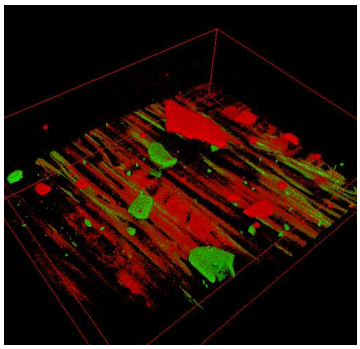
Multiphoton microscopy: an efficient and promising imaging technique for *in situ* study of historical artifacts



Gaël Latour[1,*], J.-P. Echard[2], M. Didier[2], M.-C. Schanne-Klein[1]

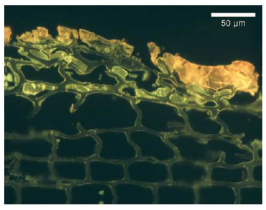
[1] Laboratory for Optics and Biosciences (LOB), École Polytechnique, CNRS, INSERM, Palaiseau, France

[2] Laboratoire de recherche et de restauration, Musée de la musique, Cité de la musique, Paris, France

*** currently at** Paris Sud Univsersity, Laboratory IMNC, Orsay, France

Artworks: why optical techniques ?

- **Sampling and then analysis**



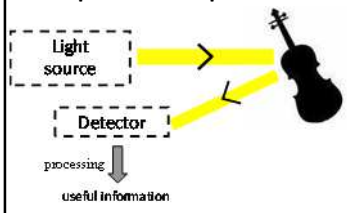
Fluorescence microscopy
Cross-sectional view of Stradivari's "Provigny" varnish

Echard *et al., Ang. Chem.* (2010)

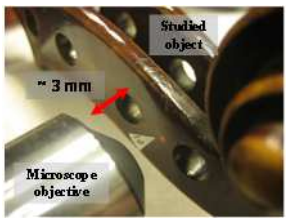
⇨ destructive

but, gold standard for analysis
➔ complete information

- **Optical techniques**

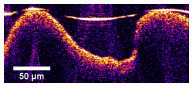


Advantages:
- non contact
- non destructive
- without sampling
- without preparation
- « real time » analysis

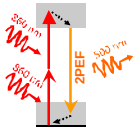


Goal of the work:
- developping **3D optical** imaging technique with **micrometer scale resolution**
- performing a **characterization** of the nature of the materials

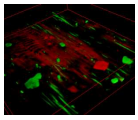
Content



✓ Optical coherence tomography
- interest of full-field OCT
- attempts toward spectroscopic information



✓ Multiphoton microscopy
- principle and experimental setup



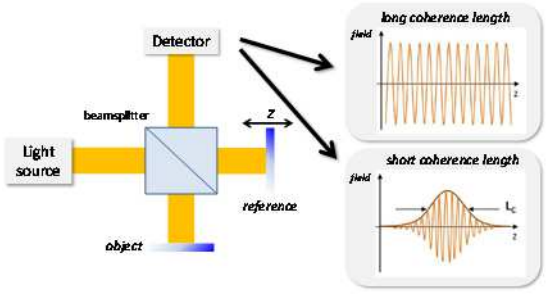
✓ Multiphoton microscopy: potential for artworks
- study of various materials
- stratified layers
- study of a historical violin

Optical Coherence Tomography (OCT)

1990: First application in biomedical imaging — Huang et al., *Science* (1991)

2004: First applications in cultural heritage field — Yang *et al.*, *Achaeometry* (2004); Targowski *et al.*, *Studies Cons.* (2004); Liang *et al.* , *Opt. Express* (2004)

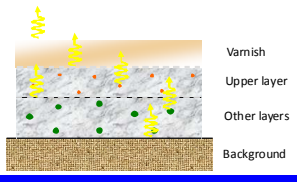
Principle: interferometry with a low temporal coherence length light source



Axial resolution

$$\delta z \propto \frac{1}{\Delta\lambda}$$

Source of contrast: any variation of refractive index
↳ interfaces, scattering particles…



3D micrometer scale OCT: full-field OCT

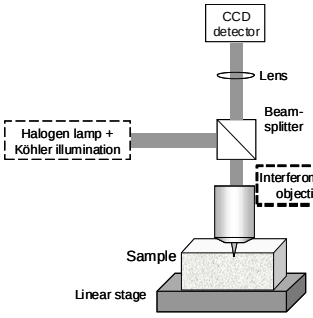
Fourier-domain OCT

- ✓ real-time longitudinal image (B-scan)
- ✓ axial resolution around 3-10 µm
- ✓ lateral resolution around 10 µm

