

Article

Monitoring of Wool Stretching Process Using Polarized Second Harmonic Generation

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Abstract

Wool fibers undergo significant structural changes during industrial stretching, which directly impact their mechanical properties and textile performance, making monitoring of the stretching process essential for optimizing wool products. In this study, we demonstrate the effective use of polarized second harmonic generation (P-SHG) imaging for monitoring the wool fiber stretching process. P-SHG is highly sensitive to non-centrosymmetric structures, enabling clear observation of changes in α -keratin alignment and the reconstruction of cortical interfaces during stretching. Quantitative P-SHG analysis revealed a significant decrease in the effective pitch angle (θ_e) from $54^\circ \pm 1^\circ$ to $33^\circ \pm 3^\circ$ after stretching, confirming the dipole orientation changes in keratin molecules. These findings were further validated through additional characterization techniques, including scanning electron microscopy (SEM), polarizing optical microscopy (POM), X-ray diffraction (XRD), and Raman spectroscopy (RS). The results show that the industrial stretching process of wool alters the morphology at the surface scale, enhances the alignment of macroscopic fibers, and induces a transition from α -helix to β -sheet. Our technique is simple, effective, and capable of in situ monitoring of the structural changes in wool fibers, making it highly promising for applications in the wool industry.

Keywords: wool stretching; polarized second harmonic generation; α -keratin; molecular orientation; nonlinear optical microscopy



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1. Introduction

Wool stretching is a core process in the wool processing industry, as it enhances the wool fiber's dimensional stability, tensile strength, and elasticity [1–6]. However, this process currently still mainly relies on manual experience, and coupled with the inherent differences between different types and batches of wool, it often leads to problems of insufficient stretching or over-stretching, which directly affect product quality. The performance optimization of the wool stretching process essentially originates from the transformation of its internal microstructure, with the core lying in the secondary structure transformation of α -keratin, the main component of the fibers. Existing studies have

confirmed that the conformational transformation of α -keratin from α -helix to β -sheet under stretching, as well as the accompanying changes in molecular arrangement, are the key factors leading to fundamental changes in the mechanical properties of wool [7–10]. These microstructural characteristics directly determine the core performance of the final wool products. Thus, to optimize the stretching process, it is important to gain a clear understanding of the microstructural mechanisms during fiber stretching. Such insights are essential for preventing fiber damage under high stretching ratios and improving processing efficiency.

Previous studies have linked fiber performance to structural transitions within α -helices, especially helix-to-sheet conversion and alignment changes. Conventional characterization methods such as microscopy [11,12], infrared/Raman spectroscopy [13,14], and X-ray diffraction (XRD) [15,16] can reveal morphological and structural changes but are limited to offline analysis and lack real-time monitoring capabilities. P-SHG microscopy, leveraging its capabilities for label-free detection, high spatial resolution, and quantitative structural analysis [17–20], offers an effective solution for the dynamic characterization of local microstructures in protein fibers. In recent years, the successful application of nonlinear optical imaging techniques across various protein fiber systems has further validated their distinctive advantages. In collagen fiber research, Wohlgemuth et al. employed SHG microscopy to achieve, for the first time, the visualization and quantitative analysis of strain-dependent dynamic reorganization of collagen fibers within skeletal muscle extracellular matrix, demonstrating the evolution of both intrinsic fiber alignment index and axial alignment index with increasing strain [21]. In silk fibroin fiber research, Harvey et al. demonstrated that polarized second harmonic generation (P-SHG) microscopy exhibits high sensitivity to β -sheet orientation and molecular packing structures [22]. In hair fiber analysis, Kano et al. utilized ultra-multiplex coherent anti-Stokes Raman scattering (CARS) combined with SHG/third harmonic generation (THG) microscopy to achieve label-free chemical imaging and molecular species identification of intramedullary lipids (IMLs) at sub-micrometer scales [23]. These pioneering studies establish multimodal nonlinear optical microscopy as a powerful approach for characterizing protein fiber microstructures. Nevertheless, systematic investigations into the dynamic structural evolution of wool fibers under tensile loading using P-SHG remain conspicuously lacking. Developing in situ P-SHG imaging to capture the real-time unwinding, fragmentation, and reorganization of α -helices during stretching is therefore crucial for advancing the understanding of wool fiber structure–function–mechanics relationships.

In this study, we report on the monitoring of structural changes in wool fibers during industrial stretching using P-SHG imaging. SHG images of both natural and stretched wool were acquired to visualize changes in molecular orientation. By comparing these results with SEM and RS, we demonstrated the accuracy of the P-SHG measurements. Paying attention to the P-SHG technique, it is simple, non-contact, and capable of integration into stretching equipment, making it highly promising for real-time monitoring of wool stretching in the wool industry.

