Optik - International Journal for Light and Electron Optics 169 (2018) 264–268

Contents lists available at ScienceDirect

Optik

journal homepage: www.elsevier.com/locate/ijleo

Original research article

Optical discrimination of type I and type III collagen through second order susceptibility imaging

Chiu-Mei Hsueh[a,1], Te-Yu Tseng[a,1], Hung-Ming Lin[a,1], Sheng-Lin Lee[a], Ya-Der Huang[a], Chen-Yuan Dong[a,b,c,*]

[a] Department of Physics, National Taiwan University, Taipei 106, Taiwan
[b] Center for Optoelectronic Biomedicine, National Taiwan University College of Medicine, Taipei 100, Taiwan
[c] Center of Quantum Science and Engineering, National Taiwan University, Taipei 106, Taiwan

ARTICLE INFO

Keywords:
Second order susceptibility microscopy
Second harmonic generation
Collagen
Tissue engineering

ABSTRACT

Second order susceptibility (SOS) microscopy is used as a contrast mechanism for distinguishing type I and III collagen microstructure in tissues. We found that the SOS tensor elements for type III collagen are $\chi_{zzz}/\chi_{zxx} = 1.20 \pm 0.14$ and $\chi_{xzx}/\chi_{zxx} = 0.49 \pm 0.15$ which are different from the type I collagen values of $\chi_{zzz}/\chi_{zxx} = 1.44 \pm 0.15$ and $\chi_{xzx}/\chi_{zxx} = 0.58 \pm 0.14$. In addition, relative proportion of collagen type was determined by analyzing the histogram of SOS ratio. Through the use of a Gaussian mixture model, the ratio of collagen I and III was determined. We found that in rat skin, type III collagen is present in rat skin in an average ratio of 0.36 ± 0.07. This result shows that SOS microscopy can be applied to *in vivo* label-free studies to investigate the interaction of types I and III collagen molecules and related diseases.

1. Introduction

In recent years, second harmonic generation (SHG) has been widely applied in imaging non-centrosymmetric structural motifs in biological systems. Since the three-dimensional organization of fibrous tissues often lack inherent centro-symmetry, these tissues are ideal targets for SHG imaging. In animals, commonly studied tissues include collagen and muscle. Among the various tissue constituents that have been studied, SHG from collagen fibers is of particular importance in biomedical applications. To be specific, collagen is the most abundant mammalian protein and constitutes about 30% of all human proteins. Among the more than 27 different types of collagen molecules, type I collagen is the most common and has been well studied and characterized. Collagen I is found in a wide array of tissues including skin, cornea, and tendon whereas Collagen II is the main component of extracellular matrix element in tissues such as the cartilage. Among the fibrillary collagen family, Type III collagen is found primarily in tissues exhibiting elastic properties, such as skin, blood vessels and internal organs. Deficiency in Type III collagen would result in irregularly sized collagen fibers in the dermis of the skin as well as the aortic adventitia which results in the loss of normal physiological function [1]. In addition, collagen III has been demonstrated to be crucial for regulating the fiber size of type I collagen and is significant for normal cardiovascular development [2]. A variation in the proportion of type III collagen in tissues may correlate with the obstruction of the tissue or the stage of its development [3,4]. Therefore, developing an imaging technology capable of characterizing type III collagen non-invasively is useful for studying related physiological phenomena and diseases.

In most biomedical applications of SHG microscopy, SHG intensity is used to detect the presence of noncentrosymmetric

* Corresponding author at: Department of Physics, National Taiwan University, Taipei 106, Taiwan.
E-mail address: cydong@phys.ntu.edu.tw (C.-Y. Dong).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ijleo.2018.05.039
Received 23 March 2018; Accepted 10 May 2018

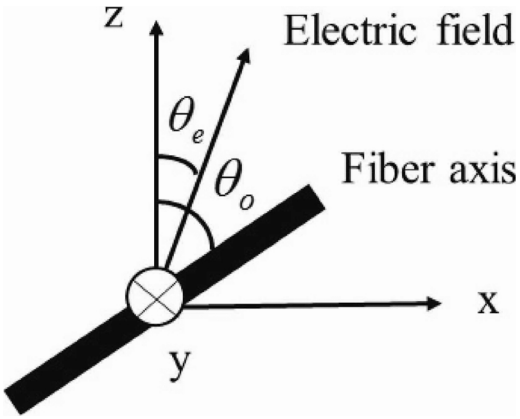

Fig. 1. Principles of second order susceptibility microscopy. By measuring the SHG intensity at different angles between the excitation electric field and collagen fiber, the second order susceptibility of collagens I and III can be determined and differentiated.