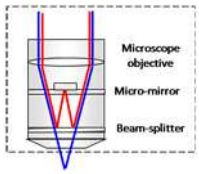
Time-domain : full-field OCT

- ✓ real-time transverse image (C-scan)
- ✓ axial resolution around 1,5 µm
- ✓ lateral resolution around 1 µm

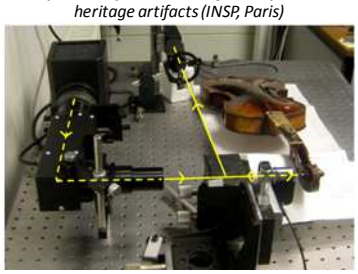
➔ **3D micrometer scale imaging**



interferometric objective



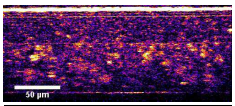
Full-field OCT for the investigation of cultural heritage artifacts (INSP, Paris)

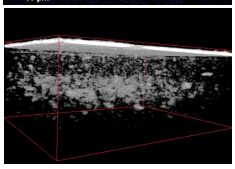


Latour *et al.*, *Appl. Opt.* (2009)

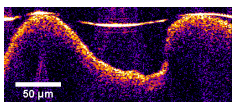
Study of artworks: full-field OCT

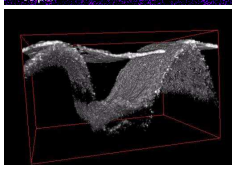
Stratified pictorial layers



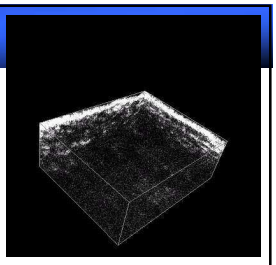


Varnished painting

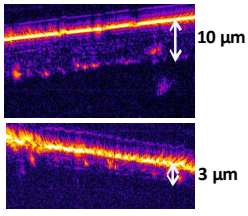




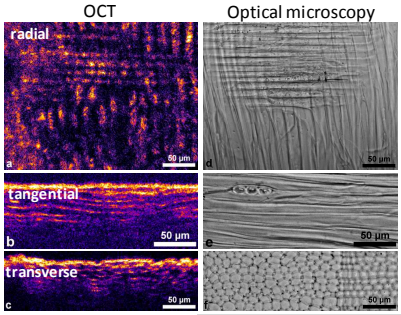
Flamed maple



Analysis of an XVIIIth century Italian violin



OCT | Optical microscopy



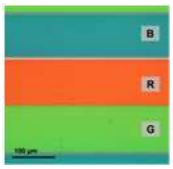
Latour *et al.*, *Appl. Opt.* (2009)

Advantage: *in situ* imaging with **micrometer scale resolution**
➔ structural information
Drawback: lack of specificity ➔ no identification possible

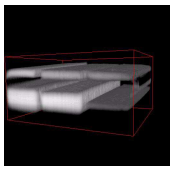
Toward spectral information from OCT data

Fourier transform of raw OCT interferograms

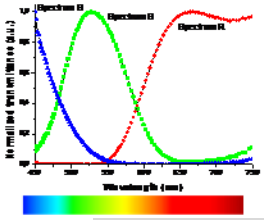
3 thin dye bands optical microscopy



tomographic image



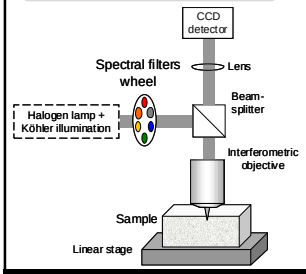
After FT of the interferograms



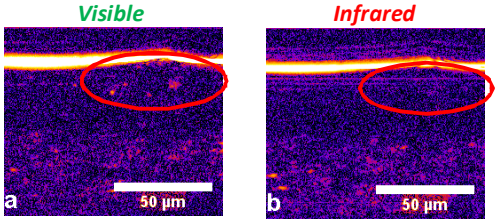
Latour *et al.*, *Opt. Comm.* (2010)

➔ **reliable only on absorbing materials**

« Multispectral » OCT



Visible *Infrared*



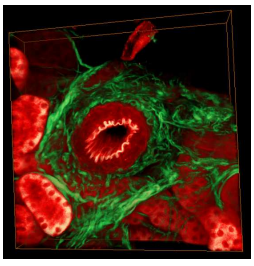
Latour *et al.*, *Proc. SPIE* **7391** (2009)