2. Materials and Methods

2.1. Materials

Two types of wool samples were used: natural wool and stretched wool. The stretched samples were prepared using a TJGD-ERDOS-02 wool stretching apparatus (Tianjin Polytechnic University, Tianjin, China). The stretching procedure involved three main processes: pretreatment, stretching, and setting, which included both physical manipulation and chemical modification. In the pretreatment step, raw wool was soaked in a solution containing 3% sodium bisulfite and 0.1% JFC at 80 °C for 5 min. The wool was then mechanically

stretched to 1.6 times its original length. In the post-treatment step, the fibers were steamed at 105 °C for 45 min, followed by immersion in 1.0% hydrogen peroxide for 12 h, and then air-dried.

2.2. Measurements

2.2.1. Polarized Second Harmonic Generation Microscopy

The nonlinear optical imaging setup is illustrated in Figure 1. A femtosecond Ti: sapphire laser (Mai Tai DeepSee, 80 fs, 80 MHz) (MKS Instruments, Andover, MA, USA) operating at 800 nm and 20 mW was used as the excitation source. The beam passed through a half-wave plate (HWP) and a polarizing beamsplitter (PBS) for power and polarization control. After reflection and scanning via a galvo-galvo mirror, the beam was focused through a scan lens (SL), tube lens (TL), dichroic mirror (DM), another HWP, and a 20× objective (NA = 0.75) onto the wool sample. Backward-propagating SHG signals were collected by the objective and filtered using a 400/10 nm band-pass filter before detection by a photomultiplier tube (PMT). When measuring P-SHG imaging, the polarization angle of the incident beam was adjusted from 0° to 180° in 20° increments for anisotropy analysis. Alternatively, the SHG signal collected by the objective lens can be directly detected by a spectrometer (Spec). We used a Princeton spectrometer (HRS-300, Teledyne Princeton Instruments, Trenton, NJ, USA) to perform spectral measurements on natural and stretched wool fibers, with an integration time of 60 s. We acquired the spectra under fixed linear polarization excitation without polarization scanning. This measurement serves solely to verify the SHG origin of the signals observed in the imaging experiments, while subsequent polarization-resolved imaging and analysis are based on the SHG intensity variations under different excitation polarization angles.

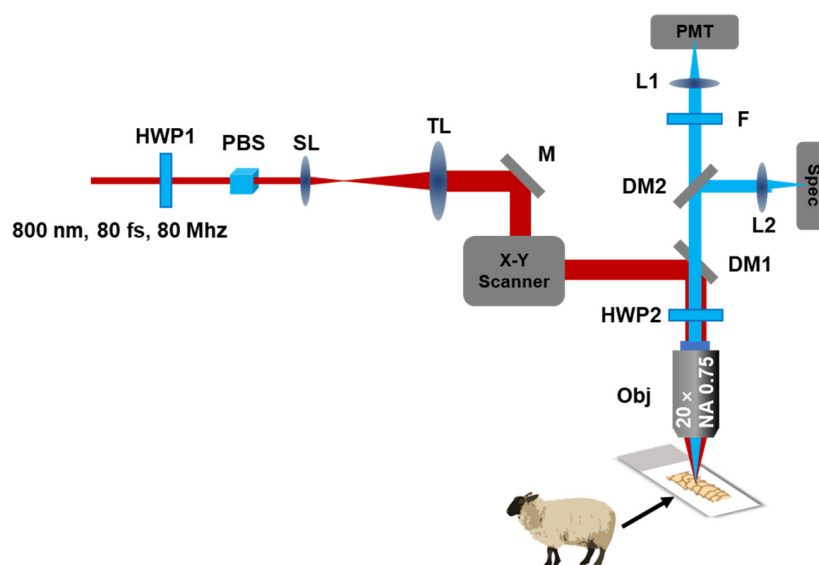


Figure 1. Schematic of the nonlinear optical imaging system used for polarized second harmonic generation (P-SHG) microscopy. HWP: half-wave plate; PBS: polarizing beamsplitter; SL: scan lens; TL: tube lens; M: mirror; DM: dichroic mirror; F: filter; L: lens; Obj: objective lens; PMT: photomultiplier tube; Spec: spectrometer.

2.2.2. Polarizing Optical Microscopy

Fiber birefringence and anisotropic structures were observed using a ZEISS Axio-scope 5 (Carl Zeiss Microscopy GmbH, Jena, Germany) polarizing microscope with a 20× objective.

2.2.3. X-Ray Diffraction

The crystalline structure was characterized using a SmartLab 9 kW X-ray diffractometer (Rigaku Corporation, Tokyo, Japan) equipped with a Cu target. Scanning was performed over a 2θ range of 5° to 80° at a speed of $10^\circ/\text{min}$ to detect changes in crystallinity and inter-planar spacing.

