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Simultaneous observation of collagen and elastin based on the combined nonlinear optical imaging technique coupled with two-channel synchronized detection method

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Abstract

Collagen and elastin are the most important proteins of the connective tissues in higher vertebrates. In this paper, we present a combined nonlinear optical imaging technique of second-harmonic generation and two-photon excited fluorescence to simultaneously observe the collagen and elastic fiber of dermis in a freshly excised human skin and rabbit aorta using a two-channel synchronized detection method. The obtained two-channel overlay image in the backward direction can clearly distinguish the morphological structure and distribution of collagen and elastic fibers. Tissue spectrum further confirms the obtained structural information. These results suggest that the combined nonlinear optical imaging technique coupled with two-channel synchronized detection method can be an effective tool for detecting collage and elastic fibers without any invasive tissue procedure of slicing, embedding, fixation and staining when two structural proteins are simultaneously present in the biological tissue.

Keywords: Collagen; Elastin; Second-harmonic generation; Two-photon excited fluorescence

Multiphoton microscopy (MPM) is becoming a powerful tool for the biological imaging of thick tissue and live animals because it can provide high contrast and optical sectioning capabilities. It relies on the nonlinear interactions between ultrafast laser pulses and biological tissues. At present, two-photon excited fluorescence (TPEF), three-photon excited fluorescence, and second and third-harmonic generation (SHG, THG) are usually the primary signal source for forming images in MPM. TPEF requires the simultaneous absorption of two photons to fulfill the energy requirement for the excitation of endogenous fluorescence species of biological tissue. Its emission spectrum has a characteristic of a broad peak. SHG process changes two near-infrared incident photons into one-energy visible photon at exactly twice the energy. It requests biological organizations have the noncentrosymmetric structures. SHG emission occurs only at the second-harmonic of the incident laser frequency and its spectrum has a narrow width. Apparent different spectral profiles make them separate each other. Although TPEF and SHG are two completely different nonlinear light-matter interaction processes, they can simultaneously occur when the ultrafast laser pulses irradiate the biological tissue. Thus, the two nonlinear

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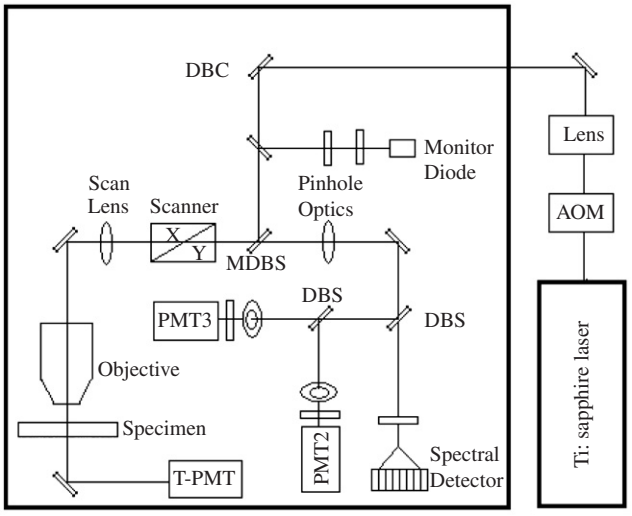

Fig. 1. The experimental arrangement is based on a Zeiss inverted confocal laser scanning microscope LSM 510 with META scanning module equipped with a mode-locked Ti:sapphire femtosecond laser (Coherent Mira 900-F).

optical responses can be detected simultaneously and used for structural and physiological investigations [1–3]. Collagen and elastin are the most important proteins of the connective tissues in higher vertebrates. Collagen has a highly crystalline triple-helix structure which is noncentrosymmetric. This makes collagen exceptionally efficient in generating the second harmonic of incident light [4]. Modifications of the collagen fibrillar matrix structure are associated with various physiologic processes, such as wound healing, cornea diseases, osteoarthritis, liver fibrosis, and cancer [5–9]. Elastin is a significant source of extracelluar matrix autofluorescence, which is responsible for the characteristic elastic properties of many tissues [10–12]. In the skin dermis, aorta, lung, liver and bladder of higher vertebrates, Collagen and elastin are usually present at the same time. So, simultaneous observation of collagen and elastin is very important in gaining structural and diagnostic information.