➔ **trade-off between spatial resolution and spectral sensitivity**

Multiphoton microscopy in biological tissues

Arcuate artery in fibrotic murine kidney

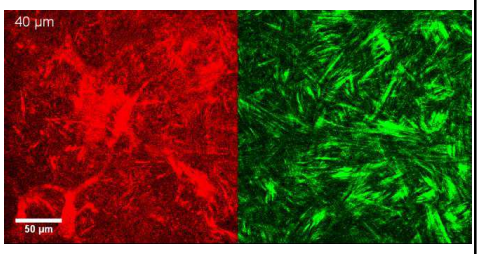
3D reconstruction



270 x 270 x 40 µm³

Human cornea

z-stack



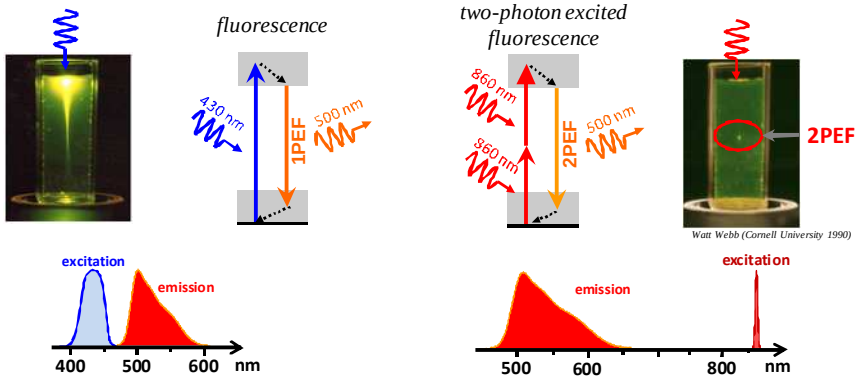
2PEF: two photon excited fluoresence

SHG: second harmonic generation

⇨ 3D specific imaging in unstained tissues

Multiphoton microscopy performed at Laboratory for Optics and Biosciences (Palaiseau, France)

Two-photon excited fluorescence (2PEF)



Spatial selection ⇩ Fluorescence confocal microscopy

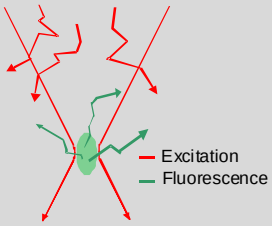
3D imaging

intrinsic optical sectoning ⇩ multiphoton or non-linear microscopy

Denk, Strickler, Webb, Science (1990)
Zipfel, Williams, Webb, Nature Biotechnology 21 (2003)

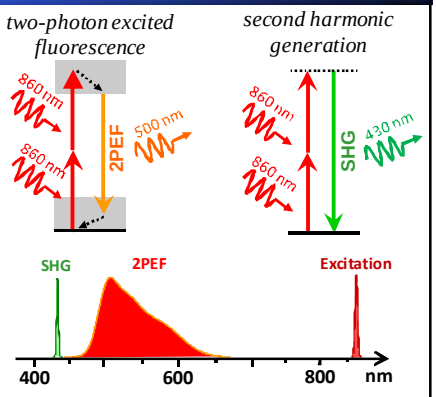
Advantages of multiphoton microscopy

Advantages



- near-IR confined excitation
  - ➔ **better penetration**
- scattered IR produce no fluoresence
  - ➔ **reduced background (or noise)**
- **preservation of sub-cellular resolution**, even in scattering media
  - ➔ **less phototoxicity and photobleaching**

key advantage: additional modes of contrast



Additional modes of contrast

- simultaneous excitation but detection at different wavelengths
- multimodal approach
- specific imaging

Other modes of contrast: *THG (interfaces), 3PEF (chromophores), CARS (chemical bonds)*

Second Harmonic Generation (SHG)