2.2.4. Raman Spectroscopy

Molecular bonding and conformational changes in wool were assessed using a Renishaw InVia confocal Raman spectrometer (Renishaw, Gloucestershire, UK) with a 785 nm laser at 50 mW power, 1200 L/mm grating, and $50\times$ objective. Spectra were recorded with a 10 s integration time and a resolution of 1 cm^{-1} .

2.2.5. Scanning Electron Microscopy

The surface morphology of wool fibers was observed using a Hitachi SU8200 field (Hitachi High-Tech Corporation, Tokyo, Japan) emission scanning electron microscope operating at 5 kV with a magnification of $\times 2000$. Wool fiber bundles were mounted onto conductive stubs, sputter-coated with gold, and examined under vacuum.

2.3. Theory

The biophysical model used here refers to SHG-active supramolecular assemblies with cylindrical symmetry [20,24,25]. This assumption is commonly applied to fibrous biological structures like collagen and myosin, where the SHG signal originates from ordered, non-centrosymmetric molecular arrangements along a dominant axis [17,26]. For wool keratin fibers, particularly the α -keratin fiber bundles within the cortical region, we assume that they are cylindrically symmetric along the main axis and oriented in-plane with respect to the imaging plane [27,28]. While the complex hierarchical organization of wool fibers and potential local symmetry breaking due to cuticle structure or microfibrillar arrangements, this cylindrical approximation is a reasonable simplification for analyzing the macroscopic polarization response of the SHG-active components, especially given the enhanced alignment upon stretching. The dependence of the second harmonic signal of this structure on the polarization of the fundamental beam input can be expressed as:

$$I^{\text{SHG}}(\phi) = C^2 \sin^2[2(\phi - \alpha)] + [A \sin^2(\phi - \alpha) + B \cos^2(\phi - \alpha)]^2 \quad (1)$$

Here, ϕ and α are the directions of the cylinder's long axis (which we assume aligns with the arrangement of the supramolecular assembly) and the angle of the basic beam polarization, respectively, defined relative to the laboratory x-axis. $A = I_0 d_{31}$, $B = I_0 d_{33}$ and $C = I_0 d_{15}$, where I_0 is proportional to the strength of the excitation field, d_{31} , d_{33} and d_{15} are the non-zero elements of the nonlinear susceptibility tensor describing the organization under the cylindrical symmetry assumption [26].

The free parameters A, B, C, and ϕ are typically obtained by fitting the experimental SHG images for each polarization α to Equation (1). It was found that another form of the model represented in Equation (1) is more suitable for fitting experimental PSHG images using a Fourier series expansion. Equation (1) can be rewritten in a more convenient form as a sum of cosine frequency components, as follows:

$$I^{\text{SHG}}(\phi) = \alpha_0 + \alpha_2 \cos 2(\phi - \alpha) + \alpha_4 \cos 4(\phi - \alpha) \quad (2)$$

Here, $\alpha_0 = C^2/2 + 3/8(A^2 + B^2) + AB/4$, $\alpha_2 = B^2/2 - A^2/2$, $\alpha_4 = (A - B)^2/8 - C^2/2$. By fitting the P-SHG images using Equation (2), we can obtain the parameters ϕ , α_0 , α_2 , and

α_4 . The parameters ϕ , α_0 , α_2 , and α_4 contain all the information related to our biophysical model (tensor elements).

To fit the model described by Equation (2), Amat-Roldan et al. proposed FF-PSHG analysis [29]. Through FF-PSHG analysis, we can not only calculate the ratios of the $\chi^{(2)}$ tensor elements but also determine the in-plane orientation of collagen (φ) and the orientation of the dominant axis of the hyperpolarizability tensor (θ_e), which is related to the helical pitch angle of α -helix keratin. The helical pitch angle can be derived from the ratios of the $\chi^{(2)}$ tensor elements as follows:

$$\cos^2 \theta_e = \frac{B}{2A + B} = \frac{\chi_{33}/\chi_{31}}{2 + \chi_{33}/\chi_{31}} \quad (3)$$

3. Results and Discussions

3.1. P-SHG Studies

We first performed spectroscopic measurements on natural and stretched wool fibers to directly validate the SHG origin of the signals observed in our imaging experiments. The corresponding spectra are shown in Figure 2. The detected spectra revealed a characteristic narrow peak centered at 400 nm (FWHM = 5 nm), corresponding exactly to half of the excitation wavelength (800 nm), superimposed with a broad two-photon fluorescence band ranging from 405 to 670 nm. In our P-SHG microscopy setup, a band-pass filter (400/10 nm) was employed to selectively transmit signals within the 395–405 nm spectral window, thereby ensuring specific detection of the SHG signal at 400 nm while excluding the broadband fluorescence background. Consequently, the subsequent analysis and discussion in this work focus exclusively on the SHG signals.

