Laboratoire d'Optique et Biosciences (LOB)
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M2 internship / PhD project:
FDTD Modeling of Coherent Multiphoton Microscopy

Context:

Multiphoton microscopy has revolutionized three-dimensional (3D) imaging of biological tissues over the last 15 years. In addition to conventional two-photon excited fluorescence modality, coherent processes can be used to generate multimodal images and get unique functional information on intact tissues at sub-micrometer scale. These coherent techniques rely on the intrinsic nonlinear properties of biological tissues, without any staining. In particular, second harmonic generation microscopy (SHG) is now the gold standard technique for *in situ* visualization of unstained fibrillar collagen because of its high specificity for this key protein of connective tissues [1]. Similarly, third harmonic generation microscopy (THG) detects interfaces and optical heterogeneities, such as lipidic domains or biominerals, and provides highly contrasted 3D structural images of unstained biological tissues, such as brain tissue and whole embryos [2]. However, **these harmonic modalities have non-trivial contrast mechanisms because of their coherent nature** and extracting as much information as possible about the sample from the images – for example by changing the excitation polarization - is an active research field [3,4]. Notably, **current modeling approaches neglect optical aberrations created by mismatches in linear indices** and cannot reproduce some experimental measurements, preventing accurate quantification of the images. To address this, we recently developed a **Finite Difference Time-Domain (FDTD) pipeline for THG microscopy of isotropic media** [5]. Indeed**,** FDTD takes into account the nanometer-scale structure of biological samples and can thus reproduce experimental results in complex heterogeneous tissues (See fig 1).

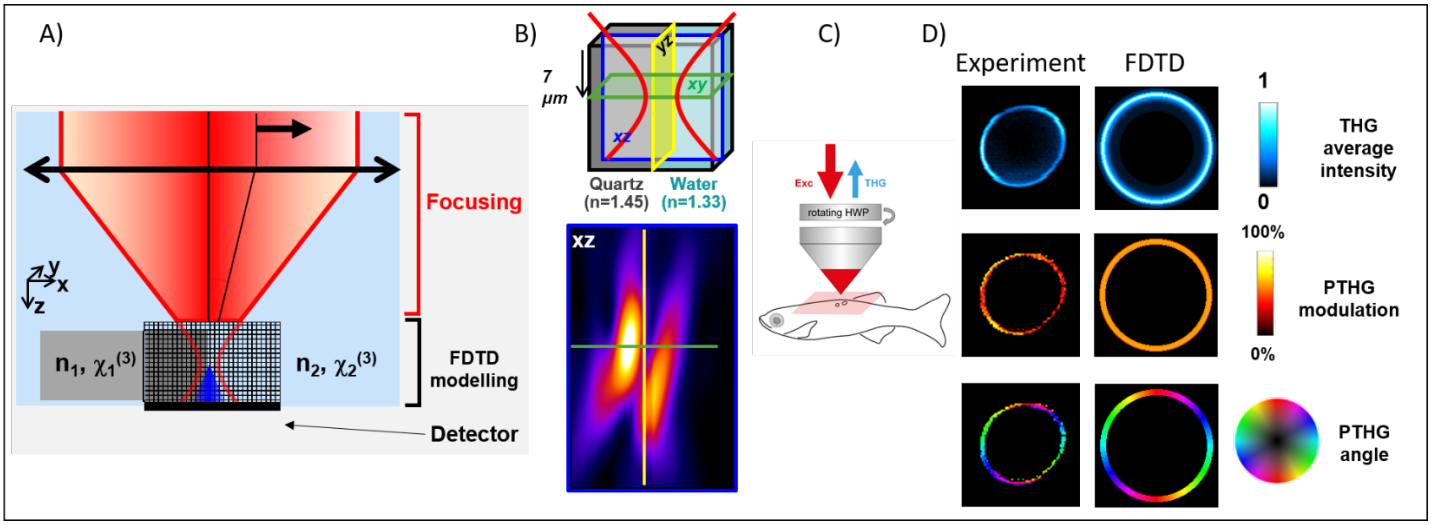

Figure 1: FDTD for THG Microscopy: FDTD simulations are performed in the focal volume of a high-NA objective (A) Introducing different media, such as water and glass leads to aberrations, which can be reproduced accurately (B), and coherent nonlinear signals from samples such as the zebrafish (C) can be reproduced, even their polarization response (D) (adapted from [5])

