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

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Characterization of coatings (stratigraphy, composition) is of the utmost importance for the understanding and conservation of historical artifacts. Optical Coherence Tomography (OCT) has been for a few years a well-established as an *in situ* three-dimensional (3D) imaging tool.[1] In particular, full-field OCT allows 3D imaging with a micrometer scale resolution, necessary for the characterization of wood structure, the fine determination of the stratigraphy of thin layers and the imaging of scattering particles such as fillers or pigments.[2] Nevertheless, the discrimination of the various components is strongly limited with OCT despite some attempts in spectral selectivity[3] and spectros.[4]

Multiphoton microscopy (MPM), also called non-linear optical microscopy, appears to be a promising alternative imaging technique for investigating cultural heritage artifacts. This technique performs 3D imaging with micrometer-scale resolution based on an intrinsic optical sectioning. A key advantage of MPM is its multimodal capability with different modes of contrasts that are directly linked to the chemical nature of the materials. Two-Photon Excited Fluorescence (2PEF) is emitted by a wide range of materials in historical artifacts and spectral discrimination of different fluorophores is possible. Second Harmonic Generation (SHG) signals are emitted by non-centrosymmetric structures. We showed that plaster particles exhibit strong SHG signals when they are composed of bassanite crystals. SHG signal was also detected from crystalline cellulose within the wood cell walls.

Stratified layers composed of compounds widely used as artists' materials were studied by MPM and the different components were specifically detected and located within the stratigraphy. We were able to discriminate gelatin-based films from sandarac films and to perform 3D imaging of cochineal lake pigments. In the case of two stratified layers composed of cochineal lake pigments and plaster, the different fillers were clearly distinguished (2PEF versus SHG). This technique also allowed precise measurement of the thickness of each layer. Finally, we demonstrated that MPM can be used for *in situ* investigation of a historical violin. To conclude, this study demonstrates that multimodal MPM is an efficient and promising technique for 3D *in situ* investigation of historical artifacts and woods.

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