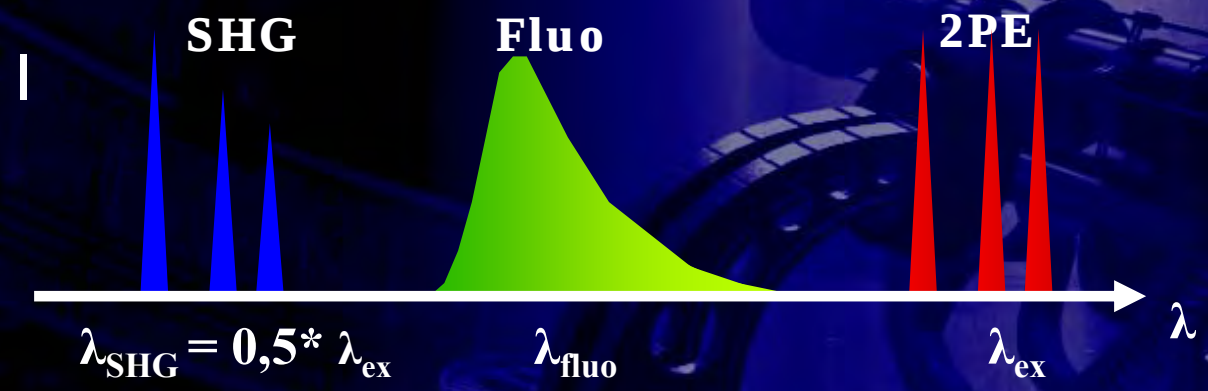


Apoptosis (live/dead)

