Characterization of excitation beam on second-harmonic generation in fibrillous type I collagen

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Abstract Following our established theoretical model to deal with the second-harmonic generation (SHG) excited by a linearly polarized focused beam in type I collagen, in this paper, we further quantitatively characterize the differences between SHG emissions in type I collagen excited by collimated and focused beams. The effects of the linear polarization angle (α) and the fibril polarity characterized by the hyperpolarizability ratio ρ on SHG emission has been compared under collimated and focused beam excitation, respectively. In particular, SHG emission components along the i axis ($I_{2\omega,i}$) ($i = x, y, z$), the induced SHG emission deviation angle γ_{ij} , and the detected SHG signals ($I_{2\omega,ij}$) in the ij plane by rotating the applied polarizer angle ϕ_{ij} have been investigated ($i = x, y, z, j = y, z, z$). Results show that under our simulation model, SHG emission in the xy plane, such as $I_{2\omega,x}$, $I_{2\omega,y}$, γ_{xy} and $I_{2\omega,xy}$ varying as polarization angle (α) under collimated and focused light, presents no significant difference. The reverse of the fibril polarity has induced great impact on $I_{2\omega,x}$, γ_{xy} and $I_{2\omega,xy}$ in both collimated and focused light. $I_{2\omega,x}$ and γ_{xy} show similarity, but $I_{2\omega,xy}$ at $\alpha = 30^\circ$ demonstrates a slight difference in focused light to that in collimated light. Under focused light, the reverse of fibril polarity causes obvious changes of the collected SHG intensity $I_{2\omega,xz}$ and $I_{2\omega,yz}$ at a special polarization angle $\alpha = 60^\circ$ and γ_{xz} , γ_{yz} along α .

Keywords Collimated beam · Focused beam · Polarization (α) · SHG intensity

1 Introduction

Second-harmonic generation (SHG) is a nonlinear optical effect first recognized by Franken et al. in crystals in 1961, the year shortly after the demonstration of the laser [1]. In this

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process, two near-infrared incident photons are scattered by a material with noncentrosymmetric structural features into one emerging visible photon, which is at exactly half the excitation wavelength (twice the energy). This well-known nonlinear optical effect was then identified in biological systems; fibrillous collagen was identified about 10 years later in 1971 [2].

In such a nonlinear optical process, as it occurs by scattering rather than absorption and re-emission as in two-photon fluorescence processes, there is no energy loss during excitation to emission; SHG thus has unique advantages such as no photobleaching and no phototoxicity caused in the specimen. SHG is the intrinsic signal induced by the interaction of photons on the optical properties of specimen itself, no additional staining on fluorochrome is required for its enhancement. Meanwhile, in light of the coherence preservation of the excitation light, SHG usually carries the directionality information that is associated with the characteristics of the specimen [3, 4].

SHG signals therefore have been widely exploited for imaging in biological tissues, especially in fibrillous collagen type I, one of the strongest SHG producers of biological specimens. SHG was first noticed by Fine and Hansen in fibrillous collagen type I early in 1971 based on the use of linearly polarized collimated light [2]. In 1978, Gannaway and Sheppard successfully demonstrated an SHG phenomenon through a microscope [5]. Since then, more researchers have been interested in taking advantage of the microscopic SHG signals to achieve three-dimensional high-resolution optical imaging in biological specimens. A great number of experiments of microscopic SHG imaging through fibrillous collagen type I have been conducted [6, 7]. At the same time, a series of theories of dealing with SHG based on linearly polarized focused light have been developed [8–10]. Yet, in a theoretical model to deal with polarization effects of focused light on SHG emission in fibrillous collagen type I, the type of biological specimen with nonplanar (but linear) homogenous distribution of scatterers (dipoles) has not been well established. On the contrary, such effects studies in collimated light have been well explored [11, 12]. Accordingly, how focused light influences SHG in fibrillous collagen type I has not been comprehensively compared to that of collimated light before. Therefore, based on our newly established theoretical model to deal with the SHG excited by a linearly polarized focused beam in type I collagen [13], in this paper, we further characterize the differences between SHG emissions in type I collagen excited by collimated and focused light. In particular, the effects of the linear polarization angle (α) and the fibril polarity characterized by the parameter of hyperpolarizability ratio (ρ) on SHG emission have been compared under collimated and focused beam excitation, respectively.

2 Theory of SHG in type I collagen under collimated and focused beam

Based on the structural features of type I collagen, it is assumed to be composed of highly organized fibrils that have a cylindrical rod-like shape. In this paper, the collagen fibrils are hypothetically of zero thickness and are composed of many infinitesimally small subunits (dipoles), each possessing C_6 symmetry. A schematic coordinate system to describe SHG emission from such fibrils is shown in Fig. 1. The subunits (dipoles) align in the x direction and extend along the x direction. The x axis is thus also the polar symmetrical axis of the dipoles that conforms to cylindrical symmetry. The incident excitation field $\vec{E}_\omega$ propagates toward the z axis with a linearly polarized direction in the xy plane and a polarization angle α from the x axis. We suppose that the effective volume density N_V of dipoles and the effective

Fig. 1 Schematic diagram to characterize SHG emission from type I collagen fibrils under linearly polarized beam of collimated light and focused light, respectively. Collagen fibrils composed of dipoles are assumed to be aligned along the x direction. The light is linearly polarized in the xy plane ($\vec{E}_\omega$) with angle α from the x direction and propagates in the z direction. Collimated light trace is represented by a dashed line, and the solid line represents a focused light trace

excited volume V under collimated and focused beams are the same, which means that the total number of dipoles $N = N_V V$ that contribute to the generation of second-harmonic light is identical for both sets of conditions.