Response @ molecular level

Induced polarization

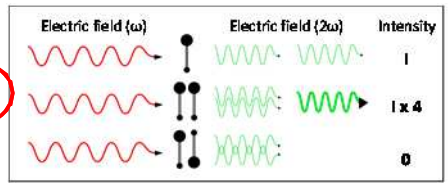
$$\vec{p} = \alpha \vec{E} + \beta \vec{E}\vec{E} + \gamma \vec{E}\vec{E}\vec{E} + \ldots$$

linear response

non-linear response
strong excitation necessary

non centrosymmetric medium ➔ $\beta \neq 0$

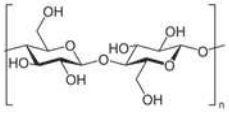
Response @ macro-molecular level



SHG ⇔ **non-centrosymmetric** and **dense** distribution of « harmonophores »

cellulose

polysaccharidic chains

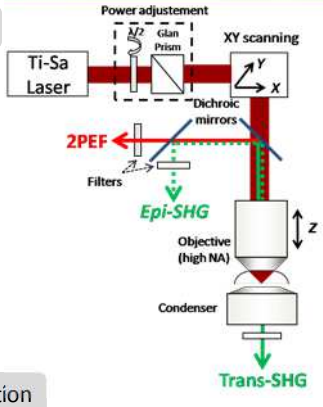


hemi-hydrate calcium sulphate (bassanite)

pseudohexagonal monoclinic crystal
➔ non-centrosymmetric structure

Multiphoton microscopy: setup

Setup

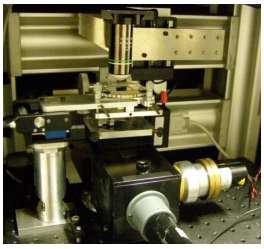


Resolution @860 nm

20x (air, 0.75 NA) ➔ 0.45 µm x 1.6 µm

Acquisition time:

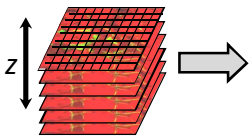
~1 s / image (500 x 500 pixels)



Multiphoton microscope @LOB

Acquisition

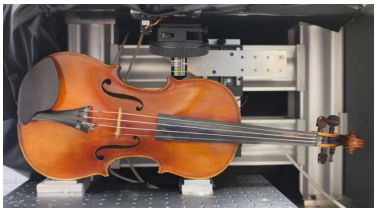
Stack of 2D images *vs.* z



3D reconstruction

2PEF SHG

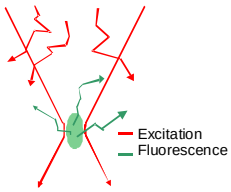
+ possibility to perform **mosaic**



Multiphoton microscopy: considerations about power

Power measured under the objective: 8-20 mW

Excitation wavelength: 860 nm (**near IR**)
most of the materials are transparent in this spectral range
no absorption ➔ no photodamage



2PEF: absorption only in the focal volume
≠
fluorescence: absorption along the excitation beam

Pulsed laser: average power **very low**
high peak power ➔ deterioration possible but immediately visible

Materials and method

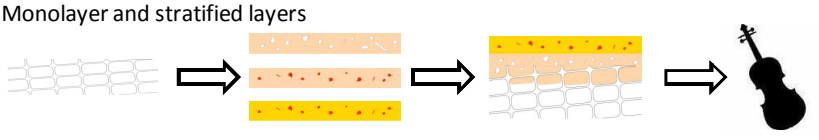
Aim: determining the potential of multiphoton microscopy for the investigation of artworks
↳ in particular for the study of wooden musical instruments

- **Materials**

Wood	Fillers	Binders
Maple wood	*plaster, cochineal lake*	*gelatin, sandarac*

- **Samples**

Monolayer and stratified layers

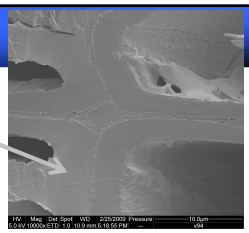


- **Method**

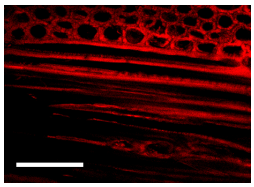
Comparative imaging with transmitted light microscopy

Multiphoton microscopy: wood

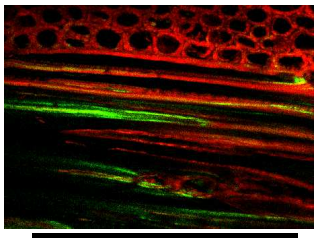
Two main components of plant cell wall: **2PEF**: lignin
SHG: cellulose



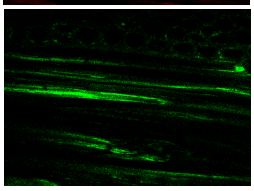
2PEF (GG5 filter)