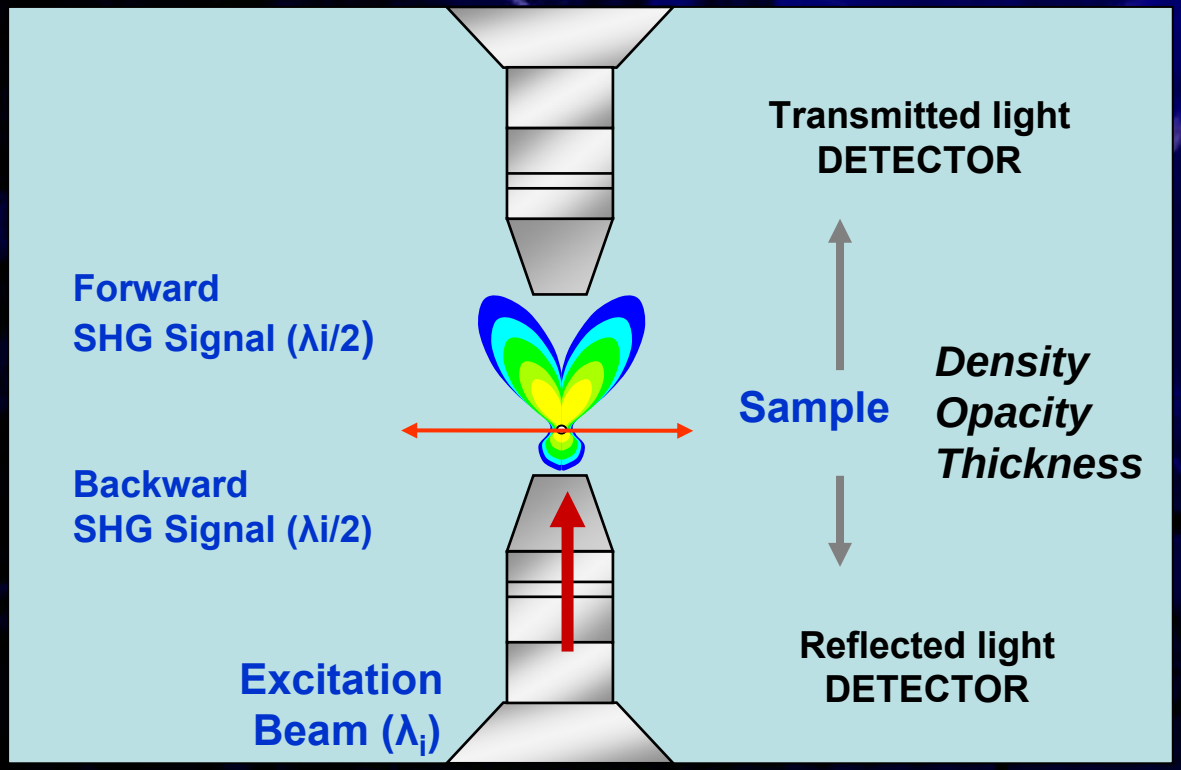


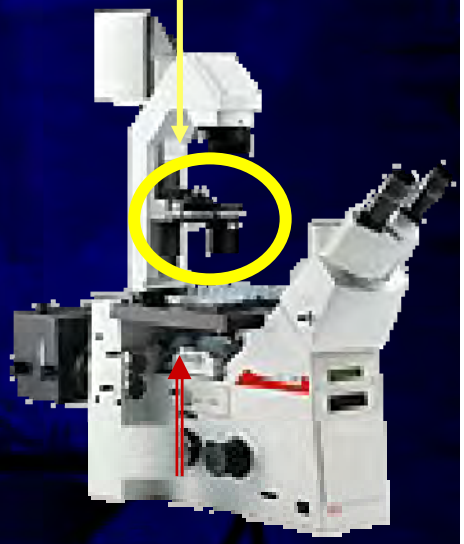
Chondrocyte in aliginate bead
Depth : 1600 µm

• SHG : Second Harmonic Generation



PMT (Transmitted Light)
BP Filter 400 ± 25 nm
(=800/2)
LP Filter 700 nm (IR)



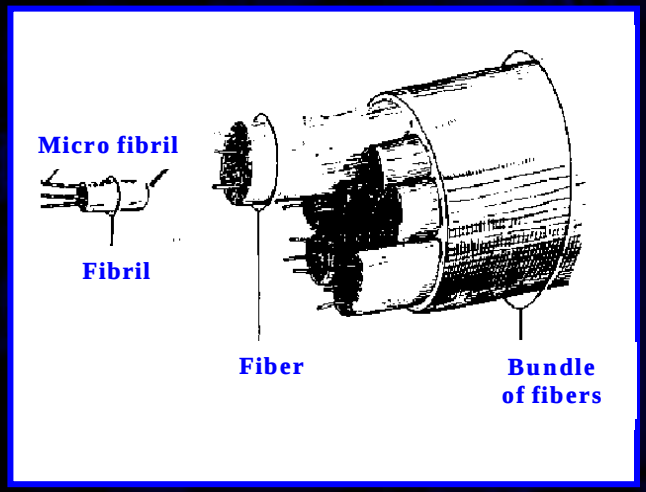


Excitation
Beam (λ_i)

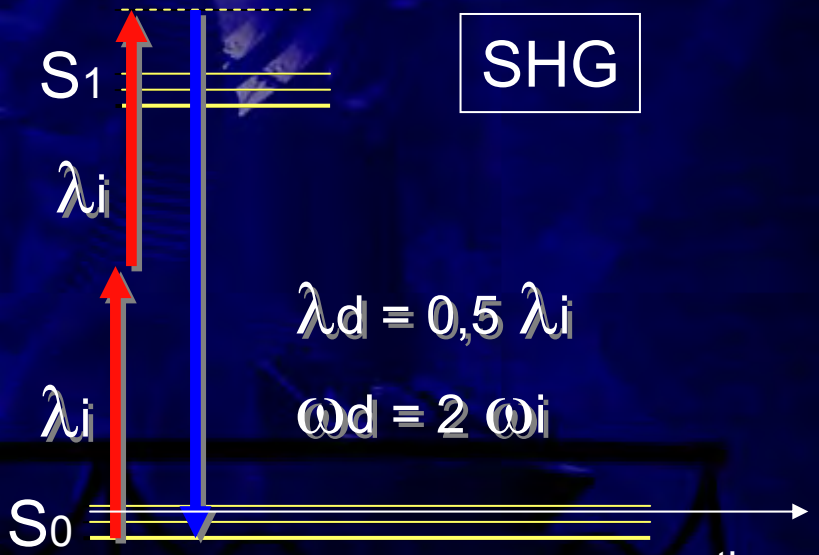
• SHG collagen : high non linear susceptibility

