Laboratoire d'Optique et Biosciences
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M2 internship + PhD project in cultural heritage science

Polarization-resolved Second Harmonic Generation for the investigation of collagen degradation in parchments.

Non-invasive and quantitative investigation techniques are highly sought-after to establish the degradation state of cultural heritage artefacts and assess the relevance of conservation conditions or restoration processes. This is crucial for ancient parchments that are a precious testimony of the past and a key source of information for historians, since they were the main writing support material in the Middle Ages in Western Europe. However, up to now, the degradation state of parchments is assessed, when possible, by thermal techniques which are destructives. Multiphoton microscopy has revolutionized three-dimensional (3D) imaging of biological tissues over the past 10 years. Notably, second harmonic generation (SHG) microscopy enables imaging of collagen fibrils (10-300 nm diameter) without any labelling and with unequalled sensitivity in intact tissues [1]. This is directly related to the property of SHG to be nonzero only in dense and non-centrosymmetrical materials. On the contrary, SHG signals vanish for centrosymmetric materials such as gelatin, which is the ultimate degradation state of collagen in parchment. Accordingly, SHG microscopy provides structural information about the 3D organization of the fibrillar collagen within parchments and other skin-based artefacts. Additionally, we have combined SHG with polarimetry, which enables measurements of collagen orientation in each pixel and thus provides *in situ* quantitative mapping of collagen 3D organization [2]. We have also shown theoretically and experimentally that polarization-resolved SHG (P-SHG) is highly sensitive to the disorder of the collagen fibrils within the focal volume, at sub-micrometer scale [3].

This project focuses on the implementation of advanced NLO microscopy imaging for quantitative *in situ* mapping of parchment degradation by introducing two parameters: the ratio of two-photon excited fluorescence (2PEF) over SHG signals (I_{2PEF}/I_{SHG}), which probes the loss of the non-centrosymmetric organization of fibrillar collagen, and the anisotropy parameter extracted from P-SHG measurements, which is sensitive to the sub-micrometer scale disorder [3]. Model samples from contemporary parchments will be artificially aged in order to mimick parchment degradation and will be characterized by NLO microscopy to monitor the collagen degradation. Similar experiments will be performed on isolated collagen fibers in order to understand the physico-chemical processeses that occur during degradation. To that end, a dedicated chamber, which can be controlled in temperature and humidity, will be placed under the microscope objective to record the evolution of the nonlinear signals.

This project will benefit from the experimental and numerical expertise of the advanced microscopy group at the LOB and from a close collaboration with L. Robinet, leader of the team Leather and Parchment from the Centre de Recherche sur la Conservation (Museum National d'Histoire Naturelle de Paris). The master internship can be followed by a PhD.

Related recent publications (see also http://www.lob.polytechnique.fr/) :
[1] Bancelin et al, Nat. Commun. 5 (2014) - 10.1038/ncomms5920
[2] Raoux et al, Light Sci Appl 12 (2023) - 10.1038/s41377-023-01224-0
[3] Schmeltz et al, Sci. Adv. 7 (2021) - 10.1126/sciadv.abg1090

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