Optik - International Journal for Light and Electron Optics 169 (2018) 392–396

Contents lists available at ScienceDirect

Optik

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

Original research article

Measurement of wavelength-dependent second order susceptibility tensor elements in types I and II collagens

Chiu-Mei Hsueh[a,1], Hung-Ming Lin[a,1], Chun-Ping Huang[a], Chen-Yuan Dong[a,b,*]

[a] Department of Physics, National Taiwan University, Taipei 106, Taiwan
[b] Molecular Imaging Center, National Taiwan University, Taipei 106, Taiwan

ARTICLE INFO

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

ABSTRACT

In this work, we measured the second order susceptibility (SOS) tensor ratios of χ_{zzz}/χ_{zxx} and χ_{xzx}/χ_{zxx} between 725–875 nm in type I (rat tail tendon) and II (rat trachea) collagen. In this wavelength range, χ_{zzz}/χ_{zxx} and χ_{xzx}/χ_{zxx} were found to be frequency-independent. Specifically, χ_{zzz}/χ_{zxx} and χ_{xzx}/χ_{zxx} are around 1.4 and 0.7 for collagen I; 1.2 and 0.4 for collagen II, respectively. Therefore, the near-infrared wavelength range of 700–800 nm is sufficiently far from the collagen resonance at 247 nm in order for the SOS tensor ratios to be wavelength independent. Our results suggest that microscopy imaging based on the use of SOS as a contract mechanism can use the simultaneous illumination of different excitation wavelengths with different polarizations for faster implementation of second order susceptibility microscopy.

1. Introduction

Second harmonic generation (SHG) has emerged as a power imaging modality for a variety of biological tissues. Through the use of SHG intensity as a contrast mechanism, SHG microscopy has proven to be effective in imaging biological superstructures lacking an inversion symmetry. Both animal (collagen, muscle) and plant tissues (cellulose) have demonstrated to be effective harmonophores useful for SHG imaging [1–3]. In biomedicine, SHG microscopy has been applied to systems such as skin photoaging, extracellular matrix environment of tumor, liver fibrosis, cornea infection, and keratoconus [4–9]. In most applications, imaging contrast is based on the SHG intensity at half the wavelength of the excitation source, with the Stoke-shifted fluorescence emission at longer wavelength than that of the SHG signal. As a result, non-centrosymmetric tissue components such as collagen can be imaged with the SHG signal while fluorescence can be used to visualize tissue components such as the cytoplasm. While SHG intensity imaging alone is able to image a single species of harmonophores, the presence of two or more types of harmonophores would render SHG intensity ineffective in distinguishing among the harmonophores. For example, in tissue engineering applications involving cartilage where evaluating the relative content of types I and II collagen is important for quality control of the engineered tissues, similar morphological features of the two collagen types would require immunohistochemical techniques for distinction. However, it has been demonstrated that the use of second order susceptibility (SOS) tensor elements as a contrast mechanism would enable label-imaging of collagens I and II [10].

* 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.2018.05.038
Received 23 March 2018; Accepted 10 May 2018

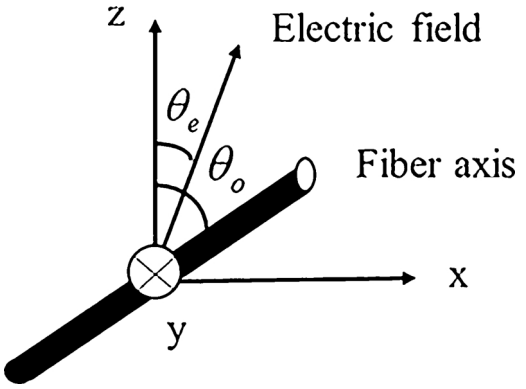

Fig. 1. Cylindrical model of a harmonophore showing the relationship between the orientations of external electric field and the harmonophore.