In this paper, using a two-channel synchronized detection method, we present a combined nonlinear optical imaging technique of SHG and TPEF to

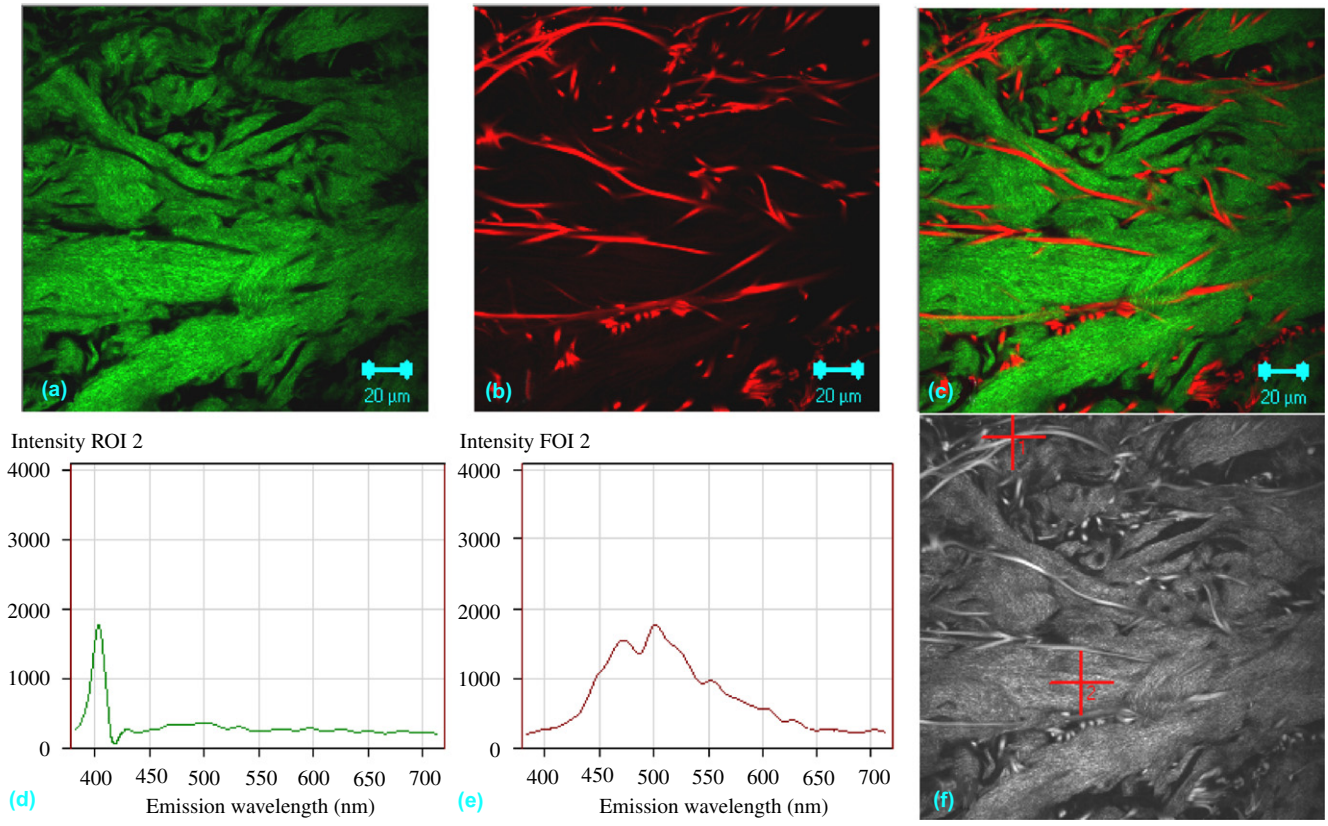

Fig. 2. Panels (a)–(c) show the characterization of a combined nonlinear optical image of collage and elastin in the dermis using a two-channel synchronized detection method. The images of panels (a) and (b) are separately obtained from the channels with a narrow detection spectral range (387–409 nm) and a broad detection spectral range (450–600 nm), respectively. Panel (c) is the overlay image of (a) and (b). Panels (d)–(f) present the image and the corresponding spectrum using the META detection. Panels (d) and (e) exhibit the spectral properties in site 1 and site 2 of panel (f), respectively. Each image covered an area of 206.8 × 206.8 μm.