- Main tissular component giving rise to SHG : Collagen
- Importance of orientation and arrangement of molecules
- Quadratic dependence on number of molecules

- Quasi instantaneous Generation (fs)→ Coherent Signal
- No exogenous dye→ Diminution of cytotoxic and phototoxic effects
- No absorption process, no photobleaching (≠ Fluorescence)
- Quadratic dependence on excitation power



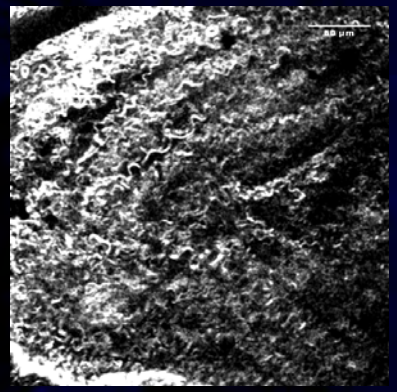
- Crystalline triple-helical structure (left)
- Non-centro symmetric structure



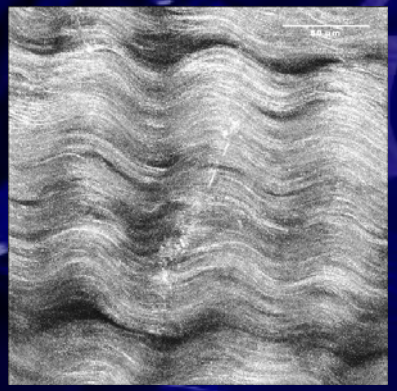
Non linear Diffusion

- **SHG : 3D network for functional collagen**

Skin mouse

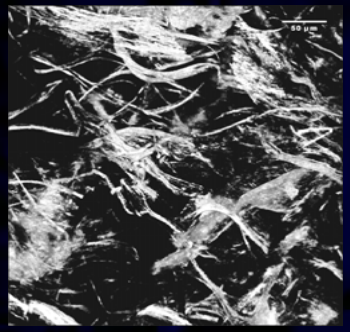


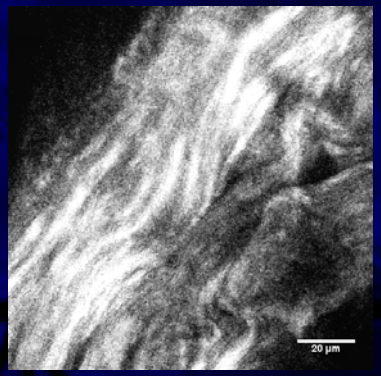
Rabbit Tendon



Rabbit muscle

Collagen I sponge

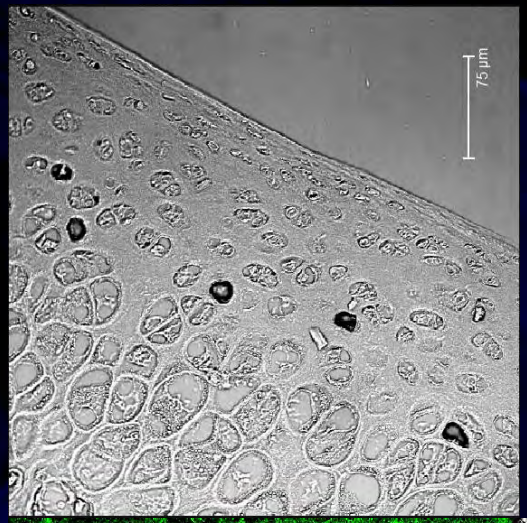




Inter-costal cartilage (sternum)

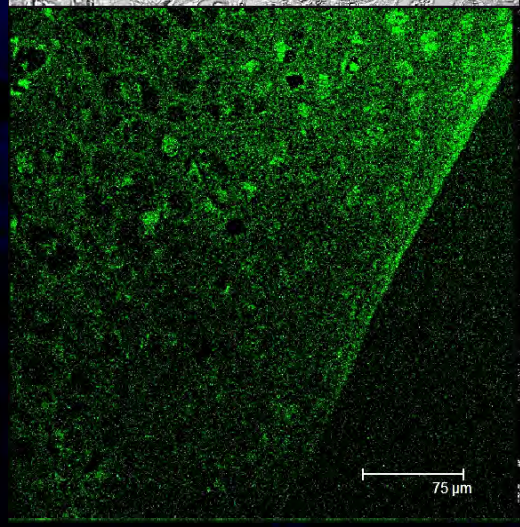
• Cartilage SHG imaging : quantitative evaluation