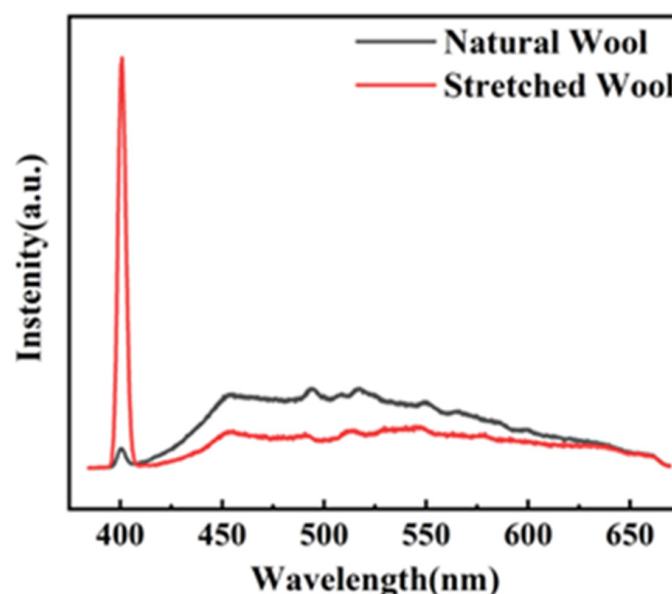


Figure 2. Spectra of natural and stretched wool fibers.

To investigate the anisotropy, alignment, and molecular ordering of keratin fibers in wool before and after stretching, we recorded the natural and stretched wool using SHG (Figure 3A). To show the surface changes, we first took bright-field images of natural and stretched wools in Figure 3A(I,II), revealing that the surface morphology and scale structures of the wool fibers became smoother and flatter after stretching.

Next, we imaged the fibers using SHG as shown in Figure 3A(III,IV). The generation of SHG signals in wool fibers stems fundamentally from the intrinsic non-centrosymmetric nature of their molecular architecture; however, the signal intensity remains relatively

weak due to inherent limitations in supramolecular assembly. The photophysical mechanism underlying this phenomenon can be elucidated as follows: In α -helical keratin, polypeptide chains adopt an α -helical conformation. Individual α -helix monomers exhibit right-handed helical chirality, with peptide bond dipole moments oriented along the helical axis (N $\rightarrow$ C direction), thereby generating a net polarization component. Two such monomers intertwine in a parallel fashion to form a left-handed coiled-coil dimer [30], enabling constructive superposition of constituent dipole moments and producing a measurable second-order nonlinear optical susceptibility ($\chi^{(2)}$), consequently resulting in the SHG signal. Nevertheless, upon hierarchical assembly of these dimers into protofilaments, adjacent structural units adopt an antiparallel arrangement, leading to partial cancellation of dipole moments and consequent attenuation of the macroscopic $\chi^{(2)}$ magnitude [31], which accounts for the experimentally observed weak SHG activity in wool fibers.

