Noninvasive quantitative assessment of collagen degradation in parchments by polarization-resolved SHG microscopy

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Nonlinear optical (NLO) microscopy has revolutionized three-dimensional (3D) imaging of biological tissues by offering new modes of contrast even in unstained tissues. Notably, two-photon excited fluorescence (2PEF) and second harmonic generation (SHG) signals can be recorded simultaneously in two detection channels to probe different tissue components. Since few years, some studies have shown the potential of this technique for the study of cultural heritage artefacts. 2PEF signals are emitted by a wide range of materials (fluorophores) in historical artefacts with specific absorption and emission fluorescence spectra [1]. SHG signals are specific for dense and well aligned structures and thus enable the visualization of fibrillar collagen without any labelling and with unequalled sensitivity and specificity [2]. In contrast, SHG signals vanish for centro-symmetric materials such a gelatin, which is obtained upon degradation (denaturation and hydrolysis) of collagen. Accordingly, SHG microscopy provides structural information about the 3D organization of the fibrillar collagen within parchments and other skin-based artefacts [3].

In this context, polarization-resolved SHG (P-SHG) microscopy has been shown to provide a more accurate characterization of the 3D organization of collagen because the SHG signal is higher when the excitation electric field is parallel to the dipoles accounting for the nonlinear optical response. The acquisition of a series of SHG images recorded with linear incident polarizations of various orientations thus provides the orientation of collagen fibrils in every pixel, using appropriate data processing based on a tensorial analysis of collagen response.

In this study, we implement advanced NLO microscopy imaging for quantitative in situ mapping of parchment degradation by introducing two parameters: the ratio of 2PEF to SHG signals (I_{2PEF}/I_{SHG}), which probes the loss of the non-centrosymmetric organization of fibrillar collagen and the anisotropy parameter extracted from polarization-resolved SHG (P-SHG) measurements, which is sensitive to the sub-micrometer scale disorder [4]. We first rigorously validate this method on artificially aged model samples by comparing NLO to thermal measures. Thanks to the measurement of the collagen degradation state by thermal analyses (DSC), we show that P-SHG anisotropy parameter probe the first steps of degradation corresponding to a slight disorder of the collagen fibrils, while the further steps, when the hierarchical organization of the fibrillar collagen is lost, are revealed by an increase of the I_{2PEF}/I_{SHG} parameter. We then analyse and map the conservation state of some historical parchments from Chartres’ library, even in valuable areas, near to the text, where sampling is prohibited [4].

References
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