2.1 Electric dipole moment of the single dipole induced by fundamental electrical field

The overall electric dipole moment (or polarization) induced by a fundamental electric field is [14]

$$\vec{\mu} = \vec{\mu}_0 + \alpha \cdot \vec{E}_\omega + \frac{1}{2!} \beta \cdot \vec{E}_\omega^2 + \frac{1}{3!} \gamma \cdot \vec{E}_\omega^3 + \dots \quad (1)$$

Here, $\vec{\mu}_0$ is the permanent dipole moment and the frequency-dependent parameters α , β , and γ are the linear polarizability and first and second hyperpolarizability, respectively. The contribution to SHG is related to the third term by a single dipole (scatterer) in the fibrils, which can be described as [15]:

$$\mu_{2\omega,i} = \frac{1}{2} E_\omega^2 \sum \beta_{ijk} \varepsilon_j \varepsilon_k \quad (2)$$

β_{ijk} represents the first hyperpolarizabilities. The subscripts ijk refer to three axes in an orthogonal coordinate system; ε is the polarization direction. Because of Kleinman and cylindrical symmetry, only two terms in β related to μ remain:

$$\mu_{2\omega,x} = \frac{1}{2} \left(\beta_{xxx} E_{\omega,x}^2 + \beta_{xyy} E_{\omega,y}^2 \right) = \frac{1}{2} \left(\beta_{xxx} E_\omega^2 \cos^2 \alpha + \beta_{xyy} E_\omega^2 \sin^2 \alpha \right) \quad (3)$$

$$\begin{aligned}\mu_{2\omega,y} &= \frac{1}{2} (\beta_{xyy} E_{\omega,x} E_{\omega,y} + \beta_{xyy} E_{\omega,y} E_{\omega,x}) \\ &= \frac{1}{2} (\beta_{xyy} E_{\omega}^2 \cos \alpha \sin \alpha + \beta_{xyy} E_{\omega}^2 \sin \alpha \cos \alpha) = \frac{1}{2} \beta_{xyy} E_{\omega}^2 \sin 2\alpha\end{aligned}\quad (4)$$

where $E_{\omega,x}$ and $E_{\omega,y}$ are the fundamental field strengths polarized in the x and y directions, respectively.

2.2 SHG emission electric field induced by effective total dipole moment

Dipole moment thus induces the second-harmonic electrical field, the configuration of $\vec{E}_{2\omega}$ is as follows [16]

$$\vec{E}_{2\omega} = \vec{\mu}_{2\omega} \frac{\omega^2}{\pi \varepsilon_0 c^2 r} \sin(\psi) \exp(-i\vec{k}_{2\omega} \cdot \vec{r}) \hat{\psi}. \quad (5)$$

We define $\eta = \frac{\omega^2}{\pi \varepsilon_0 c^2 r}$, where ε_0 is the free-space permittivity, c is the speed of light, ω is the frequency of the fundamental beam. The direction of the vector $\vec{k}_{2\omega}$ denotes the SHG emission direction. ψ represents the angle between the $\vec{x}$ axis and the emission direction $\vec{r}$ of SHG. $\sin \psi$ thus illustrates the projection relationship between the direction of the excited SHG dipole moment $\vec{\mu}_{2\omega}$ and the emitted electric field $\vec{E}_{2\omega}$ of SHG, and it is defined by the solid angle in Cartesian coordinates:

$$\sin \psi = (\sin^2 \theta \sin^2 \varphi + \cos^2 \theta)^{\frac{1}{2}}, \quad (6)$$

where θ is the angle between the direction of SHG propagation and the direction of propagation of the incident beam (z axis), and φ is the angle between the emission plane (which is defined as the plane constituted by the direction of incident beam propagation and the direction of SHG propagation, as shown in Fig. 1) and the xz plane.

2.2.1 SHG electric field strength from collagen fibrils when illuminated by a collimated laser beam

When collimated light is applied, emitted SHG propagates in the direction of incident beam with no deviation, thus $\psi = \pi/2$. As a result, for the whole quantity N of excited scatterers (dipoles), the induced SHG electrical field is

$$\vec{E}_{2\omega} = N \vec{\mu}_{2\omega} \frac{\omega^2}{\pi \varepsilon_0 c^2 r} = N \eta \vec{\mu}_{2\omega} \quad (7)$$

Correspondingly, according to Eqs. 3 and 4, the electric field of SHG along the x - and y -axes can be respectively written as

$$E_{2\omega,x} = N \mu_{2\omega,x} \eta = \frac{1}{2} N \eta E_{\omega}^2 (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \quad (8)$$

$$E_{2\omega,y} = N \mu_{2\omega,y} \eta = \frac{1}{2} N \eta E_{\omega}^2 \beta_{xyy} \sin 2\alpha \quad (9)$$

2.2.2 SHG electric field strength from collagen fibrils when illuminated by a focused laser beam

The single electric dipole moment induced by the fundamental electrical field has the same expressions as those demonstrated in Eqs. 3 and 4; however, under the focused laser beam case, the driving field $\vec{E}_\omega$ has a more complicated form, which can be well approximated by a three-dimensional (3D) Gaussian profile in the focus area as follows:

$$\vec{E}_\omega(x, y, z) = -i\vec{E}_\omega^{(0)} \exp\left(-\frac{x^2 + y^2}{w_{xy}^2} - \frac{z^2}{w_z^2} + i\xi k_\omega z\right) \hat{\epsilon} \quad (10)$$