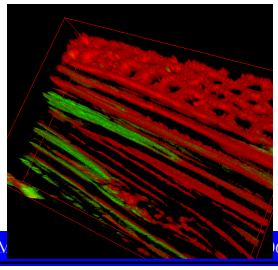
Merged 2PEF and Forward-SHG



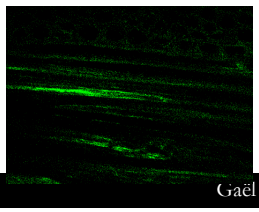
Forward-SHG



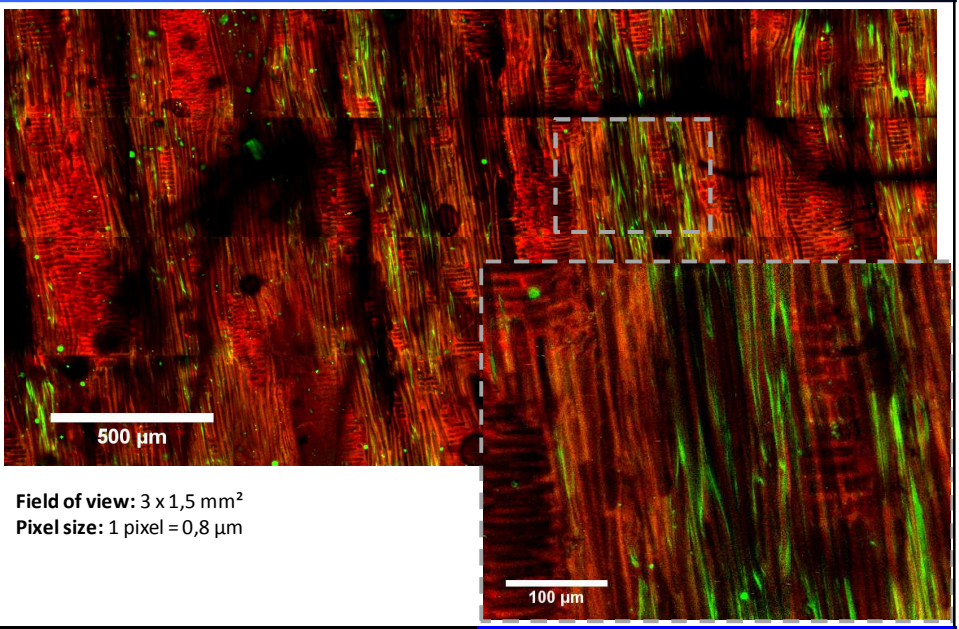
3D reconstruction



Backward-SHG



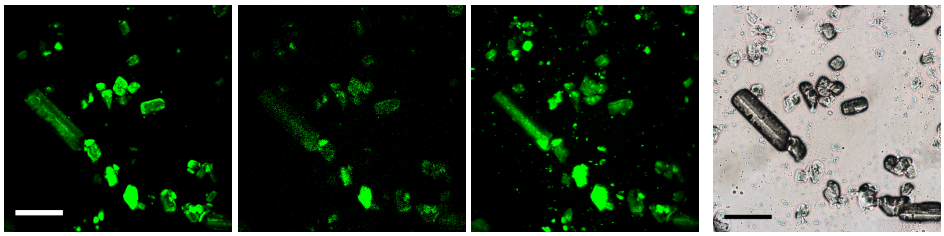
Multiphoton microscopy: wood



Field of view: 3 x 1,5 mm²
Pixel size: 1 pixel = 0,8 µm

SHG from plaster

Forward-SHG | Backward-SHG | B-SHG sum of z-slices | transmitted light microscopy



3 types of calcium-sulfate particles

anhydrite $CaSO_4$

dihydrate *(gyspum)* $CaSO_4.2H_2O$

→ centrosymmetric crystals
→ **SHG silent**

hemihydrate *(bassanite)* $CaSO_4.0,5H_2O$

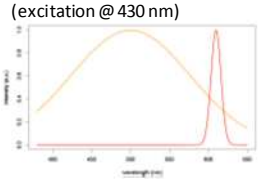
X-ray diffraction analysis

→ non-centrosymmetric crystals
→ **SHG emitter**

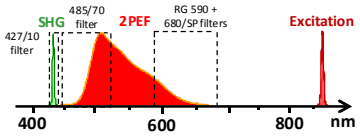
Latour *et al., Opt. Express* (2012)