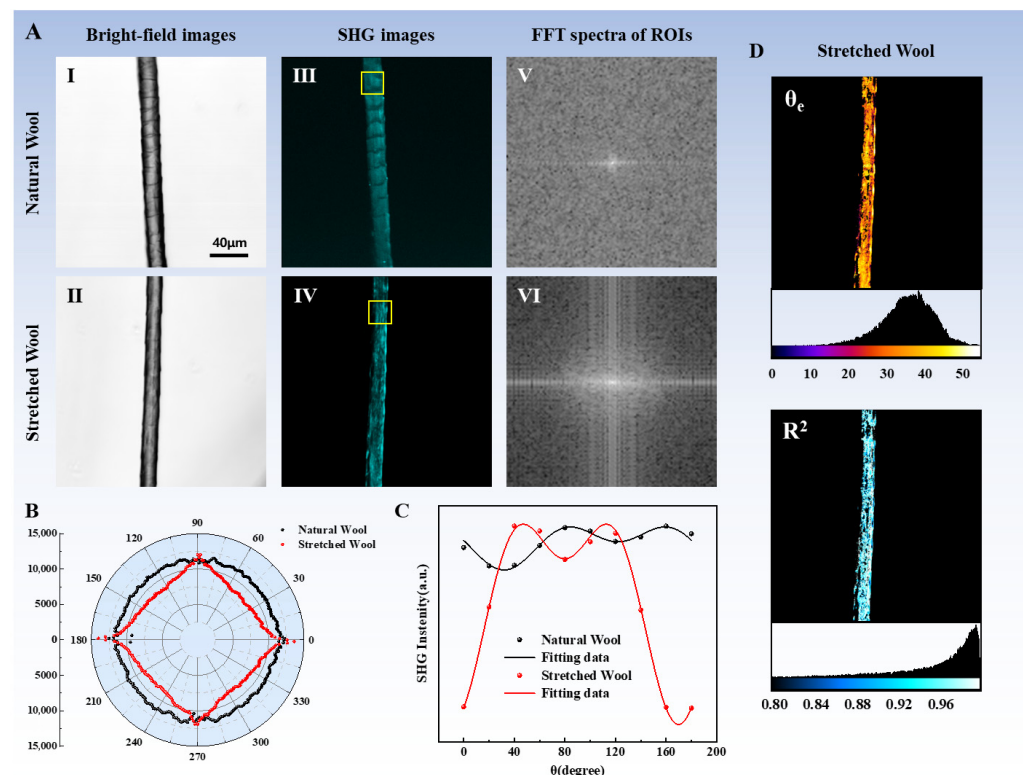


Figure 3. (A) Nonlinear optical images of wool fibers before and after stretching, as well as fast Fourier transform (FFT) spectra of selected regions of interest (ROI, Yellow square area). The first column (I,II) presents bright-field images, the second column (III,IV) displays the SHG images, and the third column (V,VI) shows FFT spectra of the selected ROIs. (B) Polar plots of the FFT spectra of the natural and stretched wool fibers. (C) Curve showing the relationship between the incident light polarization and SHG intensity measured within the ROIs of the natural and stretched wool fibers. (D) Evaluation images of CollagenFit fitting stretched wool fibers: θ_e and R^2 .

As illustrated in Figure 3A(IV), the significant enhancement of SHG signals in stretched wool fibers originates from a synergistic structural modulation involving both oxidation and mechanical stretching, wherein mechanical stretching serves as the predominant physical factor governing molecular orientation and conformational transitions, while oxidation primarily functions as a chemical facilitator for structural fixation and auxiliary processing. Specifically, mechanical stress induces the extension and linearization of keratin molecular chains along the direction of stress, substantially improving the axial alignment order of molecular dipole moments. This enhanced orientational order effectively amplifies the axial component of the macroscopic second-order nonlinear susceptibility ($\chi^{(2)}$), thereby

establishing the direct structural foundation for SHG enhancement. Previous investigations on structural proteins with analogous architectures, such as collagen and silk fibroin [18,21], have demonstrated that mechanical stretching can increase the frequency doubling strength by enhancing the orientation distribution of nonlinear scatterers. Our findings substantiate this perspective and further confirm that industrial stretching processes profoundly influence the SHG response and optical anisotropy of wool fibers. Such stretching not only facilitates the conformational transition from α -helix to β -sheet structures but, more critically, drives the highly ordered axial arrangement of both α -helical and β -sheet domains, resulting in supramolecular architectures with pronounced structural anisotropy that substantially amplify optical signal generation. In this process, moderate oxidation treatment (e.g., hydrogen peroxide processing) can break the dense disulfide bond network in wool [32], reduce the cross-linking constraints between molecular chains, and thus promote protein chain rearrangement and orientation at the molecular level. Literature reports indicate that oxidative stress can induce alterations in collagen fibrillar organization, manifesting as increased D-band periodicity and modified anisotropy factors [33]. In the wool system, oxidative cleavage of disulfide bonds (yielding thiol or sulfonic acid moieties) generates the necessary structural prerequisites for molecular chain slippage and reorganization under tensile forces. Nevertheless, the marked enhancement in signal intensity is primarily attributable to the stretching-induced reorientation of keratin molecules and the resultant alignment of dipole moments, which form highly ordered, non-centrosymmetric configurations along the fiber axis, thereby enhancing constructive interference in second harmonic generation. Mechanical stretching constitutes the direct driving force for establishing such anisotropic supramolecular arrangements, whereas oxidation serves merely as an auxiliary means that enables structural reorganization through the breaking of chemical bonds.

Notably, although the overall SHG signal increases after stretching, its spatial distribution becomes less uniform, as shown in Figure 3A(IV). This may result from non-uniform tensile stress across the fiber: edge regions are more fully straightened than central regions, where slight residual crimping may remain, leading to stronger SHG signals at the periphery and weaker ones at the core. In addition, macroscopic straightening cannot completely eliminate microscopic entanglements such as twists or kinks among fibrils, which reintroduce local disorder and disrupt fibril alignment, further causing differences in SHG signals. These microscopic heterogeneities and local structural variations, such as those introduced by the cuticle structure and the intricate packing of microfibrils, can indeed lead to local symmetry breaking. Such local symmetry breaking can influence the local nonlinear optical response. However, our P-SHG analysis, particularly the quantitative assessment of effective pitch angle (θ_e), primarily reflects the average orientation and conformational state of the α -keratin fibril bundles within the cortical region, which are the dominant contributors to the SHG signals. The observed signal non-uniformity not only reflects the microscale differences in stress transfer but also provides a nonlinear optically sensitive early diagnostic basis for damage assessment and processing optimization of wool fibers.

