CORRELATIVE MULTIPHOTON / AFM-IR IMAGING OF NATIVE AND DENATURED COLLAGEN.

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Collagen is the main component of connective tissues such as skin, cornea, tendons, bones and arteries. This protein is characterized by triple helical domains, which spontaneously align to form fibrils. Heat treatment or other degradation processes can induce the denaturation of collagen fibrils that ultimately forms gelatin (more or less proteolyzed simple helices). The reference technique for *in situ* visualization of label-free fibrillar collagen in biological tissues is second harmonic generation microscopy (SHG) [1]. On the other hand, gelatin exhibits no SHG signal because of its centrosymmetric organization, but two-photon excited fluorescence signals (2PEF), which can be detected simultaneously to SHG in a multiphoton microscope [2]. To further characterize collagen samples at the scale of the collagen fibers (~1-5 µm) or fibrils (~100 nm), an Atomic Force Microscope (AFM) is coupled to infra-red (IR) illumination to collect IR absorbtion spectra with give access to the chemical information with nanometer scale resolution (AFM-IR). The objective of this work is to have a better insight into the optical and chemical signature of the collagen denaturation by correlating multiphoton microscopy and AFM-IRIR [3].

First, we have induced a local denaturation of collagen fibrils in a small region of interest in order to visualize the same collagen in its fibrillar and denatured states. A specific cantilever is used to directly heat the sample with the AFM tip. The comparison of IR spectra measured locally before and after denaturation shows the onset of an absorbing band at 1725 cm^{-1}, which is therefore specific to denatured collagen [3]. Second, we have correlated this IR mapping with multiphoton imaging. Multiphoton images acquired before and after the collagen denaturation under the AFM tip show that the denatured collagen no longer exhibit an SHG signal, but a 2PEF signal that is specific to the denatured collagen. This correlative study of collagen denaturation thus takes advantage of the chemical specificity of IR nanospectroscopy, a technique limited to thin samples, to get a thorough characterization of multiphoton signals that can be visualized *in situ* in thick intact biological samples.

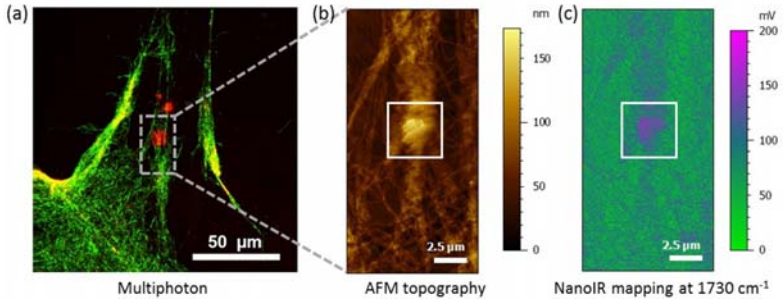

Figure 1: Correlative measurement of (a) multiphoton signals (2PEF in red color, SHG in green color), (b) AFM topography and (c) AFM-IR mapping at 1730 cm^{-1} after thermal denaturation of collagen fibrils with the AFM tip (white square).

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