$\vec{E}_\omega^{(0)}$ is the electric field strength at the central point of the focused beam. $k_\omega = 2\pi n_\omega / \lambda_\omega$ is the wave vector in a specimen with refractive index n_ω . ξ is the wave vector reduction factor due to focusing that accounts for a reduction in axial momentum by conversion to lateral momentum within the focus. w_{xy} and w_z are the focal ellipse in the lateral and axial directions, respectively. According to Eq. 5, the distribution of the SHG electric field induced by whole excited dipoles N at an observation point (r, θ, φ) is

$$\begin{aligned} \vec{E}_{2\omega}(\theta, \varphi) &= \left(\sqrt{\frac{\pi}{2}}\right)^3 N_V w_{xy}^2 w_z \eta (\sin^2 \theta \sin^2 \varphi + \cos^2 \theta)^{\frac{1}{2}} \cdot \vec{\mu}_{2\omega}^{(0)} \\ &\quad \times \exp\left\{-\frac{k_{2\omega}^2}{8} \left[w_{xy}^2 (\sin \theta \cos \varphi)^2 + w_{xy}^2 (\sin \theta \sin \varphi)^2 + w_z^2 (\cos \theta - \xi')^2\right]\right\} \\ &= N\vec{E}_{2\omega}^{(0)} A(\theta, \varphi), \end{aligned} \quad (11)$$

where

$$N = \left(\sqrt{\frac{\pi}{2}}\right)^3 w_{xy}^2 w_z N_V \quad (12)$$

$N = VN_V$, and $V = \left(\sqrt{\frac{\pi}{2}}\right)^3 w_{xy}^2 w_z$ is the active SHG volume [17].

$$\vec{E}_{2\omega}^{(0)} = \eta (\sin^2 \theta \sin^2 \varphi + \cos^2 \theta)^{\frac{1}{2}} \cdot \vec{\mu}_{2\omega}^{(0)} \quad (13)$$

$$\begin{aligned} A &= \exp\left\{-\frac{k_{2\omega}^2}{8} \left[w_{xy}^2 (\sin \theta \cos \varphi)^2 + w_{xy}^2 (\sin \theta \sin \varphi)^2 + w_z^2 (\cos \theta - \xi')^2\right]\right\} \\ &= \exp\left\{-\frac{k_{2\omega}^2}{8} \left[w_{xy}^2 \sin^2 \theta + w_z^2 (\cos \theta - \xi')^2\right]\right\} \end{aligned} \quad (14)$$

$$\xi' = \xi \frac{n_\omega}{n_{2\omega}}, (k_\omega = 2\pi n_\omega / \lambda_\omega \text{ and } k_{2\omega} = 2\pi n_{2\omega} / \lambda_{2\omega}) \quad (15)$$

2.3 The amplitude and direction of SHG intensity

The intensity of SHG produced has the following relation with the electric field:

$$I_{2\omega} = \frac{1}{2} n_{2\omega} \epsilon_0 c |E_{2\omega}|^2 \quad (16)$$

2.3.1 SHG intensity illuminated by a collimated beam

Based on Eqs. 8 and 9, the x and y components of the SHG intensity under collimated beam excitation can be easily obtained as

$$\begin{aligned} I_{2\omega,x} &= \frac{1}{2} n_{2\omega} \varepsilon_0 c |E_{2\omega,x}|^2 \\ &= \frac{1}{8} n_{2\omega} \varepsilon_0 c \eta^2 N^2 E_\omega^4 (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha)^2 \end{aligned} \quad (17)$$

$$\begin{aligned} I_{2\omega,y} &= \frac{1}{2} n_{2\omega} \varepsilon_0 c |E_{2\omega,y}|^2 \\ &= \frac{1}{8} n_{2\omega} \varepsilon_0 c \eta^2 N^2 E_\omega^4 (\beta_{xyy} \sin 2\alpha)^2 \end{aligned} \quad (18)$$

The total SHG intensity thus has the following relationship with the linear polarization angle α under the collimated beam:

$$\begin{aligned} I_{2\omega} &= I_{2\omega,x} + I_{2\omega,y} \\ &= \frac{1}{8} n_{2\omega} \varepsilon_0 c \eta^2 N^2 E_\omega^4 [(\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha)^2 + (\beta_{xyy} \sin 2\alpha)^2] \end{aligned} \quad (19)$$

2.3.2 SHG intensity illuminated by a focused beam

Due to the distribution of the SHG electrical field in three dimensions in the emission space, the total SHG intensity under the focused beam is the integration in solid angle as

$$\begin{aligned} I_{2\omega} &= \frac{1}{2} n_{2\omega} \varepsilon_0 c \iint |E_{2\omega}(\theta, \varphi)|^2 \sin \theta d\theta d\varphi \\ &= \frac{1}{8} n_{2\omega} \varepsilon_0 c \eta^2 N^2 \Theta E_\omega^{(0)4} [(\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha)^2 + (\beta_{xyy} \sin 2\alpha)^2], \end{aligned} \quad (20)$$

where

$$\Theta = \iint A^2(\theta, \varphi) (1 - \sin^2 \theta \cos^2 \varphi) \sin \theta d\theta d\varphi. \quad (21)$$

Accordingly, three components of SHG intensity based on the coordinates x -, y -, and z -axes have been obtained (as for collimated light, we represent the excited SHG by the x and y components; for focused light, we present it in our previous paper [13] by the components perpendicular (s) and parallel (p) to the emission plane). In order to compare the SHG by focused light to that from collimated light, we project the components in the p and s directions to the x , y , and z directions. This detailed derivation process is revealed in the [Appendix](#).