To quantitatively assess the structural changes in wool fibers before and after stretching, fast Fourier transform (FFT) analysis was performed on the regions of interest (ROI) selected from the SHG composite images shown in Figure 3A(V,VI). Compared to single-angle SHG images, polarization-averaged SHG composites provide a more reliable representation of overall fiber orientation. Using the Polar Transformer plugin in ImageJ 1.54g, the FFT images were converted from Cartesian to polar coordinates (q, θ), and radial integration from the center was performed to extract the angular distribution of Fourier intensities. The resulting polar plots are shown in Figure 3B. The unstretched wool fibers exhibit weak, isotropic distributions with no dominant orientation, corresponding to their randomly

coiled and disordered structure. In contrast, the stretched fibers show a pronounced intensity increase along 0° and 180° , indicating improved alignment along the stretching axis and enhanced nonlinear polarization. This approach provides a sensitive and quantitative method to evaluate fiber orientation and processing effects, offering valuable insights for optimizing fiber treatment in textile manufacturing.

Figure 3C presents the polarization-resolved SHG responses of wool fibers before and after stretching, along with the corresponding fitted effective pitch angle θ_e . By fitting the polarization-dependent SHG intensity curves within the ROI using Equation (2) and calculating θ_e via Equation (3), we obtained $\theta_e \approx 54^\circ \pm 1^\circ$ ($R^2 = 0.94$ of the fitting curve) for natural wool fibers and $\theta_e \approx 33^\circ \pm 3^\circ$ ($R^2 = 0.99$ of the fitting curve) after stretching. The pitch angle of wool fibers decreases after stretching, indicating that the dipole orientation changes. Figure 3D shows the pitch angle θ_e and R^2 of stretched wool fibers calculated using CollagenFitJ [34]. For stretched wool fibers, the effective pitch angle (θ_e) obtained from CollagenFitJ analysis is approximately 38° . This result is basically consistent, within a reasonable margin of error, with the value ($33^\circ \pm 3^\circ$) we obtained by fitting Equation (2) in selected representative regions. The slight differences between the two mainly arise from the different spatial scales of the analysis methods: in this study, a randomly chosen square region was analyzed, whereas the CollagenFitJ plugin performs a global average over the entire image, which is more susceptible to the influence of fiber edges or local structural heterogeneities. Nevertheless, the consistency in trend and magnitude between the results of both methods fully validates the reliability of the P-SHG analysis approach used in this study.

3.2. SEM, POM and XRD Studies

The surface morphologies of natural and stretched wool fibers are shown in Figure 4A. Panel (I) displays an SEM image of natural wool, while Panel (II) shows an image of stretched fiber. The surface of natural wool exhibits characteristic ring-like or semi-ring scale structures, with compact overlapping scale layers. This morphology contributes to the wool's distinctive frictional properties and felting behavior. After stretching, significant morphological changes are observed: the fibers elongate axially and contract radially, the surface becomes smoother, and the spacing between adjacent scales increases. In some cases, noticeable delamination or lifting of scale edges is evident. These features indicate structural disruption and rearrangement in the outer layer of the fiber under tensile stress.

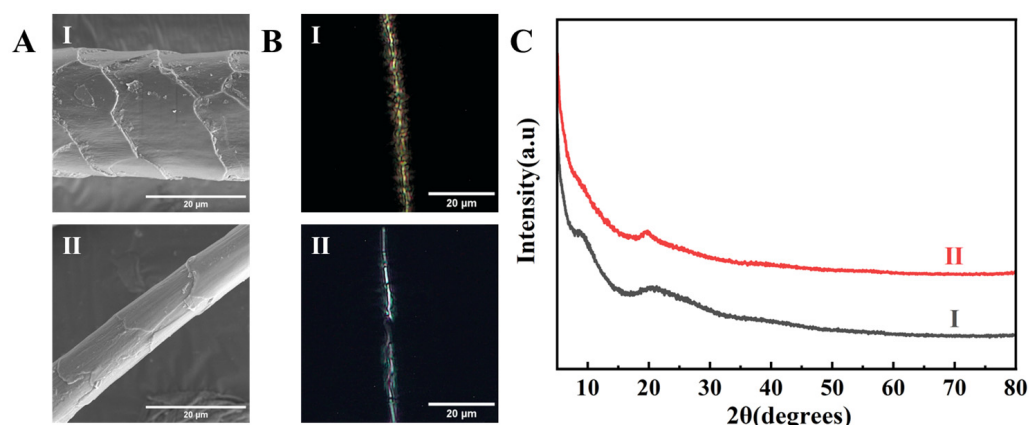


Figure 4. (A) SEM images of (I) natural and (II) stretched wool. (B) POM images of (I) natural and (II) stretched wool. (C) XRD of (I) natural and (II) stretched wool.