structures within tissues. However, in the case of collagen or other fibrous structures, SHG intensity imaging alone is insufficient to characterize tissue specimens containing more than one type of fibrous structures, especially when they have similar morphological features. In the case of type III collagen, one approach to differentiate collagen III from type I collagen is its weaker SHG signal [5,6]. However, this method often requires additional staining for confirmation and variation in collagen content can lead to difficulty in quantifying the presence of the two types of fibrillary collagen. In this work, we will attempt to differentiate types I and III collagen through the use of the ratios of second order susceptibility tensor elements. Previously, we have demonstrated the feasibility of differentiating collagen microstructures of types I and II collagens through the use of second order susceptibility (SOS) as the contrast mechanism [7].

2. Experiment step

In SOS microscopy, a series of SHG images at different polarization angles of the excitation laser source are acquired (Fig. 1). The images can then be analyzed by fitting the excitation polarization-dependent intensity profile to a model which regards the collagen fiber as a cylinder. In this case, the polarization dependent SHG intensity is given as

$$I_{SHG} = c\{[\sin^2(\theta_e - \theta_o) + (\chi_{zzz}/\chi_{zxx})\cos^2(\theta_e - \theta_o)]^2 + (\chi_{xzx}/\chi_{zxx})^2 \sin^2(2(\theta_e - \theta_o))\} \quad (1)$$

where χ_{zzz}/χ_{zxx} and χ_{xzx}/χ_{zxx} are the two ratios of the second order susceptibility tensor elements. Furthermore, θ_e and θ_o are the respective angles of the external electric field and collagen fiber relative to the z axis; and c is a proportional constant. Our approach will be used to investigate type III collagen content and to determine the proportion of collagen III in rat skin samples.

The SOS microscope is home-built and is based on a commercial inverted microscope (TE2000U, Nikon, Japan). The excitation source is a titanium-sapphire laser (Tsunami, Spectra Physics, Mountain View, CA) operating at 780 nm A set of half-wave and quarter-wave plates was used to compensate for the depolarization effect caused by the primary dichroic mirror (720SP, Semrock, Rochester, USA) and to control the orientation of linearly polarized laser source onto the specimen. Backward SHG (BSHG) signals were collected by the focusing objective (S Flour, 40x, NA 0.8, WI, Nikon) and filtered by the band-pass filter (HQ380/40, Chroma Technology). Each image of 35 × 35 μm^2 area was scanned at 256 × 256 pixels resolution by using the water immersion objective [7].

For the purpose of comparison with the SOS microscopy results, the tissue specimens were also labeled by the use of the primary antibodies for type I and type III collagens: monoclonal mouse anti-human collagen I (Cat. No. C2456, Sigma, St Louis, MO; 1:500 dilution) and polyclonal rabbit anti-human collagen III (Cat. No. ab7778, Abcam, Cambridge, UK; 1:500 dilution). The secondary antibodies used were goat anti-mouse IgG conjugated with Alexa Fluor 594 (Cat. No. A11005, Invitrogen; 1:1000 dilution) and goat anti-rabbit IgG conjugated with Alexa Fluor 488 (Cat. No. A11008, Invitrogen; 1:1000 dilution). Negative controls were achieved by omitting the primary antibody. The single photon fluorescence images were collected with the digital camera (DXM 1200C, Nikon, Japan) attached to the fluorescence microscope (Nikon TS100, Japan). Alexa Fluor 594 was illuminated by a 50 W mercury arc lamp with an excitation filter (EX 510–590 nm), dichroic mirror DM 580, and the barrier filter BA590 was used to image type I collagen. Similarly, Alexa Fluor 488 was illuminated by the same lamp but with a different excitation filter set (EX 450–490 nm), dichroic mirror 505, and the band pass filter HQ525/50 (Chroma Technology) was used to visualizing the immunofluorescent labeling of collagen III.

3. Experimental results

We acquired backward SHG intensity (BSHG) image (Fig. 2A) from a frozen section of the rat skin and compared it with the immunofluorescence double stained for type III (Fig. 2B) and I (Fig. 2C) collagens. As can be seen from the images, although