Nonlinear Microscopy Imaging of collagen fibers using a polarimetric approach

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Second harmonic generation (SHG), also known as frequency doubling, is a quadratic nonlinear optical process induced by coherent light within nonlinear materials, which can be synthesized molecular assemblies or biological structures such as tissues. It has been demonstrated that polarized second harmonic signals contain structural information from the sample, in particular on the organisation of molecular assemblies (symmetry, orientation, ..). In our experimental setup, an infra-red excitation from a femto-second laser has been used to probe a collagen tissue obtained from a mouse tail. We are also working on optimising pulse shaping signals to increase structural contrasts in SHG imaging.

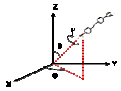
Principle

Second Harmonic Generation (SHG) polarized signals contain information on the sample structure.

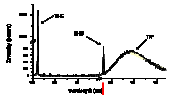
Susceptibility $\chi^{(2)}_{IJK} = \sum_{ijk} \underbrace{(I.i)_{(\theta,\phi,\psi)}(J.j)_{(\theta,\phi,\psi)}(K.k)_{(\theta,\phi,\psi)}}_{\text{Orientation and disorder information}} . \underbrace{\beta_{ijk}}_{\text{Molecular scale information}}$

I, J, K: macroscopic axes

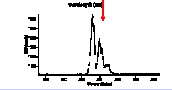
i, j, k : microscopic axes



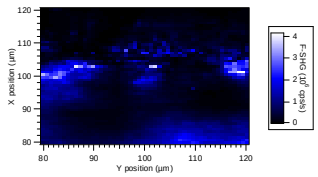
• Single frequency excitation : $I_I^{2\omega} = \left| \sum_{JK} \chi^{(2)}_{IJK} . E_J(\omega) . E_K(\omega) \right|^2$



• large excitation spectrum : $I_I^{2\omega} = \left| \sum_{JK} \int \chi^{(2)}_{IJK} . E_J(\omega - x) . E_K(\omega + x) . dx \right|^2$



Collagen Results

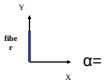


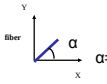
Sample:

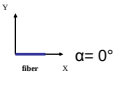
Collagen type I fiber extracted from a mouse tail (collaboration Peter Winlove, Exter University, UK).

Experiments:

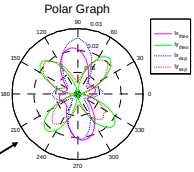
Investigations have been made in three different positions of the fiber to determine the microscopic organization of the collagen.



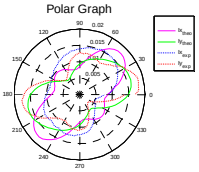




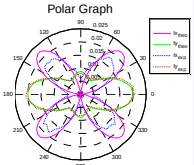
Polar Graph



Polar Graph



Polar Graph



Fits results

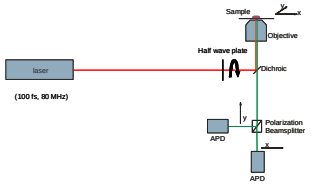
$$\chi^{(2)} = \begin{bmatrix} \chi^{(2)}_{XXX} & \chi^{(2)}_{XYY} & \chi^{(2)}_{XXY} \\ \chi^{(2)}_{YXX} & \chi^{(2)}_{YYY} & \chi^{(2)}_{YXY} \end{bmatrix} = \begin{bmatrix} 1 & \frac{-5}{3} & \frac{1}{3} \\ \frac{1}{3} & \frac{-1}{3} & \frac{-5}{3} \end{bmatrix}$$

• The symmetry is seen to be different from a cristalline collagen fiber (symmetry group C6).

• Here we look at an ensemble of a collagen fibers in a complex environment.

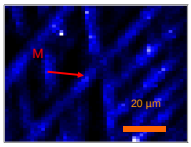
Setup and sample calibration

Polarimetric set-up



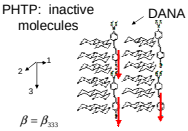
• The PHTP/DANA (1D molecular crystal) sample has been used as a calibration sample for our set-up.

• Our work has been focused on the study of the polarimetric response of a collagen fiber to determine its microscopic molecular organization.