Quantitative analysis of SEM images reveals a reduction in fiber diameter from above $20\ \mu\text{m}$ (natural wool) to below $15\ \mu\text{m}$ (stretched wool). The scale layers become thinner,

and the overall fineness of the fibers improves, resulting in enhanced softness. These changes are closely related to molecular chain realignment and cortical compaction induced by stretching.

Polarizing optical microscopy images of wool fibers before and after stretching are shown in Figure 4B. Natural wool exhibits a characteristic scale layer morphology, where ring-like or tile-shaped scales cover the fiber surface. These scales have relatively thick, curled edges that contribute to pronounced surface roughness. Under polarized light in Figure 4B(I), this multi-layered and irregular structure causes complex refraction and reflection, resulting in alternating light and dark textures along the fiber axis with a serrated appearance. The cortical layer displays birefringence due to the crystalline α -helical microfibrils, whereas the disordered cuticle layers exhibit weak birefringence, appearing as dark contours that highlight surface irregularities.

After stretching, the internal and surface structures of wool fibers undergo significant reorganization. The fibers become more elongated, and the cuticle scales tend to align more uniformly along the fiber axis. As shown in Figure 4B(II), the stretched fibers exhibit increased axial alignment and optical uniformity, with enhanced interference brightness and stronger birefringence, reflecting improved molecular orientation. These observations are consistent with the P-SHG results, further supporting the mechanism of α -keratin chain rearrangement and microstructural ordering induced by mechanical stretching.

The crystallinity of a fiber is a key parameter that reflects its aggregated molecular structure and significantly influences its thermal, optical, and mechanical properties, as well as dimensional stability, light resistance, and thermal aging performance [35]. As a natural biopolymer, wool exhibits both crystalline and amorphous regions. According to the two-phase model [36], crystalline protofibrils are dispersed within an amorphous matrix and cannot be physically separated. To evaluate the crystalline structure of wool fibers before and after stretching, XRD analysis was performed, and the results are shown in Figure 4C. The natural wool exhibited diffraction peaks near $2\theta \approx 9^\circ$ and 21.5° , corresponding to contributions from both α -helical and β -sheet structures. In contrast, the stretched wool fibers displayed a sharper, more pronounced peak around 19.4° , while the peak near 9° diminished significantly. This suggests that stretching induces partial unfolding of α -helices into more extended β -sheets, leading to increased crystallinity. Additionally, the main diffraction peak shifted from 21.5° to 19.4° , indicating an increase in interplanar spacing caused by tensile stress during processing. Higher crystallinity reflects more orderly molecular chain alignment, which correlates with improved mechanical properties such as tensile strength and elastic modulus, as well as enhanced thermal insulation performance.

3.3. Raman Spectroscopy Analysis of Secondary Structure Transition

We performed Raman spectroscopy to monitor the structural changes in wool fibers before and after stretching. Figure 5 shows the normalized Raman spectra, calibrated to the CH_2 bending band at 1453 cm^{-1} . The main bands observed include amide I ($1650\text{--}1680\text{ cm}^{-1}$), amide III ($1230\text{--}1300\text{ cm}^{-1}$), and C–C backbone stretching ($890\text{--}960\text{ cm}^{-1}$). Peaks at 1657 cm^{-1} and 1673 cm^{-1} correspond to α -helix and β -sheet structures, respectively. Compared to natural wool, the stretched samples showed a decrease in the 1657 cm^{-1} band and an increase at 1673 cm^{-1} , indicating a conformational transition from α -helix to β -sheet.

Gaussian fitting of the amide I region (Figure 5B) revealed that the α -helix content decreased from 54.1% to 28.7%, while the β -sheet content increased from 19.1% to 32.0%. The reduction in full width at half maximum (FWHM) after stretching indicates increased crystallinity, consistent with the XRD findings. Additionally, the Raman peak near 935 cm^{-1} ,

associated with the α -helical C–C backbone stretching, showed a marked intensity decrease, further confirming the loss of helical order.