$$\begin{aligned} I_{2\omega,y} &= \frac{1}{8} n_{2\omega} c \varepsilon_0 \eta^2 N^2 E_\omega^{(0)4} \iint A^2(\theta, \varphi) \{ [\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\ &\quad + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha] \cos \theta \cos \varphi - [-\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\ &\quad + \beta_{xyy} \cos \varphi \sin 2\alpha] \sin \varphi \}^2 \sin \theta d\theta d\varphi \end{aligned} \quad (22)$$

$$\begin{aligned}
I_{2\omega,y} = & \frac{1}{8} n_{2\omega} c \varepsilon_0 \eta^2 N^2 E_{\omega}^{(0)4} \iint A^2(\theta, \varphi) \{ [\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\
& + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha] \cos \theta \sin \varphi + [-\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\
& + \beta_{xyy} \cos \varphi \sin 2\alpha] \cos \varphi \}^2 \sin \theta d\theta d\varphi,
\end{aligned} \quad (23)$$

$$\begin{aligned}
I_{2\omega,z} = & \frac{1}{8} n_{2\omega} c \varepsilon_0 \eta^2 N^2 E_{\omega}^{(0)4} \iint A^2(\theta, \varphi) \{ -[\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\
& + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha] \sin \theta \}^2 \sin \theta d\theta d\varphi
\end{aligned} \quad (24)$$

2.3.3 Polarization direction of SHG emission

In this paper, the polarization direction of the emitted SHG is to be expressed through three deviation angles γ_{ij} relative to the i axis in the Cartesian coordinate system, where when i is taken to be x , y , and z , j is correspondingly taken to be y , z , and x , respectively. In the ij plane, the maximum SHG intensity appears at the deviation angle γ_{ij} , given by

$$\tan \gamma_{ij} = I_{2\omega,j} / I_{2\omega,i} \quad (25)$$

If a polarizer is applied that is parallel in the ij plane and has an angle ϕ_{ij} with the i axis based on Malus's law, the collected SHG emission signals as the angle of polarizer ϕ_{ij} will be

$$I_{2\omega,ij} = (I_{2\omega,i} + I_{2\omega,j}) \cos^2(\phi_{ij} - \gamma_{ij}) \quad (26)$$

3 Comparison of SHG emission excited by collimated and focused beam

3.1 Influence of the linear polarization angle α

In this section, first, we discuss SHG emission intensity components along the x -, y -, and z axes as the linear polarization angle α excited by the collimated and focused beams.

To do this, we introduce a parameter ρ as the ratio of hyperpolarizability as $\rho = \beta_{xxx} / \beta_{xyy}$, and at the same time, we suppose $\rho = 2.6$ and $\beta_{xyy} = 1$. Meanwhile, $\frac{1}{8} n \varepsilon_0 c \eta^2 N^2 E_{\omega}^4 = 1$ in collimated light and $\frac{1}{8} n \varepsilon_0 c \eta^2 N^2 E_{\omega}^{(0)4}$ in focused light are defined for simplicity and comparison.

Figure 2a demonstrates the SHG intensity $I_{2\omega,x}$, $I_{2\omega,y}$, and $I_{2\omega}$ as the polarization angle α excited by the collimated beam when $\rho = 2.6$, based on Eqs. 17, 18, and 19. We notice that $I_{2\omega,x}$ has two symmetrical maximum values at $\alpha = 0^\circ$ and 180° as well as two symmetrical minimum values at $\alpha = 90^\circ$ and 270° , while $I_{2\omega,y}$ shows four maximum values at $\alpha = (2n + 1) \times 45^\circ$ ($n = 0, 1, 2, 3$) and four minimum values at $\alpha = n \times 90^\circ$ ($n = 0, 1, 2, 3$). $I_{2\omega}$ has two maximum and two minimum values at the same excitation angles α as those of $I_{2\omega,x}$. It is clear that $I_{2\omega,x}$ has a more dominant contribution than that of $I_{2\omega,y}$ to the total SHG intensity $I_{2\omega}$.

In the case of an excitation beam that is focused, SHG emission is three-dimensional, in which case there is an additional component along the z direction except components confined within two-dimensional xy plane as those in collimated case. The cause is due to

Fig. 2 Effects of the polarization angle α on the SHG emission intensity along the x direction ($I_{2\omega,x}$), the y direction ($I_{2\omega,y}$), and the total component ($I_{2\omega}$) under collimated light when $\rho = 2.6$ (a). Effects of polarization angle α on the SHG emission intensity along the x direction ($I_{2\omega,x}$), y direction ($I_{2\omega,y}$), z direction ($I_{2\omega,z}$), and the total component ($I_{2\omega}$) under focused light when $\rho = 2.6$ (b)

the appearance of the additional angle modulation term $A(\theta, \varphi)$ as demonstrated in Eq. 14, which results in an off z axis propagation direction of SHG emission. Based on Eqs. 22, 23, and 24, SHG intensities $I_{2\omega,x}$, $I_{2\omega,y}$, $I_{2\omega,z}$ and $I_{2\omega}$ are shown as a function of α (Fig. 2b) after excitation by a focused incident beam. We notice that $I_{2\omega,x}$ and $I_{2\omega,y}$ keep the similar shapes as those in collimated beam under rotation of the polarization angle α . However, the maximum values appearing at $\alpha = 0^\circ$ and 180° as well as the minimum values appearing at $\alpha = 90^\circ$ and 270° of $I_{2\omega,x}$ no longer coincide with those of total SHG intensity $I_{2\omega}$ observed in the collimated excitation beam, since $I_{2\omega,z}$ now contributes to the total SHG intensity $I_{2\omega}$. $I_{2\omega,z}$ varies less steeply along α and its strength is much smaller than $I_{2\omega,x}$.

