

Multimodal non-linear optical microscopy for tissue characterization and diagnostics

Abstract

Modern optics and spectroscopy offer promising solutions for tissue imaging at the subcellular level as it can provide morphological and functional information without the use of exogenous labels. Among NLO microscopy techniques, second-harmonic generation (SHG) microscopy is a coherent microscopic modality able to provide background-free images of anisotropic hyperpolarizable repetitively patterned biological molecules, such as collagen, muscle, or microtubules. During the last years, SHG microscopy has been successfully applied to the imaging of non-centrosymmetric molecules inside cells, cellular membranes, brain, and biological tissues. The capabilities of SHG microscopy in providing background-free high-resolution imaging of collagen-rich tissues has already been demonstrated in several tissues. Even though a single collagen molecule has high hyperpolarizability, when arranged in bundles in fibrous structures, an extremely strong SHG signal emerges, enabling selective high-resolution imaging in connective tissues. SHG microscopy can be used for the investigation of structural organization and fibrillar orientation of collagen in human dermis, cornea, fibrosis, and thermally-treated samples. Further, the investigation of collagen organization in the tumour microenvironment can be related to invasiveness as a useful prognostic indicator. This imaging approach offer several advantages with respect to standard histopathological examination for tissue diagnostics. However, a lot of work needs to be done before this methodology is recognized as a diagnostic standard, including training pathologists to the new kind of images, reducing the cost for laser sources required for these techniques, standardizing the acquisition and analysis processes. Nevertheless, we believe that in the near future these techniques could find a stable place in a medical setting. Here, we present the potential offered by this imaging technique, employed alone or in tandem with other NLO microscopy techniques, in providing a label-free characterization of both morphology and functionality on biological tissues.