Rat Articular Cartilage

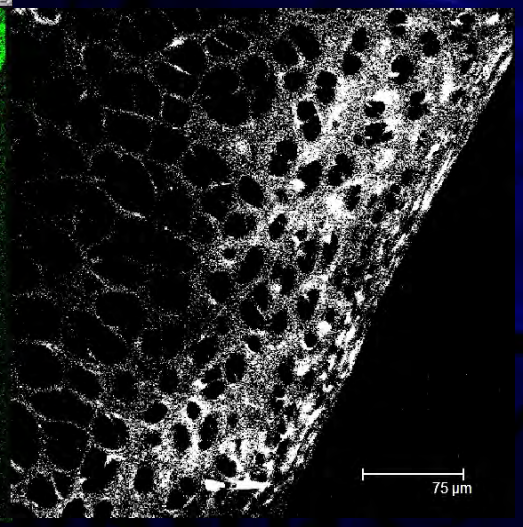


LT

AF



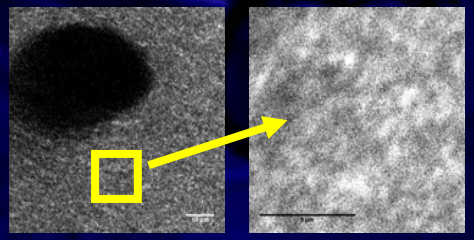
SHG



Textural analysis ?
3D Organisation ?

Denaturation
Degradation
Synthesis

Haralick's analysis



Gray levels : SHG - Collagen

Haralick :

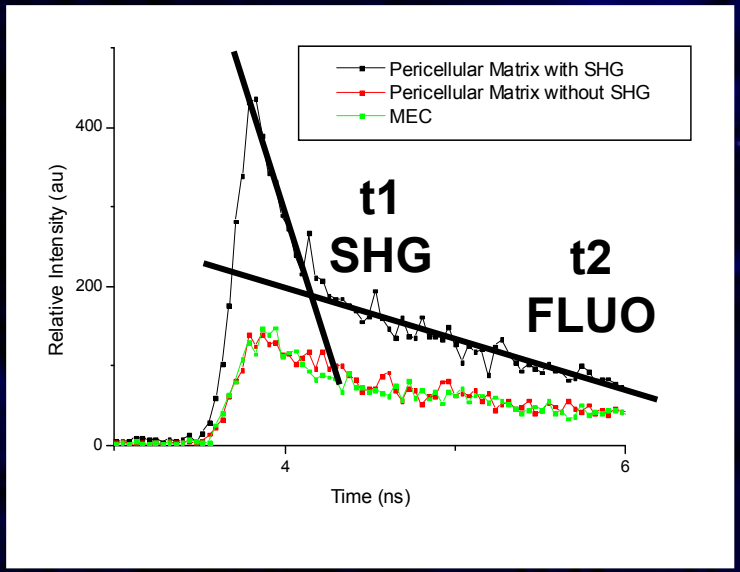
Textural analysis based on cooccurrence matrix

9 parameters of textural elements:

- **Homogeneity**
- **Size**
- **Linearity**
- **Contrast**
- **Variance**
- **Second Angular Moment ...**

• Micro- TCSPC- SHG imaging : time decay in matrix

Fluorescence Lifetime Imaging Microscopy
Biexponential adjustment of Decay curves