Figure 3a shows the influence of the fundamental polarization angle α ($0^\circ \sim 360^\circ$) on the SHG polarization deviation angle γ_{xy} in a collimated beam. It is noticed that γ_{xy} changes periodically as a function of α . γ_{xy} has its maximum deviation degree of 21° at $\alpha = 60^\circ$, 120° , 240° , and 300° and the minimum deviation degree $\gamma_{xy} = 0^\circ$ at $\alpha = 0^\circ$, 90° , 180° , and 270° . Figure 3b shows the influence of α on γ_{ij} in the case of focused beam. The influence

Fig. 3 Effects of excitation polarization angle α ($0^\circ \sim 360^\circ$) on the SHG polarization deviation angle γ_{xy} in a collimated beam when $\rho = 2.6$ (a). Effects of excitation polarization angle α ($0^\circ \sim 360^\circ$) on SHG polarization deviation angle γ_{xy} , γ_{xz} , and γ_{yz} in a focused beam when $\rho = 2.6$ (b)

of α on γ_{xy} is similar to that under the collimated beam case. In the xz plane, the polarization angle α has an even and negligible effect on the SHG deviation angle γ_{xz} . In the yz plane, four maximum angles γ_{yz} (around 86°) appear when polarization angle α is $0^\circ, 90^\circ, 180^\circ$, and 270° and when the polarization angle α is $60^\circ, 120^\circ, 240^\circ$, and 300° . γ_{yz} presents four minimum angles (around 15°). We notice that α has different impact range on γ_{yz} , γ_{xy} , and γ_{xz} , and γ_{yz} is the most significantly affected; its change range is about 70° , while that of γ_{xy} is about 21° and that of γ_{xz} is almost zero. In addition, we also notice that α has a nonsymmetrical effect on both γ_{yz} and γ_{xy} from x and y axes. When α is off from the x axis, the deviation angle γ_{yz} and γ_{xy} decreases or increases slowly to the peak value, while when α is off from the y axis, the deviation angle γ_{yz} and γ_{xy} decreases or increases dramatically to the peak value. In other words, the turning points of α locate far away from the x axis and closer to the y axis rather than in the middle of the x and y axes.

Finally, in practice, SHG emission signals are usually collected by applying a polarizer in front of the detector. To understand the influence of the polarizer direction ϕ_{ij} on the collected SHG signals for reference to practical applications, the collected SHG signals varying as the polarizer direction ϕ_{ij} rotating from 0° to 360° under different fundamental polarization angles $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° have been investigated.

◀ **Fig. 4** **a** SHG intensity $I_{2\omega}$ as a function of the rotation of the angle ϕ_{xy} of the polarizer under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° in collimated beam when $\rho = 2.6$. **b** SHG intensity $I_{2\omega,xy}$ as a function of the rotation of the angle ϕ_{xy} under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° in focused beam when $\rho = 2.6$. **c** SHG intensity $I_{2\omega,xz}$ as a function of the rotation of the angle ϕ_{xz} under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° in focused beam when $\rho = 2.6$. **d** SHG intensity $I_{2\omega,yz}$ as a function of the rotation of the angle ϕ_{yz} under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° in focused beam when $\rho = 2.6$

Figure 4a demonstrates the SHG intensity $I_{2\omega,xy}$ ($= I_{2\omega,x} + I_{2\omega,y} = I_{2\omega}$) as a function of the rotation of the angle ϕ_{xy} of the polarizer under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° in collimated beam. We notice that when the fundamental polarization α is along the x axis ($\alpha = 0^\circ$) or the y axis ($\alpha = 90^\circ$), SHG emission has a similar mode where the maximum SHG intensity appears at $\phi_{xy} = 0^\circ$ and 180° and no SHG emission collected at $\phi_{xy} = 90^\circ$ and 270° . However, the strength of collected SHG signals at $\alpha = 90^\circ$ is much weaker than that when $\alpha = 0^\circ$, which indicates that SHG has the most efficiency when the illumination electric-field polarization is orientated parallel to the principal axis of the fibrils ($\alpha = 0^\circ$). As the illumination beam polarizes at $\alpha = 30^\circ$ and 60° , the collected SHG signals present a slight peak intensity deviation of 9° and 21° , respectively, from the fibrils axis.

Figure 4b–d demonstrates the SHG intensity $I_{2\omega,xy}$, $I_{2\omega,xz}$, and $I_{2\omega,yz}$ as the rotation of the angle ϕ_{xy} , ϕ_{xz} , and ϕ_{yz} of the polarizer under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° , respectively, in focused beam. In the xy plane (Fig. 4b), collected SHG emission mode as the rotation of angle ϕ_{xy} is similar to that under collimated beam, but the derivation angle of the peak intensity has a slight change to be 8° under focused beam rather than 9° in collimated beam at $\alpha = 30^\circ$. Figure 4c shows the collected SHG emission $I_{2\omega,xz}$ as the rotation of the polarizer ϕ_{xz} in the xz plane. It demonstrates that the SHG peak intensity that could be collected has an almost constant deviation at any polarization angle α , which is 4° at $\alpha = 0^\circ, 90^\circ$, and 5° at $\alpha = 30^\circ$ and 60° from the fibrils axis (x axis) towards the z axis. The SHG emission $I_{2\omega,yz}$ in the yz plane is demonstrated in Fig. 4d, where we notice that the SHG peak intensity appears at the direction almost along the z axis ($\phi_{yz} = 86^\circ$) when the fundamental polarization α is along the x axis ($\alpha = 0^\circ$) or y axis ($\alpha = 90^\circ$). The SHG peak intensity deviates from the y axis at 30° and 14° at polarization $\alpha = 30^\circ$ and $\alpha = 60^\circ$ respectively.

3.2 Influence of the fibril polarity characterized by hyperpolarizability ratio ρ

β is the hyperpolarizability of the collagen fibrils which is related to the electronic transition in the material; thus, the ratio $\rho = \beta_{xxx}/\beta_{xyy}$ is a reflection of the biological structure and chemical features of the collagen. It has been proved that ρ increases with the increasing of age and $\rho = \beta_{xxx}/\beta_{xyy}$ has been predicted to vary between -3 to 3 in collagen [18]. The negative value of ρ suggests that the polarity of the fibrils reverses from the excited electrical field. In this section, to understand the influence of the fibril polarity on SHG emission under collimated and focused beam, the negative value of $\rho = -2.6$ is applied for the exploration of SHG emission.