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Aim:

This PhD project aims at **developing an improved Finite Difference Time-Domain (FDTD) pipeline for simulating realistic sample geometries for a variety of coherent nonlinear processes** (SHG, THG…). In particular this improved pipeline will have to **consider materials which have non-diagonal nonlinear responses that mimic the anisotropy of real materials more closely.** This is crucial to model SHG, since SHG is only possible in anisotropic material, as well as THG from myelin sheaths in brain.

Method:

Starting from an existing pipeline using a commercial FDTD software developed for nanophotonics (Lumerical), the first task will be to optimize the convergence and speed on some known geometries so as to be able to study a broad range of conditions. The second step will be to take into account anisotropic materials. This must be implemented for second-order (SHG) and third-order (THG) susceptibility tensors corresponding to a variety of crystalline symmetries. The last step will focus on real materials and try to reproduce experimental results generated in the same team at LOB. Notably, the SHG response of **collagen in cornea** will be simulated in order to extract the sub-nanometer scale structure of collagen in this complex tissue from polarization-resolved experimental images. Similar approaches may be also applied to **collagen in bone or to myosin in muscle and in heart**. Regarding THG processes, simulation of the polarimetric THG response of **myelin sheaths in brain** will be performed in order to quantify demyelination in multiple sclerosis. The same approach will be applied to biominerals such as otoliths in zebrafish and kidney stones from human patients.

Expected results:

While this project is primarily a theoretical and computational project, it will rely on experimental data generated in the same team at LOB : projects of E. Beaurepaire (p-THG in biominerals), M-C Schanne-Klein (p-SHG in cornea, p-THG in kidney stones), W. Supatto (p-SHG in myosin), C. Stringari (p-THG in myelin).
The main expected results are the following ones:
- New code to include non-diagonal tensorial responses in FDTD method
- New pipeline for simulating numerically realistic sample geometries for a variety of second and third-order coherent processes (SHG, THG…)
- Simulation of p-SHG images of cornea and extraction of sub-nanometer structure of collagen from experimental images of human healthy and pathological corneas
- Simulation of p-THG images of myelin and extraction of quantitative myelin structural data from experimental images of multiple sclerosis murine models
- Simulation of p-THG images of zebrafish otoliths and human kidney stones and follow-up of crystalline structure from experimental images

Research environment

This project will take place in the “Advanced Microscopies” and “Nanoimaging” groups at the Lab for Optics and Biosciences (LOB), located on the Polytechnique campus in Palaiseau. The LOB is an interdisciplinary lab with both physicists and biologists who develop together new approaches in optics, computational physics, image analysis, cell/developmental biology and biophysics to study biological systems.

Student profile

Master in physics with major in computational physics or master in applied mathematics with strong interest in biophysics or Engineer with majors in physics or applied mathematics

Bibliography (see also Marie Claire’s webpage or Nicolas’ webpage)

[1] S. Bancelin et al, "Determination of collagen fibril size via absolute measurements of SHG signals". *Nat. Commun.* **2014**
[2] N. Olivier et al. "Cell lineage reconstruction of early zebrafish embryos using label-free nonlinear microscopy." Science 329 (5994) **2010**
[3] J. Morizet et al. "High-speed polarization-resolved third-harmonic microscopy." *Optica* 6 (3) **2019**
[4] C. Schmeltz et al, "CD-SHG microscopy probes the polarity distribution of collagen fibrils" *Optica 7* (11) **2020**
[5] J. Morizet et al. “Modeling nonlinear microscopy near index-mismatched interfaces”, *Optica* 8 (7) **2021**

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