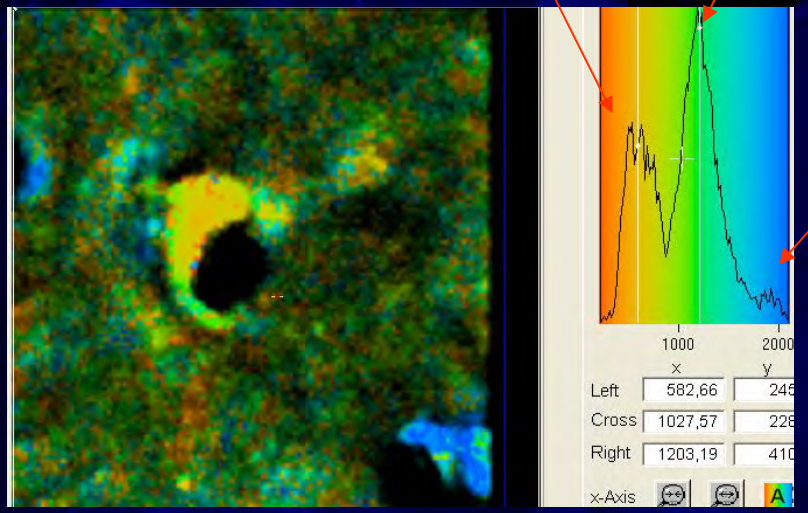


Fluorescence Decay Curves

SHG + Fluorescence Signal

Autofluorescence + Fluorescence Signal

Alexa 488 Fluorescence Signal



Mean Fluorescence lifetime (τ_m)
Color coded image

• SHG - TCSPC imaging : distribution of first component

MSC in sponge
TGF-BMP

Intensity

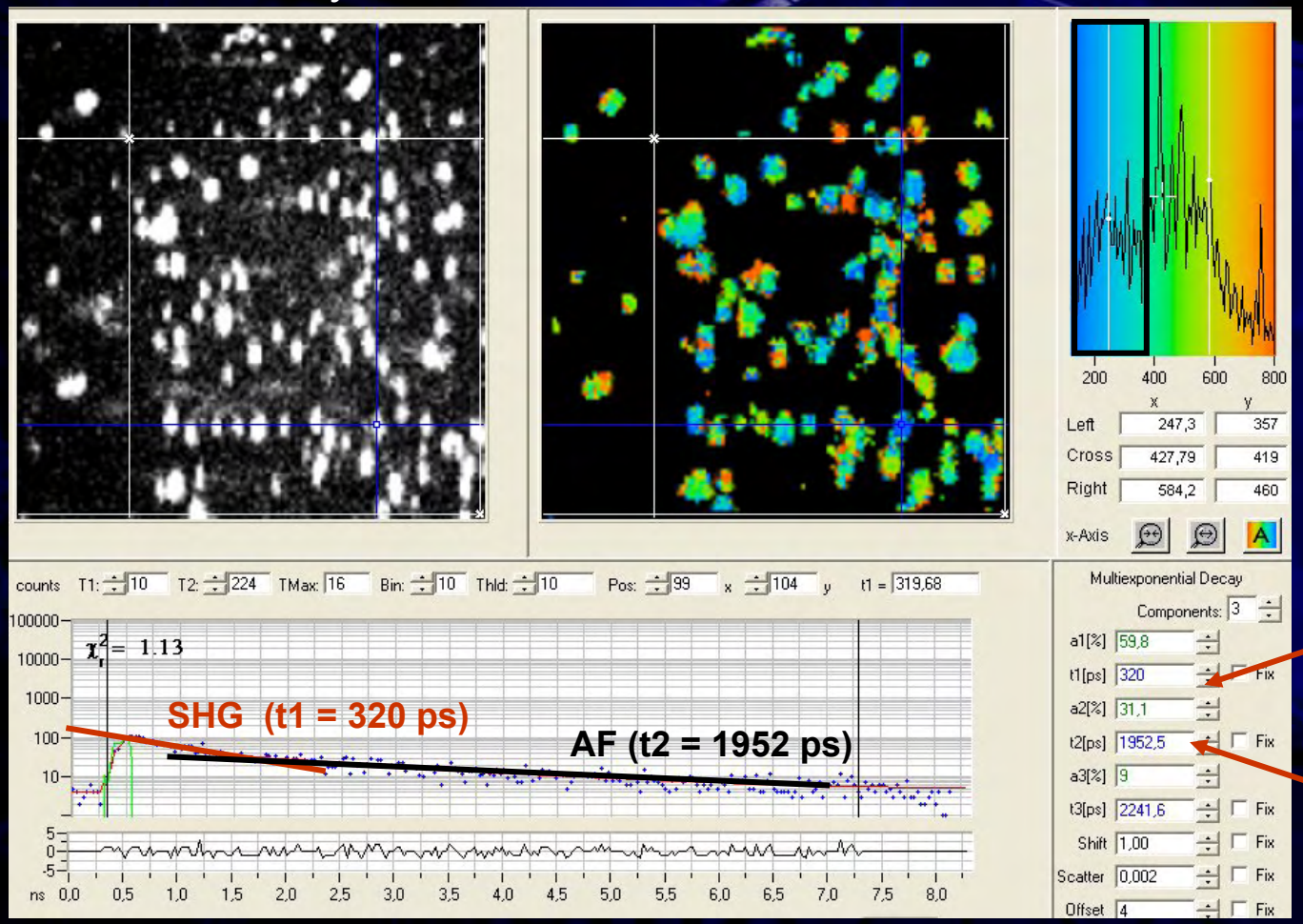
Lifetime

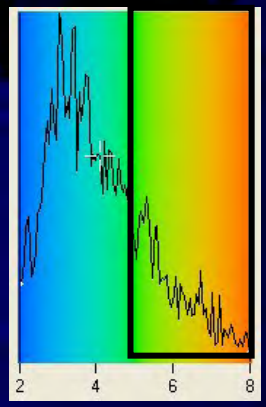
t1

SHG AF

t2/t1

AF SHG





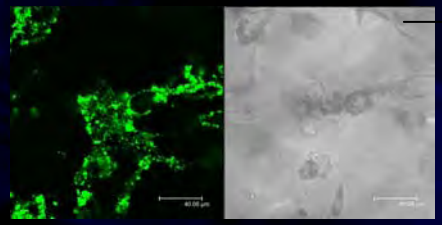
descriptor
Proliferation /
Synthesis

t1
SHG collagen
diffused

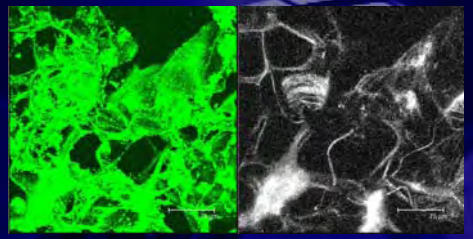
t2
AF cells
reflected

• SHG Multimodalities Imaging : Limitations in Microscopy ?

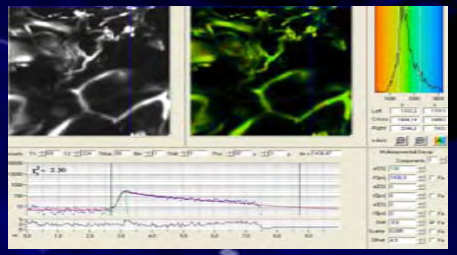
MSC in Col1 sponge – Mab anti-Col2 (alexa-488)
Coll. A.Pinzano – C. Henrionnet – P. Gillet



Fluo 2D
LT



Fluo3D
SHG



Index
SHG/AF

Limitations for medical applications : labelling / cutting / depth penetration

- Destructive assay for deep imaging or (cutting)
- Invasive protocol for labelling : exogenous probes,natural protein in tissue (elastine, collagene)
- New advances : Functional Imaging (physiological condition)

