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Refractive index dispersion properties of collagen for microscopic second-harmonic generation

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ABSTRACT

We theoretically simulate second-harmonic generation (SHG) in collagen under linearly polarized focused laser beam. With this model, the effects of numerical aperture (NA) and refractive index dispersion on SHG emission have been investigated. Dispersion properties of collagen are significant under incident wavelength in the visible range. Our results show that the efficient SHG is obtained by controlling the NA, and the higher NA is a necessity when the dispersion effect is considered. Our theoretical simulation results provide useful clues for experimental study of microscopic SHG emission in collagen excited by focused beam.

1. Introduction

In recent years, multiphoton fluorescence microscopy has gained significant popularity in bioimaging applications [1]. The combination of non-linear optical spectroscopy with scanning microscopy generates innovative tools, for example, second-harmonic generation (SHG) microscopy [2], coherent anti-Stokes Raman scattering (CARS) microscopy [3], and third-harmonic generation (THG) microscopy for biology and materials science, such as collagen and muscle fibers [4].

In the case of collagen, SHG imaging has been widely investigated because of its particular relevance of microscopic applications onto biological imaging [5,6]. The fibrils within the collagen fiber are non-linear scatterers or dipoles, which have non-vanishing second-order susceptibility due to lacking inversion symmetry, and have been shown to be effective in generating second-harmonic signals. Therefore, the theory of SHG is usually studied in which the radiated scatterers are spatially ordered. A common geometry is that of collagen fibrils are distributed uniformly within collagen fiber. Typically, collagen is illuminated by a laser beam, resulting in the radiated SHG in well-defined forward or backward emission [7]. If the laser beam is unfocused or weakly focused, both the driving laser field and the radiated SHG field can be treated as simple collimated beams with well-defined wavevector.

In contrast, when SHG is intended to provide a microscopic image resolution, the driving laser beam must be focused to a small spot size. For 3D observations, a confocal optical system composed of a couple of objective lenses with a high numerical aperture (NA) and a pinhole is widely applied. The radiated SHG can no longer be considered unrelated to the illuminated area. SHG in collagen by focused laser beams has been well established theoretically and experimentally [8,9]. Since SHG is a coherent process, the Gouy shift plays an important role in the efficiency of harmonic generation, which needs to fulfill the phase matching (PM) condition [10]. However, accurate prediction of the PM condition is not sufficient to predict the accurate propagation direction due to refractive indices of collagen fiber is sensitive to the incident wavelength. The driving wavelength becomes one of most critically factors on the radiated distribution of SHG signals. No dispersion is inappropriate in this case, and one must resort to a refined description of PM in the high efficient SHG emission.

In this paper, we provide a detailed theoretical simulation of SHG emission from collagen using a focused excitation beam. The sensitivity of emission angle to the refractive index of collagen has been proposed. Our research here serves as a follow-up to provide complete theoretical and experimental support.

2. Theory of SHG emission

The electric field of a linearly polarized focused light of angular frequency ω propagating in the z-direction and polarized in the $\hat{\varepsilon}$-direction ($\hat{\varepsilon}$ is a unit vector in the x–y plane) may be approximated by [11]

$$\vec{E}_\omega(x,y,z) = -i\vec{E}_\omega^{(0)} \exp\left(-\frac{x^2+y^2}{w_{xy}^2} - \frac{z^2}{w_z^2} + i\xi k_\omega z\right)\hat{\varepsilon}, \quad (1)$$

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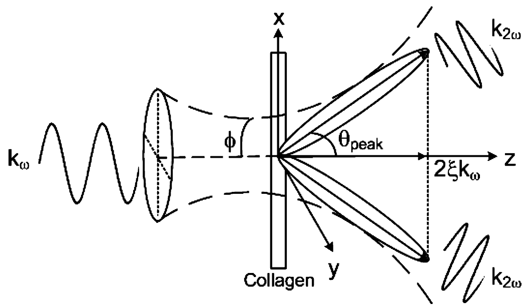

Fig. 1. Schematic diagram defines the fundamental and second-harmonic field radiated in a direction of wavevector k_ω and $k_{2\omega}$, respectively.

where $\vec{E}_\omega^{(0)} = \vec{E}_\omega(0, 0, 0)$ is the complex amplitude of the driving field at (0,0,0). w_{xy} and w_z are the $1/e^2$ radii of the focal ellipse in the lateral and axial directions, respectively. ξ represents the effective reduction in the axial propagation vector $k_\omega = 2\pi n_\omega/\lambda_\omega$, where n_ω is refractive index at the incident wavelength of λ_ω.

The radiation pattern of SHG critically depends on how the scatterers within focused volume are spatially distributed due to coherence of harmonic wave. Referring to Fig. 1, we consider here the uniformly distributed fibrils (non-linear scatterers or dipoles) within collagen fiber which is assumed along the *x*-direction. The second-harmonic far field radiation is excited based on the collagen focused by a Gaussian beam through a microscopic objective with the numerical aperture of $NA = n_\omega \sin\phi$ [8],

$$\vec{E}_{2\omega}(\theta, \varphi) = N\vec{E}_{2\omega}^{(0)} A(\theta, \varphi), \tag{2}$$

where $N = (\pi/2)^{3/2} w_{xy}^2 w_z N_V$. N defines as the effective total number of dipoles that contribute to the generation of second-harmonic light. $N = VN_V$, and $V = (\pi/2)^{3/2} w_{xy}^2 w_z$ is the active SHG volume.