Figure 5a shows the SHG intensity $I_{2\omega,x}$, $I_{2\omega,y}$, and $I_{2\omega}$ as a function of the polarization angle α excited by collimated beam when $\rho = -2.6$. In this situation, $I_{2\omega,x}$ is the component that has been affected more obviously compared to that of $\rho = 2.6$. The shape of $I_{2\omega,x}$ as α changes becomes more complicated; two additional peak values of $I_{2\omega,x}$ appear at, $\alpha = 90^\circ$ and 270° ; there are no at $\alpha = 0^\circ$ and 180° . Accordingly, the total SHG intensity presents two peaks of the same size of $I_{2\omega,x}$ at $\alpha = 90^\circ$ and 270° instead of two minimum values when $\rho = 2.6$. Also, in the focused beam case when $\rho = -2.6$ as shown in Fig. 5b, $I_{2\omega,x}$

Fig. 5 **a** Effects of fibril polarity ($\rho = -2.6$) on SHG emission intensity along the x direction ($I_{2\omega,x}$), y direction ($I_{2\omega,y}$), and the total component ($I_{2\omega}$) under collimated light at excitation polarization angle $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° . **b** Effects of fibril polarity on SHG emission intensity along the x direction ($I_{2\omega,x}$), y direction ($I_{2\omega,y}$), z direction ($I_{2\omega,z}$), and the total component ($I_{2\omega}$) under focused light at excitation polarization angle $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90°

is heavily affected. The intensity distribution at different polarization angles α becomes different from that at $\rho = 2.6$, where two peak intensities suddenly appear at $\alpha = 90^\circ$ and 270° . Nevertheless, the fibril polarity has a similar influence on $I_{2\omega,x}$ under collimated and focused beam conditions.

Figure 6a shows the SHG polarization deviation angle γ_{ij} (in the xy plane) induced by excited polarization angle α ($0^\circ \sim 360^\circ$) when $\rho = -2.6$ in the collimated beam. We notice that in the collimated case, the reverse of fibril polarity to the excited electrical field has little impact on the overall shape of the SHG polarization deviation angle γ_{xy} along all the excited polarization angle α ; however, the maximum deviation angle γ_{xy} reaches to 90° instead of 21° . In the focused beam case, as shown in Fig. 6b, also, the fibril polarity has an impact on the SHG maximum deviation angle γ_{xy} , which reaches to 90° . Fibril polarity has no apparent impact on γ_{yz} except to cause a decrease in the minimum value from 14° to 4° , while it does have significant impact on the SHG polarization deviation angle γ_{xz} , where

Fig. 6 Effects of fibril polarity ($\rho = -2.6$) on the SHG polarization deviation angle γ_{xy} in collimated beam as excitation polarization angle α varies from $0^\circ \sim 360^\circ$ (a). Effects of fibril polarity ($\rho = -2.6$) on SHG polarization deviation angle γ_{xy} , γ_{xz} , and γ_{yz} in focused light as excitation polarization angle α varies from $0^\circ \sim 360^\circ$ (b)

γ_{xz} has a similar pattern as γ_{xy} , a fully different shape compared to the flat curve when $\rho = 2.6$.

Figure 7a demonstrates the influence of fibril polarity ($\rho = -2.6$) on collected SHG intensity distribution $I_{2\omega,xy}$ by rotating of the angle ϕ_{xy} of the polarizer under $\alpha = 0^\circ, 30^\circ, 60^\circ, 90^\circ$ in collimated beam. When $\alpha = 0^\circ$ and 90° , the fibril polarity has no visible impact on $I_{2\omega,xy}$. ϕ_{xy} changes from 9° to 15° at $\alpha = 30^\circ$ and from 21° to 90° at $\alpha = 60^\circ$ from $\rho = 2.6$ to $\rho = -2.6$. The collected SHG intensity distribution $I_{2\omega,xy}$, $I_{2\omega,xz}$, and $I_{2\omega,yz}$ as rotation of the polarizer angle ϕ_{ij} in the corresponding xy , xz , and yz planes in the case of focused beam have been demonstrated in Fig. 7b–d, respectively. As shown in Fig. 7b, we notice that the fibril polarity in the focused beam has a similar impact on $I_{2\omega,xy}$ as that in the collimated beam when $\alpha = 0^\circ$ and 90° as well as $\alpha = 60^\circ$. However, the angle of polarizer ϕ_{xy} corresponding to the collected peak SHG emission shifts from 8° to 13° at $\alpha = 30^\circ$ in focused light rather than 9° to 15° in collimated light. Comparing Fig. 7c to Fig. 4c, we see that when $\alpha = 60^\circ$, $I_{2\omega,xz}$ is the one that has been influenced greatly by the fibril polarity, the corresponding ϕ_{xz} for the collected peak SHG emission shifts from 5° to 85° . By the comparison of Fig. 7d to Fig. 4d, we realize that fibril polarity has no influence on $I_{2\omega,yz}$ at

◀ **Fig. 7** **a** SHG intensity $I_{2\omega}$ as the rotation of the angle ϕ_{xy} of polarizer under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° in collimated beam when $\rho = -2.6$. **b** SHG intensity $I_{2\omega,xy}$ as the rotation of the angle ϕ_{xy} under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° in focused beam when $\rho = -2.6$. **c** SHG intensity $I_{2\omega,xz}$ as the rotation of the angle ϕ_{xz} under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° in focused beam when $\rho = -2.6$. **d** SHG intensity $I_{2\omega,yz}$ as the rotation of the angle ϕ_{yz} under $\alpha = 0^\circ, 30^\circ, 60^\circ$, and 90° in focused beam when $\rho = -2.6$

polarization angle $\alpha = 0^\circ$ and a slight influence at $\alpha = 90^\circ$ that causes a shift of $\phi_{yz} = 86^\circ$ to $\phi_{yz} = 85^\circ$. In addition, the reverse of the fibril polarity causes the shift of ϕ_{yz} from 28° and 14° to 30° and 4° at $\alpha = 30^\circ$ and 60° , respectively.