→ *Multiscaled imaging : Macroscopy*

- **New advanced Macroscopy : Confocal - Multiphoton-SHG**

CLSM

+

Macroscope

=

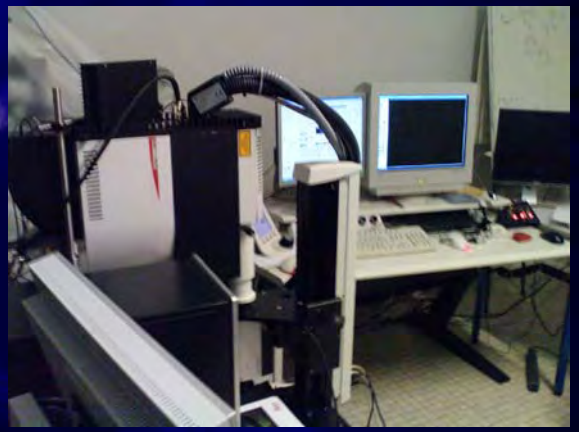
Confocal Macroscope

Multiphoton Laser

+

Confocal Macroscope

=



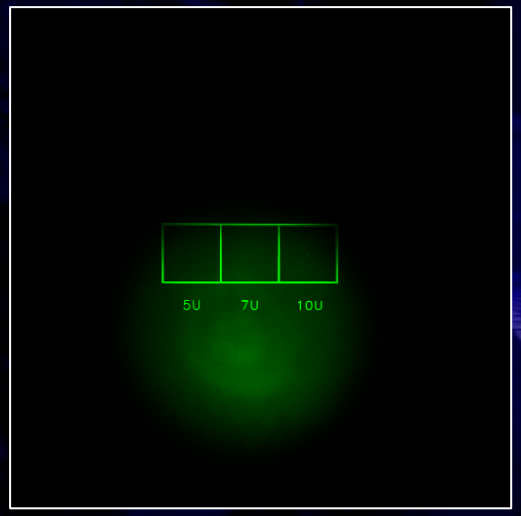
Macroscope Multiphoton

FLIM

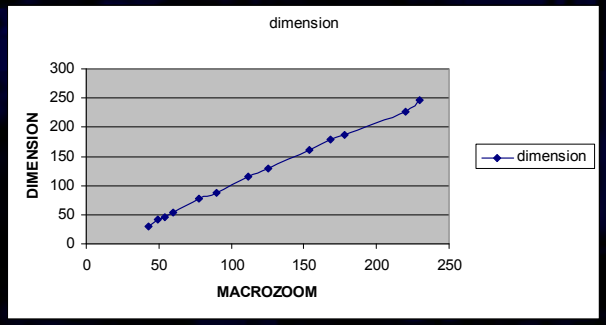
SHG

- **Metrology : MacroConfocal–MP**
- **Appel à projet CNRS 2008-2009 - prototype PTIBC**

488 nm



Macrozoom linearity – (20µm-5cm)

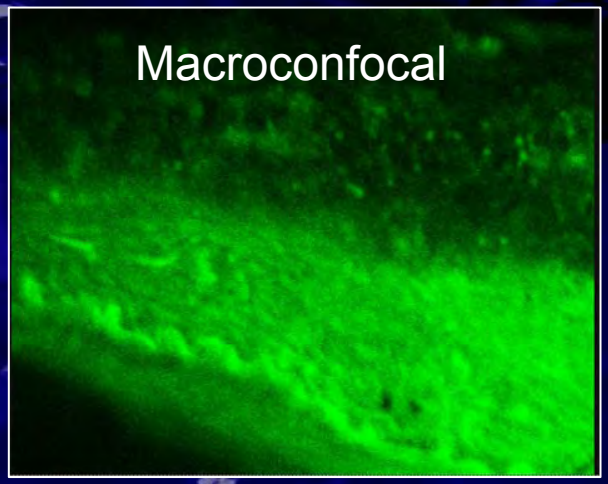


Profil Macro 2x
BE0, ZC 32, ZM 575

Rabbit carotid (autofluorescence)