PHTP: inactive molecules

DANA : SHG-active molecules



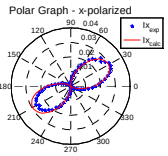
1D molecular crystal (collaboration Tübingen University)

• polarimetric measurements at point M to find the nanometric scale orientation of the molecules.

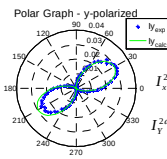
• angles of incident polarization α : 0°to 360°

• SHG signals measured along two analysis directions X and Y.

Polar Graph - x-polarized



Polar Graph - y-polarized



$$I_X^{2\omega} = \left|P_X^{2\omega}\right|^2 = \left|\chi^{(2)}_{XXX} E_X^2 + \chi^{(2)}_{XYY} E_Y^2 + 2\chi^{(2)}_{XXY} E_X E_Y\right|^2$$

$$I_Y^{2\omega} = \left|P_Y^{2\omega}\right|^2 = \left|\chi^{(2)}_{YXX} E_X^2 + \chi^{(2)}_{YYY} E_Y^2 + 2\chi^{(2)}_{YXY} E_X E_Y\right|^2$$

➡ Fitting α-dependent SHG response allows deducing :

- macroscopic coefficients χ_{IJK} with (I,J,K) = (X,Y)
- crystal order (here: 1D)
- local orientation (here: , θ = 90°, ϕ = 31° ψ = 0°)

Collagen Properties

Description:

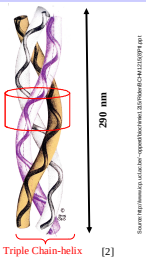
A collagen molecule is a long peptide chain (290 nm in length). It is a fibrous protein with glycine, proline, and hydroproline as the basic constituants with a number of other amino acids present in variable proportions. [1].

Characteristic:

- Triplet Gly-X-Y repetition where X and Y can be any amino acid and are often proline and hydroxyproline, respectively [2].

Most important Collagen types:

-Type I: skin, bone, tendon.
-Type II: cartilage, intervertebral discs.
-Type III: fetal skin.

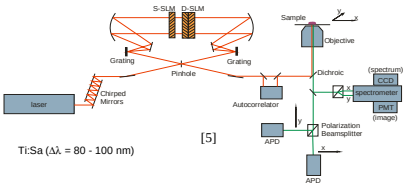


Triple Chain-helix [2]

Towards Structural Contrast Imaging By Polarization and Phase Optimization -Perspective

Perspectives:

The possibility to **extinguish** some **species** in the sample and to **brighten others**. The aim of this work is to increase the non linear optical imaging contrast and to optimize polarimetric signals by using **an optimization algorithm** which can optimise the polarization and the phase of the incident field $E(\omega)$



[5]

Assumptions:

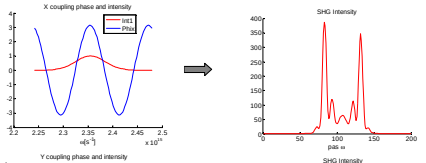
1. The field: $E(\omega) = \begin{bmatrix} E_X^{\,0}(\omega) e^{i.\varphi_X(\omega)} \\ E_Y^{\,0}(\omega) e^{i.\varphi_Y(\omega)} \end{bmatrix}$ [4]

2. The amplitudes have a gaussian shape

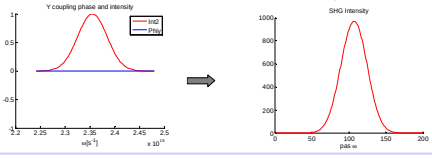
Phase and polarization shaping allow the control of the SHG responses from X and Y coupling independently [3].

Illustration:

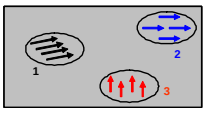
X coupling:



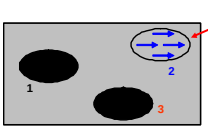
Y coupling:



Exemple: Three species are represented in the sample. Our goal is to optimize the polarimetric signal of the species 2.



How to increase the contrast?



The species 2 should have the maximum polarised signal

• Existence of 3 different species in the sample.

• Goal: Optimization the signal of the species 2 to increase the contrast.

• To optimise the signal of the species 2, the intensity of the species 1 and 3 are decreased down to zero.

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