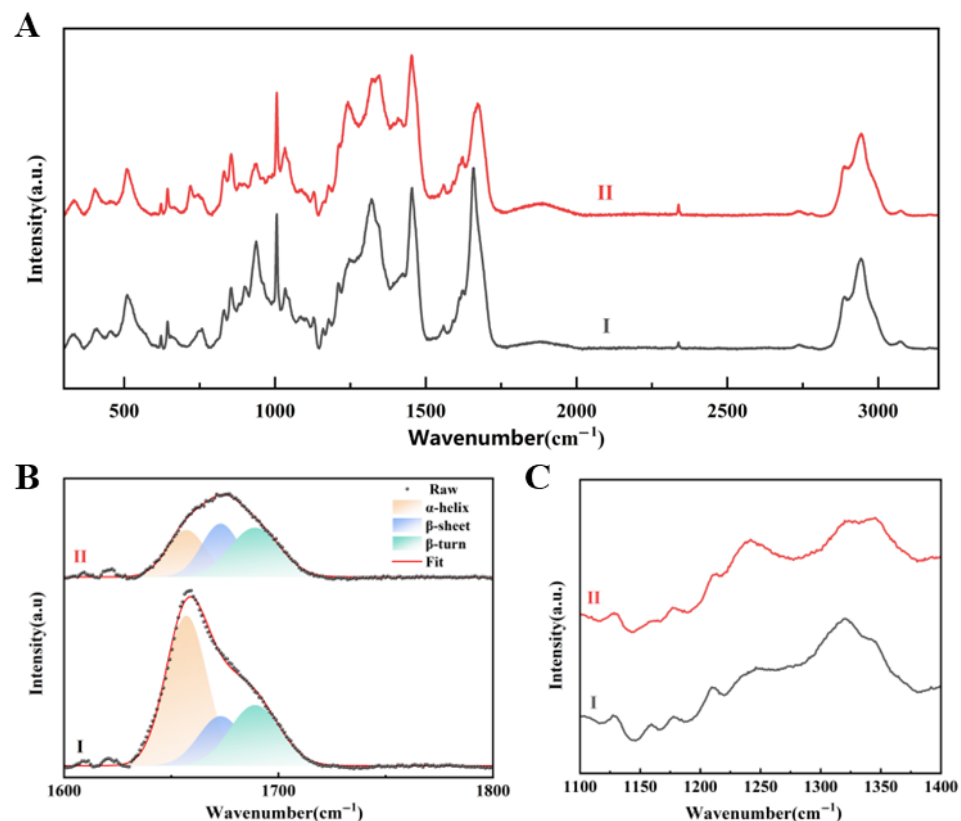


Figure 5. (A) Raman spectra of wool fibers: (I) natural and (II) stretched wool. (B) Comparison of the amide I region: (I) natural and (II) stretched wool. (C) Comparison of the amide III region: (I) natural and (II) stretched wool.

Disulfide bond behavior was analyzed via the 510 cm^{-1} band, corresponding to g–g–g conformation. Slight intensity reduction after stretching suggests that sodium bisulfite may cleave disulfide bonds, followed by reformation under oxidative treatment in new stretched conformations. This bond rearrangement contributes to fiber fixation and shape retention.

In the amide III region (Figure 5C), we observed a peak shift from 1245 cm^{-1} (disordered structures) to 1240 cm^{-1} (β -sheet), supporting the conclusion that stretching induces β -sheet formation. These observations are in agreement with previous studies (e.g., Jeffrey S. Church) [37].

4. Conclusions

In summary, this study confirms the effectiveness of P-SHG microscopy in monitoring the stretching process of wool fibers through the quantitative evaluation of molecular orientation evolution. Specifically, the effective pitch angle θ_e decreased significantly from $54^\circ \pm 1^\circ$ to $33^\circ \pm 3^\circ$, directly demonstrating the substantial reorganization of keratin molecules along the fiber axis. This structural evolution arises from the synergistic effect of mechanically induced molecular orientation ordering (the dominant mechanism) and disulfide bond breakage and molecular rearrangement mediated by oxidation (the auxiliary mechanism). Their coupling enables highly ordered structure formation from the molecular scale to the macroscopic level, thereby significantly enhancing SHG signal intensity. In subsequent studies, we aim to further deconstruct the independent contributions of these mechanisms through control experiments. Compared with traditional offline characterization techniques (such as XRD, SEM, and FTIR), P-SHG technology demonstrates significant

advantages, including non-invasive in situ detection, submicron spatial resolution, and quantitative analysis of molecular orientation, and it is particularly useful for assessing the stretching state of wool fibers through the θ_e parameter. Currently, we are working to expand the application of P-SHG technology in imaging complex systems, with the goal of developing simple and universal in situ monitoring solutions for industrial production processes. It should be noted that the cylindrical symmetry model employed in this study primarily describes the supramolecular assembly of α -keratin within the cortical region. Although local microstructures (such as cuticle structure and microfibril bundles) may lead to symmetry breaking, this approach can still reflect the orientation changes in fibers during stretching. In conclusion, this study provides an effective SHG-based approach for non-destructive, high-resolution characterization of wool fiber structural evolution, offering significant scientific value and application potential.

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