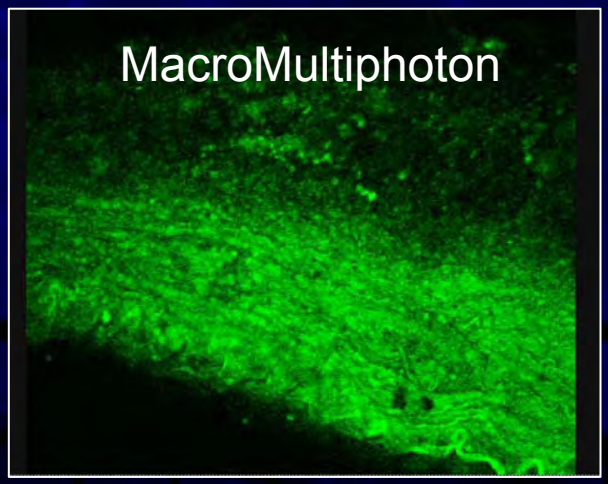
Macroconfocal

488nm



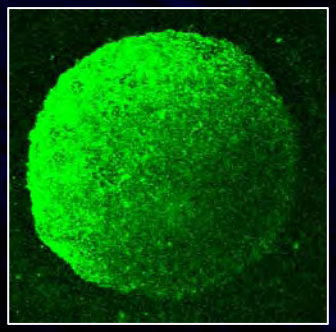
MacroMultiphoton

800 nm

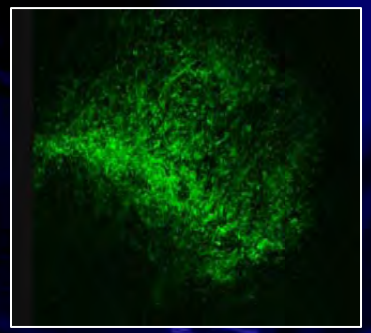


• MacroTCSPC-SHG : Col synthesis by MSC in sponge

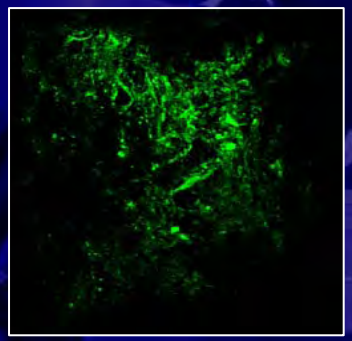
Free Cell (pure col1)



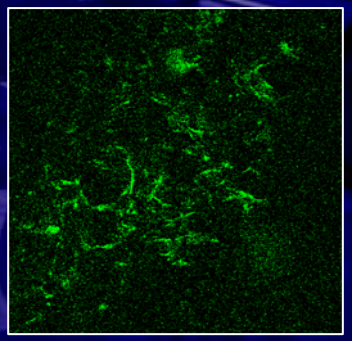
7 mm
Zoom 1,8
Macro 240x



500 µm
Zoom 18x
Macro 340x

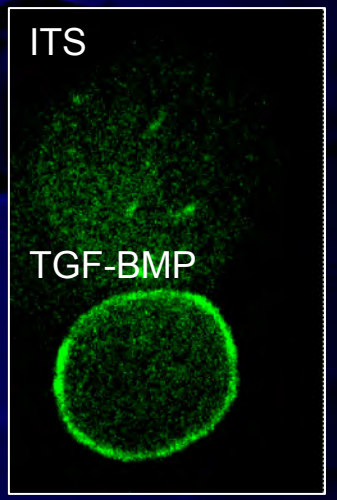


46 µm
Zoom 32x
Macro 545x



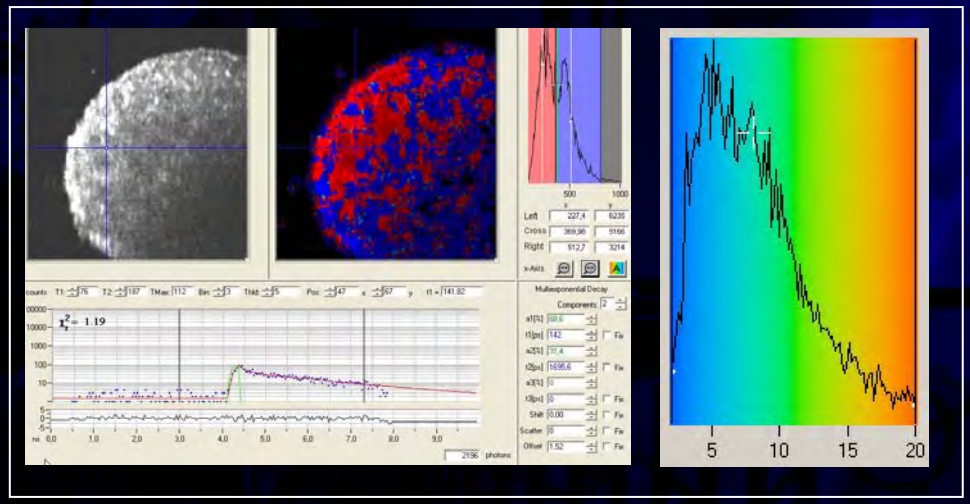
46 µm
Zoom 32x
Macro 545x

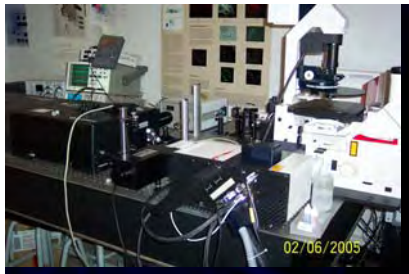
MSC



Zoom Macro 27x

Sponge of Col1





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