4 Discussion and conclusions

Base on our established theory of SHG emission in collagen type I as the variation of the excitation polarization angle α under focused light, in this paper, we further characterize the differences between SHG emissions in type I collagen influenced by excitation polarization angle α and fibril polarity excited by collimated and focused beams, respectively.

The comparison of the emission SHG excited by collimated beams and focused beams demonstrates the following results. When the illumination is collimated, SHG propagates along the direction of the incident beam; thus, the SHG electric field is confined to be polarized in the xy plane, and two components of SHG emission along the x and y directions are included. However, when the illumination is focused, SHG propagates along a defined solid angle, which can be split into components along three axes; an extra component along the z axis exists in the focused beam.

On SHG emission components along the x and y directions, focusing induces a change of the relationship of $I_{2\omega,x}$ with the total SHG emission $I_{2\omega}$, where the maximum SHG emission at $\alpha = 0^\circ$ and 180° as well as the minimum SHG emission at $\alpha = 90^\circ$ and 270° of $I_{2\omega,x}$ are no longer coincident with $I_{2\omega}$. Obviously, $I_{2\omega,z}$ has an additional contribution to $I_{2\omega}$. Here, we should mention that Tiaho et al. [19] have their experimental conclusion that SHG has the maximum magnitude when the polarization angle of incident light is 40° , while our simulation results show that SHG has its maximum magnitude when the polarization angle is 0° . The apparent discrepancy is due to the difference of the prerequisite conditions. In the paper by Tiaho et al., they introduce a concept of the effective orientation angle θ_e of the harmonophores, which means that the angle was formed by the scatterers (dipoles) with the fibril axes. It is around $50\text{--}60^\circ$. In our theoretical simulation study, as we assume that the scatterers (dipoles) are distributed uniformly along the fibrils axes, based on this concept, the effective orientation angle is 0° . Because the initial polarization direction of the dipoles is different, the maximum SHG emission direction is different.

On the xy plane, the deviation angles γ_{xy} of the SHG emission along excitation polarization angle α has a similar pattern under collimated and focused beams where the maximum deviation angle $\gamma_{xy} = 21^\circ$ appears when α locates off the x axis 60° and the minimum deviation angle $\gamma_{xy} = 0^\circ$ appears when α locates along the x and y axes. The deviation angles γ_{xz} and γ_{yz} in the focused beam show different affected patterns by excitation polarization angle α . α has a non-symmetrical affect on γ_{yz} from the x and y axes. When α is off from the x axis, γ_{yz} decreases slowly to the peak value, while when α is off from the y axis, γ_{yz} decreases dramatically to the peak value. On the other hand, α has almost uniform influence on γ_{yz} .

The collected SHG emission signals vary as the variation angle ϕ of the polarizer as well as the polarization angle α . In xy plane, the variation pattern under all demonstrated cases

of polarization angle α shows similarity under collimated and focused beams, which shows that the collected SHG emission signals have peaks locating at two symmetrical angles ϕ_{xy} . However, there is a slight difference. The angle ϕ_{xy} correspondingly shifts from 0–9–21–0° in collimated light, yet 0–8–21–0° in focused light at different polarization angles α of 0–30–60–90°. In the xz and yz planes, the collected SHG emission signals show different patterns of the shift angles ϕ_{xz} and ϕ_{yz} in focused beam.

The fibril polarity has caused significant influence on $I_{2\omega,x}$ in both collimated and focused light cases. Two additional peak values of $I_{2\omega,x}$ appear at $\alpha = 90^\circ$ and 270° with no peaks appearing at $\alpha = 0^\circ$ and 180° . No visible effect is found on $I_{2\omega,y}$ and $I_{2\omega,z}$ in collimated light and focused light. Also, fibril polarity has induced the maximum value of γ_{xy} to increase from 21° to 90° in both cases. Moreover, in the focused light case, fibril polarity causes dramatic changes of γ_{xz} and affects the minimum values of γ_{yz} , decreasing them from 14° to 4° .

Generally, the effect of fibril polarity on $I_{2\omega,ij}$ is different at different polarization angle α in both collimated and focused light case. The reverse of fibril polarity has slight different influence on $I_{2\omega,xy}$ at $\alpha = 30^\circ$ comparing that in focused light to that in collimated light, where the peak intensity of $I_{2\omega,xy}$ shifts from 8° to 13° in focused light and 9° to 15° in collimated light. However, at $\alpha = 60^\circ$, it has a significant influence on $I_{2\omega,xy}$ in both collimated light and focused light, which induces the peak intensity of $I_{2\omega,xy}$ shifting from 21° to 90° . Influence that is more obvious occurs on $I_{2\omega,xz}$ and $I_{2\omega,yz}$ at $\alpha = 60^\circ$ in focused light.