$$\vec{E}_{2\omega}^{(0)} = \frac{\eta}{r}(\sin^2\theta \sin^2\varphi + \cos^2\theta)^{1/2} \cdot \vec{\mu}_{2\omega}^{(0)}, \tag{3}$$

$$\vec{\mu}_{2\omega}^{(0)} = \begin{pmatrix} \mu_{2\omega,x}^{(0)} \\ \mu_{2\omega,y}^{(0)} \\ \mu_{2\omega,z}^{(0)} \end{pmatrix} = \frac{1}{2}\vec{E}_\omega^2(0, 0, 0)\beta = \frac{1}{2}\vec{E}_\omega^{(0)2}\beta, \tag{4}$$

$$A = \exp\left\{-\frac{k_{2\omega}^2}{8}[w_{xy}^2(\sin\theta\cos\varphi)^2 + w_{xy}^2(\sin\theta\sin\varphi)^2 + w_z^2(\cos\theta - \xi n_\omega/n_{2\omega})^2]\right\} = \exp\left\{-\frac{k_{2\omega}^2}{8}[w_{xy}^2\sin^2\theta + w_z^2(\cos\theta - \xi n_\omega/n_{2\omega})^2]\right\}. \tag{5}$$

The angular modulation term A directly defines the propagating structure of SHG with the radiated vector $k_{2\omega} = 2\pi n_{2\omega}/\lambda_{2\omega}$, where $n_{2\omega}$ is refractive index at the incident wavelength of $\lambda_{2\omega}$.

3. Effects of NA on SHG emission

In order to obtain high resolution, a strongly focused excitation beam is often used in SHG microscopy, and the active volume from which harmonic is generated is sharply confined near the focal center. The beam radius at the focal position is used as measures of the spatial resolution. To an excellent approximation, the focused excitation beam amplitude may be considered to have Gaussian profiles in the lateral and axial directions:

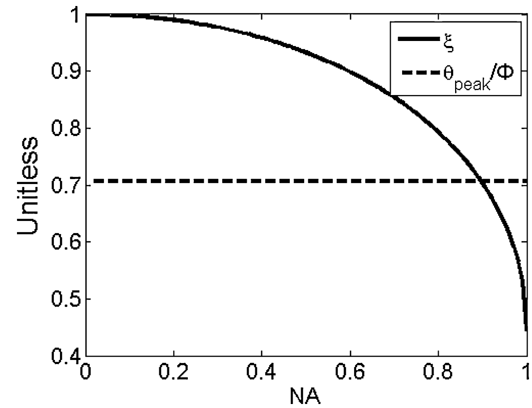

Fig. 2. Variations of the wavevector reduction factor ξ and of the ratio θ_{peak}/ϕ as a function of the NA of a focused microscopic objective.

$$w_{xy} = \begin{cases} \dfrac{0.320\lambda_\omega}{\text{NA}} & \text{NA} \le 0.7 \\ \dfrac{0.325\lambda_\omega}{\text{NA}^{0.91}} & \text{NA} > 0.7 \end{cases}, \tag{6a}$$

$$w_z = 0.532\lambda_\omega \left[\frac{1}{n_\omega - \sqrt{n_\omega^2 - \text{NA}^2}}\right]. \tag{6b}$$

Both of two characteristic lengths are related to NA, which directly influences spatial resolution. In spite of these advantages, the tightly focused optical system with a higher NA yields phenomena substantially different from those of a weakly focused one. Besides the NA impact, here, λ_ω is the wavelength of an incident wave, n_ω is the refractive index of collagen at λ_ω. In this section, we consider the case of no dispersion $n_\omega = n_{2\omega} = 1$, and the wavelength of incident beam $\lambda_\omega = 2\lambda_{2\omega} = 800$ nm.

The reduced effective wavevector of focused beam is interpreted as a consequence of the Gouy shift across the focal center. The properties of the propagation of electromagnetic wave is controlled by reduction factor ξ, which is numerically evaluated to be $\cos(\phi/\sqrt{2})$, where ϕ is the focusing angle of NA, as shown in Fig. 2 with solid line. ξ is approximately less than 1, therefore, the reduced effective wavevector near focused volume can be written as ξk_ω. Additionally, a comparison of θ_{peak} and ϕ as a function of NA is represented with dash line. We also observe that θ_{peak}/ϕ is roughly constant and less than 1. As a result, a collection angle, which is equal to the focused excitation angle ϕ, is sufficient enough to collect all the SHG light. Moreover, the angular radiation distribution A critically depends on ξ and exhibits two symmetric lobes at $\theta_{peak} = \pm\cos^{-1}(\xi)$. This differs from the usual geometry used for studying SHG excited by collimated laser beam [12]. To demonstrate the profile of emission, SHG signals under NA = 0.1 and NA = 1.0 have been compared as shown in Fig. 3. At lower NA, SHG emission concentrates along small angles $\pm 3^\circ$, regarding as a forward harmonic propagation which also can be observed by collimated beam. Instead, at higher NA, SHG emission propagates along two forward directed lobes at an off-axis angle close to $\pm 29^\circ$. No matter at high or low NA, the max value of SHG radiation satisfies the condition of PM, $k_{2\omega}\cos\theta_{peak} - 2\xi k_\omega = 0$, as shown in Fig. 1, corresponding to momentum conservation along the axial direction. As discussed by Boyd and Kleinman, the strongly focused SHG exhibits essentially different features from the weakly focused case, and, in particular, the SHG intensity depends strongly on the sign of the condition of PM [13]. PM plays a significant role in defining not only the total power emitted by SHG but also its angular distribution.