2PEF from cochineal lake and sandarac

Fluorescence emission properties
(excitation @ 430 nm)



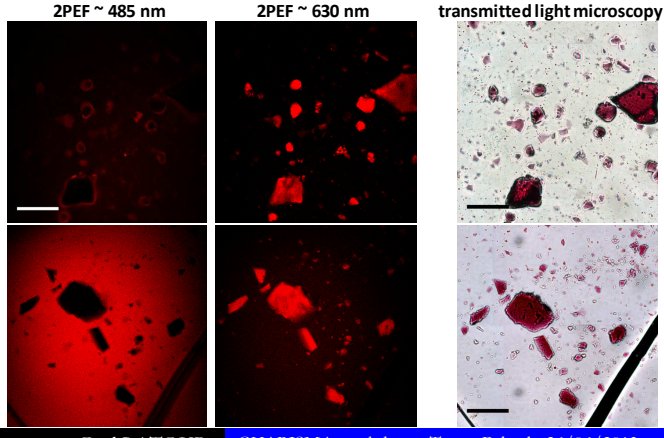
Emission filters for 2PEF detection



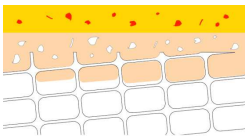
2PEF ~ 485 nm | 2PEF ~ 630 nm | transmitted light microscopy

Cochineal lake pigments in gelatin-based film

Cochineal lake pigments in sandarac film



Multiphoton microscopy: stratified layers

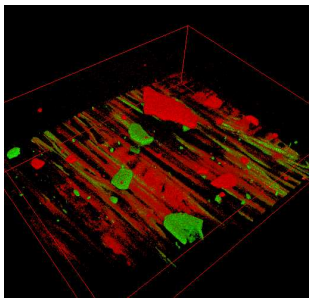


cochineal lake in sandrac film

plaster in gelatin-based film

wood

3D reconstruction

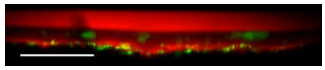


Transverse imaging along the depth

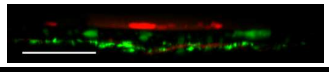
2PEF ~ 485 nm **2PEF** ~ 630 nm **SHG**

Axial reconstruction

2PEF ~ 485 nm
SHG



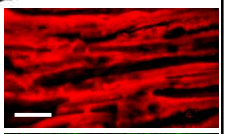
2PEF ~ 630 nm
SHG



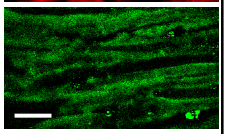
Latour *et al., Opt. Express* (2012)

Multiphoton microscopy: violin

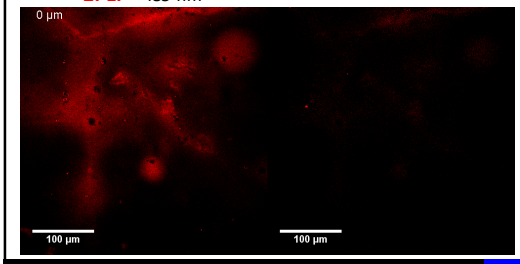
2PEF (485/70)



Backward-SHG

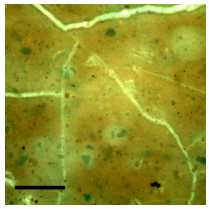


2PEF ~ 485 nm **2PEF** ~ 630 nm

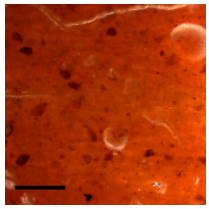


***in situ* microscopy**

luminescence



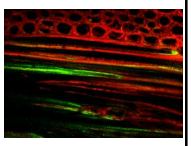
dark field at surface



Conclusion and perspectives

Study of wood

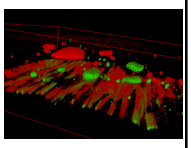
localization of lignin *vs.* cellulose



Perspectives
- polarization-resolved SHG ➔ structure of crytalline cellulose
- aged wood
- relationship with mechanical properties ➔ acoustic properties

Multimodal and specific imaging

2PEF: potential spectral discrimination
SHG: signal from plaster (only for bassanite)



Perspectives
- determination of the type of plaster in Italian gesso
- fluorescence properties of various materials
- study of a historical artwork
  Cité de la Musique

Powerful technique for other materials encountered in cultural heritage artefacts ???

Aknowledgments

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latour@imnc.in2p3.fr