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Appendix

We apply a matrix to represent SHG components along parallel (p) and perpendicular (s) directions of emission plane to the x , y , and z direction:

$$\begin{pmatrix} E_{2\omega,x} \\ E_{2\omega,y} \\ E_{2\omega,z} \end{pmatrix} = \begin{bmatrix} \cos \theta \cos \varphi & -\sin \varphi \\ \cos \theta \sin \varphi & \cos \varphi \\ -\sin \theta & 0 \end{bmatrix} \begin{pmatrix} E_{2\omega}^p \\ E_{2\omega}^s \end{pmatrix}, \quad (27)$$

$$E_{2\omega,x} = E_{2\omega}^p \cos \theta \cos \varphi - E_{2\omega}^s \sin \varphi, \quad (28)$$

$$E_{2\omega,y} = E_{2\omega}^p \cos \theta \sin \varphi + E_{2\omega}^s \cos \varphi, \quad (29)$$

$$E_{2\omega,z} = -E_{2\omega}^p \sin \theta \quad (30)$$

while

$$\begin{aligned}
 \vec{E}_{2\omega}^{(0)}(\theta, \varphi) &= \begin{pmatrix} E_{2\omega}^{(0),p}(\theta, \varphi) \\ E_{2\omega}^{(0),s}(\theta, \varphi) \end{pmatrix} = \eta \vec{M} \cdot \vec{\mu}_{2\omega}^{(0)} = \eta \vec{M} \cdot \frac{1}{2} \vec{E}_{\omega}^{(0)2} \beta \\
 &= \frac{\eta}{2} E_{\omega}^{(0)2} \begin{pmatrix} \cos \theta \cos \varphi & \cos \theta \sin \varphi & -\sin \theta \\ -\sin \varphi & \cos \varphi & 0 \end{pmatrix} \begin{pmatrix} \beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha \\ \beta_{xyy} \sin 2\alpha \\ 0 \end{pmatrix} \\
 &= \frac{\eta}{2} E_{\omega}^{(0)2} \begin{pmatrix} \cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha \\ -\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \varphi \sin 2\alpha \end{pmatrix}.
 \end{aligned} \tag{31}$$

Therefore,

$$E_{2\omega}^{(0),p}(\theta, \varphi) = \frac{\eta}{2} E_{\omega}^{(0)2} [\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha], \tag{32}$$

$$E_{2\omega}^{(0),s}(\theta, \varphi) = \frac{\eta}{2} E_{\omega}^{(0)2} [-\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \varphi \sin 2\alpha], \tag{33}$$

$$\begin{aligned}
 E_{2\omega}(\theta, \varphi) &= N E_{2\omega}^{(0)} A(\theta, \varphi) \\
 &= \frac{\eta N A(\theta, \varphi)}{2} E_{\omega}^{(0)2} \begin{pmatrix} \cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha \\ -\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \varphi \sin 2\alpha \end{pmatrix},
 \end{aligned} \tag{34}$$

$$E_{2\omega}^p(\theta, \varphi) = \frac{\eta N A(\theta, \varphi)}{2} E_{\omega}^{(0)2} [\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha], \tag{35}$$

$$E_{2\omega}^s(\theta, \varphi) = \frac{\eta N A(\theta, \varphi)}{2} E_{\omega}^{(0)2} [-\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \varphi \sin 2\alpha]. \tag{36}$$

Finally,

$$\begin{aligned}
 E_{2\omega,x} &= E_{2\omega}^p \cos \theta \cos \varphi - E_{2\omega}^s \sin \varphi \\
 &= \frac{\eta N A(\theta, \varphi)}{2} E_{\omega}^{(0)2} \\
 &\quad \{ [\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha] \cos \theta \cos \varphi \\
 &\quad - [-\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \varphi \sin 2\alpha] \sin \varphi \},
 \end{aligned} \tag{37}$$

$$\begin{aligned}
E_{2\omega,y} &= E_{2\omega}^p \cos \theta \sin \varphi + E_{2\omega}^s \cos \varphi \\
&= \frac{\eta NA(\theta, \varphi)}{2} E_{\omega}^{(0)2} \{ [\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha] \\
&\quad \times \cos \theta \sin \varphi + [-\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) + \beta_{xyy} \cos \varphi \sin 2\alpha] \cos \varphi \},
\end{aligned} \quad (38)$$

$$\begin{aligned}
E_{2\omega,z} &= -E_{2\omega}^p \sin \theta = \frac{\eta NA(\theta, \varphi)}{2} E_{\omega}^{(0)2} \{ -[\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\
&\quad + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha] \sin \theta \}.
\end{aligned} \quad (39)$$

As a result, we can derive emission SHG intensity in the x , y , and z direction components

$$\begin{aligned}
I_{2\omega,x} &= \frac{1}{8} n_{2\omega} c \varepsilon_0 \eta^2 N^2 E_{\omega}^{(0)4} \iint A^2(\theta, \varphi) \{ [\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\
&\quad + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha] \cos \theta \cos \varphi - [-\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\
&\quad + \beta_{xyy} \cos \varphi \sin 2\alpha] \sin \varphi \}^2 \sin \theta d\theta d\varphi,
\end{aligned} \quad (40)$$

$$\begin{aligned}
I_{2\omega,y} &= \frac{1}{8} n_{2\omega} c \varepsilon_0 \eta^2 N^2 E_{\omega}^{(0)4} \iint A^2(\theta, \varphi) \{ [\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\
&\quad + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha] \cos \theta \sin \varphi + [-\sin \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\
&\quad + \beta_{xyy} \cos \varphi \sin 2\alpha] \cos \varphi \}^2 \sin \theta d\theta d\varphi,
\end{aligned} \quad (41)$$

$$\begin{aligned}
I_{2\omega,z} &= \frac{1}{8} n_{2\omega} c \varepsilon_0 \eta^2 N^2 E_{\omega}^{(0)4} \iint A^2(\theta, \varphi) \{ -[\cos \theta \cos \varphi (\beta_{xxx} \cos^2 \alpha + \beta_{xyy} \sin^2 \alpha) \\
&\quad + \beta_{xyy} \cos \theta \sin \varphi \sin 2\alpha] \sin \theta^2 \} \sin \theta d\theta d\varphi.
\end{aligned} \quad (42)$$

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