2. Experiment step

The use of second order susceptibility (SOS) as a contrast mechanism in microscopy is based on a cylindrical model of the harmonphore (Fig. 1).

In this model, the second harmonic generation intensity I_{SHG} varies according to the equation

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

in which θ_L and θ_F are the respective angles of the excitation polarization and harmonophore orientation. χ_{zzz}/χ_{zxx} and χ_{xzx}/χ_{zxx} are ratios of second-order susceptibility tensor elements and c is a constant [1,10]. Therefore, by measuring the angular dependence of SHG intensity, ratios of second-order susceptibility tensor elements can be extracted and used for image contrast purposes. In this manner, second order susceptibility microscopy (SOSM) has been demonstrated to be effective in the label-free imaging of types I and II collagens.

In SOSM, orientation of excitation polarization, angle of the harmonophore, and the two SOS tensor ratios represent four independent variables that need to be determined for imaging. With the harmonophore orientation fixed, SHG intensity needs to be measured at a minimum of four excitation polarization angles for the SOS tensor ratios to be determined. The requirement of a minimum of four independent measurements of SHG intensity means that SOSM is inherently time-consuming. A significantly more efficient implementation would be to illuminate the specimen simultaneously with four polarizations of excitation sources at different wavelengths. Provided that SOS tensor elements are frequency-independent, simultaneous measurement of SHG intensities at four wavelengths would allow ratios of SOS tensor elements to be determined (through the use of Eqn. (1)).

Through a semi-classical model, the SOS tensor elements can be derived as

$$\chi_{ijk} \propto \frac{1}{D_i(2\omega)D_j(\omega)D_k(\omega)} \quad (2)$$

where $D_i(\omega) \equiv \frac{1}{\omega_{\circ i}^2 - \omega^2 - i\gamma_i\omega/m}$. In this model, $\omega_{\circ i}$ and γ_i are the resonant frequency and damping coefficient along the i axis, ω is the driving frequency of external electric field, and m is the electron mass [11]. Therefore, if the harmonophre's resonant frequency $\omega_\circ$ is much larger than that of the electric field ω along the j and k axes, and the SHG frequency 2ω along the i axis, then χ_{ijk} becomes frequency independent. In the case of collagen, strong absorption is found to be between 200–250 nm with weaker absorption in the 250–350 nm range, with resonance reported to be at 227 nm [12,13]. Therefore, it is not clear that the typical wavelength around 800 nm and the associated SHG signal at 400 nm is sufficient to maintain the frequency independence of χ_{ijk}.

In this study, we measured the SHG intensity at different excitation polarization angles and different wavelengths in the 700 and 800 nm range. By fitting of the experimental results, Eq. (1) was used to determine the tensor elements, χ_{zzz}/χ_{zxx} and χ_{xzx}/χ_{zxx}. The rat tail tendon and trachea specimens were prepared and imaged as previously described [10]. For SOS imaging, we used a home-built multiphoton system based on an inverted microscope (TE 2000U, Nikon, Japan). The excitation source is a titanium-sapphire (ti-sa) laser (Tsunami, Spectra Physics, Mountain View, CA) pumped by a diode-pumped, solid-state (DPSS) laser (Millennia Pro, Spectra Physics). The ti-sa laser has nominal pulse width of 100 fs and its repetition frequency is 80 MHz.

A combination of half-wave and quarter plates were used for depolarization compensation to ensure that upon reflection from the primary dichroic mirror (720sp, Semrock Rochester, USA), the laser remains linearly polarized. For focusing, a water-immersion objective (S Fluor 40x, NA 0.8, WI, Nikon) was used for focusing the excitation wavelengths between 725–875 nm. Collection of the backward SHG was also performed by the focusing objective. After passing through the primary dichroic, the SHG signal was further processed by additional dichroic filters (405dcxr, 495dcxr, 435dcxr, Chroma Technology) and bandpass filters (HQ380/40, HQ410/